Immunology

Historical Milestones

The concept of forbidden foods that should not be eaten goes back to the Garden of Eden and apart from its religious meanings it may also have foreshadowed the concept of foods that can provoke adverse reactions. Thus we could say that allergic diseases have plagued mankind since the beginning of life on earth. The prophet Job was affected by a condition that following the rare symptoms described by the Holy Bible might be identified as a severe form of atopic dermatitis (AD). The earliest record of an apparently allergic reaction is 2621 B.C., when death from stinging insects was first described by hieroglyphics carved into the walls of the tomb of Pharaoh Menes depicting his death following the sting of a wasp. In 79 A.D., the death of the Roman admiral Pliny the Elder was ascribed to the SO_2-rich gases emanating from the eruption of Mount Vesuvius. Hippocrates (460–377 B.C.) was probably the first to describe how cow's milk (CM) could cause gastric upset and hives, proposing dietetic measures including both treatment and prevention for CM allergy. He also coined the term "ασθμα," meaning breathlessness. Subsequently, Thucydides described that during the plague afflicting Athens from 430 to 429 B.C., "those people who recovered from the disease rarely developed the illness a second time and never mortally," an observation that was verified by Panum in 1847 [407]. The Roman poet Lucretius (98–55 B.C.) stated that "what is food to one, is a bitter poison to others." Galen (129–202) was the first to describe allergy to goat's milk and also evidenced the fifth cardinal sign of inflammation, that is the loss of function or *functio laesa* not previously described by Celsus (first century B.C.). Perhaps an example of tolerance could be that of Mithridates (132–63 B.C.), who reportedly acquired immunity against poisons by assuming progressively increasing doses of each poison, a precursor of the oral desensitization by taking small incremental amounts of noxious foods. The concept of immunity, deriving from the Latin *immunitas* (meaning being exempt from), a century ago was linked by early immunologists to the resistance of an individual to infections; therefore immunology probably began its march as acquired immunity [33]. The concepts of immunology have a long history and began primarily as a branch of microbiology. Fracastoro wrote in *De contagione et contagiosis morbis*, published in 1610, that "an infection is the same in both the carrier and newly infected" and postulated the existence of "imperceptible" germs. Since then, several important discoveries have launched a renaissance of research into the field of immunology. Experimental immunology began in 1798 with Jenner [237], Pasteur developed killed and attenuated vaccines, and Miescher later discovered DNA (deoxyribonucleic acid) [33]. In 1875 Cantani Sr stated that the cause of diabetes mellitus was to be sought in a missing *ferment* which in the healthy metabolized glucose. He also demonstrated that dehydration should be cured by *fluid rehydration*. In 1890, Ehrlich expressed the concept of autoimmunity as horror *autotoxicus* [137], amply skimming the etiopathogenetic mechanisms. At the turn of the century, von Pirquet coined the term "allergy" from "αλλος" and "εργος" (meaning altered reaction) [611] and therefore included the development of protective immunity [33], coupled by Coca and Cooke in 1923 with the term "atopy," from "ατοπος" (meaning out of place and thus abnormal) [88]; thus many episodes based on the mechanisms of cause and effect were brilliantly documented [33]. In 1921, Prausnitz and Küstner demonstrated the presence of "reagins" in the serum of allergic patients [436]; in the same year a "Textbook of Immunology" was first published in Italy [75]. In 1966, the Ishizakas attributed a scientific meaning to reagins by identifying IgE as the carrier of reaginic activity in the sera of hay fever sufferers [228]: the first case of immunodeficiency (ID) was reported in 1952 (Chap. 22).

The Immune System

Definitions. The *immune system* was presumably evolved by animals during evolution as a means of self-preservation in a world teeming with microorganisms. It is not selective and immune responses ensue against foreign substances regardless of whether they are bacterial products or not. Therefore the immune system has become exquisitely specialized and highly complex, to protect the host from potentially noxious environmental agents (antigens). The introduction of foreign substances into the host may have an adverse effect on a variety of cells; hence this system synthesizes highly specialized molecules (antibodies), also generating selected cells,

called cytokines, adhesion molecules, chemokines, and the like. Related cells and cell products are consequently a defense system designed to interact with foreign agents to protect the host from any external injury [34].

- *Immunity* is the complex of cellular and/or humoral events following the entry of foreign substances (non-self) into the host. These events are overall free of adverse effects for the majority of subjects, therefore defined as normal.
- *Immunology* is the study of the ways used by the host to maintain homeostasis in the internal environment when confronted with non-self.
- *Allergy* currently implies all forms of hypersensitivity with detrimental consequences for the host and type I, IgE-mediated or cell-mediated reactions.
- *Atopy* is a term underlining the personal or familial hereditary aspects of allergic reactions and is associated usually in childhood or adolescence with increased production of IgE antibodies and/or altered specific reactivity in response to ongoing exposures to allergens, usually proteins.

A thoroughly functioning immune system serves three main functions: (a) defense against invasion of microorganisms and foreign substances, ingested, inhaled or achieved by mucosal contact, or by parenteral injection; (b) homeostasis fulfilling universal requirements of multicellular organisms to preserve uniformity of a given cell type, as well removing worn-out "self" components; and (c) surveillance devoted to perception and destruction of mutant cells [33]. Therefore, inability to relate to *unum* is intrinsic to atopic diseases that depend on hyperproduction of IgE antibodies being IgE-mediated. This is a genetic characteristic, although the mode of transmission is still a matter of debate, and the fact of being atopic does not result automatically in the development of clinical manifestations. Atopy is also polymorphous, manifesting in various forms, starting out as AD or food allergy (FA), to further develop into allergic rhinitis (AR) and/or asthma.

According to the classic theory of clonal selection (Chap. 2), the crucial function of the immune system from the first days of life is to distinguish self from non-self, for example, between what belongs to or is closely correlated to the organism (tolerance to self antigens) and what is foreign, with the goal of eliminating the latter, independent of its potential pathogenicity. This is the quintessential dichotomy of immunology: the *self versus non-self discrimination*. We shall see in Chap. 2 that theories regarding self/non-self are still valid (as long as they are not interpreted literally), with the exception of some recent studies that have clarified how the so-called *neonatal window* period represents an ontogenetic window only as far as tolerance induction is concerned [60]. Immunity is acquired at the first contact with non-self (*antigen recognition*), it is *specific* for a given foreign substance and *acquired,* since it is able to respond to molecules not encountered before. Following an initial antigen stimulus, finally familiar with self and non-self, a series of reactions results, sometimes as if it were reacting to invaders. In virtue of such events, the integrity of the organism, which becomes protected from successive entries of potentially pathogenic substances, is maintained. This is put into practice by the immune memory enabling the immune system to memorize previous exposures to a particular antigen, in a way analogous to natural memory that recalls past experiences. Amongst all defense mechanisms put into action by the host against any external aggressor, we find a network of organs, tissues, cells and molecules responsible for immunity: the *integrated immune system* [36]. The interconnected and coordinated responses following the entry of foreign substances are globally known as *immune reactions.* Relatively small modifications in the delicate and complex molecular and cellular structures of the immune system may cause a functional disequilibrium that is responsible for a cascade of organic perturbations that may become evident alterations, such as atopic diseases varying in nature and severity and primary immune deficiency (PID) [33].

Ideally, the immune reactions determine schematically ordered responses articulated on six levels: processing, presentation and recognition of non-self, cellular activation, elaboration of biologically active chemical substances, mediators, and cellular cooperation. The final outcome of the encounter between host and a foreign invader is now recognized as dependent upon an integrated network of multidirectional communication pathways and signaling, where the mechanisms of memory, effector responses and consequent regulation of secretory proteins and soluble molecules such as the interleukins (ILs) are clarified [34]. Antigen contact starts to form those elements necessary for its recognition, including the cells emitting signals, ILs transducing such signals and target cells receiving them by binding to appropriate receptors; following transduction and amplification of signaling, a detectable physiological response occurs [34]. Thus, immune responses usually culminate in the elimination of provoking agents. The specificity of antigen recognition, as defined in molecular terms, is entrusted to three structures: the variable (V) region of Igs (immunoglobulins), MHC (major histocompatibility complex) V regions and TcR (T-cell receptor) chains. A fourth component of molecular recognition, the cluster of differentiation (CD) markers, differentiates subsets of T from B lymphocytes (from *lympha*, water) and other cell populations [34].

Systems of Immunity

Two general systems of immunity with specialized roles in defending against infection have been selected during evolution [114]:

- *Innate immunity,* an ancient form of host defense, also called natural or congenital immunity, is an attribute of

Table 1.1. Comparison of the innate and acquired immune systems

Properties	Innate immune system	Acquired immune system
Cellular components	Macrophages, PMN, eosinophils, NK cells, DCs, IFN-producing cells, γδ T cells, $CD8^+$ T cells	T and B lymphocytes, macrophages, DCs, $CD4^+$ T cells, $CD8^+$ T cells
Soluble components	Enzymes (lysozyme, complement, etc.), acute-phase proteins, interferon, collectins, defensins, chemokines	Antibodies, interleukins
Memory	No	Yes
Physical barriers	Skin and mucous membranes	None
Recognition	++	+
Self–non-self discrimination	Yes	Yes
Specificity	No	Yes
Speed	tast	slow

Modified from references [115, 184, 537].

every living organism, present at birth, that is, before exposure to foreign agents and consisting of several non-specific factors. Innate responses occur to the same extent as the infectious agent is encountered. Not significantly modified after an encounter with non-self substances, it is void of both a fine-tuned discrimination of such substances and an increased activity following repeated encounters, thereby demonstrating that it does not possess memory. The phagocyte cells (macrophages, neutrophils and monocytes) and alternative complement pathway, although void of specificity, are essential as primary elements of defense against a large number of infectious agents.

- *Acquired immunity,* also called adaptive immunity, becomes involved when the first level of defense fails to fully prevent infection, exemplifying a recent evolutive process, distinct by a particular specificity for offending antigens and by memory. Unlike innate immunity, it is elicited or stimulated by exposures to intruders that escaped early elimination by the innate immune system, insofar as it is armed with a versatile discriminating capacity and potentiated by a successive encounter with such agents.

Table 1.1 [114, 184] shows the major differences between these two types of immunity: the effector mechanisms of innate immunity are activated immediately after infection and rapidly control the replication of infecting pathogens, so the infection is restrained until lymphocytes can accomplish their action. It takes 3–5 days for a sufficient number of clones to be produced and differentiated into effector cells, which allows time for pathogens to damage the host [353]. The greatest difference is that acquired immunity, to compete with genetic variability of microorganisms, has lost the cardinal characteristic seen in innate immunity, that is, the ability to distinguish between potential pathogens and harmless substances. However, innate immunity may have an additional role in determining which antigens the acquired immune system responds to and the nature of that response [148].

Acquired Immunity

As Table 1.1 shows, acquired immunity involves T and B lymphocytes, antibodies and ILs, distinguished schematically into humoral and cell-mediated immunity (CMI), each equipped with various functions, partly different and partly overlapping. Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Specialized cells, the antigen-presenting cells (APCs), display the antigen to lymphocytes and collaborate with them in the response to the antigen [537].

Humoral-mediated immunity, above all responsible for primary defense against bacterial infections, is passively transferable by serum or plasma, being mediated by antibodies with a specific aptitude for reacting with the configurations responsible for its production, which is typical of B lymphocytes, which in humans differentiate in mammalian bursal equivalent tissue and acquire features of B cells from plasma cells with an endoplasmic reticulum (ER) characterized by an abundant RNA (ribonucleic acid). Specific antibodies are responsible for the reactions of immediate hypersensitivity, cytotoxicity, Arthus reaction and, by means of Fc receptors, phagocytosis.

CMI, as well as being active in the defense against viral infections, is implicated in some cases of autoimmune disease and is characteristically associated with effector–target cell interactions involved in antimicrobial immunity, rejection of allografts, immune surveillance and rejection of tumor cells. This specific immunity is transferable by lymphoid cells and not by serum, where T lymphocytes play a fundamental role [481].

Delayed-type hypersensitivity (DTH) is a typical CMI reaction [33], first discovered by Jenner [237].

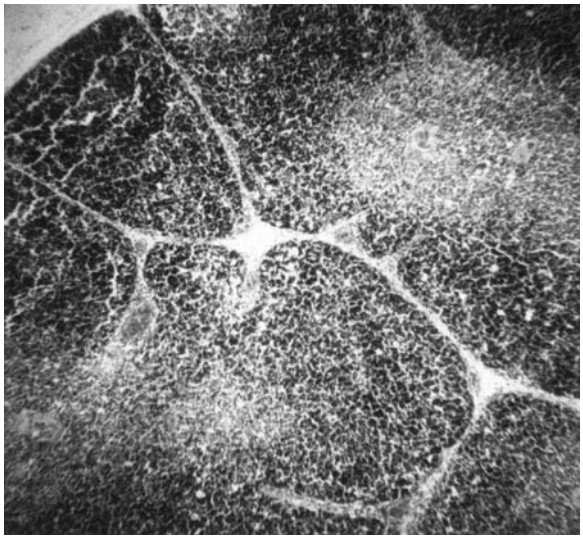
Organs and Cells of the Immune System

Several organs and tissues participate in the host defense and are classified into [33, 36, 47]:

- *Primary lymphoid organs* (forming the central lymphoid organs) where both T and B lymphocytes mature into antigen-recognizing cells and developing lymphocytes acquire antigen specific receptors. These cells develop from pluripotent stem cells (SC) *in bone marrow and only during fetal life in the liver* and then circulate throughout the extracellular fluid. B cells reach maturity within the bone marrow, but T cells must travel to the thymus to complete their development. An important and key role in homing-related responses and in the regulation of cell trafficking of stem/progenitor cells (SPC) is played by SDF-1 (stromal cell derived factor-1) bound to G-protein-coupled CXCR4 (chemokine receptor4). Supernatants of leukapheresis products activate SDF-dependent actin polymerization and significantly enhance the homing of human cord blood (CB) and bone marrow-derived CD34 cells in a NOD (nucleotide-binding oligomerization domain)/SCID mouse (severe combined immunodeficiency (ID). CXCR4 plays a critical role in the trafficing of other tissue/organ specific SPCs expressing CXCR4 on their surface, such as during embryo/organogenesis and tissue/organ regeneration [277, 654]. They are represented in mammals by the thymus, bone marrow and only during fetal life by the liver.
- *Secondary lymphoid organs* (forming the peripheral lymphoid organs) including lymph nodes, spleen, tonsils and linings of the digestive, respiratory and genitourinary tracts, skin, conjunctiva and salivary glands. These organs of different size, disseminated throughout the organism, build structures where antigen-driven proliferation and differentiation occur and lymphocytes circulate and recirculate. There T cells interact with foreign configurations by cytotoxicity or releasing nonspecific mediators, with the non-complementary help of ILs, and store, amplify, and disseminate information about macromolecules encountered in various parts of the body [33].

The primary lymphoid organs include the thymus and the bone marrow.

The *thymus* is a lymphoepithelial organ located in the anterosuperior region of mediastinum deriving from the endoderm of the third and fourth pharyngeal pouches. In the 6th week of fetal life, primitive mesenchymal and neural crest cells seed epithelial structures. Remarkably, parathyroids develop about the same time as the same pouches. During fetal development, the thymus size increases, then reaches its greatest relative weight shortly after birth, and its greatest absolute weight at puberty when contrary to other lymphoid organs, it progressively involutes and is replaced by adipose tissue. However, even if lymphocyte numbers progressively decrease, they do not disappear altogether. Apparently, a few fragments are adequate to ensure a compatible T cellularity in peripheral lymphoid tissues. By 8 weeks of gestation, $CD7^+$ hematopoietic SCs migrate from the yolk sac and fetal liver and later from the bone marrow via the bloodstream and enter the thymus through the epithelial cell linings of the cortex. The hematopoietic SC activity is regulated by C/EBPα (CCAAT-enhancer binding protein α) [679]. It has not yet been clarified whether these traveling stem cells are already committed to differentiate along the T-cell (T = thymus-derived) lineage or become committed only after entering the thymus and what excites them to such a gland. It has been hypothesized that these precursors may express certain as yet unidentified surface markers that selectively bind to their corresponding ligands on the thymic vascular endothelial cells. For this reason, the thymus is the central organ where the precursors, bone marrow-derived prothymocytes, undergo intense proliferation and differentiation, as well as do macrophages, epithelial and dendritic cells (DCs) [454]. The gland consists of two lobes surrounded by a thin capsule of connective tissue extending into the lobe, thus forming septa, partially dividing parenchyma into lobules (Fig. 1.1, a) [454]. The peripheral zone of each lobule forms the cortex with immature proliferating cells. The cortex is further subdivided into a superficial and a deeper zone, with a more central area forming the medulla containing more mature cells and Hassall's corpuscles, an aggregate of epithelial cells. While the thymus has a cortex and a medulla, there is no germinal center (GC), or plasma cells, as in normal situations [33]. The thymus, independent of antigen stimulation, may be viewed as a lymphoid organ controlling all peripheral lymphoid organs, by means of expanding immunocompetence of the lymphocytes associated with CMI. The thymus exercises its function in time by secreting several soluble hormones (thymosin, thymopoietin, etc.): in fact, epithelial thymic cells secrete a series of polypeptides (thymic hormones and ILs), contributing to the maturation of T lymphocytes, with which they can interact directly, influencing their differentiation [33]. During fetal cell development, the requirement for STAT5 (signal transducers and activators of transcriptions) in thymopoiesis is developmental stage specific. STAT5 is required for IL_7R-regulated TCR gene transcription, but it is not necessarily essential for TCR gene rearrangement, denoting that factors other than STAT5 activated by the IL_7R-JAK pathway control TCR locus accessibility to VDJ recombinase [246]. One of the major roles of STAT3 in the G-CSF signaling pathway is to augment the function of C/EBPα (CCAAT enhancer binding protein-α), which is essential for myeloid differentiation. Moreover, co-operation of C/EBPα with other STAT3-activated proteins is required for the induction of some G-CSF responsive genes [386]. The close connection between epithelial cells and lymphocytes is confirmed by the presence of large epithelial cells (*nurse cells*) in the superficial cortex, each containing 20–30 blasts in



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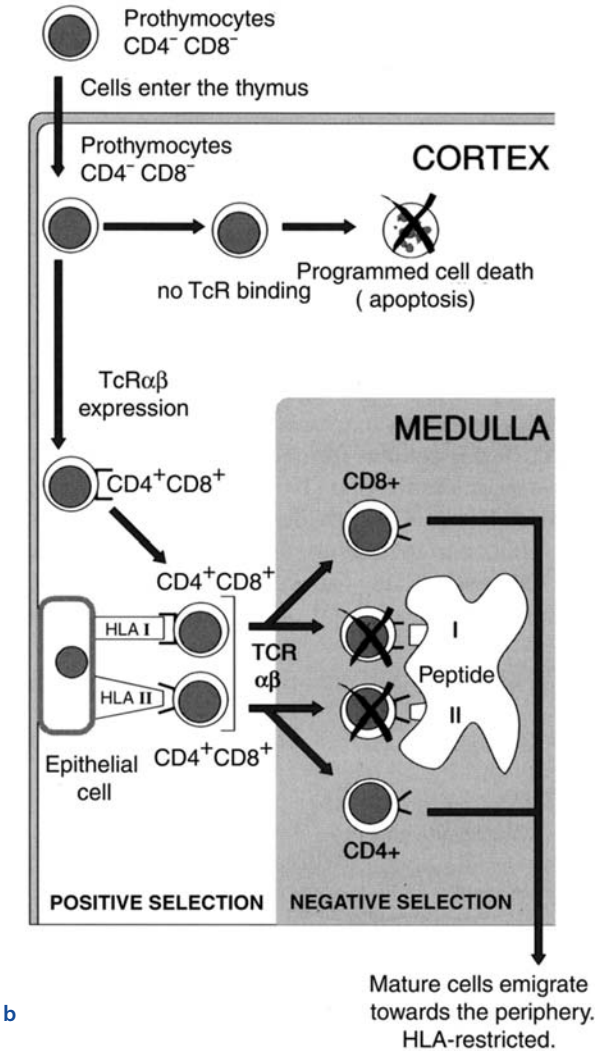


b

Fig.1.1. **a** The thymus of a normal newborn. Connective tissue septa, traversed by large blood vessels, divide the thymus into lobules, the very dense peripheral cortex is clearly distinguished from the more central, less dense medulla. **b** Positive and negative selection in the thymus

division. Prothymocytes (pro-T) express CD2 and CD7, immature T cells of the cortex, smaller (pre-T), such as CD1 and once migrated into the medulla, CD3, CD4, CD8, CD38 and CD71 (transferrin receptor) present not only on T cells, but also on cells of other lineages undergoing proliferation. A low number of $CD4^+8^+$ (DP, double positive) T cells located in the medulla learn how to discriminate self from non-self in a process known as education; they interact with stem cells expressing MHC molecules, then $CD4^-8^-$ (DN, double negative) T cells acquire a $CD4^+8^-$ or $CD4^-8^+$ (MP, mono-positive) phenotype. Targeted mutations of CD80 and CD86 substantially reduce the proliferation and survival of DN T cells in the thymus, since the DN development in the thymus is subject to modulation by the CD80–CD28 costimulatory pathway [682]. The earliest immune maturation of any lymphocyte compartment in humans is represented by αβ and γδ TcR and most likely reflects the importance of these cells in controlling pathology due to common environmental challenges. A significant difference between the γδ and αβ T cell lineages is the much earlier activation and conversion to memory of the γδ T cells, which illustrates the central role that γδ T cells have in adressing Ag challenge from birth onward. γδ T cells are distinguished by expression of Vδ1 vs Vδ2 δ-chains. The majority of Vδ2 cells display signs of early activation in neonates. Even in infants <1 year most of Vδ2 nonnaive cells stain for perforin and produce IFN-γ after short-term stimulation, yet nearly all naive Vδ2 cells disappear from blood by 1 year of life. Vδ1 cells predominate during fetal and early life, but represent the minority of γδ cells in healthy adolescents [111]. As regards TcR, only cells with an αβTcR-CD4/CD8 coreceptor combination that is able to bind the same MHC class I or class II molecules will fully mature. Cells with a mismatched combination will die [263]. Positive selection is conditioned by activation of the MAPK (mitogen-activated protein kinase) cascade, although it does not involve $CD4^-8^-$ (DN) T cells [10]. Most lymphocytes not surviving the thymic selection process die by apoptosis (from "απωπτοσις," cellular suicide): they are engulfed and digested by macrophages of the corticomedullary junction within 3–4 days of their last cell division [192] (Fig. 1.1 b). In the deep cortex, T cells interact with macrophages, FCDs and epithelial cells, which during a differentiation process modify expression of cytokeratins such as skin keratinocytes; medullary T cells are found in Hassall's corpuscles [225]. Several immune deficiencies are characterized by selective deficiency of T cells [33].

The *bone marrow* is a structure present in man and in higher mammals considered to be a primary lymphoid organ functionally equivalent to avian bursa. The location of pluripotent hemopoietic stem cells eventually differentiating into stromal cells guides hemopoiesis both by direct cellular contacts with developing lymphocytes (precursors) and by other blood cells, also under the control of growth factors. Many ILs are involved

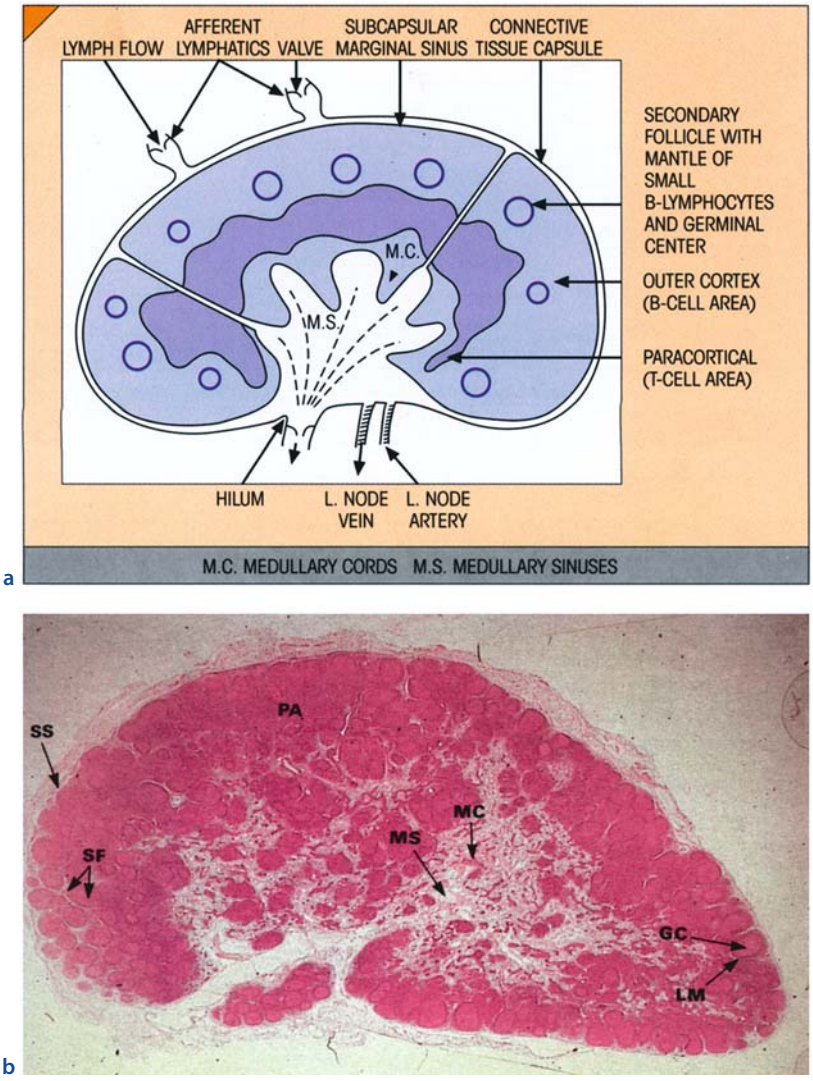
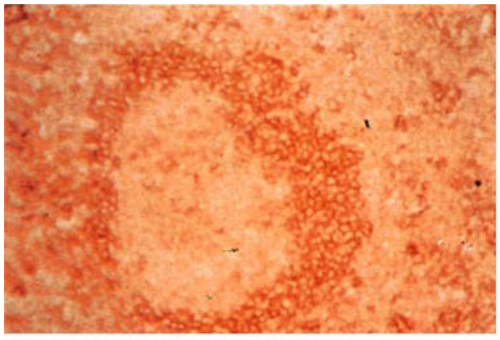


Fig.1.2. **a** Schematic structure of human lymph node. **b** Human lymph node (low magnification). **c** Secondary lymphoid follicle showing GC surrounded by a mantle of B lymphocytes. In the center there are few IgD-positive cells; both areas contain IgM-positive B cells. Lymphocyte mantle stained with anti-human IgD antibody. **d** Section of lymph node medulla stained in methyl green (DNA)/pyronin (RNA) to show the basophilic (*pink*) cytoplasm of plasma cells with abundant ribosomes. **e** Section of lymph node medulla showing macrophages lining the medullary sinus following the uptake of red dye. **f** Lymph node of a mouse immunized with pneumococcal polysaccharide antigen, thymus-independent, revealing a prominent stimulation of SFs with GC. **g** Green stain (methyl/pyronin) of a lymph node draining site of skin stained with the contact sensitizer oxazolone, with the generalized expansion and activation of the paracortical T-cell area, which is clearly noted, since T blasts are strongly basophilic. **h** Lymph node section from a congenitally athymic (nude) mouse showing paracortical depletion with failure of T-cell development. *LM* lymphocyte mantle with SF, *MC* medullary cords, *MS* medullary sinus, *PA* paracortical area, *PC* plasma cells, *PN* primary node, *SF* secondary follicle, *SM* sinus macrophage, *SS* subcapsular sinus

in different pathways; among them IL_4 produced by T cells regulates proliferation of B lymphocytes (B=bone marrow-derived or bursal equivalent). In mammals, B cell maturation occurs in bone marrow: precursor cells (pro- and pre-B; see Chap. 2) multiply when in contact with primitive reticular cells, which generate ILs necessary for cellular multiplication and maturation. As for T cells in the thymus, a great number of B lymphocytes die through apoptosis, while mature B cells leave the bone marrow traversing the walls of venous sinuses [469]. Then they are transported by the circulation to secondary lymphoid organs, where they encounter and respond to invading antigens.

Secondary lymphoid organs include lymph nodes, spleen, tonsils and MALT.

Lymph nodes are lymph filters placed at the junctions of sympathic vessels. They are aggregates of lymphoid tissue strategically positioned to form a complete network throughout the body and carry out the basic functions of filtering foreign material, favoring antigen-dependent differentiation of lymphocytes, and localizing and preventing the spread of infectious processes. Apart from collagenous capsules surrounding lymph nodes, two cortical zones are recognizable within ganglia parenchyma, a more external (cortex or B-cell area) and a deeper one (paracortex or T-cell area) and a central medulla, where parenchyma is organized into medullary cords interdigitating with medullary sinuses. T and B cells, plasma cells and several macrophages are found there (Fig. 1.2) [470]. B cells populate three principal areas, the GCs, with dark and light zones, the follicular mantle zone, including $CD5^+$ and $CD5^-$ with features of virgin cells, and the marginal zone with several subsets also present in the spleen, subepithelial areas of the tonsils and the dome region of Peyer's patches (PP) [63]. B memory cells coming from tonsils colonize subepithelial areas and directly present antigen to T cells by rapid up-regulation of CD80 and CD86 [304]. T lymphocytes reside in T-cell-dependent areas, the deep cortex or paracortical zone together with interdigitating DCs (IDC) and high endothelial venules (HEV) present in lymphoid tissues and possessing cubic epithelial cells



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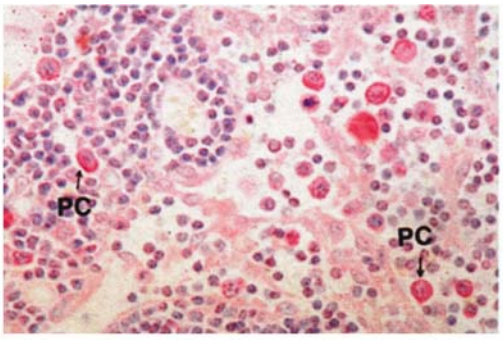

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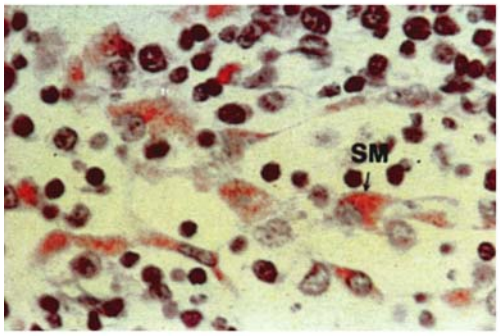

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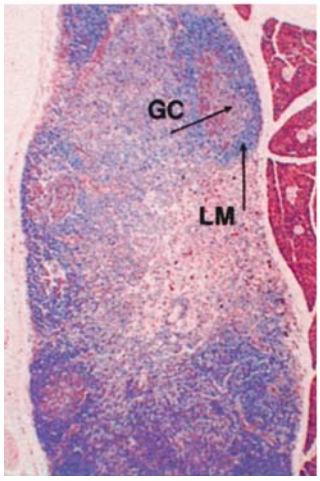
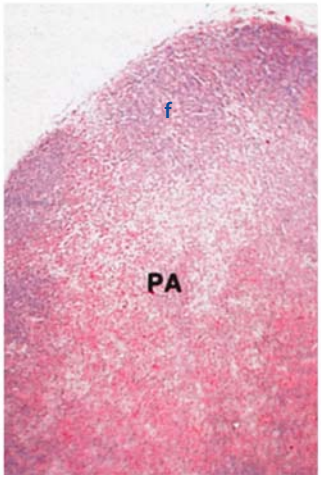
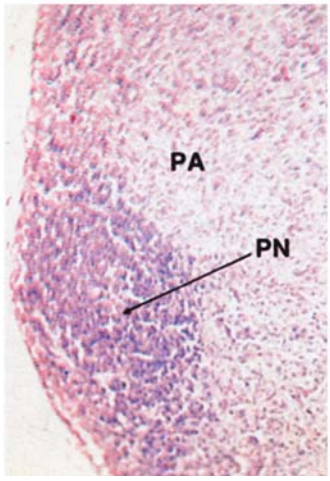




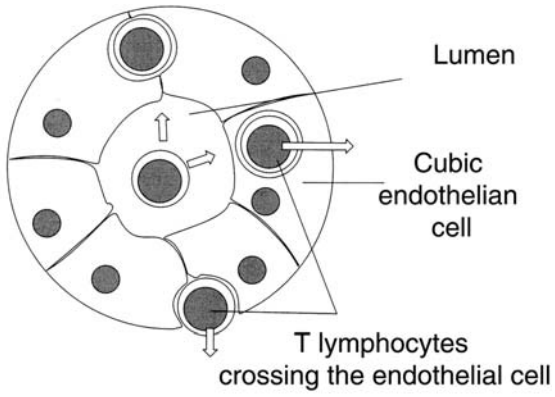


f–h

Fig.1.2. (Continued)

(Fig. 1.3). By crossing HEVs, non-antigen stimulated, virgin lymphocytes reach lymph nodes [454], where the paracortical area following antigen stimulation is hypertrophied and contains T lymphocytes and ICDs that are APCs. A specific subset of DCs (originating from circulating CD11c + DCs), which strongly stimulate T cells but not B cells, was recently identified in GCs [191]. Finally, the medullary layer, which is made up of cords separating lymphatic sinuses, contains essentially macrophages and plasma cells.

The *spleen*, in addition to being a filter eliminating senescent or worn-out cells, foreign particles and macromolecules from the circulation, is the only lymphatic tissue specialized in filtering blood as well as in filtering and processing antigens transported into the bloodstream (Fig. 1.4). Similar to lymph nodes, it is divided both from a functional and structural point of view into B and T zones. There are no lymphatic vessels, since blood enters the splenic parenchyma at the hilum via the splenic artery and follows along trabeculae until smaller arterial branches become surrounded by sheaths of lymphocytes, the white pulp. This zone contains lymphoid cells aggregating in lymphoid follicles or lymph nodules, and in *periarteriolar lymphoid sheaths* (PALS), which are similar to the cortex of lymph nodes. The pulp surrounding periarteriolar sheaths is called the marginal zone. In the peripheral area, cortical lymphocytes aggregate into follicles (as can be found in all peripheral lymphoid organs), distinguished into two types, primary and secondary follicles, also designated as GCs (see also "B Lymphocytes"). GCs also contain B cells and macrophages; B cells can also be found in red pulp, which is formed by venous sinuses, reticular fibers (Billroth's cords) and vessels [454]. The PALS mainly

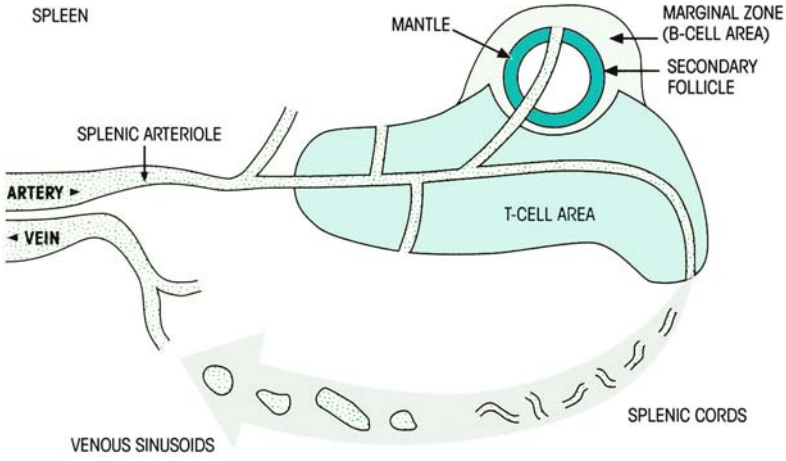


T lymphocytes		Endothelial cell
CD44	⟷	Hyaluronate
CD62L	⟷	Sialic acid
CD29/49	⟷	CD 106
CD11a	⟷	CD54, 58, 102

Fig.1.3. High endothelial venules

contain mature T lymphocytes with a CD4 phenotype in 65% of cases and CD8 in the remaining 35%. GCs and cells in active proliferation are present; the larger and medium-sized cells predominate in the central part of the GC, while the smaller cells localize at the periphery and form the mantle [454]. The surrounding follicles and marginal zone are composed mostly of B cells in addition to monocytes, plasma cells, red cells, platelets, DCs and several macrophages active in phagocytosis. The main immune function of the spleen is to initiate immune responses against polysaccharide antigens (PSA) traveling in the blood and fixed on the surface of follicular DCs (FDCs), differently from lymph nodes where immune responses to antigens take place if antigens enter via afferent lymphatics [33, 36]. The marginal zone is a natural reserve of memory B cells [48].

The *tonsils* (lingual, palatine and adenoidal) are important immune organs, containing many primary and secondary follicles and GCs, with morphology and cellular composition identical to those in lymph nodes. They are strategically placed for initiating immune responses, as they are continually in contact with inhaled or ingested antigens (Chap. 15).



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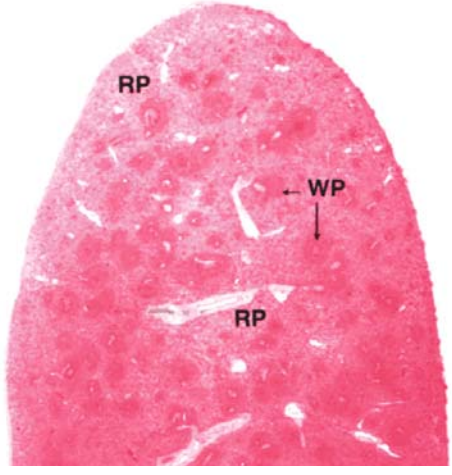

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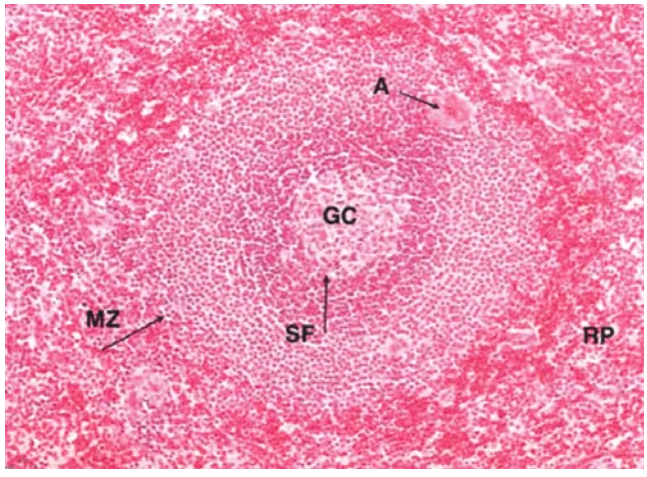


c

Fig.1.4. a Spleen: diagrammatic representation. **b** Low-power view showing lymphoid white pulp (*WP*) and red pulp (*RP*). **c** High-power view of a secondary follicle (*SF*) with germinal center (*GC*) and lymphocyte mantle (*M*) surrounded by marginal zone (*MZ*) and the red pulp (*RP*). Adjacent to the follicle is an arteriole (*A*) surrounded by periarteriolar lymphoid sheath (*PALS*, T cells)

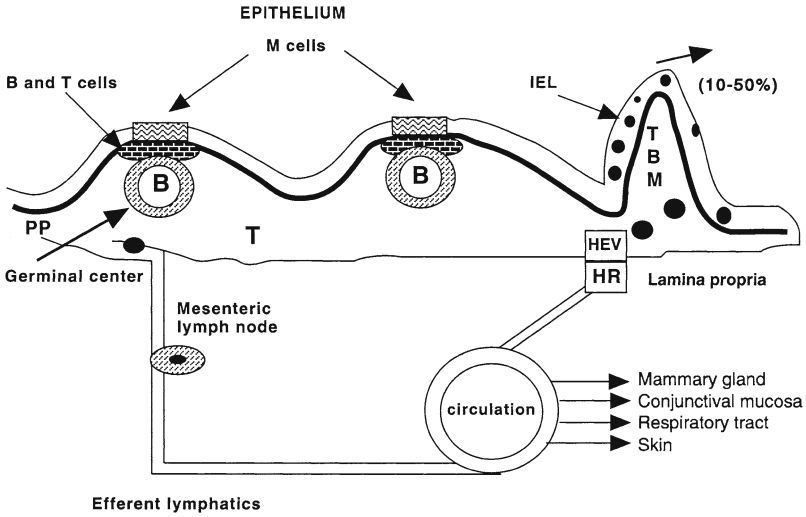


Fig.1.5. Schematic representation of recirculation of lymphocytes showing the relationships with MALT sites. After antigen presentation, T lymphocytes of the organized MALT leave the Peyer's plaques (*PP*) via efferent lymphatics and reach the circulation via mesenteric lymph nodes. Cells derived from the gut preferentially home back to the gut lamina propria via specialized homing receptors (*HR*) and the *HEV*. Cells originating from the gut can also reach other MALT sites: GALT, BALT, SIS, NALT, CALT (see text). *T, B* T and B lymphocytes, *M* macrophages, *IEL* intraepithelial lymphocytes, *HEV* high endothelial venules, *HR* homing receptors. (Modified from [546])

Mucosa-associated lymphoid tissue (MALT) is made of lymphocytes aggregated to form follicles in the lamina propria underlying basement membranes of mucosal epithelia of the respiratory, gastroenteric, cutaneous and urogenital systems. The most prominent of such tissues are termed GALT and BALT (gut- and bronchus-associated lymphoid tissue) (Fig. 1.5) [548]. Such distant anatomical sites not only share common immune effectors such as secretory IgA antibodies (sIgA), but they are also interrelated by a traffic of lymphoid cells.

GALT is represented by PPs in the mucosa of the outer wall of the terminal ileus (up to 200 PPs are present in mammals) and by the appendix, with a similar division into B- and T-dependent areas. Lymphoid tissue is also diffusely distributed in the lamina propria of intestinal villi and crypts and among epithelial cells of gut mucosa. GALT may intervene in differentiating stem cells into B lymphocytes that are mainly committed to IgA synthesis throughout the MALT. Primed B lymphocytes within PPs travel via mesenteric lymph nodes (where they differentiate into mature IgA B cells) and thoracic duct lymph, and subsequently to the systemic circulation, and finally they return mostly to reside at intraepithelial sites in the mucosa and to some extent to the spleen or to more distant sites such as BALT. Studies have shown that T lymphocytes may leave intravascular space via homing receptors on specialized HEVs where vascular adhesion molecules may direct their traffic [548].

BALT is structurally and perhaps functionally similar to GALT, associated with both upper (nasal mucosa) and lower (lungs) respiratory tracts. Lymphocytes are organized into lymphoid aggregates and follicles. They are commonly placed along main bronchi in all lobes that are found especially at bifurcations of bronchi and bronchioli; the so-called M (*microfold*) cells are found in both GALT, over the PP dome, and BALT overlying follicles (see Chaps. 9 and 11).

SIS (skin immune system). The term highlights the skin's immune characteristics, where intraepidermal lymphocytes, DCs, Langerhans' cells (LCs), keratinocytes, etc. are found.

Subsequently, additional lymphoid structures, including *NALT (nasal-associated lymphoid tissue),* corresponding to Waldeyer ring and *CALT (conjunctiva-associated lymphoid tissue)* have been characterized.

Cells of the Immune System

Two Families of Lymphocytes

Both T and B cells can be defined as two lines of immunocompetent cells, morphologically indistinguishable but functionally distinct, having a different origin. The immune response has indeed different functional aspects according to the reciprocal involvement of T or B cells; therefore, this response can be regarded as having two distinct components, the T system of lymphocytes producing immune or sensitized lymphocytes, and the B system of lymphocytes producing Igs. For their identification there is an internationally defined nomenclature, the group of CD antigens (CD followed by a number) (CD1–CD342) (Table 1.2) [4, 6, 16, 23, 34, 49, 70, 82, 85, 102, 103, 109, 112, 120, 132, 147, 158, 175, 242, 262, 343, 373, 407, 428, 437, 442, 484, 501, 515, 559, 571, 584, 608, 604], denoting that a cluster of antibodies will react with a particular antigen (see "Afferent Phase of Immune Response").

These effector cells of specific immunity share two cardinal features:

- They can be induced in that they have the ability to be activated by antigens evoking their formation and can be stimulated to proliferate and differentiate, so as to generate effector and memory cells.

Table 1.2. Leukocyte cell surface proteins: cluster of differentiation (*CD*) and their immune expression

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD1a (R4, Leu 6, VIT6, gp49)	Myeloid progenitors, DCs, LCs, activated T cells	49	Presentation of lipid and glycolipid antigens, antigen presentation to T γ/δ
CD1b (R1, NUT2, 4A76, gp45)	Thymocytes, DCs, LCs, T subpopulation	45	CD1a to C1b, noncovalently bound to β_2-microglobulin, include forms of non-HLA class I-like molecules, with a potential role alternative to known APCs
CD1c (R7, PHM3, M241, gp43)	Thymocytes, DCs, LCs, T subpopulation	43	Presentation of lipid and glycolipid antigens, αβ TcR
CD1d (R3)	Leukocytes, epithelium		Presentation of hydrophobic nonpeptide antigens, αβ TcR
CD1e (R2)			Presentation of lipid and glycolipid antigens, αβ TcR
CD2 (9.6, 35.1, T11, LFA-2)	Murine B, mature T and NK cells, thymocytes	47–58	Associated with CD3ε and ζ, promotes intercellular adhesion via the CD58 ligand, of CTLs to target cells, of T cells to endothelial cells and APCs, signal transduction
CD2R (T11.3, VIT13, D66)	Activated T and NK cells	50	Epitope of restricted CD2, activates thymic, T and NK cells
CD3-α (UCHT1, Leu 4, T3)	Thymocytes, T cells	25–28, 21,	Invariant part of TcR (5 chains), signal transduction for T cell activation
		20, 16, 22	Lineage-specific marker, Ig-SF
CD4 (91.D6, Leu 3, T4)	Thymocytes, T subpopulation, monocytes	59	Th marker, CD3 co-receptor, stabilizes HLA class II–TcR complexes, HIV receptor, signal transduction in association with $p56^{lck}$, Ig-SF
CD5 (T1, UCHT2, T101, gp67)	Mature (murine) B cells and T cells, thymocytes	67	Costimulation of T cells, binds CD 32, CD38, CD45RA, CD45RO, CD72, increases the pool of 2nd messengers, SRCR- SF
CD6 (T12, T411, gp100)	B and T subsets, thymocytes	100–130	Interacts in the TcR-mediated activation of T cells, binds CD166; SRCR-SF
CD7 (3A1, 4A, CL1.3)	Human fetal liver, thymocytes, T and NK cells	40	Signaling, early T-lineage marker, activates T and NK cells, FcR for IgM, Ig-SF
CD8 (α chain: T8, Leu 2a, T811; β chain: T8; gp32)	Most thymocytes, CTLs, intraepithelial lymphocytes, some DCs	34	Stabilizes HLA class II–TcR complexes, coreceptor for HLA class I CTL-restricted, signal transduction, Ig-SF
CD9 (PHN200, FMC56, p24)	Pre-B cells, monocytes, basophils, platelets, activated B and T cells	22–27	Adhesion of pre-B cells, possible role in signal transduction mediated by interaction with GTP-binding proteins, activation and aggregation of platelets, binds CD41/CD61, TM4-SF
CD10 (J5, NEP, BA-3, gp100, CALLA)	B precursors, TN thymocytes, PMNs, fibroblasts	100	Zn-dependent neutral endopeptidase, putative role in B-cell development

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD11a (MHM24, LFA-1, CRIS3, CR1)	Myeloid precursors, monocytes, macrophages, neutrophils, eosinophils, B and T cells	180	β2 Integrin (αL chain), receptor for CD50, CD54 and 102, mediates cell–cell leukocyte adhesion to endothelium (CD54/102), of CTL to target cells, heterotropic among B, T and monocytes and homotropic of neutrophils via CD50
CD11b (Mo1, MAC-1, CR3, gp155/95)	Monocyte-macrophages, neutrophils, NK cells	165	β2 Integrin (αM chain), receptor for CD54, iC3b and fibrinogen, modulates adhesion of neutrophils/monocytes and extravasation via CD54
CD11c (L29, B-LY6, BL-4H4, CR4, gp150/95)	Monocyte-macrophages, neutrophils, NK cells, subsets of activated B and T cells	150	β2 Integrin (αX chain), receptor for CD54, iC3b and fibrinogen, neutrophil/monocyte adhesion to endothelium, receptor for phagocytes
CDw12 (M67)	Eosinophils, monocytes, PMNs, basophils	90–120	Requires further analysis
CD13 (MY7, MCS-2, TÜK1, gp150)	Myeloid progenitors, monocytes, granulocytes	150–170	Aminopeptidase N: inactivates active peptides, receptor for coronaviruses
CD14 (Mo2, VIM 13, MoP15, gp55)	Eosinophils, monocytes, PMNs, basophils	55	Inhibits IgE synthesis on monocytes, receptor for LPS, suggested role in Gram– pathogen clearance, polymorphism in the flanking region, receptor for endotoxin, LPS/LPS binding complex
CD15 (My1, VIM-D5)	Eosinophils, monocytes, PMNs, basophils, LCs	–	Oligosaccharide; binds CD62E; modulates PMN adhesion and phagocytosis
CD15 s (sLe^x)	Granulocytes, NK, T and B cells, monocytes	–	Sialyl-Lewis, binds CD6E, CD62P and CD62L
CD15u			Sulfated CD15, carbohydrate structures
CD16a (BW209/2, HUNK2,3 GB)	Immature fetal T and B cells, monocytes, NK cells, PMN subsets, macrophages	50–65	FcγRIIIa, associated with TcR-ζ and FcεRI-ɣ, promotes signal transduction for NK cells (ADCC) and macrophages (phagocytosis), IgSF
CD16b	Granulocytes	48–60	FcγRIIIb, IgSF
CDw17 (GO35, Huly-m13)	Granulocytes, PMNs, monocytes, eosinophils	–	Lactosylceramide, possible role in phagocytosis, signaling
CD18 (MHM23, M232, 11H6)	All leukocytes	95	Integrin, β2 chain of CD11a, b, c, binds CD54, participates in cell adhesion
CD19 (B4, HD37)	Pan B-cell and pre-B precursors, FDCs	95	Membrane protein: B-cell activation and proliferation, part of signal transduction complex including CD21, CD81 and Leu-13, IgSF
CD20 (B1, IF5, p37/32)	Pan B-cell except pre-B and plasma cells	33, 35, 37	Modulates ionic channels, B-cell activation and proliferation, associated with CD53, CD81, CD82 and HLA

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD21 (B2, HB5, CR2, p140)	Mature B cells, FDCs, normal thymocytes	145	See CD19; binds C3d, EBV and CD23, which is implicated in IgE synthesis; intracellular domain contains potential PKC and PTK sites; RCA
CD22 (HD39, S-HCL1, To15, gp135)	B cells mature, pro- to pre-B cells	130/140	Homologous to MAG, the α-form mediates adhesion to monocytes and RBCs, the β-form to T cells via CD45RO and to B cells via CD75, the cytoplasmic domain has potential PKC sites and 6 tyrosine residues; IgSF
CD23 (Blast-2, MHM6, gp45/50, FcεRII)	Low-affinity IgE receptor (see text)	45	C-type lectin, ligand of CD21, inducible by IL_4 and IL_{13}, modulates cytotoxicity of eosinophils and macrophages, may prevent apoptosis of GC B cells
CD24 (VIBE-3, Ba-1, HSA, gp41/38)	Immature T, B/T cells, PMNs, eosinophils, FDCs	38–70	T-cell costimulation, adhesion and signaling, supports CD80 for T-cell growth
CD25 (7G7/B6, 2A3, Tac p55)	Activated B cells, T, NK, monocyte-macrophages	55	Integrin, IL_2R α chain, associating with β (CD122) and γ chain induces proliferation and activation of T, B and NK cells, and macrophages; CCP-like
CD26 (134–2C2, TS145, gp120)	Memory CD45RO, T and NK cells, macrophages	110	Dipeptidyl-peptidase IV (substrate is the V3 loop of HIV-1 and HIV-2); triggers T cells, to which anchors adenosine deaminase (ADA); binds collagen, fibronectin
CD27 (VIT14, S152, T18A, gp55)	T cells, B cells, subsets of thymocytes, NK cells	55	CD70 ligand, costimulatory signaling activating T cells; TNFR-like protein
CD28 (9.3, KOLT2, B7, gp44)	T-cell subsets, resting T cells, plasma cells	44	Binds CD80 (CD86); costimulatory signal for T cells distinct from TcR signal; IgSF
CD29 (K20, A-1A5)	T, B and NK cells, all leukocytes, monocytes, platelets, endothelial cells	110,130	VLA-β, β1 chain integrin, binds collagen, laminin and fibronectin, or heterodimer of CD49 modulates cell–cell and adhesion to cellular matrix
CD30 (Ki-1, Ber-H2, HSR4)	Activated T, B and NK cells, Reed-Sternberg cells	105	TNFR-like protein for MCP, enhances HIV replication in CD4 T cells, delivering a death signal, expresses Th2-like ILs, binds CD153; TNFRSF, and NGFRSF
CD31 (SG134, TM3, HEC-75, gp140, PECAM-1)	Platelets, monocytes, macrophages, neutrophils, B, naive T and NK cells, Lateral endothelium	140	Platelet antigen GPIIa, $\alpha_V\beta_3$ integrin, binds CD51/CD61, regulates adhesion to endothelium and transmigration of monocytes, PMNs, T and NK cells; IgSF
CD32 (CIKM5, 41H16, 2E1, ex CDw32)	Monocytes, B cells, all leukocytes, platelets	40	FcγRII, implicated in the phagocytosis of neutrophils and monocytes; IgSF
CD33 (My9, H153, L4F3, gp67)	Monocytes, all leukocytes, myeloid precursors	67	Sialoadhesin, binds sialylate glycoproteins

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD34 (My10, Bi-3C5, ICH-3, gp105–120)	Myeloid precursors, vascular endothelium, immature hematopoietic cells	105–120	Sialomucin, lymph node HEVs, CD62L-independent leukocyte adhesion to HEVs, receptor for CD62L, early marker of hematopoietic stem/progenitors cells
CD35 (TO5, CB04, J3D3, CR1)	Monocytes, some B and NK cells, erythrocytes, macrophages, neutrophils, eosinophils, FDCs	250	Receptor for C3b and C4b, RCA, reversible complement inactivation; supports phagocytosis of monocytes and neutrophils, APC putative role on DCs
CD36 (5F1, CIMeg1, ESIVC7, gp90)	Monocyte-macrophages, DCs, platelets, B cells	88	Platelet antigen GPIV (IIIb), binds thrombospondin, collagen, LDL receptor for macrophages (phagocytosis of apoptotic cells)
CD37 (HD28, HH1, G28–1, gp52–40)	Mature B, T and myeloid cells, PMNs, monocytes	40–52	Role in signaling, intercellular traffic with CD53, CD81, CD82, HLA-class II with function of ion channel; modulates B cell activation and proliferation (?), TM4-SF
CD38 (HB7, T10, p45)	Early and activated T, B cells, myeloid progenitors	45	Signal transduction, and cell adhesion
CD39 (AC2, G28–2, gp 80, gp70–100)	T, B and NK cells, monocytes, vascular endothelium	78	Directs NK cell activation, may deliver activating signals from B cells to T cells
CD40 (G28–5, gp50)	Normal and neoplastic B cells, FDCs, macrophages, epithelial and endothelial cells, keratinocytes	50	Ligand for CD40L, important in B cell differentiation and activation, GC formation and isotype switching, prevents apoptosis, TNFRSF
CD40L (see CD154)			
CD41 (PBM6.4, PL 273)	Platelets, megakaryocytes	125/22, 105	Platelet antigens GPIIb/IIIa, mediates platelet aggregation; β3 integrin; associates with CD61; binds fibrinogen, fibronectin, vitronectin, vWF and thrombospondin
CD42a (FMC25, BL-H6, GR-P, gp23)	Platelets, megakaryocytes	22	Platelet antigen GPIX
CD42b (PHN89, AN51, GN287)	Platelets, megakaryocytes	135, 25	Platelet antigen GPIbα
CD42c	Platelets, megakaryocytes	22	Platelet antigen GPIbβ
CD42d	Platelets, megakaryocytes	85	Platelet antigen GPV The a+b+c+d complex promotes platelet adhesion, binds vWF and thrombin
CD43 (OTH71C5, G19–1, gp95)	Hematopoietic precursors, CD4, pre-B, leukocytes	115	Sialomucin, leukosialin; T cell proliferation, costimulation, and adhesion
CD44 (H-CAM, Pgp-1, gp80–95)	Fetal and immature adult thymocytes, memory T	80–95	Binds hyaluronic acid, primes T cell activation, adhesion, HEV homing, apoptosis

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD44R (CD44-restricted or variant)	Expressed in different epithelia	110–250	Possible role in DC migration, binding and presentation of chemokines
CD45 (T29/33, BMAC 1, LCA, T200)	Expressed on all leukocytes	180–220	Tyrosine phosphatase, ligand for CD22; signal transduction for T and B cells
CD45RA (G1–15, F8–11–13, gp220)	Naive T cells, T CD8 subset, B and NK cells	205, 220	T200 with expression restricted to T cells, isoform with A exon
CD45RB (PTD/26/16)	Naive T cells, T, B and NK cells	205, 220	T200 with expression restricted to naive T cells, isoform with B exon
CD45RC			CD45 restricted epitope
CD45RO (UCHL-1, gp180)	Granulocytes, monocytes, T cells	180	T200 with expression restricted to memory T cells, isoform without A/C exons
CD46 (HULYM5, J4B, MCP, gp66/56)	Myeloid precursors, hematopoietic cells and non-myeloid precursors, T, B cells, all leukocytes	56/66	Membrane cofactor protein, prevents C3 breakdown
CD47 (BRIC 125, BRIC 126, gp47–52)	Myeloid precursors, T, B cells, all leukocytes	47–52	β1 integrin associated protein, signaling molecule facilitating adhesion, antigen associated with Rh group
CD47R ex CDw149 (MEM-133)	B and T cells, neutrophils, eosinophils, monocytes	120	Unknown
CD48 (WM68, BCM1, OX-45, Blast-1)	distributed to hematopoietic cells and nonhematopoietic T, NK, capillary, endothelial cells, fibroblasts	45	Binds CD2, involved in T cell adhesion to APCs and their costimulation, may be required by γδ cells for antigen recognition; IgSF
CD49a	T, NK, capillary, endothelial cells, fibroblasts	200–210	α1 Chain associated with CD29 to form VLA-1, binds laminin and collagen I, IV
CD49b (Gi14, CLB/thromb4)	Platelets, B and T cells, endothelia, fibroblasts	155–165	α2 Chain associated with CD29 to form VLA-2, receptors for GPIa, laminin and collagen I-IV, regulates the expression of metallo-proteinase-1
CD49c	B cells, fibroblasts, keratinocytes, epithelia	145–150	α3 Chain associated with CD29 to form VLA-3, binds laminin, collagen and fibronectin
CD49d (B5G10, HP2/1, HP1/3)	Myeloid precursors, thymocytes, NK cells DCs, B- and T-cell lineages	150, 80, 70	α4 Chain associated with CD29 to form VLA-4, binds fibronectin, CD106, MAdCAM-1, role in T cell adhesion/migration to lymph nodes and homing to HEVs
CD49e	Platelets, epithelia, endothelia, thymocytes, monocytes, myocytes, T and B cells	160, 135/25	α5 Chain associated with CD29 to form VLA-5, binds fibronectin and GPIc, T cells, involved in cell adhesion and migration mediates proliferation and differentiation

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD49f (GoH3)	Widespread: T cells, thymocytes, monocytes, platelets, epithelial cells, eosinophils	150, 130	α6 Chain associated with CD29 to form VLA-6, binds laminin; the different α6 isoforms have a distinct developmentally regulated distribution in various tissues
CD50 (101–1D2, 140–11, ICAM-3)	T, B and NK cells, monocytes, PMNs, LCs	116–140	Binds CD11a/CD18, promotes signal transduction and costimulation on T cells; signaling via CD50 stimulates β1 and β2 integrin function on T cells; IgSF
CD51 (13C2, 23C6, NKI-M7)	Platelets, endothelia, monocytes, macrophages	125–24	Complex with CD61, binds fibronectin, vitronectin (α chain) and vWF; role in platelet aggregation, cell-cell adhesion (via CD31) and γδ costimulation
CD52 (O97, YTH66.9, Campath-1)	B and T lymphocytes, monocytes, macrophages	25–29	Target of complement-mediated lysis, may contribute to T lymphocyte depletion and GvHD prevention
CD53 (MEM-53, HI29, HI36, gp32–40)	Granulocytes, B and T lymphocytes	32–40	Bound to phosphoinositol
CD54 (RR7/7F7, ICAM-1)	B (and T) cells, endothelia, epithelia, DCs, fibroblasts	90–115	Protein implicated in B-cell activation, ligand of CD11a/CD18, CD43 and monocytes, Rhinovirus, mediates leukocyte adhesion to epithelium in inflammation sites and in T-cell interactions with APCs and target cells; IgSF
CD55 (BRIC110, BRIC128, DAF)	All leukocytes, activated T cells, endothelium	70	Bound to phosphatidylinositol, RCA, binds CD97 and C3b/C3bBb and C4b/C4b2a convertase, thus accelerating the decay of the C3 convertase and C5
CD56 (Leu19, NKH1, L185, gp220/135)	NK, T and Schwann cells, neurones, astrocytes	200–220	Isoform of NCAM, NK-cell marker, putative role in tissue architecture (during embryogenesis) and in non-restricted cytotoxicity; IgSF
CD57 (Leu 7, L183, L186, gp110)	NK and T cells (CD8), B subpopulation	110	Binds CD62-L, -P, NNK-1, role in non-HLA-restricted cytotoxicity after activation
CD58 (BRIC5, G26, TS2/9, LFA-3)	Myeloid precursors, erythrocytes, all leukocytes	55–70	Role in APCs and T-cell interactions via CD2, bound to phosphatidylinositol; IgSF
CD59 (MEM-43, YTH53.1, p18, gP18)	Many hematopoietic cells and nonhematopoietic cells, T cells	18–20	MACIF, 2nd ligand of CD2, binds C8, C9, phosphatidylinositol, signaling
CD60 (M-T32, M-T21, M-T41)	T-cell and NK-cell subsets, platelets		Carbohydrate with NeuAc-NeuAc-Gal sequence modulates T-cell activation
CD60a			GD3, carbohydrate structures
CD60b			9-*O*-acetyl-GD3, carbohydrate structures

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD60c			7-*O*-acetyl-GD3, carbohydrate structures
CD61 (CLB thromb/1, BL-E6)	Platelets, megakaryocytes, monocytes	105	β3 Chain integrin, receptor for vitronectin (β chain), fibronectin, fibrinogen; platelet antigens GPIIb/IIIa, platelet aggregation, heterodimer with CD41 CD51
CD62E (ELAM-1, LECAM-1, E selectin)	Vascular endothelium	110	C-type lectin, binds ESL-1, promotes tethering and rolling of monocytes, some memory T cells, and for neutrophils also the adhesion to endothelia with CD15s
CD62L (LAM-1, LECAM-2, L selectin)	All circulating leukocytes, except some memory cells	70–90	C-type lectin, migration of lymphocytes to lymph nodes and leukocytes to sites of T cells inflammation, mediates T-cell tethering and rolling and adhesion to HEVs, binds CD34, ESL-1, GlyCAM-1, and MAdCAM
CD62P (gp140, PADGEM, P selectin)	Activated platelets and endothelial cells	140	C-type lectin binds CD162, adhesion of platelets to neutrophils and monocytes, as well as tethering and rolling of leukocytes on activated endothelium
CD63 (CLB gran/12, gp53)	Activated platelets, PMNs, monocyte-macrophages	53	Platelet-activating antigen, associated with con CD9, CD81, VLA 3–6; TM4-SF
CD64 (Mab22, Mab32.2, gp75)	Monocyte-macrophages, DCs, neutrophils	75	FcγRI; high-affinity receptor for IgG Fc, promotes ADCC; IgSF
CD65 (VIM2, VIM8, HE10)	Granulocytes, monocytes, myeloid leukemia cells		Ceramide-dodecasaccharide 4c
CD65 s	Sialylated form of CD65		
CD66a (BGP-1, CEA)	Granulocytes, epithelial cells	140–180	Binds CD62E, adhesion molecule for neutrophils, signaling role; IgSF
CD66b (ex CD 67, CGM6, p100)	Granulocytes	95–100	Adhesion molecule, can activate neutrophils, signaling; IgSF
CD66c (NCA, CEA)	Granulocytes, epithelial cells	90	Binds CD62E, adhesion molecule, capable of activating neutrophils
CD66d (CGM1, CEA)	Granulocytes	30	Adhesion molecule, can activate neutrophils
CD66e (CEA)	Colon epithelium, colon carcinoma	180–200	Adhesion molecule
CD66f	Myeloid lineage cells		
CD67 now CD66b			
CD68 (EBM11, Ki-M7, Ki-M6, gp110)	DCs, PMNs, monocyte-macrophages, basophils	110	Marker of macrophages, lysosomal protein implicated in endocytosis

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD69 (MLR3, VEA, gp 34/28, AIM)	All activated leukocytes, neutrophils, platelets	85	C-type lectin involved in signal transduction for lymphocytes, apoptosis of eosinophils and in early events of T γδ cell cytotoxicity
CD70 (Ki-24, HNE 51, HNC 142)	Activated B and T cells, Reed-Sternberg cells	75, 95, 170	CD27 ligand, implicated in T-cell activation, TNRSF
CD71 (138–18, 120–2A3, T9)	Proliferating, activated T and B cells, macrophages	95	Receptor for transferrin and proteins binding Fe, activated on proliferating cells
CD72 (J3–109, BU-40, BU-41)	pan-B including progenitors, macrophages	43–43	C-type lectin, CD5 ligand, elicits B-cell activation/proliferation, inducible by IL_4
CD73 (AD2, 1E9.28.1, gp69)	B- and T-cell subsets, endothelial/epithelial cells	69	Ecto-5′-nucleotidase, may induce T-cell activation, B-cell interactions with FDCs
CD74 (LN2, BU-43, BU-45)	HLA class II, mature B cells, T cells, monocytes	41–35–33	Invariant chain associated with HLA class II prevents binding of foreign peptides
CD75 (LN1, HH2, EBU-141)	GC and mature B cells, T-cell subpopulation	53	GC B cells, putative ligand for CD22 supports B-B cells interactions
CD75s			α-2,6-Sialylated lactosamines, carbohydrate structures
CD76 (HD66, CRIS-4, ex CD76)	Mature B cells, T-cell subpopulation	53–87	Directs mantle zone and extrafollicular B cells in tonsil areas
CD77 (424/4A11, 424/3D9)	Activated GC and centrofollicular B cells, FDCs	–	Globotriaosylceramide (Gb3), may control transmembrane signals → apoptosis
CDw78 (Anti Ba, Leu21, 1588)	Resting and activated B cells, macrophages	–	Can inhibit or enhance B-cell activation
CD79a (mb-1, Igα)	Specific for B-cell ontogeny, BcR complex	33	BcR component, important for signal transduction comprising ITAM; IgSF
CD79b (B29, Igβ)	Specific for B-cell ontogeny, BcR complex	39	BcR component, important for signal transduction, comprising ITAM; IgSF
CD80 (B7–1, BB1)	Activated B and T cells, DCs, macrophages	60	Early activation marker, binding to CD28 and CD152 (CTLA-4) regulates IL_2 gene expression and activates T lymphocytes
CD81 (TAPA-1)	All B, T, NK cells, FDCs, thymocytes, eosinophils	26	Part of complex including CD19 and CD21: cross-linking CD81 is thought to have a role in signal transduction; TM4-SF
CD82 (R2, IA4, 4F9)	B, T, NK cells, macrophages, monocytes, platelets	60	Participates in T-cell activation, probably in signal transduction; TM4-SF
CD83 (HB15)	GC B cells, B cells, circulating DCs, LCs	43	Mature DC specific marker, may function in antigen presentation; IgSF
CDw84 (GR6, BPC6)	Mature B and T cells, platelets, macrophages	68–80	May be a signaling molecule

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD85 (VMP-55, GH1/75)	Mature circulating and neoplastic B cells	120, 83	ILT/LIR family, dendritic cells (DC)
CD86 (FUN-1, BU63, B7–2)	Activated, mature circulating B cells, DCs, monocytes	80	Rapid expression on activated B cells; interacts with CD28 and CD152 (CTLA-4) to regulate IL_2 gene expression and prevent T-cell anergy; IgSF
CD87 (UPA-R)	Activated PMNs, monocytes, NK and T cells	35–39	Binds vitronectin, receptor for urokinase, role in leukocyte extravasation
CD88 (C5aR)	Monocytes, granulocytes, NK cells, DCs, microglia	43	G-protein-coupled, C5a receptor on phagocytes
CD89 (Fcα-R)	Neutrophils, monocyte-macrophages, eosinophils	45–70	Fc receptor for serum/secretory IgA, signal transduction for phagocytes; IgSF
CD90 (Thy-1)	CD34 subset of bone marrow, CB and fetal liver hematopoietic stem cells, HEV endothelium	25–35	Associated with CD45, participates in T-cell recirculation, adhesion, activation, in cell–cell modulation and cell signaling; IgSF
CD91 (α_2M-R)	Monocyte-macrophages, non hematopoietic cells	600	Binds LDL, receptor for α2M-R mediator of endocytosis
CD92 (CTLL1, GR 9)	Monocytes, granulocytes, B, T, epithelial cells	70	Unknown
CDw93 (GR 11)	Monocytes, granulocytes, endothelial cells	110	Unknown
CD94 (kp43)	γ/δ T subsets, human NK cells and αβ CD8	70–43	Implicated in signal transduction for NK and T cells; the CD94 receptor (43kD) can inhibit HLA class I molecules; CD94 with a new associated protein (94AP) forms a NK receptor involved in the recognition of HLA-A, HLA-B, HLA-C molecules
CD95 (Fas/Apo-1)	Thymocytes, activated B and T cells	36–45	Transduces apoptosis signal, role in T-cell clonal deletion, TNFRSF
CD96 (TACTILE)	Activated T and NK cells	160	Promotes T and NK cells activation; IgSF
CD97 (GR1, BL-KDD/F12)	Monocytes, granulocytes, NK cells	74, 80, 89	Receptor for CD55 (?) and TNF; TNFR
CD98 (4F2, 2F3)	Monocytes, B and T cells, several cell lines	85, 40	Actin-associated, modulates intracellular Ca^{++} levels and cell proliferation (?)
CD99 (E2, MIC2)	All hematopoietic cells, thymocytes, T and B cells	32	T-cell rosette formation with erythrocytes, DP thymocytes adhesion to T cells
CD99R (CD99-mAb restricted)	B and T cells	32	Restricted CD99 hematopoietic cells
CD100 (BB18, A8, GR3, ST-003, -005)	Most hematopoietic cells, activated B, T, NK cells	150	Semaphorin, associated with CD45, PBMC proliferation, role in T-cell adhesion
CD101 (BB27, BA27, GR14, ST-004)	Monocytes, granulocytes, mucosal T cells	140	Inhibition of T-cell proliferation, possible role in T-cell signaling, IgSF

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD102 (ICAM-2)	Lymphocytes, monocytes, vascular endothelia, NK cells, HEVs, platelets	55–65	Binds CD11a/CD18 on endothelial cells, mediates T-cell recirculation, trafficking and activation, and stimulates leukocyte activity in inflammation; IgSF
CD103 (HML-1, M290)	Intraepithelial T cells, activated CD8 T cells	175–105	Integrin, $\alpha_E\beta_7$ subunit, binds E-cadherin, role in mucosal lymphocyte adhesion
CD104 (β1)	Epithelia, monocytes, T cells, keratinocytes	150–125	β4 Chain integrin, binds laminin
CD105 (GR7, CELL-CAM)	Endothelia, PMNs, activated monocyte-macrophages	90	Endoglin, receptor for TGF-β1 and TGF- β3; TGF-βR type III and ectoATPase
CD106 (VCAM-1, INCAM-110)	IL-activated vascular endothelia, bone marrow stromal cells, embryonic tissues, APCs	90–110	Binds CD49d/CD29; lymphocyte migration, recruitment, activation/stimulation by APCs, PBMC and eosinophil adhesion stimulated by endothelial ILs; IgSF
CD107a (LAMP-1)	Activated platelets, T cells, neutrophils	110	Lysosome-associated protein
CD107b (LAMP-2)	Activated platelets, T cells, neutrophils	120	Lysosome-associated protein
CD108 (SEMA7A)	Splenic T lymphocytes, some stromal cells	80	Human blood group antigen
CD109 (8A3, 7D1, PAF)	Endothelial cells, T cells, activated platelets	170/150	Possible role in activation, proliferation and signal transduction
CD110 (MPL, TPO-R)	Platelets		
CD111 (PRR1/Nectin1)	Myeloid cells		
CD112 (HVER, PRR2)			
CD113 (PRR2)	Myeloid cells		
CD114 (CSF3R, G-CSFR)	Monocytes, granulocytes, endothelial cells, platelets	130	G-CSF receptor; CSF3R
CD115 (CSF-1R, M-CSFR)	Monocyte-macrophages and progenitors, placenta	150	M-CSF receptor (CSF-1) promotes phagocyte proliferation/ differentiation
CD116 (GM-CSF)	Macrophages, eosinophils, PMNs, DCs, fibroblasts	75–85	Binds GM-CSF, with higher affinity if coexpressed with CD131, supports proliferation and differentiation; class CSFR
CD117 (SCF-R, c-kit)	TN thymocytes, hematopoietic progenitors	145–150	SCF receptor, signal transduction, differentiation and adhesion; IgSF
CD118 (IFN-α/βR)	Broad cell expression	–	Receptor for IFN-α and IFN-β; CSFR
CD119 (IFN-γR)	Macrophages, B, T, NK and epithelial cells	90	IFN-γα chain receptor induces macrophage activation, B-cell differentiation; A CSFR

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD120a (TNFR.I, p55)	Granulocytes, epithelial cells, FDCs, monocytes	55	Type 1 TNFR, up-regulates leukocyte adhesion molecules and inflammation; TNFRSF1A
CD120b (TNFR.II, p75)	Granulocytes, myeloid cells, monocyte-macrophages	75	Type 2 TNFR, signaling triggers biological effects TNFRS1B
CD121a (IL_1R 1)	Thymocytes, fibroblasts, T cells, endothelial cells	75–85	Type 1 IL_1R, promotes cell growth in synergy with IL_2 and IL_4, protects against intracellular pathogens, inhibits the IL_{12} protective effects; IgSF
CD121b (IL_1R 2)	Neutrophils, monocytes, bone marrow and B cells	68	Type 2 IL_1R; IgSF
CD122 ($IL_2R\beta$)	Activated T cells (α, β, γc), $\beta\gamma$ chain on NK cells, CD8 T cells	70–64	β Chain of IL_2R, responsible for all the effects induced by IL_2, γc chain could prevent induction of anergy, the complex IL_2 + CD122 + CD25 forms a high-affinity receptor for the activation of thymocytes, B, T and NK cells and macrophages, incrementing the NK cytolysis and Ig synthesis; CKR-SF
CD123 ($IL_3R\alpha$)	Bone marrow stem cells, megakaryocytes, granulocytes		$IL_3R\alpha$ CKR-SF (cytokine receptor-superfamily)
CD124 (IL_4R)	Mature B and T cells, hematopoietic precursors, fibroblasts, epithelial and endothelial cells, pre-B, B and T lymphocytes	130–150	Activates B cells increasing CD23 and IgM expression; is a switch factor implicated in IgE regulation. Associates with $IL_{13}R\alpha$ to form $IL_{13}R$ complex; CKR-SF
CDw125 ($IL_5R\alpha$)	B cells, eosinophils, basophils	80	Combines with CDw131 to form the high-affinity receptor for IL_5; CKR-SF
CD126 ($IL_6R\alpha$)	T and B cells, monocytes, fibroblasts, hepatocytes	60	α Subunit, forms with CD130 the high-affinity receptor for IL_6; CKR-SF
CD127 (IL_7R, $IL_7R\alpha$)	Thymocytes, T/B cell progenitors, mature T cells, monocytes, lymphoid and myeloid cell lines	68–64	The two chains associate to form the high-affinity receptor for IL_7, triggers proliferation of pro- and pre-B cells and immature T-cell growth; CKR-SF
CDw128 (IL_8R)	NK cells, neutrophils, monocytes	58–67	Receptor for IL_8 and α chemokines
CD129 (IL_9R)	T and B cells, macrophages, megakaryoblasts	64	Receptor for IL_9, 40% homology with $IL_2R\beta$, inhibits apoptosis; CKR-SF
CD130 (gp130, SIG, IL_6R)	All hematopoietic cells, many other cell lines	130–140	β Subunit; signal transduction chain for IL_6 receptors; CKR-SF
CD131	Widespread on myeloid cell types	120–140	Common β subunit associated with α subunit of IL_3R (CD123), IL_5R (CDw125) and GM-CSFR (CD116) ; class BCSFR (see Table 1.5: it links to IL_6R)

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD132	B, T, NK cells, monocytes, PMNs, macrophages	65–70	IL_2R γc (common) chain, subunit of IL_2R, IL_4R, IL_7R, IL_9R, $IL_{15}R$, $IL_{21}R$ and similar signaling components of receptors; associated with JAK3; CKR-SF
CD133 (AC133)	Stem/progenitor cells		Prominin1 (PROM1)
CD134 (OX40)	Expressed on activated T cells and macrophages	50	Adhesion of activated T cells to vascular endothelial cells via gp 34, TNFRSF4
CD135 (Flt3, Flk2)	All hematopoietic stem cells, B-cell progenitors	130	Tyrosine kinase receptor, growth factor for hematopoietic progenitors, IgSF
CDw136 (MSP-R)	Tissue macrophages, some epithelial cell lines	180	Receptor for macrophage-stimulating protein (MSP-R)
CD137 (4–1BBL)	B and T cells, macrophages		T-cell activation and differentiation, distinguishes between IgE- and non-IgE-mediated atopic dermatitis and asthma, TNFRSF9
CD138	B cells		Glycosaminoglycan, binds fibronectin, collagen, thrombospondin
CD139	B cells, granulocytes, monocyte-macrophages		
CD140a (PDGF-Rα)	Endothelial cell lines		Receptor for PDGF α chain; CKR-SF
CD140b (PDGF-Rβ)	Monocytes, PMNs, fibroblasts, smooth muscle cells	160	Receptor for PDGF β chain, tyrosine kinase directing signal transduction
CD141	PMNs, monocyte-macrophages, endothelia, platelets, smooth muscle cells, keratinocytes, megakaryocytes	75	C-type lectin, thrombomodulin
CD142	Monocytes, vascular endothelial cells, fibroblasts, keratinocytes, stromal cells	45–47	Serine protease cofactor, binds factors VIIa and Xa coagulation factor III
CD143 (ACE)	Endothelial cell lines		Peptidylpeptidase, binds angiotensin
CD144	Vascular endothelial cell lines		Cadherin-5 or VE-cadherin, binds β-cadherin
CDw145	Endothelial cell lines (?)		
CD146 (MUCI8, 5-endo)	All leukocytes, endothelial cells, platelets	50–60	Likely adhesion molecule; IgSF
CD147	Widespread cell diffusion		Neurotelin, basigin; IgSF
CD148 (HPTP-η)	Granulocytes, monocytes, DCs, Kupffer cells	240–260	Proteintyrosinphosphatase (PTP)
CD150 (SLAM, IPO-3)	B and T cells		SLAMF1, IgSF
CD151 (PETA-3)	Macrophages, platelets		TM4-SF

SLAM signaling lymphocytic activation molecule.

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD152 (CTLA-4)	Activated cytotoxic T cells molecule, modulates lymphocyte homeostasis		CTLA-4; 70% similarity with CD28, binds CD80 and CD86, costimulatory; killer cell Ig-like receptors IgSF
CD153 (CD30L)	Activated T cells, B cells, neutrophils, macrophages		CD30L; cross-linking supports T-cell proliferation and IL production; TNFSF5
CD154 (CD40L, T-BAM, gp39)	T and NK cells, basophils, mast cells, eosinophils production; CD154 deletion results in the ID with hyper-IgM	33	Ligand for CD40, activates B cells, costimulates T-cell proliferation and IL production, CD154 deletion results in the hyper-IgM syndrome, TNFSF5, belongs to the family of TNF-α and -β, NGF, and of Fas, CD27, CD30 ligands
CD155 (PVR)	Monocytes	80–90	Poliovirus receptor; IgSF
CD156 (ADAM-8, MS2)	Neutrophils, monocytes	69	Metalloprotease, binds peptidase, possibly involved in leukocyte extravasation
CD156b (TACE/ADAM17)	Adhesion structures		Snake venom-like protease
CD157 (BST-1, MO-5)	B and T lines, monocyte-macrophages, granulocytes	50	Unclear function in myeloid cells
CD158e, i, k (KIR family)	NK cells		NK receptors specific for class I HLA; IgSF
CD159a (NKG2A)	NK cells		Killer cell lectin-like receptor subfamiliy C
CD160 (BY55, NK1, NK25)	NK cells		
CD161 (NKR-P1)	NK cells		Killer cell lectin-like receptor subfamiliy B, binds NK cells
CD162 (PSGL-1)	Myeloid cells, granulocytes, monocytes	120	Sialomucin, binds CD62P
CD162R (PEN5)	NK cells		
CD163 (M130)	Tissue macrophages, LPS-stimulated monocytes	110	Unknown function; SRCR-SF
CD164 (MGC-24)	Epithelium, monocytes, bone marrow stromal cells	80	Potential adhesion molecule
CD165 (GP37/AD2)	T cells		Potential adhesion molecule
CD166 (ALCAM)	Activated T cells and monocytes, epithelium, fibroblasts	100–105	Adhesion molecule binding CD6; IgSF
CD167 (DDR1)	Adhesion structures		Discoidin
CD168 (RHAMM)	Adhesion structures		
CD169	Adhesion structures		Sialoadhesin
CD170 (Siglec-5)	Adhesion structures		
CD171 (L1)	Adhesion structures		
CD172a (SIRP α)	Adhesion structures		

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD173	Carbohydrate structures		
CD174 (Lewis y)	Carbohydrate structures		
CD175 (Tn)	Carbohydrate structures		Tn antigen (T antigen novelle)
CD175s	Carbohydrate structures		Sialyl-Tn
CD176 (TF)	Carbohydrate structures		
CD177 (NB1)	Myeloid cells		
CD178	Cytokine/chemokine receptors		Fas ligand (CD95L), TNFSF6
CD179a	B cells		Vpre-B
CD179b (×5)	B cells		
CD180 (RP105)	B cells		
CD183	Cytokine/chemokine receptors		CXCR3
CD184	Cytokine/chemokine receptors		CXCR4
CD195	Cytokine/chemokine receptors		CCR5
CDw197	Cytokine/chemokine receptors		CCR7
CD200 (OX2)	Thymocytes, B cells, T cells		
CD200R	Monocyte/macrophage, DCs		
CD201 (EPC R)	Endothelial cells		
CD202b	Endothelial cells		Tie2 (Tek)
CD203c (NPP3/PDNP3)	Myeloid cells		Expressed on blood basophils but not on other blood leukocytes
CD204	Myeloid cells		Macrophage scavenger R
CD205 (DEC205)	Dendritic cells		
CD206	Dendritic cells		Macrophage mannose R
CD207	Dendritic cells		Langerin
CD208 (DC-LAMP)	Dendritic cells		
CD209 (DC-SIGN)	Dendritic cells		
CDw210 (IL_{10} R)	Cytokine/chemokine receptor		$IL_{10}RA$, $IL_{10}RB$
CD212 (IL_{12} R)	Cytokine/chemokine receptor		$IL_{12}R\beta$

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD213a1	Cytokine/chemokine receptor		$IL_{13}R\alpha1$
CD213a2	Cytokine/chemokine receptor		$IL_{13}R\alpha2$
CDw217	Cytokine/chemokine receptor		$IL_{17}R$
CD220	Non-lineage molecules		Insulin R
CD221 (IGF1R)	Non-lineage molecules		
CD222	Non-lineage molecules		Mannose-6-phosphate/RIGF2 R
CD223 (LAG-3)	Non-lineage molecules		Lymphocyte activation gene 3
CD224	Non-lineage molecules		γ-Glutamyl transferase
CD225 (Leu13)	Non-lineage molecules		
CD226 (DNAM-1 PTA1)	T cells		
CD227 (MUC.1)	Non-lineage molecules		
CD228	Non-lineage molecules		Melanotransferrin
CD229 (Ly9)	Non-lineage molecules		
CD230	Non-lineage molecules		Prion protein
CD231 (TALLA-1/A15)	Non-lineage molecules		
CD232 (VESPR)	Non-lineage molecules		
CD233 (Band 3)	Erythroid cells		
CD234	Erythroid cells		fy-Glycoprotein (DARC)
CD235a	Erythroid cells		Glycophorin A
CD235b	Erythroid cells		Glycophorin B
CD235ab	Erythroid cells		Glycophorin A/B cross-reactive mabs
CD236	Erythroid cells		Glycophorin C/D
CD236R	Erythroid cells		Glycophorin C
CD238 (Kell)	Erythroid cells		
CD239 (B-CAM)	Erythroid cells		
CD240CE (Rh30CE)	Erythroid cells		Cross-reactive mabs
CD240D (Rh30D)	Erythroid cells		

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD240DCE (Rh30D/CE)	Erythroid cells		
CD241 (RhAg)	Erythroid cells		
CD242 (ICAM-4)	Erythroid cells		
CD243 (MDR-1)	stem/progenitor cells		
CD244 (2B4)	NK cells eosinophils		Elicites ERK, activates NK cells and causes eosinophils to release EPO, IL_4 and IFN-γ
CD245 (p220/240)	T cells		
CD246	T cells		Anaplastic lymphoma kinase
CD247	T cells		ζ Chain of TcR

CDw128 has been divided into CDw128a (CXCR1, IL_8RA) and CDw128b (CXCR2, IL_8RB). The main cells are underlined following the Immune Receptor Supplements [4, 6, 262] and International Workshops on Human Leukocyte Differentiation Antigens [501]. Data from the 5th International Workshops on Human Leukocyte Differentiation Antigens [453], Immune Receptor Supplement, 1st [4] and 2nd ed [5], 1997, CD antigens 1996 [257], CD Designations, 7th HLDA Workshop [331] and new CD designations (2002) [691] and from [4, 6, 16, 23, 34, 47, 70, 82, 85, 102, 103, 109, 112, 120, 132, 147, 158, 175, 242, 262, 343, 407, 428, 437, 442, 484, 501, 515, 559, 571, 584, 608, 688, 691]. The 8th HLDA Workshop has extended the CD nomenclature up to CD339 (S. Zola pers. com., March 5, 2005). Abbreviations are in the list.

- They are committed to specific, initial antigen recognition by interacting with a restricted part of the macromolecule called epitope by means of specific membrane receptors present on both B and T cells [326].

There are two major sets of lymphocytes. The antibodies produced by B lymphocytes play a crucial role in interacting with extracellular antigens, when found outside the cell (for example, when viruses are encountered in the blood), whereas these antibodies are ineffective against endocellular antigens, because overall they are unable to penetrate the cells. Accordingly, T lymphocytes are probably originally derived to ensure a high specificity in the critical phase of immune responses to pathogens of various sources. *Antigen recognition takes place in a different way for each phenotype,* since T cells respond to antigens only when encountered inside or on the target cell surface, thereby showing that T-cell antigen recognition is fundamentally different from B-cell antigen recognition. Such properties are gradually acquired during ontogenesis when lymphocytes undergo a series of differentiation events, each characterized by sequential expansion or regression of the genes coding for expression of membrane proteins and glycoproteins (gps) [35, 481, 647].

Structure and Molecular Framework

Igs or antibodies are molecules of specific immunity, present in soluble form in biological fluids or as membrane receptors at BcR (B-cell receptor) and TcR surface. The immune system consists on the whole of $\approx 10^{12}$ T cells and of $\approx 10^{20}$ B cells: in the bloodstream ≈80% of lymphocytes are T, 10%–15% are B cells and 10%–15% are *null cells* known as LGLs (*large granular lymphocytes*), non-T non-B since they lack markers of B and T cells. The Ig N-terminal part has a binding site or paratope providing specific recognition. The C-terminal transmits biological signals following epitope-paratope binding. TcR, BcR, and Ig polypeptide chains are composed of subunits, each made up of ≈100 amino acids, bound to disulfide (-S-S) bonds, termed domains or regions. Such striking similarities are also found in molecules belonging to the *Ig superfamily (IgSF):* although these proteins are found in diverse tissues, they all appear to share a common primordial origin.

B Lymphocytes

B cell precursors, the first cells to be appraised in ontogenesis, originate from hemopoietic bone marrow stem cells and complete developmental and maturative processes that began in the fetal liver. Briefly, the earliest distinguishable cells in the B repertoire are known as pro-B cells and pre-B cells with no surface Igs but with surrogate light (L) chains. At this stage the cells express RAG-1 and RAG-2 (recombination-activating genes),

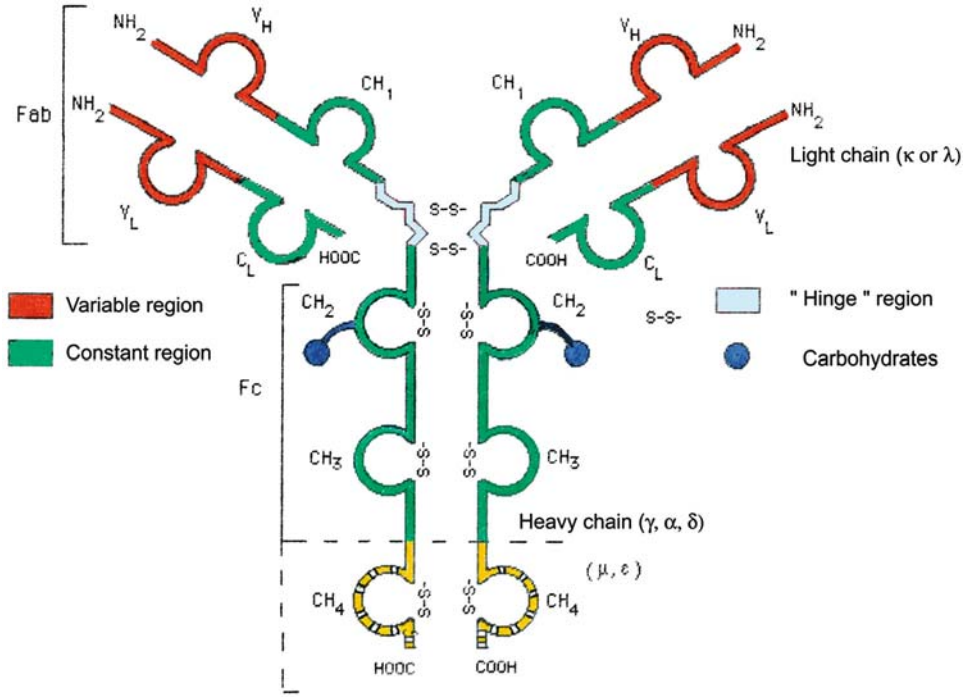


Fig. 1.6. Immunoglobulin basic structure. VH + VL Antigen binding site, which is located in the V (variable) domain. CH_1 + CL Covalent and noncovalent binding between the H (heavy and L (light) chains; spacer between the antigen binding site and effector functions. Intrachain -S-S bonds regulate the molecular flexibility and determine the spatial conformation. CH_2 C1q fixing site (complement activation). CH_2 + CH_3 Binding site for Fc receptor of monocytes, macrophages, neutrophils, streptococcus A protein, syncytial trophoblasts (placenta crossing). CH_3 binding site for Fc receptor of lymphocytes and mast cells

whose deficiency is responsible for one form of SCID; affected patients are unable to produce functional lymphocytes bearing antigen receptors [508]. The next cells in the B-cell lineage bearing monomeric surface IgM (IgMs or mμ) are referred to as immature B cells. In the following stages of maturation, a mature B cell expresses IgM, IgD, and Fc receptors (FcR) for IgGs. Up to this point, all maturative steps take place in the bone marrow and are antigen-independent (Chap. 2). It has been suggested that bursal epithelial cells might produce polypeptides, triggering maturation of lymphoid stem cells into immunocompetent B cells, similarly to what occurs in the thymus. These short-lived lymphocytes colonize typical zones of lymph nodes and the spleen. Each cellular clone synthesized in a GC produces isotypes of the same class, specific for a given antigen. During the evolution of immune responses, there is a switch in Ig classes from IgM to IgG, IgA and IgE. If IgG and IgA antibodies are produced last, this could account for an associated defect of the two Ig classes. Babies with X-linked Hyper-IgM Syndrome (HIgMS) have little or no IgG and IgA [96, 544].

The Igs are structurally similar. A typical antibody molecule has a Y-shaped configuration when viewed schematically (Fig. 1.6) and consists of a basic unit of four polypeptide chains, two identical L and two identical H (heavy) held together by interchain -S-S bonds also present on single chains and by hydrogen bonds. As the figure shows, NH_2 and COOH indicate amino and carboxy terminals, respectively. L chains are common to the diverse Igs, which instead differ from their H chains that determine class type, hence IgA H chains are α, those of IgG are γ, etc. Each L chain is made up of two domains: a V region that varies from antibody to antibody and a constant (C) region, essentially identical among L chains of a given type (Fig. 1.7). For this reason, the molecules are practically identical, except in amino-terminal domains where variability differentiating V domains of both L and H chains is most pronounced [18]. In man, C regions are encoded by one of four C_H genes for H chains and by one of two C_L genes for L chains (Lλ and Lκ). H chains therefore consist of four C and one V domains and L chains of one C and one V region. Both chains, organized in sequence, are distinguished by their succession of amino acids: L chains consist of 211–217 amino acids and H chains of 450 for γ and α chains, and of 614 for μ and ε chains [3]. One noteworthy characterization of Igs lies in their *bifunctional aspects,* in that they bind antigens and, in addition, elicit biological processes that are independent of antibody specificity. Each of these functions is localized to a different part of protein: C domains have various effector activities (complement activation, opsonization, etc.) via IgG, IgE and IgG_4 FcRs, while V do-

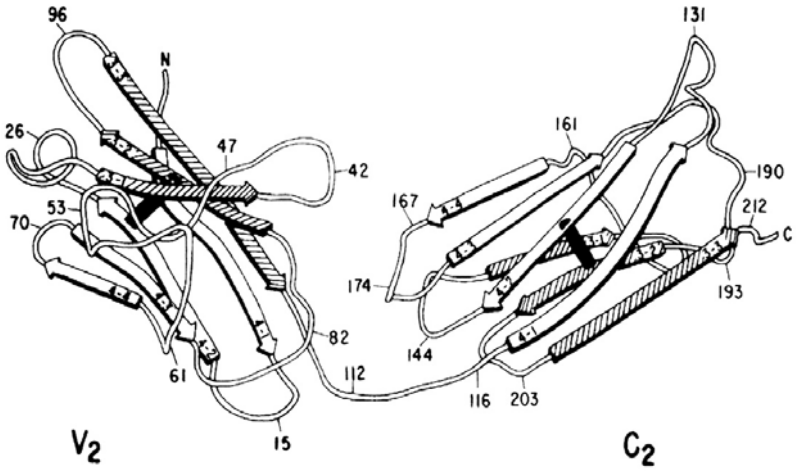


Fig. 1.7. Structure of V and C domains of a light chain. The -S-S bridges in each domain are indicated by *thick black lines*. The hypervariable regions that form the antibody combining site or paratope correspond to the loops around positions 26, 53 and 96

mains are committed to the specificity of antigen recognition. V regions within any group are not uniformly variable across their whole 110 amino acid span. Instead, the greatest amount of variability of V domains of each chain (genes V_H and V_L) is concentrated in three regions of both L and H chains called hypervariable regions: the less variable stretches interspersed within these hypervariable regions are called *framework regions* (FR) of 15–30 amino acids. The six hypervariable regions, which are each 9–12 amino acids long, participate in antigen binding and form a region that is complementary in structure to antigen epitopes, accordingly designated *complementarity determining regions* (CDRs) principally involved in antigen contact, three in V_H domains and three in V_L domains [86]. H chains γ, α and δ are formed by four C_H (C_H1, C_H2, C_H3 end C_H4) domains, μ and ε by five. Only γ, α and δ chains have a hinge region located between two C_H domains; however, for the other two chains there is the C_H2 region. The C domain of H chains is dictated by one gene (C_γ, C_μ, C_ε, etc.) dictating isotypes [474].

To understand the diversity of antibody repertoires, recent evidence shows that coding information of a typical human gene, that is the sequences coding regions to be transduced into amino acid sequences, does not exist as intact, functional genes. Therefore a single, uninterrupted DNA stretch in germ cells is broken up along the chromosome and dispersed along the DNA strand into multiple, shorter discrete entities (exons), widely separated by noncoding DNA regions (introns). Eventually, exons coding for V domains may be broken down in still smaller segments, each lacking some features needed for proper RNA splicing and unable to function separately [470]. Consequently, a V domain of a V_L chain is encoded by two separate DNA gene segments, exons separated by introns: the first 95 amino acids of V_κ domains are dictated by a V exon, the shorter exon J_κ (joining) codes the remaining 13 amino acids (96–108) of C-terminal domains [414]. H chains are dictated by V_H, J_H, D_H (diversity) and C_H exons; the D exon, absent in L chains, dictates a few amino acids and is in charge of the higher H chains variability compared to L chains [3]. An L chain gene is assembled from three types of gene segments: V_L and J_L are transferred on their genome (total inherited DNA) and integrated with a C_L segment. This machinery, known as *gene rearrangement*, necessary for transcription of gene information (mRNA synthesis) is employed only for genes dictating Ig L and H chains or TcR. After genes are transcribed into RNA, introns are removed from transcripts and exons are joined together by RNA splicing. As will be seen later, this rearrangement is the core of the capability shown by the immune system to recognize an incredible variety of antigen structures in nature [18].

Genetic Rearrangements for Ig Synthesis

Antibody molecules are encoded by three independent groups of genes. Two genes dictate λ chains: one comprises V_λ and C_λ genes, κ comprises V_κ and C_κ, genes, while the third group dictates H chains and has V_H and C_H genes. Before synthesizing Ig chains, however, Ig genes must be assembled within a genome of differentiating cells (rearrangement). Briefly, a V region gene can be located in a DNA position of an inherited chromosome (*germline*), and can then move to another position on the chromosome during lymphocyte differentiation. For example, five J segments of a κ chain are clustered near C exon, whereas at least 30–35 different V segments lie scattered widely over many DNA kilobases (kb). Rearrangements follow an order fixed in advance: H chain first, then L κ, finally L λ, encoded by chromosomes *14q32*, *2p12* and *22q12*, respectively (Fig. 1.8), with resulting deletion of intervening genetic materials and consequent realignment according to a configuration calling for Ig generation [186].

In the rearrangement of H chains, selection of one each from ten D, five J genes, and ≈200 or more V regions will produce a tripartite gene complex VDJ recombination for V_H regions. VDJ recombination is the process by which the V region exons encoding the antigen recogni-

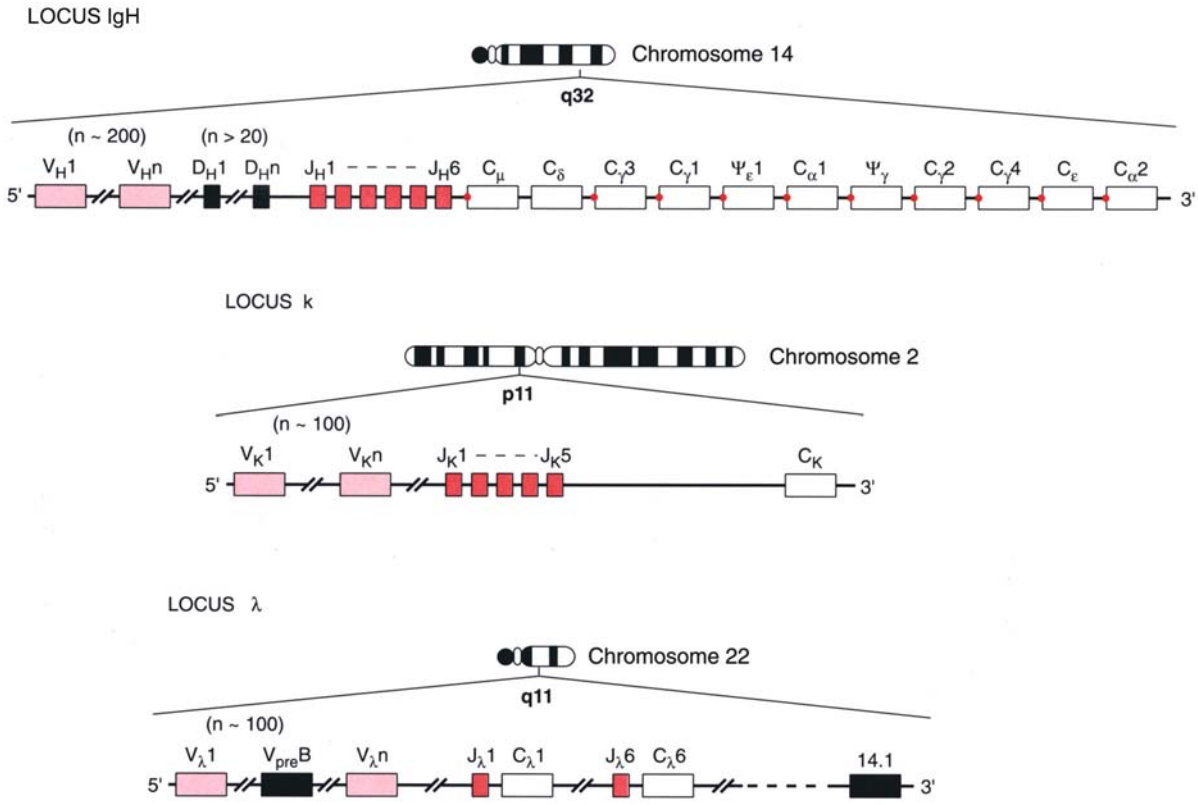


Fig. 1.8. Schematic representation of chromosomes 14, 2 and 22

tion sites of receptors expressed on B and T lymphocytes are generated during early development via somatic assembly of component gene segments [128]. During maturation of B and T lymphocytes, the first rearrangement involves H *locus* with the juxtaposition of D and J genes (DJ rearrangement) by the deletion of intervening DNA. This rearrangement occurs in a very early stage of maturation, when precursors are not yet clearly committed to B lineage; indeed it is also observed in 20% of T cells [474]. The second rearrangement involves subsequent translocation of DJ segments to selected V region genes (VDJ rearrangement) (Fig. 1.9), which occurs in pre-B cells, already committed to B lymphocyte family. As a consequence, the VDJ segment joins the C_H gene [591]. However, recombination can be somewhat imprecise, since during this process several nucleotides may be removed from or added to a junction, so combination of VDJ segments of H chains results in a large number (10 D_H × 4 J_H × 200 V_H) of possible sequences (that is, antibodies). This reshuffling process, known as *combinatorial joining,* is the prevalent source of protein diversity. For example, when organizing κ genes, as there is only one Cκ exon, all κ proteins must have identical C domain sequences, while cells can choose among several Vκ and Jκ segments, with the result of joining such segments in diverse combinations. Thus, a large number of different V region sequences can result and when combined with 50 κ chain V domains could form 400,000 different V_H genes. Considering a necessary heterogeneity of gene segments and combining 400,000 with 200 V_κ and four J_κ genes (200×4) × 10 equal to flexibility of VJ rearrangement, we have *at least* $3{\times}10^8$ *different antigen-binding sites*. Such an outstanding recombinant capacity is further increased by somatic mutations to 10^{10}–10^{15} [18]. Nonetheless, even using a relatively small amount of gene segments, the immune system can secrete an impressive antibody diversity through combinatorial joining [414].

In the most common scenario, a VDJ segment joins first with C_μ genes, and subsequently with C_γ, C_ε, C_α genes, with synthesis of a complete H IgM chain, etc. Without an associated L chain, surface expression is not possible and only cytoplasmic μ is found (pre-B cells). Isotype switching requires DNA rearrangements. Consequently, a single V gene is associated in successive phases with different C genes on the same chromosome, thereby allowing each cell to produce antibodies of different isotypes with the same paratope. Several components that are involved in this process have been identified. Among them, a complex system of enzymes and other proteins jointly known as *recombinase activity* appear to be necessary for gene rearrangements. Thus the enzyme VDJ recombinase catalyzes recombination processes, during which intervening DNA is usually excised. However, since similar sequences are found also

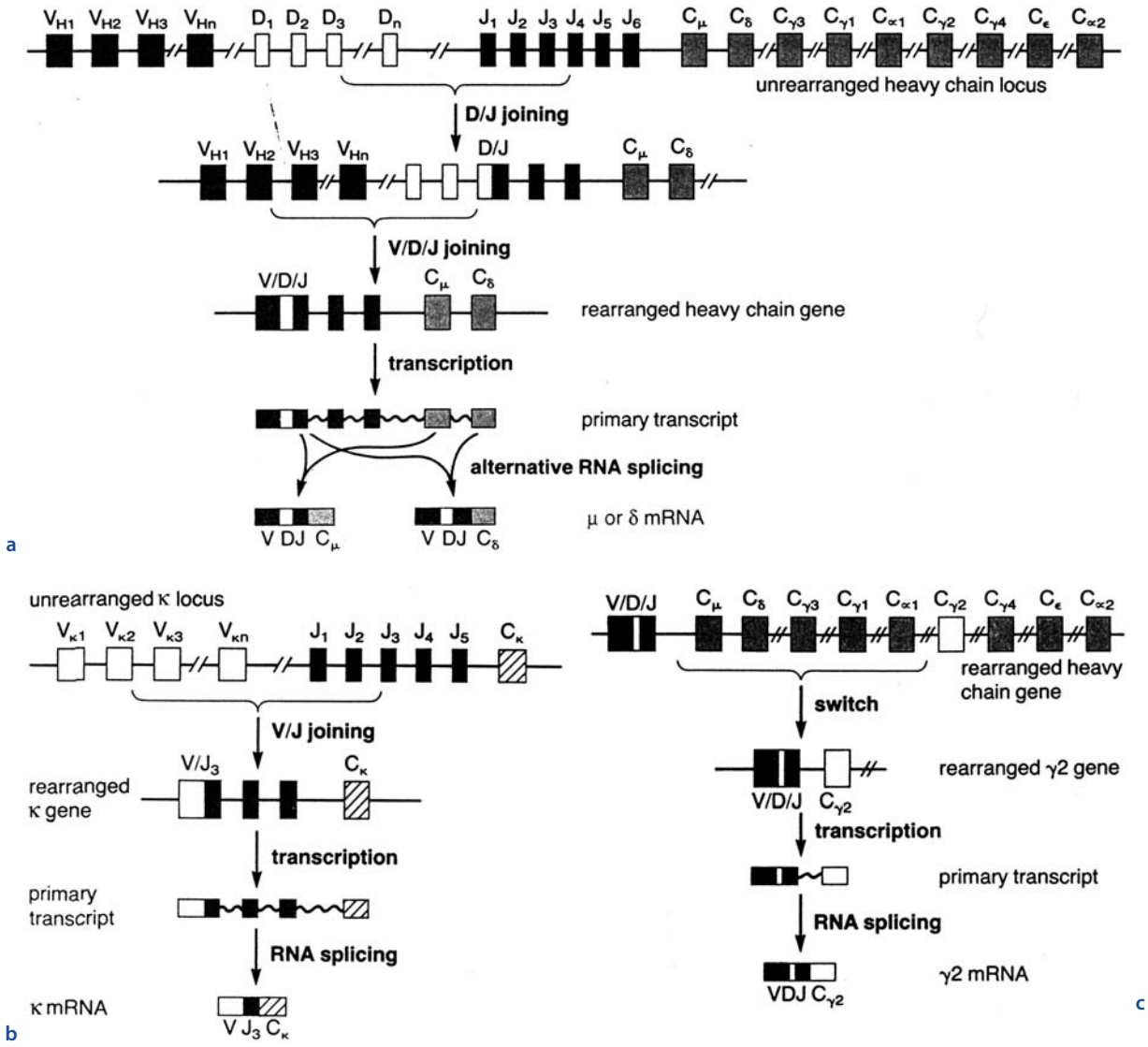


Fig. 1.9. **a** Schematic depiction of transcription, rearrangement and splicing of an H chain (Hμ or Hδ). **b** Schematic depiction of transcription, rearrangement and splicing of an L chain (Lκ or Lλ). **c** H-chain isotype switching

in TcR T-cell regions, it appears that VDJ recombinase is used by both B and T cells. VDJ recombination proceeds via precise DNA cleavage initiated by the RAG proteins at short conserved signal sequences [128]. Whatever their precise role, the coordinated expression in pre-B and pre-T cells is essential for the rearrangement of Ig genes and TcR αβ chains, but RAG activity is switched off in mature lymphocytes [299]. Mutations of such genes consisting in imprecise gene segment joining of coding sequences during VDJ recombination appear to cause SCID with B and T cells severely impaired [299].

After hierarchical H-chain rearrangement, L-chain rearrangement occurs (Fig. 1.9): when a D segment is absent, V domain is encoded by VJ genes (VJ rearrangement), with subsequent juxtaposition to respective C segment; λ genes are rearranged only in the event of a nonproductive κ rearrangement. This procedure continues until efficient V genes are generated for H and L chains and ends with synthesis of functional polypeptides.

Rearrangements are carefully orchestrated, following the principle of *allelic exclusion,* that is, in each B cell is transcribed the gene product of only one of each chromosome pair. The gene located on the second chromosome typically is not used, to prevent contemporary synthesis of H chains with differing V domains in any given cell; it is an *allele*, a term designating two genes or two or more alternate forms of a single gene occupying the same *locus* [591]. The gene on the second chromosome is not rearranged unless a nonproductive rearrangement occurs; when rearrangements are nonproductive on both chromosomes, cell death ensues, there-

by clarifying why the same B lymphocytes within their entire life span can produce only a single type of L chain (κ or λ) [474].

Precombination of gene segments implies further elements of diversity, due to either an imprecise DNA rearrangement of nucleotides that are believed to be inserted at V–J junction, which increases junctional diversity of L chains, or of H chains by mechanisms amplifying diversity, including the possible joining of two D genes, with or without inversion of one rearranging gene segment, as well as disparities in recombination of chromosome 14 [263]. Another very important mechanism generating variations is somatic mutations, which are able to introduce a heterogeneity even higher in nucleotide sequences. Insertions of such nucleotides are carried out by the enzyme terminal-deoxynucleotidyl-transferase (TdT), a terminal-independent DNA polymerase expressed in B lymphocytes early during their development, but also in thymocytes of all species. The variations in gene sequences resulting from imprecise joining or from insertion of N regions, via addition of nucleotide sequences, absent in germline, provide a supplemental diversity at VDJ junctions designated "N-region diversity." Moreover, these processes affect sequences within each VDJ exon coding for CDR3 of L- or H-chain V domain. Hence, TdT can be viewed as another component of the VDJ recombinase system [263]. This implies that all these mechanisms engendering a disproportionate antibody diversity leave an unknown fraction of B cells to aberrant Ig gene rearrangements, probably >50% [47]. Recent data show that TcR and Ig V, D, and J gene segments are flanked by conserved *recombination signal sequences* (RSS), consisting of a heptamer and a nonamer separated by a nonconserved spacer of either 12 or 23 nucleotides. The 12–23 base pair rule first postulated to explain Ig gene rearrangement also governs TcR gene recombination. Virtually, during a rearrangement, a gene with a flanking sequence containing a 12-base pair spacer can only join to a gene whose flanking sequence has a 23-base pair spacer and vice versa, thereby elucidating the precise order of Ig gene transcriptions [337].

In conclusion, because of the very efficient DNA usage displayed in gene rearrangements, as well as RNA transcription and joining machinery described above, B lymphocytes can produce an isotype switching conserving antigen specificity, although utilizing an assembly of about 300 gene segments, there would be several million different antibodies in the same subject [3]. Once the recombination process is completed, C region genes of both H and L chains are associated with the VDJ or VJ complex. In this way, the VDJ complex becomes joined to C region genes of other isotypes located downstream on the same chromosome. The conclusion of such rearrangements is the production of different isotypes of the same antibody. Such antibodies capable of recognizing a specific antigen will be on the whole clone, on the whole progeny originating from a single B cell precursor. In that way, each lymphocyte clone can respond only to antigens able to bind to its unique pair of H and L chains, and all antibodies synthesized by an activated clone are directed against that particular antigen, as in the case of B lymphocytes, or has receptors ready for the antigen, as in the case of T lymphocytes [470].

On the other hand, papain digestion splits Ig molecules yielding three fragments of roughly similar size (50 kD), two monovalent Fab (fragment antigen binding) fragments with a whole L chain and half H chain (retaining ability to combine with antigen), and an Fc fragment (fragment crystallizable) comprising carboxy-terminal portions of both H chains. On B cell membrane, Igs are disposed as follows: the tail of Y is the Fc fragment, while the arms correspond to Fab fragment (Fig. 1.6).

Pepsin digestion yields a single large fragment called $F(ab)'_2$, roughly corresponding to two -S-S linked Fab fragments with bivalent antigen-binding activity. Antibody ability to fix on several cell types with Fc region of H chains takes place via FcRs, present on different cells. FcR molecules appear to exemplify a point of contact and cooperation between humoral and CMI. The main FcR for H chains of different Igs are summarized in Table 1.3 [186, 486].

In addition to the variability related to paratope diversity for antigen molecules, under appropriate circumstances antibodies deliver three main forms of Ig epitopes as defined by their location on antibody molecules, that is isotype, allotype, and idiotype determinants [18].

The term *"isotype"* is often used as equivalent to the terms "class" and "subclass;" isotypes are Ig epitopes codified by identical gene segments found in all healthy individuals of the same species. Individuals of a different species have different isotypes, since each isotype is located on a distinct gene locus on the pertinent genome. Antibody isotypes are defined principally in relation to H- or L-chain C regions and in some instances to V_H and V_L invariable regions, as are Ig epitopes that characterize classes, subclasses and types of H and L chains. As a general rule, isotypes are recognized by antibodies produced by different species.

The *allotypes,* or genetic markers, a form of variation in Ig structure, are allelic forms of the same protein as a result of different forms of the same gene at a given locus. They are located in C regions of both L and H chains: allotypes of L chains are named Km, those of H chains Gm, Am, and Mm. Inherited as dominant mendelian traits, they stem from genetic differences in amino acid sequences of C genes (*genetic polymorphism,* GPM). Allotypes are accepted in some countries as legal evidence in paternity disputes. As in other allelic systems, variants are not present in all healthy subjects and therefore distinguish Igs of one subject from Igs of another, as do blood groups.

The *idiotypes* (from Greek "ιδιος," personal or private), are as many as B-cell clones, 10^8 in adults [186], and are formed by single idiotopes building a given paratope with its characteristics and binding idiotypes

Table 1.3. Immunoglobulin (*Ig*) receptors

Receptors	CD	Immunoglobulins	Cells
FcαR	89	IgA	Neutrophils, lymphocytes, monocyte-macrophages, eosinophils
FcεRI	–	IgE	Mast cells, basophils, eosinophils, LC
FcεRIIa	23	IgE	B cells
FcεRIIb	23	IgE	B and T cells, eosinophils, LCs, NK cells, platelets
FcγRI	64	IgG_1 +++, IgG_3 +++, IgG_4 ++	LCs, monocytes, neutrophils
FcγRII	32	IgG_1 +, IgG_3 +	B, T and NK cells, monocytes, macrophages, eosinophils, neutrophils, platelets, basophils, LC, FDC, epithelial and stromal cells
FcγRIII a, b	16	IgG_1 +, IgG_3 +	T and NK cells, neutrophils, macrophages

FcαR consists of an α chain linked to IgA and a dimeric chain FcγR for transduction of signals [470].
Modified from [186, 470].
LC Langerhans' cells, *FDC* follicular dendritic cells.

to antibody specificity [469]. Being determined by antibody V regions in hypervariable regions, *they are not linked to the class*: each idiotype is defined by association of V_H and V_L domains and therefore distinguishes one V domain from the others. There are two types, those associated with paratopes are located very near CDRs and those recognizing structures outside the binding site are carried by hypervariable and V regions, respectively. Based on such definitions, we can see two ways to evaluate V regions: by serologic aspects (idiotype) or by antigen-binding properties (paratope) [58].

The IgSF (Table 1.4) [4, 23, 34, 109, 139, 186, 323, 557, 693] (Fig. 1.10) is formed by polygenic groups of genes and single genes, structurally correlated but not necessarily functionally, but whose structural features recall those of Igs; they belong to families sharing an evolutionary homology, probably originating from a few ancestral genes. It is presumed that V regions of such molecules may have a role in intercellular interactions, or as receptors for soluble ligands [186].

T Lymphocytes

T cells especially, but also B cells and other immune cells, synthesize ILs, proteins with a molecular weight (MW) of 15–25 kD governing intensity and duration of immune reactions, mediating cellular proliferation and maturation, and also wound healing (Tables 1.5–1.8) [4, 14, 34, 45, 50, 52, 55, 66, 68, 78, 92, 98, 118, 119, 126, 128–130, 140, 141, 152–154, 156, 158, 167, 172, 181, 213, 219, 220, 240, 245, 249, 258, 272, 278, 318, 326, 372, 377, 383, 387, 398, 399, 412, 413, 421, 426, 437, 455, 461, 474, 480, 481, 487, 517, 523, 526, 528, 532, 534, 560, 562, 607, 641, 669, 693]. In parallel, there is a leukocyte superfamily (Table 1.9) [27], less extensively represented as the IgSF.

Table 1.4. Constituents of the IgSF

H and L chains
Class I and II HLA molecules (correlated)
β_2-Microglobulin
TcR, CD3, CD2, CD7, CD4 and CD8
BcR, CD79a, CD79b
Poly-IgR (receptor of IgM and IgA antibodies), a fragment of poly-IgR constitute the SC of sIgA
Adhesion molecules, integrins, selectins, etc. (see below)
Additional molecules: CD1, CD16, CD19, CD22, CD28, CD32, CD47, CD48, CD58, CD64, CD66a-e, CD80, CD83, CD86, CD89, CD90, CD96, CD101, CD117, CDw150, CD152, CD155, CD158 a, b, CD166
Receptors of: IL_1 (IL_1R, I and .II, CD121a and 121b) IL_6 (IL_6R, CD126) GM-CSF (GM-CSFR, CD116) M-CSF (M-CSFR, CD115) PDGF, etc.

Table 1.4. (Continued)

Adhesion molecules as part of the IgSF		
Molecules	**Ligands**	**Distribution**
CD1	?	Thymocytes and DCs
CD2	CD58 (LFA-3], CD48, CD59	T and NK cells, endothelial and epithelial cells
CD3/TcR	Nominal antigen	T cells
CD4	HLA-II	T cells, monocytes
CD7	?	T and NK cells
CD8	HLA-I	T and NK cells
CD22	CD45RO	B cells
CD28	CD80, CD86	T cells and plasma cells
CD31 (PECAM-1])	CD31, heparin	T cells, endothelial cells, monocytes, platelets, neutrophils
CD48	CD2	Hematopoietic and not hematopoietic cells
CD50 (ICAM-3)	CD11a/CD18	T cells, monocytes, neutrophils
CD54 (ICAM-1)	CD11a/CD18, CD11b/CD18, CD43	Activated T and parenchymal cells, NK cells, endothelial and epithelial cells, fibroblasts, monocytes, chondrocytes, DCs
CD56 (N-CAM)	CD56	T, B, NK cells, endothelial and epithelial cells, keratinocytes, DCs
CD102 (ICAM-2])	CD11a/CD18	Endothelial cells (high expression), epithelial, T, and NK cells, monocytes, DCs, platelets (low expression)
CD106 (VCAM-1)	CD49d/CD29, $\alpha_4\beta_7$	Endothelial cells, monocytes, DCs, fibroblasts, stromal medullary cells, myoblasts
HLA-I	CD8	APCs
HLA-II	CD4	APCs
Ig	Nominal antigen	B cells
MAdCAM-1	CD62L	Mucosal and lymphoid HEVs

CD2, CD48, CD58 form a family, although they are located in pericentric loci of two different chromosomes: 1p13 for the first two and *1q21–23* for the third one; a correlated family is CEA, with CD66b (ex CD67) and CD66e (NCA).
Data from [4, 6, 23, 34, 109, 139, 186, 323, 557, 693] and International Workshops on Human Leukocyte Differentiation Antigens [501].
APCs antigen-presenting cells, *CEA* carcinoembryonic antigen, *DCs* dendritic cells, *ICAM-1, 2, 3* intracellular adhesion molecules-1, 2, 3, *IgSF* immunoglobulin superfamily, *LFA-3* lymphocyte function-associated antigen-3, *MAdCAM-1* mucosal addressin cell adhesion molecule-1, *N-CAM* neural cell adhesion molecule, *PECAM-1* platelet endothelial cell adhesion molecule, *SC* secretory component, *VCAM-1* vascular cell adhesion molecule-1.

A tentative nomenclature may divide ILs into five main types:
a) Interleukins (IL_1–IL_{33})
b) Hematopoietic growth factors (CSF)
c) IFN
d) TNF
e) Growth factors: EGF (epidermal growth factor), FGF (fibroblast growth factor), PDGF (platelet derived growth factor), TGF, etc.
ILs are released from several inflammatory effector cells [151] (Table 1.5):
1) From *Th1 T cells* IL_2, IL_3, IL_{12}, IL_{18}, IL_{22}, IL_{23} IL_{27}, IL_{32}, TNF-α (tumor necrosis factor-α) and -β, IFN-γ, and GM-CSF
2) From *Th2 T cells* IL_3, IL_4, IL_5, IL_9, IL_{10}, IL_{11} IL_{13}, IL_{18}, IL_{25}, IL_{27}, IL_{31}, GM-CSF, whose genes are associated with the chromosome 5 gene cluster in region q31–q33, together with IL_6, IL_9, IL_{12}, IL_{13}, M-CSF and CD14 (IL_4, IL_5, IL_9, IL_{11}, IL_{13}, IL_{16}, IL_{17}, IL_{25} induce asthma)
3) From Th1 and Th2 lymphocytes IL_{14}, IL_{28}, IL_{29}, GM-CSF, IFN-γ, BaDF, NGF
4) From B lymphocytes IL_{10}, IL_{12}, IL_{14}, IL_{19}
5) From endothelial cells GM-CSF, TNF, IL_1, IL_3, IL_6
6) From fibroblasts GM-CSF, G-CSF, M-CSF, IL_1, IL_3, IL_6, IL_{15}
7) From hemopoietic cells IL_{23}
8) From mast cells IL_3–IL_6, GM-CSF and TNF-α
9) From monocyte-macrophages IL_1, IL_6, IL_8, IL_{10}, IL_{12}, IL_{15}, IL_{18}, IL_{19}, GM-CSF and TNF-α
10) From NK cells GM-CSF, M-CSF, IFN-γ, IL_2, IL_{15} and TNF-α
11) From PBMCs IL_{24}
12) From skin and trachea IL_{20}
13) From thymus and medullary stromal cells IL_7, IL_{11}.

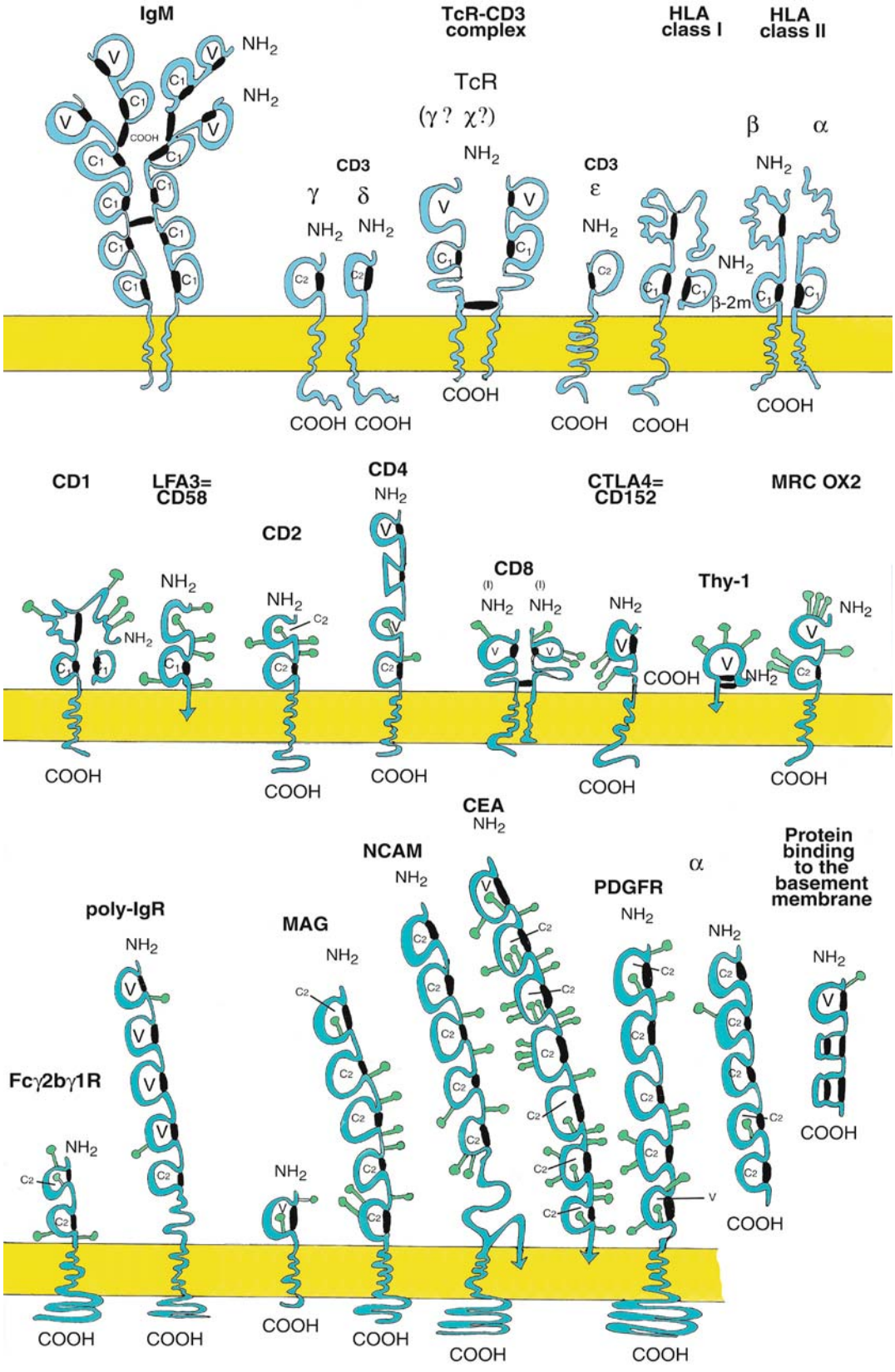


Fig. 1.10. Immunoglobulin superfamily (IgSF). Protein binding to the basement membrane. A model is shown for each molecular type from one species, the *circles* show sequence segments that form Ig domains. α *1Bgp* non-cell surface glycoprotein, β_2-*m* β-microglobulin, *CEA* carcinoembryonic antigen, *MAG* myelin associated glycoprotein, *poly-IgR* immunoglobulin receptors, *NCAM* neural adhesion molecule, *PDGFR* platelet-derived growth factor receptor = CD140, *Po* myelin protein, *Thy-1* and *MRC OX2* brain/lymphoid antigens

Table 1.5. Main characteristics of interleukins (*ILs*; cytokines)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_1	α and β (17 and 15–17; *2q13–21*) and IL_1RA [27] with a structure similar to that of IL_1, however, competes for binding of type I receptors $IL_1α$ Sources: Several different cell types, especially monocytes and macrophages, T and B cells, NK cells pluripotent stem cells: proliferation T lymphocytes, primarily Th2, but also Th1: stimulate to express IL_2 B and NK cells: activation, differentiation and proliferation Macrophages: activation Basophils: differentiation $IL_1β$ Sources: T and B lymphocytes, dendritic cells (DCs), endothelial, and epithelial cells, fibroblasts, etc. Effects: Induces acute-phase responses of liver (thus playing an important pro-inflammatory role) Stimulates the production of IL_2, IL_3, IL_6, IFN-γ by activated, antigen-specific T lymphocytes IL_1 and IL_{18} have an analogue signaling pathway, are key molecules in both the innate and adaptive immunity, and are members of a larger family of related receptors, some of which contribute to host defense TRAF6 participates in IL_1 signaling
IL_1R	$IL_1α$ and β bind with about the same affinity to the same cell surface receptors IL_1R-activating kinase (IRAK), leads to translocation of NF-κB
IL_1R type-I	(80) T and endothelial cells, fibroblasts, hepatocytes and other cells, has an extended amino acid cytoplasmic tail and after binding IL_1 transmits signals intracellularly
IL_1R type-II	(6) Defined as inactive, can function as a precursors of soluble forms binding IL_1
IL_2	(15–20; *4q26–28*) Sources: Exclusively Th1, NK, B cells, and mast cells Effects: Stimulates antigen activation of TcR T lymphocytes stimulated by IL_1 (CD4 and cytotoxic cells = TCT, NK cells and TCT IL-activated cells = LAK): Growth, activation, and differentiation B lymphocytes a) Proliferation and differentiation b) Isotypic selection of IgG_2 Basophils and macrophages: differentiation, increase of TNF-α expression and/or production of IL_3, IL_4, GM-CSF (colony-stimulating factor) and IFN-γ
IL_2R	Formed by three chains: α (55; 10) one affinity-modulating subunit, and β (75; 22) and γ (64; X), two essential signaling subunits Mediates the functions and activities of IL_2 (see above); resting NK cells express its β and γc receptor; the β chain associates with JAK1 and γc associates with JAK3 JAK1/JAK3 (and STAT3/STAT5): induce IL_{15}-like activities $IL_2Rγ$ gene (*Xq13*) encodes the γc-chain of the IL_2, IL_4, IL_7, IL_9, IL_{13}, IL_{15} receptors that are essential for the development of T and NK lymphocyte subsets
IL_3	(14–30; *5q31–33*, where linkage to human asthma has been demonstrated) Sources: Bone marrow stromal cells, activated Th cells, mast cells, keratinocytes Effects: Hematopoietic stem cells (all lines): growth factor and differentiation B and T cell early precursors: growth factor Basophils and mast cells (mostly of mucosal type): proliferation, differentiation, activation, degranulation Basophils: mediator release NK cells and LAK: stimulation Eosinophils: pro-inflammatory alterations

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_3R	Has α and β chains, the β chain is common to IL_5R and GM-CSFR
IL_4	(18–20; *5q31–33* on gene cluster) Sources: Progenitor cells, B and Th2 subsets, and mast cells Effects: Hematopoietic stem cells: differentiation, proliferation B and T lymphocytes: activation, proliferation and differentiation B lymphocytes a) Isotype switching of IgG_1 and IgE antibodies b) Up-regulation of HLA class II and of FcεRI for IgE antibodies c) Induction of ε-germline transcription in B cells T lymphocytes: a) Differentiation of naive T cells to Th2 cells producing Th2-like ILs b) Proliferation of Th2 cells c) Stimulation of antigen activation of TcR Th1 lymphocytes: inhibits switch of T cell differentiation from Th0 to Th1 and IFN-γ production Eosinophils: transendothelial migration Macrophages: inhibit activation Mast cells: growth factor (in experimental animals, in synergy with IL_3), up-regulates FcεRI and CD54 Basophils: up-regulates FcεRI Mononuclear phagocytes: up-regulates FcεRII and HLA class II Neutrophils: activation (see also IL_{13}) Fibroblasts: up-regulates chemokine production Epithelial cells: up-regulates mucin gene expression Endothelial cells: up-regulates CD106 Additional effects: Is thought to exert its effects through the FcεRI present on several cell lines (see text) Induces the expression of FceRII by eosinophils, monocytes and platelets Down-regulates the production of IL_8 by monocytes, of $IL_1α$, IL_6 and TNF-α by macrophages and of IL synthesis by Th1 subsets Activates STAT3 and STAT6
IL_4R	(CD124) (*16p12*) hematopoietic precursors, B and T cells, fibroblasts The receptors signaling triggers the activation of JAK-1 (via the receptor α) and JAK-3 (via the common γc chain, part of this receptor Stimulates T-cell growth, and B-cell activation to promote IgE isotype switching and T–B interactions, induces CD8 cells to produce IL_4
IL_5	(25–50; *5q31–33* on gene cluster) Has α and β chains, the β chain is shared by IL_3 and GM-CSF receptors, explaining the overlapping biological activities Sources: Th2 subsets, mast cells and eosinophils Effects: Activated B lymphocytes: a) Differentiation b) IgA and secretory IgM (sIgM) production c) Expression of IL_6 receptors T lymphocytes: a) Cytotoxic activity b) Expression of IL_2 receptors c) Antigen activation of TcR Eosinophils: a key role in augmenting activation, differentiation, vascular adhesion (CD11b), life span *in vitro* (eosinophilopoietic IL) and IgA-mediated degranulation Basophils: histamine release
IL_5R	(60) The α chain (CD125) (60 kD) has non-binding β chain (CD131) (95 kD) shared with IL_3R and GM-CSFR α chains to form the high-affinity IL_5R [62] Distribution: eosinophils and basophils Binding to IL_5 promotes growth and differentiation of eosinophil precursors, activating mature cells

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_6	(21–28; *7q15–21*) Pleiotropic cytokine induced by IL_1, IL_2, TNF, PDGF and IFN and inhibited by IL_4 and IL_{13} Forms a family with IL_{11}; they have overlapping effector profiles and multimeric receptor complexes in which the promiscuous gp130 molecule serves as a signaling subunit Sources: T and B lymphocytes, monocytes, macrophages, fibroblasts, keratinocytes, hepatocytes, epithelial and endothelial cells Effects: Th2 cells: synergize with IL_1 or TNF to promote growth of immature thymocytes and activation of mature T cells B cells: a) Growth and differentiation b) Ig production: synthesis of IgA, of IgG subclasses, and of IgE induced by IL_4 NK cells: activation TCT: differentiation Additional effects: Antiviral activity Hepatocytes: synthesis of acute phase proteins up-regulating the gene transcription Interacts with CSFs in the proliferation and cell differentiation of pluripotent stem cells Inhibits the inducer and effector phases of delayed-type hypersensitivity
$IL_6R\alpha, \beta$	The α chain is CD126 (80 kD), and the β chain CD130 (130 kD) (gp130), high-affinity IL_6R is formed with CD130 Distribution: resting or activated B cells, plasma cells, T cells, monocytes, fibroblasts, hepatocytes, neural cells $IL_6R\beta$ has signaling chains for receptors for IL_{11}, keratinocytes, LIF, oncostatin M (OSM), and ciliary neutrophil factor (CNF) Overlapping actions: induction of differentiation and proliferation of hematopoietic precursors, and of acute phase proteins
IL_7	(25; *8q12–13*) Sources: Chiefly thymic, bone marrow and stromal cells, but also human keratinocytes Effects: T precursors: proliferation and differentiation of early thymocytes Mature T cells: enhancement to produce IL_2 and IL_2R IL_2 and IL_6 synergize with IL_7 effects on T cells TCT and LAK cells: generation, proliferation and activation Pro- and pre-B lymphocytes: proliferation and differentiation of progenitor cells Megakaryocytes: maturation Monocytes: IL_1, IL_6 and TNF secretion $\gamma\delta$: Proliferation
IL_7R	CDw127 (68 kD) associated with the γ common chain of CD132 (64 kD) to form the high-affinity IL_7R The common γc chain is part of this receptor Distribution: immature thymocytes, pre-B cells, mature T cells, monocytes, $\gamma\delta$ IEL Induction/promotion of immature T-cell growth, expression of CD25 on T cells, proliferation of pre-B/B cells, and monocyte activation, critical for T lineage but not for B lineage development
IL_8	(8–10; *4q12–21*), neutrophil-activating peptide (NAP) Sources: Activated monocytes and macrophages, in addition to monocytes, endothelial and epithelial cells, T lymphocytes, fibroblasts, keratinocytes, hepatocytes, and chondrocytes IL_8 production is stimulated by LPS, IL_1, TNF and virus

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Effects: Neutrophils: a) Potent chemoattractant b) Activation and induction of degranulation, respiratory burst and adhesion to endothelium via CD11b/CD18 T lymphocytes: chemotactic activity and directed migration Basophils: inhibits histamine release
	Additional effects: Together with TGF-β inhibits IgE synthesis Promotes angiogenesis by blood vessels
IL_8R	(44–59/67–70; 2) (CDw128)
	Distribution: CD8 T cells, neutrophils, monocytes, NK-cell subsets
	Receptor of α chemokines
IL_9	(32–39; *5q31–33*) growth factor for clones of CD4 cells which also produce it
	Sources: T lymphocytes (especially Th2, but not CD8) and B lymphocytes, mast cells, and eosinophils, and potentially involved in allergy and asthma
	Effects: Mast cells, megakaryocytes: enhances proliferation Basophils: proliferation, increases the sensitivity to IL_3 B lymphocytes: enhances IL_4-induced production of IgE antibodies
	Additional effects: Enhances the synthesis of IL_6 Induces IL_{22} activity Substantial promoter of myeloid and erythroid precursor
IL_9R	(CD129) T and B cells, macrophages, megakaryoblasts
	The common γc chain is part of this receptor
IL_{10}	(19; *1q32*), several IL synthesis inhibitory factor (CSIF)
	The genes for IL_{10}, IL_{19}, IL_{20}, IL_{22}, IL_{24}, IL_{26} are found within a 200-kb region of chromosome 1q32, whereas genes encoding the two other IL_{10} family members, IL_{22} and IL_{26}, are found within 30 kb of each other and less than 100 kb from the IFN gene on chromosome 12q15; the family can be further divided into two groups. IL_{19}, IL_{20}, and IL_{24} belong to one group, whereas IL_{10}, IL_{22}, and IL_{26} form another group. Moreover, IL_{19}, IL_{20}, and IL_{24} use the common $IL_{20}R2$ chain for signaling, and IL_{10}, IL_{22}, and IL_{26} may also share a common R2 chain
	Pleiotropic IL with important immunoregulatory functions whose actions influence activities of many of the cell types in the immune system
	Sources: $IL_1β$, IL_6, IL_8, IL_{12}, G-CSF, GM-CSF, IFN-γ, TNF-α produced by activated monocytes, IL_4 and IL_5 by Th2 subsets, leukocytes and skin
	IL_2 and IFN-γ produced by Th1 subsets, thus eliciting the generation of IL_4
	$IL_1α$ and β and TNF-α by Th1 and NK cells
	IFN-γ and TNF-α by NK cells
	Negatively regulates its own synthesis by monocytes
	IFN-γ inhibition results in the switch of T cell differentiation from Th0 to Th2
	B cells, Th1, Th2 and suppressor T subsets, DCs, mast cells, monocyte-macrophages, eosinophils and keratinocytes
	Effects: Macrophages: inhibition of differentiation and expression of HLA class II and adhesion molecules Monocytes: inhibition of differentiation into macrophages CD4 lymphocytes: Inhibits IL_8-induced migration Down-regulates Th1 responses, thus suggesting an anti-inflammatory activity

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Suppressor CD8 T cells: Favors differentiation and chemotaxis Inhibitory factor at the maternofetal interface (Chap. 2) TCT precursors: enhances the number and the function B lymphocytes: has a significant effect opposite to the SCIF activity stimulating: a) Growth of progenitors of the erythroblast series and of mast cells b) Differentiation and expression of HLA class II c) Isotype switching to IgA_1 and IgA_2 secretion along with TGF-β, and to IgG_1, IgG_3 and IgG_4 On IL synthesis, in particular inducing IFN-γ on NK cells by IL_{12} On APCs function down-regulating HLA class II expression, an effect reversed by IL_4 On production of reactive oxygen and nitrogen species d) Isotype switching to IgE e) Modulation of IL_4-induced B-cell IgE production in favor of IgG_4 f) Inhibition of IgE-dependent mast cell activation ILs: inhibits $IL_1\alpha$, $IL_1\beta$, IL_2, IL_6, IL_6, IL_{18}, TNF-α Langerhans' cells (LCs): tolerogen effects in addition to suppressing their APC function STAT1, 3, 5: activation, such as IL_{22}
$IL_{10}R1$	(110 kD) Distribution: thymocytes, B cells, mast cell and macrophage cell lines After binding to B cells induces B-cell proliferation, differentiation and isotype switching to IgA secretion with CD40L = CD154 and TGF-β
$IL_{10}R2$	Intact second chain of the human IL_{10} R complex, which may be shared by receptors for the other IL_{10} homologs A distinct feature of the $IL_{10}R$ complex is that both chains can independently bind ligand, whereas in the IL_{10} and IFN–R complexes, only one chain (the R1 chain) can bind ligand in the absence of the other: in all of these receptors, the second (R2) chains are necessary for signaling through the JAK-STAT complex Recognizes IL_{10} and IL_{22}
IL_{11}	(23; *19q13.3–q13.4* and *7* centromeric region) Sources: Bone marrow stromal cells Effects: Hematopoietic progenitor cells of megakaryocytes and macrophages/monocytes: growth factor B lymphocytes: increases Ig secretion independently of T lymphocytes Hepatocytes: synthesis of acute phase proteins cooperating with IL_1 and IL_6 Modulates Th1/Th2 IL production from activated $CD4^+$ T cells, and augments T-cell IL_{10} elaboration Additional effects: Acts synergically with IL_3 in megakaryocyte differentiation Sharing several biological activities with IL_6, it is likely that they use similar signal transduction mechanisms
$IL_{11}R$	Belongs to the receptor family, also including IL_6R, etc.; IL_{11} initiates signaling via binding to a unique IL_{11} receptor chain ($IL_{11}R$). Isoforms of $IL_{11}R$ have been described that contain $IL_{11}R1$ and do not contain $IL_{11}R2$, a cytoplasmic domain. The binding of IL_{11} to $IL_{11}R$ forms a complex that binds to and induces gp130 molecule homodimerization, resulting in the assembly of the active $IL_{11}R$ trimer
IL_{12}	(*5q31–33*) Heterodimeric IL manifests some effects almost reciprocal to those of IL_{10}; an IL_{12}-family is composed of IL_{23} and IL_{27} Has two covalently bound 35- and 40-kD chains (and thus designated p35 and p40 subunits), encoded by two separate genes regulated independently and inactive if expressed separately – the p40 subunit is expessed in large excess of the p35 subunit – p35 is among the early inducers of Th1 responses, while it inhibits Th2 lymphocytes – when p35 associates with p40, a soluble member of the cytokine receptor superfamily, it forms IL_{12}, which induces Th1 differentiation and the release of IFN from Th1 and NK cells – CD40 cross-linking up-regulates IL_{12} p40 mRNA in monocytic and B-cell lines and in human monocyte-derived DCs – the two subunits act as signal transducer by providing a cytoplasmic STAT4 binding sit which enables STAT4-mediated responses to IL_{12} to occur DCs; The gene encoding the p40 subunit is located in the region of chromosome 5 – the duo $IL_{12}p35/IL_{12}p40$ is called IL_{12} p70

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Sources: B cells and monocyte-macrophages (via CD40–CD154 interactions) Effects: Allergen-specific T cells Crucial role in selective switch of T cell differentiation into Th0 or Th1 Stimulation of adhesion molecules Th1-inducing and Th1-maintaining B lymphocytes Induction of STAT1 activation, T-bet expression and IgG2α class switching NK cells a) Activation, proliferation and cytotoxicity b) Expression of CD2, CD56 and CD11a c) Stimulates production of IFN-γ from Th1 cells and NK cells NK cells, macrophages and Th1 subsets: production of IFN-γ – p40 is expressed by some cells such as nonprofessional APCs and only after activation Has homology with the soluble receptor of IL_6 Costimulates proliferation of peripheral lymphocytes Promotes cell-mediated immunity (CMI) Protection against infectious diseases
$IL_{12}R$	(α chain or R1, 120 kD, and β chain or R2, 140 kD) Sources: CD4, CD8 activated T cells, NK cells, and B cells Effects: Stimulates their proliferation as well as the peripheral hematopoiesis R2 is a key Th1 commitment step when naive Th precursor cells commence differentiation into Th1 cells
$IL_{12}Rβ1$	(*5q31–33*) Encodes the p40 subunit
IL_{13}	(9–17; *5q31*) The gene encoding IL_{13} is only 25 kb upstream of the IL_4 gene and in the same orientation Has a receptor different from that of IL_4 possibly a subunit in common with IL_4R Sources: CD4 (Th 0, 1 and 2) and CD8 T cells stimulated by antigens, monocytes and mast cells Effects: Up-regulates HLA class II and adhesion molecules such as CD11b, 11c, 18, 28, 49e Down-regulates CD16, CD31, and CD 64 Manifests no growth effects on T cells, unlike IL_4 Human B lymphocytes: a) Expression of CD23 (also on monocytes), CD71, CD72, sIgM and HLA class II b) Class switching to IgE and IgG_4 c) Expression of ε-germline transcripts independently of IL_4 even if two to five times less powerful Monocyte-macrophages: IL_4-like effects (inhibits HIV replication in monocytes and ADCC in both cells) Additional effects: Has a persisting activity unlike IL_4, which is activated only 8–12 h Together with IL_4 induces VCAM-1 = CD106 on endothelial cells Its polymorphism is associated with high total serum IgE levels and increased risk of atopic asthma
$IL_{13}R$	Appears to be heterotrimer, and formed by $IL_4Rα$, $IL_2Rγ$ and $IL_{13}Rα1$- or $IL_{13}Rα2$-binding Associates with CD124 to form the $IL_{13}R$ complex; the common γc chain is part of this receptor Distributed to human B cells, endothelial cells, several non-hematopoietic cells, including monocyte-macrophage populations, B cells, basophils, eosinophils, mast cells, fibroblasts, smooth muscle, and airway epithelium Crucial role in inducing human B-cell proliferation and class switching to IgE in presence of CD40; suppresses the induction of inflammatory ILs, activates STAT6

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_{14}	(53) Sources: T and B lymphocytes Effects: Activated B lymphocytes: a) Expands clones b) Suppresses antibody secretion c) Enhances selected subpopulations d) Promotes long term growth in vitro Monocytes: release of ILs
$IL_{14}R$	Binding of IL_{14} increases expression of $IL_{14}R$, as well as in intracellular cAMP, DAG and Ca^{++} levels
IL_{15}	(*15*; *4q31*) Sources: Stromal cells, monocyte-macrophages, fibroblast cell lines, epithelial cells, and in various tissues such as muscle, placenta, etc. Effects: T lymphocytes: growth factor Powerful chemoattractant Stimulates proliferation sharing its functions with IL_2R β and γ chains and the only α chain possessed T- and NK-cell clones: proliferation LAK, NK and CTL cells: activation and differentiation B cells: proliferation and differentiation Human tonsillar B cells stimulated by CD154: proliferation and Ig synthesis γδ subpopulations: proliferation and differentiation Mast cells: stimulation STAT3/STAT5 and JAK1/JAK3: phosphorylation Additional effects: IL_{15} presence in several fetal tissues suggests a role in differentiation and maturation of fetal immune system Shows several biological activities similar to IL_2, for example the capacity to stimulate T-cell proliferation and induction of cytotoxic T lymphocyte and IL-activated killer cells
$IL_{15}R$	(58–60) Forms a new family with CD25; the common γc chain is part of this receptor Sources: T-cell lines, macrophage lines, diffused in non-lymphoid cells and tissues Induces proliferation and differentiation of activated B cells
IL_{16}	(50–60) Sources: Activated CD8 lymphocytes, airway epithelial cells Effects: T lymphocytes: chemotactic action binding to CD4 receptor Eosinophils: chemotaxis Additional effects: Expression of HLA class II and IL_2R Suppresses HIV replication
IL_{17}	Has 5 members, $IL_{17}A$, $IL_{17}B$, $IL_{17}C$, $IL_{17}E$ (IL_{25}), and $IL_{17}F$ (ML-1) Sources: a) CD4 memory and CD8 lymphocytes and TcR-αβ b) Spleen $CD4^+$ cells and neutrophils c) IL_{15} able to induce IL_{17} release from purified spleen $CD4^+$ cells and airway neutrophils and could lead to IL_{17} production following bacterial infection Effects: T cells: proliferation Fibroblast: proliferation Stimulates production and expression of ILs by macrophages, epithelial and endothelial cells, and fibroblasts

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
$IL_{17}R$	(98, 22) Sources: Triple negative thymocytes, T-cell clones and cell lines Effects: Enhances T-cell proliferation induced by PHA, activation of NF-κB, IL_6, IL_8 and expression of CD54; the soluble form instead inhibits T-cell proliferation and IL_2 production
IL_{18}	(24; *11q22.2–22.3*) Initially described as an IFN-γ-inducing factor; is closely related to the IL_1 family in terms of structure and pro-inflammatory properties Sources: Activated macrophages, airway epithelial cells, Kupffer cells, dermal keratinocytes, osteoblasts, adrenal cortex cells, and intestinal epithelial cells Effects: Activation of both CCL and CXCL chemokines Activates NF-κB, expresses Fas ligand, induces human HIV Synergizes with IL_{12} and anti-CD40 to induce B cells to express IFN-γ Stimulates T cells for IFN-γ production and growth promotion, also in the absence of T cell antigen receptor engagement Stimulates T-cell proliferation and potentiates the Th1-driving capacity of IL_{12}p70 With IL_1 is a key molecule in both the innate and adaptive immunity and is member of a larger family of related receptors, some of which contribute to host defense Also enhances Th2 cytokine production (IL_4 and IL_{13}) and regulates IgE production in vivo in the absence of allergen
$IL_{18}R$	Recruits IRAK and IL_1R-activating kinase and NF-κB-inducing kinase (see above) One of the IL_{18}R chains is the IL_1R-related protein, a member of the IL_1R/Toll-like receptor (TLR) superfamily
IL_{19}	(*1q32*) Shares 21% amino acid identity with IL_{10} and binds to IL_{20}R Signals through a receptor complex that is also utilized by IL_{20} and IL_{24} Two distinct IL_{19} mRNA species differ in their 5′-sequences IL_{19}, IL_{20}, IL_{22}, and probably also IL_{24} could form a single subfamily of helical ILs Sources: B cells and monocytes Effects: Signaling via STAT3, induce JAK-STAT signal transduction pathway through a specific receptor complex IL_4 and IL_{13} potentiate IL_{19} gene expression in LPS-stimulated monocytes GM-CSF directly induces IL_{19} gene expression in monocytes
$IL_{19}R$	
IL_{20}	(*1q32*) a new member of the IL_{10} family Sources: Skin (where its overexpression leads to skin abnormalities) and trachea Effects: Activates a STAT3-containing signal transduction pathway Skin differentiation and keratin expression Pro-inflammatory effects on keratinocytes and possible central role in the epidermal response to inflammation Stimulates chemotaxis and antimicrobial activity of myeloid cells
$IL_{20}R1$	Both its subunits are expressed in keratinocytes throughout the epidermis, recognizes IL_{19} and IL_{24}
$IL_{20}R2$	Also on certain endothelial and mononuclear cells, recognizes IL_{10} and IL_{22}
IL_{21}	(4q26–q27), IL_9-induced factor maps to the same locus as the IL_2 gene, separated by roughly 180 kb Sources: Activated peripheral T cells

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Effects: Proliferation and maturation of NK cells populations from bone marrow Proliferation of mature B-cell populations costimulated with anti-CD40 Proliferation of T cells with or without anti-CD3 costimulation in concert with IL_2, IL_{15} and, to a lesser extent, with IL_7
$IL_{21}R$	(*16p11*) With the first exon situated just 39 kb from the $IL_4R\alpha$ locus, the common γc chain is part of this receptor Sources: Stromal cells in the bone marrow or thymus Resting B cells Cell lines of B, T, and NK lineages Human $CD23^+$ and $CD56^+$ Associated with JAK1, JAK3 and Tyk2
IL_{22}	(*12q15*), or IL-TIF, another member of the family of IL_{10} homologs, located on human chromosome *12q*, where several *loci* potentially linked to asthma and atopy have been identified by genetic studies, particularly in the *12q13.12–q23.3* region. More precisely, it is located on chromosome *12q15*, at 90 kb from the IFN-γ gene, and at 27 kb from the IL_{26} gene, which codes for another IL_{10}-related cytokine. In the mouse, the IL_{22} gene is located on chromosome 10, also in the same region as the IFN-γ gene Sources: Th1 cells, IL_9 Pleiotropic effects: Activates STAT1, 3, 5; as IL_{10}, stimulates monocytes to make TNF Can activate IFN-γ-like biological responses such as HLA class I induction and STAT activation Target tissue is the liver, where it induces acute-phase reactants and is active in the innate immunity In keratinocytes IL_{22} activated STAT3 and directly increased the expression of β-defensin and β-defensin 3
$IL_{22}R$	(1) Complex composed of two subunits, the $IL_{22}R\alpha$ chain and the second $IL_{10}R\beta$ chain: both chains are required for signaling, each chain alone is capable of binding IL_{22}
$IL_{22}BP$	(*6q24.1–25.2*). This receptor, named IL_{22} binding protein, inhibits IL_{22} activity by binding IL_{22} and preventing its interaction with the functional $IL_{22}R$ complex, but $IL_{22}BP$ often fails to block IL_{22} activity Both chains of the $IL_{22}R$ complex are ligand binding chains; however, none of them is capable of transducing IL_{22}-signaling alone. Both chains are necessary to assemble the functional receptor complex able to induce signaling after binding IL_{22}
IL_{23}	A p19-p40 heterodimer structurally related to IL_6, G-CSF, and the p35 subunit of IL_{12} sharing homology with members of the IL_6/IL_{12} family of ILs, also combining the IL_{12p40} subunit with its subunit p19, requires interaction with $IL_{12}R1$ and $IL_{23}R$ with a cytoplasmic STAT4 binding domain Source: Hemopoietic cells, bone-marrow-derived DCs Effects: Th1 activation T-cell memory Activates $IL_{17}E$ and STAT4 in blast T cells Induces exclusively the proliferation of naive and CD45RO memory T cells and of related IFN-γ
$IL_{23}R$	Associates with $IL_{12}R\beta1$ in a combined deficiency
IL_{24}	(*1q32*) (melanoma differentiation-associated factor MDA-7) Sources: PBMCs and melanoma (MDA-7) Induced by: IFN-β and mezerein Effects: Signaling by STAT3 Targets: tumor cells Biological effects: Tumor and apoptosis inhibition

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_{25}	Also called $IL_{17}E$ Sources: Th2 cells Effects: Induces Th2-like IL_4, IL_5, and IL_{13} gene expression through a subset of APCs expressing high levels of HLA class II Amplifies systemic and localized allergic-type inflammatory responses by its actions on several cell types Increases serum IgE, IgG, and IgA levels, induces blood eosinophilia and pathological changes in the lungs and digestive tract
IL_{26}	(*12q15*) (= AK155) (a factor discovered after overexpression in human T lymphocytes after transformation by the simian rhadinovirus herpesvirus saimiri) Sources: T cells Mast cells after FcεRI cross-linkage Effects: IL_{26} and IL_{19} bind to the $IL_{20}R$ complex, composed of cytokine receptor family 2–8/$IL_{20}R\alpha$ and $IL_{20}R\beta$ (type I). IL_{26} or $IL_{10}R$ 2 chains and $IL_{20}R1$, but not IL_{21}, bind to the receptor complex, composed of $IL_{22}R$ and $IL_{20}R\beta$ (type II) Enhances secretion of IL_8 and IL_{10}, and cell surface expression of CD54 Activates the JAK/STAT signaling pathway, inducing a rapid tyrosine phosphorylation of STAT1 and STAT3 in cells expressing $IL_{20}R1$ and $IL_{10}R2$ IL_{26} may play a role in local mechanisms of mucosal and cutaneous immunity
$IL_{26}R$	Formed by a combination of two receptor subunits, $IL_{20}R1$ and $IL_{10}R2$, which dimerize to generate the receptor engagement and results in phosphorylation of STAT1 and STAT3
IL_{27}	p28 protein, a further member of the IL_{12} family involved in Th1 initiation A heterodimer composed of two chains, p28 (IL_{30}) and EBI3, analogous to $IL_{12}p40$ and $IL_{12}p35$ (p28-related protein), respectively, and a p40-related protein The heterodimer is expressed by APCs Sources: Activated APCs, macrophages and DCs Effects: Involved in Th1 initiation Triggers clonal proliferation of antigen-specific naïve but not memory $CD4^+$ T cells and synergizes with IL_{12} in IFN-γ production by naïve $CD4^+$ T cells Activates STAT-1, -2, -3, and -5, JAK-1 and -2 in naïve $CD4^+$ T cells Promotes polarization towards a Th1 phenotype with expression of IFN-γ IL_{27} stimulation induced phosphorylation of STAT-1 and expression of T-bet and $IL_{12}RB2$ in naïve $CD4^+$ T cells. Together with IL_{12}, IL_{27} augmented IFN-γ secretion in naïve $CD4^+$ T cells Suppresses $CD4^+$ T cell proliferation and Th2-II-like production Activates Th0 and Th1 cells Activates STAT-1 and induces T-bet expression and IgG2a in stimulated B cells, together with WSX-1 limits innate and adaptive components of type 2 immunity at mucosal sites
$IL_{27}R$	One subunit is WSX-1 Its engagement results in IFN-γ production
$IL_{28}A$	(19) IFN-λ2 mediates antiviral activity in cells in response to viral infection
$IL_{28}R$	Includes receptors for type I and type II IFNs (IFN-αR1, IFN-αR2, IFN-γR1, and IFN-γR2) and receptors for $IL_{10}R$, $IL_{20}R$, and $IL_{22}R$
$IL_{28}B$	(19) IFN-λ3 mediates antiviral activity in cells in response to viral infection
IL_{29}	(19) IFN-λ1 mediates antiviral activity in cells in response to viral infection
IL_{30}	(16p11) a member of the long-chain 4-helix bundle IL family, and EBI3, form the IL_{27} heterodimer Termed p28 because of its molecular mass

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_{31}	The oncostatin M receptor (OSMR) is part of receptor complexes for OSM and IL_{31} a four-helix bundle IL Sources: Preferentially Th2 cells Effects: Skin tropism Signaling events are triggered by JAKs constitutively binding to membrane-proximal receptor regions
$IL_{31}R$	Gp130-like receptor (GPL) recruits JAK1, JAK2, STAT-1, STAT-3, STAT-5 signaling pathways, as well as the Pi3 kinase/AKt cascade
IL_{32}	Exists as four splice variants Sources: Human peripheral lymphocyte cells after mitogen stimulation, human epithelial cells by IFN-γ, and NK cells after exposure to the combination of IL_{12} and IL_{18} Effects: Induces human TNF-α, and IL_8 in THP (Th precursors) monocytic cells Activates nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK); may play a role in inflammatory/autoimmune diseases
IL_{33}	Sources: Member of the IL_1 family Effects: Mediated via IL_1R ST 2 Activates NF-κB and MAP kinases Drives production of Th2-associated ILs
G-CSF	(18–22; *17q11–21*) Granulocyte colony-stimulating factor Favors growth of myeloid progenitor cells
GM-CSF	(22; *5q31–33*) Sources: Activated T cells, monocytes, macrophages, fibroblasts, bone marrow stromal cells Effects: Stimulates growth of hematopoietic precursor cells, mostly of granulocytes/monocytes; modifies the functions of mature granulocytes Enhances neutrophils (inhibiting migration), macrophages and eosinophils (paraeosinophil IL) Induces phagocytosis, production of eicosanoids, ADCC, platelet production by megakaryocytes Promotes growth and maturation of LCs to APCs
IFN α, β, γ (interferons) produced by Th1 cells;	Common properties Hematopoietic stem cell growth B cells: proliferation NK cells, TCT: activation Macrophages: phagocytosis and accessory activities (IFN-γ > IFN-α, β) Additional effects: IL_1 and IL_2 synthesis (IFN-γ > IFN-α, β) Induction of HLA molecules of class I (IFN-γ > IFN-α, β) and class II Production of FcR Production of Igs Antiviral activity (IFN-γ < IFN-α, β): inhibition of viral replication and of tumor growth Differentiation of leukemia cells of promyelocytic and monoblastic origin Additional actions (IFN-γ > IFN-α, β) Differentiation of erythroleukemia cells Antibody production Influence on CMI
IFN-α	(16–27) (*9p22*) Sources: Mainly monocytes, macrophages, and lymphocytes, secondarily B cells, NK cells Stimulates macrophages and B differentiation Has activities similar to IL_{12} inducing the differentiation of allergen-specific T cells into Th0 or Th1 cells Inhibits virus-infected cells

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IFN-β	(20) Sources: Macrophages and granulocytes, and also fibroblasts Effects: On B proliferation
IFN-γ	(20×25; *12q22–24*) Sources: Th1 and T γδ lymphocytes, CD8 cells, activated NK cells and macrophages Has a central role in the immune response, since stimuli activating T lymphocytes induce IFN-γ synthesis Effects: T lymphocytes a) Inhibits Th2 proliferation inducing their shift to Th1 b) TCT and NK cells: induction and proliferation B lymphocytes a) Differentiation inhibiting IgE antibodies production either directly or down-regulating FcεRI expression by B cells and of CD23 by IL_4 b) Expression of IgG Fc Activated B lymphocytes: proliferation and differentiation, in synergy with IL_2 Additional cells: Monocytes, macrophages, mast cells, DCs, fibroblasts, and T lymphocytes: induces or increases the expression of HLA class II Eosinophils: increases the cytotoxic activity and the adhesion to endothelium via the expression of CD54 Macrophages and neutrophils: activates and enhances their phagocytic and bactericidal activities Macrophages: induces chemotaxis and increases survival Mast cells: prolongs the life span Neutrophils, monocytes and macrophages: activation to promote ADCC reactions NK cells: activation Pluripotent stem cells: growth and activation Additional effects: Antagonizes the action of IL_4 and inhibits the IL_4-induced IgE production by B lymphocytes Inhibits secretion of IL_{10}
IFN-κ	Expressed in epidermal keratinocytes Sources: Resting DCs and monocytes Effects: Release of several cytokines from both monocytes and DCs Inhibits inducible IL_{12} release from monocytes
IFN-λ 1–3	Correspond to $IL_{28}A$, $IL_{28}B$ and IL_{29}
IFN-λR1	One of the two receptors utilized by all three IFN-λ proteins, the other is $IL_{10}R2$
IFN-τ	Suppression of proliferation and inhibition of IgE production
IFN-ω limitin	Sources: Mature T lymphocytes in spleen and thymus Bronchial epithelial and salivary duct cells Effects: Induces apoptosis in pre-B cell lines Reduces the proportion of CD45R-positive cells Enhances the antigen-induced cytotoxic lymphocytes Suppresses the antigen-induced T-cell proliferation
IFN-αβR	Consists of two subunits, IFNAR-1 and IFNAR-2
IFN-γR	Receptors 1 (*6q23–24*) and 2 Expressed on nearly all cell types Coupled to the JAK-STAT signaling pathway Mice lacking this receptor or STAT1 display a profound disruption of both innate and adaptive immunity

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
M-CSF	(45–70; *5q31–33*) Monocyte/macrophage-colony stimulating factor Promotes proliferation and differentiation of monocyte/macrophages and the activation of NK cells
TGF-α	(5, 6) transforming growth factor, eosinophil generation
TGF-β	(12.5–25) the TGF-β family contains five homologous members, TGF-β 1 and 3 are encoded by single genes on chromosomes 19.1, and 14, respectively; TGF-β may be a Th3 IL Sources: Activated B and T cells, macrophages, platelets; has opposing actions: a) Promotes proliferation of fibroblasts and collagen synthesis, increases IgA production by B cells b) Inhibits almost all other cells, including T and B lymphocytes, blocks IgM and IgG synthesis, IL_1R production and HLA molecules expression c) Eosinophil generation
TGF-βRI	Recognizes Smad-2 and -3 that transduces TGF-β-triggered signals together with Smad-TGF-βR
TGF-βRII	Causes recruitment and phosphorylation of TGF-βRI and formation of a receptor complex
TGFR-1	CD120a
TGFR-2	CD120b
TNF α, β	Tumor necrosis factors Effects: Express HLA class I and II and manifest antiviral activity Epithelial cells: induce the proliferation by G-CSF Endothelial cells: interact to produce CD54 Monocytes: stimulate both motility and production of IL_6 and IL_8 Neutrophils: potent activators and adhesion-inducing, promote chemotaxis and degranulation B cells: modulate immune responses mediated by IL_4
TNF-α	(17–51) (*6p21.3*) Sources: Monocyte-macrophages, mast cells, PMNs, endothelial, NK and activated T cells Effects: Macrophages, neutrophils, eosinophils: activation and expression of HLA class I and adhesion molecules B cells: activation Hepatocytes: synthesis of acute phase proteins Muscle cells: induces endotoxic shock Additional effects: Favors delayed-type and contact hypersensitivity Manifests an inhibiting effect on LCs
TNF-β	(20–25) Sources: Activated B and T (Th1] lymphocytes Effects: On neutrophils and on the proliferation and differentiation of B lymphocytes Favors the expression of adhesion molecules
TNFR-SF	Includes CD30, CD40, CD95, CD97

Several other molecules have been described: *BaDF* basophil differentiation activating factor, *EAF* eosinophil activating factor, *ECEF* eosinophil cytotoxicity enhancing factor, *EGF* epidermal growth factor, FGF fibroblast growth factor; *MIF* (monocytes) migration inhibiting factor, *NGF* nerve growth factor, *PDGF* (30–32) platelet derived growth factor, *SCF* stem cell factor.
IL_4R, IL_7R, IL_9R, $IL_{13}R$, $IL_{15}R$, and $IL_{21}R$ have the common γc chain; cytokine receptors are dealt with also in Table 1.2.
EBI3 Epstein-Barr virus-induced gene 3, * chemokines, see below, *WXS-1* WSXWS (Trp-Ser-X-Trp-Ser).
Data from [4, 14, 34, 45, 50, 52, 55, 66, 68, 78, 92, 99, 118, 119, 126, 128–130, 140, 141, 152–154, 156, 158, 167, 172, 181, 213, 219, 220, 240, 245, 249, 258, 272, 278, 318, 326, 372, 377, 383, 387, 398, 399, 412, 413, 421, 426, 437, 455, 461, 474, 480, 481, 487, 517, 523, 526, 528, 532, 534, 560, 562, 607, 641, 669, 693] and PubMed ID: 14764690.

Table 1.6. Regulation of isotype switching and HLA expression by some ILs

ILs	Immunoglobulins	HLA class
IL_3		II
IL_4	IgG_1 and IgE	II
IL_5	IgA	I
IL_{10}		II
IL_{12}	IgE	
IFN-α and -β		I/II
IFN-γ	$IgG_{2α}$	I/II
TGF-β	IgA	II
TNF-α and -β		I

Table 1.7. Synergic activities of ILs

Synergy	Cytokine synergic effects
IL_1, IL_3, IL_5	Production of granulocytes, eosinophilopoiesis
IL_2, IL_4	Enhancement of T lymphocytes
IL_2, IL_5, IL_6	Synergy with IL_4 and IL_{13} in promoting IgE production
IL_2, IL_{12}	Generation of TCT and LAK
IL_3, IL_{11}	Megakaryocytopoiesis
IL_3, IL_4, IL_{10}	Differentiation of B lymphocytes, production of mast cells
IL_3, IL_6	Hemopoiesis
IL_3, IL_4, IL_{11}	Cooperation in several functions
IL_3, IL_9	Promotion of the growth of some mast cell lines
IL_4, IL_6	Hemopoiesis
IL_4, IL_{13}	Isotype switching ε
IL_5, NGF	Production of basophils/mast cells and eosinophils
IL_{10}, IL_2, IL_4	Growth of immature thymocytes
GM-CSF, NGF	Production of granulocytes

Table 1.8. Activity of ILs and chemokines in atopic diseases

Effects	Cytokines and chemokines	Activity
IgE regulation	IL_4, IL_{13}	ε Isotype switching
	IL_4	Generation of IL_4 producing CD4
	IL_2, IL_5, IL_6	Synergy with IL_4 and IL_{13}
	IFN-γ, TGF-β	Inhibit IL_4 and IL_{13}
	IL_{12}	Enhances production of IFN-γ by T cells and NK cells
IgA regulation	TGF-β	α Isotype switching
Eosinophils	IL_3, IL_5, GM-CSF, RANTES*, MIP-1α*, eotaxin, MCP-3* IL_1, TNF	Eosinophilopoiesis Eosinophil chemotaxis and activation Eosinophil activation
Mast cells: development and activation of GM-CSF	IL_3, IL_9, IL_{10}, NGF, H-CSF	Mast cell growth factors Inhibits mast cell proliferation
	MIP-1α*, MCP-1*, MCP-3*, RANTES*	Basophil chemotaxis, histamine release
	IL_8	Inhibition of histamine release
Inflammation	IL_1, IL_4, IL_6, IL_8, GM-CSF, G-CSF, TNF, IFN-γ	Activation of neutrophils
	IL_1, IL_3, IL_5, TNF, GM-CSF	Activation of eosinophils
	IL_1–IL_4, GM-CSF, M-CSF, TNF, IFN-γ	Activation of macrophages
Anti-inflammatory	IL_{10}, TGF-β	Inhibit IL production and T cell and/or monocyte function

EBI3 Epstein-Barr virus-induced gene 3, * chemokines, see below.
Data from [4, 14, 34, 45, 50, 52, 55, 66, 68, 78, 92, 99, 118, 119, 126, 128–130, 140, 141, 152–154, 156, 158, 167, 172, 181, 213, 219, 220, 240, 245, 249, 258, 272, 278, 318, 326, 372, 377, 383, 387, 398, 399, 412, 413, 421, 426, 437, 455, 461, 474, 480, 481, 487, 517, 523, 476, 528, 532, 534, 560, 562, 607, 641, 669, 693] and PubMed ID: 14764690.

Table 1.9. Leukocyte surface molecule superfamilies

Superfamily domains	Examples	Common functions in immune system
Complement control proteins	CD21, CD35, CD62P	Control of complement cascade
IL receptors	$IL_2R\beta$ (CD122), $IL_6R\alpha$ (CD126)	Growth factor receptors
Epidermal growth factor (EGF)	CD62L, CD62P	Cell surface ligand binding
Fibronectin type II	Mannose receptor	Polyvalent functions
Fibronectin type III	Integrin β4 (CD104), IL_7R (CDw127)	Polyvalent functions
Immunoglobulin V set	IgV, TcRV, CDw90	Adhesion, recognition
Immunoglobulin C1 set	β_2M, HLA class I α3 domain	Adhesion, recognition
Immunoglobulin C2 set	CD2 domain 2, CD3ε	Adhesion, recognition
Integrins	CD11/CD18, CD49	Adhesion
Lectin C-type	Mannose receptor, CD23, CD62L	Carbohydrate binding
Lectin S-type	CD11b/CD18	Carbohydrate binding
Leucine-rich glycoprotein repeats	CD42a, CD42b	Protein–protein or –lipid interactions
Link	CD44	Hyaluronic acid/chondroitin sulfate binding site
LDL receptor	LDL receptor	Lipoprotein binding, NN function
Ly-6	CD59	NN
HLA	Class I α1, α2, II α1, β1 domains	Recognition
NGF receptor	CD27, CD40	NN
Rhodopsin (serpentine receptor)	IL_8R (CDw128), C5aR, CD5, CD6	G-protein coupled receptor
Somatomedin	PC-1	NN
Transmembrane 4 pass	CD9, CD37, CD53	NN
Phosphotyrosine phosphatase	CD45	Signal transduction
Tyrosine kinase	M-CSFR, c-kit (CD117), lck	Signal transduction

Modified from [27].
IL interleukins, *LDL* low-density lipoproteins, *M-CSFR* Monocyte/macrophage-colony stimulating factor receptor, *NN* not known, *PC-1* plasma cell surface antigen-1.

PubMed ID: 12734330 has given a full description of IL_{30}: according to the HUGO Gene Nomenclature Committee, the symbol p28 (IL_{27}) should be used. It was finally identified as one subunit of $IL_{27}R$ [664].

Recently, the IFN family has been enriched by several new acquisitions (Table 1.5): type I IFN family contains IFN-α, IFN-β, IFN-κ, IFN-λ, IFN-ω, IFN-τ and IFN-ζ. Limitin is IFN-ω, an IFN-like IL that has approximately 30% sequence identity with IFN-α, IFN-β, and IFN-τ [398, 399]. IFN-κ, like IFN-β, induced the release of several ILs such as IL_{28} and IL_{29} from both monocytes and DCs, without the requirement of a costimulatory signal [390]. TLR-9 (Toll-like receptor-9) stimulation by CpG (cytosine-phosphate guanine) DNA induced the expression of all IFN-α, -β, -ω and -λ subtypes in PDCs (plasmacytoid dendritic cells), whereas TLR-4 stimulation by LPS (lipopolysaccharide), or TLR-3 stimulation by poly I:C, induced only IFN-β and IFN-λ gene expression [88]. A new IFN, STAT1-induced FLN29, might be involved in the termination of LPS signaling [335]. Three genes on human chromosome 19 have been found to encode distinct but similar proteins, which are called IFN-λ1, IFN-λ2 and IFN-λ3, and are designated as $IL_{28}A$, $IL_{28}B$ and IL_{29}, respectively. It is suggested that these ILs are functionally referred to as type III IFNs because of their unique primary sequence homology and receptor usage [88]. A distinct receptor complex is utilized by all three IFN-λ proteins for signaling and is composed of two subunits, a receptor designated as IFN-λR1 and a second known as $IL_{10}R2$, which signal through the JAK-STAT pathway [274, 519].

This receptor mediates the tyrosine phosphorylation of STAT1, STAT2, STAT3, and STAT5 (signal transducers and activators of transcriptions). Activation of this receptor by IFN-λ can also inhibit cell proliferation and induce STAT4 phosphorylation, further extending functional similarities with type I IFNs [88]. T lymphocyte origin is from pluripotent stem cells, initially arising during embryonic development from hemopoietic tissues of the yolk sac, then the early stages continue in the

fetal liver. Unlike B cells, maturation does not occur *in situ,* but from bone marrow-derived progenitors undergoing maturation in the thymus, where the greater part of cells multiply and differentiate into immunocompetent lymphocytes. In the thymus, functionally competent cells are exported into peripheral lymphoid compartments in accordance either with thymus ability to produce different soluble factors or with particular intercellular interactions. Early in development, thymocytes express several cell surface molecules, including CD2 and CD7 (CD7 deficiency is described in Chap. 22), but lack both DN CD4/CD8 and CD3. Precisely in the thymus, via a series of intermediate steps, DN cells change into DP $CD4^+8^+$ thymocytes. As thymocytes mature into T cells, they express increasing levels of TcR, finally turning into $CD4^+8^-$ and $CD4^-8^+$, becoming MP. Therefore, during early stages of thymus phases, thymocytes express indifferently both CD4 and CD8; the association of either subset with TcR will show whether cells recognize MHC class I or II molecules, thus regulating subsequent differentiations [255]. IKK (inhibitor of κB kinase)-induced NF-κB (nuclear factor κB) activation, mediated by either IKK1 or IKK2, is a pivoral factor for the generation and survival of mature T cells, and IKK2 has a crucial role in regulatory and memory T cell development [503]. NF-κB is also activated by IL_{32} [258].

T cells have been identified by their ability to bind sheep erythrocytes to form E rosettes (red cells), and more sensitive binding of monoclonal antibodies (mABs) identifies their TcR, referred to as CD2. However, the most commonly used marker for T cells is CD3 associated with TcR. Further definition of cell surface protein antigens derives from rDNA technology. The molecules expressed on lymphocyte membranes, characterizing diverse phases of the differentiation process of T subsets, allow their identification and are overall assayed by means of lymphocyte differentiation antigens designated with CD terminology (CD1–CD342) defining cellular antigens. Such markers also distinguish nonhematopoietic cells involved in both innate and acquired immunity, permitting their recognition via analysis of cell surface molecules expressed on lymphocytes during stages of cell development and/or activation. CDs constitute a group still under definition and classification [470, 501] and recently revisited [4, 262, 343, 604]. These CD cell surface markers have been grouped into T cells, B cells, and NK cells, among others. The grouping is somewhat arbitrary because essentially none of such CD markers is restricted to a single cellular lineage.

Uniformity of *nomenclature* is assessed via monoclonal antibody technology, which unlike polyclonal ones prepared from immunized animals all have the same antigen specificity, and are immunologically completely homogeneous since each antibody is synthesized from cells derived from a single clone. Briefly, a hybridoma can readily be formed by fusing a single normal B cell suspension from immunized mice to cells of continuously replicating tumor cells: hybrid cells so obtained show a unique association of antibody specificity and proliferate indefinitely. The cells can also be grown as individually cloned and screened to produce antibodies with desired specificity [414]. Periodically specialists meet in international workshops to compare specific reagents.

The CD4–CD8 dichotomy is considered out-of-date, because a few CD4 have suppressor/cytotoxic and a few CD8 have helper functions [30]. We refer to CD4 or CD8 cells to specify either helper or suppressor/cytotoxic functions, unless otherwise specified [30]. CD4 cells express a CD4 surface antigen, a monomer of 60 kD with four extracellular Ig-like domains, while CD8 is associated with CD8 molecules, αα or αβ dimers each of 34 kD, linked by -S-S bonds, both cells with a short cytoplasmic tail interacting with $p56^{lck}$ [4]. CD4 and CD8 are encoded not by MHC, but by genes on chromosomes 12 and 2, respectively; quantitative and qualitative differences make it possible to distinguish CD4 from CD8 cells present in the bloodstream with a 2:1 ratio [481]. However, CD4 and CD8 functions are at least twofold, since their extracellular portions bind to MHC molecules on the APC surface, thus acting as adhesion molecules. Another major function of CD4 and CD8 is to act as signal transducers in T cells due to their intracellular portions linked to specific kinases; thus CD4 and CD8 are phosphorylated following antigen binding to TcR [36].

Unlike B cells, T cells can interact with antigens directly, even in solution. Antigen recognition by T lymphocytes occurs only when antigens are inside or on the surface of a cell, more precisely when antigens are presented by APCs associated in man with HLA (human leukocyte antigens). Such *double* recognition of both antigens and HLA molecules is critical for T-cell activation, whether immunoregulatory or cytotoxic. We can therefore conclude that in the thymus T lymphocytes are committed to recognizing HLA antigens, and also that tolerance starts in the thymus following TcR affinity for HLA molecules, attributing to HLA a crucial role in the *recognition* process.

Originally described in mice, two phenotypes were defined showing that T dichotomy is actually the heterogeneity of CD4 cells. However in man there are three subpopulations (Tables 1.10–1.12) [52, 69, 130, 158, 220, 249, 455, 470, 650], divided into three helper (h) subsets based on different patterns of ILs they secrete [643].

These subsets are: Th1 T cells predominantly involved in DTH reactions, Th2 T cells apparently specialized in IgE-mediated reactions, and Th0 T cells [159, 455]. Th0 cells represent a heterogeneous population of effector cells, mostly naive lymphocytes that in the absence of signals clearly driving differentiation into Th1/Th2 T cells have never before been activated and thus have not acquired the ability to secrete a mature profile of ILs, modulating their effects with respect to

Table 1.10. Functional characteristics of human CD4 Th0, Th1 and Th2 lymphocytes

	Th1	Th2	Th0
IL_2	+++	–	+++
IL_3	+	++	+
IL_4	–	+++	+
IL_5	–	+++	+
IL_6	+	++	+
IL_{10}	–	++	+
IL_{11}	–	++	
IL_{12}	+++	–	?
IL_{13}	+	+++	+
$IL_{17}E$	–	++	–
IL_{18}	++	+++	
IL_{23}	++	–	
IL_{25}	–	++	?
IL_{27}	++	–	+
IL_{31}	–	++	?
IL_{32}	++	++	?
GM-CSF	+	++	+
IFN-γ	+++	–	+++
TNF-α	+++	++	++
TNF-β	+++	–	+
Necessary for development	IFN–γ	IL_4	?
Cytolytic activity	+++	±	++
Total Ig levels	+	+++	?
IgE levels	–	+++	±
IgA, IgG, IgM levels			
Relationship T/B ↓	++	++	++
Relationship T/B ↑	–	+++	±
Activation of eosinophils/mast cells	–	+++	+
Activation of macrophages	+++	–	?
Delayed-type hypersensitivity	+++	–	–
Positive immune responses	Atopic diseases, Virosis, Leishmaniasis, Leprosy	Pregnancy, Autoimmunity, Arthritis, Helminthiasis	
Negative immune responses	Autoimmunity, Arthritis, Helminthiasis	Atopic diseases, Virosis, Leishmaniasis, Leprosy	
Response to proliferation and/or production of cytokines			
IL_2	↑	↑	↑
IL_4	=	↑	?
IL_{10}	↓	↓	↓
IL_{12}	↑	↓	?
IFN-γ	=	↓	?
CD30 phenotype	–	+++	+

Data from [52, 69, 105, 119, 129, 130, 158, 220, 249, 455, 470, 650].

Table 1.11. Mechanisms of Th1/Th2 differentiation

Genetic factors	Familial predisposition toward atopy development
Allergen-specific factors	Allergens vs antigens, allergenic epitopes, physiochemical factors, dose, route of administration
Antigen processing presentation	Antigen processing pathways and cells, expression of adhesion, accessory, or homing molecules
HLA restriction/V regions used by TcR	
Pattern of cytokines	IL_4, IL_{12}

Data from [105].

Table 1.12. Differentiated production of cytokines (ng/ml) by Th1 and Th2 clones activated by CD3 and/or CD28

Clone	Activation	IL_4	IFN-γ	IL_5
Th2	–	<0.1	<0.1	<3.0
	+	31.9	0.2	39.9
Th1	–	<0.1	<0.1	<3.0
	+	<0.1	24.8	0.8

Data from [642].

the type and quantity of ILs produced in the microenvironment and the nature of responsive cells [643].

The conventional definition of a Th1 or Th2 cell depends strictly on the secretion of IFN-γ or IL_4. Th1 cells secrete IFN-γ but do not secrete IL_4, whereas Th2 cells secrete IL_4 but not IFN-γ. T cells secreting neither IFN-γ nor IL_4 are neither Th1 nor Th2 cells [470]. A subset of $CD4^+$ T-cell lines, which secrete TGF-β but not IL_4 or IFN-γ, has been termed a Th3 cell [470]. In addition to Smad-2/4 of TGF-βRI, there is Smad-7 which blocks activated receptors and interferes with phosphorylation of both Smad-2 and Smad-3 (G. Monteleone, pers. comm. 15. 2. 2005) [156]. An IL_{10}-producing subset has been termed Tr1 (T-regulatory 1) [470]. Another family, that of T-cell Ig domain and mucin domain (TIM) proteins, is identified to be expressed on T cells [320]. The quantitative difference of ILs synthesized by T cells is remarkable, as demonstrated either by production of high levels of IL_4 and IL_5 by Th2 T cells (GATA-3 is critical for expression of the IL_5 gene in Th2 cells) [678], while that of IFN-γ is significantly lower, or by nearly specular IL secretion by γδ Th1 T cells [665]. Thus, either GATA-3 or single-nucleotide polymorphisms in the IL_{18} gene might be relevant in inducing the very broad Th2 phenotype observed in atopic subjects [278]. A specific factor triggering differentiation into Th1 lymphocytes has been identified in IL_{12}, which in a dose-dependent manner increases IFN-γ levels in TcR of antigen-specific T cells while antagonizing an IL_4-induced B-cell switch to IgE production. It also appears that IFN-γ IL_{12}-mediated production is necessary to stimulate complete expression of Th1 phenotype [105]. For the outcome of CD4 T cell differentiation, the balance between T-bet (TFs T-box expressed in T cells) and GATA-3 is critical [665]. However, T-bet initiates Th1 lineage development from naive *Thp (Th precursors)* cells, both by activating Th1 genetic programs and by repressing the opposing Th2 programs [556].

CD4 T cells coming from thymus, although they can differentiate either into Th1 or Th2 T cells, are not predestined to IL production [644]: the current theory is that only at the moment of antigen presentation do uncommitted cells begin to secrete ILs, with polarization of immune processes into a model of type I or IV reactions [290]. As yet, it is not clear how CD4 T cells distinguish the antigens they encounter, because there is no proof that such antigens are endowed with specific structural features allowing CD4 to recognize them [469]. Since T-cell antigen specificity is prearranged in the thymus apparently at random, it is challenging to imagine how CD4 cells on the point of recognizing a given antigen can be programmed at this stage of development, so that they can express a defined set of ILs at the subsequent encounter. To undo the Gordian knot, the present dogma is that in any inflammatory site both Th1 and Th2 lymphocytes differentiate from common post-thymic antigen-specific ThP, while an intermediate point between ThP from one side and Th1/Th2 T cells from the other is represented by Th0 [473]. Hitherto, cell surface marker analysis for Th1-Th2 T-cell subset identification has yielded unconvincing results. Recent studies show that Th1-Th2 T cells differentiate in conformity with CD30 expression, with CD27, CD40, CD95/Fas, OX40 and other molecules belonging to the receptor superfamily of TNF/NGF (TNF-R = CD120a, CD120b and NGF-R), with low or hardly noticeable levels of Th1 and high levels of Th2 cells (and of CD8) [388]. CD30 is therefore viewed as a specific marker and if stimulated as a cofactor of T lymphocyte differentiation, preferentially toward the Th2 T-cell phenotype, which expresses CD30 on its membrane [113]. An interesting observation is that a biologically significant number of CD30 markers are present in the bloodstream of patients allergic to grasses (and not in controls) in concomitance with pollination periods [113]. A soluble CD30 (sCD30) (88 kD) is increased in serum of HIV (human immunodeficiency virus) seropositive patients [4]. Recent data show that the LAG-3 molecule (lymphocyte activation gene-3), an IgSF member binding to the nonpolymorphic part of HLA class II molecules, is selectively transcribed into activated Th1 and NK cells, since its expression is correlated with IFN-γ production by Th1 cells and not with that of IL_4 [457]. Of particular

interest is the role of the STAT family of molecules, including SH2 and SH3 (src homology 2, 3) [248], activated by an additional TF, PTK (protein tyrosine kinase) [224]. Two classes of SH2-containing inhibitory signaling effector molecules have been identified: the tyrosine phosphatase SHP-1 and the inositol phosphatase SHIP (SH2-containing inositol polyphosphate 5-phosphatase) interacting with the immunoreceptor tyrosine based inhibitory motif (ITIM) [446]. *STAT6,* activated by IL_4 and IL_{13}, *plays a crucial function related to genes regulating differentiation into Th2 T cells* [248, 523], similarly to *STAT4 phosphorylated* by IL_{12} *for Th1 T cells* [523]. Th2 T cells are thought to have a defect of STAT4 phosphorylation [401]. Thereby STAT4 and STAT6 appear to control Th1 T-cell differentiation [523], while IL_2 and IL_{15} activate phosphorylating STAT3 and STAT5, and IL_4 and IL_{12} activate phosphorylating STAT3 [240].

CD 8 T cells. IL_7 and IL_{15} are crucial for the development of $CD8^-$ T cells within the thymus, and SOCS1 (supressor of cytokine signaling-1) regulates this process by regulating both ILs [441]. CD8 T cells are the main type of effector T lymphocytes, as suppressor and cytotoxic cells. In the first case, they bind one HLA class I/peptide molecule also contacting β_2-microglobulin (β_2-m). A flexible loop of the α3 HLA domain is clamped between the CDR-like loops of two CD8 subunits in the classic manner of an antibody-antigen interaction, precluding the binding of a second HLA molecule [170]. The binding of CD8 to HLA can prime CD8 T-cell precursors that depend on IL_2 (cytotoxic T-cell differentiation factor) (Table 1.5) produced by Th1 cells to be transformed into active cells [349]. CD8 T cells thus activated secrete soluble factors suppressing both Th1 and Th2 T cells, as well as growth of Th2 T cells enhanced by IL_4, thereby down-regulating immune responses and inhibiting activation of B cells [255]. Consequently, eliminating CD8 T cells in immunized mice, the synthesis of IgE antibodies was amplified, whereas it was inhibited when CD8 T cells were deleted prior to treatment, suggesting that there are two different subsets of T suppressors, also because IL_4 inhibits CD8 T cells producing IL_2 and IL_6 and stimulates those secreting IL_4 and IL_5 [255]. CD8 T cells have two distinct patterns with different effects: *type 1 of Th1 T cells (Tc1)* and *type 2 of Th2 T cells (Tc2)* [456, 469]. CD8 Tc1 produces IFN-γ, so the highest production of IL_4 is supported by CD8 absence and anti-IFN-γ presence [349] and IL_4 may encourage Tc2 [470]. Animal data suggest that functionally distinct subsets of CD8 Tc2 T cells may play a significant role in IgE regulation [255, 349]; also in humans, such CD8 trigger isotype switching of B cells to IgE antibodies [105]. In addition, virus-specific CD8 can eventually generate IL_5 with an implicit increase of eosinophilia in airways [100], thus providing key elements to explain clinical links between viral infections and asthmatic exacerbations [105]. As we shall discuss subsequently, infantile atopy is associated with a deficiency of T suppressor cells [142] (Chap. 4).

Once activated by antigens or lectins, *cytotoxic T CD8 lymphocytes* (CTLs) play a prominent role when antibodies are unable to block cytolytic viruses, circulating or present on cell membranes. CTLs associated with HLA class I molecules via a specific receptor recognize viral peptides expressed on target cell surfaces, lysing them independently of antibodies or complement [123]. A first signal causes translocation and secretion of cytotoxic cell granules containing proteins named cytolysins, also known as *perforins* (which perforate target cell membranes) and granzymes also present in NK cell cytoplasmic granules: secretion of such Ca++-dependent proteins leads to cell lysis [322]. Typical is the granule speed when they are directionally released to make contact with target cells [189]. A second signal may induce death via a self-destructive mechanism mediated by Fas/Apo-1 (apoptosine-1) (CD95) [559], activated by contact between effector and target cells [322]. Fas and Apo-1 are identical molecules localized on murine and human cells, respectively [559]. Both are encoded by TNFRSF6 on chromosome *10q24.1* and are based on TcR-mediated recognition of target cells [189] and on increased membrane permeability of target cells: the increase in intracellular fluid polarizes to lysis [421]. The lytic process can be outlined in four steps:

- *TcR-CD8-mediated recognition* in the context of HLA class I molecules, CTL adhesion to antibody coated plasma membrane of target cells forming a CTL-target cell conjugate persisting for a few minutes and mediated by CD11a/CD18/LFA-1 (lymphocyte function-associated antigen-1) interactions with pertinent ligands CD54/ICAM-1 (intercellular adhesion molecule-1) and CD58/LFA-3 and possibly CD2/CD80 molecules
- *Irreversible programming* of lytic equipment with release of perforins, which, when in the contact area of conjugates, form 5- to 16-nm pores, upon which mobilized granules deposit their contents into intercellular clefts by exocytosis
- *Lethal hit* in unidirectional fashion mediated by the release of granule contents
- *Cellular death* by osmotic lysis, or more likely by enhanced fragmentation of nuclear DNA, a process characteristic of programmed cell death (PCD) or ritual suicide, or apoptosis [421]

The lytic process is in perspective unremitting, because cell recycling is activated by new TcR stimulations [189].

NK (natural killer) *cells* (non-T non-B) are morphologically similar to lymphocytes, but somewhat larger with a reniform nucleolus and a cytoplasm rich in large granules, assuming therefore an LGL phenotype [460]. NK cells make up about 15% of peripheral blood lymphocytes, and 3%–4% of splenic lymphocytes, while notable amounts are found in lung interstices, gut mucosa, and liver. IL_{21} and $IL_{21}R$ play a role in NK cell proliferation and maturation from bone marrow [412]. Deriving from precursor cells common to T lymphocytes residing in the fetal liver and bone marrow [193],

together with HLA-restricted CTLs, naturally occurring NK cells lyse a variety of targets with no prior specific sensitization of the host (unlike T cells), regardless of HLA gene restrictions and of antibody or complement. NK cells are thus enabled, along with macrophages and leukocytes, to act as a *first line of defense* playing a critical role in natural resistance against a variety of infectious diseases [289]. NK cells and CTLs are mutually self-regulated: NK-cell limitation of viral replication is higher in the first 3 days, contrary to CD8 lymphocytes developing from their precursors within 5–6 days. Accordingly, there is a complementary balance between innate and acquired immunity, since NK-cell cytotoxicity is temporally accomplished and replaced by that of CD8 T cells [273]. NK cells share with cytolytic cells of lower vertebrates and invertebrates both killing activity and lack of TcR and memory, thus suggesting that they are innate and primitive components of the vertebrate immune system [193]. Identified in humans, several CDs possess Fc receptors (FcR) that bind IgG (FcγR) and are activated by membrane receptors such as CD2R, CD16 (FcγRIII), CD39, CD69, and NKRP-1 (natural killer receptor = CD161) and CD94, a product of a gene in human chromosome *12p12–p13*, both members of C-type lectin superfamily [225, 320, 460, 470]. In addition to displaying cytotoxicity against autologous tumor cells, triggered by IFN-α, IL_2, IL_{12} and IL_{15}, NK cells release GM-CSF, M-CSF, IFN-γ, and TNF-α. Both activated IFN-γ and TNF-α are important for immune resistance against pathogens and regulation of hemopoiesis and immune responses, and IFN-γ also generated by other cells in response to microorganisms enhances NK cell cytolytic activity [25, 339, 460]. Increasing evidence shows that NK cells play a cardinal role in the control of microbial agents and with natural cytotoxicity of viral infections, also in absence of T and B cells, as in SCID mice [25, 130]. More precisely, the NK cell kills the abnormal target cell by inserting the pore-forming molecule perforin into the membrane of the target cell, and then injecting it with cytotoxic granzymes [115]. Furthermore, NK cells produce IL_2, necessary for their proliferation, IL_{12} [77], IFN-γ induced by IL_{15} generated by activated human monocytes [68], and VLA-4/VLA-5 (very late antigens 4 and 5) (CD49d/CD29 and CD49e/CD29) mediating NK-cell adhesion to fibronectin (FN) [64]. IL_{12} (NK cell stimulatory factor) primes in vitro Th1-specific immune responses and inhibits development of IL_4-producing Th cells [339]. IL_2 increases expression of adhesion molecules on their cell membranes [193]. Lastly, the MCP chemokines (monocyte chemotactic protein) are NK-cell major attractants, also inducing their chemotaxis [317].

One of the more intriguing aspects of NK-cell function is its possible recognition of foreign substances as non-self, allowing their recognition system to discriminate between self and non-self, to start cytotoxic activities and to produce ILs [289]. This theory is based on two models: an HLA-independent system of recognition triggered by IL_2 [289], or the *missing receptor* hypothesis focusing on immune surveillance with consequent elimination of cells failing to express HLA class I molecules [313]. The second theory has been confirmed by several experimental studies; however, at present how NK cells interact with target cells remains unanswered [667]. According to prevailing hypotheses, expression of HLA molecules could deal with a protective role from NK-mediated lysis [381]. In agreement with this model is the presence on NK cells of ≈20 receptors (NKR, natural killer receptor) specific for HLA class I antigens [4, 667]. NKRs are divided into NKAR (NK activating receptor) and NKIR (NK inhibitory receptor), among which are p50 1–3 (NKAR) and p58, p70, and p140 (NKIR), associated with IgSF, activators of HLA-C (p50 and p58), HLA-B, and HLA-A molecules, respectively [4, 381], and CD94 associated with proteins (94AP) phosphorylated by tyrosine, all implicated in HLA antigen recognition [427]. There are additional NKRs specific for HLA class I antigens, also IgSF membrane proteins and encoded by genes in human chromosome *19q13.4* [91], including the NKAT 1–4 (NK associated transcripts 1–4) family encoding transmembrane (TM) proteins with an extracellular region characterized by 2–3 IgSF domains and one cytoplasmic associated with ARAM (antigen recognition activation motif) [91].

HLA class I molecules may deliver negative signals protecting from lysis cells attacked by NK cells, and may resume cytotoxicity if target cells have reduced class I expression or abnormal peptide-HLA complexes impair recognition [320]. Thereby, NK cells are subjected to a delicate balance between activatory and inhibitory signals: only upon HLA molecule loss or reduction in number is inhibition dampened and killing enhanced [443]. A system of recognition that is characteristic of NK cells relies on the NKARs and NKIRs of these cells. The NKARs recognize a number of different molecules present on the surface of all nucleated cells, whereas the NKIRs recognize MHC class I molecules, which are also usually present on all nucleated cells. If the NKARs are engaged, a "kill" instruction is issued to the NK cell, but this signal is normally abrogated by an inhibitory signal sent by the NKIR on recognition of MHC class I molecules [287, 365]. Even if several issues remain to be adequately explained, for example, regarding specific functions of receptors so far identified and distribution of their activities, it is likely that different receptors may be used depending on the NK-cell activation state, thus releasing a negative signal that recognizes polymorphic HLA class I determinants on potential targets [667]. NK cells and CTLs may regulate functions of each other, in addition to the role of NK cells in CTL differentiation [273], but CTLs down-regulate NK-cell action expressing their inhibitor NKB1 [428], also complemented by TcR-αβ [273]. However, NK cells can express inhibitory receptors such as C-type lectins (CD94), while rodent NK cells may express those of IgSF [320].

The *LAK (lymphokine-activated killer)* cells are additional cells possibly with a cardinal role in discrimination between self and non-self [470]. LAKs display selective cytotoxicity for tumor cells not killed by NK cells with a broader spectrum, synergically expressed by IL_2 and IL_{12} and activated by IL_{15} [557, 693].

In the lytic process mediated by NK cells, we can distinguish four stages similar to those described for cytotoxic CD8 cells, the main difference regarding a shorter length of the whole procedure (4–6 h) compared to the longer one of antigen-specific CD8 lymphocytes. According to recent evidence, CTLs and NK cells not only deliver lytic messages to targets, but also bring about a substantial DNA fragmentation, a feature of CD8 cells via several secreted membrane molecules [190]. Recent reports of an adolescent and two children [43, 273] with life-threatening and relapsing infections, a quantitative and qualitative defect of NK cells [43] or no cells with NK-cell phenotype [273] and a reduced total number of CD8 T cells but with antibodies to several viruses [43], emphasize that NK cells *hold a key position in the response to infections* [460]. Dysfunctions of NK cells have been reported in Chédiak-Higashi syndrome with absence of cytolysis due to a secretory deficiency impairing granule secretion [21], in PID such as SCID, suggesting in this PID a combined deficiency of both NK and T cells, in X-linked lymphoproliferative syndrome (XLP), X-linked agammaglobulinemia (XLA), common variable ID (CVID), LAD (leukocyte adhesion deficiency), and in chronic fatigue syndrome.

K (killer) *cells,* morphologically and functionally similar to lymphocytes, are in practice null cells since they lack markers of both B and T lymphocytes. Not HLA-restricted, they have receptors for complement but not for surface Igs, and can recognize and kill target cells coated with specific antibodies by binding and being triggered via their FcRs: when target cells are killed but not phagocytosed, the process is called antibody-dependent cell-mediated cytotoxicity (ADCC). It appears that the NK-K cell system is actually one heterogeneous subpopulation also comprising LGLs, in which killing is alternatively mediated by a mechanism of NK or ADCC type [460].

A small subset of human γδ T cells fulfills in vitro either nonspecific killing or cytotoxic activities, possibly associated with CD1c molecules, with some similarity to HLA class I molecules [337]. Some activated γδ T cells can lyse infected phagocytes, being powerful K cells [110].

Lymphocyte Recirculation

T-cell recirculation illustrates the elegance of immune cell regulated migrations. Stem cells, after reaching the thymus or bone marrow via the bloodstream and maturing into B or T cells, enter the systemic circulation, peripheral lymphoid organs, and MALT, never traveling in opposite directions. Both phenotypes migrate electively between corresponding thymus-dependent and thymus-independent areas. Memory T cells leave the bloodstream via peripheral efferent vessels, especially to inflamed sites, and subsequently enter regional afferent lymphatic channels that eventually direct cells to a draining regional lymph node, circulating by lymphatics or via cortical venules into lymph node parenchyme (hence the high percentage of memory T cells) [634]. In contrast, naive cells move across afferent postcapillary HEVs into lymph nodes, directly through the HEV barrier. HEVs originate from pre-existing capillary venules (Fig. 1.3), differentiating under the influence of IFN-γ secreted by local Th1 T cells [226]. A particular feature of T lymphocytes is that they selectively bind to specific HEVs in lymphoid tissues and appear to completely ignore normal vascular endothelium, unlike in inflamed sites [226]. Moreover, between HEVs and distinct subsets of T cells there is a selective binding of finer specificity, further regulating lymphocyte homing into various lymphoid and nonlymphoid tissues [522]. In lymph nodes, encounters take place between novel antigens coming from afferent draining tissues and virgin cells. Lymphocyte traffic is not a random mixing of cells in varying tissues of the body. Yet this distinctive migration is directed by lymphocyte cell surface molecules that are receptors for ligands expressed on endothelial structures on HEV cell membranes (*homing receptors*) (Figs. 1.5, 1.11) [62]. As a result of these interactions, T lymphocytes transmigrate into mucosal tissues with immune memory of antigen sensitization, hence regulating local immunity [326]. Leaving secondary lymphoid organs and entering efferent lymphatics, lymphocytes of either phenotype recirculate via mesenteric lymph nodes and return to the blood via the thoracic duct, emptying into the superior vena cava, and crossing HEV endothelial vascular linings; they are eventually exported back to mucosal sites as memory T cells via the subclavian veins [591]. It is believed that lymphocytes migrate nonrandomly to their home: for example, naive T cells expressing CD62E that binds HEV can also migrate into skin T cells, as well as memory T cells, if they are equipped with CLA (cutaneous lymphocyte-associated antigen), which binds to CD62E on endothelium of skin venules [634]. The contribution of adhesion molecules should be pointed out: for example, $\alpha_E\beta_7$ integrin is equal to CD103 inducible by TGF-β1 (transforming growth factor β1), binding to E cadherin directs T lymphocytes to the IEL (intraepithelial lymphocytes) group, >95% of which expresses it, thus mediating lymphocyte adhesion to mucosal epithelial cells [76]. These meaningful data provide an immune basis to analyze reactions triggered in distant target organs, so that lymphoid cells stimulated, for instance in GALT, migrate into the bloodstream and then to the gut lamina propria: *this traffic is very important for maintaining the immune surveillance within the MALT system* [326]. This also elucidates why some T lymphocytes appear to

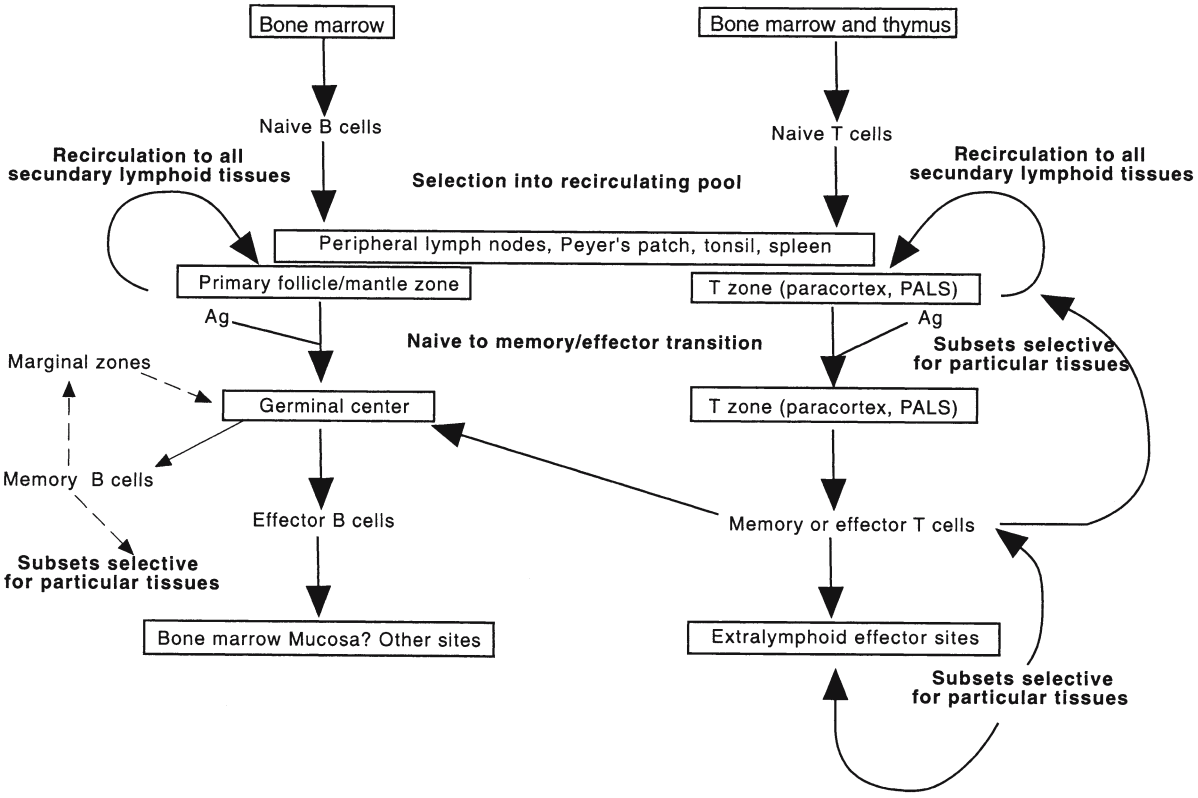


Fig. 1.11. Recirculation of lymphocytes: differential distribution of naive versus memory cells. Unlike naive cells, memory and effector T (and probably B) cells can efficiently extravasate in tertiary or extralymphoid tissues (see text). Antigen-activated B cells may home to specialized environments in the outer T zone during primary responses, or may colonize germinal center sites of hypermutation, affinity maturation, and memory cell differentiation. Less numerous, specialized lymphocyte subsets, such as γδ T cells or IELs, may be targeted from their origin in the thymus or bone marrow directly to reproductive, cutaneous, intestinal, or other tertiary sites. Extralymphoid tissue sites of selective homing include skin, lung, intestinal lamina propria, and synovial tissues. *Ag* antigen, *IEL* intraepithelial lymphocytes, *PALS* periarteriolar lymphoid sheath. (Modified from [62])

patrol throughout the body (*recirculation*) and their traffic is greatly enhanced if an inflammatory response develops. The toxic action carried out by viruses can alter both homing and recirculation of T cells until inactivation, notably of lymphocytes specific for that particular virus (Chap. 22). The immunocompetent memory cells recirculate uninterruptedly, since encounters with particular antigens and triggering of immune reactions are facilitated on the one hand, and on the other localization of specific cells in elective areas is supported [634]. While naive cells recirculate almost exclusively via lymph nodes and other secondary lymphoid tissues, where priming may take place, traffic of memory and effector lymphocytes is also directed to nonlymphoid tissues, including gut lamina propria, lung tissues, as well as inflamed skin, and joints (Fig. 1.11). More precisely, naive cells are excluded from effector mucosal sites because their HEVs lack MAdCAM-1, expressing inadequate levels of $\alpha_4\beta_7$ to bind it [62]. Sophisticated studies on animal models have observed that homing receptors direct traffic with a specific migratory behavior of the cells: those coming from GALT, for example, from PPs, have a preferential migration into the gut, mediated by β_7 integrins [615], and to a lower extent into other sites, and likewise for other preferential localizations (Fig. 1.12) [534]. Memory cells, once activated in specific tissues, depend on unending antigen stimulation and with remarkable selection home in to tissues related to initial exposure to foreign antigens [226]. The selective pattern of recirculation has a prerequisite of preventing inappropriate competitions between T and B cells and migrations into nonlymphoid organs [62]. Similar discrepancies involve specialized subsets making use of preferential routes, such as γδ and IEL cells, which can migrate into skin and/or gut [62]. Studies have hypothesized that an important role may be entrusted to gut-derived IELs homing preferentially to mucosal lung tissue, also effective in preventing airway infections [454]. Because of lymphocyte circulation, B_{IgA} cells of PPs following antigen sensitization home in to regional lymph nodes, where they differentiate, activate, and then return to colonize electively the lamina propria of various compartments [548], including mammary glands, where they transform into IgA-se-

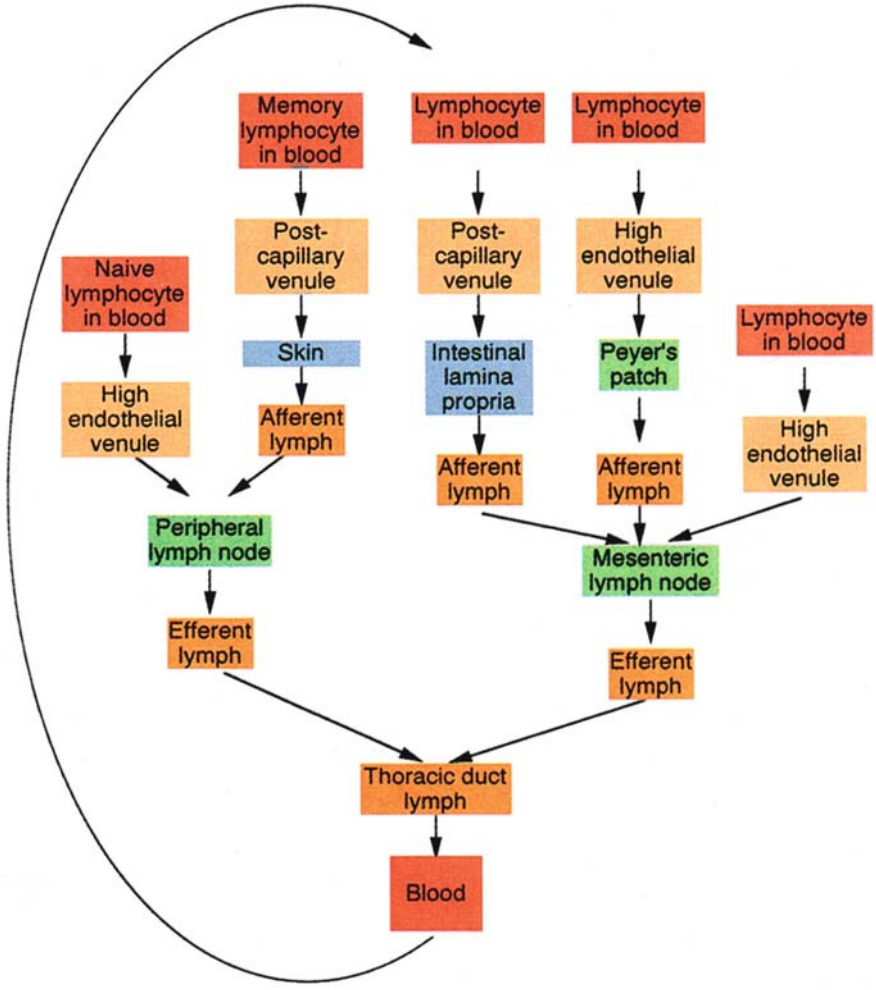


Fig. 1.12. Lymphocyte circulation routes. Patrolling the body in search of foreign antigens, lymphocytes follow circuits through both lymphoid and non-lymphoid tissues

creting plasma cells (*enteromammary axis;* Chap. 2). Analysis of B lymphocytes is limited: naive B cells are the great majority of cells recirculating between blood and lymphoid tissues [635]. As regards the volume of cell traffic, human circulating blood contains $\approx 10^{10}$ lymphocytes, with a blood transit time of 25±6 min, resulting in a 40-fold daily exchange ($\approx 5\times10^{11}$ lymphocytes) [635]. In the human thoracic duct, there is a recirculation of only $\approx 3\times10^{10}$ lymphocytes, that is about 6%–7% of the daily migration via peripheral lymphoid tissues: in rats the rate of naive and memory cells is nearly equivalent [636].

T and B Cell Receptors

T and B lymphocytes can recognize an extremely wide range of disparate foreign antigens because of the high variability of their membrane receptors, stemming from the ability of developing cells to arrange and modify genes encoding antigen receptors, even if single B cells express only one type of specificity, and the same is probably also true for T cells [225]. There are $\approx 10^{14}$ TcR and 10^{18} BcR [353] (Fig. 1.13) playing a central role in antigen recognition and activation, with structures complementary to those of specific antigens; epitope recognition by receptors is a critical event triggering immune responses. In contrast, the human genome contains 75,000–100,000 genes, most of which have no role in immune recognition [353]. Molecules coded for by HLA molecules regulate B- and T-cell differentiation, because they are involved in the host's ability to set humoral or CMI responses. More prominently, HLA molecules are necessary to signaling and their intercellular interactions contribute to distinguishing between self and non-self [470]. Specificity dominates in the molecules induced in every individual *in one or two versions,* dictated by maternal and paternal genes, whereas TcR and BcR are produced in *millions of copies* that, although adhering to a common structural plan, are different in V domains [115]. If each lymphocyte bears receptors with a unique specificity, coordinately expressed by progenitor stem cells via processes of genetic recombination, there must necessarily exist a great variety calling on the immune system to recognize an extensive spectrum of antigens. Doubtless, clonal selection interferes to naturally eliminate receptors destined to die via PCD [276, 510]. Both TcR and BcR are able to recognize a given antigen due to their unique molecular structures, which are spatially and chemically complemen-

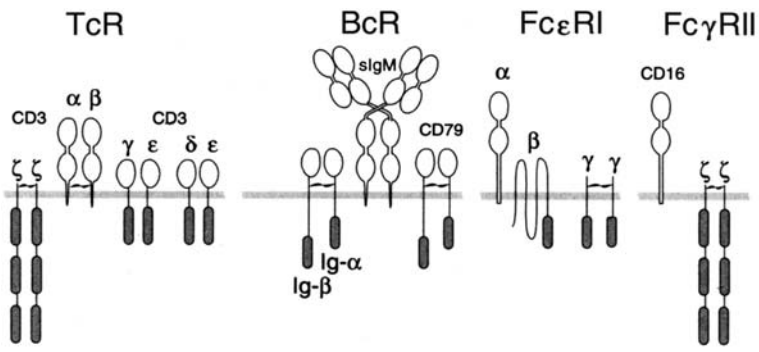


Fig. 1.13. Receptors of hemopoietic cells

tary to one another, fitting together in a "lock-and-key" relationship, thus giving the starting signal to cellular replication. The progeny of cells derived from any naive cell originate as a lymphocyte clone with cells morphologically identical to one another in nearly all respects, while each BcR or TcR expressed by cells of a given clone are also identical [185]. Lymphocytes, already triggered by binding to their specific antigens and activated, proliferate and deliver specific effector functions, thereby recognizing the intruder and successfully inactivating or eliminating it: evidence exists that cells with a specific receptor that fits better to binding sites may differentiate selectively, thereby predominating over other cells [47]. Receptor molecules mediating specific recognition have heterodimeric structures: L and H Ig polypeptides behave as BcR, while α and β chains are related to TcR. L, H, α and β chains possess V domains arranged in a specific spatial contour on available antigen surfaces and also C domains interacting with receptors on host tissues and transmitting signals to cell cytoplasm.

Therefore, BcR and TcR genes share many features and both undergo similar DNA rearrangements [18]. In addition, genes coding for BcR and TcR use a unique strategy to achieve the degree of diversity required. The set of human BcR and TcR genes is complete for all the seven *loci* – the three Ig *loci:* IgH (after the H chain), IgK (after the κ L chain) and IgL (after the λ L chain); and the four TcR *loci:* TcRα/δ on chromosome 14, TcRβ (on chromosome 7, and TcRγ on chromosome 7. The IgH cluster (431 human genes and 798 alleles) corresponds to 4 types of gene segments: V, D, J, and C. The Igκ and Igλ clusters lack D segments. All these segments contain multiple genes; in the IgH cluster, for example, there are ≈50 functional V segments. The *TcR genes* have a similar organization and in the 4 TcR *loci* there are 242 human genes and 443 allels [115, 183]. In contrast to the TcRβ and TcRδ *loci,* the TcRα and TcRγ *loci* do not contain D segments. And, as in the case of Ig genes, each TcR *locus* contains multiple V, D, and J genes; on TcRα, for example, there are 70 to 80 V genes and ≈60 J genes [115]. To such genes is committed a molecular control to increase variability via somatic and evolutive processes, but inheritable chromosomes, as discussed earlier, contain no Ig genes at all: otherwise there would be several million of these genes. For example, spermatozoon and ovum contain gene fragments that via subsequent phases of rearrangement, reorganize genome sequences so that *new genes can be created in immune cells* to continue the cycle [18]. The basic principles governing the genetic mechanism to create such a variety can be focused on DNA somatic rearrangement, versatile and casual, accompanied by deletion and an evolutionary mechanism with reference to active engagement and frequent combinations [185]. Antibodies and T-cell clones thus achieved are specific not only for antigens present now in the microenvironment, but are also able to develop different specificity to antigens not yet present, to be encountered in the future [326].

T-Cell Antigen Receptors

While nearly all developing TcRαβ thymocytes express a single TcRβ protein, many thymocytes rearrange and express two different TcRα chains and, thus, display two αβTcRs on the cell surface. The number of such dual TcR-expressing cells is surprisingly lower among the mature T cells [284]. The immature TcR consists of a β chain identical to that found in the mature TcR and a pre-T chain that contains only a C region. This segment is replaced by an α chain to form the mature TcR, and each chain consists of a V and a C region [115]. The PTK signaling and coreceptor involvement may be operating in normal thymocytes [284]. *Antigen-specific T cells have a TcR similar to membrane Igs (mIg) of B cells* and also contain V, D, J, and C segments. *CD3-TcR complex is the unity of mature T cells: TcR is able to recognize and discriminate among different foreign antigens, CD3 polypeptides have long intracytolastic tails necessary to TM transduction of activation signals* [222]. *Each TcR* molecule *is formed by two pairs of heterodimers, αβ and γδ,* each α, β, γ, and δ chain containing a V domain and a C domain, and a *CD3 complex by three distinct invariable chains known as γ, δ, ε correlated between them.* Antigen-specific T cells have a TcR similar to membrane Igs (mIg) of B cells. CD3-TcR complex is the unity of mature T cells: TcR is able to recognize and discriminate among different foreign antigens, CD3 polypeptides have long intracytoplasmic tails necessary to TM transduction of activation signals [225]. Each TcR molecule is

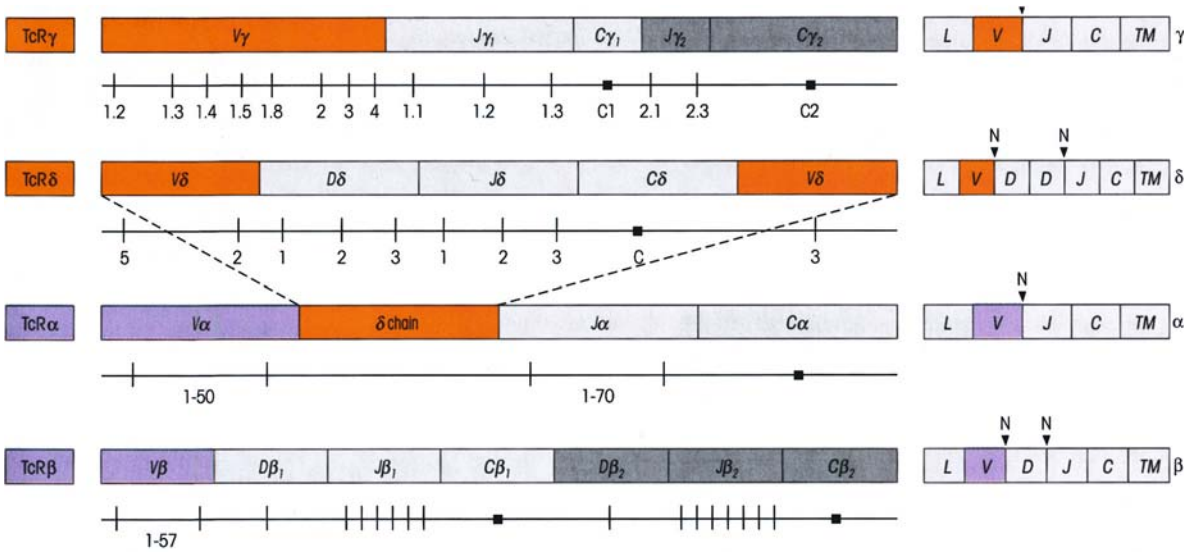


Fig. 1.14. Genes coding for TcR (for details see text)

formed by two pairs of heterodimers, αβ and γδ, each α, β, γ, and δ chain containing a V domain and a C domain. CD3 complex by three distinct invariable chains known as γ, δ, ε correlated between them. As in the antibody molecule, the V domains contain three CDRs, where the highest degree of amino acid variability is concentrated. The CDRs in the case of the αβ TcR recognize a complex formed by a peptide seated within the groove of an MHC molecule [171]. Each TcR has a single extracellular Ig-like C domain, a TM segment, a cytoplasmic tail (44–81 amino acids in length), and is completed by ζ and/or η chains. ζη chains also are invariable molecules, derived by conjunction of alternative transcripts of the same gene, and form homodimers linked by a -S-S (ζ-ζ and/or η-η) bond, or heterodimers (ζ-η), not covalently associated with five subunits (αβγδε) to form the heptameric complex. The human chain MWs are as follows: γ 25–28 kD, δ 21 kD, ε 20 kD, ζ 16 kD and η 22 kD [4].

Two T structures recognize antigen peptides bound to HLA molecules:

- In 90%–95% of T lymphocytes is found a TcR formed by two polypeptide chains α and β (αβ) (MW of 45 and 40 kD) expressed on the cell membrane in the form of 90-kD heterodimers and linked by -S-S bonds, associated on the cell surface with CD3, a clonally invariable protein, and encoded by gene segments organized in a discontinuous way on chromosomes (V, D, J and C); hence gene rearrangements are necessary. V regions are present in both chains; in addition there are J and C for α chain, and D, J and C for β chain. The α and β molecules, membrane gps belonging to IgSF (Table 1.4), are encoded by genes located on chromosome 14 (region *q11*) for α chains and on chromosome 7 (region *q32*) for β chains [337]. Use of Vα and Vβ regions of TcR from T lymphocytes is very stable, as TcR gene expression is controlled by DNA sequences, among others.
- In 5%–10% of TcRs a second heterodimeric receptor formed by a γ (45 kD) and a δ chain (40 kD) (γδ) linked by S-S bonds is expressed. The γ chain locus is on the short arm of chromosome 7 (region *p15*) and the δ chain on chromosome 14 is embedded within the α locus [222, 337] (Fig. 1.14) [470]. Human genes encoding γ chains contain exons for V, J and C regions, and gene exons for δ chain encode for V, D, J and C regions, similarly to α and β chains [18]. In humans, γ chains have two different Cγ2 exons, one encoding a cysteine residue. The γδ cells are about 80%–90% $CD8^+$ and the remaining cells $CD4^+$ [337]. In humans, both receptors are present in IELs, but αβ T cells prefer to differentiate mostly in normal epithelia homing lymphocytes [525].

The same basic principles of gene rearrangement described for Ig apply for TcRs. Also, genes encoding V and C regions of TcR chains are found on DNA varying segments, and therefore should be rearranged. However, repertoires of different TcR are believed to be at least as large as Ig molecule repertoires, also regarding antigen specificity. A remarkable degree of independence seems to dominate in the generation of αβ and γδ lineage cells from progenitor cells that, in theory, could simultaneously express a TCRγδ and a pre-TCR [180]. In peripheral lymphocytes, STAT5 is primarily required for the generation and/or maintenance of γδ T cells and TCRγδ(+) IEL [246]. Both γ and δ chains exhibit typical *fetal rearrangements* because there is essentially no N region diversity at VJ or VDJ junctions of rearranged γ and δ genes [525]. A remarkable difference between genes coding for αβ and γδ chains of TcR is a programmed regulation of their activation during ontogenesis [229], in that comprehensive rearrangements at γδ loci appear before those at αβ loci [525] in reciprocal independence [229]. *The γδ TcR is the first receptor expressed by thymocytes in utero* [279]: until day 17 of gestation, γδ cells are mainly TcR^+ subsets in murine

thymus [263]. Such TcRs appear to predominate compared to αβ TcRs in murine skin and gut epithelia [582], where γδ could play a leading role in *first-line* defense. The much earlier activation and conversion to memory of the γδ T cells is a significant differnce between the γδ and αβ T cell lineages, and illustrates the central role that γδ T cells have in adressing Ag challenge from birth onward [111], substituting for αβ cells that are scarce in mice [587], and protecting epithelia from damage caused by inflammations via selective suppression of IgE responses [279]. This effect is endorsed by normal levels of γδ cells [80]: reduction to only 500 cells [350] or suppression of γδ cells, as reported in atopic babies [495], could imply an increase in IgE levels [80, 350, 495]. As γδ TcRs develop normally in mice deficient in HLA class I and II molecules, these cells could be present in HLA defects. In addition, studies related to CDR3 of γ, δ, and α TcR chains and Ig H and L chains suggest that γδ cells may act as Igs in antigen recognition without a prior processing and presentation by professional APCs [466, 500].

In children with PIDs, studies reported CD3 γ, ε, or ζ deficiency, with TcR expression reduced on the cell membrane by about 50%–90% of T lymphocytes; ε defect is much more severe than γ defect as regards TcR expression or complete conformation, thus resulting in mature and immature T cells [263]. According to recent data, it does not seem necessary that parts of TcR αβ, γδε and ζ be present all grouped together in one complex to be expressed on the cell membrane as a functional unit: in the absence of functional γ chains, a healthy child shows that in vivo T cells may use fewer receptors than is expected (see "CD3γ Deficiency," Chap. 22). *ζ chain deficiency seems to be more severe* because of the high reduction of CD4, CD8 and TcR-αβ levels and absence of CD44 and CD25 [102].

The three CD3 chains, γ, δ, and ε, have homologous sequences of amino acids and their cytoplasmic domains comprise ITAM (immunoreceptor tyrosine-based activation motif) or ARAM, which contain tyrosine residues necessary to induce tyrosine phosphorylation in activated TcRs [3289]. TcR complex is not expressed by mature T cells and exists at their surface to carry out the whole set of T-cell functions, showing several similarities with Ig structures: it is formed by two instead of four polypeptide chains that are different from one another, linked by -S-S bonds, and consist of both V and C regions. The two polypeptide chains (similarly to Ig chains) are delineated by a C-terminal C region, the same for each TcR, and an N-terminal V region, different for each TcR. Such wide variability enables various TcR clones *to recognize a practically infinite number of invaders* [225]. On lymphocytes and in the bloodstream, there is a substantial number of TcR and BcR with different V regions; variability is acquired during somatic rearrangements between gene segments V, D, and J linked together in respective chromosomal regions. *Diversity* is generated by multiplicity of independently assorted components of V regions; however, TcRs have a greater tendency to use N-region diversity and frame shifts, whereas Igs prefer to use somatic hypermutation. TcR diversity is amplified by multiple V genes in germline, random combination, and junctional and insertional variability, thereby ensuring generation of a noteworthy structural diversity to deal with a practically unlimited universe of non-self antigens [36].

As a corollary of data discussed above, self/non-self discrimination by TcR is HLA-restricted, in that T cells and APCs must be complementary to HLA molecules: following this assumption, CTL $CD4^+$ recognize class II antigens as highly preponderant T $CD4^+$ cells, and CTL $CD8^+$ (the majority) recognize class I antigens. Therefore interactions between CD4 and CD8 and respective HLA class molecules increase efficiency of T-cell and APC interactions. CD4 and CD8, IgSF members, adhesion molecules, and signal transducers could act as receptors for nonpolymorphic HLA epitopes, contrary to TcR interacting with polymorphic epitopes [463].

B-Cell Antigen Receptors

The events following transduction of activation signals are better understood for T than for B cells. The immature pre-B cells (and pre-T cells) express preliminary versions of the antigen receptor. At this stage, the BcR comprises a pair of H chains, each with a V and a Cμ region identical to those found in the mature receptor, and a pair of surrogate L chains, termed Vpre-B and γ5. As the B cell develops, the surrogate L chains are replaced by conventional L chains, each with a V and a C region [115]. BcR consists of mIgs, more common proteins critical for antigen recognition, differing from antibodies only for an extra sequence of amino acids at C-terminal domains of H chains [214]. During embryonic and fetal life, mIgs of different isotypes alternate in B-cell populations; similarly, this occurs during clonal maturation in their adult life [3]. Igs of all classes can exist in either membrane-bound or secreted forms. Membrane forms combine with Ig L chains to make mIg; however, mIgs are retained in ER, unless associated with Igα and Igβ [414] . B cells comprise:

- *Specific mIgs:* IgM (5%–20%) and IgD (5%–10%) more frequently than IgG (1%–7%) and IgA (<5%) antibodies. These specific molecules facilitate B cell identification by immunofluorescence (IF) techniques, using antisera specific for each isotype.
- *Membrane receptors* for the Fc fragment of IgG (FcγR) [18], found also on non-B cells and monocytes (Table 1.3), possible false positivities due to IgG passive fixation on such receptors.
- *Receptors for complement* components C3d and C3b with rosette formation; receptors recognize complement when bound to either IgG or IgM antibodies (EAC rosettes).

- *CD21* is the receptor for C3d complement fragment and EBV (Epstein-Barr virus). This latter event allows microorganisms or their products to link to B cells, thus giving activating signals. The mature IgM molecule acts as the BcR for antigen, usually together with IgD BcR with the same antigen specificity. The V regions of the H and L chains each contain three CDRs. The CDRs make contact with the antigen [115]. When IgMs (with low affinity bond) play a BcR role, membrane signaling requires binding to CD45, equal to LCA (leukocyte common antigen), an important amplifier of BcR-mediated signals contained in intracytoplasmic tail domains with phosphatase activity (PTPase) [103], with several isoforms indifferently distributed between B and T cells [395].
- *Additional receptors* of mature B cells include IFN, IL_4 and IL_2. B cells present unrestricted surface antigens to cells of B lineage such as CD9, CD10 and CD23 (FcεRII).
- *B cells* have a high density of HLA class II molecules and express CD71 [96].

BcR consists of tetrameric H chains (IgH) with five isoforms: μ (73 kD), δ (67 kD), γ (50 kD), α (55 kD), and ε (70 kD), and L (IgL) with two isoforms: κ (26 kD) and λ (26 kD) [4]. mIgs of all types associate noncovalently with two heterodimers, each formed by a pair of polypeptidic chains, α and β, Igα (CD79a) and Igβ (CD79b), -S-S-linked to each other. In addition, two TM gps have an extracellular domain of Ig type (included in the IgSF), and an intracytoplasmic tail of 61 amino acids for α chains and of 48 for β chains in close contact with mIgM (mμ) [453]. Interestingly, a BcR without the T-cell CD3 complex has these proteins structurally resembling it [214]. More precisely, CD79a and CD79b cytoplasmic tails contain an amino acid motif with two tyrosine residues such as ITAM (see activation of lymphocytes), representing an important point of communication between BcRs and two types of PTKs: *src-family kinases and spleen tyrosine kinase syk* [453]. The nonreceptor PTK syk is widely expressed and has an important role in intracellular signal transduction in hemopoietic cells. It displays a leading role in BcR spectrum of activities, as demonstrated by BcR complete activation via PLCγ1 (phospholipase Cγ1) linking. In addition, syk controls signaling pathways between the two, whereas its deficiency blocks B-cell development at pre-B stages [446, 589]. A member of the src family is Bruton tyrosine kinase (btk) underlying XLA (Chap. 22). As an illustration of the SHP-1-dependent inhibition pathway, recruitment of SHP-1 to the B cell inhibitory receptor PIR-B attenuates BcR-triggered activation responses [446]. Extra BcR functional interactions are with B-cell-specific surface proteins CD19 and CD22 [453], two ligands, CD80 and CD86 (Table 1.2), also common to T cells, DCs, macrophages, etc. Their activation increases CD80 and CD86 levels [453]. CD80 expression peaks after several days, whereas CD86 expression peaks within 24 h of activation; thus higher levels are stimulated [242]. Two pairs of TM BcR-associated proteins, *BAP32/BAP37* and *BAP29/BAP31,* have been recently mapped, associated with IgM BcR and IgD BcR, respectively, in a class-specific fashion [453].

In conclusion, there are evident analogies in gene organization and structure of both receptors that suggest a common evolution from ancestral genes. All genes belong to IgSF (Table 1.4).

Immunogens, Antigens and Allergens

Definitions. Briefly, immunogens, antigens and allergens [1, 185, 428] can be defined as:

- *Immunogen:* any antigen that in a particular host can elicit immune responses and react with the relative products
- *Immunogenicity:* the capacity of provoking an immune response (B- or T-mediated)
- *Antigen:* anti(body) gen(erator), any substance recognized by TcRs or antibodies, that can trigger immune responses
- *Antibody:* selected Ig molecule containing a specific sequence of amino acids and binding specifically to antigens, inducing its synthesis
- *Antigenicity:* an epitope capacity to be recognized by specific receptors of the immune system
- *Allergen:* aller(gy) gen(erator), any foreign substance able to activate IgE synthesis
- *Allergenicity:* the ability of an allergen to elicit an IgE-mediated reaction in sensitized patients
- *Sensitization:* natural or artificial induction of an immune response, notably when it causes allergy in the host. In subsequent contacts with the same immunogen there is a quicker onset and a more severe immune response [185]
- *Panallergen:* molecule with properties shared by different species, for example, a protein with a conserved IgE-binding epitopes across species that cross-react with foods, plants, and pollen
- *Epitope:* antigenic determinant; an *allergenic epitope* marks a specific peptide domain associated with allergenic potential
- *Paratope:* antibody-combining site for epitope

Strictly speaking, all immunogens are also antigens, but not all antigens are naturally immunogens: the immune system may recognize an antigen, although it does not respond to an antigen unless it is also an immunogen. However, the terms "antigen" and "immunogen" are often employed interchangeably [185].

Immunogenicity *of a given protein* depends on epitopes present on its molecule triggering sIgE synthesis (specific IgE antibodies), thus in turn triggering allergic reactions. It also relies on the existence on the protein surface of hydrophil amino acids such as lysine, arginine, and aspartic and glutamic acids. That a non-immunogen molecule could become the target of immune responses if attached to an immunogen protein is evident with compounds with low MW, called *haptens*

(from the Greek "απτειν," to fasten) or ligands, small molecules with a restricted number of identical epitopes. Haptens are therefore antigenic but not immunogenic, and can combine with only one type of antibody, but are unable by themselves to elicit immune responses, owing to their low MW. To become an immunogen, the free hapten must bind a *carrier,* either serum proteins or epidermal proteins: for a single hapten more than one carrier may exist [185]. Anti-hapten antibody responses require cooperation between subsets of B and T cells first recognizing the hapten and second the carrier. Current examples are drugs, metal contaminants such as Ni, Cr, Cu and β-lactamines, natural constituents of vegetable origin, including balsams, fucomarins, lactones and terpenes, to be found in fruits, vegetables and aromatic plants [18].

Antigens are soluble or corpuscular substances, mostly made up of proteins, each formed by one, two or more chains composed of different combinations of about 20 amino acids linked to one another. Every chain is rolled and folded; hence when it is extended, parts distant from each other come into contact when protein structures fold [12]. The term "antigen specificity" refers to antigen binding only to antibodies thereof activated. This specificity has a crucial value in the immune system: notwithstanding complexity of molecular structure, any given antibody including IgE will recognize and bind not to the whole molecule, but to the epitope, a limited portion of such a molecule formed by a few amino acids arranged in sequence or close by because of chain folding. Antigen–antibody reactions always denote a primary, dynamic fixation, based on noncovalent forces; hence they are relatively low and dependent on steric complementarity between epitopes and antibody. Epitopes often behave as haptens [18].

Epitopes and Paratopes

The *epitope* is a molecular structure with a diameter of 2–3 nm representing *the antigen part electively recognized by* a given antibody (that is *the paratope*) or TcR, thereby determining immune reaction specificity. Chemically, it is composed of five to seven amino acids active in molecules of globular proteins and glucide units of lateral chains of polysaccharides (PS). A part of the epitope reacts with Fab of L chain, the other with antibody H chain, being the fundamental part of a protein molecule recognized by binding either to T cells or Fab fragments [1]. Another molecule, the *agretope,* also formed by amino acid residues interspersed in primary sequences, binds to both TcR and paratope [11] and to a HLA-DR molecule of new synthesis localized in a hypervariable β1 region, the *desetope* (Fig. 1.15) [577]. Each TcR chain supplies three CDRs [in particular CDR3 with (D) J sequences as Igs interact with epitopes] contributing to the antibody combining site, the paratope, three-dimensional space defined by the folding of new

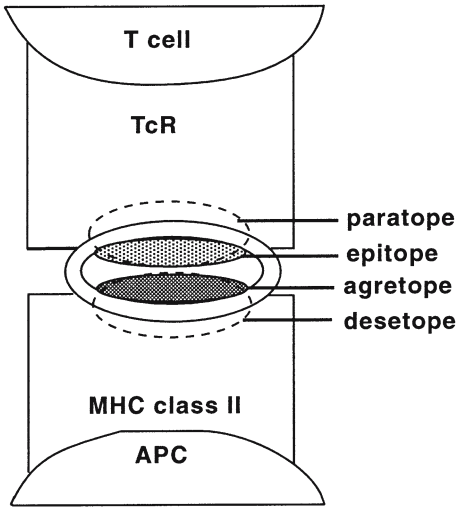


Fig. 1.15. Interactions between T cell, TcR and APC before antibody production. The epitope of the immunogenic peptide binds to TcR (and the paratope region), while the agretope binds to the Ia antigens of APC. The desetope is the part of HLA molecule to which the agretope binds. *APC* antigen-presenting cell, *TcR* T cell receptor

polypeptide chains. H chain regions corresponding with V_H and C_H1 domains and whole L chains forming Fab (Fig. 1.6) are closely located to form paratope walls. In conclusion, one H and one L chain together form a paratope, therefore a single four-chain unit is bivalent, since it contains two combining sites [469]. The paratope is positioned in the NH_2-terminal Fab region, whose shape changes to recognize and ensure a close fit between antibodies and complementary epitopes [459]. A *paratope* is a molecule portion determining antibody specificity, making contact with an antigen-related part, a binding pocket allowing peptides to be accommodated, thus suggesting the picture of a *key in a lock* [1, 470] (Fig. 1.16). Paratope specificity depends on amino acid sequences of hypervariable loops, an effect of minute variations of genes encoding for this antibody portion. Analyses of antibody terminal parts have demonstrated a three-dimensional flexibility of the paratope calling antigens for binding, with spatial conformational changes made on peptide binding, absent in unliganded Fabs. As Fig.1.17 illustrates, in liganded Fabs a prominent groove connected to a deep pocket is formed to fit peptides, adding a prominent channel to encompass extended portions of bound peptides [459]. Affinity between these two structures depends on the strength of attraction and repulsion existing between them. Mutation of an amino acid residue forming part of an epitope or paratope can greatly increase or weaken such affinity [459]. Structurally, there is also flexibility in epitopes, permitting antigens to interact with antibodies, thus widening the likelihood of antigen–antibody encounters of sufficient affinity to generate immune responses [459]. The immune system utilizes such properties to

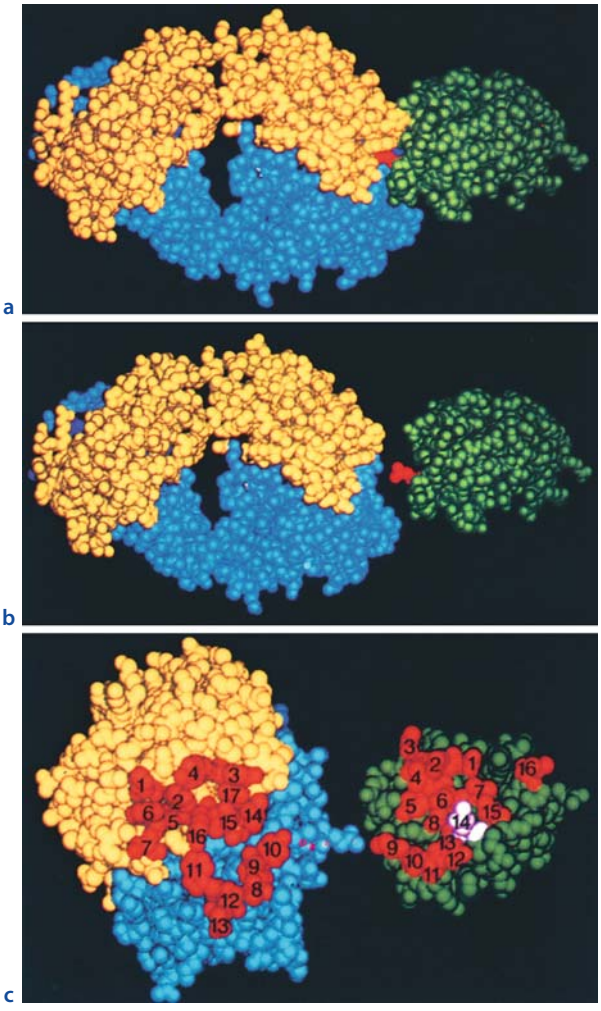


Fig. 1.16 a–c. Structure of the antigen-antibody interface. **a** Space-filling model showing Fab and lysozyme molecules fitting closely together. Their interactions form an antigen–antibody complex. The antibody H chain is shown in *blue*, the L chain in *yellow*, lysozyme in *green*, and glutamine 121 in *red*. **b** The Fab and lysozyme models have been pulled apart to show that protuberances and depressions of each are complementary to each other. **c** End-on views of the paratope (*left*) and lysozyme epitope recognized by antibody (*right*), formed from **b**, by rotating each molecule approximately 90° around a vertical axis. Contact residues on both antigen and antibody are shown in *red*, except for glutamine 121 in *light purple*

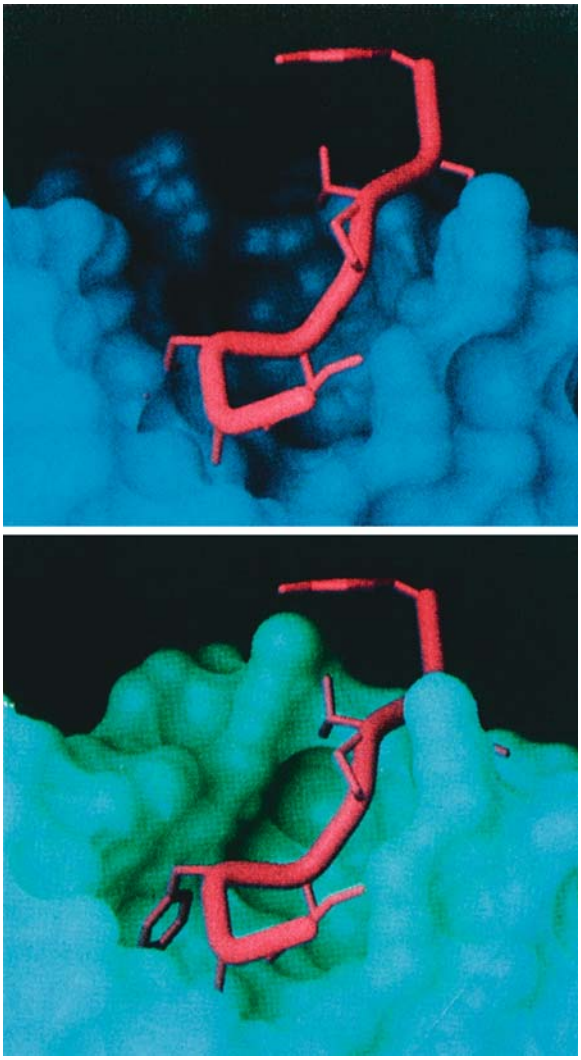


Fig. 1.17. Shape of the binding pockets of the unliganded (*above*) and liganded Fab (*below*). The unliganded Fab appears as an open, basin-shaped pocket, and in the liganded Fab a prominent groove connected to a deep pocket is formed to fit the peptide antigen

amplify antibody affinity during immune responses (affinity maturation and antigen selection). However, microorganisms may use such changes to evade host immune recognition by a variety of strategies, including shifts in critical surface antigens, as is the case of HIV, of influenza virus, etc. [337].

Epitopes are classified into *B- or T-reactive epitopes*. *B-epitopes* are recognized by B cells and binding sites of specific antibodies, whereas T-epitopes correspond to small peptide fragments (8–12 amino acids) recognized by TcR in the context of HLA molecules on antigen linear surfaces [368]. Several B- and T-epitopes have been characterized, but antigen-specific T cells appear to be distinct from those individuated by IgE antibodies [672]. The total number of separate B or T epitopes on antigen molecules, identical or different, is called *valence*, overall proportional to foreignness, molecular size and chemical complexity, which represent the factors influencing immunogenicity of a given antigen [185]. We shall see that an immune response also depends on the dose and mode by which foreign invaders enter the body. As a rule, macromolecules are the strongest immunogens, usually having more epitopes; however, in a given subject and under the influx of particular circumstances, only one or a few are recognized by BcRs and/or TcRs, since they are targets of immune responses. Macromolecules with one type of epitope are

named monospecific, but their greatest part has many independent epitopes with different specificities. Some large proteins have been found to contain as many as 50 separate T-cell epitopes. However, even small molecules can have bivalent structures: for example, the human hormone glucagon, which is only 29 amino acids long, contains separate B- and T-cell epitopes [185]. In addition to the events described, T-cell epitopes show the exquisite specificity required to activate CD4 T cells, which in turn are needed for B-cell responses against nearly all antigens. The first requirement for a molecule to be an immunogen is to contain at least one T-cell epitope: consequently, molecules comprising only B-cell epitopes (such as haptens or amino-terminal parts of glucagon) may serve as targets for antibody responses, being unable to elicit such responses autonomously [185]. Even if, in theory, T-cell epitopes do not bind to IgE antibodies, small peptides may do so and subsequently block (as monovalent haptens) or stimulate basophils [496]. These observations are of substantial importance, since identification of IgE-binding B-cell epitopes may:

1. Increase both specificity and sensitivity of diagnostic tests by including molecules with epitopes of this type
2. Distinguish B-cell from T-cell epitopes
3. Elucidate trigger mechanisms of IgE-sensitized metachromatic cells [496]

The epitopes of a protein are of two types [12, 185]:

- *Sequential,* or linear, or segmental epitopes (usually T-cell epitopes), determined by primary structures, wholly positioned sequentially in linear sequences of a protein or PS antigen, a segment of amino acid sequences: immunogenicity is the result of covalent linkage of these residues to one another, which cannot move significantly apart. By definition, such epitopes are more resistant and generally remain unchanged following heat or enzyme denaturation and may be left untouched by enzymes not specific for amino acid bonds present within epitopes.
- *Conformational,* or discontinuous epitopes (usually B-cell epitopes), are far apart in primary sequences and critical residues are brought close together via folding of antigen chains in normal three-dimensional steric configurations, so that they may encompass residues widely distant from one another along protein sequences, positioned also nearby, but juxtaposed in tertiary structures. Immunogenicity is due to molecular configuration. Normally, such epitopes are scarcely resistant; hence they are lost when antigens are denatured and fail to refold appropriately [13, 414] (Fig. 1.18).

Consequently, since B epitopes are mostly conformational, they can be more easily eliminated, while sequential T epitopes are more resistant. Epitopes of this type have acquired relevance for their use in specific immunotherapy (SIT) and preparation of protein hydrolysates. *Spatial conformation* plays a key role, since *accessibility* of epitopes is a prerequisite for binding to immunocompetent cells and for immunogenicity. In the case of atopy, IgE antibodies recognize one specific B-cell epitope in peptide tertiary structures, whose three-dimensional conformation can mask additional epitopes that will not bind to antibodies. On the contrary, T lymphocytes recognize sequential epitopes with a few amino acids located on antigen linear sequences [368]. T- and B-cell epitopes are distinct by their topographical position being located at different points, with T-cell epitopes positioned in primary structures, whereas B-cell epitopes lie in secondary and tertiary structures. Due to such differences, following heat denaturation, conformational shifts may eliminate certain epitopes on a protein, not affecting the integrity of sequential ones, and enzyme hydrolysis may alter both types. Physicochemical manipulations therefore do not alter all conformational epitopes, yet they may unmask epitopes hidden within a native protein three-dimensional structure, which may become immunogenic and accessible to IgE antibodies for binding while causing new ones to form [1, 12]. Actually, polypeptides without flanking amino acids can originate from hydrolysis, apt to combine with antibodies [591]. Instead, hydrolytic reduction of -S-S bonds of β-lactoglobulin (βLG) does not influence reactivity with IgE antibodies of native molecules or of fragments of tryptic hydrolysis, but reduces IgG reactivity [351]. These studies have revealed the structural bases of βLG antigenicity and immunogenicity: βLG immunogen epitopes are comprised in a relatively restricted part of the molecule, while those reactive with IgG appear to be correlated with conformational sites [351]. However, the distinction between immunogen and antigen epitopes is as yet scarcely known [402]. Individual residues within single epitopes, due to characteristics of either antigens or those specific to patients, are called *immunodominant,* thus are recognized

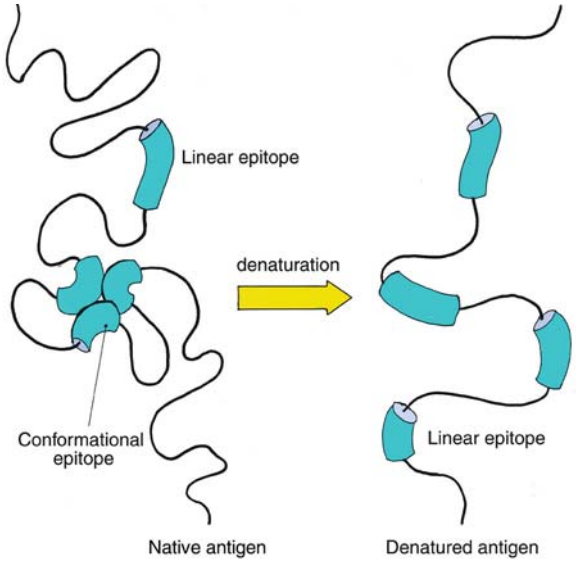


Fig. 1.18. Conformational and linear epitopes

Table 1.13. Main properties of human immunoglobulins (*Ig*)

Ig class	Heavy chains	Light chains	MW (kD)	Serum level (mg/dl)	Presence in secretions	Serum half-life (days)
IgA	α	κ/λ	160 (sIgA 390)	200±50	+	6
IgD	δ	κ/λ	175	3–4	–	3
IgE	ε	κ/λ	190	0.03	±	2
IgG	γ	κ/λ	150	1,250±300	–	23
IgM	μ	κ/λ	900	150±50	±	5

by most T or B lymphocytes induced by whole proteins. However, certain immunodominant, linear epitopes are represented by peptides with four to nine amino acids [511]. A single antibody may also react with an antigen other than the one causing its formation, as well with *different epitopes on the same molecule or carried by different molecules*: this is called cross-reactivity. Consequently, *allergenicity does not depend on the size of a given protein, but on the number of immunoreactive epitopes, similar or not* [185]. In practice, the same molecule can bind several different epitopes, either two combining with and cross-linking the same IgE molecule, or six, eight, or even ten nonidentical epitopes; whereas sIgE can bind more strongly to an allergen different from the one that stimulated its synthesis, but with characteristics similar to the first one [1].

In this context, children sensitive to Der p (*Dermatophagoides pteronyssinus*), for example, may produce sIgE binding to allergens of a species they have never met in the environment. Accordingly, following exposure to only one species of mites, children recognizing these common epitopes may produce antibodies that will recognize and respond to other species with this epitope, even if such species are absent in the environment where children live [671]. Additional studies support that different proteins may have common epitopes, thereby explaining cross-reactivities between different proteins within a given food as well as between different related foods, or between pollens, vegetables and fruits [1] (see p. 164). Likewise, if there are epitopes common to different substances with the same amino group in *p* (para) position on the benzene ring, patients sensitive to a drug with such a group should prudentially avoid other substances within the same chemical group. In conclusion, *epitopes identical or nearly identical can be found on antigen molecules of different origin or species*: an epitope common to two or more allergens triggers cross-reactions between substances with a similar structure, or between metabolites alike from an immunochemical point of view. As a consequence all molecules containing an epitope provoke allergic reactions in patients sensitive to another, even in the absence of a previous exposure and/or sensitization.

Antibodies

Immune responses are characterized by Ig production (Fig. 1.6): there are nine different isotypes correlated with nine C_H genes and two C_L genes: IgA_1, IgA_2, IgD, IgE, IgG_1, IgG_2, IgG_3, IgG_4, IgM, belonging to five major classes [3]. All Igs are glycoproteins and contain 3% to 13% of carbohydrates, depending on the class of antibody. The carbohydrate is essential in maintaining the Ig structure [115]. Tables 1.13 and 1.14 [544] summarize their main characteristics, including MW and half-life, and Table 1.15 [545] presents Ig levels at various ages compared with adult values; other authors prefer geometric means (GM). Isotype switching has an indubitable final significance, with every isotype having specialized biological properties, as follows:

- *IgG* comprise 75%–80% of serum Igs, being the only class crossing the placenta from mother to fetus, where it is found and is responsible for neonate protection during the first months of life. The levels decrease progressively, leading to a transient hypogammaglobulinemia at approximately 4–6 months of age, while normal adult values are attained only at about 4–6 years. Molecules are further divided into four subclasses: IgG_1 (70%), IgG_2 (20%), IgG_3 (8%), IgG_4 (5% of total). Besides the Hγ chain, subclasses have different properties, regarding for example, binding to serum complement and activating its alternative pathway, and adhesion to macrophages. IgG_2 has a lower placental passage, and IgG_4 is the only IgG unable to fix complement by the classic pathway, in addition to having a poor affinity for phagocytes. Abnormal levels of one or more IgG subclasses have been reported in children with severe chronic asthma, or recurrent respiratory infections (RRI) whether associated with asthma or not, of allergic type or not, with selective IgA deficiency (IgASD) or other PIDs, but not in otherwise healthy subjects (Chap. 22). Therefore, IgG antibodies represent the more differentiated phase of antibody responses with variations of subclasses according to encountered pathogens (or persistence of antigen stimuli). IgG is also an opsonizing antibody, although with lesser potency than IgM, reacting with epitopes on microorganisms via its Fab portions. However, the Fc portion for which many phagocytes bear

Table 1.14. Main biological properties of human Ig classes and IgG subclasses

	IgG_1	IgG_2	IgG_3	IgG_4	IgM	IgA	sIgA	IgE
Placental transport	+	+	+	+	–	–	–	–
Complement activation								
Via classic pathway	++	+	+++	–	+++	–	–	–
Via alternate pathway	–	–	–	–	–	+	?	?
Presence in secretions	–	–	–	–	–	–	++	+
Agglutination	+	+	+	+	++	–	–	–
Opsonization	+	+	+	+	++	–	–	–
Virus neutralization	+	+	+	+	+	–	+	–
Hemolysis	+	+	+	+	++	–	–	–
Bacterial lysis	–	–	–	–	+	–	–	–
Degranulation of MC	–	–	–	?	–	–	–	+++
Fixation to macrophages	+	–	+	–	–	–	–	–

Data from [544].
MC metachromatic cells.

Table 1.15. Levels of IgG, IgM, IgA and total immunoglobulins (mean±1 SD) in sera of normal subjects by age (mg/dl)

Age	IgG	% of Adult level	IgM	% of Adult level	IgA	% of Adult level	Total Ig
Newborn	1,031±200 (645–1,244)	89±17	11±5 (5–30)	11±5	2±3 (0–11)	1±2	1,044±201 (660–1,439)
1–3 Months	430±119 (272–762)	37±10	30±11 (16–67)	30±11	21±13 (6–56)	11±7	481±127 (324–699)
4–6 Months	427±186 (206–1,125)	37±16	43±17 (10–83)	43±17	28±18 (8–93)	14±9	498±204 (228–1,232)
7–12 Months	661±219 (279–1,533)	58±19	54±23 (22–147)	55±23	37±18 (16–98)	19±9	752±242 (327–1,287)
13–24 Months	762±209 (258–1,393)	66±18	58±23 (14–144)	59±23	50±24 (19–119)	25±12	870±258 (398–1,586)
25–36 Months	892±183 (419–1,274)	77±16	61±19 (28–113)	62±19	71±37 (19–235)	36±19	1,024±205 (499–1,418)
3–5 Years	929±228 (569–1,597)	80±20	56±18 (22–100)	57±18	93±27 (55–152)	47±14	1,078±245 (730–1,771)
6–8 Years	923±256 (559–1,492)	80±22	65±25 (27–118)	66±25	124±45 (54–221)	62±23	1,112±293 (640–1,725)
9–11 Years	1,124±235 (779–1,456)	97±20	79±33 (35–132)	80±33	131±60 (12–208)	66±30	1,334±254 (966–1,639)
12–16 Years	946±124 (726–1,085)	82±11	59±20 (35–72)	60±20	148±63 (70–229)	74±32	1,153±169 (833–1,284)
Adults	1,158±305 (569–1,919)		99±27 (47–147)		200±61 (61–330)		1,457±353 (730–2,365)

Levels of total serum IgE are in given in Chap. 6.
Modified from [545].

receptors delivers opsonizing properties; similarly it plays a critical role in ADCC, focusing NK cells on their targets [36]. To sIgGs (specific IgGs) and STS-IgGs (short-term sensitizing IgGs) has been credited a sensitizing activity mostly belonging to IgG_4, that turned out to mediate *similar-reaginic reactions but not of the IgE*

type, competing with IgE antibodies to bind to allergens [514]. In allergen molecules, IgE antibody binds to sIgE epitopes, while IgG antibody can bind to IgG-specific epitopes localized in the same point of the molecule or elsewhere: namely, IgG may react with the same epitope as IgE antibodies or the two Igs may react with two quite different epitopes on the same molecule [401]. IgGs are erroneously called *blocking antibodies* since they inhibit effects of IgE antibodies in the Prausnitz-Küstner test [1]: IgGs probably act as such, without interfering with sIgE epitopes to provoke an in vivo synthesis of IgE antibodies to a given allergen. However, not being reagins, IgG antibodies cause diagnostic perplexities due either to negativity of allergic tests in individuals with typical symptoms of immediate hypersensitivity or to a paradoxical situation of the same antibody acting in pathogenic and protective ways [1], an issue to be dismissed among the hypotheses put forward [13]. In summary, no direct challenge can reproduce clinical disease or tissue reactivity ascribed solely to specific IgG_4 antibodies to challenged allergens [13]. From a clinical point of view, in FA diagnosis measurement of IgG antibodies is superfluous because healthy subjects following a prolonged allergen exposure also have frequent elevations of serum IgG antibodies [13], while in respiratory allergy such levels are identical in patients whether they be atopic or not (Chap. 11). Characteristically, IgGs increase during SIT, although correlations between their rise and clinical results are unstable (Chap. 13). A high amount of IgG_4 was found in complexed IgG anti-IgE antibodies, recognizing at least two epitopes located within Cε2-Cε3 and Cε4 domains. IgG antibodies binding FcεRI located within interdomain regions might potentially cross-link IgE bound to FcεRI and blocking it, since only one of two ε sequences binds to FcεRI [514]. That an effector function of such weight is recognized by anti-IgE allows us to conclude with good reliability that anti-IgE antibodies play a modulator role, so essential during activation of metachromatic cells as to stimulate basophils to release histamine, a fact related not to IgG antibody levels or to subclasses, but to epitope specificity [513]. Administered intravenously (IV), Ig are highly beneficial to children with PIDs and several other affections.

- *IgA* antibodies prominent in the MALT are the sole antibodies in secretions (aside from a small percentage of IgM), hence it is called sIgA. IgA occurs as monomers, dimers, trimers and polymers. IgA monomers have no agglutinating properties (unlike sIgA) but can bind to antigens, predominant in serum, while dimeric IgA is quantitatively the most abundant in secretions. Two dimers are held together by the same J chain and associated with a polypeptide, called *secretory component* (SC), localized on the long arm of chromosome 1. SC is a fragment of epithelial cell poly Ig receptors and a component of high significance because it protects mucosal sIgA from proteolytic degradation, literally wrapping round $C_\alpha 2$ domains of both sIgA subunities, even if -S-S bonds are for only one subunit [186]. The two subclasses, IgA_1 and IgA_2, represent 12% and 3% of total serum Igs, respectively. sIgA is actively transported across epithelial cells, *covering as a film* and protecting mucosal surfaces from microorganisms, preventing organism attachment to cells and clearing them off by phagocytosis. IgA can activate complement via the alternative pathway, not inducing bacterial lysis mediated by the classic pathway, and possess bactericidal activity against Gram– organisms, but only when lysozyme is present, interestingly, in the same secretions containing sIgA [36]. Table 1.15 shows IgA levels slowly ascending, and sIgA levels quickly produced. IgA antibodies present in colostrum and breast milk protect at-risk infants (Chaps. 2 and 24). IgASD is the most frequent PID.
- *IgE* (in which the E is erythema), also termed reagins with a half-life of 2–4 weeks on metachromatic cell membranes, are normally present in serum at the lowest concentration, <0.001%, of total circulating Igs. For this reason, IgE concentration is expressed in international units (IU), one IU being equal to 2.4 ng/ml. IgE levels increase considerably in atopic diseases, hyper-IgE syndrome (HIgES), infections with parasites [471], pediatric AIDS, and other diseases. High levels saturate mast cell receptors and pass into the circulation, where detectable levels are extremely elevated compared to normal subjects [471]. IgE antibodies come from plasma cells distributed primarily in lymphoid tissues of respiratory and gastrointestinal tracts, derived from pre-B cells from a differential lineage originating from IgM B lymphocytes and via the following passages: $B_{IgM-IgD}$ – $B_{IgM-IgD-IgE}$ become IgE B lymphocytes. Like other Igs, IgE has four polypeptidic chains, but two chief immunobiological characteristics: an ε chain 11 kD greater than homologous chains, with five domains, one V and four C, one more than other Igs, and is cytophilic, having a propensity to bind to metachromatic cells with Fc fragment, of which FcεRI is a receptor (Table 1.3). FcεRI extends from the C-terminal part of Cε2 to the N-terminal sequence of Cε3 domains, while the FcεRII binding site, the second receptor, is localized in the N-terminal part of the Cε3 domain close to FcεRI [553]. Since IgE molecules have a bent form, their convex surface nearest the membrane (Cε3) binds to FcεRI, thereby *making remote a binding site for another receptor* (Fig. 1.19, showing also FcεRII). This explains why FcεRI has only an α chain, its affinity for only an ε chain, and why there is only one receptor [553]. IgE molecules have no subclasses, similarly to IgM and IgD molecules [186].
- *IgM* is present early in primary immune responses to most antigens. Small IgM amounts in secretions (sIgM) also contain SCs. Being a pentamer, covalently linked by -S-S bonds with J chain, it *does not pass through the placenta*. Moreover, the IgM molecule is the most efficient agglutinating and complement-fixing Ig (by classic pathway). Because of their pentameric form, IgM antibodies can form macromolecular bridges between epitopes that may be too distant from each other to be

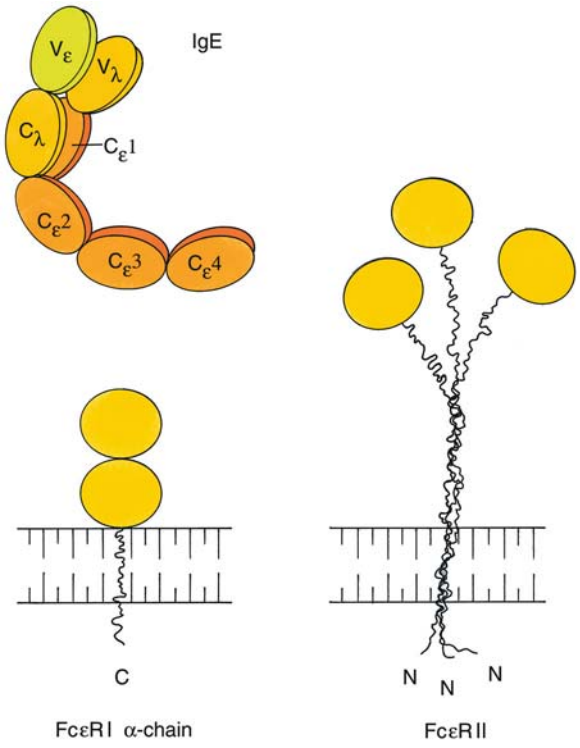


Fig. 1.19. IgE and its receptors. FcεRI α-chain. Schematic representation of IgE and its receptors FcεRI and FcεRII drawn to the same scale. IgE is illustrated as a drawn molecule, whereas the FcεRI α-chain is drawn erect. The other chains have been omitted to simplify the diagram

bridged by monomeric IgA antibodies. Also, because of their multiple valences, they are best suited to combine with antigens containing more epitopes, as related to PS or cellular antigens [36]. Together with IgD, IgM is the most common Ig expressed on B cell surfaces (and virgin B cells). IgM is the first Ig class synthesized by both the fetus and newborn: high fetal IgM levels are indicative of congenital or perinatal infections [186]. IgM is the isotype synthesized in noticeable amounts by children and adults as a primary antibody response after immunization or exposure to T-dependent and T-independent antigens; therefore a role of IgM as regulator of immune responses via a specific receptor of Th lymphocytes has been postulated. IgM antibodies are significantly increased in IgASD and particularly in HIgMS.

- *IgD* is present in serum in very small amounts. Its presence on B-cell membranes during certain stages of development may suggest either an involvement in B cell maturation or a prominent role in immune tolerance. However, IgD functions are mainly unknown 186].

Idiotypes and Anti-idiotypes

V regions of antibodies may have two different functions:

- *Recognizing, with the paratope,* one or more epitopes present on an antigen or on different antigens
- *Acting as an antigen* with an idiotype leading to anti-idiotype antibodies [18]

Such antigen functions are committed to idiotypes, present in V_H and V_L regions of antibody molecules.

Definitions

- *Public or cross-reacting idiotypes,* expressed by antibodies produced by all individuals of the same race, are directed against a single epitope or against nonidentical antigens. V gene mutations altering paratopes without modifying idiotypes determine the sharing of a public idiotype from antibodies with different specificity.
- *Private or individual idiotypes,* present in a single clone, expressed irregularly by subjects of the same species, therefore exclusive of a given individual, directed against a single epitope.
- *Anti-idiotypic antibodies* are complementary antibodies, directed against the structure of an antigen-recognizing antibody, which can prospectively trigger production of a network of anti-anti-idiotypic antibodies and so on.
- The *internal image of the epitope* is a structurally identical idiotype cross-reactive with epitopes of foreign antigens.
- *Regulatory idiotype,* present on a relatively high number of Ig molecules and/or T lymphocytes, where it can function as a base of a regulatory receptor-specific system.

In other words, a V region of an antibody contributing to paratope expression and antigen recognition has idiotypes against which the organism reacts, instinctively inducing anti-idiotype antibodies against sIgE; anti-idiotype antibodies are normally formed during immune responses. In some instances, a fraction of an anti-idiotype may exemplify a facsimile (or an internal image) of the nominal epitope that triggered the original reaction [47]. Figure 1.20 summarizes a wide panorama of idiotype activities.

In Network Theory, the Nobel laureate Niels Jerne [238] proposed a network of idiotype–anti-idiotype interactions as a regulatory mechanism of immune responses, the expression of each idiotype being suppressed by complementary anti-idiotypes. The foreign antigen approach, disturbing pre-existing homeostasis sustained by an equilibrium between idiotype and anti-idiotype, generates an immune response by T and B cells, activating *anti-idiotype responses* [238], either humoral (antibodies against idiotypes of soluble Igs) or CMI (T cells with TcR specific for hypervariable regions of both TcR and BcR) [474] (Fig. 1.20). Some anti-idiotype antibodies may competitively block binding to paratopes of corresponding antigens, interacting with epitopes or directly with paratopes (*associated idiotypes*), thus eliciting an antigen–antibody reaction wholly similar to classic reactions induced by antigens [454]. Other anti-idiotypes, without such selective inhibition,

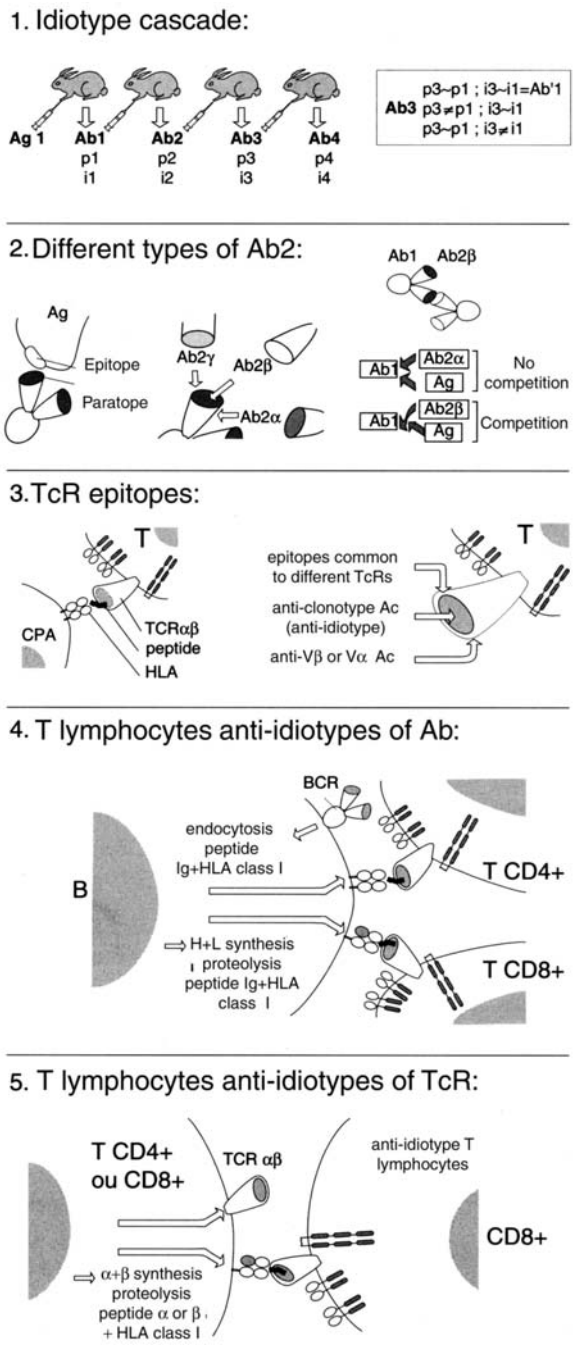


Fig. 1.20. *1*: Idiotype cascade. It was observed that antibodies with different specificities can share the same idiotypes. *Ag* antigen, *Ab* antibody. *2*: Different types of Ab2α. Ab2βs, internal images of Ag, bind to Ab1 at a site corresponding to the paratope, thus inhibiting Ab1-Ag binding. *3*: TcR epitopes. The anti-clonotype Abs are directed against the hypervariable regions of Vα and Vβ domains forming the TcR binding-site: anti-Vα or Vβ Abs recognize epitopes defined by sequences common to members of the same family, while other Abs recognize epitopes common to TcRs with different specificity. *4*: T lymphocytes anti-idiotypes of Ab. *5*: T lymphocytes anti-idiotypes of TcR

may instead bind to amino acid sequences of the paratope FR region, which do not contribute to antigen binding (*not associated idiotypes*) [454]. As it was demonstrated, anti-idiotype antibodies can bind directly to Ig idiotype determinants, regulating their expression [58]. Also for T cells there is evidence of T-cell idiotypes (clonotype markers) and of interactions between B-cell and T-cell idiotypes [58]. In addition, anti-idiotype antibodies specific for TcR clonotype markers have been observed, in turn interrupting the network of anti-idiotype antibodies produced in certain diseases during a normal immune response: since T cells can have varying phenotypes, a potential exists for augmenting or suppressing immune responses via idiotype networks involving T cells [58]. For example, T-cell idiotypes may be recognized by B cells (antibodies) or by peptides associated with T cells (TcR) presented by HLA class I or II molecules. Similarly, T cells can recognize B idiotypes presented as HLA-associated peptides [236] (Fig. 1.20). Additionally, although antibody responses to target antigens are characterized by formation of several hundred different antigen–antibody molecules, idiotype–anti-idiotype reactions are much more restricted depending on common amino acid sequences, in close contact with paratopes of different antibodies. According to these studies, cross-reactivity is frequently observed among various antibodies in anti-idiotype responses to target antigens. In other words, if paratopes able to recognize a given idiotype exist, classic epitopes should present it together with idiotypes already existing in the molecule [236].

Anti-idiotypes can regulate sIgE responses but cannot cause antibody responses; however, miming functionally original epitopes, the so-called idiotypes *internal image* of antigen *or network antigens* (in the sense given by Kohler) [268] can behave as *surrogate* antigens, or as epitopes in terms of structural affinity, like antigen molecules, thus increasing antibody responses. Figure 1.21 [58] shows another of Jerne's theories, in which idiotypes can mimic structures of apparently unrelated antigen molecules, binding to receptors specific for that antigen and inhibiting immune responses or, on the contrary, up-regulating them by replacing some antigen functions. The paratope of antibody 1 is complementary to a structure on the immunizing antigen; in turn the paratope of antibody 2 is complementary to that of antibody 1, and thus antibody 2 can resemble a structure on the immunizing antigen. In the case of insulin and of anti-insulin antibodies (Fig. 1.21), the paratope of antibody 1 is complementary to the epitope, antibody 2 is an anti-idiotype vs anti-insulin antibodies (antibody 1), and can bind to the insulin receptor and even stimulate glycolysis [58].

The circuit thus far described may have envisioned an idiotype network. Examining part A1 of the figure, we consider two B cells, B1 and B2, whose complementary surface Igs form an idiotype–anti-idiotype pair. Thereby, B1 cell binds to an Ig V region on the B2 cell surface,

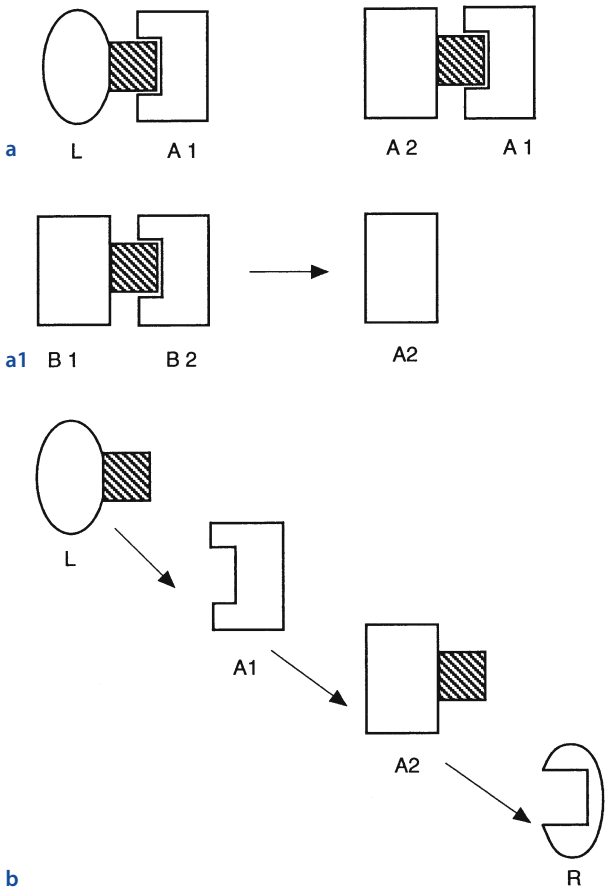


Fig. 1.21. **a** Diagram of the complementary relation between antibody 1 (*A1*, idiotype) and antibody 2 (anti-idiotype). **b** Because of the complementarity with the ligand (*L*), *A2* can bind to the receptor (*R*) for L. (Modified from [58])

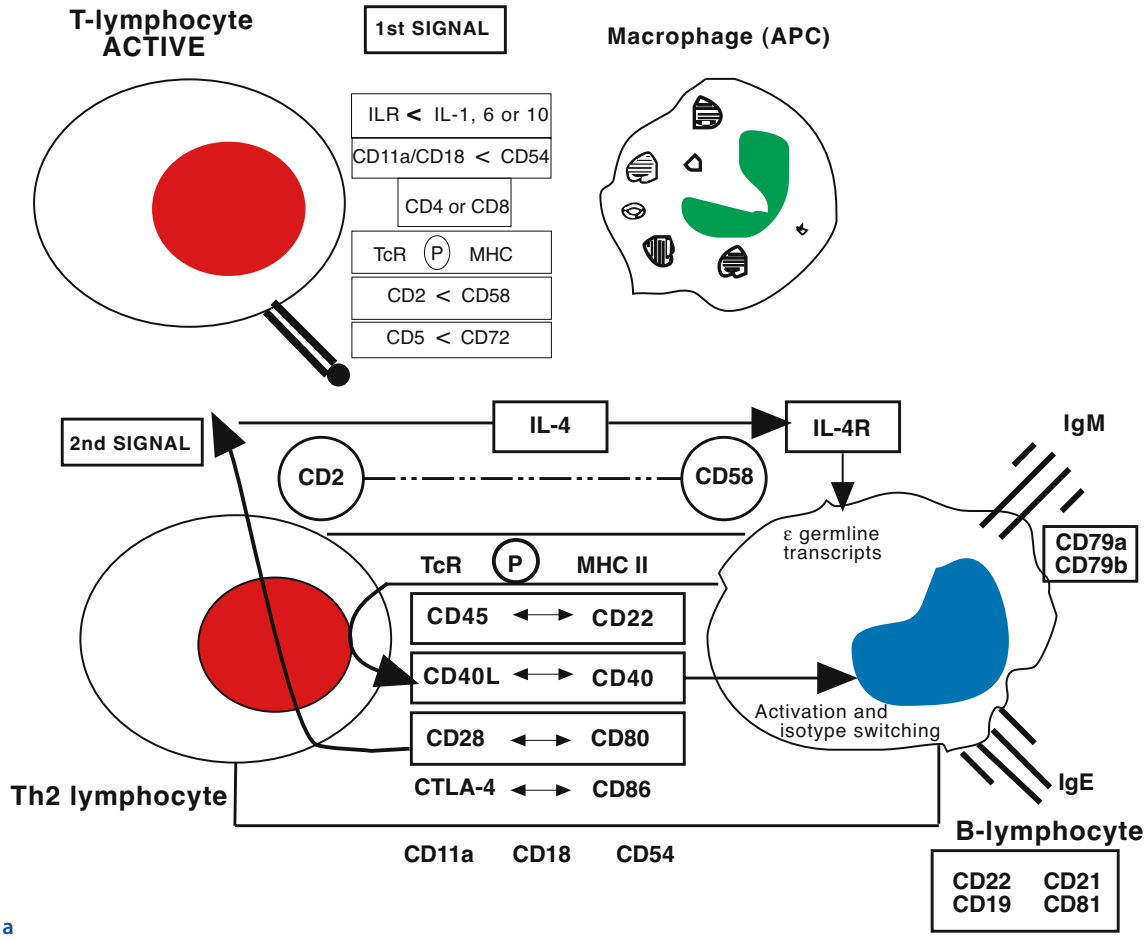
stimulating the cell to secrete antibody 2: in this way an idiotype secreted during immune response to a given antigen can yield a corresponding anti-idiotype. In several idiotype–anti-idiotype systems, anti-idiotypes can act as anti-receptor antibodies. Among hormones, besides insulin, we find thyroid-stimulating hormone, prolactin, glucagon, etc.; among neurotransmitters are found acetylcholine, catecholamines, endorphins, reovirus, etc. In all these instances, the V region of antibody 2 represents the internal image of the external antigen. It may seem odd that an antibody is disguised as an antigen, but among 10^8 three-dimensional V regions there must be a kind of mimicry of other molecules [58].

Interesting applications of these studies are observed in laboratory animals: the basic principle is that in different species immune responses elicit formation of antibodies expressing a common idiotype. For example, oral immunization with heterologous antibodies modulates both systemic and mucosal anti-Ig responses: after administering murine IgA antibodies to rabbit females, murine anti-idiotype anti-antibodies are found in the serum and colostrum [683]. It is striking that murine monoclonal anti-idiotype antibodies, specific for the rye grass pollen allergen *Lol p 4*, are able to inhibit binding of murine, rabbit, and even human antiserum. Since *anti-Lol p* antiserum can inhibit idiotype–anti-idiotype interactions, accordingly the monoclonal antibody in question has been characterized as an internal image anti-idiotype of the antigen [683]. Further experiments have demonstrated that immunizing adult mice with anti-idiotype antibodies against poliovirus are activated antibodies that effectively neutralize upcoming viruses. An interesting study (Chap. 2) following this line of research has revealed that the CB of offspring of agammaglobulinemic mothers or with IgASD was able to inhibit binding of poliovirus antigens to anti-poliovirus antibodies: a suggested rationale is a probable reactivity against CB anti-idiotype anti-antigen antibodies.

Current acquisitions on immune system regulations are still a matter of debate. At least two control systems are concerned, one provided by the suppressor cell network and the other by the idiotype cell network. The first network can be activated by original immune stimuli that regulated the system. CD8 T cells may be specific for a given clone or may down-regulate T-cell responses in a nonspecific way. Consequently, the same complex molecule can trigger helper T cells, effector T cells, and specific or nonspecific suppressor T-cell responses. Several ILs can also contribute to these results, modulating CD4 or CD8 T cells [469]. Idiotype regulation depends on recognition by the immune system that an immune reaction took place. In this event, antibody production in response to an antigen stimulus and pertinent antigen–antibody interactions trigger a response by a second series of IgE-producing B cells. The second-generation antibodies react with idiotypes on the original paratope producing the first-generation antibody and block its production. Anti-TcR antibodies are also formed and down-regulate T-cell responses in a similar way, while CD8 T cells can operate against idiotypes. Such intervention of cells and responses is essential to avoid that *a failure, even partial, of any regulatory systems may result in active immune responses* [469].

Apoptosis or PCD

If a thymocyte fails to produce any functional α chain, it cannot be selected and eventually dies of apoptosis; if a T-cell cannot be activated because the second signal is absent, it may be a target of a distinctive event known as *clonal anergy* (Fig. 1.22a) [138, 160, 177, 457]. Consequently, mature lymphocytes are rendered functionally unresponsive, resulting in *tolerance,* for example because CD28/CD80–CD86 [446] or CD40–CD154 (CD40L) costimulatory signals are lacking, which instead amplify such signals [37, 160]. Signaling via the CD122 γ chain can prevent induction of T-cell anergy



a

Fig. 1.22. **a** Schematic representation of the interactions arising during antigen presentation and the effects of T and B cells in the lymph node cortex (T zone) (see text). *CTLA-4* CD152, *CD40L* = CD154. (Modified from [138, 160, 177, 436]). (Fig. 1.22 b, c see next page)

[4]. *Clonal deletion* applies to particular clones of autoreactive, immature T cells that are physically destroyed during ontogeny to maintain self-tolerance [30]. Figure 1.22b shows that a CD4 T-cell associated with a HLA molecule recognizes a self-antigen and is eliminated instead of being activated (PCD) [138, 185]. Costimulatory signals are crucial also for T naive cells: when such signals are absent, the first stimulation by TcR renders them ignorant, refractory to further stimuli, and following a likely second stimulation, they are exposed to an inappropriate activation; therefore they may remain functionally inactive (anergy) or die (PCD) (Fig. 1.22c). Clonal deletion and negative selection also take place in immature B lymphocytes. For example, *limitin* (IFN-ω) (Table 1.5) produced by mature T lymphocytes in spleen and thymus as well as by bronchial epithelial and salivary duct cells suppresses the proliferation of pre-B cells [402]. Figure 1.23 shows positive and negative selections developing in GCs [309]. PCD occurs during embryogenesis, in the thymus during cell immune maturation, and at the end stage of immune responses [115, 276, 510]. It was also shown that PCD is an active form of genetically programmed cell suicide, not provoking inflammatory reactions [192]. More precisely, PCD is a physiological apparatus essential for normal development and homeostasis of multicellular organisms, a sophisticated defense mechanism to remove potentially dangerous cells, including self-reactive cells, virus-infected cells, and tumor cells, aiming at restoring a previous equilibrium [542]. CD4 and CD8 T cells, after their maturation into effector cells, die in 95% of cases, not only for precise regulation of cell numbers, or to maintain cellular homeostasis, but to protect T cells from continued secretion of potentially harmful amounts of ILs [400] and to leave a stable pool of long-lived memory cells [7]. Apoptosis occurs by *ex novo* activation of specific genes acting as PCD inducers/activators, or whose expression coincides with cell entry into apoptotic pathways (Tables 1.16, 1.17) [192, 400]. Here Fas/Apo 1 (CD95)-induced apoptosis is an important

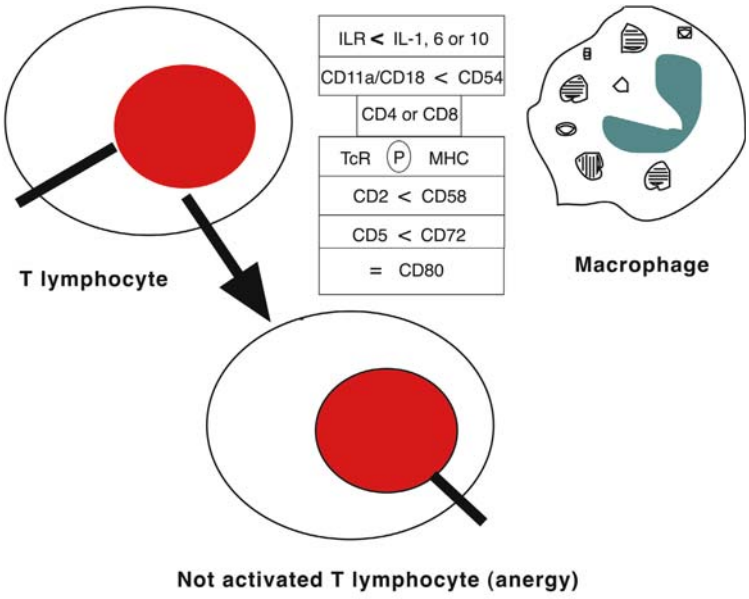
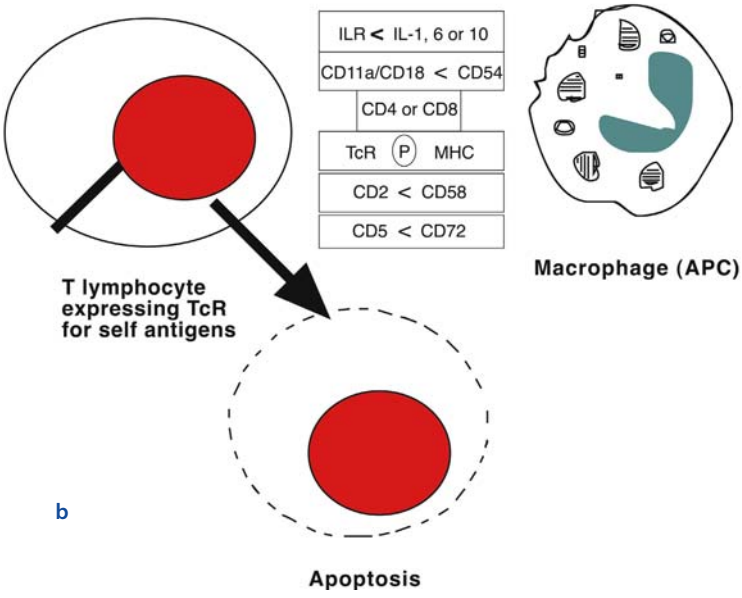


Fig. 1.22. b *Upper part*: Clonal anergy. CD4 T cell recognizes the antigen presented by APC; however, there is no CD80–CD28 interaction and it remains inactivated. *Lower part*: Clonal deletion. CD4 T cell recognizes a self-antigen; however, instead of being activated it is eliminated (apoptosis). **c** Effect of the intervention or not of costimulatory signals: on the *left* there is normal binding between peptide-HLA-TcR associated with the binding of a costimulatory ligand (*CL*) to a costimulatory receptor (*CR*), on the *right*, ignorance, anergy and apoptosis result because of the lack of costimulation. (Modified from [138, 160, 177, 457])



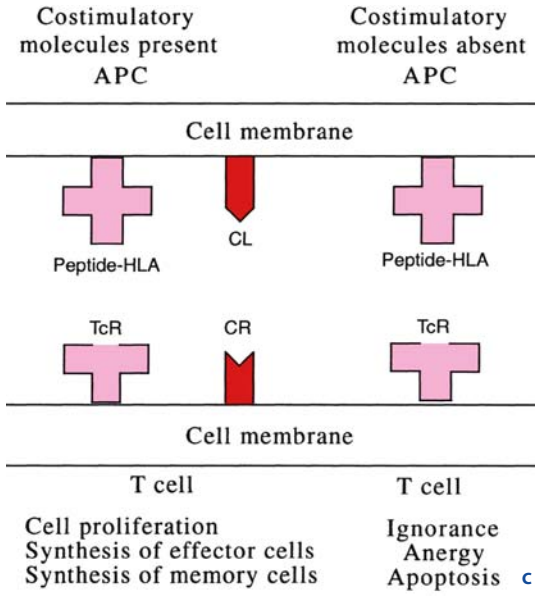


Table 1.16. Genes of the apoptotic pathway, either induced or expressed during apoptosis

Gene	Gene products	Probable function
c-*fos*	Transcriptional factor	Induces apoptosis when continuously expressed
c-*myc*	Transcriptional factor	Activates an apoptotic program
Fas/APO-1	Membrane receptor	Ligand binding induces apoptosis
Grb-3	Transduction factor	Expressed in some tissues during apoptosis
ICE/pr ICE	Cysteine protease	Initiates the active phases of apoptosis
nur 77	Nuclear receptor	Expression during apoptosis starts gene transcription
p-53	Transcriptional factor	Induces apoptosis upon DNA damage or loss of Rb function
RP2	Membrane receptor	Expressed during early stages of apoptosis
TG	Transglutaminase	Accumulates in apoptotic cells
TRP M2	Clusterin, SGP-2	Expressed in some tissues during apoptosis

Modified from [192].

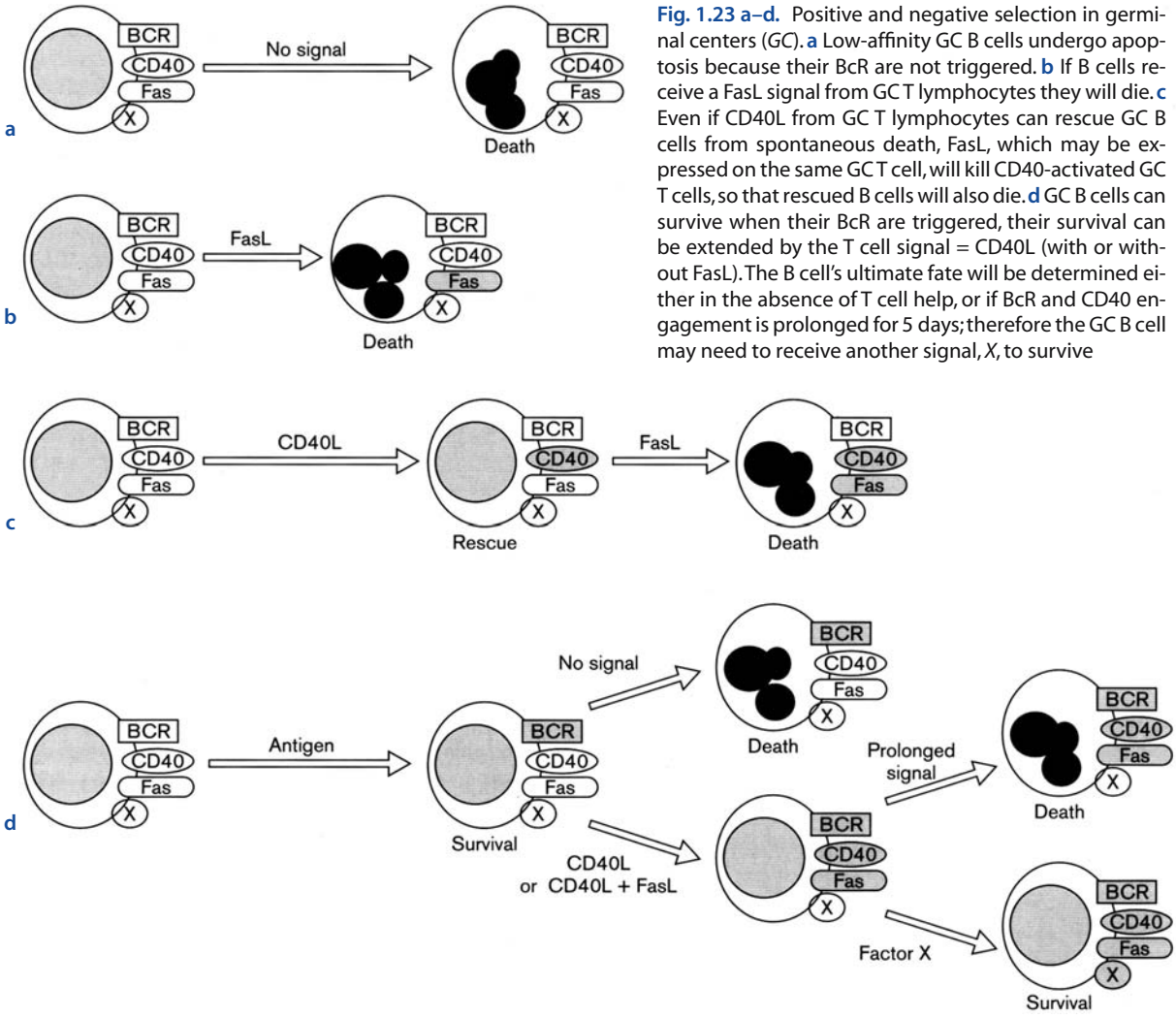


Fig. 1.23 a–d. Positive and negative selection in germinal centers (*GC*). **a** Low-affinity GC B cells undergo apoptosis because their BcR are not triggered. **b** If B cells receive a FasL signal from GC T lymphocytes they will die. **c** Even if CD40L from GC T lymphocytes can rescue GC B cells from spontaneous death, FasL, which may be expressed on the same GC T cell, will kill CD40-activated GC T cells, so that rescued B cells will also die. **d** GC B cells can survive when their BcR are triggered, their survival can be extended by the T cell signal = CD40L (with or without FasL). The B cell's ultimate fate will be determined either in the absence of T cell help, or if BcR and CD40 engagement is prolonged for 5 days; therefore the GC B cell may need to receive another signal, *X*, to survive

Table 1.17. Genes interacting with Fas

Gene	Probable function
CAP-3	Signal transducer of Fas
CAP-4	Signal transducer of Fas
FADD/MORT1/CAP1,2	Signal transducer of Fas
RIP	Signal transducer of Fas

Modified from [415].

regulatory mechanism in T cells. CD95 is in the first place as well as additionally involved proteins, including ICE ($IL_1\beta$-converting enzyme), and other proteases of the family, which may be common effectors of cell death [400]. Other genes code for products preventing cell entry into PCD pathways or regulate cell survival (Table 1.18) [192]. A third option is the inhibition or induction mediated by viral genes (Table 1.19) [192], clearly a strategy to ensure virus survival until the genome has replicated to establish a successful infection. More recently, the protein kinase RIP (receptor-interacting protein) has been included. Although the best role of RIP is in TNF signaling and NF-κB activation, it contains a death domain and it is capable of causing apoptosis upon cleavage [26]. From the picture shown by the tables, this multiform apoptosis pattern is unfolded: at variance with necrosis, cells shrink, both nucleus and cytoplasm condense, dying cells often release intercellular elements bound to membrane fragments, rapidly phagocytosed and engulfed by neighboring cells to remove possible noxious contents, and activated nucleases extensively degrade chromosomal DNA into small oligonucleotide fragments [542].

Studying cell-death defective (ced) species, three genes were shown to play a central part in PCD: *ced-3, ced-4 and ced-9*. The first two promote cell suicide; instead, ced-9 prevents the process started by ced-3 and ced-4, thus inhibiting apoptosis [542]. The mammalian counterpart of ced-3 is ICE, and that of ced-9 is proto-oncogene *bcl-2* (B cell lymphoma-2), mainly located within outer mitochondrial membranes, the ER, and nuclear membranes [267]. *The bcl-2/ced-9 gene prevents*

1.18. Genes of the apoptotic pathway: inhibitors and survival regulators

Genes	Gene products	Probable function
bcl-2 (ced 9)	Radical trap (?)	Inhibits apoptotic program(s)
Regulators of bcl-2 (with structural homology with bcl-2)		
A1	bcl-2-Related protein	Presumed inhibitory function
bax	bcl-2-Related protein	Inhibits bcl-2 activity
bcl-xB	bcl-2-Related protein	Inhibits apoptotic program(s)
bcl-xL	bcl-2-Related protein	Inhibits apoptotic program(s)
bcl-xS	bcl-2-Related protein	Inhibits bcl-2 activity
MCL-1	bcl-2-Related protein	Presumed inhibitory function
abl	Protein tyrosine kinase	Inhibits apoptotic program(s)

Modified from [193].

C cytochrome mitochondrial release, necessary to start apoptosis, and interacts with ced-4 inhibiting its function [661]. Expression of gene product par-4 correlates with severe impairment of cell proliferation and apoptosis via inhibition of PKCζ (protein kinase Cζ) enzymatic activity more probably of atypical PKC isoforms, also impairing MAP kinase (mitogen-activated protein) activation [655], whereas PI3K (PI-3-kinase) *generates survival signals* [412, 684]. The PKC family consists of serine/threonine-specific protein kinases that transduce a cascade of signals especially derived from the hydrolysis of PIP2 (phosphatidylinositol-bisphosphate). The classical PKC (cPKC) α, βI, βII and γ isoforms require diacylglycerol (DAG) and Ca^{++} for activation, whereas PKC δ ε γ and η isoforms, along with the related protein PKCμ, need DAG but do not require Ca^{++} [114]. In a recent study, CD43 ligation led to membrane translocation and boosted the levels of membrane-bound PKC isoenzymes, mainly of the PKCζ, PKCα/β, PKCε and θ, and PKCμ isoforms. Following CD43 ligation PKCθ activation induced CD69 up-regulation via an ERK (extracellular signal-regulated kinase)-dependent kinase pathway, promoted the AP-1, NF-κB activation and an ERK independent pathway promoting NFAT (nuclear factor of activated T cells) activation. Consequently, PKCθ was found to play a key role in the co-stimulatory functions of CD43 in human T cells [114]. bcl-2 was identified as a mammalian homolog to the antiapoptotic ced-9 in *Caenorhabditis elegans,* but mutations in the bcl-2-like gene ced-9 as well as DRP-1 (dynamin-related protein-1) and BH3-only protein EGL-1 may block its mitochondrial fragmentation [235]. Studies on this nematode by the 2002 Nobel laureates have shown that it has a fixed number of cells, 959, and if the number is altered, it is because too many cells die or, conversely, too many proliferate. In humans, AIDS could be provoked by excess PCD and autoimmune disease by insufficient PCD. At least 19 bcl-2 family members have been identified in mammalian cells, which possess at least one of *four conserved motifs* (BH1–BH4) [684]. The bcl-2 family members can be subdivided into three categories according to their function and structure: *antiapoptotic members,* such as bcl-2, bcl-XL, bcl-w, Mc1–1, and A1 (Bf1–1); *proapoptotic molecules,* such as Bax, Bak, and Bok (Mtd); and the BH3-only proteins, Bid, Bad, and Bim, which are called BH3-only proteins because of 4 bcl-2 homology regions, and share only the third [684]. *Galectin-1* (Gal-1) and *galectin-3* (Gal-3) are

Table 1.19. Viral genes whose expression inhibits or induces apoptosis

Genes	Gene products	Probable function
Inhibition		
BHRF-1 (EBV)	bcl-2 Related protein	Inhibits apoptosis
LMW5-HL (swine fever)	bcl-2 Related protein	Inhibits apoptosis
E1B (adenovirus)	p19 K	Inhibits apoptosis
E1B (adenovirus)	p55 K	Inactivates p53
crmA (cowpox virus)	Protease inhibitor	Inhibits ICE
p35/IAP (baculovirus)	p35/IAP	Inhibits apoptosis
ICP 34.5 (herpes simplex)	ICP 34.5	Inhibits apoptosis
E6 (papilloma virus)	E6	Inactivates p53
Induction		
E1A (adenovirus)	E1A	Inhibits RB
E7 (papilloma virus)	E7	Inhibits RB

Modified from [193].

β-galactoside-binding proteins with pro- and anti-apoptotic properties, respectively (Chap. 18). Some CTLs play a role of effector cells during apoptosis, as we have seen: this is the result of perforin action [69] or of activation and concurrent transcription of Fas and its counterreceptor CD178 or Fas ligand [388]. In humans, Fas gene is located on the long arm of chromosome 10, spans 12 kb, comprises nine exons, and in mice is expressed in tissues enriched by mature lymphocytes, except for DN [388]. A further study documented a TNF role in the apoptosis of activated CD8s [681], thereby confirming the higher inclination of Th1 T cells to effectively modulate killing by Fas binding [69]. A main pathway involves the signaling pathway of TNFR/CD95 activating both PCD and TF nuclear factor κB (NF-κB) (also activated by IL_1), the two events occurring independently [599], via recruitment of multifunctional FADD (Fas-associated death domain) and TRADD (TNFR-1-associated death domain) molecules [82]. PCD provides death receptor 3 (DR3) that most likely participates in lymphocyte homeostasis [82]. Activation of NF-κB is also regulated by the NF-κB phosphorylation via the IKK complex. The IKK complex, consisting of two kinases, IKK1/α and IKK2/β, and the NF-κB essential modulator (NEMO)/IKKγ regulatory subunit, mediates NF-κB activation by most known stimuli [503]. Another essential component of NF-κB activation is NIK (NF-κB-inducing kinase) whose interacting protein, TNAP (TRAF or TNFR-associated factor and NIK-associated protein) specifically inhibits NF-κB activation induced by TNF-α, TNFR1, TRADD, RIP, TRAF2, and NIK but does not affect IKK1- and IKK2-mediated NF-κB activation [217]. Overexpression of a new molecule, NIBP (NIK and IKKβ-binding protein), potentiates TNF-α-induced NF-κB activation [218]. TRAF6 interacts with TIR domain-containing adaptor inducing IFN-β (TRIF) through the TRAF domain of TRAF6. However, disruption of TRAF6-binding motifs of TRIF impaired its association with TRAF6, thus resulting in a reduction in the TRIF-induced activation of NF-κB [491]. TRAFs as components of the IL_1 signaling pathway mediate signaling by interacting with TNFR, rather than with TLR, thus playing a role in cellular processes such as apoptosis [327]. The scenario is not complete, since TRAF2 and TRAF3 have been shown to play opposing roles: a positive one in the standard pathway that activates NF-κB through IKKβ, but a negative role in the uneven pathway that activates NF-κB via IKKα, roles of TRAF proteins possibly linked to their ability to synthesize different forms of polyubiquitin chains [656]. The apoptosis–associated induction of the ubiquitin–proteasome pathway components and the proteasome activity shows that the proteasome plays an important role in the successful execution of apoptosis. Inhibiting either the proteasome activity or the increase in proteasome 26S gene expression or its upstream PI3 kinase activity results in an inhibition of NF-κB translocation thereby suppressing apoptosis [527]. There are many Fas variants: soluble Fas (sFas) can block apoptosis induction [600] as well as crmA – inhibiting ICE/ced-3 (Table 1.19). A second pathway depends on mitochondrial participation by releasing apoptogenic factors: cytochrome c catalyzes the oligomerization of APAF-1 (apoptotic protease activating factor 1), which recruits and promotes the activation of apoptosis proteins (IAPs) such as procaspase-9. These proteins interact via CARD-CARD (caspase recruitment domain interactions) [599]. Procaspase-9, in turn, activates procaspase-3, leading to apoptosis, but is prevented by members of the bcl-2 family, but cells also contain natural IAP inhibitors, the caspases (aspartate-specific cysteine protease), a family of cysteine proteases, which were found both in baculovirus and in human cells (XIAP, c-IAP1, and c-IAP2). IAPs can act as direct inhibitors of the two death effectors, caspase-3 and caspase-7, and are able to suppress the activation of two initiator caspases, caspase-8 and caspase-9 [418, 599]. Studies performed on the activity of effector caspase 3 and on the initiator caspases 2, 8, and 9 revealed that, in the absence of RIP, the activity of these caspases decreases, indicating that RIP-associated apoptosis is caspase-dependent [26]. Thus, Fas, TNF-related apoptosis-inducing ligand (TRAIL) and TNFRs can initiate cell death by two alternative pathways, one based on caspase-8 and the other dependent on the RIP kinase [211]. NF-κB is an essential regulator of immune cell survival, critical for the activation of T and B lymphocytes, and is a central coordinator of innate and adaptive immunity [470].

The apoptosis machinery in B lymphocytes may be different, generally immature cells with IgMs or IgDs or cells that have formed an extra paratope [37]. Also, B cells with poor affinity for antigens, or autoreactive, are destined to a rapid apoptosis and are phagocytosed by macrophages leaving nuclear residues forming tingible bodies [254]. The *bcl-2/ced-9 gene blocks PCD* in B cells and *cells provided with bcl-2* [400]. CD23 may prevent apoptosis of GC B cells [4]. Mounting evidence suggests that autoimmune diseases and viral infections, for example, may be associated with failure to undergo PCD, as well as others characterized by inappropriate cell destruction, AIDS as a first example [542].

The HLA System

The HLA system is the human version of the MHC called H2 in mice. HLA, first discovered in the 1950s, was recognized by its major influence in transplant rejection. Subsequent studies have revealed that HLA is in mammals a single gene region with a pivotal role in antigen recognition and control of immune response (Ir); some Ir genes are also mapped within its structure [329]. The chromosome region containing genes coding for self/non-self discrimination is a highly polymorphic complex region of about 4,000 kb located on *chromosome 6 short arm* (6 in region *p21.3*) (Fig. 1.24). HLA is

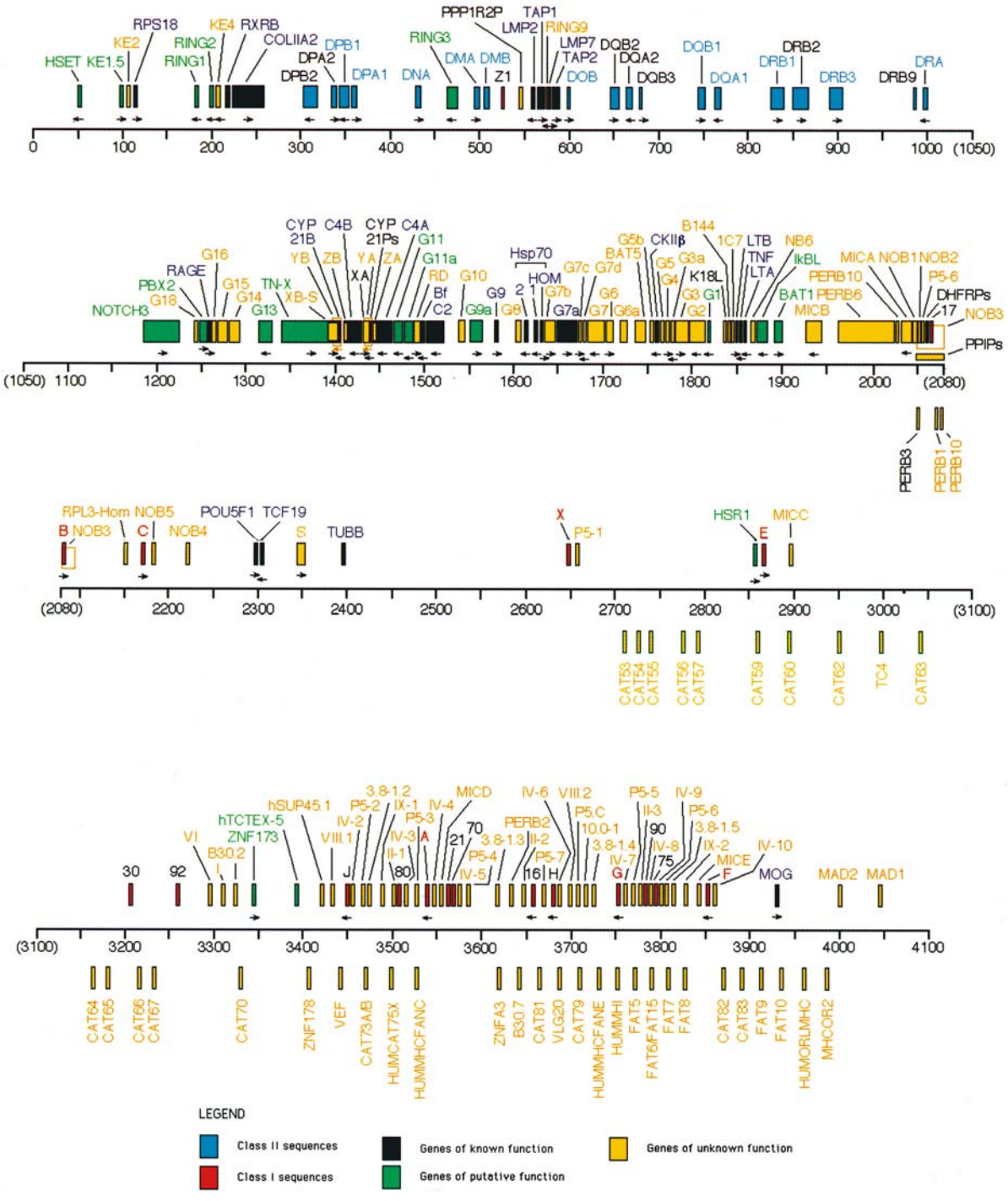


Fig. 1.24. Scheme of human HLA

referred to as complex because its genes are located on a single chromosome, and all genes can therefore be transmitted to children as one unit [18]. In humans this is the best known histocompatibility system as defined at the genetic level, comprising hundreds of genes connected together (>40% of which encode leukocyte antigens) and characterized by structural and functional diversions [264]. *Histocompatibility* is the property of accepting cells or tissue grafts between individuals [329]. Because of their close linkage, the combination of alleles at each locus on a single chromosome is usually inherited as a unit, except for infrequent cases of recombination, and is referred to as the *haplotype*. Although during evolution there may have been variations, the haplotypes of various mammals and birds hitherto studied are fundamentally similar. A key task

carried on by these molecules is to present antigens or peptides deriving from enzyme cleavage to TcRs. That peptides associate with HLA molecules and are recognized as a single entity demonstrates either the phenomenon of restriction [688] or the genetic control mediated by products of HLA genes present on all nucleated cells. HLA class II genes, expressed in a constitutive way in some cells, including B and T cells, macrophages, and DCs, stimulated by specific activators such as IFN-γ in endothelial and epithelial cells, form a trait with high genetic variability, so that proteins encoded by the above-mentioned genes usually differ from one individual to another [329].

In mice, chromosomal segments controlling immune phenomena are divided into five regions: K and D regions encode serologically defined (SD) antigens, found in all nucleated cells and called *HLA class I molecules.* Ir genes (in mice antigen-associated with the I region of the H2 complex and in man DR- and D-related) are present on APCs, more restricted as regards their distribution and defined as *HLA class II molecules;* the S region controls synthesis of complement components. K and D regions are recognized by CD8 T cells during transplant rejection. It was also shown that following a viral infection, murine CD8 cells will lyse only infected target cells derived from a line that is genetically identical with the same K- and D-region molecules as the original stimulating cells [33].

HLA includes *loci* A, B, C (class I region) and D (class II region); lymphocyte-defined (LD) antigens are called HLA-DR, -DP and -DQ. Each *locus* is composed by a series of alleles determining the corresponding gene products (HLA antigens) on cell membranes. According to recent nomenclature, alleles with confirmed sequences of amino acids or nucleotides have a specific designation, including the name of *locus* and four arabic numbers: the first two designate more closely related specificity and the last two the allele number (Appendix 1.1) [640]. The number is preceded by an asterisk: e.g., HLA-DRB*0401 stands for allelic variant of 0401 of gene 1 [264]. The nomenclature of different HLA specificities has grown very large, including more or less completely defined *locus* numbers of alleles: 287 HLA-A, 527 HLA-B, 147 HLA-C, 6 HLA-E, 1 HLA-E, and 15 HLA-G, making a total of 980 class I alleles, 454 HLA-DR, 79 HLA-DQ, 128 HLA-DP, 10 HLA-DM, 16 HLA-DO, for a total of 649 class II alleles [640]. Some genes HLA-C, HLA-D, HLA-E, HLA-F, HLA-G are less characterized [640]. There are 54 class I genes and MICA pseudogenes; TAP 1 and 2 (transporter associated with antigen presentation 1 and 2) are 10 in number [640], while MICB, MICC, MICD and MICE do not yet have a fixed function [469]. MICA and MICB are ligands for the activating CD94: CD94R may inhibit class I molecules [329]. As Appendix 1.1 shows, not all alleles have serologically defined specificities. Inclusion of different designations of HLA alleles and specificities comes from using serologic methods instead of molecular reactions recently employed for typing such as *in situ* hybridization, PCR (polymerase chain reaction), RFLP (restriction fragment length polymorphism), etc. For example, HLA-B27 was associated 30 years ago with several autoimmune disorders [329], now encompasses several subtypes, from B*2701 to B*2709 [640].

HLA and encoding genes form three categories, classes I, II and III, membrane gps expressed on all nucleated cells. Class I and II molecules are members of IgSF (Figs. 1.25–1.27) [454]. Class I and II molecules can be differentiated according to their structure, tissue distribution, and function [329]:

- *Class I genes,* located in the more distal region from centromere, encoded by main HLA-A, HLA-B and HLA-C *loci,* include a polymorphic H chain, a 45-kD α chain, in close, noncovalent association on the membrane with a nonpolymorphic 12-kD L chain, a β_2-m encoded by a single gene located on chromosome 15. A molecular three-dimensional structure shows H chains divided into six regions: three extracellular globular domains, α1, α2, an Ig-like domain, α3, a short extracellular connecting peptide N-terminal, a hydrophobic TM region (25 amino acids), and a hydrophilic intracytoplasmic C-terminal tail (30 amino acids), while L chain forms only an extracytoplasmic Ig-like domain. A molecular part furthest from the membrane embraces a deep groove or cleft, *the peptide-binding site,* made up of segments of α1 and α2 domains with α-helical sides and an irregular β-sheet base. A single α3 domain has sequences interacting with CD8 cells.
- *Class II genes,* positioned in a more proximal region to the centromere, controlled by at least three HLA-DR, HLA-DP, HLA-DQ subloci, consist of two polypeptide domains, a 33-kD α and 28-kD β, noncovalently linked. This pattern resembles that of class I, but with five domains, since each chain contains two extracellular globular regions (α1 and α2, or β1 and β2), not covalently linked. The peptide binding groove has a structure similar to that of class I molecules. Also in this case the cleft chemical surface, distinct from class II molecule GPM, determines specificity of antigen binding. Class II is restricted to immunocompetent cells presenting processed antigens to CD4 cells and is necessary for interactions between immunocompetent cells. Additional class II-related molecules are eight HLA-DOA alleles [469]. The designation of class II *loci* on chromosome 6 consists of three letters: the first (D) indicates the class, the second (M, O, P, Q, or R) the family and the third (A or B) the chain (α or β, respectively).
- *Class III genes,* placed between classes I and II loci, encompass a heterogeneous mixture of genes, including classic pathway complement components (C2, C4a, C4b), properdin factor P of the alternate pathway, enzyme β-21 hydroxylase, molecules of the heat shock protein family (HSP70-1 and -2), and TNF-α and TNF-β. Many class III genes are involved neither as transplant antigens nor in antigen presentation [329, 337].

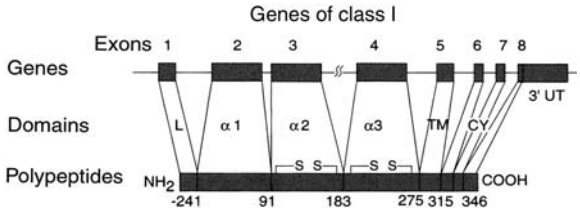


Fig. 1.25. Diagrammatic representation of HLA class I genes and molecules

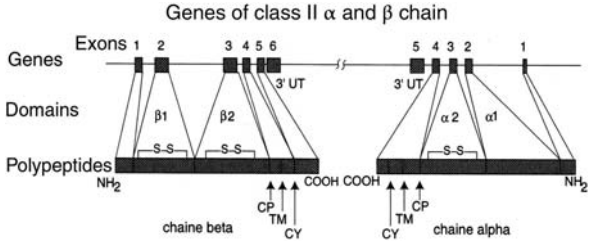
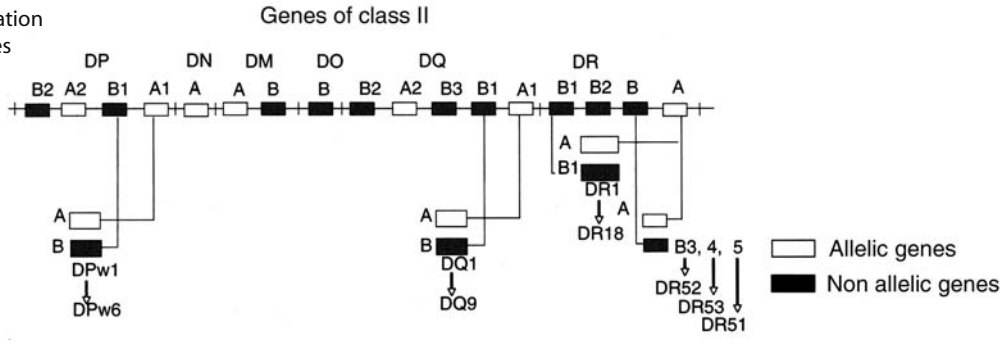


Fig. 1.26. Diagrammatic representation of the genes of α and β chain of HLA class II molecules

Fig. 1.27. Organization of HLA class II genes



Other genes encoding proteins involved in antigen processing machinery have been identified interspersed among class II genes, between HLA-DQ and HLA-DP, such as six TAP-1 and four TAP-2 alleles (Appendix 1.1), and two LMP2 and LMP7 genes (low-MW polypeptides 2 and 7) [329].

The Ir genes, mostly mapped to HLA antigens, encode a synthesis of class II molecules, that is HLA-restricting elements recognized by CD4 cells. Owing to allelism of the above-mentioned molecules, different individuals respond differently to the same antigen when responses are HLA-restricted, that is, only when antigen presentation is in the context of class II specific molecules. In the absence of Ir alleles, there is no production of antibodies against specific antigens; hence individuals have inherited HLA antigens that in peptide presentation to TcR are markedly effective in eliciting synthesis of antibodies different from IgE antibodies. It was speculated that Ir genes are expressed on macrophages and not on T cells. Actually, the genetic control related to interactions between T cells and macrophages is localized in HLA I regions and products encoded by genes of such regions are defined as HLA-DR [198]. Only 10%–50% of macrophages isolated from peritoneum and spleen are provided with these antigens, and it seems that only HLA^+ cells look after antigen exposure [12]. To better define *genetic restriction,* it refers to different specificity for different HLA molecules, since not all T-cell subsets use the same HLA molecules, each having a capacity of response limited to some HLA components, since both class I and II contain recognition sites for CD8 and CD4 coreceptors on T cells [463].

According to the restriction concept, the HLA molecule has a key role in binding to immunogenic peptides deriving from processing foreign proteins: bimolecular HLA–peptide complexes expressed on APC membranes are the ligand recognized by T cells. CD4 cells recognize only peptides expressed on membranes of actively phagocytic APCs (10^5 molecules/cell) [337] associated with HLA class II antigens. Suppressor-CTLs are restricted to associating with HLA class I molecules, expressed by most nucleated cells of the body (between 10^4 and 5×10^5 molecules/cell), while the HLA C region contacts CD8 coreceptor [463]. HLA class II antigens bind to peptides with sufficient affinity by means of amino acid sequences of their hypervariable parts situated in α-1 and β-1 domains; similarly, TcRs recognize HLA–peptide complexes. Instead, T cells, with a γδ TcR that is often DN, represent an exception to restriction in that, in addition to class II antigens on DCs [558], they recognize bacterial proteins as well as heat shock proteins (HSP) in an HLA-independent way [279]. Centrality of HLA molecules in tripartite interactions with processed antigens and TcRs is exemplified by the repertoire of antigen specificities recognized by T lymphocytes.

Main features distinguishing class I and II molecules are GPM, association and codominance [18, 329]:

- *The major part* of HLA genes is characterized by an almost unique GPM, which has been shown to be even more extensive by rDNA technology. Each gene has multiple alleles, leading to a great number of likely combinations on each chromosome (haplotype): the number of genotype combinations is $>10^{10}$. Besides these alleles, there are many variants in the general population, so

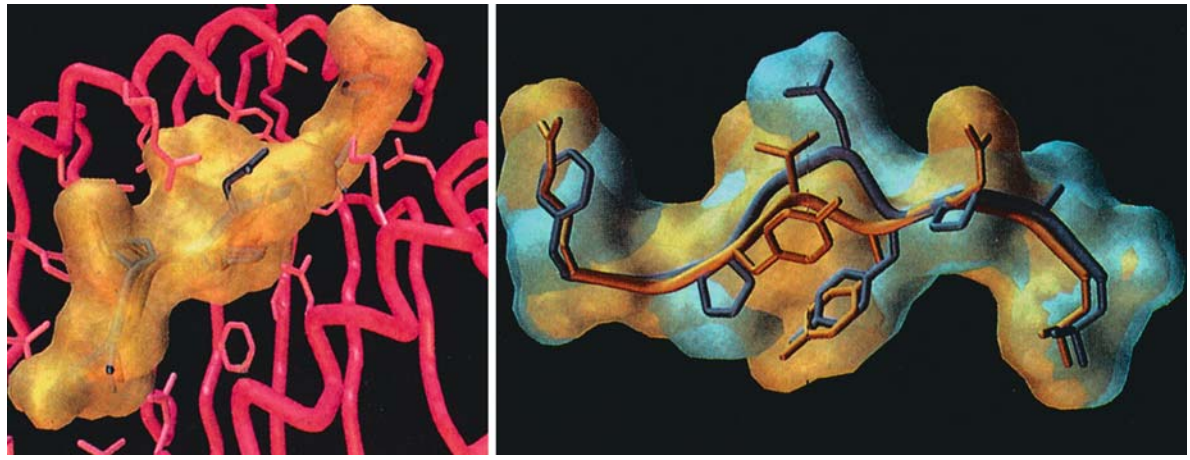


Fig. 1.28 a,b. A viral peptide buried in a HLA class I molecule (**a**), and together with another peptide (**b**) in the extended configuration they assume when complexed with HLA class I molecule [161]

that the majority of subjects are heterozygous (HZ) for different *loci*. Therefore, an extensive GPM makes wholly different HLA molecules expressed by genetically distinct individuals, that is each one has his own genetic set of HLA molecules, shared only by a few individuals of the same population. GPM is also the basis for rapid graft rejection between genetically different individuals.

- *Association* means that all genes are transmitted en bloc by HZ parents to their children. When paternal haplotypes are a and b and maternal ones c and d, each child inherits from both haplotypes, that is ab, ac, bc and bd; thus the chances of a child having the same two haplotypes are = 25 %. A recombination between two haplotypes (cross-over) can be in germ cells, paternal or maternal, creating a new haplotype. The recombination rate is 1 % in HLA-A and -B and between -B and -D.
- *Codominance* means that each cell expresses HLA proteins transcribed from paternal and maternal chromosomes. One point worth noting is that only one chromosome is used to form Ig and TcR molecules, the unique occurrence of allelic exclusion. Each individual is commonly HZ for each *locus* and her/his HLA group is composed by several letters as described above, plus two numbers corresponding schematically to the alleles transmitted by both parents.
- Moreover, *some gene combinations* can have an unexpected frequency, because individuals of a heterogeneous population can have two genes segregating together, with a frequency markedly different (higher or lower) than the predicted frequency. *Linkage disequilibrium* is a phenomenon common to almost all complex genetic systems, the tendency of specific alleles of linked genetic *loci* to be inherited together (as a unit or haplotype) on the same chromosomal region far exceeding that expected by chance association, functionally interacting between themselves.
- *GPM* is a strategy for generating diversity of HLA molecules; hence it contrasts with the unique strategy of gene rearrangement of TcR and BcR. Accordingly, each lymphocyte expresses only slightly different receptors, whereas every cell of an individual expresses the same HLA molecules, but different from HLA molecules expressed by genetically unmatched subjects. GPM also contrasts with principles of allelic exclusion and thus with the genetic principles governing receptors: class I and II molecules are codominant, that is, each cell expresses HLA proteins transcribed from both maternal and paternal chromosomes, consequently confirming GPM [36].
- As regards the respective functions and properties, class I and II molecules have a similar general structure, although not identical: both seem to have a wide GPM and bind to peptide fragments subsequently recognized by TcRs. Perhaps due to different types of processing, class II molecules appear to present a heterogeneous group of peptides for a given epitope, instead of only one well-defined epitope as is normal for class I molecules.
- GPM of HLA *loci* and some alleles of different *loci* tend to associate between themselves; this inclination has been understood as necessary for human immune system diversity or functioning [463].

Several ILs can stimulate HLA molecules: IFN-γ is one of their most potent activators, whereas TNF-α, TGF-β and IFN-α and -β functions remain obscure [579]. IFN-γ induces activatory signals for CIITA (class II transactivator) expression [539], which is more of an essential regulator for expression of HLA class II genes than their direct modulator: CIITA is defective in a form of primary HLA deficiency [539].

Many peptides bound to HLA molecules may not be invariably presented to TcRs, in part because cells expressing TcRs reacting with peptides derived from a given individual are often removed during T-cell development in the thymus, either clonally deleted, or dying by apoptosis as formerly alluded to [579]. The set of HLA alleles inherited by an individual is exceedingly tiny compared to diverse TcR repertoires. In theory, TcRs are

restricted to recognize a very limited subset, while HLA molecules can bind to a large collection of foreign and self-derived peptides: this issue appears to be paradoxical since HLA molecules can bind a multitude of peptide fragments in a very restricted way, with an affinity at least 1,000-fold higher than that of TcR. Of course, each HLA molecule could bind this large group of peptides, but what governs the ability of a peptide to bind one HLA haplotype rather than another? [18]. It might also be that a very small number of HLA–peptide complexes are required at the APC surface to generate immune responses [36]. As we see from Fig.1.28 [161], class I molecules in concomitance with the encounter with molecules to be scrutinized present, as previously described, the special conformation with binding grooves destined to reception of already processed peptide fragments [161]. In particular, as evidenced by crystallographic studies, conserved pockets at both ends of the peptide-binding groove accommodate their N-terminals via extensive H_2 binding, thus warranting a correct orientation of peptide-binding and its closed nature at both ends [264]. In the middle of the groove there is a deep pocket providing structural complementarity (like a gem in a ring) for one of the peptide amino acids called the *anchor* (the amino acid residue recurring more frequently). The peptide antigen appears to be anchored by residues either at or near the end of the peptide, thus enhancing the specificity of HLA–peptide binding [344]. The single substitution of an amino acid of an exposed peptide residue, although changes do not seem to be critical for the above binding, is sufficient to abrogate TcR recognition. Since HLA molecules are very unstable, only peptides with an allele-specific anchor residue may provide sufficient stability, while a small decrease in affinity has the wide biological effect of increasing the dissociation rate of bound peptides, hence preventing adequate time for presentation to T cells [161]. The pocket of class II molecules is open-ended, allowing larger peptides to bind [264].

Initial Phase of the Immune Response

Functions of HLA Molecules and Antigen Processing

Antigen presentation is defined as processing or cleavage of foreign antigens consisting in transformation from a native form to a nonnative form to yield peptides complexing with HLA molecules, thereby associating HLA products only with fragments of presenting antigens, and not with intact antigens themselves, drawing attention to the analysis of proteolytic events lying upstream of presentation events.

A remarkable contribution to understanding this step of immune responses comes from studies on related viral attitudes. As discussed in the preceding section, HLA class II molecules interact with CD4 cells, class I molecules with cytotoxic CD8 cells (Fig. 1.29) [591]. These mechanisms entail recognition of peptide fragments deriving from intracellular microbial antigens, which sometimes expose native proteins to the host surface, and thus to a cytolytic attack [591]. Some viruses in particular can infect every type of human cell, so that to respond to aggressions T cells must recognize viral peptides associated with class I molecules. When other pathogens degraded via phagocytosis are active, this process implies T cells being reactive with exogenous peptides associated with class II molecules [337]. Such programming has a remote ontogenetic origin, since TcRs were planned by the immune system to recognize even small fragments of viral antigens, sometimes of only eight to ten amino acids, hence virtually indistinguishable from the related peptides of human cells. Consequently TcR was diversified to react with such fragments only if associated with HLA products [457]. Prerequisites are therefore processing outside peptides and assembling molecules for one or more HLA alleles. To degrade a protein into small fragments, both endogenous and exogenous antigens should be first broken down into vesicles with acid pH, to be associated with HLA alleles on the cell surface. Processing clarifies why T cells, unlike B cells, fail to recognize protein conformational epitopes, but only linear ones, and why they cannot distinguish between native and denatured proteins of the same antigen [474].

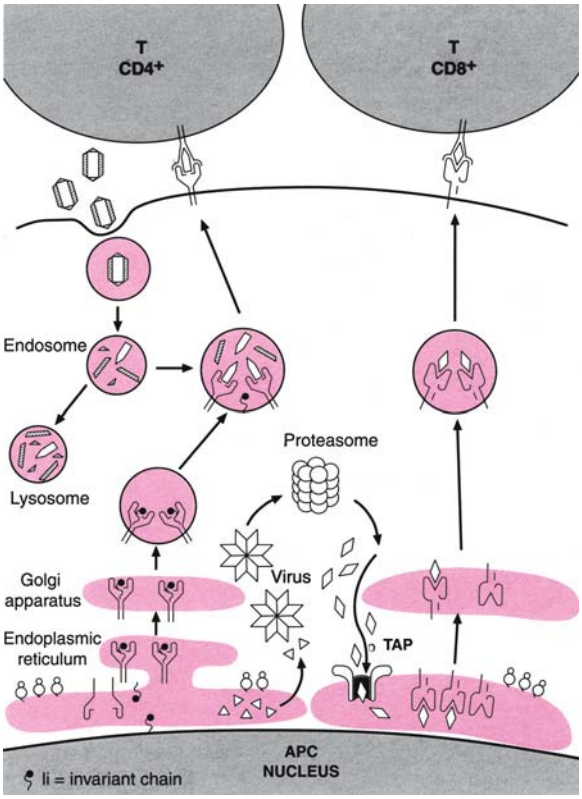


Fig. 1.29. Antigen processing and presentation to T lymphocytes

Various methods are employed by cells for antigen processing as follows:

- *Phagocytosis* delineates the most prominent function of mononuclear phagocytes at the point of assuming such denomination, and antigen processing has a pivotal role in inducing immune responses: during the different steps, among fragments subjected to processing, those cleaving in the endosomes and with affinity for HLA molecules escape a complete denaturation, bind to HLA alleles, and are then transported to macrophage surfaces [540]. On the contrary, after an exhaustive proteolytic cleavage in *lysosomes,* peptides with no HLA association are excreted outside the cell surface through a process of exocytosis.
- *DCs and B lymphocytes* cannot use phagocytosis, but a more common process called pinocytosis, by which fluids or very small particles (diameter <10 nm = 100 Å) are taken into the cell.
- *B cells* can employ antigen-specific Igs (IgMs or IgDs), a much more effective procedure because the antigen concentration necessary is 1,000-fold less than that required by pinocytosis. However, despite the advantage of close linking with B lymphocytes, this process is really restricted to a comparatively limited number of these cells [384].

Exogenous peptides generated in acid vesicles bind to class II restricted T cells [391], whereas endogenous peptides synthesized by cytosol and ER are recognized by class I restricted T cells [372]. When processing of a non-virus-infected cell (modified, for example, in a vaccine) takes place in acid vesicles, the resulting peptides are presented to CD4 lymphocytes associated with class II molecule. However, if the same antigen infects the cell, processing moves over the cell cytosol/ER, then antigens are presented in association with CD8 lymphocytes and HLA class I molecules. Following these two different procedures, CD4 and CD8 lymphocytes of an individual can recognize different epitopes of a given antigen [47]. Moreover, as a number of experiments have demonstrated, the structural characteristics of peptides do not determine the binding to one or to another HLA class, but it can be hypothesized that distinctions arise from two different ways of processing and presentation [369, 391].

Now the way by which HLA molecules acquire peptides is the center of attention, rather than the origin of peptides presented by HLA products. As regards class I molecules, before entering an exocytic system, processing of antigen material in the cytosol is usually attributed to *proteasomes,* an ATP-dependent complex of peptidases, proteolytic enzymes encoded by LMP2 and LMP7, a pathway probably operative in converting native antigens into peptide fragments then translocated into the ER to associate with class I molecules [377]. Unfolded or worn-out polypeptides in the ER are retrotranslocated into the cytosol where the SC factor (SCF) (Fbs1, 2) proteins distinguish native from unfolded glycoproteins and ubiquitin targets the worn-out proteins for dumping [648]. These proteins unfold with the help of other specialized molecules, the chaperones, and the polypeptide chains are then fed into the proteasomes, where unassembled or defective proteins in the cytosol are degraded into peptides [264]. The ubiquitin-proteasome system is a fundamental machinery in the cell [520] and has been shown to be involved in the virion budding process of several viruses, particularly of rhabdoviruses [198]. In the presence of the proteasome inhibitor MG132, the entering viruses accumulated in both the endosome and denser lysosome, suggesting that the ubiquitin-proteasome system is involved in the virus transfer from the endosome to cytoplasm during the virus entry step. Understanding the sensitivity to the ubiquitin-proteasome inhibitors may be used to distinguish the early steps of viral entry [656]. Studies of *Thermoplasma acidophilum* [316] have shown that proteasomes, with a barrel-shaped structure, consist of two inner rings, each consisting of seven β subunits, and two outer rings, each made up of seven α subunits, all different [316]. Proteasomes interact with either ER membranes or TAP-1 and TAP-2 [293] encoded by polymorphic genes of class II and associated with H chain α1 and α2 domains [443] (Fig. 1.30). TAP-1 and TAP-2, closely located to genes encoding LMP2 and LMP7 inducible by IFN-γ [293], import into the ER *lumen* selected peptides, which may attach to a newly synthesized β chain of the class I molecule encoded by the β_2-m gene [263]. Possibly the peptide NH_2 terminus is also trimmed [361]. The peptides are necessary for a correct assembly of HLA class I molecules. Class I molecules first assume a peptide-receptive conformation, then release peptides to be delivered to the cell surface for antigen presentation to CTLs with CD8 markers [337]. However, subsequent analyses have shown the polymorphism of TAP and LMP genes that map between DPB1 and DQB1 within class II genes [372]. So it seems reasonable to imagine that different epitopes of the same antigen are presented to T cells of different subjects. Support for this hypothesis will lead to evident implications, such as individual differences in immune responses to specific antigens [28], in addition to differences in tolerance induction, predisposition to autoimmunity, and disorders associated with HLA molecules [28]. HLA class I molecules could also bind to exogenous antigens loaded by macrophages and DCs [39]. The principal routes are as follows: release of antigens acquired from endocytic or phagocytic vesicles returning immediately to normal pathways, or antigen digestion in a vesicle by using normal class I mechanisms; pertinent examples in support of both hypotheses derive from inhibitors of processing pathways such as brefeldin A and chloroquine [39]. When TAP-2 is deficient, assembly of class I molecules cannot be completed.

As reported by studies on the routes of class II molecule intracellular traffic, newly synthesized HLA products are collected in the ER where they are delayed for

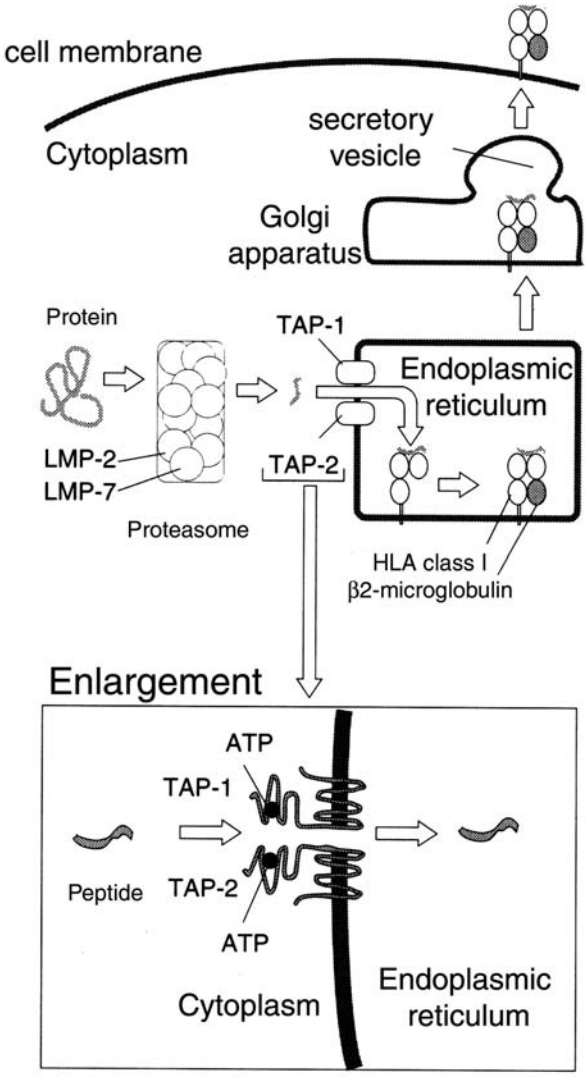


Fig. 1.30. Biosynthesis of HLA class I molecules

1–3 h, apparently not for recycling back to the cell surface, but for peptide loading to take place. Molecules leave it only when linked with three pairs of αβ heterodimers of the same ER, transiently aggregated to three copies of the TM invariant chain (li=CD74) [61]. The agglomeration of nine chains (nonamer) without contacting peptides in the ER is transported across trans-Golgi and post-Golgi networks to an endocytic compartment, MIIC (MHC class II-loading compartment) [443]. The li chain appears to protect class II dimers from binding cytosolic peptides imported into the ER for class I binding [61]. The li region, important in binding class II molecules and in preventing premature peptide binding, corresponds to CLIP (class II associated invariant chain peptide), which binds HLA-DR3 in sites normally occupied by peptide antigens [61]. In *Golgi networks,* after glycosylation peptides are internalized into the cell by endocytosis, an APC encounter with foreign peptides occurs in endosomal or prelysosomal compartments where peptides are internalized, then extruded in intra- and extracellular compartments [378]. HLA class II peptide presentation can be inhibited by lysosomotropic agents such as chloroquine and NH_4Cl, which raise the pH, and by inhibitors of endosomal proteases, with no effect on class I endogenous peptides [378]. While in transit to endocytic routes, the li chain subjected to the compartment acid pH is degraded by endosomal proteases and CLIP is removed from DR3 by HLA-DM [182]. As the intact li chain, CLIP inactivates the groove by binding directly to it [61]. Consequently, HLA molecules can bind processed antigen peptides, and peptide–class II-αβ complexes can be transported to cell surfaces: HLA molecule antigen binding assures sufficient stability, allowing it to reach cell membranes to activate the correct T cells [372].

In conclusion, the intracellular routes followed by HLA class I molecules and respective antigens traverse structures different from those crossed by both antigens and HLA class II products, thus avoiding their reciprocal encounter.

Antigens presented in association with class I molecules bind preferentially to peptides usually 8–11 residues long (their grooves are restricted in length and are closed at both ends) [337], whereas class II molecules can accommodate much longer peptides, with 11–17, and up to 25 amino acids [337]; therefore both ends of a class II molecule-bound peptide have been assumed *to protrude out of the groove* [482], thus showing a higher heterogeneity at NH_2 and COOH termini [337]. Accordingly, peptides with 13–17 amino acids associated with class II molecules can stimulate T-specific clones [482], considering that the theoretical number of different peptides formed by nine amino acids is 5.1×10^{11} [337]. Selectivity of HLA molecules implies that only some of them have grooves with sufficient length to hold processed peptides [482]. It is critical that a different structure is appointed for trimming of longer peptides, which could be:

- In *ER precursors,* associated with class I molecules [144]
- In accordance with *binding sites,* or the same peptide, not associated with class I molecules [482]
- *Within the cytosol,* where peptides may be immediately re-exported [361], or where there may be envisaged a proteolytic structure specialized in reducing peptides to fitting size for HLA before transport in the ER
- *Stress protein gp96* may function as a chaperone for peptides not fitting HLA molecules [286]

On the contrary, class I molecules need no trimming, being unable to hold longer peptides.

Antigen processing also has particular features: if amino acid chains or peptides are rolled up and folded, when the chain is extended, distant parts can be brought together via folding of protein structures, leading to conformational epitopes. Furthermore, unlike B cells directly interacting with antigens, being able to recog-

nize epitopes contained in intact and normally folded protein molecules, T cells recognize only epitopes present in denatured and linear molecules. Besides splitting, in macrophage intracellular vacuoles an unfolding takes place, which makes accessible previously hidden, totally or partially, immunogen peptide parts [443]. Small amino acid sequences equipped with the structural characteristics essential for complex formation with HLA molecules are often found inside of rising protein molecules and become available for binding to HLA products only after *unfolding of protein structures* is accomplished: fragments containing the critical amino acid sequences are transferred into the macrophage membrane surface, ready for subsequent antigen presentation [12].

Therefore, if antigen processing consists in the *conversion of an antigen from the native to a non-native form*, a process carried out by APCs expressing HLA antigens, peptides must make contact with TcR as well as bind to an HLA molecule. A most likely hypothesis is that processing involves changes into antigens provided with a conformational freedom to form a secondary structure permitting both epitope and agretope to form. Both are composed of small amino acid sequences, at least *two or three residues* interspersed into the primary sequence of native proteins: *recognition is facilitated* by an α-helical conformation of peptide chains, in other words the helicoidal spatial arrangement. Residues making contact with T cells and HLA molecules, respectively, during enzyme splitting segregate to opposite sites of the α-helix one group forming the epitope and the other the agretope. However, although in many instances the whole amino acid sequence of several allergens was successfully disclosed, it is poorly understood which sequences are responsible for IgE binding, probably because IgE antibodies have different requirements as regards the conformation of the peptides to bind [12].

Subsequent studies have proposed a new type of antigen processing, a noncytosolic pathway, typical of class I molecules, also suggesting that peptides internalized into phagosomes are hydrolyzed by proteinases in endocytic compartments, but it is uncertain how these peptides bind to class I molecules, thus more mechanisms may be operative [467]:

- In the first type, HLA class I molecules from plasma membranes or newly synthesized may enter the phagosome, bind peptides and transport them to the cell.
- In the second type, class I molecules in macrophages may recycle between the cell surface and endocytic compartments.
- In the third type, the li, although commonly used in class II transport, can associate with newly synthesized class I molecules and direct their transport into endocytic vesicles [467].

The role of this exogenous pathway may be in innate immunity, again with three different types:

- In the first type, the immune system may have the privilege to detect and monitor pathogens surviving in phagosomes, not eliminated by CD4, permitting CTLs to destroy infected cells, in turn CTLs can secrete IFN-γ, which would stimulate macrophages to kill the microbes.
- In the second type, antigens are imported from somatic cells, poor stimulants of naive T cells lacking CD80, CD86 and CD54, into professional APCs; such exogenous pathway APCs can trigger primary T-cell responses since they express high levels of HLA class I and II molecules, in addition to costimulatory and adhesion ligands, and trafficking into lymphoid organs at that time.
- In the third type, the exogenous pathway potentially stimulates CTL immunity using protein-based vaccines: as a rule antigens in extracellular fluids are not presented in association with class I molecules on mast cells [467].

Cells of the Immune System Participating in Immune Responses

Phagocytes, so called for their ability to ingest and digest living or inert particles, have a common origin in the bone marrow, from progenitor cells, or colony-forming unit-spleen (CFU-S), or -thymus (CFU-T). CFU-S can generate myeloid, lymphoid and erythroid cells (Fig. 1.31) and, in the presence of stimulating factors (M-CSF, GM-CSF) (Table 1.5), mixed colonies of polymorphonuclear leukocytes or PMNs and macrophages. Mononucleated phagocytes, having achieved their maturation in the bone marrow, pass into the bloodstream for a short period of time, which leave to enter the tissues through capillary walls by diapedesis in response to chemotactic factors released in inflammatory processes (see "Innate Immunity", p. 152). Strictly speaking, phagocytes are granulocytes and monocyte macrophages with the particular uptake of vital strains, as numerous other cells are widely distributed throughout the body (macrophages of alveoli, spleen, lymphoid tissue, and histiocytes, Kupffer cells, osteoclasts, chondroclasts, mesangial cells of the kidney and microglial cells of the brain), and constitute the reticuloendothelial system (RES), comprising other phagocytes in a broad sense (Fig. 1.32) [470].

Monocytes (Fig. 1.32, a–c) derive from bone marrow promonocytes under the action of specific mediators (CSF). They have a diameter of 9–15 µm, and a round, oval or indented nucleus in an eccentric position with one or two prominent nucleoli [609]. The first cells to circulate in blood, but only for about 1–2 days, they migrate via blood-vessel walls, then are discharged into various tissues where, because of the stimuli of differentiation signals typical of each tissue, differentiate into resident macrophages. In certain tissues they become fixed or may enter internal cavities. During this period, the resting cells are activated by IFN-γ, a process also increasing the transcription of new genes [474]. Moreover,

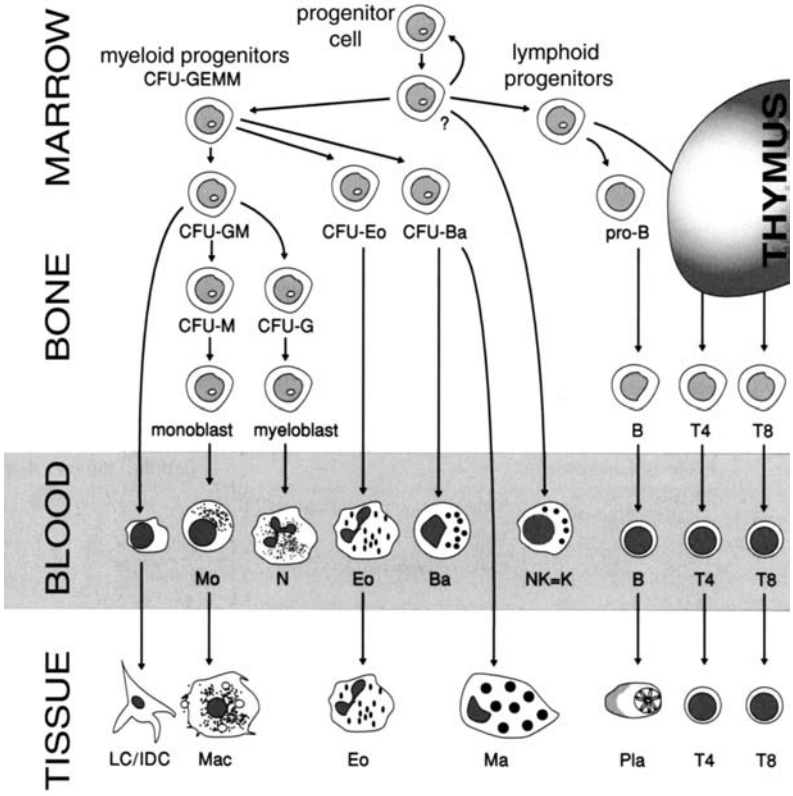


Fig. 1.31. Hemopoiesis: myeloid and lymphoid differentiation and the tissue compartment in which they occur. *Ba* Basophils, *IDC* interdigitating dendritic cells, *LC* Langerhans' cells, *CFU* colony forming unit, *Eo* eosinophils, *CFU-GM* colony forming unit-granulocyte-macrophage, *Mac* macrophages, *Mo* monocytes, *N* neutrophils, *Pla* plasma cells, *T4* CD4, *T8* CD8

IFN-γ-primed monocytes exposed to LPS showed enhanced phosphorylation of IRAK (IL_1R-associated kinase) and increased NF-κB DNA binding activity [49]. Mature cells, before differentiating lose CD34, an early marker of hematopoietic progenitor cells and of other immune cells, and CD62L ligand, whose delayed maturation could be, for example, an SCID marker. Monocytes express normal levels of CD13 and CD33, and high levels of $CD14^+/CD11c^+$ useful for their identification, in addition to a greater number of membrane receptors for IgG Fc fragments. CD14 engagement on monocytes could also inhibit human Ig synthesis, including IgE antibodies [608]. The phenotype and function of monocytes are modulated by several ILs and include IL_1RA, IL_2, IL_3, IL_4, IL_7, IL_8, IL_{10}, IL_{12}, IL_{13}, IL_{15}, IL_{16}, IL_{18}, IL_{19}, GM-CSF, TNF-α and -β, IFN-γ [73, 609]. The TNF and IL_{12} induction was dramatically increased in IFN-γ-primed monocytes [49]. The release of these ILs by monocytes can be modulated by different infectious and noninfectious stimuli. Monocytes also yield MCP 1–3 (see later) several ILs, such as IL_1RA, IL_8, TNF-α and -β [73], and MIF (monocyte migration inhibiting factor) [73, 609]. As IgE antibodies bind to their receptors, they acquire bactericidal and cytotoxic properties producing $O_2^{\bullet -}$ (superoxide), apparently due to activation of NADPH (reduced nicotinamide-adenine dinucleotide phosphate)-oxidase [115]. They are endowed with CD23 [115] and FcεRI, with a stronger binding to IgE [347], also becoming capable of engulfing complexed IgE-peptides subsequently presented to T lymphocytes [347]. In atopic patients they provide the RNA with IL_{13} transcripts [219].

Macrophages [540, 593] (Fig. 1.32, d, e) have variable dimensions (15–50 μm) and probably a structural and functional heterogeneity like mast cells. They are particularly active, together with PMNs, in defense against infections, playing a pivotal role in immune responses via a wide range of functions performed (Table 1.20) [68, 540, 609]. Although referred to by a variety of names depending on the location, they have mostly common features: in addition to being APCs, they are avid phagocytes engulfing any bacteria, cellular debris, or foreign particulate materials in the area. Stimulated by the ILs produced during the recognition phase, they are attracted into a site of injury and acquire the phagocyte's typical aspect. Macrophages undergo several morphological, functional and metabolic changes, with a parallel increase in size, adhesion, endocytosis (both pinocytosis and phagocytosis), lysosomal enzymes and chemotaxis, as well biochemical-metabolic activities, enhancing their equipment of enzymes of the respiratory chain, the Emden-Meyerhof way, and hexose-monophosphate shunt [540]. Thus positioned along capillaries, macrophages readily make contact with and engulf invading antigens and pathogens, which are broken down with the aid of the cited enzymes into simple amino acids, glucides and other substances, for a subsequent excretion or recycling, and eventually remove them from circulation [47]. These cells also express HLA class I and II molecules for CD4 and CD8. Due to receptors for IgG

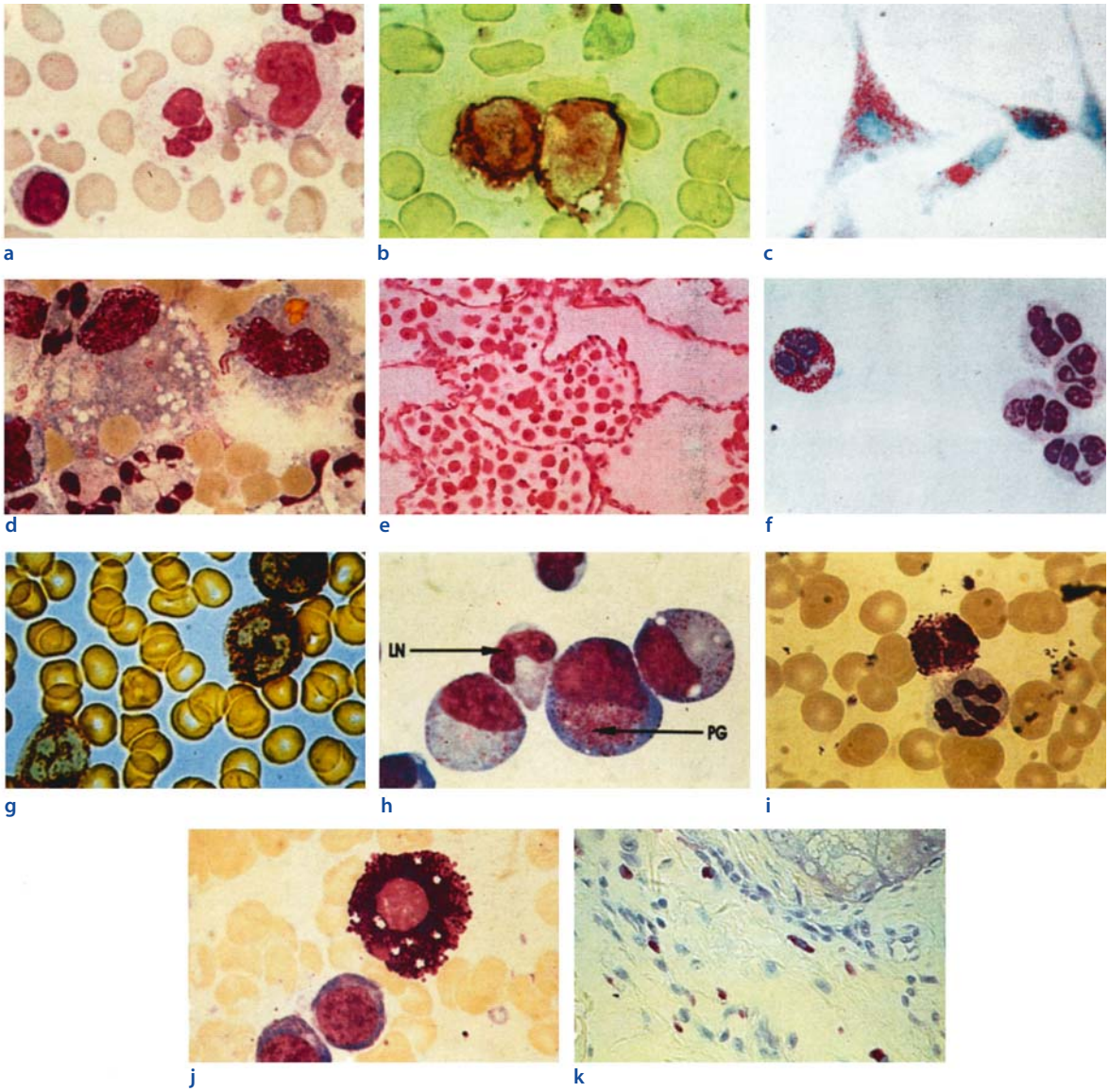


Fig. 1.32 a–k. Cells of the immune system. **a** Monocyte showing a horseshoe-shaped nucleus and moderately abundant pale cytoplasm. Note the three multilobed polymorphonuclear neutrophils (*PMNs*) and the small lymphocyte (*bottom left*). **b** Two monocytes with vacuolated cytoplasm. The small cell with focal staining at the top is a T lymphocyte. **c** Monocytes in monolayer cultures after phagocytosis of mycobacteria (stained *red*). **d** Inflammatory cells showing a large active macrophage in the *center* and phagocytosed red cells and prominent vacuoles. To the *right* is a monocyte with horseshoe-shaped nucleus and cytoplasmic bilirubin crystals (hematoidin); several multilobed PMNs are clearly delineated. **e** Numerous plump alveolar macrophages within air spaces in the lung. **f** Four PMNs (neutrophils) and one eosinophil. The multilobed nuclei and the cytoplasmic granules are clearly shown, those of the eosinophil being heavily stained. **g** A PMN neutrophil showing cytoplasmic granules. **h** Early neutrophils in bone marrow. The primary azurophilic granules (*PG*) originally clustered near the nucleus move toward the periphery and as the cell matures, the neutrophil-specific granules are generated by the Golgi apparatus. The nucleus gradually becomes lobular (*LN*). **i** Basophil with heavily stained granules compared with a neutrophil (*bottom*). **j** Mast cell from bone marrow with a round central nucleus surrounded by large darkly stained granules. Two small red cell precursors are shown in the *bottom*. **k** Tissue mast cell in skin stained with toluidine *blue*. The intracellular granules are metachromatic and stain *reddish purple*. Note the clustering in relation to dermal capillaries

Table 1.20. Secretory products of macrophages

Enzymes
Arginase
Angiotensin converting enzyme
Lipoproteinlipase
Lysozyme
Neutral proteinases Alveolar macrophage elastolytic metalloproteinase Collagenase specific for collagen of basal membrane (type IV) Collagenase specific for interstitial collagen (types I–III) Collagenase specific for pericellular collagen (gelatinase) (type V) Cytolytic proteinase Elastases metallo-dependent Plasminogen activator Stromelysin
Acid hydrolases Glycosidases Lipases Nucleases Phosphatases Proteases and peptidases Others
Plasma proteins
α_2-Macroglobulin
Apolipoprotein E
Fibronectin
Inhibitor of α_1-proteinase
Tissue inhibitor of metalloproteins
Transcobalamin II
Coagulation factors Factors V, VII, IX, X Thromboplastin
Complement components C1–C9 Factor B Factor D Factor H (β_1H) (C3 convertase inactivator) Factor I (C3b inactivator) Properdin
Reactive O_2 species
H_2O_2
Superoxide anion
Others
Bioactive lipids
6-Ketoprostaglandin $F_{1\alpha}$
12-Hydroxyeicosotetranoic acid
LTC
PGE_2
Tromboxane B_2
Others

Table 1.20. (Continued)

Nucleotides
Adenosine
cAMP
Guanosine
Thymidine
Uracil
Factors regulating cell functions
IL and IL-like
Activin
Erythropoietin
Fibroblast growth factor
Insulin-like growth factor
Heparin-binding growth factor
Inhibiting factor for leukemic cells
Platelet factor 4
G-CSF
GM-CSF
IFN-α and -β
IL_1
IL_6
IL_8
IL_{10}
IL_{12}
IL_{15}
IL_{18}
M-CSF
MCP, MIP 1 and 2 and other chemokines
PDGF
CCL chemokine receptor 1
Receptor of IL_1 antagonist
TGF-α and -β
TNF-α
Factors promoting the proliferation of: B cells Endothelial cells T cells Fibroblasts
Factors inhibiting the proliferation of: *Listeria monocytogenes* Tumor cells

Data from [68, 540, 609].

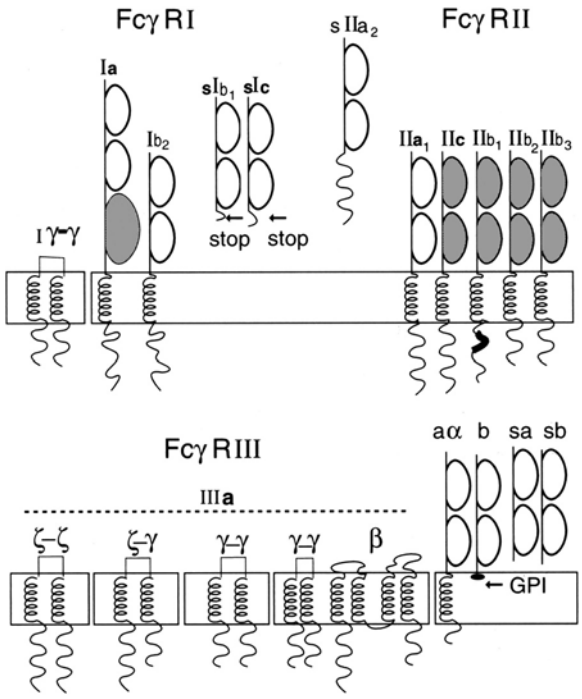


Fig. 1.33. Structure and properties of Fcγ receptors. *R* receptor

Fc fragments, they attract potential targets to be processed later, modified by enzyme digestion and subsequently presented to T cells. They possess membrane receptors for [540, 609]:

- *Igs:* IgG = FcγR, with three distinct types, FcγRI (CD64), FcγRII (CD32), and a low-affinity receptor (FcγRIIb) (Table 1.3; Fig. 1.33), in addition to the specific Fc receptor, which promotes phagocytosis of particulate antigens such as antibody-coated bacterial antigens, a phenomenon called opsonization.
- *Complement* (C3b, C5a).
- *Hematopoietic growth factors* (M-CSF, GM-CSF), lipoproteins, peptides and PS.
- *Membrane receptors* the best characterized of which are FcεRII (bind to IgH chains) and CR3/CD11b (fix iC3b) [445].

Macrophages are versatile secretory cells: they have ≈ 100 receptors on their surface, including complement proteins, active O_2 radicals, bioactive lipids such as PGE_2, PAF (platelet-activating factor), nucleotides and arachidonate metabolites and several enzymes including lysozyme, acid hydrolases, neutral proteases, and enzyme inhibitors (Table 1.20). Macrophages generate CCL chemokines, including MIP-1α and -1β (macrophage inflammatory protein-1α and -1β) and MCP-1 [540, 609]. Like monocytes, they are activated by IFN-γ and MIF, in certain conditions acquiring cytotoxic or suppressive properties, in addition to contributing to lymphocyte responses as accessory cells, releasing ILs such as TNF, IL_6, IFN-γ via IL_1 and IL_{12}, as well as IFN-α and TGF-β [488]. Macrophages synthesize IL_{18} as an IFN-γ induction factor [669]. In turn, IFN-γ induces in macrophages IL_{12} and $IL_{12}R$, whose activity depends on Th1 T-cell activation [124]. IL_{15} can also be a macrophage endogenous product [67]. Four ILs instead deactivate these cells: IL_4, IL_{10}, IL_{13} and TGF-β [124]. Correspondingly, macrophages are pluripotent cells supporting opposite actions depending on their local microenvironment: for example, the response to IL_2 can activate T-cell proliferation or suppression and CMI [124]. Probably, CD28 expression by IL_{12}-producing macrophages can lead to generation of Th1 T-cell responses [387], anticipating that CD28 binding to CD80 represents a costimulatory signal for T lymphocytes. Because the number of monocytes and macrophages can increase 3- to 100-fold at a site of inflammation, the regulatory and effector roles of these cells become even more prominent after an inflammatory response has begun. Also prominent is the portfolio of chemokines that attract these cells to a site of inflammation, which include CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22 [87].

Granulocytes live only a few days; have the main function of ingesting and kill any non-self substance. C/EBPα is essential for their development [678]. Cells of granulocyte lineage, PMNs, eosinophils, and basophils are distinct according to cytoplasmic granule staining. Granulocyte surface markers are shown in Table 1.21 [470] and compared with metachromatic cells.

Neutrophils (Fig. 1.32, d, f, g, h) are the most numerous leukocytes in the bloodstream (half-life, 4–10 h), with a normal adult having >100 billion PMNs present daily, a number that can increase to almost 1 trillion when the host is stressed by infections. PMNs carry on

Table 1.21. Surface markers of neutrophils or PMN and eosinophils compared with metachromatic cells

Cells	Cell markers								
	CD88	CD35	CD11a	CD11b	CD49d	CD32	CD16	FcεRI	FcεRII (CD23)
Neutrophils	+	+	+	+	+	+	+	–	–
Eosinophils	+	+	+	+	+	+	±	+	+
Basophils	+	+	+	+	+	–	+	+	–
Mast cells	+	+	+	+	–	–	+	+	–

Modified from [470].

the following activities: chemotaxis, phagocytosis, degranulation, and opsonization [360]. PMNs are involved in the immune inflammation in concert with eosinophils and platelets, also determining within such processes the delayed phase of allergic reactions. Prolonged ADAM17 (a disintegrin and a metalloproteinase 17) expression during neutrophil effector functions and apoptosis may play a role in both the induction and downregulation of neutrophil activity [617]. Primed PMNs in the presence of tissue injury due to microbial agents produce factor(s) which inhibit some of the cell's antimicrobial functions contributing to immune dysfunction, while the factor(s) produced by unprimed PMNs facilitate antimicrobial countermeasures [407]. High mobility group box 1 (HMGB1) protein increases the nuclear translocation of NF-κB and enhances the expression of proinflammatory ILs in human PMNs. These effects appear to involve the p38 MAPK, PI3K (phosphatidylinositol 3-kinase), and ERK1/2 pathways. However, the mechanisms of HMGB1-induced neutrophil activation are distinct from endotoxin-induced signals [407]. They have receptors for:

- *Igs:* IgG=FcγRII and FcγRIII (CD16) and IgA=FcαR (CD89), while PMNs appear to lack IgE receptors.
- *Complement:* C3b, C3a, C5a; C5a is different in structure and affinity from that of eosinophils.
- *LTB_4* (LTB_4), GM-CSF, and G-CSF.

As APCs, PMNs have only limited potential as they synthesize only HLA class I molecules. PMNs have a rich equipment of proteins and mediators, and release several immunoregulatory ILs, modulating both cellular or humoral immunity (Table 1.22) [73, 315]. During acute inflammations, IL_1 enhances T-cell activation, also inducing IL_6, IL_8 and GM-CSF. Recruited early to injured sites, PMNs release M-CSF, hence activating the more slowly invading monocytes [315].

PMNs are activated by NAP (neutrophil activating factor)/IL_8 released by PBMCs (peripheral blood mononuclear cells) and then recruited to inflamed sites by NCF (neutrophil chemotactic factor) and LTB_4, utilizing CD11/CD18 integrin ligands of CD54 (ICAM-1) and CD102 (ICAM-2, intercellular adhesion molecule 1,2) to adhere to vascular endothelium (margination) [23]. PMNs infiltrate injured sites ingesting whatever foreign protein or cellular debris they encounter, including bacteria and CIC (circulating immune complexes)-IgG by virtue of specific receptors, releasing enzymes with lytic action responsible for the maintenance of immune inflammation [135]. When activated, the enzyme NADPH oxidase attached to the cytoplasmic membrane produces monovalent hydroxyl radicals with oxidant properties and oxidized metabolites [663]. During phagocytosis and especially when antigens adhere to the PMN surface and are therefore not phagocytosed, PMNs undergo a respiratory burst, degranulate and fully mobilize their secretory vesicles. Their cytoplasmic membrane fuses with the intracellular granules, finally emptying their enzyme content into the cell lumen

Table 1.22. Proteins and other mediators produced by PMNs

Efferent mediators
CR1
CD11b/CD18
FcR
Class II MHC
Plasminogen activator
IFN-α
PAF
LTB_2
Heat shock proteins
Fibronectin
PGE_2
Afferent mediators
M-CSF
$IL_1\beta$
IL_1RA
IL_3
IL_6
IL_8
GM-CSF
IFN-α
TGF-β
TNF-α

Modified from [73, 315].
CR complement receptor, *IL_1RA* IL_1 receptor antagonist.

[135]. The secondary granule proteins (SGP) are secreted in a hierarchical manner: first gelatinase-containing granules, then specific granules, and last azurophilic ones [135] (Table 1.23 [315], Fig. 1.32 g). In the later stages of myeloid development, the SGP genes are coordinately upregulated, and members of the C/EBP family of TFs, in particular C/EBPα and C/EBPη, play specific and unique roles in upregulating their expression [257]. Among the many granule enzymes (>20), MPO (myeloperoxidase), elastase, collagenase and gelatinase play a prominent role in cytotoxicity, also attacking and degrading above all connective tissues [630]. The mature granules also contain Igs, complement proteins, clotting factors, cationic proteins and defensins (see "Innate Immunity" p. 152). The CCAAT-enhancer binding protein (C/EBP) family of nuclear TFs is implicated in the regulation of terminal myeloid differentation. In particular, C/EBPα and C/EBPη play specific and unique roles in upregulating SGP expression [257, 646]. The abnormal PMN accumulation in states of acute inflammation consists of several processes taking shape in sequence:

Table 1.23. Constituents of specific granules and azurophil granules of human neutrophils

Specific granules	Azurophil granules	
Histaminase	Myeloperoxidase	Elastase
Collagenases	Acid phosphatase	Histonase
Binding vitamin B_{12} protein	β-Glucosaminidase	Lysozyme
Laminin receptor	5′-Nucleotidase	Cationic proteins
C3b receptor	α-Mannosidase	BPI
Receptor of formylated peptides	Arylsulfatase	Defensins
Cytochrome b558	α-Fucosidase	Glycosaminoglycans
Lactoferrin	Neuraminidase	Chondroitin sulfate
Flavoproteins	Cathepsin D	Heparin sulfate
Lysozyme	Cathepsin G	

Modified from [315].
BPI bactericidal/permeability inducing protein.

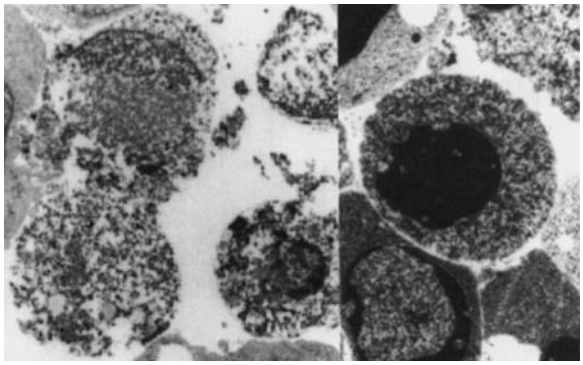


Fig. 1.34. Necrosis and apoptosis: ultrastructural aspects. Cells undergoing necrosis (*left*) or apoptosis (*right*)

endothelium and PMN activation, adhesion, diapedesis and phagocytosis [315]. Besides their essential defensive functions, PMNs are coming under scrutiny whereby they may cause excessive injury to host cells, since a potent cytotoxicity in acute inflammation is potentially damaging. PMN persistent accumulation involves an excessive secretion of O_2 toxic radicals and proteases, with amplification of tissue injury following LTB_4, LTC_4 (leukotriene C_4) and TXA_2 (thromboxane A_2) synthesis [630]. It has been suggested that such negative regulation is favored by the density reduction observed in allergic subjects [364]. Aged neutrophils undergo *apoptosis* [276]: endogenous activations of endonucleases allow PMNs to be recognized and engulfed intact by macrophages to avoid dispersion of cytotoxic products [510] (Fig. 1.34). Early neutrophil influx into the airways after allergen challenge is mediated by IL_1, IL_{18} and p38 MAPK and can be reduced by inhibiting either IL or p38 MAPK. However, both the neutralization of these ILs and reduction of PMN number do not modify the later development of eosinophilic airway inflammation or the BHR (bronchial hyperreactivity) insurgence, thus suggesting that the early and transient neutrophil response fails to play a direct role in the development of allergen-induced BHR. The effects of inhibiting p38 MAPK in decreasing BHR indicate activities independent of its prevention of PMN accumulation [569].

Eosinophils (Fig. 1.32f, 1.35a) [680], present in peripheral blood at a mean concentration of 300–400 cells/m^2 from 0 to 2 years and of 200–250 cells/m^2 from 4 to 21 years, survive longer than other granulocytes (half-life, 6–12 h). Eosinophils migrate into the thymus during the neonatal period, localize to the corticomedullary region and attain maximum levels by 2 weeks of age. A second influx of eosinophils to the medullary region is observed at 16 weeks of age, when the thymus begins to involute [477]. Even after leaving the bone marrow together with CD34, they continue the synthesis process and can return into the bloodstream from tissues; however, compared to PMNs, they are inconclusive as phagocytes and less efficient at intracellular killing [631]. Eosinophils are present in allergic, immunological, parasitic disorders and hypereosinophilic syndromes. They can be activated either *in vivo* or *in vitro* gy diverse agonists, such as Igs, lipid mediators, and ILs [371] and from CD69, CD44, and CD54 [670]. On activation, they probably kill parasites mainly by releasing cationic proteins and reactive O_2 metabolites into the extracellular fluid [623]. Eotaxin induces a rapid concentration-dependent activation of ERK2 and p38 in eosinophils, and the activation of these kinases is required for eotaxin-induced eosinophil chemotaxis, actin polymerization, and degranulation. It is therefore proved that eotaxin plays an integral role in the development of eosinophilic inflammation in asthma and allergic inflammatory diseases [247]. Key eosinophil regulatory ILs such as IL_5 and the eotaxin subfamily of chemokines regulate eosinophil production and localization at baseline and during inflammatory responses

Table 1.24. Surface molecules of human eosinophils (excluding Ig receptors and complement components)

Adhesion molecules
CD11a/CD18
CD11b/CD18
CD11c/CD18
CD15
CD31
CD44
CD49a/29
CD49b/29
CD49c/29
CD49d/29
CD49e/29
CD49f/29
CD62E
CD62L
CD62P
CDw65

Ig supergenes
CD9
CD13
CD24
CD43
CD45
CD50
CD54
CD102

Ig supergenes (Continued)
CD46
CD47
CD50
CD59

Inducible molecules
CD4
CD16
CD23
CD25
CD54
CD64

Receptors
Adenosine
β_2-Adrenoreceptor
FMLP
GM-CSF
IFN-γR
IL_3R
IL_5R
IL_8R
LTB_4
PAF
TNF-αR

Modified from [197].
FMLP formylmethionyl leucylphenylalanin.

[477]. *In vitro* studies and those on BALF (bronchoalveolar lavage fluid) show that the regulation of eosinophil production is highly dependent on GM-CSF, IL_3 and IL_5 [550]. Such ILs play a pivotal role in promoting eosinophil maturation, remarkably influencing both differentiation in the bone marrow and activation in the tissues [61]. GM-CSF and IL_3 particularly increase the number of precursor cells prolonging their survival, IL_5 acting primarily as a selective chemotactic factor mediates their maturation and triggers degranulation and adhesion [34]. GM-CSF and IL_5 further activate eosinophil cytotoxicity and oxidative metabolism and, added to culture with CD34, increment notably their number, making the cells functionally mature on the 21st day [515]. Since during intense responses eosinophils can undergo cytolysis, the detection of cationic proteins is a useful marker of their involvement (Chap. 7). Eosinophils can be engaged to express HLA class II molecules and act as APCs [632]. Eosinophils also express CD48, CD58, CD84, CD244/2B4, but not CD [371] on their surface. Table 1.24 [197] summarizes the most significant data on surface molecules and Tables 1.5–1.8 detail IL effects on eosinophils playing a vital part in inflamed lesions of allergic disorders. Accordingly, eosinophils yield [631]:

- Fc receptors for Igs: both IgE receptors, FcεRI and FcεRIIb, present chiefly on the hypodense phenotype, with an affinity comparable to that of FcγRII (CD32) for IgG antibodies and FcαR; IL_4 and other unidentified factors amplify FcεRI α chain expression on eosinophils [573], making them capable of diffusing their cationic proteins.

- Receptors for complement components: C1q, C3a, C3b/C4b (CR1=CD35), iC3b (CR1, CR3) and C5 are different from neutrophil receptors. C3a, C4a and C5a are called *anaphylatoxins,* a term derived from anaphylaxis-like responses produced when such peptides were injected in experimental animals [159].
- Receptors for GM-CSF, IL_3, IL_5 and IL_8 are further potential sources of IL_1, IL_4, IL_6, TGF-α, TNF-α and MIP-1α [46, 477]. Signal-transduction molecules implicated in these IL-mediated priming responses include Lyn, JAK2, PTK, and p21 [46].
- A functional CD244R cross-linking on the surface of eosinophils which elicits ERK, activates NK cells, and causes eosinophils to release EPO, IL_4 and IFN-ɣ can contribute to eosinophil effector functions in both Th1- and Th2-like responses, thus indicating a broader role for eosinophils in health and disease [255].
- Receptors for chemokines, including eotaxin, MCP1–4, MIP-1α and β (now called CCL3, CCL4), and RANTES (regulated on activation normal T expressed and secreted), now called CCL5 [687].

Whether eosinophils generate additional ILs is unclear, since circulating cells, unlike BALF cells, fail to express ILs [550]; however, they can secrete, as no other cell does, the *CD40–CD154 couple* [175], with substantial repercussions on B cell isotype switching to IgE phenotype, in addition to synthesizing IL_4, especially in the airways [397].

Eosinophils contain substantial intracellular quantities of several granule- and vesicle-associated IL receptors, including IL_4R, IL_6R, and $IL_{13}R$ as well as CCR3. A temporal coincidence of $IL_4Rα$ and IL_4 mobilization from granules into the vesicles was combined with a clear association of IL_4 with secretory vesicle membranes, thus suggesting that eotaxin-mobilized $IL_4Rα$ functions as a transporter for IL_4 via the secretory pathway. The intracellular ILRs localization possiby extended to granules of innate immune system cells. Mast cells and neutrophils may play a role in the secretion of granule-derived ILs from both these cells comparable with the ILs recognized in eosinophils. This suggests that several additional ligand-binding receptor chains such as an ILR chain specific for eosinophil-derived ILs and chemokine secretion may provide a crucial component of the regulatory mechanisms governing specificity of rapid, stimulus-induced release of preformed immunomodulatory ILs from human eosinophils, as well as other innate immune cells containing granule stores of preformed ILs and chemokines [532].

Eosinophils also express:

- $β_2$ integrins facilitating their migration from blood into normal and inflamed tissues such as CD11c (p 150,95), the adhesion molecules CD54, its receptor CD11a/CD18 and VCAM-1 (vascular cell adhesion molecule), CD106, and IL-induced endothelial protein (appears 6–12 h after stimulation), playing a critical role in eosinophil adhesion to activated vascular endothelium and epithelium, their extravasation, tissue localization and interplay with other cells [197].
- The surface molecule CD4 identified on T4 lymphocytes binding to HIV-1 glycoprotein 120 (HIV-1 gp120). So far the role of CD4 is less clear; however, it might act as a signal transducer, as demonstrated by the capacity of its three ligands (HIV-1 gp120, bivalent monoclonal anti-CD4 antibody and chemotactic factor of leukocytes) to trigger eosinophil migration but neither their degranulation nor their superoxide formation [593].
- GM-CSF, IL_3, IL_5, TNF-α, and RANTES positively regulating the last two properties [210].
- HLA-DR class II synthesized by mature cells stimulated by GM-CSF, IL_3, IL_4, IFN-ɣ [594], thereby mediating interactions of other APCs with $CD4^+$.
- Eicosanoids, released in particular conditions from membrane phospholipids, including LTB_4, LTC_4 prostaglandins (PG) such as PGE_2, and PAF, substances with a well-known bronchoconstrictor action, bradykinin, H_2O_2, $O_2^{\bullet -}$ and several enzymes. They include peroxidase and additional enzymes with oxidoreductive power, among which histaminase (inactivating histamine) is the best known, digestive enzymes (proteases, nucleases, other hydrolases such as kininase), anti-inflammatory enzymes, aryl sulfatase B specifically inactivating LTs, one of the main mediators of bronchospasm and immune inflammation, phospholipase D (PLD) inactivating PAF, catalase, collagenase, β-glucuronidase, and non-enzymatic molecules of which plasminogen is the most notable [470].

Eosinophils are also active in immune reactions with a *destructive armamentarium at the tissue level,* initiating highly damaging actions committed to cationic proteins and O_2 radicals. Histamine released by skin mast cells and other chemotactic factors, PAF in the first line, C5a, IL_{16} and various chemokines such as eotaxins 1, 2, 3 [435] selectively recruit eosinophils to the site of inflammation where they release cationic proteins and other mediators [581]. Whether cells are activated during their migration is not clear, but certainly the involvement of chemotactic factors and interplay with the extracellular matrix (ECM) expedite their progression, including signals transmitted from adhesion molecule receptors, which, all findings considered, involve cell activation and mediator release [625].

Diversely from other granulocytes, eosinophils have typical secondary granules, containing four distinctive cationic proteins. All of them yield toxic actions at the cell level, *directly damaging host cells and tissues.* MBP (MBP-1 and MBP-2; major basic protein) of 14 kD, elaborated also by basophils and rich in reactive sulfydryl groups, has no inflammatory property, but via a direct cytotoxic mechanism, has adverse effects extended to mono- and multicellular parasites and to human cells, including bronchial epithelium. MBP produces pomphoid reactions, *induces ciliostasis,* activates neutrophils and platelets and neutralizes heparin, pro-

vokes degranulation of metachromatic cells and histamine release, making a relevant contribution to the perpetuation of inflammation. A likely cause of its accumulation in AD lesions is the IgE-mediated delayed reaction [593]. In asthmatic patients, it provokes *BHR*, inhibited in vivo in animal studies by a specific antiserum [294]. MBP is the crystalloid core of secondary granules, while the matrix surrounding it contains other cytotoxic proteins: all are among the more destructive mediators, not released by extrusion from the entire granule (as in the case of mast cell reaction), but following a process of granule exocytosis, unlike PMNs, to kill foreign substances internally [337]. About 90% of granule proteins are represented by EDN (eosinophil-derived neurotoxin), or EPX, of 18.6 kD, cytotoxic and neurotoxic, interfering with CMI. EPO (eosinophil peroxidase), consisting of two 15- to 55-kD polypeptides, carries on the same routine as EDN, interferes with coagulation and fibrinolysis, degranulates metachromatic cells and has ribonuclease properties. ECP (eosinophil cationic protein), 18–21 kD, more cytotoxic than EDN and up to tenfold more potent than MBP, degranulates mast cells and has a peroxidase activity [631]. Release of such proteins is selective, because EPO release takes place subsequently to the others, perhaps in different stages of activation. The remaining 10% of proteins are formed by hexagonal and bipyramidal Charcot-Leyden crystals (CLC), present also in basophils. CLCs, first observed in 1872, belong to the type-S lectin superfamily; they can neutralize natural lung surfactants, causing atelectasis [631]. Both eosinophil activation and release of cationic proteins are IgA-mediated [581], via production of IgA antibodies endorsed by IL_5, SC binding, and consequent IgA-mediated eosinophil degranulation [572]. If ECP reduces IgA production and alters oral tolerance this could confirm that eosinophil detrimental activity is a prominent pathological feature at the expense of mucosal surfaces. Evidence suggests that eosinophil damaging activity is also committed to their oxidative products, such as reactive O_2 radicals, triggered by PAF and to a higher extent by C5a, while EPO will oxidize a variety of substrates in the presence of H_2O_2 (Fig. 1.35b) [680], with resulting production of other potent O_2 radicals, among which is singlet O_2 ($^1O_2$) [680]. PAF deriving from T lymphocytes is also active on mature cells and on the most potent chemotactic factor for eosinophils also due to O_2 uptake, $O_2^{\cdot -}$ release and iC3b increase in binding capacities [673].

Recent studies have critically revisited eosinophil phenotypic changes, showing that peripheral cells may be distinguished on the basis of their *hypodensity* (of sedimentation), chiefly under GM-CSF, IL_3, IL_5 [631], C5a and PAF effects [680] (Fig. 1.35c), but such cells are also found in normal nonatopic subjects [649]. Furthermore, in addition to speed cell survival, the hypodense phenotype can more easily bind to IgE antibodies, thereby appearing more metabolically active with IL_3 intervention and above all with IL_5 [367]. Certainly such change is not synonymous with activation [680], of which a more sensible parameter is LTC_4 synthesis under GM-CSF, IL_3 and IL_5 stimulation [631]. Eosino-

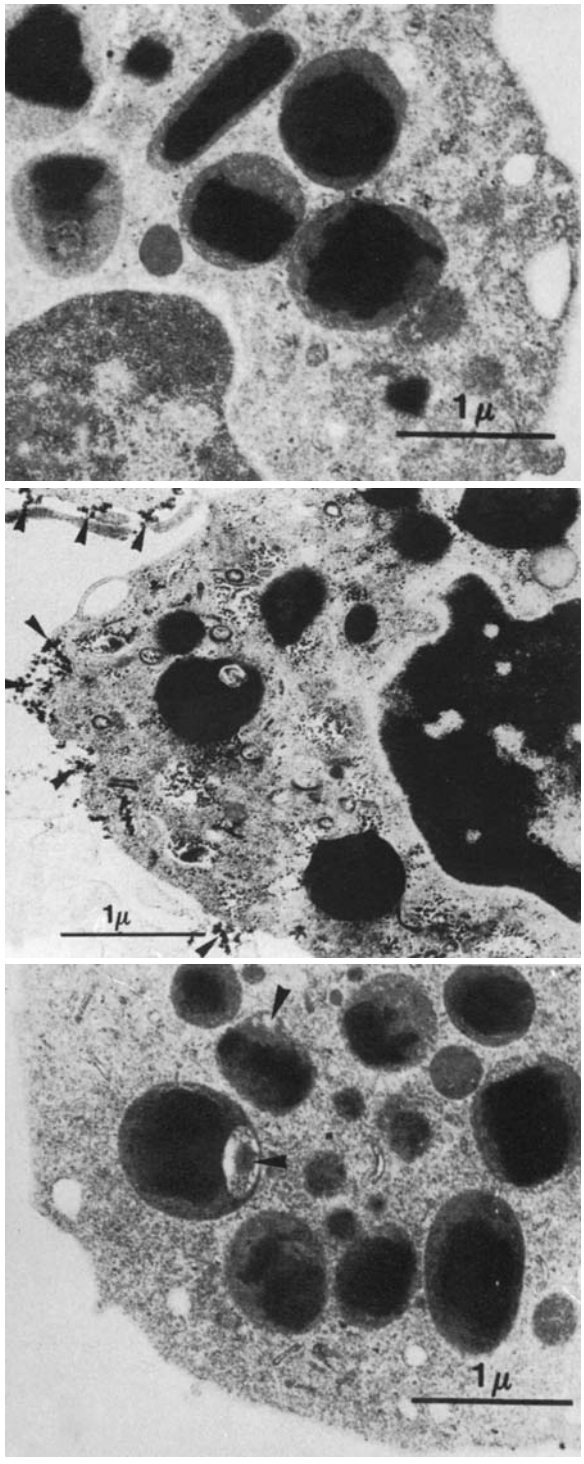


Fig. 1.35 a–c. Ultrastructural aspects of eosinophil activation (electron microscopy, EM). **a** Unstimulated eosinophils. **b** Eosinophils stimulated with PAF: *arrowheads* indicate production of H_2O_2. **c** Eosinophils stimulated with PAF and C5a: *arrowheads* indicate *hypodense cells*

phil exposure to activating ILs leads to the development of these hypodense eosinophils, with a specific gravity of <1.085 g/ml. Increased numbers of hypodense eosinophils are found in many allergic disorders, including AR and asthma [46]. In children these hypodense cells may be *truly immature* (Chap. 11): these findings could explain why ECP levels, activation markers, are not augmented in children compared to adults [367]. Studies have given rise to the hypothesis that hypodense cells are of two types, one with features similar to normodense cells [631].

Mast cells and basophils (*metachromatic cells*), cells prominently involved in immediate and late reactions, have in common cytoplasm granules containing histamine, heparin, serotonin or 5-hydroxytryptamine (5-HT) and kinins. Immature progenitors of human mast cells (SCF=c-kit=CD117) are present in fetal liver cells, bone marrow and CB, enter a peripheral tissue still without secretary granules and cell surface FcεR1, and then complete their differentiation probably with multipotential capabilities in connective tissues and mature beneath epithelial tissues and in areas adjacent to blood and lymph vessels, and near or within nerves [168, 509]. Basophils differentiate and mature in the bone marrow and circulate in the bloodstream (CDw17), but only rarely in connective tissues [341]. Basophils develop largely under the influence of IL_3, a process that is increased by TGF-α [509]. IL_3 present in cultures with CD34 promotes basophil and mast cell development up to 16%–28.5%; however, addition to the medium of GM-CSF and IL_5 reduces the rate to 3%–15% in favor of eosinophils [515]. Mast cells, which Ehrlich called "overfed cells" because their cytoplasm is filled with granules [136], abound in lymphoid organs, connective tissues of most organs, in particular on the epithelium and airway lumen, sensitive to IgE-dependent stimuli, while in blood and lymph there are instead basophils [593]. Mast cells and basophils migrate toward the chemokine gradient at a site of inflammation but stop and accumulate at the site where allergen concentration is high [580]. Both cell types, real biochemical "powder-kegs," interact with allergens, degranulate and release mediators with different pharmacological actions, and provoke clinical manifestations of immediate hypersensitivity, such as vascular permeability increase, smooth muscle contraction, epithelial mucus secretion, chemotactic action on eosinophils, and platelet aggregation. Costimulation via FcεRI engagement with IgE/antigen and CCR1 engagement with human rCCL3 synergistically enhance the degranulation of metachromatic cells at the site where the cells accumulate, thus playing important roles in the orchestration and focusing of the allergic response. The progression toward chemoattractants requires actin cytoskelton rearrangement and polarization such as formation of leading edge and membrane ruffles [127]. However, FcεRI engagement affects CCL3-mediated actin reorganization and chemotaxis of CCR1 cells [580]. Moreover, Rac signaling and/or phosphatidylinositol (PI)4,5-bisphosphate synthesis by PI 4-phosphate 5-kinase I was required for membrane ruffling [127]. Only inflammatory cells provided with cytoplasm granules containing histamine and FcεRI [358], together with other cells, also express FcεRII a and b and can up-regulate and aggravate tissue inflammation [509]. Both cells are important sources of CD16 (FcγRIII), C5aR and CD35 [470]. In concert with adhesion molecules and receptors, they mediate the binding to other cells and to ECM gps, including CD49d and 49e/CD29 (β1 integrins), basophils add CD11a, CD11b, CD11c/CD18 (β2), and mast cells CD51/61 (β3) [196]. Metachromatic cells possess high-affinity receptors for IgE (FcεRI) and thereby become coated with IgE antiboidies [175]. *Activated metachromatic cells produce IL_4 and express CD154, with the immediate consequence that IgE antibody synthesis may also occur in peripheral tissues* [174], even independently of T cells. Therefore these cells are important in AD, asthma, and AR in which allergen binding to the IgE cross-links the FcεRI [347]. Several differences call for distinguishing these cells from functional, ontogenetic, histochemical points of view, and the quantity and quality of released mediators: Table 1.25 [166, 168, 340, 567] shows that differences prevail over analogies also regarding the immune stimuli activating them (Table 1.26) [340].

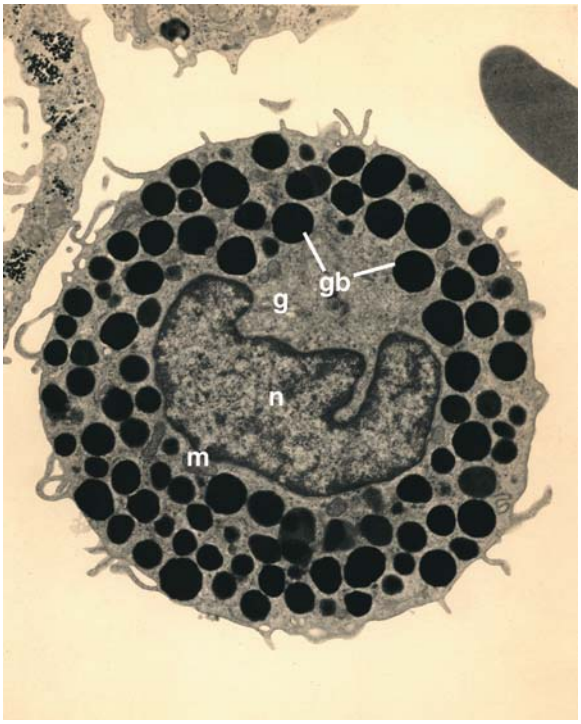


Fig. 1.36. Basophil, EM (×18,500). *n* Multilobed nucleus, *m* mitochondrion, *gb* basophil granules, *g* Golgi apparatus

Circulating *basophils* (Fig. 1.32 i, 1.36) have concentrations of 20–45 cells/mm^3, = 0.3%–0.6% of leukocytes, 6–60×10^5 FcεRI/cell (twice as much as mast cells), 0.04 pg/cell of tryptase [166, 168] (250- to 875-fold less

Table 1.25. Differences among mast cells and basophils

Characteristics	Mast cells	Basophils
Origin of precursors	Bone marrow	Bone marrow
Site of maturation	Connective tissue	Bone marrow
Mature cells in the circulation	No	Yes (<1 % of blood leukocytes)
Mature cells recruited from the circulation	No	Yes (immune inflammation)
Mature cells normally residing in connective tissues	Yes	No
Form	Round	Irregular
Cell diameter	10–15 μm	5–7 μm
Nucleus	Round or oval; eccentric	Bilobed or multilobed
Granulations	0.1–0.5 μm	10–15 μm
Cytoplasmic membrane	Less regular	More regular
Cell surface	Cytoplasmic protrusions	Smooth
Life span	Weeks or months	Days
Preliminaries to degranulation	The granules fuse intracellularly	Fusion of individual granules
Degranulation procedure	Chains of connected granules are released via newly formed channels to cell membrane	Fusion to cell membrane with extrusion of the granule
Cytoplasmic granules	Numerous and relatively small granules	Relatively few and large granules
Major granule contents	Histamine, chondroitin sulfate, neutral acid hydrolases, heparin, MBP	Histamine, chondroitin sulfate, proteases, neutral proteases, MBP and CLC
Mediators and other molecules	PGD_2, TNF-α, thromboxane A_2 (TXA_2), LTB_4, LTC_4, 5-HETE, PAF, NCF, ECF	PGD_2, LTC_4, LTC_4, TXA_2
LTC_4	60 ng/10^6 cells	60 ng/10^6 cells
PGD_2	60 ng/10^6 cells	0.006 ng/10^6 cells
TXA_2	5 ng/10^6 cells	0.005 ng/10^6 cells

CD	Receptors	Mast cells	Basophils
Interleukins			
25	IL_2R	–	+
116	GM-CSFR	–	±
117	SCFR, c-KIT	++	–
119	IFN-γR	?	±
121b	IL_1RII	–	+
123	IL_3R	–	+
124	IL_4R	–	+
125	IL_5R	–	+
128	IL_8R	–	±
Immunoglobulins			
NC	FcεRI	+	+
32	FcγRII	–	+

Table 1.25. (Continued)

CD	Receptors	Mast cells	Basophils
Complement			
11b	CR3	–	+
11c	CR4	–	+
35	CR1	–	+
88	C5aR	–	+
Adhesion molecules			
09		++	++
11a		–	+
18		–	+
29		+	+
31		–	+
41		+	–
43		++	++
44		++	++
49c, d, e/29		+	+
50		±	+
51		+	–
54		±	+
58		+	+
61		+	–
102		±	±
104		–	+
Additional receptors			
13		–	+
45		–	+
47		–	+

The receptors/antigens not expressed by both cell types are not shown.
Modified from [166, 168, 331, 376, 567].
NC not classified.

than TC and T mast cells) and ≈1 pg/cell of histamine, 25% of mast cells [341]. Although basophils account for only 0.5% to 1% of peripheral leukocytes their participation is emphasized in all allergic diseases, and the presence in tissues is correlated with affection severity. Despite their short life span and their reduced percentage, they characteristically increase in number in delayed-type responses, matching the rise of histaminemia [46, 332]. Circulating human basophils co-operate with eosinophils by playing a significant role in promoting allergic inflammation through the release of pro-inflammatory mediators [including ECP, MBP, histamine-releasing factor (HRF), IFN-γ, IL_4, and IL_{13}], capable of potentiating or priming histamine and LTC_4 release [46], as well as CD203c on blood cells exposed to recombinant allergens (RAs) [200] and represents a basis for a sensitive novel allergy test (Chap. 6). Basophils have several receptors (Tables 1.25, 1.26), and ILs as well as adhesion molecules regulate their chemotaxis [567]. Several chemokines induced chemotaxis at different potencies: eotaxin > SDF-1 > RANTES MCP-1 >> MIP-1 [239]. Their passage from blood vessels to inflamed tissues is correlated with the availability of specialized chemotactic factors, among which are factors of leukocyte derivation, kallikrein, C5a, specific antigens [528] and CD62L [625]. CD44 and CD54 are constitutively expressed on basophils, CD69 expression is preferentially and strongly upregulated by IL_3 [670]. Exposure of basophils to priming stimuli increases their sensitivity to FcεRI mediated-activation [46]. Basophil recruitment and activation may be facilitated by the CXCR4-SDR-1 receptor ligand pair [239]. The action of

Table 1.26. Immune stimuli activating human basophils and mast cells (*MC*)

Stimuli	Basophils	Lung MC	Skin MC
IgE-mediated			
Antigens	++	+	+
Anti-IgE	++	+	+
Anti-FcεRI	++	+	+
HRF	+	–	–
A protein	++	–	–
Fv protein	++	+	+
L protein	++	+	+
Not IgE-mediated			
C5a	+	–	+
IL-3	+	–	–
SCF	–	+	+
TNF-α	–	–	+
MBP	+	–	–
MCP-2	+	–	–
MCP-3	+	–	–
MCP-4	+	–	–
MIP-1α	+	–	–

Modified from [331].

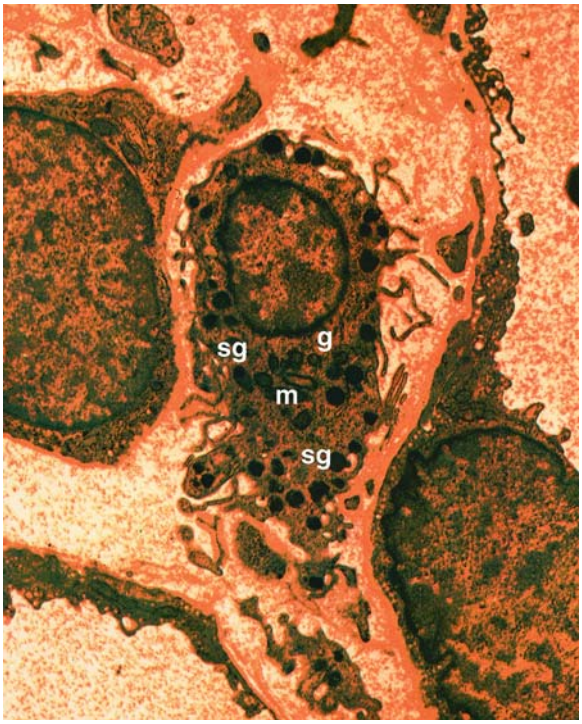


Fig. 1.37. Mast cell, EM (×26,000). *g* Golgi apparatus, *m* mitochondrion, *sg* secretory granules

anti-IgE, and in sequence by growth factors including GM-CSF, IL_3, IL_5, NGF, IL_8, IL_{18}, IFN, PAF and C3a, drive basophils to release histamine [46, 567, 669]. IL_{18} also stimulates IL_4 by basophils [669]. Histamine release was induced by MCP-1 SDF-1 > eotaxin > RANTES > MIP-1 [239]. In this process, the PAF role is Ca^{++}-dependent and -independent of GM-CSF and IL_3, which up-regulate PAF activity [92]. IL_3 increases adhesion to endothelium and induces basophils either to produce IL_4 priming these cells at the level of membrane IgE [55], even if there are measurable IL_4 levels in cells devoid of IL_3, although tenfold less [506], or activating MCP-1 triggering their degranulation with dose-dependent histamine release [22], an effect inhibited by several CXCL and CCL chemokines [281]. The tendency to release histamine is genetically controlled, but in a way different from IgE production: it may be particular of allergic subjects, in whom it should be considered as a biological feature favoring the progression to chronic inflammation [331]. Several disease states also result in a concordant relationship between serum IgE and basophil FcεRI expression [485]. Studies have focused on new aspects of releasability (Chap. 11), a parameter not yet defined from a biochemical point of view, although it regulates proinflammatory mediator release and IL release from effector cells, including mast cells and eosinophils [332]. *Basophils represent a prominent source of IL_4, above all considering that IL_4 stimulates peripheral monocytes to synthesize IgE antibodies* and maybe also additional ILs regulating immune responses of other cells, thus amplifying inflammation. The role of IL_4 produced by basophils is also reflected at the Th2 T-cell level [507]. IL_4 present on endothelial surfaces participates in regulating eosinophil adhesion and selective transmigration as well; therefore eosinophil accumulation in inflammatory sites can be propitiated by activated basophils [506]. A recent study shows that the ability of TLR2 ligands to target basophils not only for IL_4 but also for IL_{13} secretion could have relevance to *in vivo* findings [41], *yet IL_{13} early in ontogeny* [182] so that they could play an important role in promoting and amplifying the Th2-dependent responses manifest in allergic disease [506]. The best characterized TRL2 ligand, peptidoglycan, directly activated basophils for IL_4 and IL_{13} secretion and greatly increased IL and mediator release in response to IgE-dependent activation [41]. Increased spontaneous basophil histamine release improves with food avoidance in children with FA (Chap. 10).

Each mast cell (Figs. 1.32 j, k, 1.37) bears on its surfaces $10–30\times10^5$ FcεRI receptors able to bind to Fc fragments, leaving free the Fab one, which is provided with the binding site for antigens, probably within the Cε3 domain [168]. An IgE molecule binds to one FcεRI receptor and vice versa: as a result, parallel to a high number of receptors, *only one or a few ng of IgE are enough to start mast cell activation.* Mast cells are also capable

of presenting antigens to T cells, resulting in their activation, in either an HLA class I or class II-restricted and polarizing T cells toward the Th2 phenotype via the effects of IL_4 and IL_{13} [347]. Mast cell precursors arise from pluripotent bone marrow-derived stem cells, circulate in the blood and lymphatics, and migrate into tissues, in which they reach phenotypic maturation [347]. Mast cells are stategically located at perivascular sites to trigger inflammatory responses [343]. Increased vascular permeability induced by mast cell-derived tryptase and chymase, and degradation of ECM components by enzymes such as matrix metalloproteinase 9 (MMP-9) which has been shown to be released from mast cells on activation by T cells also following a possible autocrine regulation by mast cell TNF-α, may further expedite cell migration through barriers including the vascular wall and the blood–brain barrier [347]. These cells, usually absent from blood, are scattered in connective tissue sites throughout the body, and especially around blood vessels and nerve endings in a variable number, between 7,000 and 20,000 cells/mm^3 of tissue [168]. Molecules such as HLA class I and II, CD28, CD54 (ICAM-1), CD154, β_2-integrins, and TLRs (TLR1 to TLR4, TLR6 and TLR9 [333]) allow mast cells to interact with other inflammatory cells, thus orchestrating an immune response [347]. MIP-1α may be a costimulatory signal operating via CCR1 for mast cell-mediated immediate hypersensitivity reactions [357]. A unique role for mast cells is to produce and release a vast array of mediators such as vasoactive amines, products of arachidonic acid (AA) metabolism, and several proinflammatory, chemoattractive, and immunomodulatory ILs that may contribute to immune reactions by affecting cell growth, recruitment, and function [476] (see also Innate Immunity). By using complementary DNA microarrays 1–2 hours after cross-linking with FcεRI, 2,530 genes exhibited 2–200-fold changes in expression and mast cells were shown to produce 18 ILs, including 130–529 pg of $IL_{11}/10^6$ cells and TNF-α, 13 chemokines, including two CXCL (IL_8, Gro2) and lymphotactin), ten CCL chemokines and several adhesion molecules [494]. Every human cell isolated from nose, lung, skin and intestine contains on average 2, 2.5–10, 4.6 and 1–2 pg of histamine, respectively [402]. Mast cells have been identified as a potential source of MBP [376]. Moreover, IL_{11} mRNA was co-localized with MBP in inflammatory cells in the subepithelial layer of the airway in subjects with severe asthma [354]. This raises the possibility that both mast cells and eosinophils represent sources of IL_{11} in asthma [354, 494]. Studies in rodents have revealed two mast cell phenotypes, called T or TC, being associated either with mucosal and connective tissue or with both tryptase and chymase (35 and 4 pg/cell) or, respectively, only tryptase (10 pg/cell) [168]. Apparently, T mast cell maturation, but not the TC phenotype, requires the help of IL_3 and IL_4 generated by T lymphocytes. Mast cells produce several ILs in response to cross-linking with FcεRI (Tables 1.25, 1.26), including TNF-α and IL_4 [509] and in vitro IL_1, IL_3-IL_6, GM-CSF, IFN-ɣ, which do not elicit histamine release as for basophils, as well as chemokines such as MIP-1α and -1β [168]. IL_1, IL_3, GM-CSF, MCAF (monocyte chemotactic and activating factor), = MCP-1 and RANTES; IL_4 and TNF-α induce adhesion molecules on vascular endothelium in injured sites, a first step for inflammatory cell migration such as lymphocytes and granulocytes [420]. Also, the expression on the mast cell surface of integrins linked to the FN receptor causes their activation [420]: FN binds to β1 integrins including CD49a, CD49c, CD49d, CD49e, and CD49f/CD29, playing a fundamental role when both IgE and antigen levels are low in local microenvironments [420], whereas FcεRI aggregation to cells adherent to FN specifically amplifies the phosphorylation of such proteins [195]. Signal transduction obtained in such a way by FcεRI looks like that of TcR/CD3 in T cells. We mention that antigen binding to FcεRI-IgE brings about the degranulation of metachromatic cells, thus initiating reactions of immediate hypersensitivity; however, mast cells can undergo forms of non-IgE-mediated signals from their environment (Chap. 10).

Table 1.27. Tissue prevalence (%) of T and TC mast cells

	T mast cells	TC mast cells
Skin	12	88
Conjunctiva and nasal epithelium	100	0
Small intestine mucosa	98	2
Small intestine submucosa	13	87
Bronchial epithelium	99	1
Bronchial subepithelium	77	23
Alveoli	90	10

Modified from [227].

Table 1.27 [227] summarizes several differences between T and TC mast cells on the basis of tissue prevalence. In humans, somewhat different phenotypes have been recognized; in addition, mast cells residing in the airways contain mostly tryptase, and those of other locations both proteases [227]. Their predilection to occupy tissues that interface the external environment makes them well represented in inflamed tissues under T-cell functional control through IL_3 (proliferation) and IL_4 (maturation) [593]. Under the influence of IL_3, IL_4 and NGF, T mast cells may assume characteristics of the TC phenotype; therefore, one may consider the distinction into two types nearly obsolete, with both phenotypes potentially coexisting in the same site, although in different proportions. Furthermore, it has been suggested that mast cells in unrelated locations respond to allergens with the same pattern of mediators [227]. As a consequence, mast cells are strategically positioned to

detect rapidly inhaled or ingested allergens, expressing a chronic array of proinflammatory mediators, without neglecting PAF and chemotactic factors such as LTB_4, NCF, and ECF (eosinophil chemotactic factor).

Appendix 1.2 summarizes receptors and surface molecules expressed from eosinophils, basophils and mast cells [593].

Platelets too have a virtual role in the pathogenesis of allergic disease [405], in addition to a typical role in coagulation processes. Classically thought to originate in the bone marrow from cytoplasm of megakaryocytes, it has recently been suggested that actually megakaryocytes travel to lung vessels, where they are physically fragmented into small clumps of granules, each of which is a platelet. These are the smallest blood cells, anucleated (2 µm in diameter), with a half-life of about 10 days. Platelets have been shown to express HLA class I molecules on their surface, IgG receptors (CD32), IgE (CD23), vitronectin (CD51), CD9, CD17, CD31 (GPIIa), CD36 (GPIIIb), CD41a (GPIIb/IIIa), CD42a–d (platelet antigens), CD49f, CD60, CD61 and CD63 (Table 1.2). Human platelet antigens (HPA) are at least 15: PHA 1–15 [298]. When activated, together with aggregation they undergo morphological modifications, secrete PAF, cytotoxic cationic proteins and free radicals. Moreover, platelets release 5-HT from dense bodies, which like histamine produce contraction of smooth muscles, increase vascular permeability and produce proteolytic enzymes and cationic substances from α granules with equal effects on blood vessels, in addition to chemotactic factors including PF4 (platelet factor 4), PDGF, 12-HETE, NO (nitric oxide), TGF-α and -β, albumin, β-thromboglobulin, eicosanoids (PGG_2, PGH_2, TXA_2, and CD62P), allowing binding to fibrinogen, FN and CD51. Consequently, platelets, although confined in the vascular compartment, also acting by diapedesis, can release mediators active in inflammatory extravascular foci. However, their specific role in inflammatory reactions is not well defined as it is for other cells: if activated, they also have chemotactic and phagocytic properties and contribute to immune reactions, releasing growth and coagulation factors, vasoactive amines and lipids as well as acid and neutral hydrolases. Following platelet aggregation, abnormal agglomerates develop and recruiting and entrapping leukocytes may contribute to the start of an endovascular occlusion. The human PMN adhesion to vascular endothelial cells was increased by the platelet presence. This effect was endothelial cell dependent and involved platelet activation. Thus platelet participation in cell recruitment occurs at the circulation level and might involve leukocyte priming for subsequent adhesion and transmigration into tissues [430]. Platelets produce enzymes cleaving C5, thus resulting in C5a, with a marked chemotactic activity for neutrophils: this is most likely the establishment of an active form of cooperation for the production of new mediators. C5a can prime mast cell degradation, while C5b-9 participate in the non-lytic platelet activation [680]. Platelets interact with the immune system via FcεRII (in 10%–30% of healthy and in 50%–60% of atopic subjects), FcγRII (CD32), the VLA-2, -5 and -6 integrins, ensuring adhesion mechanisms among immunocompetent cells and CD62P [65]. Activation of FcεRII elicits PAF production and an IgE-dependent platelet activation, which is not expressed as the classic aggregation, but by secretion of O_2 toxic radicals, triggered by SP, CRP (C-reactive protein), IFN-γ and TNF. Also prominent is the portfolio of chemokines that attract these cells to a site of inflammation, such as α-chemokines (CXCL), β-family (CCL), including eotaxin, Gro-α, RANTES, TARC, macrophage-derived chemokine (MDC), and SC-derived factor 1 (SCDF1), and chemokine receptors, such as CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22, activate platelets to give Ca(++) signals, aggregation, and release of granule content [87]. The inappropriate platelet activation materializes with eosinophil recruitment on the sites of immune inflammation, TGF-β secretion with mitogen activity towards bronchial smooth muscles, PF4 released during asthmatic attacks and PDGF triggering fibroblast proliferation. So platelets can be involved in the onset and perpetuation of structural alterations underlying subepithelial fibrosis, which contribute to emphasize BHR. Two series of experiments account for what we discussed earlier: platelets from patients with asthma from ASA (acetylsalicylic acid) or NSAIDs (nonsteroidal anti-inflammatory drugs) incubated with SP, PCR, IFN-γ and TNF start the release of O_2 radicals. Thrombocytopenia is congenital in Wiskott-Aldrich syndrome (Chap. 22) [405].

Additional Cells

The principal *APCs* expressing class II determinants [541] are DCs (in the skin LCs), macrophages, Kupffer cells, endothelial cell, enterocytes, monocytes with FcεRI [384], and B cells. All these cells, provided with HLA class II molecules, constitutive or inducible by bacteria and macrophage IFN-γ, collaborate with T lymphocytes (as well as among themselves), in different procedures according to the microenvironment and antigen type. DCs present antigens and virus in extralymphoid tissues and B cell toxins, virus, and bacteria in the spleen, while macrophages focus their attention on intracellular pathogens [49]. A differential type I IFN gene transcription was induced in monocyte-derived DCs and PDCs stimulated by specific TLR agonists. TLR-9 stimulation by CpG DNA induced the expression of all IFN-α, -β, -ω and -λ subtypes in PDCs [88]. Activated TcR-γδ can secrete ILs efficient in the activation of several cell families, also inducing a functional maturation of professional APCs with the accessory aid of several molecules, among which is CD154, hence facilitating the recruitment of antigen-specific TcR-αβ [110].

Dendritic Cells

Myeloid DCs are crucial APCs for primary T-cell responses: tissue-resident immature DCs are excellent at internalizing and processing antigen, but they exhibit a low ability to stimulate naive T cells [88]. DCs encompass a heterogeneous group of cells present either in lymphoid tissues such as thymic DCs and FDCs or in parenchymal organs such as IDCs, circulating and/or cutaneous LCs. Moreover, several chemokine receptors in $CD4^+$ lymphocytes are primed by DCs (see Chemokines) [88]. LCs arise from bone marrow precursors that colonize peripheral tissues through the blood or lymph, according to recent data, a line common to macrophages [424], which modulated by GM-CSF and TNF-α [221, 546] leads to two precursors identified by CD1a and CD14, both maturating into LCs and, respectively, DCs or macrophages depending on IL influence [74]. Activation causes DCs to up-regulate the CSMs expression (CD80 and CD86) on their surface. CSMs provide the signals necessary for lymphocyte activation in addition to those provided through the antigen receptor [115]. Circulating conventinal DCs coexpress and IFN-γ most potently favors activating CD32a, whereas soluble anti-inflammatory concentrations of monomeric IgG express inhibitory CD32b, both isoforms of IgG FcγR II (CD32). Ligating complexed human IgG to CD32a *matures and activates DCs* in proportion to the frequency of CD32a expression. However, coligation of CD32b significantly abrogates all of these immunogenic functions. These findings have important implications for understanding the pathophysiology of CIC disease and for optimizing the efficacy of therapeutic mAbs [48]. DCs produce a wealth of ILs: immature DCs exhibited higher amounts of IL_1, TNF, TGF-1, and MIF mRNA/protein than mature DCs. After differentiation, DCs up-regulated the levels of IL_6 and IL_{15} mRNA/protein and synthesized *de novo* mRNA/protein for IL_{12} p_{30} and p_{40} and IL_{18}. CD1a precursors generate cells expressing Birbeck granules and E cadherin characteristic of LCs, while CD14 progenitors mature into CD2, CD9, CD14, CD68 and factor XIIIa, specific of dermal DCs [74]. DCs, described as localized in the suprabasal layer of epidermis, represent in the adult only 2%–8% of epidermal cells, sharing with dendritic lymph nodes several phenotypic and functional features [454]. Although immature bone-marrow-derived DCs did not stimulate naive allogenic T cells, mature DCs elicited a mixed population of Th1 (mainly) and Th2 cells. The DC subset may contribute significant polarizing influence on Th differentiation and the CD subset 1 may exert Th1 polarization by IL_{12} production and STAT4 activation [366]. Growth and differentiation of LCs and their migration delineate a crucial step in the immune surveillance of foreign antigens invading the host [221, 546]. LCs via afferent lymphatics reach the paracortical areas of regional lymph nodes as FDC with APC function; thereby they are the first cells to trap antigens, which are

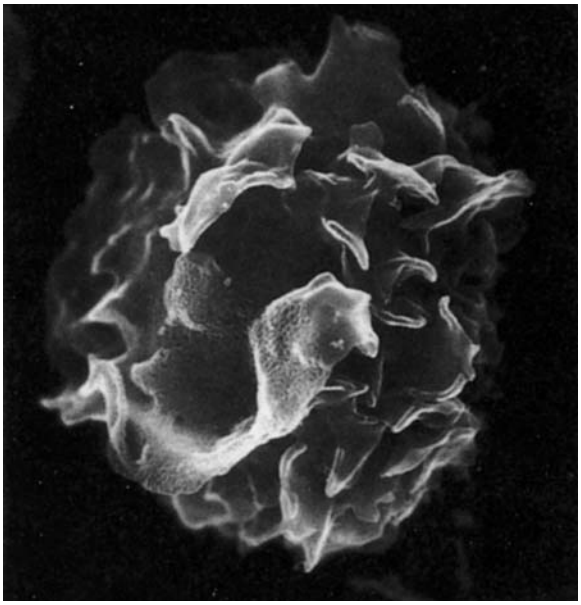


Fig. 1.38. Langerhans' cells appearing as veiled cells

then internalized and processed at the level of target cells [541], then presented in draining lymph nodes to upcoming T cells stimulated by the same DCs [546]. In comparison, DCs are strategically located below the M cells of PPs, thus sampling antigens in vivo and migrating to T-cell areas of the same PP or mesenteric lymph nodes, where they present antigen to naive T lymphocytes [253]. LCs migrate quite rapidly after having taken up a peptide, present a rounded phenotype with long cytoplasmic protrusions rhythmically moved, hence assuming the aspect of *veiled cells* [541] (Fig. 1.38). FDCs returned in paracortical areas as APCs, having a poor expression of HLA class II molecules, present the same peptide processed over several days [541] or months [37], also contributing to long-term maintenance of memory B cells [37]. FDC maturation due to an increased presence of CD80 or CD86, and enhanced by CD40, is stimulated until FDCs encounter T cells [424]. To understand the role of DCs in antigen presentation and processing, we mention that DCs select potential antigens taking up microbial glycoconjugates by means of specialized receptors. An in vitro model has demonstrated that PBMCs, in GM-CSF and IL_4-dependent cultures, develop into DCs that are extremely efficient as APCs, a property lost when treated with TNF-α [487]. LCs are thought to play a key role in enhancing immunogenicity since their first identification, because they *express FcεRI binding to IgE,* and pick up antigens in vivo even before presentation, and like they other APCs process antigens, degrading them into peptides that become approximately six to eight amino acids in size with a low MW [384]. The evidence that *LCs also possess FcεRII* [40] implies a major role in view of their significant activity shown in atopic diseases [546]. Table 1.28 [208, 424] summarizes their markers, denot-

Table 1.28. Surface markers of LC

Markers	Skin
CD1a	+/++
CD2	
CD4	
CD8	
CD11a	
CD11c	
CD14	–
CD15s	
CD18	
CD23	
CD29	
CD32	
CD34	
CD40	+++
CD45	
CD49f	
CD50	
CD54	
CD59	
CD80	+
CD86	++
CGRP	
FcεRI	
HLA-DP	++
HLA-DQ	++
HLA-DR	+++

See CDs in Table 1.2.
Data from [208], the skin markers from [424].
CGRP calcitonin gene-related peptide.

ing the skin LCs [424]. The LC ability to stimulate primary responses is up-regulated in the epidermis where they migrate in association with CD15s and CD62E and express *in loco* the E cadherin to bind to keratinocytes [566]. Cells very sensitive to UV action lose the capacity of presenting antigens to T cells after irradiation, an effect modulated by IL_{10} secreted by keratinocytes [461].

Afferent Phase of Immune Response

Antigen Processing and Presentation

Antigen processing and presentation are among the key events between a foreign protein penetration into the host via mucosal, skin or blood routes and its recognition by immunocompetent cells. The immune response results from a complex network of subpopulations of different cells interacting via soluble proteins, the ILs, most of which are involved in either inactivating or activating the expression of immune effector functions (cytokine cascade) [34]. This picture is integrated by a variety of actively trafficking cells such as lymphocytes, APCs, adhesion molecules, etc. [28]. T lymphocytes cooperate in the induction of immune responses influencing the up-regulation of B cell progenitors of IgE-secreting cells (Fig. 1.39). Antigen recognition and activation are neither consequent processes nor are they homologous: antigens can be recognized by the immune system without inducing mandatory immune responses, as in nonatopic subjects who instead yield Th1-cell clones [601, 606].

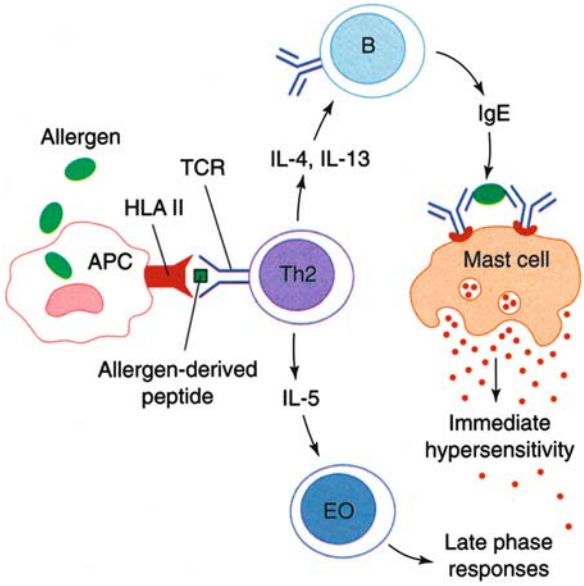


Fig. 1.39. Role of CD4+ cells in the immune response. Upon entry in the body, allergens are taken up and processed by APC, after presentation, HLA class Ii restriction and TcR usage of allergen-specific Th2-like cells, B cell progenitors of IgE-secreting cells are up-regulated. If IgE are produced, an immediate Th1-type response may ensue, but Th2-like cells may activate eosinophils, thus resulting in a late-phase response. *APC* antigen presenting cell, *B* B cell, *EO* eosinophil, *Th2* Th2 T cell, *TcR* T-cell receptor

CD4 cells do not recognize intact antigens, but interact only with previously processed native, exogenous antigens associated with class II HLA molecules, unlike CD8 and B cells recognizing endogenous peptides associated with class I molecules [12, 372]. γδ T cells recognize antigens differently, especially small molecules, but the functional consequences remain to be elucidated [109]. CD1 comprising five different proteins (Table 1.2) present lipids or glycolipids of microbial origin to T lymphocytes. However, their role in vivo is not yet clear [115]. HLA-E, HLA-F, HLA-G, HLA class I-like, representing differentiation antigens, have a limited tissue

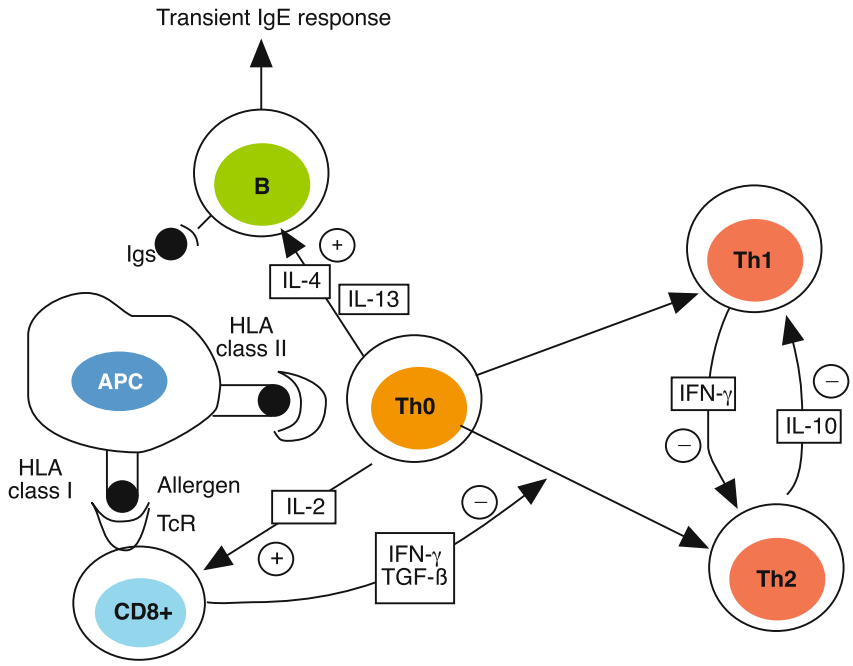


Fig. 1.40. Selection of Th1-mediated protective immunity toward allergens during primary immune response via class I $CD8^+$ HLA-restricted immunodeviation. Cell shading reflects the presence of peptides bound to class I or II HLA presented to $CD8^+$ or Th0 T cells, respectively. *Igs* surface immunoglobulins. (Modified from [212])

distribution and polymorphism [47]. HLA-G has been recently characterized as TAP-associated, which directs its expression and binding to nonameric peptides [293]; HLA-G expression on target cells protects from NK-mediated lysis interacting with NKR [419], of special significance pertaining to maternofetal tolerance.

Classic studies have shown that immune responses consist in the production of antibodies, up-regulated stimulating CD4 lymphocyte activation, but may be down-regulated when CD8 cells predominate. Non-self substances that have gained access to sites patrolled by the immune system enter automatically in contact with it, thus activating a complex mechanism of cellular activity aimed at its destruction and at restoration of preexistent homeostasis. Therefore, immunogen peptides encountering lymphocytes of an atopic individual for the first time trigger a multiple pathway of cell interactions ensuring that B lymphocytes differentiate into antibody-secreting cells [601]. As soon as an invader is identified by two different immune effector mechanisms, antibodies (humoral effector limb) and TcR (cell-mediated effector limb), the foreign antigen is captured by APCs, the peptide-HLA complex is recognized by TcR, then internalized and processed in fragments subsequently exposed on the cell surface in association with class II HLA molecules. Thus the interaction between antigen-specific T and B cells (cognate interaction) and consequent IL release result in the activation of lymphocytes [290]. A theory of two signals is suggested also for the T cells: accordingly, Th0 T cells receive the first signal from the TcR triggered by pathogen-derived antigenic peptides bound to HLA class II molecules on APCs, which indicates the peptide molecular identity. Signal 2 delivered from costimulatory molecules (CSM) comprises contact-dependent and humoral signals and transmits the information about the DC-activating property of invading pathogens, reflecting its pathogenic potential. The combination of signal 1 and signal 2 results in Ag-specific activation of naive Th cells and their development into effector/memory cells [290]. A Th0 signal might provide a further refining cadence, whereby the IL milieu produced by DCs provides naive T helper cells with a Th1- or Th2-polarizing signal at the time of priming [212]. Activated T cells differentiate into Th2 T-cell clones, which secrete low amounts of IL_4, thought to play a crucial role in IgE isotype switching. The resulting IgE low levels can be captured by high-affinity receptors on the mast cell surface [290]. Thereby the first exposure to an immunogen leads to the production of antigen-specific IgE and priming of the immune system (*primary immune response*), an event preventing a second encounter via the $CD8^+$ class I HLA-restricted immune deviation (Fig. 1.40) [212]. In immunologically healthy neonates and infants, such initial responses, an integral part of normal immune responses, are self-limited and gradually resolve, after weeks or months, despite continuous allergen exposures, due to the development of tolerance [250]. In healthy, uncommitted subjects, membrane-bound IgG forms immune complexes with allergens. IgG is tethered to the membrane by binding the Fc fragment of FcεRIIb. When an allergen binds both IgE and IgG, the activating FcεRI is brought together with the inhibitory FcεRIIb, thereby silencing the FcεRI-mediated activation pathway [259]. According to this model, following subsequent exposures, CD4 clones from nonatopic individuals have a Th1 profile, whereas in atopic patients they can lead to an immediate hypersensitivity reaction (*secondary immune response*). Antigen persistence or reexposure leads to ongoing antibody production, which is outstanding, rapid, more specific

and enduring with different functional features, dominated by Th2 T cells and IgE, an expression of immune memory [212]. When receptor-bound IgE is cross-linked, release of potent biochemical mediators and further IL_4 production induce uncommitted T cells recruited at a site of allergen re-entry to differentiate into a Th2 phenotype, hence amplifying immune reactions [250]. Th2 T cells, IL_4-derived IgE production and IL_5-triggered serum and tissue eosinophilia result in *a vigorous IgE response* and a severe clinical response [212]. T cells, eosinophils, metachromatic cells provided with IL_4 and CD40L, ILs and adhesion molecules and their interactions are the major players around which atopic diseases evolve [177].

Antigen Capture and Processing

Antigen capture by APCs can occur via three distinct mechanisms [488]. The first is macropinocytosis [555], a type of fluid phase endocytosis, uptake of large vesicles (1–3 μm) mediated by membrane ruffling driven by actin cytoskeleton [488]. In DCs this constitutive mechanism calls for a continuous internalization of large volumes of fluid (1,000–1,500 μm^3, a volume close to one cell/h), whereas macrophages and epithelial cells need to be stimulated by growth factors [555]. The second mechanism is mediated via the mannose receptor (MR), a 175-kD C-type lectin, which on human cultured DCs modulates endocytosis of $>10^5$ molecules of mannosylated proteins per cell/h [488]. Furthermore, a membrane protein of murine DCs, structurally homologous to macrophage MR, internalizes peptides, delivering them to a multivesicular endosomal compartment provided with HLA class II molecules, of which DCs synthesize elevated levels: in this model, the signaling process initiated by T cells is up to 100-fold more efficient [488]. The third mechanism is mediated by FcγRII, also expressed by DCs [290]. B cell clones bear high-affinity mIgM and mIgD on their membranes ready for antigen epitopes and antibodies and their BcRs fulfill two functions: signal release leading to B cell activation and antigen uptake and delivery to processing compartments [290]. In addition, nonprofessional receptors are surface molecules able to occasionally capture antigens and effectively present viral proteins bound to surface receptors [396].

Presentation and Recognition

Antigen recognition from T cells with a TcR complementary to peptide-HLA association triggers the first phase of *T-cell activation and consequently the immune response* [28]. For this purpose, both the epitope and agretope that bind to an HLA molecule are critical (Fig. 1.15). The peptide–HLA complex exposed to the CD3–TcR complex of antigen-specific T cells is expressed on the cell membrane in the *fitting pocket* of HLA class II molecules (a *genetic restriction* mechanism) (Fig. 1.22). Such complexes are as firm and as high as the peptide affinity for the hypervariable part of HLA molecules; such adhesion is mediated by CD2, CD11a/CD18, CD54, CD58, and other integrins and selectins [534]. The cells expose peptide fragments assembled with HLA class I or II molecules, so that they are examined by circulating T cells, which, although relatively few in view of the great number of potential antigens, and of their great diversity, are conditioned to recognize those they encounter [34]. The affinity for peptides depends on the amino acid sequence of hypervariable regions and consequently on the HLA molecules that everybody inherits. TcR and HLA interactions are characterized by a high-sensitivity and low-affinity paradox, which is only a small number of TcRs that interact with APCs [595]. On the contrary, APCs are fit for almost any foreign invader encountered by the immune system, thus raising a very intriguing question of how so few receptors can transduce an activation signal [225]. It remains to be elucidated how as few as 80–100 HLA–foreign peptide complexes on the cell surface (which may express as many as 10^5 HLA molecules) are sufficient to trigger a T-cell response [47]: the answer lies in the capacity of a single peptide–HLA complex to serially engage and trigger up to ≈ 200 TcR, amplifying the signal according to T-cell biological responses [595]. C3b plays a critical role in at least two phases of recognition, as shown by the T clone response to presentation of C3b–Ig complexes. The uptake of such complexes is helped by the interactions with complement receptors virtually present on all APCs; furthermore, C3 covalent binding to specific antigen peptides can define, during the processing, which part of the molecule is selected as epitope presented to T cells [359].

Recognition of immunogenic proteins in their natural configuration is not sufficient to stimulate B lymphocytes to differentiate into plasma cell IgE, since switching steps requires T lymphocyte cooperation, which materializes via IL_2 production; so B cells and APC interaction within TcR presentation of peptide–HLA complexes involve antigen specificity and consequently B lymphocyte capacity to bind to and present peptides, even if their extracellular number is reduced. At the level of peripheral lymphoid tissues, where antigen concentration is greater, other APCs are involved in antigen processing and presentation background. The first step of B-cell activation requires signals generated upon recognition of antigen by the BcR as well as additional signals provided by cognate interaction with T cells, including the CD40–CD154 interaction [201]. Following peptide–HLA complex recognition on the B-cell surface by TcR, T cells deliver appropriate activating signals to B cells (*cognate help*); hence T cell–B cell cooperation begins, class II-restricted and antigen-specific, with formation of tightly associated conjugates [457]. Such conjugates result from peptide–HLA complex presentation from B cells; fur-

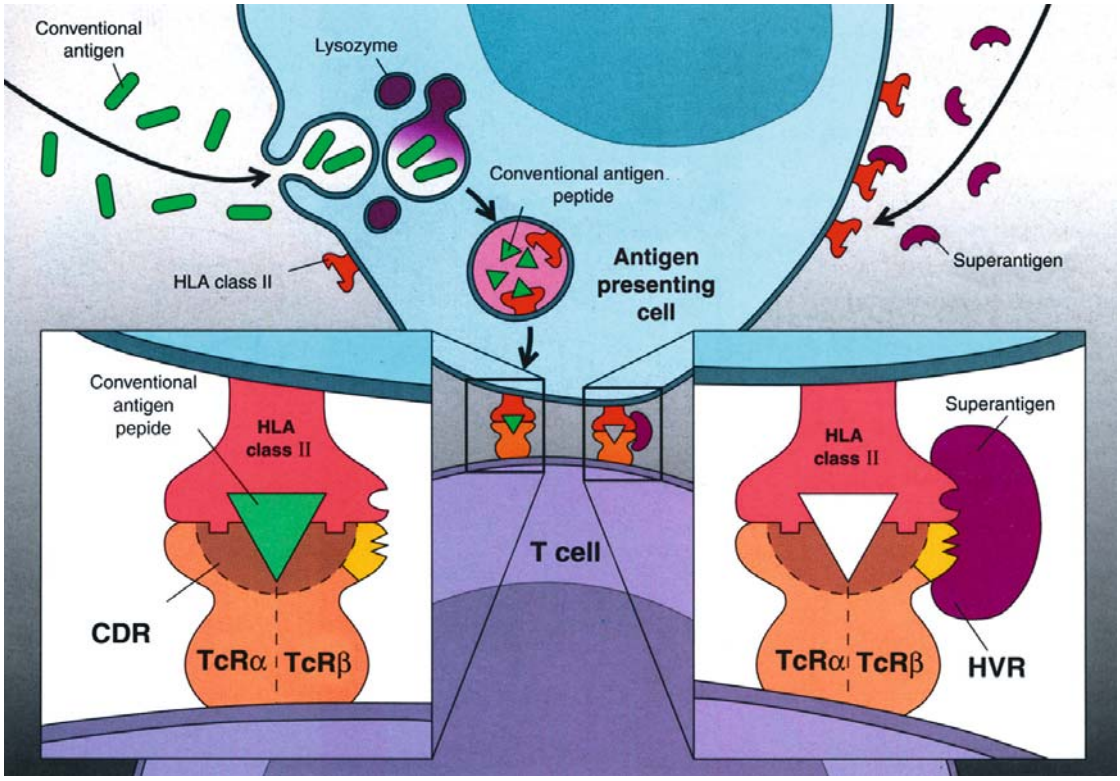


Fig. 1.41. Superantigen abbreviated presentation. Superantigens sidestep the usual pathways of antigen presentation (*left*), but are presented intact on the outside of the HLA peptide-binding groove (*right*) and activate T cells. Superantigen is recognized by a side face of TcR-Vβ, which encompasses a HVR that has been designated HVβ-4. *CDR* complementarity determining regions, *HVR* hypervariable region

Table 1.29. Bacterial superantigens

Superantigen	Toxin (name and abbreviation)	MW (kD)
Staphylococcus aureus	Enterotoxin A=SEA	27.8
	Enterotoxin B=SEB	28.3
	Enterotoxin C1=SEC1	26
	Enterotoxin C2=SEC2	26
	Enterotoxin C3=SEC3	28.9
	Enterotoxin D=SED	27.3
	Enterotoxin E=SEE	29.6
	Toxic shock syndrome toxin-1=TSST-1	22
	Exfoliating toxin A=ExFTA	26.9
	Exfoliating toxin B=ExFTB	27.3
Streptococcus pyogenes	Erythrogenic toxin A=SPEA	29.2
	Erythrogenic toxin B=SPEB	27
	Erythrogenic toxin C=SPEC	24.3
	Protein M	22
Mycoplasma arthritidis	Mycoplasma arthritidis mitogen=MAM	26
Pseudomonas aeruginosa	Exotoxin A	66
Clostridium perfringens	Clostridium perfringens toxin=CPET	34

Modified from [474].

Table 1.30. Prominent characteristics of superantigens (*SAs*) interacting with B lymphocytes (B superantigens, *BSA*)

SAs activate a large percentage of B lymphocytes, about 40% of human polyclonal IgM binds to SPA
SAs interact with the major part of components of V_H– gene family: SPA binds to a high rate of V_H3+IgM
SAs trigger B lymphocytes in vitro; SPA delivers activation signals to IgM V_H3+, thus triggering Ig differentiation; HIV-1 gp120 selectively induces Ig secretion by V_H3+ IgM
SAs also induce in vivo changes in B lymphocytes; it has been suggested that during HIV-1 infection the B V_H3+ cells are initially up-regulated, and then highly down-regulated
SAs interact with regions of the V_H gene domain; for SPA binding a motif between residues 75 and 84 of FR3 is involved, outside the conventional paratope
SA binding activity experiences age-related alterations

Modified from [692].
FR framework region, *SPA Staphylococcus aureus* protein A.

ther enhancement of cognate interactions depends on CD54 binding to CD11a/CD18 and CD4 to monomorphic domains of class II proteins [391]. Engagement of both the BcR and CD40 results in synergistic activation of B cells [201]. CD4 T cells bind to antigen-specific B lymphocytes; the associative recognition induces B-cell activation, clonal expansion, and differentiation, while cell division goes on as long as T cells stimulate it. Mature plasma cells are generated and secrete specific receptors, the mIgs, which bind to antigens present in the bloodstream [457]. Previous studies suggested that binding to TRAF2 and/or TRAF3 but not TRAF6 is essential for CD40 isotype switching and activation in B cells [232]. More recently a model was presented in which Btk contributes to the enhancement of the CD40 response by TRAF2 in a BCR-activated protein kinase D (PKD)-dependent manner [201].

Superantigens (SA) are antigens able to select subsets of T cells during thymic ontogenesis, playing an important role in T cell development: an example is given by bacterial toxins, some of which can be mitogen for some T cell subsets. Such SAs bypass key antigen processing and recognition steps in T cell activation, by binding more or less exclusively to lateral exposed surfaces of HLA class II molecules and TcR determinants of the Vβ region (HVβ-4), that is, not to a normal paratope. As a result of this sidestep they are able to activate greater proportions of lymphocytes, not $1/10^4$ or $1/10^5$, as with usual antigens, but whole clones up to 30% of T lymphocytes, thus amplifying their activity, and functioning as a bridge between T cells–HLA and accessory cells [275]. Figure 1.41 shows a polyclonal activation of T cells, which recognize both conventional peptides with Vα and Vβ regions, and SAs essentially with an area of the Vβ region [275]. Table 1.29 [474] details the different types of microbial SAs. NKB1 inhibitor receptor, expressed by many T cell clones and engaged by their HLA class I ligands on potential target cells, protects against cytotoxicity induced by bacterial SAs [427]. An additional means of interaction between T and B cells can occur, whereby molecules termed B-SAs (BSA) can bind directly to human BcR of a given variable V gene family [647]. This mechanism requires contributions from the FR loop away from CDRs; hence this loop is less favorably placed for antigen contact and has a greater potential for unconventional binding (Table 1.30) [605].

Lymphocyte Activation

Like many other cells of the body, T and B cells exist for most of their life span in a quiescent state or a G_0 state. To proliferate, the cells must re-enter the G_1 phase, where several proteins undergo a substantial process of biosynthesis, so these cells grow in size and prepare for DNA synthesis. In the S stage, DNA synthesis and replication of each chromosome bring about two matching sister chromatids. The subsequent G_2 and M (mitotic) phases involve the two sister separations, generation of two new nuclei, and final division of the cytoplasm to produce two daughter cells: growth factors and different environmental stimuli are required for cell cycle progression, depending on the cell type [337]. There are several *functional differences* between T and B cells and recent work has focused on their mutual interactions: T lymphocytes have a variety of signals allowing them to leave the circulation and enter tissues to reach the site of antigen exposure, both because they constitute the prevalent portion of peripheral lymphocytes and they have the central feature to recirculate. Instead, B cells encounter preferably native macromolecules *in situ,* in specialized organs and tissues; however, in an antigen-independent phase of B-cell development, it is likely that B cells do not require interactions with antigens, which will be ultimately recognized by soluble antibodies subsequently synthesized [481]. Both T and B cells need to be stimulated before acquiring the capacity of responding to specific antigens, T cells by their clonally restricted TcR and B cells by Igs, or in T-independent polyclonal systems or molecules with mitogenic properties, both experimentally and physiologically [326]. We also note some biochemical similarities in B and T cell activation: as an antigen binds to an APC, a series of defined events occur over a period of several hours. Within a few seconds, the phosphorylation of cell proteins takes place, mostly associated with CD3ε and ζ and CD79a and b receptors and membrane phospholipid cleavage. A cascade of protein activation in regulated sequence and the rise of Ca^{++} levels occur. As a result of these early activation events, TFs such as NFAT 1, 2 and NF-κB are activated to enter the B cell nucleus and promote transcription of nontranscribed specific genes. In

T cells, the most important genes include ILs and IL receptors, while B cells start to transcribe Ig genes. Within about 48 h, DNA is synthesized and cells undergo division [36, 47].

Role of T Lymphocytes

CD3, the nonpolymorphic part of the TcR complex, is a signal transducer in T cells whose activation with IL_1 contribution elicits both proliferation and activation of cell subsets. CD4 stimulation by HLA molecules and IL_1 drives IL_2 and IL_6, an intervention defined as a *synergistic promoter* [34], and additional metabolic processes lead to a final activation and proliferation of CD4 cells, of cytotoxic CD8 and, as a result, of B lymphocytes, which become antibody-secreting cells [206]. For definitive proliferation, CD3 must be escorted by accessory stimuli, one expressed by BcR, the membrane protein CD80, recognized by CD28/CD152 receptor = CTLA-4 (CTL-associated antigen-4) [242]: we stress that when appropriate signals are absent, clonal anergy ensues (Fig. 1.22 a, b).

During the processes of presentation and activation, the trimolecular complex made up of TcR-αβ/CD4 and peptide–HLA transmits signals to cells, as discussed earlier. Due to TcR binding to extracellular V regions, modulated by CD3ζ, also following second-messenger generation, TcR transduces signals initiating biochemical and conformational changes. Intracellular signals generated by TcR and transmitted to T cells appear, therefore, to be critical for proper T cell maturation and activation [276]. Transduction of activating signals by CD3-CD28 costimulation initiates multiple signaling cascades that lead to the activation of several TFs, including the activation of NF-κB family members [276]. TcR-CD3 stimulation alone is not sufficient to optimally activate NF-κB because its requires *Bc110, a CARD* associated with CARMA1 (CARD11) [504], a member of the *CARD family* also including CARMA2 (CARD14) and CARMA3 (CARD10) [621]. The CARD family also encompasses CARD/NOD a member of the *ced-4 superfamily* also including APAF-1, mammalian NOD-LRR (leucine rich repeat) proteins and CARD15/NOD2, which in turn act in LPS recognition and activate NF-κB [79] that is depressed in patients with Crohn's disease [621]. Prominent in this context is *G protein* participation with a manifold role in cellular signal transduction coupling an array of receptors at the cell surface with a variety of intracellular effectors exposed to the plasma membrane's inner surface that couple a large family of receptors to effectors, such as adenylcyclase, PLC, and ion channels [292]. The large heterotrimer G proteins have 21 Gα subunits, which are related to small G proteins, plus five Gβ and six Gγ that exist as a single complex (Gβγ). Gαs are stimulated by GAPs (GTPase-activating proteins) and Gβγ by RGS (regulators of G protein signaling) [231]. In the resting state, guanosine diphosphate (GDP) is tightly bound to Gα. When a membrane receptor is activated by binding of a first messenger, this causes GDP to dissociate from Gα and be rapidly replaced by guanosine triphosphate (GTP). GPT binding leads the Gα subunit to dissociate from Gβγ, each of them can independently transmit signals, hence activating effector cells (active state). In a subsequent phase, hydrolysis of GTP to GDP inactivates Gα, allowing it to reassociate with Gβγ (inactive state) and reset stable heterotrimers. Gβγ subunit binding to several components of the Gα subfamily could open up a new *communication pathway among second messengers* [292]. Considering this activity, G proteins oscillate between GPT- and/or GDP-bound states, and regulate diverse processes, including signal transduction [24]. G proteins also belong to a superfamily comprising a number of receptors; however, the amount of G proteins bound by a given receptor is reduced, practically restricting to one G protein signaling to one receptor. One of the best characterized among signal transduction systems is an increased formation and accumulation of intracellular cAMP (cyclic adenosine monophosphate) as a result of β-adrenergic receptor stimulation; further receptor/ligand interactions enhance Gα3 activity due to Gα3/GTP dissociation from Gβγ. Correspondingly, the activation of the enzyme chain of membrane adenylyl cyclase catalyzes cAMP synthesis in Mg ion presence. Returning G protein to its initial conformation the enzyme is inactivated, while cAMP is converted to noncyclic inactive 5-AMP by cAMP-PDE (phosphodiesterase) constitutive activity; otherwise other receptors activate another G protein, Gi, which binds to adenylyl cyclase to block enzyme activity [326].

Direct evidence suggests that, depending on the type of related cells, cAMP, a second messenger present only inside the cells, plays a role in enzyme phosphorylation, Ca++ levels increase, also affecting both gene expression and further endocellular processes [292]. In addition, the CD3ζ cytoplasmic domain interacts with two families of tyrosine kinases such as *PTK of the src and syk* (intracytoplasmic) *families* (Tables 1.31, 1.32) [157, 214, 222, 240]. Some PTK src are associated via the SH2 domain with ARAM or ITAM sequences of intracellular regions of γ δ ε chains of CD3 as well as α and β (CD79a and CD79b) of BcR and ζ or γ of CD16. To fulfill G protein effects on CD4/CD8 T cells [292], within a few seconds GTP is hydrolyzed to GDP; a cytoplasmic tyrosine kinase, ZAP70 (ζ-associated protein 70), belonging to the syk family, becomes active upon attachment to the TcR–CD3 complex [213] and in turn activates PLCγ1 [462]. The important result of PKC is PLCγ1 activation, which then acts to hydrolyze PIP2 into IP3 (inositoltrisphosphate) and DAG [462]. IP3 and DAG serve as second messengers of the T-cell activation process: IP3 with a short half-life rapidly increases cytoplasmic Ca++ levels; however, in the absence of additional signals there is no activation [337]. DAG has been shown to activate PKC, a process leading to its translocation to the

Table 1.31. Kinases and receptors (*R*)

Receptors associated with kinase domains	
Tyrosine kinase	CSF-R, EGF-R, M-CSFR, PDGF-R, SCF-R, insulin-R
Serine/ threonine kinase	Activin-R eg:TGF-β-R
Receptors associated with cytoplasmic kinases	
Src-family kinases (blk, fgr, fyn, hck, lck, lyn, src)	TcR (fyn, lck); BcR (blk, fyn, lck, lyn); FcR (fgr, lyn); CD4 (lck); CD8 (lck); CD19 (lyn)
Syk-family kinases (syk, ZAP70)	TcR (ZAP70), BcR (syk); FcR (syk)
PI 3 kinase	CD28
Tec-family kinases (btk, itk, tec)	CD28 (itk); BcR, pre-BcR (btk?)
JAK-family kinases (JAK 1, 2, 3, tyk 1, 2)	Receptors of all IL (except IL_1, IL_8, IL_{11}, IL_{14}); EGF, G-CSF, GM-CSF, all IFN; M-CSF; PDGF, growth hormones, erythropoietin

Data from [157, 214, 222, 240].

Table 1.32. Cytokines, receptors and signaling

Receptors	Activated JAK	Activated STAT
IL subfamily sharing the γ chain		
IL_2R	JAK1, JAK3	STAT3, STAT5
IL_4R	JAK1, JAK3	STAT6
IL_7R	JAK1, JAK3	STAT5
IL_9R	JAK1, JAK3	?
$IL_{13}R$	JAK1, JAK3	STAT6
$IL_{15}R$	JAK1, JAK3	STAT5
$IL_{21}R$	JAK1, JAK3	?
Subfamily of GM-CSF receptor sharing the gp140β chain		
IL_2	JAK1, JAK2	STAT5, STAT6
IL_5	JAK1, JAK2	STAT5
GM-CSF	JAK1, JAK2	STAT5
Subfamily of IL_6 sharing gp130		
IL_6	JAK1, JAK2, Tyk2	STAT1, STAT3
IL_{11}	?	?
CNTF	JAK1, JAK2, Tyk2	STAT1, STAT3
LIF	JAK1, JAK2, Tyk2	STAT1, STAT3
Oncostatin M		
IL_{12}	JAK2, Tyk2	STAT3, STAT4
IFN receptors		
IL_{10}	JAK2, Tyk2	
IFN-α/β STAT3	JAK1, Tyk2	STAT1, STAT2,
IFN-γ	JAK1, Tyk2	STAT1α/STAT1β
Receptors with single chain		
G-CSF	JAK1, JAK2	STAT3
GM-CSF	JAK2	STAT5α
EGF	JAK1	STAT3
EpoR	JAK2	STAT5
TpoR	JAK2	STAT5
EpoR	JAK2	STAT5
GH	JAK2	

Data from [157, 214, 222, 240].

cell surface and to a cascade of downstream events, also resembling the biochemical way employed for EGF transduction signals, which activates phospholipase C (PLC) instead of PLCγ1 [454].

The increase in Ca++ concentrations by the second messenger cascade activates calmodulin, which regulates several protein kinases and phosphatases, including calcineurin (CN). This event, together with PKC phosphorylation of serine residues of the CD3 γ chain, and of tyrosine residues of the ζ chain, plays a major role in T cell activation as well as in gene transcription coding the IL_2R α chain (G_1 phase). CN also regulates NFAT activity and contains a binding site for one of its components (Fig. 1.42) [457]. In particular, CD3ζ phosphorylation appears to be *the signal for ZAP70 binding to CD3 ARAM* [462]. The significance of ZAP70 in such processes is demonstrated by its deficiency in a form of SCID characterized by the absence of CD8 T cells [544], emphasizing its prominence in T lymphocyte intrathymic selection and not only in mature cell activation. CD45 (CLA) is associated with T- and B-cell activation processes: for example, CD4-mediated signals are enhanced by cross-linking to CD45, with a rapid rise in Ca++ levels, an effect mediated by Ca++-independent PTPase activity of two domains within the CD45 cytoplasmic tail [584]. On the contrary, CD45 direct interactions with the TcR–CD3 complex could lead to dephosphorylation of CD3ζ and a down-regulation of the response [337]. CD45 is also able to dephosphorylate and activate members of the src family of kinases, a likely basis for the requirements of antigen-induced receptor signaling [395, 584]. CD45 activation of the src-family members before stimulation is consistent with the belief that src are the first tyrosine kinases required for antigen-induced signaling [395].

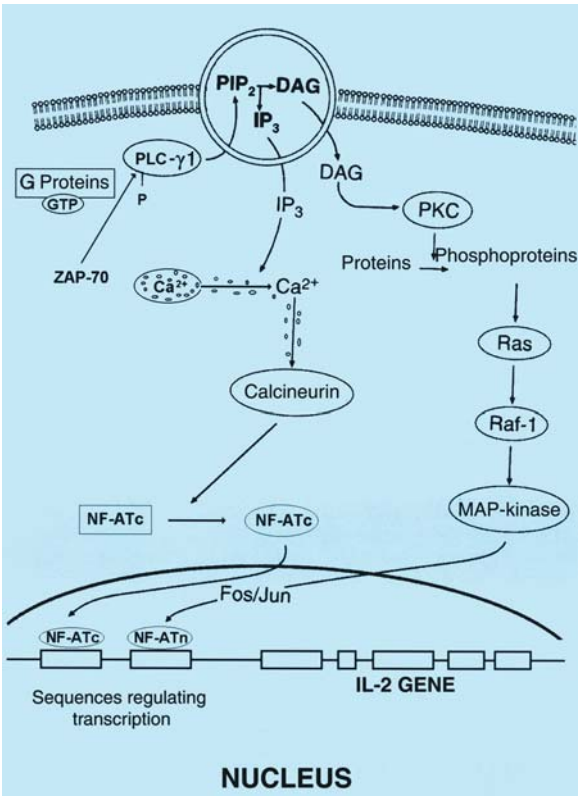


Fig. 1.42. Schematic representation of the morphofunctional aspects of T and B lymphocyte activation signals

Costimulatory Molecules

Recently stressed is the part played by CSMs such as CD2 present on T cells and its ligand CD58 on B cells. CD2 enhances antigen recognition drawing TcRs into zones of cell–cell contacts also arranging the opposing cell membranes of both T cells and APCs at the optimal distance, thus promoting TcR–peptide-HLA interactions. Thus CD2 can allow lower affinity TcRs to be utilized, thereby increasing the size of the mature T-cell repertoire [109]. CD58 for B cells stimulated by IL_4 and CD2 for T cells can provide a second signal for isotype switching to IgE; CD58 can only cross-bind to anti-CD58 lower than that of CD40 [243]. CD2 could also mediate an alternative pathway of T activation if aggregated to the TcR–CD3 complex [109]: it is postulated that molecules different from CD40L are involved in IgE synthesis. Further molecules are CD28 (expressed by the B-cell majority and CD4 as well as by ≈ 50 % of CD8 T cells) and CD152 is = CTLA-4 (present only on activated T cells), whose interactions with CD80 and CD86, main ligands for CD28/CD152, represent a very important costimulatory membrane signal for T-cell activation and are crucial for both proliferation and differentiation of T-cell effector functions [243]. Studies have also hypothesized that both ligands could follow a different model in regulating T-cell differentiation, CD86 to Th2 T cell production with IL_4 predominance and CD80 to Th1 T cell phenotype [279]. Skin CSMs are keratinocytes coming on stage when external factors shift the immunological balance toward an epithelial sphere of influence. Cells expressing CD80 can have a pivotal role in triggering immune responses, delivering costimulatory signals to resting T cells and modulating their maturation into Th2 T cells [279]. Lack of such a strategy can depend on intrinsic differences of class II HLA molecules (that is, li reduction), rather than on defects of CSM potential [381].

Role of B Lymphocytes

At first glance, the B cell first stage of activation is in the T cell area, the splenic PALS, then proliferating cells form GCs (Fig. 1.43). In the superficial cortical zone there are primary follicles, lymphoid aggregates of uniform cellular density, with mature resting B lymphocytes probably not yet stimulated by antigens, and secondary follicles containing GCs, proliferated in response to antigen stimulation. GCs are characterized by a *central dark zone* proximal to T areas, containing many rapidly dividing B cells failing to express surface Igs, the centroblasts [37], and a *basal light zone* filled with centrocytes, non-dividing B Ig^+ lymphocytes [254]. The GC reaction reaches a peak volume by day 10–12 after immunization, when PALS (Fig. 1.11) start to decline; without further antigen stimulation GCs also gradually regress until they wane around 4 weeks after immunization [37]. In GCs there are macrophages with phagocytic activity and IDCs deriving from tissue homologous cells, among which are also DCs. Naive B cells come into contact with antigens presented by DCs in the GC light zone: within a few hours B cells interact with specific CD4 cells; their proliferation reaches the apex by day 5, followed by their migration into lymphoid follicles or other peripheral sites [454]. Exponential proliferation of a given B clone leads to thousands of antibodies/min in 3–4 days that are secreted outside GCs [254]. During the course of primary response, isotype switching occurs in centroblasts, and somatic mutations accumulate in V_H and V_L regions [63]; by day 10 of the response, GCs are clearly divided into dark and light zones [37]. In the dark zone, at about 2 days B blasts differentiate into mIg-negative centroblasts which collect at one pole adjacent to the FDC network, which a little later fills up with centrocytes [37]. Studies suggested that in the apical light zone centroblasts differentiate into memory cells (small lymphocytes) or plasma cells with T-cell cooperation [256]. When FDCs form their protrusions embracing B cells to present bound antigens to BcR, the process is exhausted and plasmocytes migrate into the medulla and eventually the bone marrow, where they undergo a terminal differentiation [256]. Interestingly, light zone centrocytes re-enter the dark zone, join the centroblast population and reinitiate proliferation,

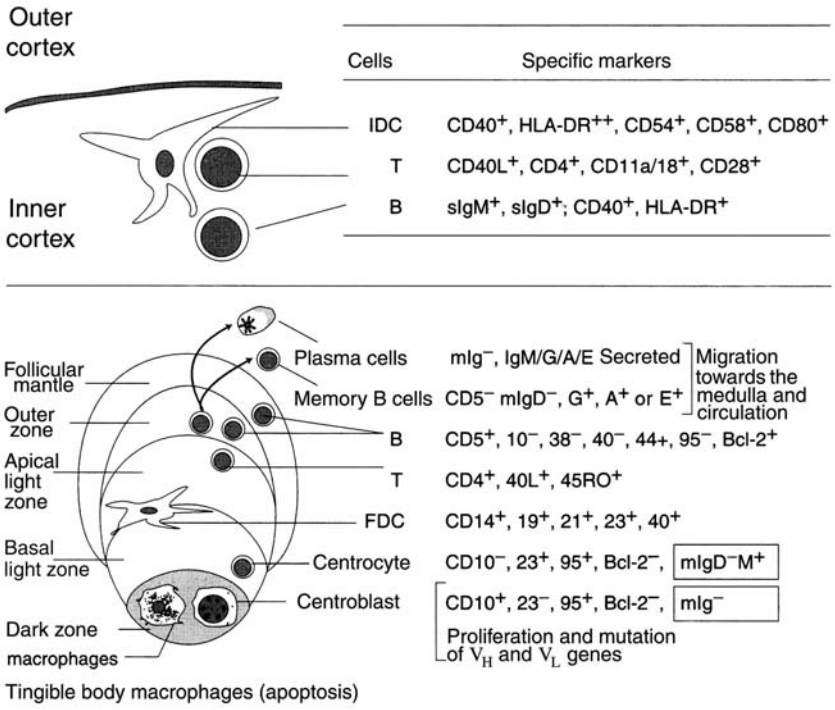


Fig. 1.43. Formation and structure of germinal centers (GC). Tingible body macrophages (apoptosis). *FDC* follicular dendritic cells, *IDC* interdigitating dendritic cells

whereas T–B collaboration in the light zone is necessary to maintain active GC reactions [256]. A principal purpose of GC formation is to direct VDJ rearrangement, and mutated Igs are first observed on day 7–10 of primary responses, coincident with GC polarization and CD86 expression on centrocytes [254]. T-cell–B-cell interactions involve signaling via CD40 and CD154 (CD40L), found in the outer zone of tonsillar GCs [71]. Inhibition of this signaling pathway also impairs GC formation [71]. Markers and/or participants in the activation process are CD19 and CD20 expressed at all stages of differentiation, CD21 (CR2) and CD22 expressed only by mature B cells; IgE^+ antibodies instead express CD5 ligand of CD72, CD32, CD38, CD45RA and CD45RO (Table 1.2). CD19 interactions with BcR markedly lower the threshold (100 antigen receptors per cell, 0.03% of total) to enable B cell activation [70], as they are consequently processed, degraded into peptides, and transported to the cell surface associated with HLA molecules [384]. Independently of signals mediated or not by T cells, BcR internalization does not require ITAM participation [453]. B cell activation by CD79a and CD79b involves triggering src and syk, which form molecular mechanisms able to transduce activation signals generated by interactions between antigen and epitope [408]. However, so that the B cell functions as an effective APC, CD80 and CD86 coexpression is necessary, while it is absent in resting B cells. Upon BcR cross-linking, PTK src are activated, tyrosine residues are phosphorylated in the ITAM, while syk is required for BcR communication with PLCγ1, IP3 generated via PIK3 activation, and Ca^{++} release [453]. Analogous to ZAP70, in addition to tyrosine kinase regions, src and syk carry a SH2 domain with high affinity for phosphorylate tyrosine residues, binding those from CD79a and CD79b [453]. Two pathways are involved in IL_4-mediated Cε transcription: the one associated with PLCγ1, leading to PKCδ translocation with cooperation of IP3, PIP2 and DAG, and the other based on PIK3 and PKCζ [659]. A growing body of evidence indicates that FcγR in B cells inhibits their activation and Ig production [337]. If properly glycosylated, CD45 may interact with CD22, an important step for cell–cell adhesion [395], which has been shown to regulate the B cell phosphatases, also regulating T cell activation. As a result of this sequence of events, 12 h after the antigenic stimulation, the blasts increase in size and, *if they receive appropriate signals from T cells,* proliferate and differentiate in plasma cells [36]. As we have mentioned, B cells can be at the center of *antigen-independent* responses, which occur early in the B cell developmental pathway and can be induced by the association of H chains with CD79b in B cells that develop to the pre-B cell stage even in the absence of L chain synthesis [408].

Expression of Genes and Transcriptional Activity

Phosphorylation of several membrane and cytoplasmic proteins corresponds to a transient stage during which both translocation of TFs and expression of new genes are modulated. It plays an important role in intercellular transduction of signals: studies on animal mast cells have shown that phosphorylation of tyrosine residues is an essential component of the signals deriving from

Table 1.33. Cytokine usage of JAK and STAT proteins

ILs	JAK1	JAK2	JAK3	Tyk2	STAT1	STAT2	STAT3	STAT4	STAT5	STAT 6
Antigen (BcR)					+		+			+
Angiotensin		+		+	+	+				
IL_1										
IL_2	+		+				+		+	
IL_3		+							+	
IL_4	+		+							+
IL_5		+							+	
IL_6	+	+		+	±		+			
IL_7	+	+							+	
IL_8										
IL_9	+		+	+	+					
IL_{10}	+			+	±		+		+	
IL_{11}										
IL_{12}	+			+				+		
IL_{13}	+		+	+						
IL_{14}										
IL_{15}	+	+	+				+		+	
IL_{16}										
IL_{17}										
IL_{18}										
IL_{19}							+			
IL_{20}							+			
$IL_{21}R$	+		+	+						
IL_{22}					+	+	+		+	
$IL_{22}R$							+			
CNTF	+	+		+	+		+			
CSF1					+				+	
EGF	+				+		+			
Epo		+							+	
G-CSF	+	+			+		+			
GM-CSF		+							+	
HGH		+			+		+			
IFN-α	+		+	+	+		+			
IFN-β				+	+	+	+			
IFN-γ	+	+		+	+		+			
LIF	+	+		+	+		+			
OsM	+	+		+	+		+			
PDGF	+	+		+	+		+			
Thrombopoietin	+			+	+				+	

Data from ICI.

IL_6R is homologous to the p40 subunit of IL_{12}, which in turn may produce gp130 dimers; however, signaling takes place above all through the activation of a peptide homologous to gp130. G-CSFR is homologous to the p130 chain of IL_6. EpoR has a high degree of homology with the$IL_2R\beta$ chain. Updated from the Institute of Clinical Immunology.

CNTF ciliary neurotropic factor, *EGF* epidermal growth factor, *Epo* erythropoietin, *GH* growth hormone, *IL* interleukin, *LIF* leukemia inhibitory factor, *PDGF* platelet derived growth factor, *Tpo* thrombopoietin.

FcεRI [35, 195]. Aggregation of polyvalent antigens to the FcεRI–IgE complex results in tyrosine phosphorylation of several protein substrates, including β and γ subunits of FcεRI, and proteins such as $p72^{syk}$, $p53/56^{syn}$, $pp60^{sc\text{-}src}$, PKCγ, $p95^{svav}$, paxilline, pp105–115 and $pp125^{FAK}$: FN adhesion of cells of basophil lineage, when FcεRI is absent, reduces phosphorylation to only the last three proteins [195, 196]. Another example is NF-κB associated with IκB-α (inhibitory κBα), an inhibitor and multiform protein induced by Bc110 [24, 621], probably to prevent inadvertent tissue detriments [303]. In vitro studies show that IκB-α phosphorylation by PKCζ [118], since it does not lead to protein degradation [224], allows the NF-κB–IκB-α complex dissociation and NF-κB activation by PKCζ translocation [118] into the nucleus and fixation on κB regulatory sequences [54]. TRAF6 is thought to activate a member of the MAPK family, which directly or indirectly leads to the activation of IKK1 and IKK2. Both kinases phosphorylate IκB on serine residues, thus targeting IκB for degradation and releasing NF-κB [345]. A TF of the NFAT family, including c-*jun* and c-*fos* dimers that together form a potent transcriptional activation complex, binds via NF-κB to specific DNA-regulatory sites of many IL genes in T cells [252]. CN then dephosphorylates NF-κB, passing from a pre-existing state, NFATp, to the cytosolic state, NFATc, beyond which there are NFAT3, NFAT4 genes, etc. [207]. NFATc is then translocated into the nucleus and binds to regulatory sequences in position 5' of the promoter region of some genes, for example of IL_2 [252]. During the 30 min following ligand interactions with membrane receptors, there is the expression of proto-oncogene c-*fos*, c-*jun* and c-*myc*: their products bind to regulatory structures [454]. The association of c-*jun* and c-*fos* dimers, products of *immediate-early genes,* generates the heterodimer TF AP-1 (activating protein-1) [252]. At the T-cell level, the coordinated fixation of several TFs on regulatory elements, such as NFATc, NF-κB, the fos/jun proteins and AP-1 disposed upon a site from −300 to −63 bp upstream of the IL_2 promoter leads to the pertinent gene transcription and IL synthesis [444]. IL_2–IL_2R binding yields a progression signal allowing the complex internalization and lymphocyte progression from G_1 stage to S stage of cell cycle and DNA replication, accompanied with CD71 and HLA-DR expression [444]. Similar processes regulate IL_2R α chain and other receptor transcription, IL_4, IL_7, IL_9, IL_{13}, IL_{15}, *whose α chain is a functional component of* IL_2*-Rγ* [99, 269, 395, 483, 693] and of IL_5, IL_6, IL_{10}, the IFNs, GM-CSF and TNF-α [222, 444]. In particular IL_2, IL_4, IL_5 and TNF-α are under NFAT influx stimulated by calmodulin [588]. Recently, the GATA family of transcription factors has been characterized, which bind to DNA sequences through a highly conserved C4 zinc finger domain. Six members (GATA-1–GATA-6) of this family have been identified in avians, with homologs in mammals and amphibians [342]. Based on their expression profile, the GATA proteins may be classified functionally as hemopoietic (GATA-1–GATA-3) or non-hemopoietic (GATA-4–GATA-6) [342]. GATA-3 is expressed primarily in T lymphocytes and in the embryonic brain. Functionally important GATA-3-binding sites have been identified in TcR genes and the CD8 gene [342]. Significantly, Th2 cells contain GATA-3 protein in a constitutive fashion, which increases upon stimulation of the cells by antigen or cAMP, whereas Th1 cells express very little or no GATA-3 at the basal level [678]. T-bet, a TF member of the T-box family expressed in T cells is necessary to induce T cells to differentiate into Th1 cells and for Th1 cells to produce IFN-γ. Since IFN-γ induces T-bet expression, it is possible that IFN-γ affects T-bet expression by Th1 cells. Mice lacking T-bet do not have a functional Th1 response in vivo [556], and recent studies stress that T-bet expression is down-regulated in asthmatic patients [155]. Further, T-bet enhances IFN-γ secretion and suppresses IL_4 secretion in γδ cells, and GATA-3 fails to counterbalance T-bet-mediated IFN-γ production, accounting for the default synthesis of IFN-γ by these T lymphocytes [665].

Signal Transduction

The importance of post-receptor signals transduced by members of the IL receptor superfamily attached to JAK (Janus-family kinase) 1–3 and Tyk-2 activated family members connecting the receptors with the STAT factors is now apparent [214]. The STAT family in turn also includes erythropoietin, G-CSF, and the IFNs [214]. In the Tables 1.32, 1.33 we show two aspects of IL interactions with both JAK and STAT proteins. STAT specificity, phosphorylated and activated by different ILs, is mediated by STATs present within target cells and by affinity of such proteins to the JAK–Tyk complex [222]. In addition, IL_4-mediated expression of pertinent genes activates the JAK modulating STAT phosphorylation, three exons of which dictate the SH2 region necessary to its activation, of IL_4-NFAT (NFAT activated by IL_4), and other signaling pathways: in particular STAT6 binds to DNA sequences controlling the expression of IL-4-induced Th2 T cell response [562] (Fig. 1.44) [457], absent in mice with disrupted STAT6 genes [523]. Mice devoid of STAT6 fail to produce both IgE antibodies and Th2 lymphocytes in response to IL_4 or IL_{13} [248]. A differential expression of the IL_4 gene in Th1 and Th2 T cells is associated with a diverse regulation of NFAT binding to IL_4 CLE0 (consensus lymphokine element-0), mediated via a different TF regulation in Th1 and Th2 lymphocytes [642]. GATA-3 protein is expressed in both immature and mature T cells, but in a constitutive fashion in Th2 cells, which increases upon stimulation of the cells by Ag or cAMP. In contrast, GATA-3 is selectively suppressed in Th1 cells; thus *GATA-3 may function as a more general regulator of Th2 ILs expression* [678]. An alternative signal transduction system in activated T cells, formed by JAK-1, JAK-3, STAT3 and STAT5, is asso-

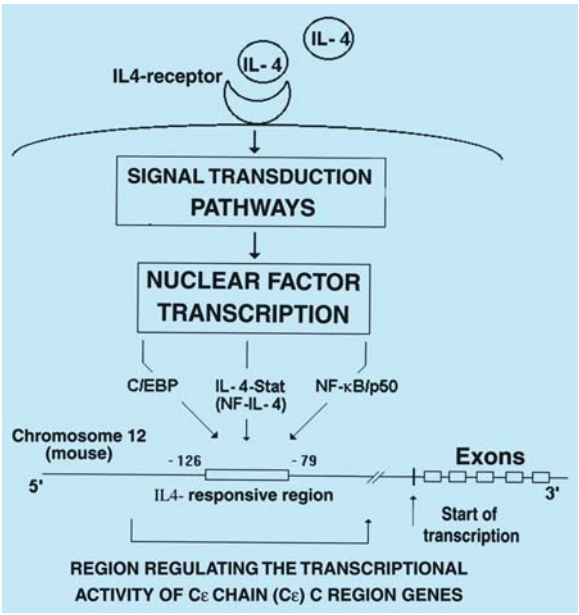


Fig. 1.44. Schematic representation of IL_4-mediated regulation of genes controlling the Cε chain synthesis of IgE antibodies

ciated with IL_2 and IL_{15} [240], JAK1 and -2, STAT1, -2, -3 and -5 by IL_{27} [245], also including IL_2R, $IL_{15}R\alpha$ and -β [557] as well as the receptors sharing the IL_2R γ chain [404]. Studying intracellular signals has led to the identification of three immunosuppressors of T lymphocyte activation: cyclosporine A (CsA), FK-506 (tacrolimus), and rapamycin (RAP) [505]. CsA and FK-506 bind, respectively, to a cyclophilin and the proteins binding to FK-506 (FKBP): the complexes thus formed bind to CN-dependent phosphatase 2B activity [505]. The CsA/FKBP/RAP-mediated inhibition of NFAT-dependent IL_2 gene transcription is overcome by CN overexpression [341]. CN is therefore essential in the lymphocyte signal transduction pathway, also leading to metachromatic cell degranulation [341].

Activation and Immunosuppression of B and T Lymphocytes and of Other Cells

The *lectins,* carbohydrate-binding gps, are active either substituting IgE antibodies on mIgs or cross-linking their H chains to carbohydrates expressed on various cells, and activating mast cell degranulation by an aspecific binding, and are also known as mitogens (proliferation inducers). Mitogen in vitro stimulation of lymphocytes is believed to mimic fairly closely specific antigen stimulation. B and T cells are activated by different mitogens: mouse B cells by LPS (lipopolysaccharide), human B and T cells by PWM (pokeweed mitogen), and human and mouse T cells by Con-A (concanavalin A) and PHA (phytohemagglutinin) [470].

Table 1.34. Absolute size of the main, age-related lymphocyte subpopulations (median + 5th–95th percentiles)

Lymphocyte	Neonate	7 d–2 m	2–5 m	5–9 m	9–15 m	15–24 m	2–5 y	5–10 y	10–16 y
Lymphocyte Absolute size	4.8 (0.7–7.3)	6.7 (3.5–13)	5.9 (3.7–9.6)	6.0 (3.8–9.9)	5.5 (2.6–10.4)	5.6 (2.7–11.9)	3.3 (1.7–6.9)	2.8 (1.1–5.9)	2.2 (1.0–5.3)
CD19 (%)	12 (5–22)	15 (94–260)	24 (14–29)	21 (13–35)	25 (15–39)	28 (17–41)	24 (14–44)	18 (10–31	16 (8–24)
Absolute size	0.6 (0.04–1.1)	1.0 (0.6–1.9)	1.3 (0.6–3.0)	1.3 (0.7–2.5)	1.4 (0.6–2.7)	1.3 (0.6–3.1)	0.8 (0.2–2.1)	0.5 (0.2–1.6)	0.3 (0.2–0.6)
CD3 (%)	62 (28–76)	72 (60–85)	63 (48–75)	66 (50–77)	65 (54–76)	64 (39–73)	64 (43–76)	69 (55–78)	72 (55–83)
Absolute size	2.8 (0.6–5.0)	4.6 (2.3–7.0)	3.6 (2.3–6.5)	3.8 (2.4–6.9)	3.4 (1.6–6.7)	3.5 (1.4–8.0)	2.3 (0.9–4.5)	1.9 (0.7–4.2)	1.5 (0.8–3.5)
CD3/CD4 (%)	41 (17–52)	55 (41–68)	45 (33–58)	45 (33–58)	44 (31–54)	41 (25–50)	37 (23–48)	35 (27–53)	39 (25–48)
Absolute size	1.9 (0.4–3.5)	3.5 (1.7–5.3)	2.5 (1.5–5.0)	2.8 (1.4–5.1)	2.3 (1.0–4.6)	2.2 (0.9–5.5)	1.3 (0.5–2.4)	1.0 (0.3–2.0)	0.8 (0.4–2.1)
CD3/CD8 (%)	24 (10–41)	16 (9–23)	17 (11–25)	18 (12–28)	18 (13–26)	20 (11–32)	24 (14–33)	28 (19–34)	23 (9–35)
Absolute size	1.1 (0.2–19)	1.0 (0.4–1.7)	1.0 (0.5–1.6)	1.1 (0.5–2.2)	1.1 (0.4–2.1)	1.2 (0.4–2.3)	0.8 (0.3–1.6)	0.8 (0.3–1.8)	0.4 (0.2–1.2)
CD4/CD8	1.8 (1.0–2.6)	3.8 (1.3–6.3)	2.7 (1.7–3.9)	2.5 (1.6–3.8)	2.4 (1.3–3.9)	1.9 (0.9–3.7)	1.6 (0.9–2.9)	1.2 (0.9–2.6)	1.7 (0.9–3.4)
CD3/HLA-DR (%)	2 (1–6)	5 (1–38)	3 (1–9)	3 (1–7)	4 (2–8)	6 (3–12)	6 (3–13)	7 (3–14)	4 (1–8)
Absolute size	0.09 (0.03–0.4)	0.3 (0.03–3.4)	0.2 (0.07–0.5)	0.2 (0.07–0.5)	0.2 (0.1–0.6)	0.3 (0.1–0.7)	0.2 (0.08–0.4)	0.2 (0.05–0.7)	0.06 (0.02–02)
CD3/CD16/56 (%)	20 (6–58	8 (3–23)	6 (2–14)	5 (2–13)	7 (3–17)	8 (3–16)	10 (4–23)	12 (4–26)	15 (6–27)
Absolute size	1.0 (0.1–1.9)	0.5 (0.2–1.4)	0.3 (0.1–1.3)	0.3 (0.1–1.0)	0.4 (0.2–1.2)	0.4 (0.1–1.4)	0.4 (0.1–1.0)	0.3 (0.09–0.9)	0.3 (0.07–1.2)

Absolute counts ($\times 10^3$ cells/mm^3). Data from [93]. *d* days, *m* months, *y* years.

Table 1.35. Changes in lymphocyte major subsets and analysis as a function of age (median + 25th and 75th percentile)

Lymphocytes	Cord blood	2 Days to 11 months	1–6 Years	7–17 Years
Lymphocyte count Absolute count	12 (10–15)	9.0 (6.4–11)	7.8 (6.8–10)	6.0 (4.7–7.3)
Lymphocytes (%) Absolute count	41 (35–47) 5.4 (4.2–6.9)	47 (39–59) 4.1 (2.7–5.4)	46 (38–53) 3.6 (2.9–5.1)	40 (36–43) 2.4 (2.0–2.7)
T lymphocytes (%) Absolute count	55 (49–62) 3.1 (2.4–3.7)	64 (58–67) 64 2.5 (1.7–3.6)	(62–69) 2.5 (1.8–3.0)	70 (66–76) 1.8 (1.4–2.0)
B lymphocytes (%) Absolute count	20 (14–23) 1.0 (0.7–1.5)	23 (19–31) 0.9 (0.5–1.5)	24 (21–28) 0.9 (0.7–1.3)	16 (12–22) 0.4 (0.3–0.5)
NK cells (%) Absolute count	20 (14–30) 0.9 (0.8–1.8)	11 (8–17) 0.5 (0.3–0.7)	11 (8–15) 0.4 (0.2–0.6)	12 (9–16) 0.3 (0.2–0.4)
T cells (%)				
HLA-DR in CD3	2.0 (2.0–3.0)	7.5 (4.0–9.0)	9.0 (6.0–16) 12	(9.5–17)
IL_2R in CD3	8.0 (5.5–10)	9.0 (7.0–12)	11 (8.0–12)	13 (10–16)
CD57 in CD3	0.0 (0.0–0.0)	1.5 (0.0–2.5)	3.0 (2.0–5.0)	5.5 (3.0–10)
T cells $CD4^+$ (%) Absolute count	35 (28–42) 1.9 (1.5–2.4)	41 (38–50) 2.2 (1.7–2.8)	37 (30–40) 1.6 (1–1.8)	37 (33–41) 0.8 (0.7–1.1)
$CD45RA^+$ in CD4 (%)	91 (82–97)	81 (66–88)	71 (66–77)	61 (55–67)
Leu-8+ in CD4 (%)	91 (85–95)	90 (88–98)	91 (84–95)	87 (81–89)
T cells $CD8^+$ (%) Absolute count	29 (26–33) 1.5 (1.2–2.0)	21 (18–25) 0.9 (0.8–1.2)	29 (25–32) 0.9 (0.8–1.5)	30 (27–35) 0.8 (0.8–0.9)
$CD57^+$ in CD8 (%)	0.0 (0.0–1.0)	7.0 (4.0–9.5)	10 (6–15)	17 (12–24)
CD4/CD8 ratio	1.2 (0.8–1.8)	1.9 (1.5–2.9)	1.3 (1–1.6)	1.3 (1.3–1.4)
B cells ($CD5^+CD20^+$) Absolute count	0.5 (0.4–1.0)	0.5 (0.2–1.1)	0.5 (0.3–0.8)	0.2 (0.1–0.3)
$CD5^+$ in CD20 (%)	72 (58–79)	68 (47–76)	64 (53–77)	56 (44–64)
$CD23^+$ in CD20 (%)	35 (30–50)	50 (44–66)	61 (53–70)	63 (52–73)
Leu-8^+ in CD20 (%)	57 (23–68)	66 (40–90)	79 (57–89)	90 (83–94)
$CDw78^+$ in CD19 (%)	49 (37–64)	22 (15–37)	30 (17–45)	32 (19–50)

The absolute counts are $\times 10^3$ cells/mm^3.
Data from [142].

T cells expressing low CN levels are more sensitive to the action of CsA and/or FK-506; similarly immunosuppressive activity of CsA or of its analogs correlates with CN phosphatase 2B activity inhibition [341]. In addition, in healthy volunteers CsA caused a rapid inhibition of histamine release from basophils or a 30%–60% inhibition of their releasability [72].

Mean Values of Lymphocyte Populations and Subpopulations and of Other Immune Cells

Immunophenotyping of blood lymphocytes has become an important tool in the diagnosis of pediatric PIDs and AIDS. The increased prevalence of these disorders, as well as of pediatric asthma, frequently makes a determination of lymphocyte subsets and of pediatric BALF necessary.

Blood values determined in normal children as a function of age are reported in Tables 1.34, 1.35 [93, 142] and 1.36–1.39 [203, 440, 458]. Lymphocyte values include the median and 25th–75th percentiles, apart from one study with a 5th–95th percentile range (Table 1.34; Appendix 1.3) [93]. Table 1.35 [142] and especially in Tables 1.36–1.39 outline relative and absolute values of several lymphocyte subsets. The age range is extended to 16 years (Table 1.34), 17 years (Table 1.35) and compared to adults (Table 1.36). In a recent study the age range is extended up to 18 years [516]. We do not agree that CB values at 5 days after birth of healthy neonates (Table 1.38) [440] can serve as a reference range in the evaluation of probable PIDs and HIV infection. We recommend determining chiefly the ab-

Table 1.36. Percentage values of lymphocyte subpopulations in children at various ages and in adults (mean + 25th–75th percentile)

Age	Cord blood	1.28 (0.63–3.06)	4.25 (3.92–4.84)	9.7 (7.7–10.6)	Adults
B-lineage markers					
CD19	12.0 (3.0–29.0)	14.5 (6.0–33.0)	17.0 (4.0–38.0)	9.0 (7.0–27.0)	4.5 (2.0–6.0)
CD20	8.0 (0.0–23.0)	4.0 (0.0–47.0)	8.0 (0.0–19.0)	2.0 (0.0–8.0)	1.0 (0.0–2.0)
CD21	2.0 (0.0–10.0)	5.0 (0.0–14.0)	3.0 (0.0–28.0)	1.0 (0.0–6.0)	1.0 (0.0–2.0)
CD22	6.0 (2.0–23.0)	8.5 (1.0–30.0)	10.5 (0.0–30.0)	7.0 (2.0–13.0)	2.0 (1.0–5.0)
CD23	1.0 (0.0–7.0)	1.0 (0.0–3.0)	0.0 (0.0–2.0)	0.0 (0.0–2.0)	0.5 (0.0–6.0)
CD24	3.0 (0.0–8.0)	7.0 (1.0–14.0)	6.5 (2.0–12.0)	5.5 (2.0–22.0)	2.0 (1.0–4.0)
CD37	13.0 (4.0–29.0)	13.5 (3.0–31.0	14.5 (4.0–35.0)	10.5 (1.0–24.0)	5.0 (2.0–11.0)
CD39	1.0 (1.0–6.0)	3.0 (0.0–9.0)	4.5 (0.0–37.0)	1.5 (0.0–4.0)	1.0 (0.0–2.0)
CD40	12.0 (1.0–28.0)	14.5 (8.0–24.0)	18.0 (5.0–39.0)	9.5 (5.0–15.0)	4.0 (1.0–7.0)
HLA-DR	14.0 (8.0–29.0)	19.0 (8.0–47.0)	18.0 (10.0–35.0)	12.0 (5.0–21.0)	5.5 (3.0–8.0)
FMC7	10.0 (1.0–29.0)	10.0 (3.0–24.0)	17.5 (7.0–39.0)	11.0 (4.0–33.0)	4.0 (2.0–7.0)
IgD	7.0 (3.0–26.0)	9.5 (2.0–21.0)	6.5 (1.0–25.0)	5.0 (3.0–16.0)	1.5 (0.0–2.0)
IgG	1.5 (0.0–28.0)	2.0 (0.0–17.0)	1.0 (0.0–3.0)	0.0 (0.0–1.0)	1.5 (0.0–7.0)
IgM	11.0 (3.0–26.0)	15.4 (5.0–23.0)	14.5 (5.0–34.0)	8.5 (4.0–28.0)	3.0 (1.0–6.0)
T-lineage markers					
CD2	69.0 (36.0–81.0)	75.0 (25.0–87.0)	66.0 (42.0–82.0)	76.0 (50.0–84.0)	86.5 (71.0–92.0)
CD3	63.0 (21.0–73.0)	67.0 (53.0–84.0)	62.0 (39.0–74.0)	71.0 (58.0–78.0)	75.0 (53.0–81.0)
CD4	49.0 (16.0–58.0)	46.0 (22.0–87.0)	37.5 (29.0–51.0)	43.5 (28.0–55.0)	40.5 (29.0–62.0)
CD7	77.0 (55.0–86.0)	63.5 (44.0–83.0)	63.5 (47.0–72.0)	68.0 (58.0–84.0)	70.0 (41.0–87.0)
CD8	19.0 (13.0–29.0)	18.0 (12.0–52.0)	22.0 (11.0–33.0)	21.5 (17.0–31.0)	25.5 (20.0–43.0)
CD4/8	2.5 (0.8–4.0)	2.65 (0.4–5.4)	1.75 (1.2–4.6)	2.1 (1.0–3.2)	1.55 (0.9–3.1)
CD26	11.0 (2.0–59.0)	2.5 (0.0–10.0)	5.5 (2.0–10.0)	3.0 (0.0–7.0)	5.0 (0.0–10.0)
NK series					
CD16	15.0 (4.0–30.0)	3.5 (0.0–13.0)	6.5 (4.0–12.0)	4.5 (2.0–10.0)	6.5 (2.0–25.0)
Non-lineage marker					
CD38	75.0 (40.0–88.0)	34.0 (12.0–75.0)	24.5 (16.0–34.0)	12.5 (11.0–25.0)	8.5 (2.0–21.0)
Leukocyte common markers					
CD45	75.0 (54.0–87.0)	74.0 (12.0–81.0)	68.0 (62.0–74.0)	66.0 (41.0–91.0)	48.5 (29.0–71.0)
CD45R	68.0 (46.0–85.0)	76.0 (39.0–85.0)	63.5 (46.0–79.0)	71.0 (60.0–92.0)	54.0 (34.0–82.0)

Data from [203].

solute values, even if we also report relative values of lymphocyte subsets to allow a complete evaluation. As regards age variations, lymphocyte values decrease from 66% to 50% between 2–3 months and 5 years of age, but remain substantially stable [117], whereas CD4 values are constantly higher than CD8 values, with a reversed CD4/CD8 ratio returning to normal in adolescents when CD8 cells increase. Only one study [142] found that the CD4/CD8 ratio remained unchanged with age, a result limited to the 5- to 13-year age range [464], or was disputed [613]. *In the 1st year of life, CD8 cells are less than 50% of CD4 cells (41%) and B cells 22.5%* [142], with evidently negative consequences [647].

BALF CD4/CD8 ratios are lower than in adults [445] due to an increase in CD8 cells with a reversed CD4/CD8 ratio, which has not been observed in healthy adults [445, 458]. The case reports published (Tables 1.40, 1.41) [202, 445, 458] regard nonatopic children without acute respiratory infections aged 3 months to 10 years (mean 31 months) [458], or 3–16 years (mean, 8±3 years)

Table 1.37. Absolute values of lymphocyte subsets in children at various ages and in adults: (mean + 25th–75th percentile) $\times 10^3$ cells/mm^3

Age	0.63 (1.29–3.06)	4.08 (4.28–4.83)	7.66 (9.66–10.59)	Adults
Leukocytes	6.60 (4.50–12.80)	7.20 (5.50–8.80)	5.55 (3.00–7.20)	5.60 (3.80–9.10)
Monocytes	4.75 (3.00–9.30)	3.20 (2.00–5.60)	2.80 (1.70–4.50)	2.20 (1.20–4.80)
B-lineage markers				
CD19	0.76 (0.18–1.62)	0.58 (0.12–2.05)	0.31 (0.15–1.22)	0.08 (0.05–0.29)
CD20	0.20 (0.03–2.26)	0.26 (0.03–1.03)	0.05 (0.00–0.36)	0.02 (0.00–0.10)
CD21	0.21 (0.00–0.60)	0.06 (0.00–0.32)	0.02 (0.00–0.18)	0.02 (0.00–0.10)
CD22	0.41 (0.12–1.47)	0.38 (0.00–1.62)	0.22 (0.03–0.43)	0.05 (0.02–0.19)
CD23	0.05 (0.00–0.18)	0.00 (0.00–0.03)	0.00 (0.00–0.05)	0.00 (0.00–0.14)
CD24	0.37 (0.18–0.67)	0.16 (0.06–0.65)	0.16 (0.05–0.99)	0.04 (0.03–0.19)
CD37	0.80 (0.24–1.52)	0.56 (0.12–1.89)	0.28 (0.04–1.08)	0.09 (0.05–0.34)
CD39	0.14 (0.00–0.41)	0.06 (0.00–2.00)	0.05 (0.00–0.13)	0.01 (0.00–0.05)
CD40	0.81 (1.30–1.21)	0.74 (0.15–2.11)	0.30 (0.09–0.68)	0.09 (0.03–0.34)
HLA-DR	0.81 (0.33–1.30)	0.65 (0.24–1.35)	0.34 (0.14–0.95)	0.12 (0.07–0.38)
FMC7	0.58 (0.14–1.18)	0.64 (0.29–2.10)	0.28 (0.10–1.49)	0.09 (0.03–0.19)
IgD	0.44 (0.09–1.03)	0.17 (0.03–0.42)	0.13 (0.07–0.72)	0.03 (0.00–0.06)
IgG	0.05 (0.00–0.75)	0.00 (0.00–0.10)	0.00 (0.00–0.03)	0.02 (0.00–0.06)
IgM	0.75 (0.18–1.13)	0.39 (0.14–1.09)	0.22 (0.09–1.26)	0.06 (0.02–0.13)
T-lineage markers				
CD2	2.85 (1.28–7.16)	2.02 (1.22–4.14)	2.07 (1.41–3.70)	1.98 (1.02–3.98)
CD3	2.83 (1.83–6.60)	1.94 (1.14–4.09)	2.04 (1.22–2.86)	1.65 (0.85–3.60)
CD4	2.00 (0.94–5.05)	1.25 (0.76–2.24)	1.21 (0.77–1.80)	0.87 (0.50–1.82)
CD7	2.89 (1.77–5.21)	1.97 (1.30–4.03)	1.99 (0.99–3.08)	1.49 (0.50–2.44)
CD8	0.86 (0.51–1.86)	0.59 (0.36–1.34)	0.56 (0.32–1.36)	0.75 (0.28–1.54)
CD4/8	2.80 (1.00–5.44)	1.79 (1.50–4.64)	2.03 (1.00–3.18)	1.50 (0.85–3.10)
CD26	0.12 (0.00–0.46)	0.16 (0.06–0.22)	0.09 (0.00–0.22)	0.08 (0.00–0.29)
NK marker				
CD16	0.17 (0.00–0.47)	0.22 (0.14–0.39)	0.13 (0.05–0.45)	0.13 (0.03–0.29)
Non-lineage marker				
CD38	1.49 (0.61–3.36)	0.90 (0.38–1.19)	0.38 (0.20–0.81)	0.23 (0.03–0.42)
Leukocyte common markers				
CD45	3.42 (0.61–6.88)	2.18 (1.24–3.92)	1.82 (0.94–3.06)	0.99 (0.52–2.21)
CD45R	3.64 (2.25–7.72)	2.21 (1.18–3.47)	1.94 (1.38–3.29)	1.38 (0.55–2.40)

Data from [203].

[445] and healthy children aged 5 months to 14.6 years (median, 7.2 years) [202]. Such data derive from pooled aliquots and age-corrected volumes, where the first sample showed more ciliated cells than subsequent ones [458]; among the aliquots there is not always a significant difference [445, 458]. The analysis of the reduced CD4/CD8 ratio suggests a possible influence of the highest frequency of viral infections in the younger age groups [445].

The BALF pediatric levels of other immune cells, including macrophages, granulocytes, mast cells, etc., are summarized in Tables 1.40 and 1.41. The study in healthy children [202] examined the cells with a less invasive procedure, using a neonatal catheter (external diameter 2.6 mm), inserted prior to the start of surgery, without noting significant differences. These studies are of invaluable use in asthmatic and immodeficient children.

Table 1.38. Absolute and relative values of lymphocyte subpopulations in cord blood and venous blood (day 5): (mean + 25th–75th percentile)

CD markers	Cord blood	5 days
CD1 (%)	0.4 (0.3–0.7)	0.4 (0.2–0.6)
Absolute count	0.02 (0.01–0.03)	0.01 (0.01–0.03)
CD2 (%)	64.9 (57.0–72.8)	74.3 (65.1–87.5)
Absolute count	2.77 (2.19–3.78)	2.97 (2.49–3.95)
CD3 (%)	59.1 (52.9–67.9)	73.4 (65.6–82.9)
Absolute count	2.61 (2.01–3.36)	3.03 (2.38–3.95)
$CD3^+/CD16^+ + CD56^+$ (%)	0.1 (0.1–0.2)	0.1 (0.1–0.2)
$CD3^-/CD16^+ + CD56^+$ (%)	12.2 (7.3–17.2)	4.8 (2.3–7.0)
Absolute count	0.50 (0.26–0.88)	0.18 (0.09–0.30
CD4 (%)	44.2 (39.3–51.4)	56.9 (52.2–64.0)
Absolute count	1.93 (1.49–2.59)	2.35 (1.97–3.4)
$CD4^+/CD45RA^+$ (%)	31.8 (27.5–38.8)	47.0 (41.0–52.7)
$CD4^+/CD29^+$ (%)	8.5 (6.2–10.6)	6.6 (4.8–9.2)
Absolute count	0.92 (0.70–1.30)	0.91 (0.70–1.11)
$CD5^+/CD19^-$ (%)	61.9 (55.3–68.9)	77.8 (71.6–87.4)
Absolute count	2.68 (2.17–3.54)	3.18 (2.61–4.11)
CD7 (%)	76.8 (70.2–82.6)	81.4 (74.1–89.5)
Absolute count	3.26 (2.57–4.54)	3.38 (2.75–4.24)
CD8 (%)	21.6 (18.6–26.0)	21.2 (18.0–24.4)
Absolute count	0.92 (0.70–1.30)	0.91 (0.70–1.11)
$CD8^+/S6F1^+$ (%)	8.8 (6.0–11.7)	5.3 (3.8–8.7)
$CD8^+/S6F1^-$ (%)	12.5 (9.0–18.0)	14.9 (11.9–18.7)
CD4/CD8 ratio	1.97 (1.62–2.46)	2.74 (2.34–3.26)
CD10 (%)	0.6 (0.4–1.1)	0.3 (0.1–0.5)
CD19 (%)	12.8 (9.2–17.4)	16.0 (1.4–10.4)
Absolute count	0.62 (0.34–0.91)	0.21 (0.06–0.43)
$CD19^+/CD5^+$ (%)	0.9 (0.6–1.3)	0.6 (0.3–1.1)
CD20 (%)	13.8 (9.8–18.0)	6.1 (1.4–11.1)
Absolute count	0.62 (0.37–0.98)	0.24 (0.05–0.44)
CD22 (%)	11.6 (8.5–16.5)	5.5 (1.4–10.2)
CD23 (%)	0.6 (0.4–1.0)	0.4 (0.3–0.8)
CD57 (%)	0.1 (0.1–0.2)	0.1 (0.1–0.3)

Values for absolute cell counts are expressed as $\times 10^3$ cells/mm^3.
Data from [440].
$CD8^+/S6F1^+$ killer effector cells, *$CD8^+/S6F1^-$* suppressor effector cells.

Table 1.39. Mean and percent values of CD (lymphocyte surface antigens) in cord blood

	Percent values		Absolute values (cells/μl)	
	Mean	Range	Mean	Range
T- and NK-cell lineage				
CD1	3.8	2.3– 5.8	173	110–262
CD2	60.9	52.4–66.8	2803	1,821–3,514
CD3	57.5	50.5–63.3	2,477	1,820–3,371
TcR-αβ	57.7	48.1–61.0	2,573	1,557–3,287
CD4	36.0	28.0–42.6	1,780	904–2,320
CD8	23.0	20.0–27.4	967	673–1,248
B-cell lineage				
CD19	12.1	8.6–14.8	424	214–633
CD20	11.1	6.7–15.5	485	93–877
Activation markers and others				
CD11a	56.3	46.3–68.5	2,704	1,876–3,804
CD25	2.6	2.1– 4.5	140	75–197
CDw52	61.0	51.2–76.1	2,740	1,851–4,145
CD71	5.2	3.1– 9.3	228	164–289

Values expressed as cells/μl; 1 μl=10^6 cells/l.
Data from [269].

Table 1.40. BALF lymphocyte subpopulations from pediatric studies (BALF cells – pooled samples – % of total lymphocytes)

CD	Mean +25th–75th percentile[a]	Mean±SD[b]	CD	Mean +25th–75th percentile[a]	Mean±SD[b]
CD3 (T)	81.0 (75.5–88.0)	85.8 ±4.9	CD19	5.0 (4.0–9.5)	
CD4	27.0 (22.0–32.0)	33.1 ±12.8	CD20 (B)		0.9±1.5
CD8	45.0 (33.8–57.0)	56.8 ±13.1	CD25	2.0 (0.0–3.0)	1.9±1.3
CD4/CD8	0.6 (0.4–1.0)	0.68 ±0.44	CD57 (NK)		7.8±8.2
CD16+CD56	4.0 (1.5–7.5)		HLA-DR		1.4±1.7

[a] Data from [458]. [b] Data from [445].

Table 1.41. BALF differential cytology from pediatric studies (BALF cells – pooled samples – % of total lymphocytes)

Cell types	Mean + 25th–75th percentile[a]	Mean and range[b]	Mean and range[c]
Macrophages	91.0 (84.2–94.0)	83 (47–90)	70.07 (29.0–96.3)
Granulocytes		1.4 (0.2–11)	0.09 (0.0–0.9)
Eosinophils	0.2 (0.0–0.3)		
Neutrophils	1.7 (0.6–3.5)		0.2 (0.4–34.4)
Mast cells			0.2 (0.0–0.8)
Epithelial cells			13.4 (0.3–64.7)

[a] Data from [458]. [b] Data from [445]. [c] Data from [202].

Central Phase of the Immune Response: Synthesis of IgE Antibodies

A Two-Signal Model for Induction of IgE Synthesis

According to the two-signal theory [606], we can distinguish a first signal delivered by IL_4 and a second one provided by interactions between $TCD4^+$ and B lymphocytes [172].

First Signal

IL_4 production by Th2 lymphocytes is for B lymphocytes a crucial factor for isotype switching to IgE plasma cells. IL_4, similarly to IL_{13} [437] is sufficient to trigger in B cells the expression of ε germline transcripts containing one exon located upstream of the ε switch region, and spliced to the C_H region codified by Cε genes [176]. According to this model, such transcripts are thought to play a critical role in IgE isotype switching by increasing the ε locus opening, which thereby becomes accessible to recombination enzymes [296]. Furthermore, IL_4 acts by altering the chromatin structure of the Sγ1 region inducing γ1 and ε transcript accumulation, and B cells activate TFs binding DNA in the region 179 bp upstream of germline ε. IL_4 can therefore be sufficient to regulate some events of recombination machinery at the B lymphocyte level [177], initiating with IL_{13} transcription of the $C_ε$ gene of Ig H chain [250]. Besides, IL_4 contributes to Fcε receptor increase on B cells and LC, increases the expression of class II HLA molecules on macrophages, inhibits their IL_1 production and primes them to differentiate into DCs [296]. IL_4R (CD140), of which $IL_{13}R$ is a component [64], assists with appropriate signals inducing Cε germline transcripts and increasing their role via PLCγ1 and PI3K, which also modulates isotype switching leading to mature Cε transcription and to IgE synthesis [659]. Moreover, CD140 evokes Th2 T cell differentiation and IL_4 production mediated by CD8 lymphocytes [4]. IL_4 and IL_{13} are in some way independent, since anti-IL_4 abrogates the effects charged to IL_4, but not those that are IL_{13}-triggered [125]. Likewise, immune responses to IL_{13} can also be mediated by IL_4, whereas the contrary is not known [64]; however, cells not expressing the γc chain, as is the case of X-SCID, respond to both IL_4 and IL_{13} [64].

Role of ILs [205, 473, 606]. Besides IL_4, several ILs take part in IgE synthesis [34, 296]. When an antigen is encountered, both macrophages and accessory cells following a signal delivered by IFN-γ release ILs starting to secrete IL_1, with a substantial effect on thymocyte proliferation stimulated by a lectin, further enhancing T-lymphocyte increase, chiefly Th2 T cells. However, on antigen recognition, IL_1 binds to Th1 T-cell-specific receptors, priming them to produce IL_2 growth factor for T cells and express mRNA for IL_2 molecules [457], although IL_7 is more powerful than IL_1 and IL_6 [470].

IL_2 receptors (IL_2-R) appear on the CD4 T-cell surface. At this stage, the role of IL_2 is played by its receptor equipped with a high, mean and low affinity: the high-affinity receptor, necessary for IL_2 proliferation, consists of three subunits: α, β and γ, of 55, 75 and 64 kD, respectively [172]. The α subunit (CD25), not expressed on resting cells, with a synthesis that does not depend on antigen signals, amplified from IL_1, TNF and IL_5, controls the production of the high-affinity receptor on T cells; preventing its synthesis is inhibited by IL_2 proliferation [34, 172]. Picomolar IL_1 levels are sufficient to drive IL_2 transcription, synthesis and secretion, as well as expressing membrane receptors. IL deficiency underlying SCID demonstrates IL_2-Rγ's crucial role in intrathymic development of human T cells.

Activated T lymphocytes produce IL_3 and IFN-γ, which stimulate APC induction, while IL_4–IL_6 and IL_{10} may drive B-cell production, maturation and isotype switching; similar mechanisms on activated cells are ensured by IFN-γ synergizing with IL_2 [480]. T lymphocytes generate IL_4 but their signaling totally depends on IL_4; otherwise mast cells and/or basophils provide autonomously for its production [174].

Several ILs may play a role in modulating or contrasting IL_4-dependent IgE synthesis [177] (Fig. 1.22).

IL_6, with powerful amplifying effects on IgE responses, acts on B lymphocytes as a main factor of effector function development, with no isotype preference except IgG secretion [28]; it also plays an obligatory role in IL_4-induced human IgE production [607].

IL_2, IL_5, TNF-α and CD23 have similar effects, CD23 recognizes FcεRII, which, interacting with CD21 ligand, plays an essential role in IgE synthesis modulation [16].

IL_5 and IL_6 up-regulate IgE production, especially when IL_4 levels are suboptimal, although these ILs do not stimulate IgE synthesis together [296].

IL_8, IL_{12}, IFN-γ, IFN-α and TGF-β seem to act at different levels:

IL_8 and TGF-β inhibit IgE synthesis either in T cell-dependent or T cell-independent systems, thereby acting directly on B cells. CD14 operates with a similar mechanism [608]. TGF-β blocks ε germline expression at a transcriptional level, while inhibition by IL_8 is isotype-specific.

IFN-γ, IFN-α and IL_{12} work only in T cell-dependent systems, thus showing an indirect mechanism of suppression: all three ILs inhibit mature Cε transcript expression, whereas only IFN-γ and IFN-α have such an effect on ε germline in PBMC cultures [606].

IL_{12} mediates specific Th1 T cell immune responses and inhibits development of IL_4 producing Th2 cells [339].

PAF blocks both ε germline and mature Cε transcripts [606].

IFN-γ is a major IL_4 antagonist and suppresses IgE production by normal human lymphocytes induced by IL_4, either directly or by reducing FcR expression for IgE antibodies from B lymphocytes. IFN-α and PGE_2 also block IL_4-induced IgE production in a dose-dependent way [421]. The IFN-γ suppressive mechanism is indirect, since no inhibition of ε germline transcripts has been reported [296], therefore suggesting that IFN-γ may prevent recombination events without affecting ε-mRNA transcript expression [205]. IL_4 down-regulates IFN-γ production, but when this is driven by T cells stimulated with allogenic cells, and in mixed lymphocyte cultures, IL_4 fails to initiate IgE synthesis. By contrast adding IL_4 early to the culture, there is IgE synthesis and suppression of IFN-γ synthesis. So the selection of isotype switching is fixed by a chronological order of secretion of diverse ILs [332].

IL Role in T-Cell Preferential Activation. Also in humans, IL_4 and IFN-γ production is under the influx of a preferential activation of Th1, Th2 T cells (and Th0) cells [644] (Table 1.10) and directed, in addition to the genetic background of the individual, by antigen nature and concentration, individual APCs, and ILs produced in the microenvironment by different cells and antigen dose. IFN-γ, IFN-α, TGF-β, IL_1 and IL_{12} evoke antigen-specific T-cell differentiation into Th0 or Th1 T cells [401], whereas IFN-γ absence or low levels and IL_4 promote Th2 T-cell expansion, while IL_2 supports all three subsets [606]. T-cell stimulation directed by IL_{12} is IFN-γ- or IFN-α–dependent [401]; similarly DCs drive Th1 T-cell expansion from virgin DC^+ in an IL_{12}-dependent scenery, in the absence of IL_4 [387], with the help of CD80 [401] or CD16 [339]. IL_1 directs T cells stimulated by SEB (staphylococcal enterotoxin B) SA to differentiate into Th1 T cells [504]. Instead, IL_4, IL_{10}, and IL_{13} not only inhibit Th1 T cell growth, but also considerably reduce IL_{12} production from macrophages [606], while IL_{10} blocks IL production from Th1 lymphocytes at a transcriptional level and induces LC toleration [139]. The IL local setting therefore takes a decisive role in selecting the predominant subset: IL_4 modulates Th2 T cell differentiation, IFN-γ that of Th1 cells [644], taking into account that NK-cell deficiency, quantitative or functional, can promote poor IFN-γ production [77]. In this setting, *the Th2 T cell prevalence could be the key of aberrant and increased IL_4 and IL_5 production, and of high IgE levels* specific to severe atopic subjects [413]. This approach is confirmed by IL_{13} presence only in atopic individuals [219]. However, severity of atopic disease is not a necessary prerequisite, since the same dichotomy is observed in patients with less severe disease [219, 318], but only in patients with high IgE levels [318]. A rationale could be the CLE0, CLE1, and CLE2 presence shared by IL_3–IL_5 and GM-CSF genes expressed coordinately after antigen stimulation [588, 645]. We stress that in the CB of at-risk newborn babies an IFN-γ deficient differentiation is operative, paralleling an excessive IL_4 production (Chap. 3): this etiopathological mechanism involves the IL_3–IL_5 trio spreading aimed at activating basophils, hence emphasizing B lymphocyte transformation in plasma cells-IgE and activating eosinophils as well [473].

Therefore, in humans, a greater part of Th1 T cells could be active within DTH reactions, while Th2 T cells could promote preferential IgE, IgA, IgG_1 and IgM production (with B-cell help) and stimulate IgE antibodies with IL_4, mast cells with IL_3 and IL_4 and eosinophils with IL_5. Th1 clones carry on even cytotoxic activities against APCs, including B cells: these data suggest that such Th1 T cells eliminate not only B cells functioning as APCs, but also Ig production, hence demonstrating poor helper activity. The studies discussed above have been further emphasized by recent observations in healthy individuals on Der p 1-specific Th1 clones' cytolytic potential, but not in Th2 clones from atopic subjects [456]. The data implicit from an increasing amount of experimental results underline that activated T cells produce factors useful for B-cell growth and differentiation and a parade of ILs *crucial in immune responses* [421]. As a consequence, the selection of ILs to be secreted and T subsets to be involved is determined by the pathway of immune response. These studies appear to suggest that chronic allergen stimulations may select IL_4 predominant intervention under allergen-specific Th2 cells influx in individuals whose T cells are intrinsically prone to secreting large amounts of IL_4 on activation [176]. IL_4, strongly supported by highly IL_4-producing CD4 clones, directs B lymphocyte isotype switching to IgE production, inducing in B cells the gene recombination that represents the critical premise for B-cell differentiating activation within IgE-secreting cells (Fig. 1.22). Allergen concentration can control Th1 or Th2 phenotype development from Th0 T cells [643]: actually, in animal models low-mean doses of peptides determine IFN-γ production from Th1 T cells and almost nonexistent IL_4 levels, whereas *increasing the doses implies IFN-γ vanishing and IL_4-producing cell release* [215]. Therefore, in allergen-specific T cells there will be a fine balance between APCs (DCs) producing IL_{12} and Th2 T cells of IL_4: the antigen dose will result in critically establishing the outcome to either IFN-γ or IL_4 [401].

IgE-BF (IgE Binding Factors). According to previous explanations, several gps with affinity for IgE and the capacity to regulate its synthesis (IgE-BF) could be implied, such as the inducer factor stimulating T cells to IgE-BF synthesis, EFA (enhancing factor of allergy), analogous to GEF (glycosylation enhancing factor), SFA (suppressive factor of allergy), IgE-PF (IgE potentiating factor) and IgE-SF (IgE suppressive factor). Apparently, lymphoid cells should be able to synthesize and release IgE-BF and to develop an isotype-specific regulatory function, acting directly on B cells. IgE-PFs demonstrate affinity for lectins, for their content in mannose-rich oligosaccharides, while IgE-SFs do not have affinity for

lectins, but bind peanut agglutinins. IgE-PFs are produced by T cells when GEF is present and IgE-SFs when GIF (glycosylation inhibiting factor) is present: indeed the serum of healthy, nonatopic individuals contains IgE-SFs, while IgE-PFs were found in patients with HIgES. Post-transductional glycosylation is regulated by two factors: GIF, released by CD8 cells, which inhibits both glycosylation and IgE synthesis, and GEF, released by CD4 cells, with opposite actions. GEF is a 25-kD peptide kallikrein-like enzyme, released after stimulation with *Bordetella pertussis*, Al salts or parasite antigens, and produces a kinin activating IgE-BF production. GIF is a 15-kD derivative of phosphorylated lipomodulin capable of inhibiting PLA_2 (phospholipase A2), produced by T lymphocytes stimulated in the presence of CSs (corticosteroids) or after treatment with Freund's complete adjuvant, inhibiting glycosylation of IgE-BFs secreted from T lymphocytes expressing, therefore, an antagonist activity for GEFs. GEF should be present in atopic subjects and GIF in healthy, nonatopic subjects, a surplus of IgE-PFs and a lack of IgE-SFs foster IgE production. IgE-PF is detected when GEF is formed, with which GIF competes, whereas IgE-SF counters with IgE-PF for lymphocyte differentiation with membrane IgE in B cell IgE producers. IgE-PFs were described in the murine model, primed by T cell FcεRII$^+$: in humans they can be produced by B-lymphocyte-FcεRII$^+$, breakdown products of FcεRII (soluble FcεRII or sFcεRII) [469]. Glycosylation does not appear to be a crucial moment for the binding of FcεRII-IgE but, on the contrary, nonglycosylated IgE bind to FcεRII with an affinity tenfold greater than that of native IgE. Also, receptor glycosylation influences its activity, because carbohydrates interfere with sFcεRII release from proteases. IgE glycosylation and its affinity with FcεRII seem to act in the same direction in precluding the receptor state of solubility: IgE glycosylation interferes with its binding to FcεRII, preventing proteolytic enzyme intervention, receptor glycosylation disguises its binding site; therefore IgE glycosylation appears to be in vivo a heterogeneous process, probably subjected to regulative laws present in rodents [553].

FcεRI and FcεRII/CD23. *FcεRI* (Fig. 1.19) is a high-affinity receptor for IgE antibodies, with 325 amino acid residues, thus another key player. A tetrameric complex (4 TM polypeptides), it consists of four chains, one α, mainly extracellular containing two domains characteristic of IgSF and most closely resembling that of FcγR, FcαR and poly-Ig receptor, one β with four TM segments and two identical γ domains, mostly intracellular with evident analogies with CD3 ζ chain [553]. The cytoplasmic regions of α and β chains contain, as the ζ chain, more binding sites with tyrosine kinases, the α chain binds IgE, the β and γ are membrane proteins; the γ chains are required for signal transduction and metachromatic cell activation after their interactions with IgE. FcεRI is monovalent, each one binds only one IgE molecule and such binding triggers mast cell and basophil degranulation and release of mediators responsible for immediate hypersensitivity reactions [176, 358]. PBMCs of nonatopic individuals express the receptor in, on average, 18% of non-IgE-binding cases; the reverse is true for atopics: such differences depend on IgE levels [452]. Zhu et al [684] have reported a strategy that takes advantage of the natural capacity of FcεRIIb to inhibit the allergenic activity of FcεRI. The FcεRI-mediated activation pathways are modulated by an inhibitory receptor such as the IgG receptor FcεRIIb. The allergen-specific IgGs produced in response to immunization have formed complexes with allergens, which can, in turn, form a bridge between FcεRI and FcεRIIb with 320 amino acid residues. Both receptors are expressed on mast cells and basophils; the Fc fragment of IgG in the immune complex binds to FcεRIIb, whereas the allergen binds to IgE, which is already bound to adjacent FcεRI. The formation of this bridge induces the aggregation of activating FcεRI with inhibitory FcεRIIb, which inhibits the activation pathways activated by FcεRI [259, 684].

FcεRII (Fig. 1.19) or CD23 [259], a counter receptor of CD21 [16], is a *low-affinity receptor* different from FcεRI for its structure, MW (70–83 kD) and affinity of binding IgE antibodies; it is the only known antibody receptor not belonging to IgSF. The TM receptor for IgE antibodies, with the support of CD21, can perform different functions, either IgE-mediated, above all IgE synthesis regulation, or non-IgE-mediated such as B-cell survival in GCs, maturation of pre-thymocytes, proliferation of myeloid precursors, antigen presentation to T lymphocytes in association with class II HLA molecules [16] and B-cell activation [259]. CD23 is included among the cytotoxicity mechanisms and the IgE-dependent release of inflammatory mediators from eosinophils, macrophages and platelets [259]. It can also be involved in adhesion interactions with epithelial cells, due to CD62E and CD62P selectins. The latter is a membrane protein associated with granules and has a domain with analogies with liver lectins [341]. Among the ligands there are platelet CD41, CD21 and IFN-α [349]. Interacting with CD11b/CD18 of monocyte-macrophage CD21, and EBV of B cells, CD23 can increase its central role in positive IgE regulation and consequently in inflammatory diseases [16]. The two diverse molecular forms, FcεRIIa and FcεRIIb, dictated by distinct exons, differ in their intracytoplasmic portion by 6/7 N-terminal amino acids [4]. *FcεRIIa* is expressed on mature B cells and FcεRIIb on IL_4-activated cells (Table 1.3) and on monocytes of nonatopic donors [452]. CD23 is able to form dimers and/or trimers, which may account for the increased IgE avidity [384]. Its C-type lectin structure does not bind to IgE molecule carbohydrates [553].

FcεRIIa, inducible also by IL_{13} [437], seems to play a role in IgE regulation with obvious effects on atopic disease [553]. Such a receptor confined to mIgM and mIgD is no longer expressed by B lymphocytes that have undergone isotype switching.

Soluble fragments of FcεRII (*sFcεRII*), 37 kD, can be involved in IgE regulation, thus initiating both humoral and CMI responses, while fragments containing the C-terminal tail behave as IgE-BFs, and sFcεRII may provide a mechanism for sIgE activation on committed B lymphocytes activated in a maturational stage subsequent to that of IL_4. All sFcεRII retain binding specificity for IgE, but smaller fragments bind with lower affinity than intact molecules: they can stimulate growth and differentiation of several cell precursors such as plasma cells, T cells, basophils regulation and thymocyte maturation [47].

Demonstration of type II receptors has shown that allergic reactions can be due to interactions between allergens and *IgE present not only on mast cells and basophils, but also on several additional cells* [174]. We stress that a common problem of atopic patients is an unremitting overproduction of IgE and, as soon as they replete high-affinity receptors, they also occupy low-affinity receptors, as evidenced by the FcεRIIb increase observed in such patients [174].

In the animal model, IgE synthesis can be enhanced by a variety of factors, including adjuvants, insoluble molecules such as $AlOH_3$, SiO_2, certain organisms including *Bordetella pertussis* and mycobacteria, parasite extracts and perhaps gasoline residues, in addition more specifically to massive doses of X-rays, ablation of the thymus and spleen and immunosuppressive drugs. On the contrary, low doses of X irradiation or of radiomimetic drugs have the paradoxical effect of enhancing IgE synthesis by interfering with CD8 production, whereas complete Freund's adjuvant, employed to accelerate isotype switch from IgM to IgG, has little effect on IgE. However, if antigens are coupled with mycobacteria, it may even suppress IgE antibodies [45].

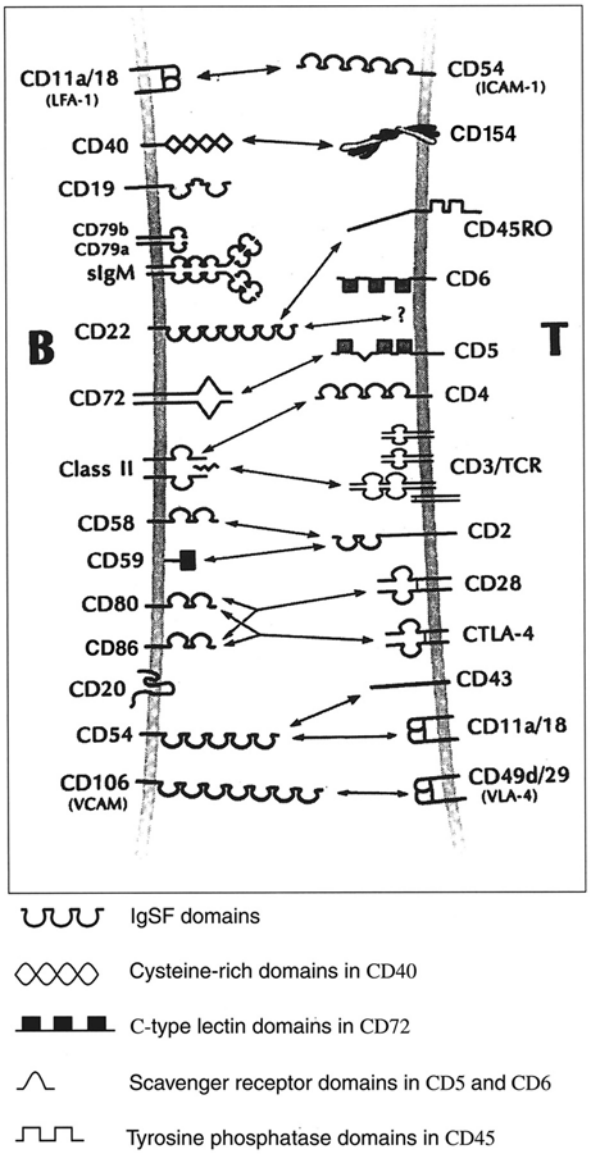


Fig. 1.45. Membrane interactions between CD4 T lymphocytes and activated B cells. *IgSF* immunoglobulin superfamily

Second Signal

The T-cell to B-cell *cognate interaction* or *associative recognition* promotes B-cell activation, proliferation and differentiation in plasma cells–IgE secretion (Fig. 1.58): T lymphocyte intervention in IgE production can be inhibited by IFN-ɣ as demonstrated in vivo from anti-IL_4 and anti IFN-ɣ antibodies [456, 473]. In this sense, activated T cells provide help for B cells stimulated by antigen molecules in two ways, either secreting ILs that regulate B-cell differentiation or resulting from an associative recognition, the HLA-dependent *cell–cell cognate interaction* (Fig. 1.45). A significant series of studies has been conducted on a tangible aspect of cooperation between T and B cells: the IgE synthesis elicited by plasma cells and IgE has led to the formation of 2,000 IgE epitope-specific/s over a few days. T lymphocytes are indispensable for IgE production and control, because B cells alone do not produce IgE, not even if stimulated [456, 473]. When *conjugated T and B lymphocytes* are cultured in close contact in the same compartment, the induction of IgE synthesis involves an associative recognition between T and B cells through a tripartite complex, allowing locus C_H rearrangement and mRNA intervention, leading to H chain formation so that IgE protein expresses ε germlines. Before this interaction, an IL profile including IL_4 in the microenvironment cannot stimulate B_{IgM} precursors to switch into B_{IgE}. However, conjugated T-B cells increase IL_4 receptor expression on B cell membranes. Consequently, Th2 lymphocytes release IL_4 and other ILs, inducing B lymphocytes to synthesize IgE [473]. The role of T cells in IgE synthesis has been further confirmed, because, in atopic patients, clones of Der p 1-specific $CD4^+$ lymphocytes occasion high IL_4 levels with a powerful amplifying effect on IgE synthesis in comparison with non-Der p

1-specific clones or with healthy individuals, similarly to CD4 clones obtained from patients with parasitosis [473]. In perspective, chronic stimulation by specific antigens seems to be able to select antigen-specific T cells in patients with T repertoires predominantly secreting high IL_4 concentrations during their activation [456].

More specific interactions between T and B cells occur following the expression of CD40L T-cell ligand, = CD154 for the CD40 molecule, surface gp of 50 kD (Fig. 1.22a), homologous to NGF and TNF-α receptors, expressed on membranes of all B cells, macrophages, FDCs and other cells able to evoke responses of activated T lymphocytes, monocytes, basophils, endothelial and epithelial cells, fibroblasts, DCs and other T cells [600]. CD154 or CD40L is similar to a 30- to 39-kD membrane gp, formed by 263 amino acids, homologous to TNF and expressed by activated CD4 T cells [446]. Subsequently to this interaction, activated B cells proliferate and acquire APC activity, generating CD80 and CD86 surface molecules, counter receptors of CD28 of T cells, which in turn also express CD152 [243]. However, CD86, because of an earlier expression, its higher levels and pro-Th2 T cells and -IL_4 orientation, may play a key part in IL_4 production. *CD40–CD154 binding amplified by CD80 and CD86 binding* [446] *is the prime stimulus for the second signal,* which plays a crucial role in responsive B cells in IgE synthesis. The ensuing IL_4 secretion and BcR binding directs gene transcription and isotype switching from IgM to IgE [132]: if CD40–CD154 are expressed on activated T cells, IL_{12} is also synthesized [524]. After the significant identification that mutations of the gene encoding CD154 on chromosome *Xq26.3–q27.1* are responsible for HIgMS, with absent IgE synthesis in the presence of IL_4 [442], studies have shown that decreased expression of CD154 inhibits the switching from IgM to IgG in 77% of patients with CVID [147] and HIgMS [442]. CD2–CD58 binding, as described above, fulfills a role in IgE induction independent of CD40–CD154: indeed the role of this pair assumes great importance in IgE production at the level of the lamina propria, where T and B lymphocytes have a major expression of CD2 and, respectively, of CD58 in comparison with their subsets in peripheral blood [600].

Second-type B-cell activating signals act in synergy with IL_4 and T cell-independent systems in the induction of IgE synthesis [205, 383, 437].

- *IL_4-dependent IgE synthesis* by *non-cognate* T–B-cell interactions has been reported in which TcR fails to recognize the HLA–peptide complex, for example, an inducible molecule associated with membranes of T $CD4^+$ clones is apparently capable of directing the B lymphocyte differentiating process toward IgE synthesis [455]. However, a latency time of 2–4 days is necessary for T-cell-activated IL_4 to deliver the signal to undergo class switching to B cells, which can drive B cells to secrete IgE independently of T cells [205]. Such data indicate that metachromatic cells select ILs able to stimulate B cells activated by T cells to produce other ILs as well as Th2 lymphocytes. Similarly, splenic non-T–non B cells produce IL_4 as a result of cross-binding to FcεRI or of exposure to IL_3 [205]. The T-cell role has been emphasized by their ability to produce ILs, thus stimulating IgE-mediated reactions by a direct action on metachromatic cells [496].
- *Stimulation with EBV and IL_4* has been shown to elicit IgE synthesis in human B cells. B_{IgE} cells obtained by activation with EBV and IL_4 contain both mature and germline Cε transcripts, whereas IL_4 alone yields only sterile μ transcripts [233].
- *IgE production* can also be promoted by direct activators of B cells, which, costimulated with rIL_4 and CD40, or anti-CD40 mABs mimicking in vitro CD154 interactions, are able to synthesize high IgE levels [233]. In nonatopic subjects, IgE synthesis is stimulated after addition of anti-CD40 and rIL_4 [294] and accelerated by adding IL_{10} simultaneously to IL_6 production [590]. However, IL_4 and anti-CD40 alone produce IgE in modest amounts (<0.1 ng/ml) [590], and IL_4 antagonizes IgE synthesis induced by B cells activated by anti-CD40 [400]. Proliferation of preactivated B cells is positively affected by IL_{10} also inhibiting Th1 cells and related ILs [480]: such processes, independent of gene restriction, could explain polyclonal IgE formation, in addition to sIgE during, for example, strong allergic reactions [176]. CD40 effects are likely to be important under physiological conditions, considering that soluble CD40 (sCD40) inhibits in vitro T-cell-dependent IgE synthesis after an appropriate second signal [132], while CD154 drives IgE production in IL_4-treated B cells [132] (Fig. 1.46) [456]. In this scenario, crucial data appear to play a significant role, since it is well known that CD154 expressed by circulating basophils and mast cells also of skin and lungs [174] and from eosinophils [175] together with CD40 [407] modulate *IgE synthesis* with the immediate implication that it takes place *not only in germinal centers of lymph nodes, but also in peripheral tissues.*
- *IgE may also be involved* in indirect mechanisms such as enhancement of APC antigen-capturing capacity: IgE-mediated presentation of a great number of allergens by interacting with FcεRI and FcεRII can activate the immune system in a continuous way, even with low allergen concentrations, thus leading to an overproduction of both IgE and Th2 cells, which in turn will induce more B cells to a switch recombination, also explaining why certain atopic patients worsen from a monosensitization to a multiple sensitization [383].
- *PBMCs* in the presence of IL_4 and hydrocortisone trigger IgE synthesis [205] and by means of FcεRI can be involved in an IgE-mediated presentation with a T-independent mechanism [384].
- *IL_{13}* is known to enhance IgE synthesis even in the absence of IL_4 [437], thus demonstrating that additional soluble mediators, as yet not well defined, are potentially involved in IgE regulation.

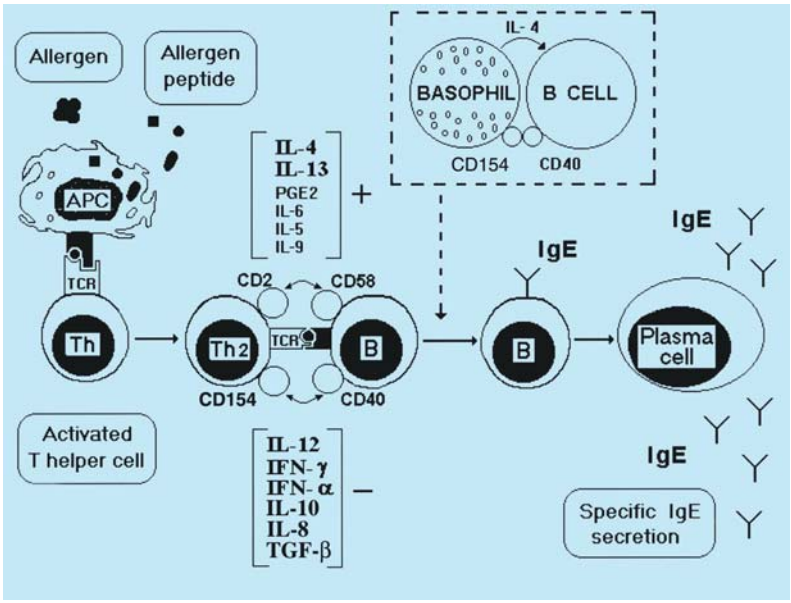


Fig. 1.46. Cellular and molecular interactions involved in the modulation of IgE antibody production. Schematic representation of the physiological aspects of IgE synthesis. (Modified from [456])

Further signals leading to isotype conversion with expression of high IgE levels are modulated by IL_5, a growth factor particularly of eosinophils, and IL_6. In a speculative way these findings suggest that IgE production can also be driven independently of T cells and that the above messengers have in common the capacity of activating the isotype switching machinery in B lymphocytes whose ε gene was activated by a transcriptional process and/or made accessible by IL_4 [176].

Immune Memory

There is wide agreement with the belief that lymphocytes preserve the memory of their first antigen encounter: when antigen is encountered again, IgE response will be more rapid and vigorous, even after decades. This reaction is due to specific CD45RO deriving from the proliferation of a specific clone following the priming, an event which induces a further expansion of production in children without an atopic background of IgG and IgA antibodies [101, 688]; such an event also explains why *vaccine booster doses* are effective. About 50%–60% of CD45 isoforms are associated with the 180-kD form, CD45RO, memory cells, and 40%–50% with the 220-kD form, CD45RA, virgin cells that are functionally inactive in the absence of antigen-driven stimulation. The RA isoform of CD45 is expressed on newly formed T cells that have not yet encountered specific antigen; during activation by exposure to specific antigen, the RA isoform is stably modulated to RO, a process that involves differential, post-transcriptional processing of the CD45 mRNA turning into CD45RO after IL_2-dependent T-cell activation. However, CD45RA and CD45RO, rather than being markers for distinct naive and memory Th cell populations [627], are probably respective markers for reversible resting and activated states; CD29 is expressed two to four times more on several adhesion molecules in addition to CD45RO cell surface than on CD45RA [23]. This cellular basis consists in antigen-specific lymphocyte expansion during primary responses, so that sensitized cells play a specific function, announcing in the bloodstream the occurred recognition, leaving a greater number of T and B cells [101]. Other memory cells undergo a rapid turnover: activated after stimulation by antigen-specific $CD4^+$ T cells are engaged to respond to a wide spectrum of APCs, and to secrete ILs and high IgG levels [326]. Memory T and B cells recirculate via peripheral blood and lymphoid organs (Fig. 1.11), and besides some cells with a short life span there are others with a long life span: their characteristics are summarized in Table 1.42 [688]. Since cytopathic viruses cause damage to fetuses and neonates when the immune protection necessary to survive is absent, particularly during a critical immunoincompetent period, mothers protect their babies passively transferring neutralizing antibodies [688]. Which signals lead to memory cell development at birth vs effector cells? One possible answer is that large antigen amounts plus costimulation in an environment dominated by inflammation may result in differentiation to effector cells, whereas the absence of an inflammatory milieu or ILs favor memory cell generation: according to this model, T cells activated in early stages of an infection would differentiate into effectors, whereas during later stages with a reduced antigen load memory T cells would prevail [7].

One theory on long-lived memory cells admits a constant stimulation of T-cell clones, according to three procedures: contact with antigens (and/or pathogens), new encounter with antigens, then with antigens bound to DCs with reconstitution of supplies [101]. According

Table 1.42. Characteristics of memory B and T lymphocytes

Characteristics	Memory B lymphocytes		Memory T lymphocytes	
	Resting	Activated	Resting	Activated
Location/migration	Recirculation blood → LN (via HEV)	Associated with persisting antigens in lymphoid organs and bone marrow	Spleen and blood possible migration blood → LN (via HEV)	Migration through tissues
Function	Secondary reaction (challenge)	Maintenance of memory, IgG levels	Secondary reaction (challenge)	Immediate killing of infected cells in periphery
Type of response	Delayed	Immediate	Delayed	Immediate
Proliferation	No	Yes	No	Perhaps yes
Site of proliferation		Germinal center		Perhaps in lymphoid organs associated with antigens
Antigen dependence	No (poor)	Yes	No	Yes
Site of antigen persistence	FDC		Unknown	

Modified from [688].
FDC follicular dendritic cells, *LN* lymph nodes.

to another hypothesis, which is gradually being widely received, memory cells do not require further contacts for their survival [7, 56, 216, 385]; hence, if a memory cell prerequisite is a persistent stimulation, one could argue that T-cell memory may not exist [56]. As a consequence, these studies have not proved that there are two types of memory, one short-term and one long-term memory, even if in 1847 a measles epidemic spared the aged infected 65 before years are the Faroe Islands [407], thus confirming earlier observations made by Thucydides. New findings are supported by demonstrations that memory CD8 CTLs persist indefinitely when associated with CD44 homing receptor, ensuring long-type memory also independently of steady antigen stimulation [291]. CD8 CTLs from a previous reaction to a given virus may in the future be reactivated and contribute to natural resistance by cross-reacting to another putatively unrelated infectious agent [512]. CD8 T-cell memory stimulation, long persisting also in the absence of the original antigen, can thereby depend not on a new encounter with known antigens, but with wholly different antigens, only if they have a cross-reaction with the first ones. Therefore CD8 long-term memory persists without antigen stimulation [216, 385]. Differential expression of memory phenotype markers CD44, CD122, and Ly6C by SOCS-1 (suppressor of cytokine signaling) IL_{15}^{-} $CD8^{+}$ lymphocytes suggests that multiple signals contributed to the memory cell differentiation program. However, the acquisition of the memory phenotype by SOCS-1-deficient $CD8^{+}$ lymphocytes does not require prior antigenic stimulation, but requires the presence of activated T cells [441].

Another particularity of CD45RO cells is to be exposed to an antigen universe precluded to virgin CD45RA cells, which can respond to antigen presented by APCs since they express increased levels of adhesion molecules [101], or on B cells exceedingly efficient at capturing even low-density antigens, unable to stimulate virgin cells [345]. Secondary immune responses are faster, with more Igs and higher affinity, provided that they are T-dependent [310]. B lymphocytes, after differentiation into effector cells or IgE producers with CD154 cooperation [71], return to a quiescent state, constituting immune memory, with mIgs and properties slightly unrelated to their ascendant with which they can interact, at the time of activation, although in a different stage of maturation; their long-term survival depends on the persistence of antigens retained in CICs by FDC networks [337].

Recent data support the so-called *decreasing potential hypothesis,* suggesting that memory T and B cells, activated after each round of stimulation, generate fewer memory cells undergoing terminal differentiation into effector cells, a phenomenon that in B lymphocytes could be more accentuated in mature cells (Fig. 1.47) [309]. Consequently, the immune system will have the resources to quickly generate large numbers of effector cells to successfully eliminate pathogens [309]. Immune memory works such that infections elicit, in most cases, a state of disease only on the first contagion, whereas subsequent contacts with the same pathogens resolve without any pathological manifestation, thanks to microorganism elimination in a rapid and sound way [474]. Another area of increasing interest suggests that it is tempting to hypothesize that *endotoxin* exposure can be used as an essential adjuvant in the induction of antigen-specific T-cell memory [303].

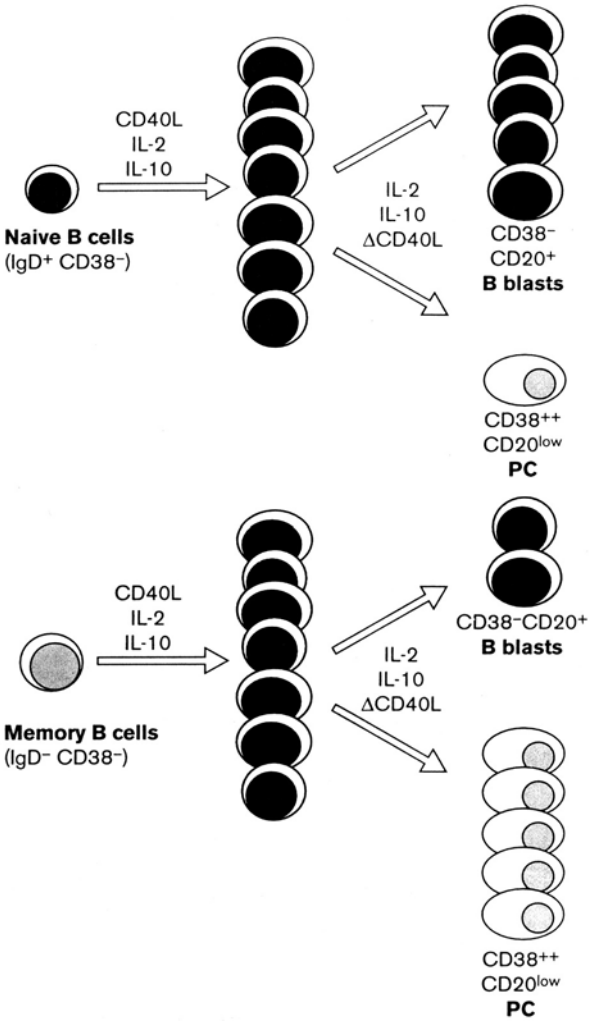


Fig. 1.47. Terminal differentiation of memory and naive B cells. Memory B cells but not naive B cells undergo terminal differentiation into plasma cells (*PC*) upon activation in vitro

Immune Responses

Not surprisingly, immunity, instead of protecting against endogenous or exogenous antigens, thus contributing to maintenance of immune homeostasis, exposes individuals to immune reactions, whose ultimate result is to damage tissues, producing clinical illness, even severe or fatal (Fig. 1.48) [34]. Such reactions are characterized in the first place by allergens according to a commonly accepted classification. Hence, hypersensitivity is a form of abnormal reactivity of the host against a non-self agent (allergen), innocuous for the majority of healthy subjects exposed to similar doses of a matching allergen. A fundamental stage of a cognitive process was the Gell and Coombs *classification system of immune reactions* [179], useful from a practical point of view for exemplification reasons. Immune reactions are particular to atopic disease and are divided into four classes, I, II, III, IV [34, 179], divided into immediate or anaphylactic, cytotoxic, IC-mediated and CMI reactions. Otherwise, the four types of reactions can overlap and almost no atopic disease is limited to only one mechanism. For example, penicillin can provoke anaphylactic shock (type I), immune hemolysis (type II), serum sickness (type III), or contact dermatitis (type IV) [470].

Immediate and Delayed Reactions

Specific studies done over the years have encouraged revisiting the Gell and Coombs historical classification. In reality, immune reactions can be subdivided into two types, immediate and delayed, according to the latency period from the appearance of allergic manifestations following the re-stimulation with the same sensitizing antigen: immediate reactions start after minutes and are IgE-mediated, while delayed reactions start after hours or days and are T-cell mediated. The main differences between these reactions, which take place expressing Th2-like ILs and, respectively, Th0 or Th1-like ILs [585], are shown in Fig. 1.49 [104]. Some perplexities have recently been aroused by focusing attention on the role of IgE antibody in DTH reactions and multifold IL interactions within inflammatory reactions. Actually, in addition to the role of IgE-mediated immediate reactions marked by effects of histamine released by mediators, recent reports have highlighted the role of DTH, characterized by release of biologically active mediators induced by IgE. The picture occurring in patients can be schematized as follows: 1. The immediate reaction; 2. A phase of relative quiescence; 3. The delayed reaction.

We underline that such distinctions have a more theoretical than practical nature, as evidenced by an almost constant association or overlapping of these two phases, not permitting a clear-cut division from a clinical point of view. Molecular studies led to recent reports on the role played by ILs, with a pattern wholly different according to reaction chronology: in acute reactions the IL_4 mRNA is very early and selectively expressed, whereas in delayed reactions IL_4, IL_5 and IFN-γ are positively regulated [105]. The practical result of the above studies is that reactions developing >24 h after allergen challenge allow for an increased number of antigen-specific T cells to invade peripheral tissues. These current investigations emphasize delayed reactions in the immune system and make it possible to elucidate the mechanisms at the base of chronic inflammation, indicating IgE heterogeneity among atopics and, indirectly, a genetically determined GPM.

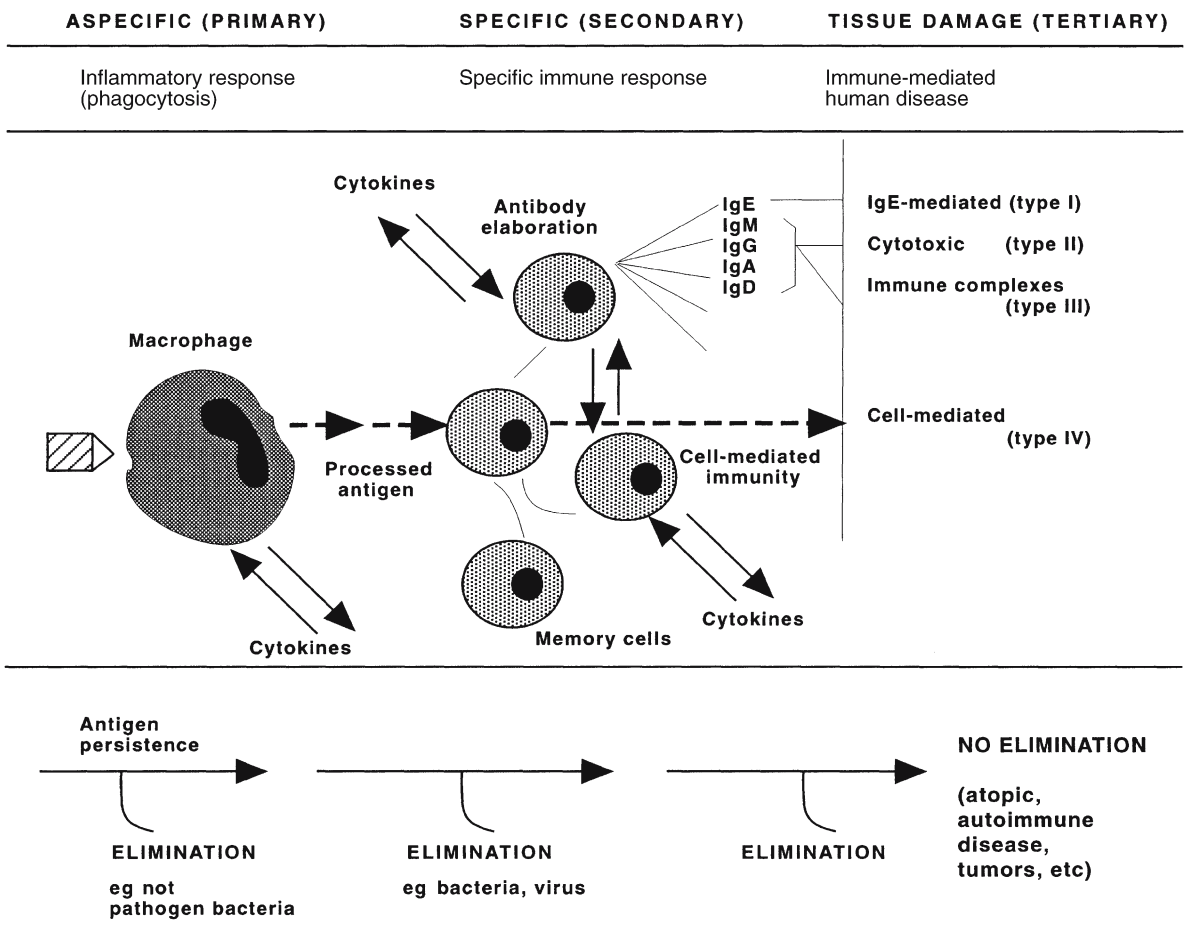


Fig. 1.48. Schematic representation of the immune reactions and the body immune capacity of eliminating foreign material. The *lower part* shows that interactions between foreign agents and the immune system may lead, depending on the antigen nature or the genetic constitution of the individual, either to elimination or persistence of the antigen with resulting alterations of immune homeostasis. (Modified from [34])

Hypersensitivity Reactions

Type I Hypersensitivity Reaction, IgE-Mediated – Anaphylactic Reactions

An immediate hypersensitivity reaction is characterized by a rapid development of clinical symptoms (a few minutes or more) when the allergen to which the patient is sensitized cross-links IgE bound to FcεRI on tissue mast cells and circulating basophils, with consequent degranulation, but IgE indosable levels are sufficient to initiate an immune reaction [367]. The sensitizing allergen, variably penetrated in the host, binds IgE fixed on the mast cell external surface. *IgE binding to FcεRI* is not wholly effective to stimulate mast cells without allergen intervention. The triggering signal of an impending immune reaction requires that the antigen is bivalent, so that it can bridge the Fab of two adjacent IgE molecules on the cell surface [349]. Signals are then activated, which, on the one hand induce transcription processes resulting in IL production, and on the other hand trigger a cascade of intracellular metabolic events, able to augment the local blood flow and to recruit a series of cells drawn to the reaction site by specific chemotactic factors.

Signaling is initiated by *mast cell activation* started by transduction signals, allowing cells to perceive changes in the extracellular environment that translate into an intracellular biochemical signal that causes an appropriate cell response (Fig. 1.50). Broad structural modifications of phospholipid components of the cell membrane follow. Biochemical events may be summarized as follows [210, 528]:

1. FcεRI cross-linking:
Interactions with cytoskeleton
2. Signal transduction:
Serine esterase activation
GPT-binding proteins
Membrane depolarization/repolarization

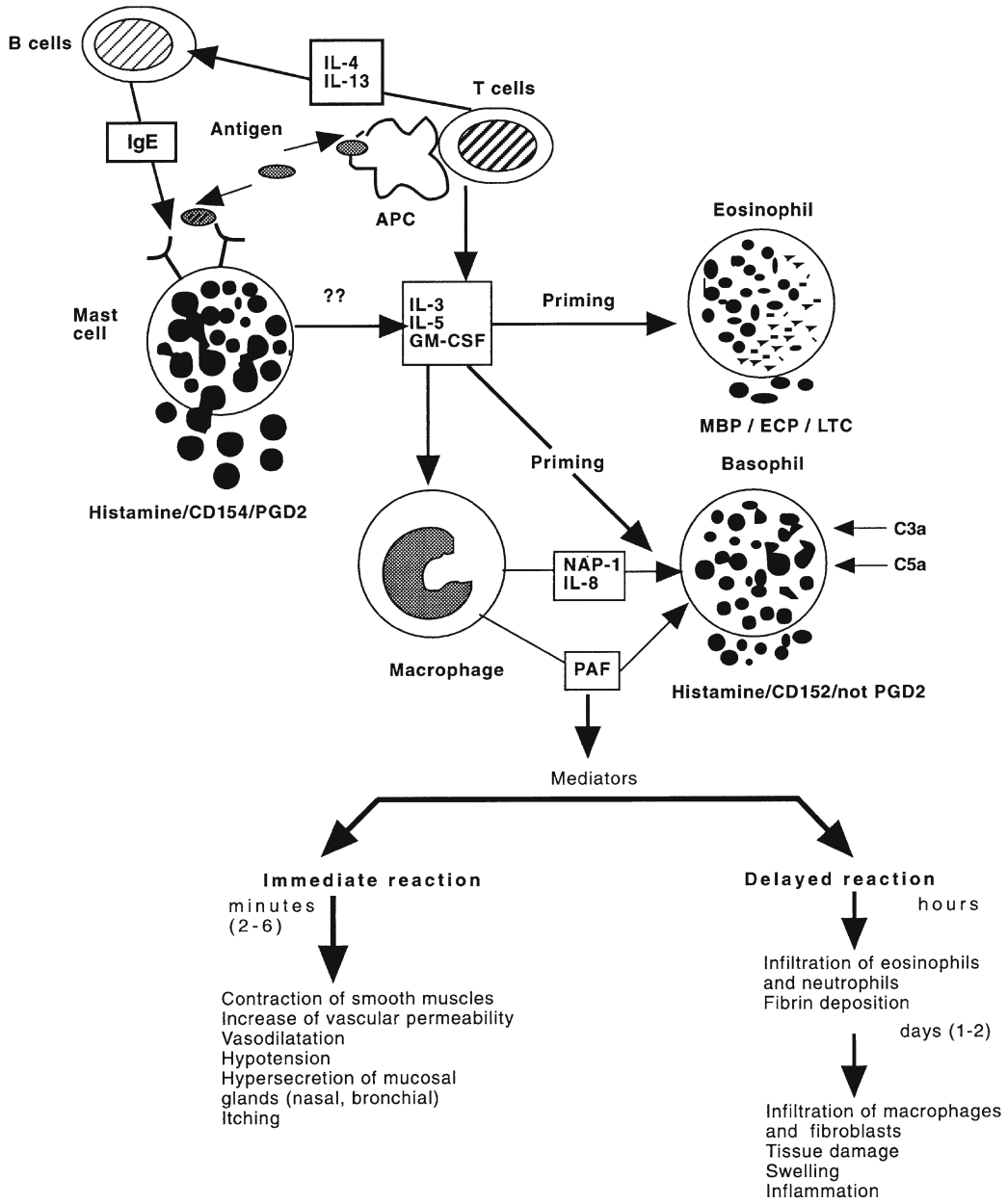


Fig. 1.49. Current hypotheses regarding the pathogenesis of immediate and late-phase reactions. Consequences of mast cell degranulation and principal differences between these reactions. *APC* antigen-presenting cell, *ECP* eosinophil cationic protein, *MBP* major basic protein, *NAP* neutrophil-activating peptide, *PAF* platelet-activating factor. (Modified from [104])

3. Signal translation and amplification (Fig. 1.51):
Development of second messengers
PLC, PLD, AA/eicosanoids, adenylate cyclase (activated by G proteins) (Fig. 1.52) with an increase in other messengers such as cAMP, cGMP (cyclic guanosine monophosphate), PKA (protein kinase A), IP3, DAG (Fig. 1.53), ion transport Ca^{++}

4. Activation of second messengers (target/effector proteins) or Ca^{++}-dependent responses:
Activation of PLC, which causes PIP2 hydrolysis, a membrane phospholipid, whose breakdown generates IP3 and DAG
Activation of PLA with AA release
Release of Ca^{++} from intracellular stores (Fig. 1.54)
5. Role of intermediate second messengers:
Activation of Ca^{++} channels and mobilization of intracellular Ca partly regulated by IP3

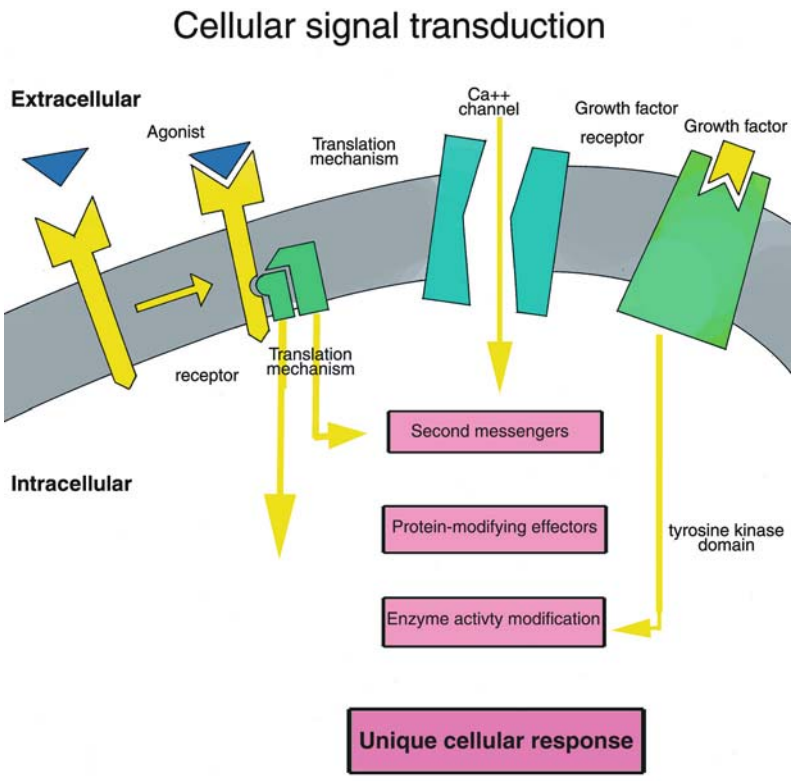


Fig. 1.50. Receptor-initiated intracellular signal transduction. Cellular signal transduction

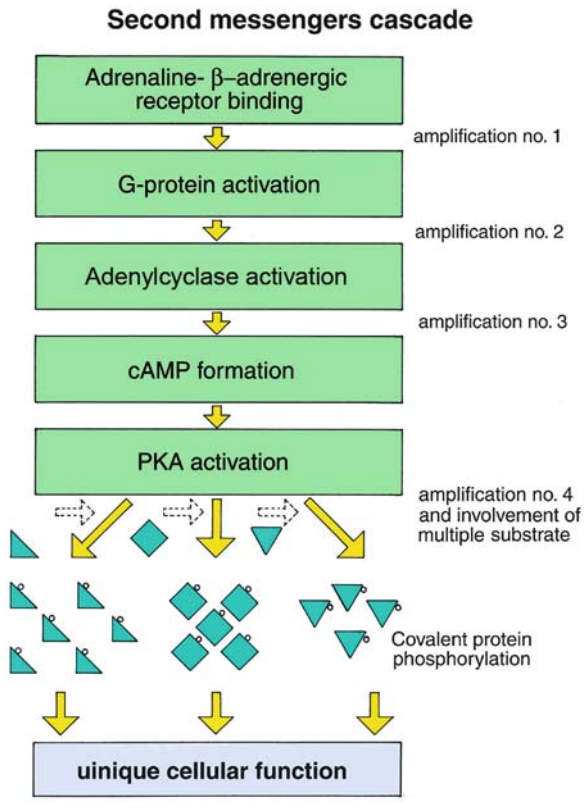


Fig. 1.51. Model of second messengers cascade. *PKA* protein kinase A

6. Effects of second messengers:
Phosphorylation and activation
Of protein kinases A and C (due to interaction with DAG)
Of calmodulin and Ca^{++}-dependent proteins altered polymerization of F-actin
7. Cellular responses:
Ca release leads to activation of glycolysis responsible for assembly of microtubules and of an ATP-dependent energy pathway due to contraction of microfilaments.
Microtubule aggregation promotes movements to the cell surface of preformed granules whose membranes fuse with plasma membranes.
Granules are then released with a mechanism of exocytosis not resulting in the loss of integrity of either the plasma membrane or granule membrane, with the support of specialized lipids called fusogens.
Granule opening leads to preformed mediator release [210, 528].

Granule release does not imply cell lysis or death. Degranulated cells regenerate and, once granule content has been revived with a de novo synthesis, are ready to resume their function.

Ca channels are of three types [29]:

- VOC (voltage-operated Ca channels), divided into L (long-lasting), T (rapid) and N (neuron) activated by different voltages
- ROC (receptor-operated Ca channels), opening in response to activation of receptors associated with these channels

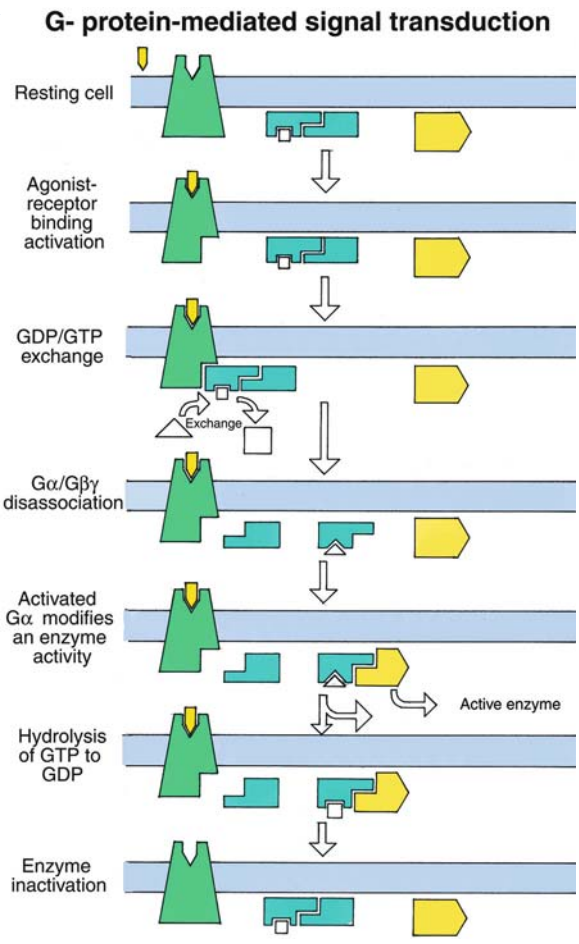


Fig. 1.52. Mechanism of G-protein-mediated signal transduction. The disassociation of the Gα/enzyme leaves Gα free to reassociate to Gβγ. *GDP* guanosine diphosphate, *GTP* guanosine triphosphate

- SMOC (second messenger-operated Ca channels)

FcεRI may activate, instead of IP3, sphingosine kinase and then sphingosine-1-phosphate, an alternative second messenger to mobilize Ca [84]. The released chemical mediators trigger the immune reaction immediate phase; meanwhile mast cells, in addition to histamine, PGs and LTs, release ILs, PAF and mediators able to induce a DTH.

Mast cell activation can also be elicited by other mechanisms [470]:

- *Immunological mechanisms:* specific IgM and IgG antibodies, anti-IgE antibodies and anaphylatoxins, MBP, IL_3, IL_5, GM-CSF, chemokines.
- *Non-IgE-mediated mechanisms* (for direct action on mast cells), such as activation of PGs, CIC-IgE, aspecific bridging due to lectins, SP, foods, drugs and a variety of chemical substances and physical agents. Mention should also be made of the degranulation effected by HRF(s) via IgE-dependent and -independent mechanisms. Non-IgE-mediated degranulation is characterized by lesser influx of Ca ions, a higher velocity (<20 s vs >5 min), a smaller LT (<18-fold) and PG (<21-fold) release and an equal histamine release in comparison with IgE-dependent degranulation [469]. However, recent results indicate that IgE-reactivity to immunoblotted human protein and IgE-dependent HRF activity

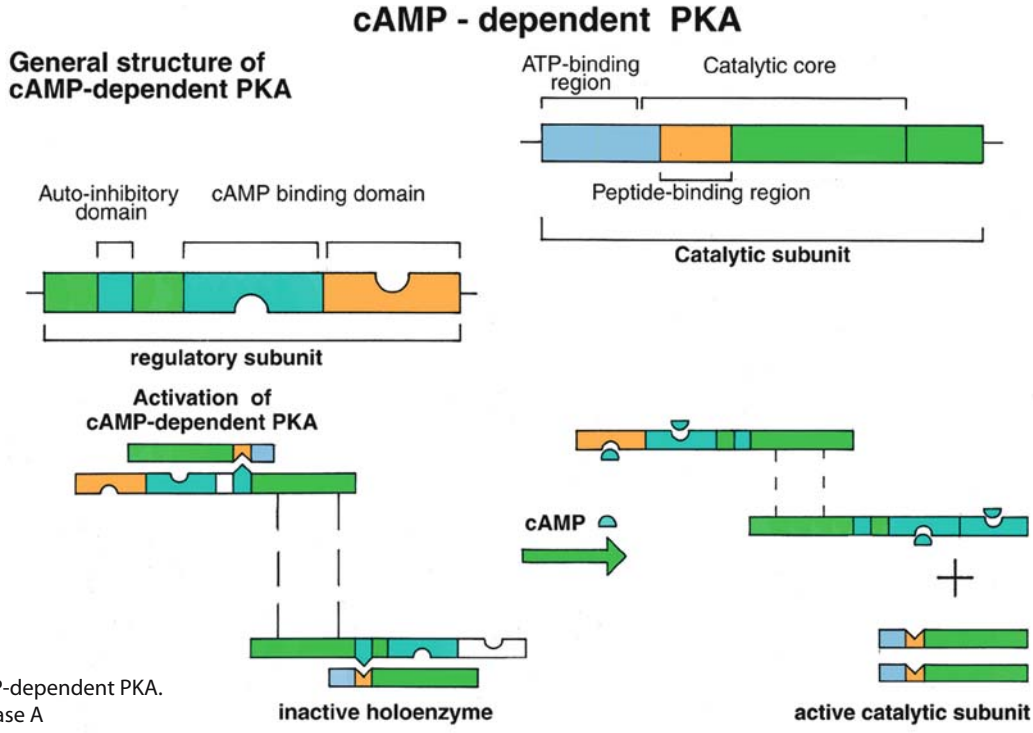


Fig. 1.53. cAMP-dependent PKA. *PKA* protein kinase A

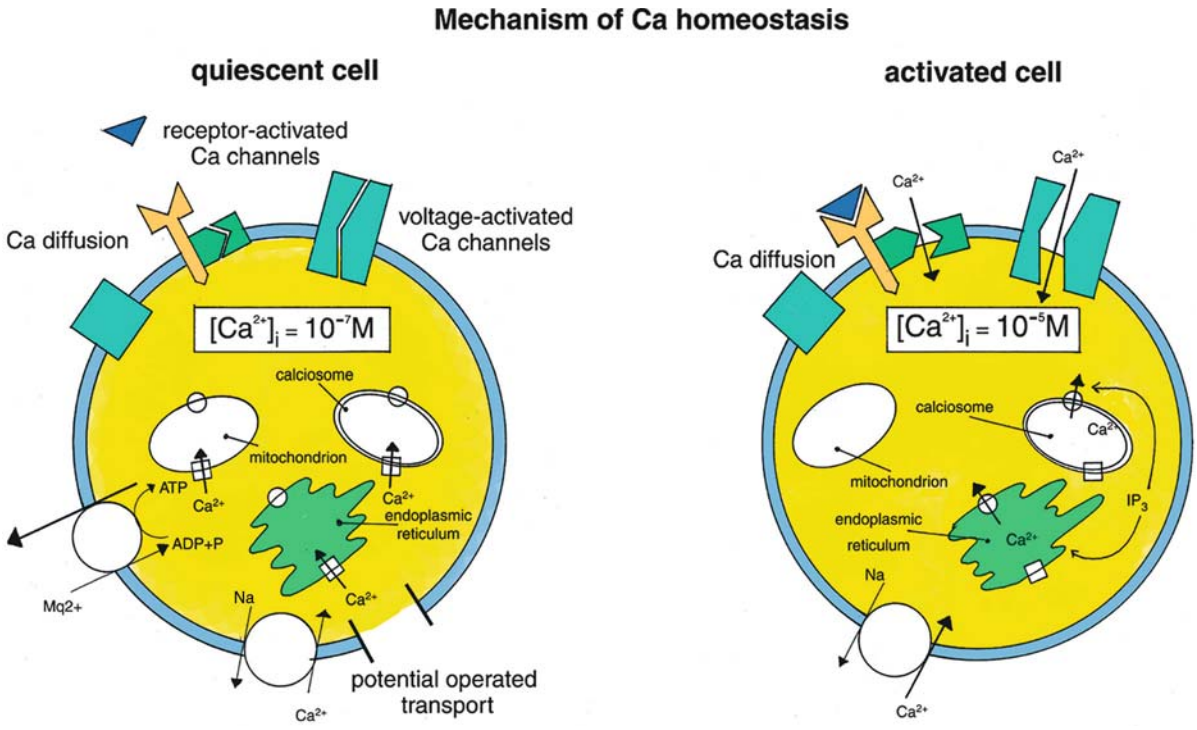


Fig. 1.54. Basic mechanism of intracellular Ca homeostasis

(IgE$^+$) are distinct entities that may co-occur in atopic patients since IgE$^+$ might be a structurally different IgE molecule which is not polymeric IgE [57].

The serum of an allergic individual can sensitize a nonallergic individual following passive transfer or Prausnitz–Küstner reaction; local hypersensitivity persists for 3–5 weeks.

Type II Hypersensitivity Reaction: Cytolytic or Cytotoxic Reactions

In type II hypersensitivity, IgG or IgM antibodies bind antigens present on target cell membranes or transported by certain molecules; antigens can be expressed on a specific cell surface (such as erythrocytes, platelets), or on exogenous (such as drugs, foods), or on haptens attached to the cell surface (CTLs and NK cells). Cytotoxic effects can be accomplished involving several effector mechanisms:

- *Activation of the whole complement cascade,* with consequent damage in various tissues, direct or indirect and subsequent cytolysis, with likely NK-cell intervention with receptors for both complement and Fc fragment of IgG. Examples include hyperacute graft rejection when a transplant recipient is preimmunized, formation of anti-erythrocyte antibodies provoking hemolysis, neutralizing enzymes, inhibiting cell receptors, etc.
- *By enhancing phagocytic cell activity* with receptors for Fc fragment of IgG, and for activated C3b are able to phagocyte target cells, such as circulating blood cells.
- *On clinical grounds* the type of reaction depends on antigen localization: on erythrocytes, hemolytic disease of the newborn from maternofetal Rh incompatibility, autoimmune or drug-induced hemolytic anemia; on several forms of leukocytes, on neutropenia, on platelet autoimmune thrombocytopenic purpura and CM-associated or drug-induced thrombocytopenia, and on other blood cell reactions due to isoimmunization.
- *The cytotoxic reaction* known as ADCC (Fig. 1.55) is performed by NK cells, macrophages and PMNs: antibodies bind to target cells through the paratope and are recognized by cytotoxic cells with FcR specific for IgG. Clinical manifestations are transfusion reactions, by Rh incompatibility, autoimmune and drug-induced [18, 454].

Type III Hypersensitivity Reaction: Immune Complex-Mediated Reactions

Circulating immune complexes of antigens and antibodies (mainly IgG) or CIC can be formed in tissues or in blood vessel walls (with an eventual deposition in the organs via the phagocyte system), overall found in antigen excess (for example, streptococcal proteins or HBsAg), with subsequent complement fixation and

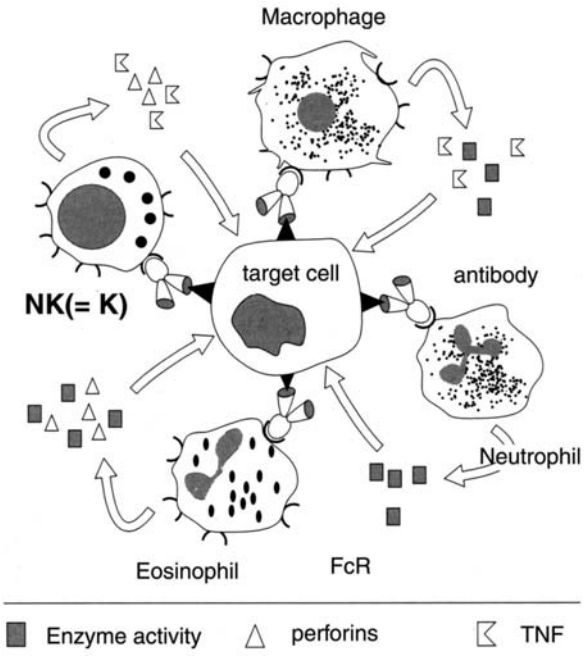


Fig. 1.55. Antibody-dependent cell-mediated cytotoxicity. *TNF* tumor necrosis factor

formation of microprecipitates at the level of small vessels. Onset of clinical manifestations is 7–14 days after primary antigen contact; following a repeated contact, the developing reaction reaches a peak intensity even in 4–6 h. When CICs are cleared in perivascular spaces, microprecipitates are formed, and if deposited in vessel walls CICs provoke vasculitis. A classic example of experimental vasculitis is the *Arthus reaction*: soluble antigen is injected subcutaneously into an animal previously sensitized to the same antigen, the antigen–antibody reaction results in high CIC local levels activating both complement and PMNs, thus provoking local inflammation. Tissue damage caused by this mechanism is in proportion to the CIC quantities and presence of vasoactive amines, which increase vascular permeability, favoring CIC deposition in tissues. First of all, there is the complement activation also increasing vascular permeability, via C2b action to activation of anaphylatoxins, and release of leukocyte lysosomal enzymes recruited by C3a, C5a, C6 and C7; then protein aggregation and lysis with vasoactive amine release; lastly activation of the prekallikrein–kallikrein system with vasoactive kinin release including bradykinin. Later complement-derived chemotactic factors recruit PMNs on the site of reaction, promoting phagocytosis and tissue damage [18].

A classic manifestation of type III immune reaction is *serum sickness,* whose relevance is presently limited, apart from some drug-induced sporadic cases (Chap. 19) or following vaccinations or sting bites. Type III reactions are also seen in infantile infections, viral (prodromic of *adenovirus* and *rhinovirus, Coxsackie,* EBV infections, infectious mononucleosis, *cytomegalovirus,* etc.), abscesses (tonsillar and abdominal abscesses, sinusitis, etc.), bacterial (mainly staphylococcus and streptococcus, mycobacteria), or chronic, in *urticarial vasculitis,* which reveals CIC with complement activation and PMN infiltration, resulting in a necrotizing vasculitis, often included in autoimmunity. We further mention polyarteritis nodosa, cryoglobulinemia, genetically determined complement deficiencies, extrinsic allergic alveolitis, allergic vasculitis such as Schönlein-Henoch purpura, nephrites and reactive syndromes from drugs and foods. Since CICs are seen both in physiological and pathological states, one cannot establish correlations between disease state and CIC levels, thus re-evaluating their pathogenetic role [18].

Type IV Hypersensitivity Reaction: CMI Reaction

A classic LPR with delayed symptoms takes up to 24–48 h after allergen contact to develop: this characteristic is different from Arthus reaction, which instead reaches the apex after a few hours. Such responses begin with lymphocytes reacting with antigens presented by APCs in association with HLA molecules. Appropriately sensitized T-lymphocytes migrate to the place where they encounter the antigen and react with target cells; while also following activation by APCs carrying antigen, other lymphocytes produce a number of ILs, often facilitating the immune response resulting in tissue damage. In reality, the scope of LPR should be that of protecting the host from intracellular parasites, such as viruses, bacteria, mycetes; however, the nature of LPR and mediator release provoke DTH.

Schematically, type I, III and IV reactions are involved in different ways in causing FA, type IV immune reaction is present in AD and in allergic contact dermatitis, type I and IV reactions in respiratory allergy, and all four in drug allergy and intolerance.

Mediators

In recent times, considerable attention has been focussed on the effector mechanisms of inflammation and has enabled us to recognize the interactions of mediators, each with potent proinflammatory properties, able to rapidly elicit the pathophysiological effects of the type I reaction. Such mediators released following mast cell degranulation are divided into primary (preformed and newly formed) and secondary (of cellular and extracellular origin) mediators [104, 210, 528, 626].

Table 1.43. Primary mediators of immediate hypersensitivity

Mediators	Structure	Origin	Functions/effects/symptoms
Preformed			
Histamine		Mast cells, basophils, platelets	VD, VP, BC, production of mucus, itching
Serotonin	5-Hydroxy-tryptamine	Mast cells, platelets, enterochromaffin cells	Specific broncho- and vaso-active effects
NCF	750 kD	Mast cells	Neutrophil chemotaxis
ECF	Tetrapeptide	Mast cells	Eosinophil chemotaxis
Proteases	Protein	Mast cells	Tissue damage, production of anaphylotoxins
Heparin	Mucopolysaccharide	Mast cells	Anticoagulant
Hydrolases	Protein	Mast cells	Function not yet clear
Newly synthesized			
PGD_2, PGE_2	AA derivatives (cyclo-oxygenase pathway)	Leukocytes, monocyte-macrophages	VD, VP, BC
LTB_4, LTC_4, LTD_4, LTE_4	AA derivatives (lipoxygenase pathway)	Leukocytes, monocyte-macrophages	Extended BC, VP
TXA_2	AA derivative (cyclo-oxygenase pathway)	Leukocytes, monocyte-macrophages	VC, BC, platelet aggregation
PAF	Phospholipid (1,000 D)	Leukocytes, macrophages, neutrophils	Tissue damage, production of anaphylotoxins

Data from [470, 626].
AA arachidonic acid, *BC* bronchoconstriction, *VC* vasoconstriction, *VD* vasodilatation, *VP* vascular permeability.

Primary Mediators

Preformed mediators, which are granule-associated with quick release, are implicated in the immediate phase of immune reaction. Among them are included: histamine, serotonin, chemotactic factors of eosinophils and neutrophils (ECF and NCF); enzymes: chymase, tryptase, callicrein, arylsulfatase, chymotrypsin, etc.; proteoglycans: heparin, etc.; acid hydrolases: β-exoglucosidase (β-glucuronidase, β-hexosaminidase, β-galactosidase), etc. (Table 1.43) [470, 626].

Histamine, from the Greek "ιστος," meaning tissue, because of its ubiquitous nature, is a dibasic vasoactive amine synthesized in metachromatic cell Golgi apparatus by decarboxylation of histidine, from where it is stored in cell granules. Normal histamine concentrations are ≤5 nmol/l in plasma, 6–24 μg/cm^3 in skin, 15–40 μg/cm^3 in lungs, and >100 mmol/l inside mast cell granules. However, histaminemia is not a valid routine test, due to its release either by basophils or mast cells and its rapid tissue turnover. Histamine has wide-ranging biological activities with an indubitable influence on allergic disease: after it is slowly dissociated from the solubilized granular matrix, histamine exercises its biological effects by binding to receptors and increases vascular permeability with subsequent fluid and plasma leakage containing C3. This is followed by tryptase and kallikrein release. Last, it drives the leukocyte diapedesis toward the site of immune reaction, where several chemotactic factors are also produced [528].

Figure 1.56 [145] shows histamine stimulated by IL_3, IL_5 and IL_8, as well as by IL_1 with consequent IL_6 production. Through negative feedback, the IL_1 increase can have an inhibiting effect on TNF-α and itself; IL_1 further potentiates histamine action on PGs and 15-HETE released by epithelial cells, while IL_3 triggers the production of histamine, IL_4 and IL_6, but has no effect on IL_2 and IFN-γ, a pattern similar to that of Th2 lymphocytes [145]. Histamine negatively influences the proliferation elicited by antigens, production of ILs and antibodies and cytolytic activity, whereas it enhances the activity of NK cells and release of mediators by other cells.

Following its release from mast cells, histamine slowly dissociates from the granule matrix to exert its large variety of effects by binding to its three receptors [210]: H_1 receptors mediate vasodilation, increase microvascular permeability acting on contractility of postcapillary venules, cause constriction of airway smooth musculature and IgE-mediated release of PGE_2 and $PGF_{2\alpha}$, generate itching, inhibit basophil chemotactic responses, while promoting that of neutrophils and eosinophils, with an increase in cAMP. Classically, *H_1 receptors* provoke the triple response consisting of *flushing, flaring*

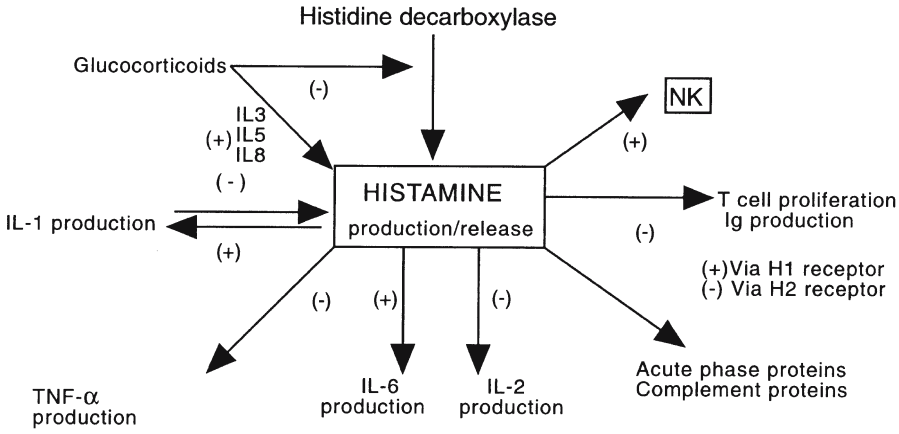


Fig. 1.56. Histamine interactions with interleukins. *Ig* immunoglobulins, *NK* NK cells. (Modified from [145])

and whealing: an intradermal injection of picomolar quantities of histamine within a few seconds yields vasodilation (flushing) by a direct effect of histamine on the microvasculature. Over the next 5–10 min the vasodilation gradually spreads away from the injection site (flaring) by an axon reflex mechanism involving antidromic neural conduction in neuropeptide-containing primary afferent nerves activated by histamine either injected or released by dermal mast cells. The final phase is the development of edema (whealing), as a consequence of contraction of endothelial cells of postcapillary venules with resulting increase of microvascular leakage, mainly due to the direct effect on blood vessel peptides released by afferent nerves.

Histamine acts on *H_2 receptors* to produce a number of negative feedback effects, including the addition of vasodilation to the triple response, reduction of C2 synthesis by monocytes and of C3 stimulated through H_1 receptors (bidirectional modulation), suppression of granulocyte-induced lysosomal enzyme release, H_2O_2 and superoxide production, and chemotactic responses from both neutrophils and eosinophils. H_2 receptors mediate gastric acid and mucus hypersecretion in the airways, the increase in cAMP cellular levels of CD8 lymphocytes and of plasma fluid leakage, as well as suppression of lymphocyte toxicity and inhibition of T-cell responses.

The concurrent stimulation of both receptors results in vasodilation, erythema, itching, bronchoconstriction, hypotension, tachycardia and headache, in addition to inhibition of chemotaxis and histamine release from basophils, and secretion of complement proteins from monocytes. However, histamine plays a positive part with H_1 receptors and a negative part with H_2 receptors, as in the paradigmatic case of APP.

H_3 receptor inhibits both histamine synthesis and release from skin and airways. Moreover, it seems that it regulates interactions between inflammatory cells and the autonomic nervous system. This receptor could play a role by inhibiting the airway cholinergic system and, in the animal model, in the control of nonadrenergic and noncholinergic bronchoconstriction, an immunoregulating action inhibiting the release of involved neurotransmitters [145].

In summary, histamine has multiple effects on various organs:

- Skin: urticaria and erythema
- Mucosa: periorbital edema, nasal congestion and itching, angioedema, pallor and cyanosis
- Upper airways: edema of oral cavity, tongue and larynx, hoarseness, stridor, sneezing, rhinorrhea
- Lower airways (with LTs and PAF): dyspnea, acute emphysema, air trapping (bronchospasm and bronchorrhea)

Among the preformed mediators there is *serotonin* (5-HT), a tryptophan metabolite and well-recognized neurotransmitter, which has effects similar to those of histamine, increasing vascular permeability and inducing airway smooth muscle constriction; 5-HT has been incriminated in the pathogenesis of migraine and has a marked pruritogenic action only if associated with PGE_2, the chemotactic mediators are the following (Table 1.43):

- NCF, distinct in two forms, one heat-stable and one heat-labile, released from mast cell granules, functions as a chemotactic factor, persisting for quite a long time, thus extending the inflammatory effects of immune reactions.
- ECF is not the principal mast cell factor and probably represents a cleavage product of a heterogeneous group of more complex oligopeptides that exhibit such activity. A more potent factor is phosphorylcholine acetyl glyceryl ether and the granule-associated enzymes.

Granule-associated enzymes include:

- *Neutral proteases,* including tryptase with a MW of 134 kD and chymase (Table 1.27). In particular TC mast cell *tryptase,* released together with histamine, cleaves C3 to yield C3a, an anaphylatoxin with degranulatory activity; the determination of tryptase levels is employed in nasal studies (Chap. 12) and in anaphylaxis diagnosis (Chap. 20). *Chymase,* at variance with tryptase, is rapidly inhibited in the extracellular environment. The evidence that it converts angiotensin I to angiotensin II and degrades bradykinin and many neu-

ropeptides suggests a role for chymase in the local control of microcirculation.

- *Additional enzymes* include kallikrein, also generated by TC mast cells, which releases several kinins with inflammatory activity from kininogen proteins including bradykinin (see "Type III Immune Reaction", p. 128), eliciting powerful effects on vasodilation, vascular permeability and airway smooth muscle. Intradermal injection provokes a triple response of a lesser degree compared to histamine not inhibited by antihistamines, equally to chymotrypsin and trypsin. Arylsulfatase has a limited capacity of regulating mediator release [418].

Proteoglycans, also of mast cell origin, found in secretory granules of many hemopoietic cells, essentially include *heparin,* with the following biological activities, in addition to the classic anticoagulant activity:

- Amplifies complement alternative pathway.
- Inhibits complement classic pathway activation.
- Stabilizes and enhances mast cell tryptase, which can express its catalytic activity.
- Potentiates C1-esterase inhibitor.
- Plays important anti-inflammatory roles by inhibiting eosinophil basic proteins provoking respiratory mucosa basement membrane thickening and airway smooth muscle hypertrophy, essential aspects of chronic asthma, whereas the inhibition caused by a powerful antidegranulating action exerted on mast cells by β_2-agonists probably leads to a breaking point in the equilibrium between inflammatory and anti-inflammatory factors (Chap. 11). Furthermore, in scarring areas, where mast cells are hyperplastic, increased heparin release improves tissue repair and the fibroblast action of structural remodeling, augments neutrophil elastase activity and recruits capillary endothelial cells into the area.

The *acid hydrolases,* present also in lysosomal granules of other inflammatory cells, include β-galactosidase, β-glucuronidase and β-hexosaminidase.

Newly synthesized mediators (membrane-derived) include [210]: PAF, PGs and LTs to which, together with TX, are ascribed DTH symptoms. Returning to mast cell degranulation, phosphatidylcholine is metabolized into lipophosphatidylcholine by PLA_2 activated by Ca, with subsequent AA release which gives rise to metabolic derivatives, the *eicosanoids.* AA is metabolized either by the 6-lipoxygenase pathway leading to LT synthesis, or by the cyclooxygenase pathway, generating PGs, PGI_2 (prostacyclin) and TX. Figure 1.57 shows how PGs and LTs derive from AA metabolism.

Biological activities of the following products of the *cyclooxygenase pathway* (Table 1.43) include:

- $PGD_{2\alpha}$: airway smooth muscle constriction, inhibition of platelet adherence and aggregation, activation of adenylate cyclase, virtual induction and increase in basophil mediator release, bronchoconstriction, facial erythema, nasal congestion
- PGD_2/I_2: vasodilation and increase in microvascular permeability, suppression of leukocyte functions
- PGE_2: bronchodilation, peripheral vasodilation and increase in microvascular permeability, inhibition of metachromatic cell mediator release, functional suppression of lymphocytes and PMNs, stimulation of pituitary function with rapid release of luteinizing hormone (LH), and conditioning of primary afferent neurones
- $PGF_{2\alpha}$: airway smooth muscle constriction, small vessel and pulmonary vasculature constriction, and stimulation of airway mucus hypersecretion
- PGI_2: pulmonary vasodilation, suppression of platelet aggregation in collaboration with NO, activation of adenylate cyclase and airway smooth muscle release, tachycardia, hypotension, facial erythema
- TX (A_2): constriction of microvasculature, stimulation of platelet adherence and aggregation [626]

Primary actions of diverse products of the *5-lipoxygenase pathway:*

- LTB_4: stimulation of leukocyte chemotaxis and activation, increased leukocyte adhesion to endothelium, functional suppression of T lymphocytes, activation of NK cells, mobilization of CA^{++} deposits, stimulation of $O_2^{\cdot -}$ synthesis, eosinophil chemotaxis and keratinocyte proliferation, infiltration of neutrophils into skin
- Cysteinyl products, powerful spasmogens of airway smooth muscles:

– LTC_4, LTD_4 and LTE_4: airway smooth muscle constriction, bronchoconstriction 200-fold to 20,000-fold more potent than histamine, vasodilation and increased microvascular permeability, mucus hypersecretion and electrolyte secretion, stimulation of gastric acid secretion, depression of myocardial contractility, mucociliary clearance impairment, inflammatory cell recruitment, and potent eosinophil chemoattractant, increased IL_1 production by monocytes, peripheral contraction, skin wheal and flare reactions, as well as LTC_4 initiation of LH release and LTE_4, BHR induction [528].

PAF, a vasoactive mediator released by numerous cells including mast cells, monocyte-macrophages, eosinophils, neutrophils, fibroblasts, vascular endothelial cells and platelets, causes a rapid sequestration of airway platelets, with TXA_2 secretion. PAF has a bronchoconstrictor activity, by recruiting and activating eosinophils and neutrophils, also because they can bind to their receptors, amplifying CD11a–CD18 and CD11b–CD18 expression and consequently both cells' adhesion to endothelium. Other biological effects include an increase in microvascular permeability, cytotoxicity, generation of $O_2^{\cdot -}$, as well as of cationic proteins and potent mediators. PAF therefore plays a prominent role in the pathogenesis of atopic disease, in addition to rapidly inducing hypotension and depressing myocardial contractility [626]. The proof of PAF pathogenicity and immunogenicity is shown by the complete defect of PAF-acetyl hydrolase, due to a mutation of exon 9, in 4% of the Japanese population, 27% of which has the heterozygote trait [535].

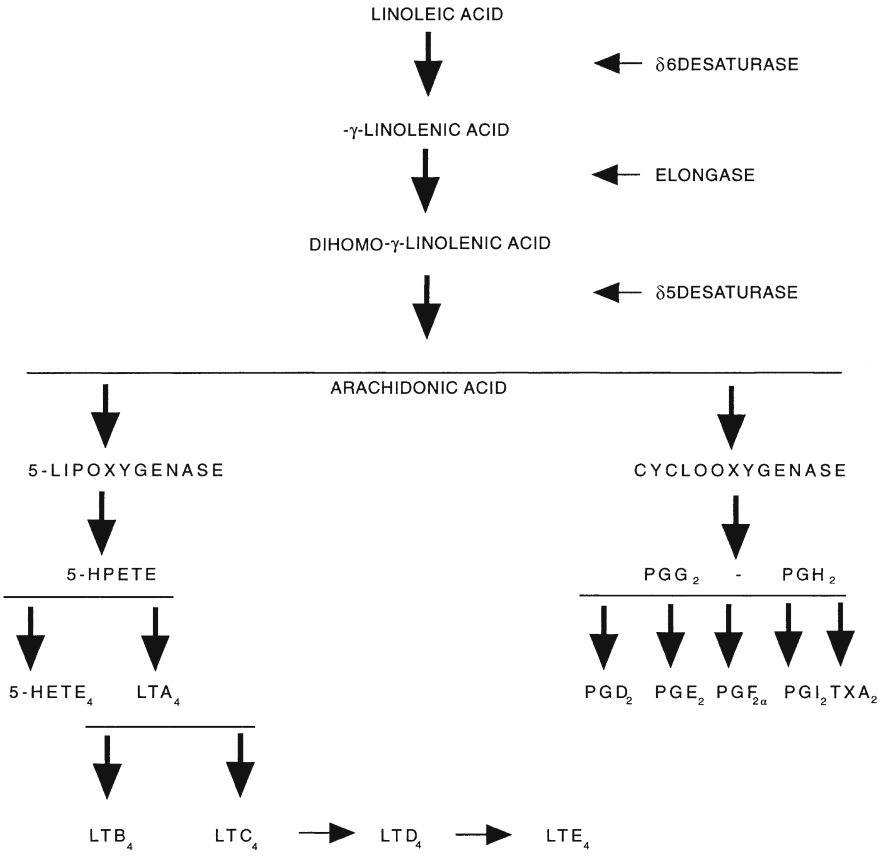


Fig. 1.57. Metabolic pathways of arachidonic acid. *HPETE* 12-hydroperoxy-eicosatetraenoic acid, *HETE* 12-hydroxyeicosatetraenoic acid, *PG* prostaglandin, *LT* leukotriene, *TX* thromboxane

Recent studies suggest that SP, a polypeptide with 11 amino acid residues, augments vascular leakage, stimulates mucus secretion in human bronchi, contracts bronchiolar smooth muscles, degranulates mast cells and is 100-fold more potent than histamine in eliciting wheal and flare reactions when injected subcutaneously [104].

Secondary Mediators

Secondary mediators are often released from primary mediators:

- *Mediators of cellular origin* released from neutrophils (toxic products of O_2, TXA_2, HETEs, LTB_4, LTC_4), eosinophils (MBP, ECP, PGE_2, HETEs, LTB_4, LTC_4), platelets (PF4, TXA_2), monocyte-macrophages, etc.
- *Mediators of extracellular origin,* from activation of complement, and of the following systems: coagulation, fibrinolytic, and kininogen-kinins

The inflammatory mediators can be also divided into:

- *Vasoactive mediators* (histamine, PGs, PAF, LTC_4, LTD_4 and LTE_4) active in the immediate phase.
- *Chemotactic mediators* (ECF-A, NCF-A, 5-HETE, LTB_4) active in the late phase (with recruitment of neutrophils, etc.) and inflammatory phase (with recruitment of eosinophils and monocytes) [626].

Several drugs inhibit mast cell degranulation and/or AA metabolism (Fig. 1.57), with very significant implications in the treatment of atopic disease [643]:

- CSs inhibit the ILs and, via lipomodulin (GIF is a fragment), inhibit PLA_2 and as a consequence AA separation from phospholipids.
- ASA and NSAIDS inhibit processes regulated by cyclo-oxygenase (they enter the cycle before the enzyme), thus stopping PG and TX formation, but not that of LTs since they augment the lipoxygenase pathway by compensation.
- Theophylline inhibits PDE-induced cAMP increase, thus down-regulating mast cell degranulation.
- β-Mimetic agents awaken the same effects because they increase cAMP levels.

Mechanisms of Cell Adhesion: Interleukins and Adhesion Molecules

As many as 51 different mostly T-cell-derived ILs of which 33 are ILs with their receptors have been identified so far (Table 1.5) as well IL receptors and signaling (Table 1.32). They comprise a heterogeneous group of intercellular regulatory proteins, with a MW of 8–140 kD ($IL_{12}R$ chain), often glycosylated, characterized by a very different biological activity and pleiotropic functions, produced singularly or associated. They were

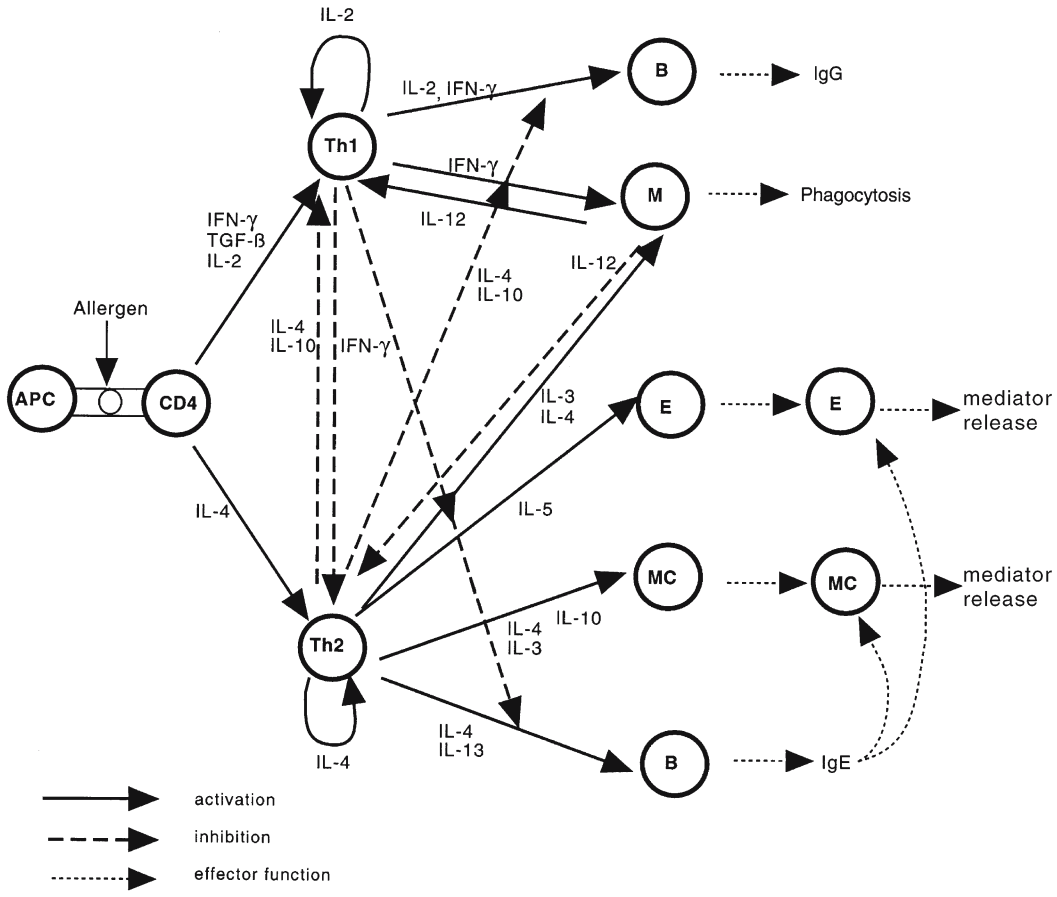


Fig. 1.58. Schematic diagram showing IL production and their activity during immune responses. *APC* antigen-presenting cells, *B* B lymphocytes, *E* eosinophils, *IL* interleukins, *M* macrophages, *MC* mast cells, *Th1*, *Th2* lymphocytes. (Modified from [619])

initially described as communication signals among leukocytes (inter-leukocytes), or soluble factors with a cellular derivation, with functions of messengers and activators of the immune system, and hence immunomodulators, regulating the growth and differentiation of immunocytes and/or many other cell types. ILs can exert a real hemopoiesis, that is, an action also on the growth and differentiation of hematopoietic cell progenitors in the area of immune inflammation where they are released, assuming a role of endogenous mediators. ILs are produced by cells exposed to inflammatory stimuli or bacterial products, or even not belonging to the immune system. They are involved in diverse stages of immune response and inflammatory reactions, greatly regulating their amplitude and duration, interacting at the cell surface with membrane receptors for which ILs have a high affinity, which transduce signals inside the cells. ILs act at picomolar concentrations and are therefore very potent, while their action is of short duration and self-limited. *A second IL cluster* has been localized on chromosome *1q32* including IL_{10}, IL_{19} and IL_{20} [45]. To IL_{12} is credited a contra-regulatory role of allergic inflammation, while a similar role could be shared by IL_{10} since it inhibits eosinophil survival [125]: these two ILs are also a pathognomonic example of opposing roles identified in the pathogenesis of atopic disease. In addition to several other ILs, the IL_{16} gene is associated with asthma, Crohn disease, and allergic contact dermatitis [59]. The numbers of IL_{16}-immunoreactive cells are increased in bronchial mucosa from atopic asthmatic patients 24 h after antigen provocation in vivo, and in allergen-induced late-phase nasal responses [283]. We have described above the relationships of human CD4 Th1 and Th2 T cells with several ILs. Recently the IL_{10} family has been shown to include IL_{19}, IL_{20}, IL_{24} (localized on chromosome *1q32*), IL_{22}, and IL_{26} (localized on chromosome *12q16*) [153]; some cardinal functions in atopic disease [457] are outlined in Fig.1.58 [619].

Recently SOCS, such as SOCS-3 induced by IL_{27} [403] and SOCS-1 and SOCS-3 genes stimulated by IL_{21} [547], have been reported to play a critical role in the inhibitory effect in a negative feedback mechanism [403]. SOCS are known to suppress DC functions and interfere with TLR4 signaling [547]. Both SOCS-1 and SOCS-3 can desensitize primary bone marrow-derived macrophages to IFN-ɣ and IL_6 signaling, respectively

[651]. Pretreatment of DC with IL_{21} inhibited LPS-induced expression of CD86 and HLA class II. Moreover, LPS-induced TNF-α, IL_{12}, CCL5, and CXCL10 production was clearly reduced in IL_{21}-pretreated cells [547]. IL_{27} inhibits CD28-mediated IL_2 production and also IL_2 responses [403].

Adhesion of immunocompetent cells between themselves, to target cells or to the intercellular matrix is a phenomenon of crucial significance in multiple immune reactions and mechanisms directing leukocyte distribution and localization. In this respect it is helpful to consider the *relationships between ILs and adhesion molecules,* which are based on vascular endothelial cells: indeed the leukocytes, when guided into circulation, must interact with such cells, which regulate leukocytes and are regulated by them. Leukocytes adhere to endothelial cells via a binding increase between leukocyte surface receptors and their *co-receptors or ligands* on the endothelial cell surface, which therefore plays a fundamental role in leukocyte migration from vascular to inflammatory sites in interstitial tissues [65]. Leukocyte adhesion to endothelial walls, chiefly of lymphocytes, is also of great magnitude for their compartmentalization and recirculation [209, 328, 534], as shown by Figs. 1.5, 1.11, and 1.58. Apart from new insights on the connections between ILs and leukocytes, the bidirectional relationships between ILs and endothelial cells are intriguing. Indeed, in addition to being targets of the action of soluble mediators of immunity, activating in vascular endothelium a large spectrum of functional interactions, endothelial cells are able to produce several ILs, some of which direct leukocyte extravasation from the circulatory system [534]. High vascular cells, the HEVs, are specialized for lymphocyte extravascular emigration into peripheral lymph nodes: lymphocytes bind selectively to HEVs, as shown by studies on the inhibition of lymphocyte–HEV adhesion done in the animal model and in humans by means of monoclonal antibodies. Therefore, it was possible to point out the role of surface structures present on HEVs. Such molecules, CD44, CD49d and others called addressins, then *selectins,* endothelial coreceptors considered to be specific for lymphatic tissues and involved in lymphocyte adhesion to endothelial cells, would bind to lymphocyte homing receptors, but with affinity for selectins present on lymphocytes [534].

Mechanisms of Cellular Adhesion

Adhesion molecules are a group of surface molecules crucial for immune system functions. In addition to promoting adhesion to the cellular matrix and modulating leukocyte adhesion to vascular epithelium and/or to ECM, they are able to direct leukocyte migration from vascular compartments to extracellular tissues, prime antigen-specific recognition by T lymphocytes, provide costimulatory signals for T-cell activation, stimulate the effector machinery for activated lymphocytes and regulate their proliferation and growth [23]. At least 70 soluble molecules and ILs are involved in lymphocyte activation and differentiation after antigen presentation. There are two different antigen-independent adhesion mechanisms between T cells on the one hand and APCs and/or target cells on the other, where CD2 (LFA-2), CD4, CD7, CD8, CD43 and CD45RO interact, as well as CD11a/CD18 adhesion molecules on T cells [328], more often Th2 T cells than Th1/Th0 cells [143]. In the first mechanism, CD2 binds CD58 and CD59 on APCs and/or B cells. In the second mechanism, CD11a/CD18 binds CD54 and CD102 expressed by APCs, where CD58 binds T-cell CD2: so both mechanisms integrate each other [23]. The largest group of these manifold and multidirectional *cell–cell adhesion molecules* encompasses integrins, selectins, etc., expressed on all surfaces and allowing cell contacts. The important molecules in the AD and asthma pathogenesis are outlined in Table 1.44 [378]: there we note the correspondence between the

Table 1.44. Adhesion molecules in AD and asthma

Adhesion molecules	AD	Asthma
Selectin superfamily		
CD62E	+	+
CD62L	+	+
CD62P	+	+
IgSF		
CD54	+	+
CD102	+	+
CD106	+	+
Integrin superfamily		
β1 Integrins		
CD49d/CD29 (VLA-4)	+	+
CD49e/CD29 (VLA-5)	–	–
β2 Integrins		
CD11a/CD18	+	+
CD11b/CD18	+	+
β3 Integrins		
GPIIb/IIIa	–	–
VNR	–	–
Others		
CD15 s (sLe^x)	+	+
CD36 (GPIIIb)	–	–
CD44 (H-CAM)	–	–
Thrombospondin	+	?
CLA	+	–

Modified from [378].

Table 1.45. β1 Integrins

Molecules	MW (kD)	Ligands	Distribution
$\alpha_1\beta_1$=CD49a/CD29 (VLA-1)	200–210	Laminin, collagen I, IV	T and NK cells, activated T, capillary E, F, HS
$\alpha_2\beta_1$=CD49b/CD29 (VLA-2)	155–165	Laminin, collagen I-IV	T and B cells, E, F, platelets, keratinocytes
$\alpha_3\beta_1$=CD49c/CD29 (VLA-3)	145–150	Laminin, fibronectin, collagen I–IV	B cells, E, F, keratinocytes
$\alpha_4\beta_1$=CD49d/CD29 (VLA-4)	150	Fibronectin, CD106, MAdCAM-1, PP	T and B cells, monocytes, NK, DC, eosinophils
$\alpha_5\beta_1$=CD49e/CD29 (VLA-5)	160	Fibronectin	Lymphocytes, monocytes, E, F, MC, basophils
$\alpha_6\beta_1$=CD49f/CD29 (VLA-6)	150–130	Laminin	T cells, platelets, eosinophils, E, monocytes
$\alpha_7\beta_1$=(VLA-7)		Laminin	Widespread
$\alpha_8\beta_1$			Widespread
$\alpha_9\beta_1$			
$\alpha_{10}\beta_1$			Thymic epithelium, endothelium, epidermis
$\alpha_V\beta_1$		Fibronectin, vitronectin	Thymic epithelium, endothelium, epidermis

Data from [4, 23, 226].
DC dendritic cells, *E* endothelial cells, *F* fibroblasts, *HS* hepatic sinusoid, *MC* metachromatic cells, *PP* Peyer's plaques.

two atopic diseases. Molecules of intracellular adhesion belonging to IgSF are listed in Table 1.4. CD54, a 90-kD gp controlled by IL_1 and TNF-α and -γ, plays a role of unquestionable importance in atopic disease. Of particular relevance is that CD11a/CD18 and CD11b/CD18 support leukocyte adhesion mechanisms binding to counter receptors of endothelial cells, including precisely CD54 and CD102 [23].

Integrins

A new chapter on the knowledge of surface molecules involved in adhesion mechanisms and leukocyte migration to tissue sites of inflammation was opened following the characterization of these gps with a heterodimeric structure made up of α and β chains, noncovalently associated, of 1,100 and 750 amino acids, respectively, important in the adherence of immunocompetent cells to tissues and cell surfaces. This preliminary stage, definite and representative, is related to antigen recognition from CD4 T cells (adhesion to APCs) or to effector activities (adhesion to target cells). Eight subfamilies of leukocyte integrins are as yet known, each made up of 24 different α subunits and a common β chain with 8 subunits distinct in β1–β8, noncovalently linked, forming many combinations [65, 534]. The α integrins play a key role in inflammatory processes, including leukocyte adhesion and migration. Their genes are located on the p arm of chromosome 16 [164].

β1 Integrins (CD29) are heterodimers composed of a common β chain, which can bind to variable α chains. They are the VLA proteins, very late antigens, because the first two appear on lymphocytes 2–4 weeks after antigen priming and are variably expressed on the leukocyte surface. VLAs are divided into various types, as is seen in Table 1.45 [4, 23, 226]. They are chiefly receptors of ECM components such as FN (VLA-5), fibrinogen, laminin (VLA-6), collagen (VLA-2), vitronectin, von Willebrand factor (vWF) and IgSF members [65, 534]. VLAs interact with a wide range of other cells: epithelial (VLA-3), mesangial (VLA-1), activated T (VLA-1, VLA-2), skin fibroblasts (VLA-1), hemopoietic lines (VLA-3), thymus, bone marrow, monocytes, leukocytes (VLA-4 and VLA-5), basement membranes (VLA-3), platelets (VLA-2, VLA-5), and liver sinusoids (VLA-1). VLAs are expressed on circulating lymphocytes and monocytes, like other VLAs on subsets and resting cells of the T phenotype. Thus they are important in lymphocyte homing, transendothelial migration to sites of inflammation, and interactions with ECM [226]. VLA-4 has distinct sites for VLA-5 and CD106, while $\alpha_4\beta_1$ could mediate pro-thymocyte migration from bone marrow to thymus [614].

β2 Integrins (CD29), characterized by a β2 common chain, and a higher immunological and immunopathological interest, are heterodimers formed by two covalently bound peptide chains expressed on PBMCs for the most part; hence they are called leukocyte integrins and regulate adhesion for PBMCs [226]. β2 Integrins have four receptors, each with two chains, α distinct and β common (Table 1.46) [4, 23], while $\alpha_d\beta_2$ is expressed by tissue macrophages [209]. However, a 30%–50% homology has been found between the two chains, denoting a common ancestral gene [23]. β2 Integrins recognize polypeptides with an Arg-Gli-Asp amino acid sequence, likely to be capable of blocking neutrophil adhesion to endothelial cells by a competitive mechanism. CD11b/CD18 and CD11c/CD18 (CR3 and CR4) mediate

Table 1.46. β2 Integrins

Molecules	MW	Ligands	Distribution
$\alpha_L\beta_2$=CD11a/CD18	180	CD50, CD54, CD102, CD62E	Widespread on all leukocytes
$\alpha_M\beta_2$=CD11b/CD18	170	CD54, iC3b, fibronectin, LPS	Monocyte-macrophages, neutrophils, NK cells
$\alpha_X\beta_2$=CD11c/CD18	150	CD54, iC3b, fibrinogen	Monocyte-macrophages, activated T cells, NK cells
$\alpha_d\beta2$	125	CD50>CD54	Macrophages, granulocytes, foam cells

Data from [4, 23].

Table 1.47. β3 Integrins

Molecules	Ligands	Distribution, effects
$\alpha_{II}\beta_3$=CD41/CD61, GP IIb/IIIa	Vitronectin, fibrinogen, fibronectin, vWF	Platelets, megakaryocytes
$\alpha_V\beta_3$=CD51/CD61	Vitronectin, fibrinogen, vWF	Endothelial cells, monocytes, platelets, T cells, LAK cells, mast cells, some B cells
VNR	Vitronectin, fibrinogen, fibronectin, vWF, leukocytes, platelets, laminin, thrombospondin	Endothelial cells

Data from [4, 11].
vWF von Willebrand factor, *VNR* vitronectin receptor.

Table 1.48. β4–β8 Integrins

Molecules	Ligands	Distribution, effects
$\alpha_6\beta_4$=CD49f/CD104	Laminin	Basal cell layer of stratified thymic epithelium
$\alpha_V\beta_5$=CD51/–	Fibronectin, vitronectin	
$\alpha_V\beta_6$=CD51/–	Fibronectin	
$\alpha_4\beta_7$ (LPAM-1)	MAdCAM-1, CD106, HEV	MALT, T-cell homing molecule, T, memory
$\alpha_E\beta_7$ (HML-1)=CD49d/β7 (CD103)	E cadherin	IEL T cells, lamina propria lymphocytes
$\alpha_V\beta_8$	CD31 ?	Cellular
$\alpha_6\beta_p$=VLA-4	Fibronectin, CD106	Cells of murine leukemia

Data from [11, 19, 226].
HML human mucosal lymphocytes, *IEL* intraepithelial lymphocytes.

phagocytosis binding CICs coated with iC3b and iC4b [337]. CD11a/CD18 (CR1) promotes CTL adhesion to target cells, CD11b/CD18 (CR3) functions as a receptor for iC3b, which has a domain with the same sequence [23], and finally CD11c/CD18 (CR4) binds to CD54 and iC3b. CD11a/CD18 and CD11b/CD18 are also involved in the LAD syndrome, fatal if not corrected by a bone marrow transplantation (BMT) [564]: five different types have been reported (Chap. 22).

β2 Integrins are distributed on a larger spectrum of cells:

- NK cells (CD11b/CD18 and CD11c/CD18)
- Leukocytes (CD11a/CD18 all, CD11b/CD18 and some CD11c/CD18)
- Monocyte-macrophages (CD11b/CD18 and CD11c/CD18) [610]

β3 Integrins (CD61) or cytoadhesins are given in Table 1.47 [4, 11]. Among *β4-β8 integrins* (Table 1.48) [11, 19, 226] is included CD104 expressed on thymocytes [166]. There are two β7: $\alpha_4\beta_7$ (CD49d/β7), which binds to MAdCAM-1, with two N-terminal domains with homology for CD54 and CD106, followed by a mucin-like region between domains 2 and 3 ending with an IgA-like domain. MAdCAM-1 further binds to both CD62L and $\alpha_4\beta_7$ [209], also mediating lymphocyte rolling and adhesion to epithelium, performing a double function of both a selectin and an integrin ligand [534]. Another β7, $\alpha_E\beta_7$, is the first to recognize E cadherin [76]. Thus, two *β7 integrins* may be important in GALT formation, since they provide lymphocyte trafficking to PPs and lamina propria [615]. Additional unclassified adhesion molecules (Table 1.49) [4, 11] are CD44 (ligand hyaluronic

Table 1.49. Additional adhesion molecules not classified among families

Molecules	Characteristics	Ligands	Distribution, effects
CD26	See Table 1.2	Fibronectin, collagen	Thymocytes, T and NK cells
CD35	See Table 1.2	C3b, C4b	E, B and T cells, phagocytes, splenic follicular CDs; on CDs possibly role in presentation of allergen, eosinophils
CD36	See Table 1.2	Thrombospondin	Monocytes, megakaryocytes, small vessel endothelium, platelets, reticulocytes
CD42a/d	See Table 1.2	Thrombin, von Willebrand factor	Platelets, megakaryocytes
CD44	See Table 1.2	Hyaluronic ac, collagen, laminin, fibronectin	B and T cells, E precursors, glial cells, monocytes, neutrophils, fibroblasts, myocytes, epithelia
CD73	See Table 1.2		Epithelial and endothelial cells
Laminin	140–400 kD	See "Integrins"	Integrity of basement membranes (embryogenesis, development)
Fibronectin	250–235 kD	Gelatin, fibrin, heparin, integrins (see "Integrins")	Adhesive and migratory events (embryogenesis, angiogenesis)
CD134	See Table 1.2	gp34	Adhesion of T cells to vascular endothelial cells, costimulation
Sialoadhesin	185 kD	Sialyl proteins	Contacts macrophage-granulocytes during hematopoiesis
VAP-1	?	?	Endothelial and dendritic cells, HEV of lymph nodes

Data from [4, 11].
E erythrocytes, *VAP* vascular adhesion protein.

Table 1.50. Selectins

Molecules	Ligands	Distribution
CD62E (E selectin)	ESL-1, CD15s, CD62L, CLA	Leukocytes, activated endothelia, epithelia, HEV of lymph nodes
CD62L (L selectin)	CD34, CD15s, GlyCAM	Resting leukocytes, peripheral lymph nodes, MALT
CD62P (P selectin)	PSGL-1, CD15s	Activated endothelial cells and platelets

All selectins mediate tethering and rolling of various cells (see text for details).
Data from [23, 610].
CLA cutaneous lymphocyte-associated antigen, ESL-1 E-selectin ligand 1, *GlyCAM* glycosylation-dependent cell adhesion molecule 1, *PSGL-1* P-selectin glycoprotein ligand 1=CD162.

acid), a homing receptor active in leukocyte extravasation to inflamed tissues [23], CD35, CD36, CD42, and others shown in the table.

Selectins

The *selectin family* is independent of integrins, mostly expressed on lymphocytes and neutrophils, and involved in the adhesion process with vascular endothelial cells; three molecules have been characterized so far, distinct by sequence and function homology (Table 1.50) [23, 610]. All belong to the C-type lectin family and share domains with regulatory complement proteins; CD62L also shares an EGF-like domain [4]. To these molecules with a slow expression (their peak is 4–6 h after IL stimulation, in TNF or IL_1, for example, the levels return to basal values after 12–24 h) also belong two molecules with a structure different from CD62E and CD62P, that is, CD54 and CD106 [610]. Selectins are typical lymphocyte homing molecules, to lymph nodes as well as to sites of inflammation [226]. CD62L expressed by 70% of circulating leukocytes is in part responsible for neutrophil in vivo recruitment to inflamed tissues. CD62E, a receptor of mononucleated cells, is on HEVs at sites of immune inflammation: IL-activated, both mediate adhesion to endothelium [4]. CD62P expressed by platelet α granules has the same preference of CD62E for endothelial cells, which express it after agonist stimulation (histamine, thrombin, etc.), returning to basal levels after 20–60 min. β2 integrins and CD62L can act in agreement with guide neutrophils to inflamed

Table 1.51. Cadherin classification

Molecules	Ligands	Distribution
Uvomorulin	Homophilic	
LCAM	Homophilic	
E-cadherin	Homophilic $\alpha_E\beta_7$	Epithelial cells (IEL)
N-cadherin	Homophilic	Neural cells
P-cadherin	Homophilic	Placental cells
V-cadherin	Homophilic	Vasal cells
VE-cadherin (CD144)		β-Cadherin

Modified from [65].
LCAM liver cell adhesion molecule.

tissues [348]. High-affinity selectin ligands are ESL-1 (E-selectin ligand 1), PSGL-1 (P-selectin glycoprotein ligand 1=CD162) related to FGF and GlyCAM-1 (glycosylation-dependent cell adhesion molecule 1) for CD62L [209]. Particularly, ESL-1 and CD162 mediate myeloid cells binding to the two selectins; CD162 also mediates CD62P-associated leukocyte binding and rolling [4].

The *cadherins* (Table 1.51) [65] are distributed in different tissues, and with their desmosomes serve as anchoring sites for the cytoskeleton to the point of Ca-dependent adhesion between adjacent cells and junction formation through which bundles of actin filaments run among cells [162]. Cadherin homophilic adhesion plays a key role in segregating embryonic tissues and in cell migration and tissue differentiation [162].

The cadherin superfamily is wide: ≈80 cadherins have been isolated. Most members are expressed in the CNS. These are homophilic adhesion molecules, and for their homophilic interactions, the ectodomains (EC) play a crucial role [657]. Over recent years *protocadherins* have emerged as a growing superfamily of molecules, with a complex picture of their structure and con-

Table 1.52. Adhesion between leukocytes and endothelial cells at the molecular level

Cells	Adhesion molecules	Ligands on endothelium
T, B, monocyte-macrophages, neutrophils, NK cells	CD11a/CD18	CD50, CD54, CD102
Monocyte-macrophages, neutrophils, eosinophils	CD11b/CD18	CD54
Monocyte-macrophages	CD62E, CD62P	CD15 s
Monocyte-macrophages, neutrophils, NK cells	CD11c/CD18, CD11d/CD18	CD106
Lymphocytes, monocytes, eosinophils	CD49d/CD29	CD106
Lymphocytes, monocytes, neutrophils, eosinophils	CD62L	CD34, GlyCAM-1

Modified from [130]. *T*, *B*, T cells, B cells.

Table 1.53. Adhesion between leukocytes and endothelial cells at the organ level

Adhesion steps	Peripheral Lymph node HEV		Peyer's plaque HEV		Gut		Skin	
Tethering								
Lymphocytes	CD62L		CD62L		CD62L		CLA	
Endothelium	CD34, GlyCAM-1?		MAdCAM, CD34?		MAdCAM, CD34?		CD62E	
Triggering								
Lymphocytes	$G\alpha_1$-coupled receptors	?	$G\alpha_1$-coupled receptors	?	$G\alpha_1$-coupled receptors	CD31/GAG	$G\alpha_1$-coupled receptors	CD31/GAG
Endothelium	chemo-attractant?	CD31?	chemo-attractant?	CD31?	MCP-1? HGF ? MIP-1?	CD31?	MCP-1? HGF ? MIP-1?	CD31?
Strong adhesion								
Lymphocytes	$\alpha_L\beta_2$		$\alpha_4\beta_7$	$\alpha_L\beta_2$	$\alpha_4\beta_7$		$\alpha_4\beta_7$	$\alpha_L\beta_2$
Endothelium	CD54–CD102		MAdCAM	CD54–CD102	MAdCAM		MAdCAM	CD54/102

GAG glycosaminoglycan.
Data from references [226, 534].

stitute the largest subgroup within the cadherin family of Ca^{++}-dependent cell–cell adhesion molecules [657]. This new family is large, 52 novel protocadherins were identified on human chromosome 5q31 [653]. Subsequently, 66 protocadherins were identified in cluster genes arrayed into two clusters [380]. Protocadherins are organized into α, β, and γ. The β genes in man are 19 and in mouse 22 [605]. The γ-protocadherins are expressed exclusively in the CNS [629]. A fourth group, δ, includes δ 1-protocadherins (comprising protocadherin-1, -7, -9 and -11 or -X/Y) and δ 2-protocadherins (comprising protocadherin-8, -10, -17, -18 and -19). Some δ-protocadherins appear to mediate weak cell–cell adhesion in vitro and cell sorting in vivo [451].

Tables 1.52 and 1.53 summarize the adhesion molecules involved in interactions between lymphocytes and endothelial cells at the molecular [226] and organ levels [226, 534].

Relationships Between ILs and Adhesion Molecules

Several ILs, mainly those with a tendency for inflammation, activate on endothelial cell membranes the expression of adhesion molecules for leukocytes and/or other circulating cells:

- IL_1, TNF-α and IFN-γ increase the expression of CD54 and CD102, CD62E, CD106, etc.; the first two molecules induce adhesion on all granulocytes, the last one only on eosinophils and basophils.
- IL_2 activates CD54.
- IL_3 fosters basophil adhesion to endothelial cells and CD11b/CD18 expression on basophils.
- IL_4 induces lymphocyte adhesion and can make up for lack of CD106 modulating its expression in the absence of CD54, CD102 and CD62E.
- IL_5 promotes CD11/CD18 expression on eosinophils.
- IL_6 expresses CD11b/CD18 on pro-monocytes, further CD11c and CD54.
- IL_7 primes CD11b/CD18 expression from T lymphocytes and of CD54 from T and B lymphocytes.
- IL_8 activates CD11b/CD18 and CD11c/CD18 on PMNs.
- GM-CSF provides neutrophils and granulocytes with CD11b and endothelial cells with CD18 [23, 323].

Chemokines

Chemokines [22, 179, 247] are more than 65 small protein molecules (8–10 kD) generated by leukocytes, monocyte-macrophages and platelets and activated endothelium, which can thus regulate leukocyte trafficking and that related to IL_8 identified as analogous to NAP-1. As their name suggests, *chemo*attractant cyto*kines* play the role of chemotactic molecules interested in integrin activation. Chemokines may be defined as small but potent leukocyte pro-inflammatory chemoattractants, cellular activating factors, and HRFs, that bind to specific G-protein-coupled seven-span TM receptors present on plasma membranes of target cells, which makes them particularly important in the pathogenesis of allergic inflammation. Chemokines are major regulators of cell trafficking and may also modulate cell survival and growth [280, 687]. Chemokines IL_8, MIP-1, MCP-1 and RANTES are known to recruit neutrophils, eosinophils, macrophages and T lymphocytes to the site of inflammation. MIP-1, MCP-1 and RANTES are direct chemoattractants of Th1 cells. These chemokines are promiscuously used by several receptors, but all three are ligands for CCR5, which is preferably expressed in type 1 cells. Therefore, it is of interest to note that the CCL-chemokines and their receptors are coexpressed in Th1 cells. The coexpression is not only Th1-cell-specific, but the similar expression pattern applies to Th2 cells that express CCR4 bearing thymus and activation-regulated chemokine (TARC) receptor. Structurally related, chemokines are divided into three groups based on the *chromosomes by which they are coded:* α = 4, β = 17, depending on whether the first two cysteine residues are separated by an amino acid (CXCL) or are adjacent (CCL), γ = 1, and δ is an exception on chromosome 16, interacting with receptors different from G proteins [9, 22, 178] (Tables 1.54, 1.55) [2, 9, 19, 22, 178, 247, 479, 574]. The proposed chemokine nomenclature is based on the nomenclature currently in use, derived from the one already assigned to the gene encoding the 4 families of chemokines [687], and includes CC, CXC, XC, or CX3C followed by L (ligand) or R (receptor) and then a number [667]:

- *CXCL α chemokines:* for neutrophils there are many specificities (Table 1.54), GROs are specific even for basophils [178]. Moreover, NAP-1/IL_8 chemoattractant of eosinophils can activate also basophils (Fig. 1.49) and act as a potent inducer of T-cell chemotactic and activating responses. From PBP (platelet basic protein) β-TG, CTAP-III and NAP-2 originate [19]. A further subdivision might be introduced, since mig, IP-10 and PF-4 lack the E-L-R (glutamic acid-leucine-arginine) residues [19].
- *CCL β chemokines:* (Table 1.55) RANTES is produced by fibroblasts to attract and activate eosinophils. LPS is known to induce RANTES and cause protein tyrosine phosphorylation [658]. RANTES is a chemoattractant selective for activated and resting T cells, including memory cells [552]. CD45RO, macrophages and eosinophils. MIP-1α and MIP-1β and MCP-1 are chemotactic for T subsets, macrophages, eosinophils and basophils; while macrophages in turn elicit several CCL chemokines [540]. In particular, MIP-1α favors activated B cells and CD8 migration and MIP-1β-activated T cell infiltration; both direct T-cell adhesion to endothelial cells, the former of CD8 and the latter of CD4 T cells. Basophils and eosinophils are among the more sensitive cells, mostly to CCLs, while MCP-3 combines the properties of RANTES and MCP-1 [247, 396].

Table 1.54. Main properties of α chemokines or CXCL chemokines

Name	Source(s)	Target cells and (biological effects)
BCA-1	Secondary lymphoid organs	B lymphocytes (chemotaxis)
ß-TG	Monocyte-macrophages, platelets	Monocytes, platelets, fibroblasts (growth), neutrophils (chemotaxis)
CK-α1		
CTAP-III	Platelets, monocytes	Fibroblasts (activation), neutrophils (chemotaxis)
ENA-78	Monocytes, neutrophils, NK and endothelial cells	T cells, basophils, neutrophils (activation and degranulation)
GCP-2	Osteosarcoma cell line	Neutrophils (chemotaxis)
GRO α, β, γ	monocyte-macrophages, fibroblasts, synovial cells and epithelial cells, hepatocytes, keratinocytes, neutrophils, T lymphocytes	neutrophils (degranulation, adhesion, activation, endothelial chemotaxis), basophils (in a reduced way), fibroblasts (growth), endothelial cells (angiogenesis)
IL_8/NAP-1	Osteoblasts, endothelial/epithelial cells, fibroblasts, keratinocytes, smooth muscle cells, astrocytes, B and T lymphocytes, monocyte-macrophages, hepatocytes, melanoma cells	Neutrophils (activation, chemotaxis, adhesion, killing), lymphocytes and NK cells (chemotaxis), basophils (chemotaxis, histamine release), endothelial cells (angiogenesis), keratinocytes (mitogenesis)
IP-10	Monocytes, endothelial cells, fibroblasts, keratinocytes, macrophages	T cells (activation, chemotaxis, integrin expression by T cells), NK cells (chemotaxis, cytolytic activity), endothelial cells (angiogenesis inhibition)
I-TAC	Monocytes, neutrophils, epithelial cells	T lymphocytes, Th1 and NK cells (chemotaxis)
MAD-2		
mig	Monocyte-macrophages, NK cells	T cells (activation and chemotaxis)
NAP-2	Platelets	Neutrophils (activation and chemotaxis)
PBP	Platelets, lymphocytes	
PF-4	Platelets, megakaryocytes, T lymphocytes	Fibroblasts, neutrophils, monocytes and T cells (endothelial cells adhesion, angiogenesis inhibition)
SDF-1α, β	Several tissues	Lymphocytes, monocytes (chemotaxis)

β-TG β-thromboglobulin, *CK-a1* α1 chemokine, *CTAP-III* connective tissue activating protein-III, *DC* dendritic cells, *ELC* EBI1 ligand chemokine, *ENA-78* epithelial cell-derived neutrophil-activating protein, *GCP-2* granulocyte chemotactic protein 2, *GRO-α, GRO-β, GRO-γ* growth-related gene, *IP-10* Inflammatory protein-10, *I-TAC* interferon inducible T-cell alpha chemoattractant, *MAD-2* monocyte adhesion dependent protein-2, *MCP 1/5* monocyte chemotactic protein-1/5, *mig* monokine inducible by IFN-γ, *MIP-1α, -1β, 2* macrophage inflammatory protein-1α, -1β, 2, *NAP-1* neutrophil activating factor-1, *PBP* platelet basic protein, *PF4* platelet factor 4, *SDF-1a/1β* stromal cell-derived factor.
Data from [2, 9, 19, 22, 178, 380, 459, 574].

MCP-1, MCP-2 and MCP-3 are also major attractants for monocytes, $CD4^+$ and $CD8^+$ T cells [317, 609]. MCP-1–4 bind to specific G-protein-coupled receptors, initiating a signal cascade within the cell. CCR2 is considered the major G-protein-coupled receptor for MCP-1 [616]. MCP-5, a 9.2-kD peptide that consists of 82 amino acid residues, has been identified in the mouse [614]. MCP-1 is efficacious nearly as much as C5a and, in conjunction with RANTES and MIP-1α, stimulates basophils to release histamine, even more so if IL_3, IL_5 and GM-CSF interfere. Furthermore, MCP-1 turns into a powerful chemoattractant of eosinophils, similarly to IL_8 if it loses the N-terminal region [628]. MCP 1–3 are active chemoattractants for NK cells [316], MCP-1 also for T cells of memory phenotype [67], whereas MCP-3 appears to be involved in the regulation of early responses to specific allergens [666]. Parallel to eotaxins [435], RANTES has an effect on eosinophil local recruitment, by acting on their locomotion similarly to C5a, and 2- to 3-fold more powerful than that of MIP-1α [658]. RANTES in IL_5-stimulated cells is an effective inducer of eosinophil transendothelial migration by a single mechanism, CD49d/CD106-dependent [133]. Although RANTES and IL_5 are elevated 24 h after antigen challenge, eosinophil recruitment and degranulation is associated only with IL_5 [552]. Unprimed mast cells are influenced to migrate by MCP-1 and RANTES, while IgE-activated cells respond to MIP-1α and PF4, but do not degranulate in response to chemokines [568]. C10, with homology to CCF-18, seems to involve only T-cell chemotaxis [19]. Positive correlations link the level of Syk expression and

Table 1.55. Main properties of chemokines: CCL, XCL and CX3CL chemokines

Name	Source(s)	Target cells and (biological effects)
β or CCL chemokines		
ACT-2		
AMAC	Activated macrophages	Naive T cells (chemotaxis)
C10		
CKβ1, 3, 4, 6–11		
DC-CK1	Dendritic cells	$CD45RA^+$ T cells, naive T cells (chemotaxis)
ELC/MIP-3β		T cells (selective)
Eotaxin-1	Nasal epithelium	Eosinophils (chemotaxis)
Eotaxin-2	Activated monocytes, basophils, myeloid progenitors	Resting T cells (chemotaxis), eosinophils, basophils
Eotaxin-3	Eosinophils	
Exodus-1/MIP-3α/LARC	DCs (regulation and migration)	
Exodus-2	Lymph nodes, airways, appendix, spleen	
FIC		
HCC-1	Normal tissues, $CD34^+$ myeloid progenitors	Monocytes (chemotaxis)
HCC-2	Liver	Monocytes (chemotaxis)
HCC-4		T lymphocytes
I-309	Monocytes, mast cells, activated T cells	Monocytes (chemotaxis)
MCP-1	Monocytes, endothelial/epithelial cells, fibroblasts, keratinocytes, smooth muscle cells, mast cells, mesothelial cells	Monocytes (chemotaxis, adhesion, phagocytosis, killing, superoxide release, arachidonic acid activity) metachromatic cells (chemotaxis, degranulation, histamine release), basophils (LCT synthesis), T cells (chemotaxis), eosinophils; macrophages (activation, secretory activity, chronic inflammation), stem cells (colony formation inhibition)
MCP-2	Monocytes, osteosarcoma cells, fibroblasts	Has half of MCP-1 activity: monocytes, T cells, eosinophils (chemotaxis), mast cells (chemotaxis, histamine release)
MCP-3	Osteosarcoma cells	Combines MCP-1 and RANTES properties: monocytes, T cells, eosinophils (chemotaxis)
MCP-4	Endothelial/epithelial cells	Lymphocytes, monocytes, eosinophils (chemotaxis)
MCP-5	Monocytes	Peritoneal macrophages (chemotaxis)
MDC/STCP-1	Macrophages, monocyte-derived DCs	Monocytes and derived DCs, activated T cells, thymic T cells, CD8 T cells, IL_2-activated NK cells (chemotaxis)
MIP-1α now CCL3	B and T lymphocytes, monocytes, fibroblasts	B and T lymphocytes, neutrophils, monocytes, NK cells, eosinophils, DC (chemotaxis); T cells (adhesion, collagenase release, integrin expression mostly by CD8 T cells), eosinophils (cationic protein release), metachromatic cells (chemotaxis and histamine release), stem cells (colony formation inhibition)
MIP-1β now CCL4	B and T lymphocytes, monocytes, fibroblasts	Monocytes (chemotaxis), T lymphocytes (chemotaxis, adhesion, integrin expression mostly by CD4 T cells), stem cells (antagonizes MIP-1α effects)

Table 1.55. (Continued)

Name	Source(s)	Target cells and (biological effects)
MIP-3α	Liver, monocytes, lymphocytes	Mononuclear, dendritic, and T cells (chemotaxis)
MIP-3β	Lymphoid tissue, activated B lymphocytes	Activated T lymphocytes (chemotaxis)
MIP-5	Liver, intestine, lymphocytes (airways)	Monocytes, T lymphocytes (chemotaxis)
MIPF-1		Resting T cells, monocytes, neutrophils
PARC	T cells (selective), lung, lymphoid tissue	T lymphocytes (chemotaxis)
RANTES now CCL5	T cells, platelets, macrophages, endothelial cells	T cells (chemotaxis, adhesion, integrin expression mostly by CD4 T cells), monocytes, DC and NK cells (chemotaxis) specific for eosinophils (cationic protein release), basophils (chemotaxis and histamine release); additional actions are similar to those of MCP-1
SLC	Lymphoid tissue, activated macrophages	T lymphocytes (chemotaxis)
TARC	Lymphoid tissue, mononuclear cells	T lymphocytes, Th2 T cells (chemotaxis)
TCA3		
TECK	Thymic dendritic cells, small intestine, liver	Activated macrophages, dendritic cells (chemotaxis)
γ or XCL chemokines		
Lymphotactin	$CD8^+$ T lymphocyte, thymocytes, NK cells	T cells (chemotaxis)
ATAC		
SCM-1	Mononuclear cells, spleen	Lymphocytes (chemotaxis, activation)
CX3CL chemokines		
Fractalkine	Endothelial cells, monocytes	T cells and monocytes (chemotaxis)

When the chemokines are without indications, the sources and the target cells are as yet unknown.
From [2, 9, 19, 22, 178, 247, 459, 574].
ACT-2 immunoactivating cytokine-2, *β-TG* β-thromboglobulin, *CK-α1*, *3 CK-β1*chemokines α1 and -β1, *DC-CK1* dendritic cell chemokine-1, *FIC* fibroblast-induced chemokine, *HCC-1* hemofiltrate CC chemokine-1, *I-309* I-309 protein, *LARC* liver and activation-regulated chemokine, *MCP-1* monocyte chemotactic protein-1, *MCAF* monocyte chemotactic and activating factor, *MIP-1α* and *MIP-β* macrophage inflammatory protein-1α and -1β, *MIPF-1*, *MIPF-2* myeloid inhibitory factor-1, -2, *PARC* pulmonary and activation-regulated chemokine, *RANTES* regulated on activation normal T expressed and secreted, *SDF-1* stromal cell-derived factor, *SLC* secondary lymphoid tissue chemokine, *STCP-1* stimulated T cell chemotactic protein-1, *TARC* thymus and activation-regulated chemokine, *TCA3* T cell activation gene 3, *TECK* thymus-expressed chemokine.

RANTES production induced by LPS. RANTES production from nasal fibroblasts stimulated with LPS is enhanced by overexpression of wild-type Syk gene transfer [658]. Several CXCL and CCL chemokines alone or together release 11 %–43 % of basophil histamine [283] and have similar activity: for example, GRO α, γ and MCP-1 are constitutively produced in human airway epithelium and bronchoalveolar macrophages [32], while IL_8 and CCL chemokines manipulate IL_2-activated NK-cell chemotaxis [334]. The *eotaxins* have a great significance in the *atopic march*: STAT-6 is required to up-regulate eotaxin-1 and eotaxin-2 expression by chronic IL_4 stimulation [687]. Multiple other STAT molecules (STAT-1, STAT-2, and STAT-3) can be recruited to various other chemokine receptors after JAK recruitment. These activated STAT molecules can then translocate into the nucleus of the chemokine-stimulated cell and directly activate (and sometimes repress) gene expression [396]. TARC, constitutively expressed in the thymus and produced by monocyte-derived DCs and endothelial cells, is a ligand for CCR4 and CCR8 and serves for the recruitment and migration of these receptor-expressing cells, and is thus responsible for the selective trafficking of Th2 lymphocytes into sites of allergic inflammation [393]. More chemokine receptors are rapidly up-regulated following T cell activation. Th1 cells express CXCR3 and Th2 CCR3, CCR4, CCR8 [687].

- *XCL γ chemokines:* only ATAC, and a lymphotactine specific for T cells are known to date [2, 9].
- *CX3CL chemokine:* only fractalkine (Table 1.55).

Table 1.56 [2, 19, 380, 396, 574, 687] summarizes the chemokine interactions.

Table 1.57 [2, 6, 247, 396, 690] shows correspondence of chromosome location, chemokine receptors, and

Table 1.56. Summary of chemokines and their interactions

Chemokines	Neutrophils	Monocytes	NK	T cells	Basophils	Eosinophils	Endothelia	Mast cells	FB	DC
CXCL										
β-TG	+									
CTAP-III	+				+				+	
ENA78	+									
Exodus-1/MIP-3α/LARC										
GCP-2	+									
GROα, β, γ	+						+			
IP-10			+	+			+			
NAP-1/IL_8	++		+	+	+		+			
NAP-2	+				+				+	
PF4	+	+							+	
SDF-1α/1β		+		+						
CCL										
C10				+						
CCF-18										
ELC/MIP-3β				++						
Eotaxin					++					
Eotaxin-2/MIPF-2				++	+	++				
I-309		+								
MCP-1		++		+	++			+		
MCP-2		++		+	+	+		+		
MCP-3		++		+		+		+		+
MCP-4		+		+		+				
MCP-5		+								
MDC/STCP-1		+	+	+						
MIP-1α	+	+	+	+ (CD8)	+	+		+		+
MIP-1β		+		+ (CD4)						
MIP-5		+		+						
MIPF-1	+	+		+						
PARC/DC-CK1				++						
RANTES		+	+	++	+	+				
XCL										
Lymphotactin				+						
CX3CL										
Fractalkine			+	+						

ELC/MIP-3β and PARC/DC-CK1 are selective for T cells, eotaxin-2/MIPF-2 and MIPF-1 act only on resting T cells, not on activated T cells, TECK is specific for T-cell development in thymus.
Data from [2, 19, 380, 396, 687].
DC dendritic cells, *FB* fibroblasts, *NK* NK cells.

Table 1.57. CXCL, CCL and CX3CL chemokine/receptor families

Systematic name	Human ligand	Human chromosome	Chemokine receptors
CXCL chemokines/receptor family			
CXCL1	GRO-α/MGSA-α	4q12-q13	CXCR2 > CXCR1
CXCL2	GRO-β/MGSA-β	4q12-q13	CXCR2
CXCL3	GRO-γ/MGSA-γ	4q12-q13	CXCR2
CXCL4 (fusin)	PF4	4q12-q13	–
CXCL5	ENA-78	4q12-q13	CXCR2
CXCL6	GCP-2	4q12-q13	CXCR1, CXCR2
CXCL7	NAP-3	4q12-q13	CXCR2
CXCL8	IL-8	4q12-q13	CXCR1, CXCR2
CXCL9	Mig	4q21.21	CXCR3
CXCL10	IP-10	4q21.21	CXCR3
CXCL11	I-TAC	4q21.21	CXCR3
CXCL12	SDF-10 /,8	10q11 .1	CXCR4
CXCL13	BLC/BCA-1	4q21	CXCR5
CXCL14	BRAK/bolekine	–	–
CXCL15	SR-PSOX	–	CXCR6
CXCL16	–	–	CXCR6
CCL chemokines/receptor family			
CCL1	I-309	17q11.2	CCR8
CCL2	MCP-1, MCAF	17q11.2	CCR2
CCL3	MIP-1α	17q11.2	CCR1, CCR5
CCL4	MIP-1β	17q11.2	CCR5
CCL5	RANTES	17q11.2	CCR1, CCR3, CCR5
CCL6	Exodus-1, LARC	17q11.2	–
CCL7	MCP-3	1 7q11 .2	CCR1, CCR2, CCR3
CCL8	MCP-2	1 7q11 .2	CCR3
CCL11	Eotaxin	17q11.2	CCR3, CCR5
CCL13	MCP-4	1 7q11.2	CCR3
CCL14	HCC-1	17q11.2	CCR2
CCL15	HCC-2/Lkn-1/MIP-1β	17q11.2	CCR2, CCR3
CCL16	HCC-4/LEC	1 6q13	CCR1
CCL17	TARC	17q11.2	CCR1, CCR3
CCL18	DC-CK1/PARC AMAC-1	9p13	CCR1
CCL19	MIP-3δ/ELC/exodus-3	2q33-q37	CCR4
CCL20	MIP-3cr/LARC/exodus-1	9p13	CCR6
CCL21	6Ckine/SLC/exodus-2	1 6q13	CCR4
CCL22	MDC/STCP-1/ABCD-1	1 7q11 .2	CCR1
CCL23	MPIF-1	7q11.23	CCR3
CCL24	MPIF-2/Eotaxin-2	1 9p13.2	CCR9
CCL25	TECK	7q11.23	CCR3
CCL26	Scya26/Eotaxin-3	9p13	CCR10

Table 1.57. (Continued)

Systematic name	Human ligand	Human chromosome	Chemokine receptors
CCL27	CTACK/ILC	9p13	CCR10
CCL28	MEC	–	CCR3, CCR10
C chemokine /receptor family			
XCL1	Lymphotactin/SCM-10/ATAC	1q23	XCR1
XCL2	SCM-10	1q23	XCR1
CX3C chemokine /receptor family			
CX3CL1	Fractalkine	16q13	

Systematic name	Human ligand	Expression
Chemokine receptor		
DARC	IL_8, GROα, NAP-2, ENA-78, MCP-1, MCP-3, RANTES	Endothelia (spleen, lungs, brain, kidneys), lymphocytes (CD45RA), Purkinje cells, erythrocytes
Viral receptors		
CMV US28	MIP-1α and -1β, MCP-1, RANTES	
HSV saimiri	IL_8	
Lipidic autacoid		
PAFR	PAF	Myeloid cells and smooth muscle cells, lymphocytes, CNS
Anaphylatoxin-formyl-peptide		
C5aR	C5a	Myeloid cells, microglia, astrocytes, mucosal epithelia, mast cells, hepatocytes
C3aR	C3a	Myeloid cells, heart, lungs, brain, intestine, some lymphocytes ?

Cell types, receptors found and known ligands	
Neutrophil	
CXCR1	IL_8, GCP-2
CXCR2	IL_8, GCP-2, GRO-α, GRO-β, GRO-γ, ENA-78, NAP-2, LIX
Eosinophil	
CCR1	MCP-3, MCP-4, MIP-1α, RANTES
CCR3	MCP-3, MCP-4, eotaxin-1, eotaxin-2, RANTES
Basophil	
CCR2	MCP-1, -2, -3, -4, -5
CCR3	MCP-3, MCP-4, eotaxin-1, eotaxin-2, RANTES
Monocyte	
CCR1	IL_8, GCP-2
CCR2	MCP-1, -2, -3, -4, -5
CCR5	MIP-1α, MIP-1β, RANTES
CCR8	I-309
	MDC, HCC-1, TECK
CX3CR1	Fractalkine
CXCR4	SDF-1

Table 1.57. (Continued)

Cell types, receptors found and known ligands	
Dendritic cell	
CCR1	IL_8, GCP-2
CCR2	MCP-1, -2, -3, -4, -5
CCR3	MCP-3, MCP-4, eotaxin-1, eotaxin-2, RANTES
CCR4	TARC
CCR5	MIP-1α, MIP-1β, RANTES
CCR6	MIP-3α (LARC, Exodus-1) MDC, TECK
CXCR4l	SDF-1
Resting T Lymphocyte	
	PARC, DC-CK-1
	Lymphotactin
CXCR4	SDF-1
Activated T lymphocyte	
CCR1	IL_8, GCP-2
CCR2	MCP-1, -2, -3, -4, -5
CCR4	TARC
CCR5	MIP-1α, MIP-1β, RANTES
CCR7	MIP-3β (ELC) PARC, SLC, 6CKine (Exodus-2)
CX3CR1	Fractalkine
CXCR3	IP-10, MIG, I-TAC
Natural killer cell	
CCR2	MCP-1, -2, -3, -4, -5
CCR5	MIP-1α, MIP-1b, RANTES
CX3CR1	Fractalkine
CXCR3	IP-10, MIG, I-TAC

CCR5 favors HIV-1 entry into target cells [82] (see Chapter 23).
DARC Duffy antigen receptor complex, *MGSA* Melanocyte growth stimulating activity , *PAFR* PAF receptor.
Data from references [2, 4, 246, 396, 690].

their different ligands: 15 for CXCL (CXCL 1–18), 28 for CCL (CCL 1–28), 1 for CX3CL chemokines and other unclassified ones [2, 4, 690]. All have seven G-protein-linked TM-spanning domains and their signaling can be typically inhibited by pertussis toxin [2], in addition to two virally encoded chemokine receptors that may be used, together with IL_8 R and IFN-R, by viruses to subvert the host immune system [2] by altering the local conditions in favor of viral persistence and replication [11]. The inhibition of chemokine receptors by pertussis toxin (PT) suggests that Gi proteins are key to the transduction of signals. Gi proteins physically associate with multiple chemokine receptors, but there is also evidence that other PT-resistant Gi proteins, such as Gq or G16, might also associate with certain receptors [465]. AA release driven by PLA2 is important for optimal cell movement toward a chemokine gradient. The roles for activated PLD are as yet unclear. Recent studies have clearly demonstrated a key role for PI3K in chemokine receptor signaling [396, 554]. This leads in turn to activation of PIP-specific PLC, PKC, small GTP-ases, Src-related tyrosine kinase, PI3K, and PKB. PLA delivers two secondary messengers, inositol- 1,4,5 triphosphate, which releases intracellular calcium, and DAG which activates PKC. Multiple phosphorylation events are triggered by chemokines. PIP-OH-kinase can be activated by the βγ subunit of G protein, small GTP-ase or Src-related tyrosine kinases [687]. The membrane tyrosine phosphatase CD45 has also been shown to regulate CXCR4-mediated activation and phosphorylation of TcR downstream effectors Lck, ZAP-70, and

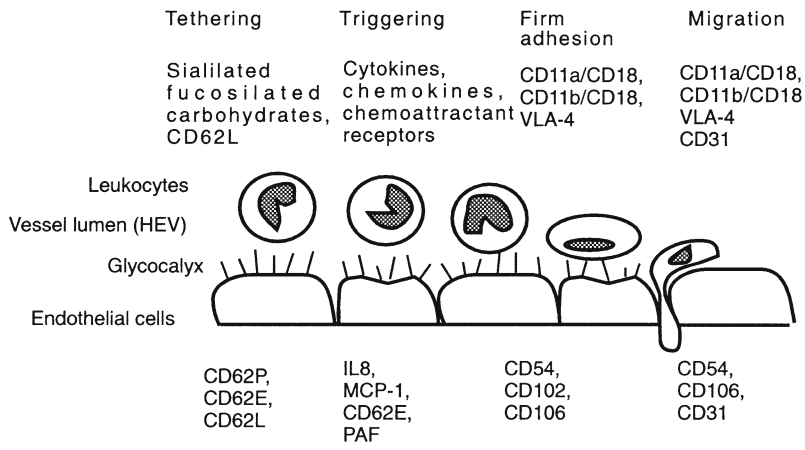


Fig. 1.59. Leukocyte trafficking and adhesion cascade. Sequential steps in leukocyte recruitment, their adhesion to endothelium and transendothelial migration (see text). (Modified from [209, 230, 328])

SLP-76. Activation of the RAFTK (related adhesion focal tyrosine kinase), a member of the related kinase family, has been shown to be induced by signaling via CXCL12 binding to CXCR4 [151]. Mitogen-activated protein kinases have also been shown to be phosphorylated and activated within 1 min after exposure of leukocytes to CCR3 ligands [247]. CXCR4 is predominantly expressed on inactivated naive T lymphocytes, B lymphocytes, DCs, and endothelial cells. SDF-1 is the only known ligand for CXCR4 [239].

The principal biological function of chemokines is contributing to leukocyte recruitment, firstly by activating integrins, as described above, and secondly by promoting leukocyte migration across endothelium and through ECM [2]. Additional functions consist in the antiviral immunity (innate immunity), hematopoiesis and angiogenesis regulation, growth and cellular metabolism [9]. The existence of so many characterized chemokines with overlapping targets is not surprising, since it is also possible that certain chemokines may have a restricted tissue expression, although their large number can actually result in a degree of redundancy [2]. However, the strong expression of IL_8 and MCP-1 by epithelial cells could also suggest that they might be key factors in leukocyte recruitment to counteract invading pathogens [141]. This notion agrees with the priming of cytotoxic CD8 T cells and NK cells by RANTES and MIP-1α [569], which can provide substantial costimulatory signals for T-cell proliferation and promotion of effector functions, also by enhancing CD80 and CD86 expression on APCs and IL_2 production from activated T lymphocytes [20]. In addition, CCL chemokines direct basophil and eosinophil migration for activation and response at DTH sites, where ILs of Th2 T cells surround and in turn stimulate the above cells, amplifying their negative effects. Nonetheless, the presently available data, even if suggestive, need to be thoroughly analyzed in the context of the pathogenetic hypotheses currently discussed.

More to the point, chemokines act in conjunction with HRFs which, like antigens, are able to trigger histamine release and to activate a large number of cells (mast cells, basophils, lymphocytes, eosinophils, macrophages, monocytes and platelets) and possibly B and T lymphocytes. By binding to sIgE, monocytes and basophils, HRFs can perpetuate histamine release, thereby inducing allergic reactions too late or too prolonged to be classified as IgE-mediated reactions. The observation that IgE of atopic subjects bind HRFs at variance with the IgE of nonatopic individuals suggests that these molecules have an unequivocal clinical weight. There is a factor inhibiting HRF (HRIF) in relation with NAP-1/IL_8 (at the same time an HRF and an HRIF), a protein of 8,000 Da derived from PBMCs, B and T lymphocytes and possibly alveolar macrophages, whose generation is augmented by normal histamine concentrations, hence suggesting a feedback mechanism inhibiting the histamine itself, which can thus regulate HRF activities. CCL chemokines are HRFs, if nothing else due to the similarity of conditions in which they are generated [687].

Leukocyte Trafficking and Migration

Adhesion processes, mediated by proteins adherent to endothelial membranes and correspondent receptors on leukocyte membranes, acquire a new impetus due to specific interactions between HEVs and lymphocytes. Transendothelial migration of activated lymphocytes from the blood into the tissues is an essential step for immune functions, which may be arrested by Zap-70 deficiency (Chap. 22). Preceded by inflammatory mediator release, causing vasodilation and blood flow deceleration, this preliminary step allowing the cascade to proceed, as mentioned in the preceding section via HEVs, influences *leukocyte rolling* [23]. Up-regulated CD62s, mainly CD62P, with a long molecular structure that extends above the surrounding glycocalyx, have the task of capturing passing leukocytes expressing appropriate receptors (Fig. 1.59) [209, 230, 328]; moreover, CD62L is localized on the tips of microvilli, a first point of contact with the endothelium [348]. Ensuing passages are orchestrated by chemokines and integrins, preceded by T-cell rolling with selectin interactions (CD15, CD62L

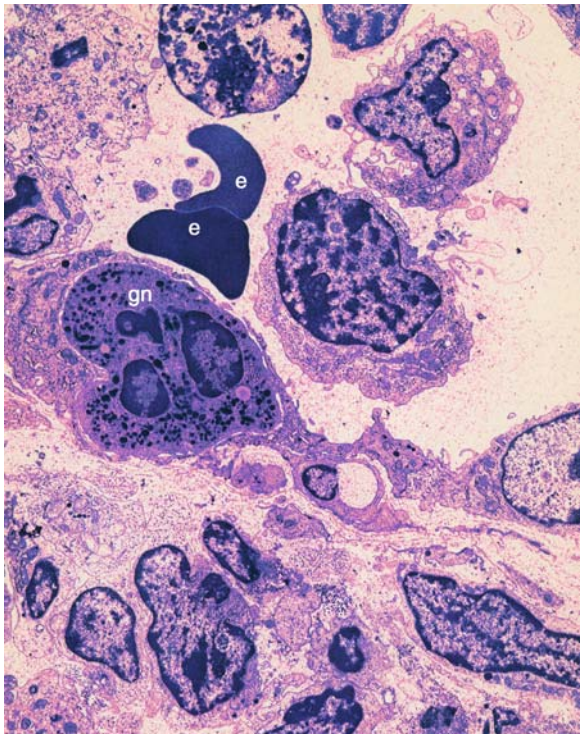


Fig. 1.60. Diapedesis. *e* Two red cells in the vessel lumen, *gn* granulocyte neutrophil while passing through the endothelial wall by diapedesis

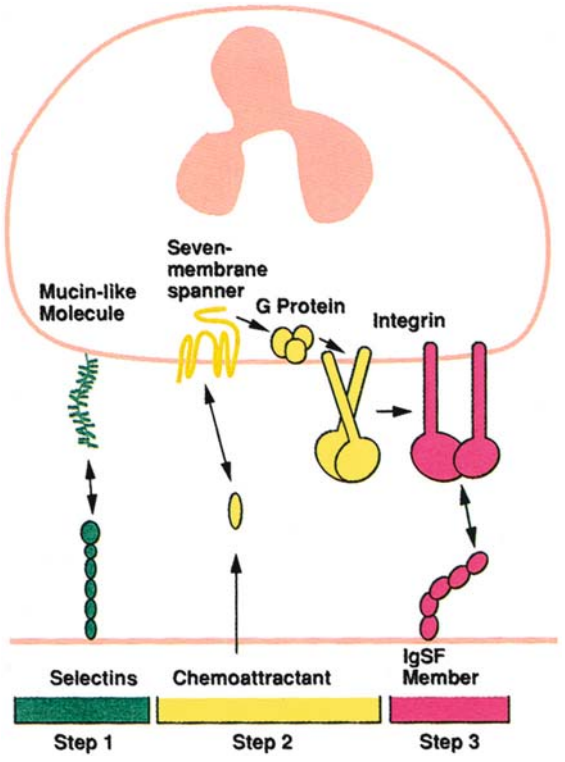


Fig. 1.61. Springer's three-step theory: how to provide the traffic signals that regulate leukocyte localization in the vasculature (for details see text). *IgSF* immunoglobulin superfamily

and CLA of lymphocytes, CD62P and CD62E on endothelium) and by integrin stimulation (activated by CD31 and chemokines). Functional activation of integrin receptors on lymphocytes, above all CD2, CD28 and PDGF, and signal transduction regulating adhesion point to a PI3K primary role, in turn activated by chemokines and receptors sensitive to G proteins [521]. After conformational modifications of the cells and integrins ensure a strong adhesiveness, lymphocytes undergo diapedesis (Fig. 1.60), and their migration between endothelial cells in extravascular spaces is followed by directional cues from chemoattractants (Fig. 1.61) [534]. The process continues within the inflamed tissues, generating additional mediators and ILs. The initiation of endothelial activation, which modulates selectins binding carbohydrate ligands, often displayed on mucin-like molecules, is responsible for the initial tethering of a flowing lymphocyte to the vessel wall and for a transient rolling along the endothelial cells (step 1). Tethering contributes to chemoattractant-mediated adhesion, resulting in integrin triggering and binding to endothelial ligands (step 2). The sound T-cell adhesion is modulated by G proteins, whose signals activate strong integrin adhesiveness, which binds IgSF on endothelium (step 3) and the transendothelial migration (step 4). Eventually T lymphocytes spread via endothelial cell–cell junctions, cross the basement membrane and migrate across lymphoid tissues [522]. A recent study indicated that activation of integrin avidity to endothelial ligands by endothelium-displayed chemoattractants (or chemokines) can take place within fractions of a seconds and can promote both reversible rolling adhesions or immediate conversion of leukocyte rolling to firm arrest on vascular ligands [187]. Obviously the adhesion required is not too strong, otherwise it would lead to cell immobilization [226] (Fig. 1.61). In step 4, lymphocytes cross intercellular HEV junctions, allowing them to enter HEVs: the entire process of lymphocyte sticking to HEVs takes only 1–3 s and step 4 10 min [454]. In humans, as many as 5×10^6 lymphocytes leak from blood via HEVs every second [454]. Even if the equilibrium among variables stabilizes with speed, relatively small changes in integrin expression or integrin–ligand affinity, or cell-substratum adhesiveness, can lead to substantial changes in migration speed [406]. Several integrins govern interactions: the initial ones are mediated by CD62L recognizing CD34 and GlyCAM-1 (Table 1.50). After activation, lymphocytes firmly adhere to endothelial cells since β2 integrins interact with IgSF members on both endothelium and G-protein-coupled receptors [162]. During stage 3, CD11a/CD18 inhibit lymphocyte migration via CD54 and CD102 HEV counter-receptors, playing the major role [534]. Then lymphocytes migrate, using β1 integrins, to

Table 1.58. Molecules active in the homing, memory and inflammatory responses of lymphocytes

Lymphocyte receptors	Ligands	Distribution	Activity
$\alpha_E\beta_7$=CD103	E-cadherin	Epithelium (unknown)	Homing
$\alpha_4\beta_7$=CD49d/CD29	MAdCAM-1	MALT, HEV	Homing
CD62L	CD34		Homing, memory
CLA	CD62E	Skin	Homing, memory
ESL-1	Unknown		Inflammation
PSGL-1	CD62P		Inflammation
CD11a/CD18	CD54, CD102		Inflammation
CD11b/CD18	Fibrinogen, CD54		Inflammation
CD11c/CD18	Fibrinogen		
CD49d/CD29	CD106, fibronectin		Inflammation

Data from [61, 209].
CLA Cutaneous lymphocyte-associated antigen, *ESL-1* CD62E ligand 1, *PSGL-1* CD62P glycoprotein ligand 1.

a chemotactic stimulus (such as a bacterial invasion) [163], while step 4 is CD31-modulated [230]. The pathways used by lymphocytes to bind endothelium depend on the site and nature of stimuli activating it:

- *IL_1 and TNF-α,* by increasing CD54 and CD106 expression on endothelium in vitro, allow lymphocytes to bind either β1 or β2 integrins [540]: IL_4 primes only CD106 expression, VCAM, hence restricting the field to β1 integrins.

There are differences regarding the involved site: the umbilical vein endothelium stimulated by IL_1 or TNF-α expresses both CD54 and CD106, whereas cutaneous microvascular endothelium responds much better to TNF-α.

Such processes are regulated by proteoglycans well expressed on endothelium, which stimulate granulocyte and T-cell adhesion by means of the chemokines IL_8 (via β2 integrins) and MIP-1β (via β1 integrins), respectively [565].

Lymphocyte recirculation is also influenced by adhesion molecules in specific tissues: one such molecule is MAdCAM-1, an IgSF member, largely restricted to gut epithelium (HEV of PPs, mesenteric lymph nodes, and endothelium of enteric mucosa) [328], where it mediates binding of a specific subset of $CD45RO^+$ cells expressing $\alpha_4\beta_1$ integrin [38]; wherever MAdCAM-1 is mentioned, its localization is in MALT [209].

CD62E mediates T-cell binding to lymph node HEVs. Such binding to mucosal HEVs is promoted by another integrin, LPAM-1, with an α chain homologous to an α4 CD49d/29 chain, in turn a CD106 receptor.

Additional molecules involved in lymphocyte homing include CLA expressed on T cells with an exclusive tropism for binding to CD62E on skin endothelium, and VAP-1 (vascular adhesion protein) also with an endothelial origin that mediates lymphocyte binding to lymph nodes and synovial membranes [328].

Lymphocyte migration into tissues has slightly diverse phases depending either on peripheral lymph node HEV, or on PPs, or skin, or intestine (Fig. 1.5). However, the CD106/receptor CD49d/29 duo plays a leading role in binding both lymphocytes and monocytes [85]. In Table 1.58 [62, 209] are shown the molecules involved in homing (and related memory) and in lymphocyte inflammatory responses: also CD62L, CD11a, b, c/CD18, $\alpha_4\beta_1$ = CD49d/CD29 (VLA-4) and $\alpha_4\beta_7$ = CD49d are included [209].

As regards *leukocytes,* the same selectivity comes on the scene: the involved molecules are CD62E and CD106, which also promotes adhesion of *eosinophils* expressing CD49d/CD29 at the surface, unlike neutrophils [624]. CD62E governs PAF production, which together with other endothelial cells with activating properties, expresses IL_1 and integrins such as CD11a/CD18, CD11b/CD18, etc. [65]. As a consequence, the CD106/receptor CD49d/29 duo represents the greater association of adhesive molecules in eosinophil recruitment and extravasation, to be directed to in vivo inflamed sites [624]. Eosinophils, following what is outlined by Fig. 1.59, supported by CD49d/CD29, adhere to endothelial cell membranes supplied with CD106 and are activated; expressing CD11a/CD18 bind to CD54 and CD102, then transmigrate into the underlying mucosa. In brief, the key points are as follows [230]:

- *Cell recruitment* mediated by T cells by means of IL_4, IL_5, RANTES and MIP-1α.
- *Selectin* (CD62E, CD62P and CD62L) and ligand (CD34, GlyCAM-1) selective expression on endothelial cells produced by ILs and mediators.
- *Integrin* (CD11a/CD18, CD11b/CD18, CD49d/CD29) selective activation induced by ILs, CCL and CXCL chemokines and chemoattractants.
- *IgSF* (CD54, CD102, CD106) selective expression induced by ILs: above all CD106 specifically expressed by

IL_4 on epithelial cells is an important regulatory moment in eosinophil adhesion to epithelium and antigen-driven migration [578].

- *TNF-α* has been demonstrated to have an important role in the expression of adhesion molecules that induce transendothelial migration of eosinophils [534].

Neutrophils in normal conditions are absent or poorly represented on endothelium, being expressed after activation. These cells in the early phase roll on activated endothelial vessel walls, accompanied by a mast cell histamine-modulated increase in vascular permeability, a process mediated by CD62P expressed on neutrophils [154]. PAF is released at these sites and in perspective can elicit, together with SP, the chemotactic movement of parallel activated neutrophils, which strongly adhere to endothelium and proceed to transendothelial migration. Lipoxin A4 modulates transmigration of human neutrophils across intestinal epithelial monolayers [90] (see also Chap. 11). In the late phase, LPS and ILs (IL_1, IFN, TNF, etc.) with inflammatory action stimulate CD62P on endothelial cells, expression of CD54, IL_8 release, and CD62L and CD15s activation on neutrophils which, via CD15s, CD62E and CD62P specific binding, continue their rolling along vessel walls, adhesion modulated by CD11b/CD18/CD54, activation and diapedesis [19, 154]. However, cell migration can be different as a consequence of an eicosanoid exposure, although neutrophil retention in a specific anatomical site could play an important role in mucosal defense [90]. Selectins mediate the initial rolling contacts of *monocytes* with the endothelium. Firm adhesion to the endothelium is the second step and involves other adhesion molecules such as CD11a, CD11b, CD18, and VLA-4 on monocyteds and CD54 and VCAM-1 on endothelial cells. The adherence to endothelial cells is modulated by endothelin-1 via the involvement of Src (p60src), JAK1-like kinases, and vascular endothelium growth factor by an increased flt-1 expression by monocyes. The final step is monocyte transmigration into the subendothelial space [609].

A primary function of *epithelial cells* is to maintain vascular integrity. Vascular injury promptly promotes epithelial cells to release their granule content (CD62P, vWF), which is quickly deposited on ECM, where it plays a crucial role in platelet adhesion to damaged sites. *Platelet degranulation* and activation of their $\alpha_{II}\beta_3$ integrin = CD41/CD61 drives further accumulation of platelets (aggregation) and modulates neutrophil and monocyte recruitment, which participate in the repair of damaged tissues. Thus platelet rolling, analogous to lymphocyte rolling, may represent an initial step in hemostasis [163].

Although knowledge on the role effectively played by such different molecules in human physiopathology is still lacking and far from yielding logically linked reference patterns, such acquisition suggests that some molecules may play a central role in normal conditions regarding homeostasis and the pathogenesis of some morbid conditions.

Interrelations with Other Organs

Several observations clearly show that the immune system interacts with other communication systems of our organism such as the endocrine apparatus and central nervous system (CNS). There are important correlations between ILs and neuropeptides: after its release from nervous tissue termination, SP is able to increase transcriptions at the gene level and synthesize and secrete IL_1, IL_6 and TNF-α, with an effect on stromal cells, GM-CSF, G-CSF and M-CSF, and consequent neutrophil, basophil, macrophage and eosinophil adhesion and activation, thus modulating the effector stages of type I and type IV reactions. Interconnections with neuroendocrine circuits are complex: SP and VIP (vasoactive intestinal peptide) are involved in the control of IL_2 production by T lymphocytes, while IL_1 and IL_1R are present in neurones and endocrine glands. IL_2 and IL_6 alter the proliferation pattern of anterior pituitary cells, as well as GH, ACTH and prolactin secretion, while IL_5, IL_7, IL_9 and TGF-β are involved in the regulation of neurodifferentiation [493]. Indirectly, an initial event such as an infection or a tissue lesion of less impact can trigger polysaccharide or ECM protein release, which stimulates tissue macrophages to release TNF-α. TNF-α-exposed endothelial cells express adhesion molecules to attract first PBMCs and then T cells to recognize APC-expressed antigens. TNF-α acting on these cells mediates IL_1 start-up, inducing phagocytes, T lymphocytes and endothelial cells to produce IL_6 [326]. The integrin-linked kinase (ILK), colocalizing with the β1-integrin subunit, is expressed in various regions of the CNS. ILK staining revealed that it is enriched in neurons and is an important effector in NGF-mediated neurite outgrowth [353]. Some ILs induce the acute-phase response (APR) in the liver, and CSs stimulate APP production, whereas they block TNF-α, IL_1 and IL_6 actions. These ILs act independently on the hypothalamus-hypophysis-adrenal (HPA) axis, but also display synergic effects: TNF-α and IL_6 act on the hypothalamus inducing fever. In addition IL_6 causes a concentration of cortisol and corticotrophin that is higher than the levels obtained with the greatest stimulation of corticotrophin-releasing hormone (CRH). How IL_6 can reach the CRH hypothalamic neurones is not known; however, it is possible that IL_6 is produced by endothelial and glial cells stimulated by TNF-α and IL_1. CNS and the endocrine system also mount inflammatory responses: for example, they generate β-endorphin endowed with a local analgesic action. CRH and probably also arginine-vasopressin have a pro-inflammatory activity; in particular CRH is found in inflamed areas, most likely produced locally by postganglionic sympathetic neurones [326]. Appendix 1.4 [326] concludes the issue of neuroendocrine interactions with the immune system: for example, prolactin amplifies IL_2 production, β-endorphins, and melanotonin, which are active on T and B lymphocytes.

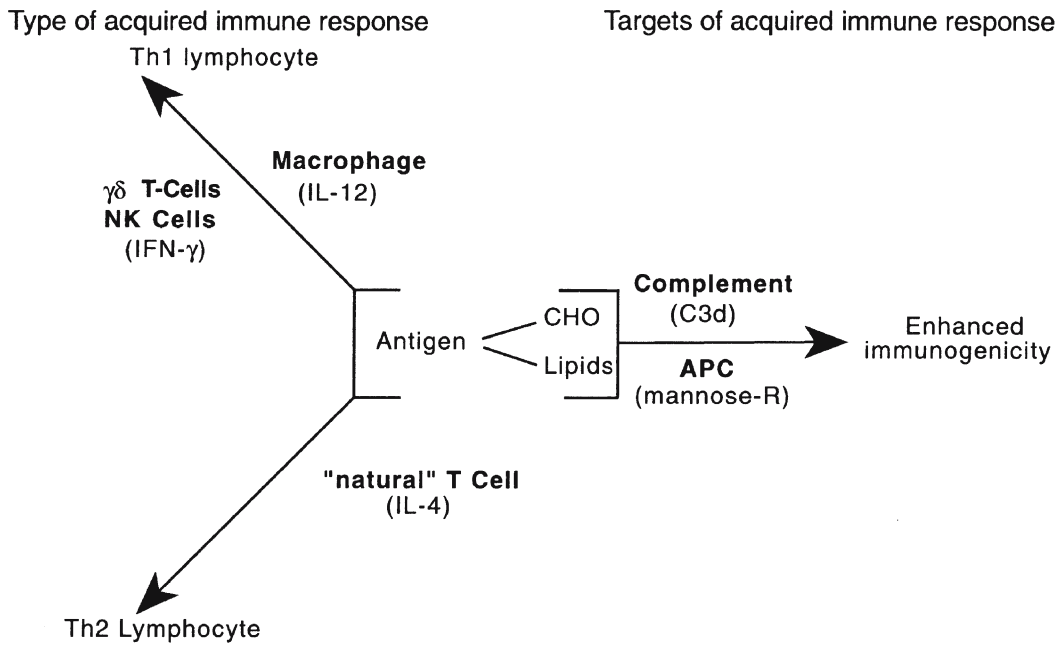


Fig. 1.62. Components of innate immunity (*in bold*), which recognizes carbohydrates (*CHO*) and lipids and instructs the acquired immune response to the antigens with which they are associated. *APC* antigen-presenting cell, *R* receptor. (Modified from [148])

Innate Immunity

Innate, or natural immunity, substantially aspecific and independent of previous contacts with pathogen agents, has evolved as the first line of defense against the constant threat of myriad microorganisms in the surrounding environment. This immunity is based on the genetic constitution of individuals. To avoid infections and prevent access of pathogens or potentially pathogenic microorganisms, present in the environment and on the body surface lying in direct contact with the environment, the host has evolved a series of *sophisticated defense systems,* closely integrated among themselves. The achievement of protective immunity against invading pathogens relies on sensing specific molecular features expressed in microorganisms and depends on the ability to elicit the pertinent type of immune response to fight a specific pathogen. Germline-encoded receptor molecules enable the cells of the innate immune system to recognize structural components conserved among classes of microorganisms [449]. The recognition of microbial products by the host innate immune system rapidly triggers appropriate responses to contain the infection and regulate the development of adaptive immunity. The exposure to microbial antigens primes several ILs and proinflammatory mediators that affect T cell differentiation and are rapidly produced by APCs, such as DCs and macrophages [366, 447]. Recent data show that IL_1R and $IL_{18}R$ are key molecules in both the innate and acquired immunity and are members of a larger family of related receptors, some of which contribute to host defense [526]. Table 1.1 shows the differences between innate and acquired immunity, which is instructed by soluble and cellular components of innate immunity to select the appropriate antigens coincidentally with strategies devised for their elimination (Fig. 1.62) [148]. Natural immunity is realized by skin, mucosal barriers and secretion protective effects, phagocyte cells, complement, and additional biological activity of nonspecific factors. Among them there are proteins coded in the germline, employed by innate immunity to recognize potentially noxious substances [148]. Such proteins are either soluble or have the form of surface receptors: for example, macrophage phagocyte particles or soluble glycoconjugates linked with the mannose receptor, a C-type lectin with a wide specificity for carbohydrates, and in addition an LPS receptor, LBP (lipopolysaccharide-binding protein) [536]. Bacteria coated with innate immune surfactants or MBP (mannose-binding protein), often including complement activated by the alternate (innate immune) pathway, are opsonized and more readily phagocytosed. In this process the receptors for antibodies and complement of macrophages and neutrophils are important, so that the coating of microorganisms with antibodies, complement, or both enhances phagocytosis. The engulfed microorganisms are subjected to a wide range of toxic intracellular molecules, including $O_2^{\bullet -}$, hydroxyl radicals, hypochlorous acid, NO, antimicrobial cationic proteins and peptides, and lysozyme [115]. LCs become activated and behave as APCs when pattern-recognition receptors on their surface recognize distinctive pathogen-associated molecular patterns on the surface

of microorganisms [345]. Such common constituents of Gram– bacteria external membrane signal infectious agents triggering IL_1, IL_6, IL_{12} and TNF-α synthesis, thus eliciting an APR [31], stimulate macrophages and other cells, as well as CD4 differentiation and growth [148]. Current data show that IL_1R and $IL_{18}R$ are key molecules in both the innate and adaptive immunity, and are members of a larger family of related receptors, some of which contribute to host defense [525] and that IL_{22} directly promotes the innate, nonspecific immunity of tissues [650]. IL_{22} induces APRs in the liver, which suggests that IL_{22} plays a role in innate immunity via induction of an inflammatory process [129]. Attractive data support the concept that potential prophylactic and therapeutic results are expected from $IL_{17}A$ and $IL_{17}F$ in host defense against bacterial infection. The IL_{25} activity associated with systemic and localized Th2 responses offers an experimental basis to modulate Th2-associated allergic diseases [249]. Some NK cells posses lectin-like membrane receptors involved in the recognition of target cells destined to cytolysis [289]. Complement is activated when the alternative pathway interacts with particles rich in carbohydrates but lacking sialic acid, or the classic pathway is stimulated by *collectins,* which bind to specific carbohydrates [210]. It is understandable why, unlike acquired immunity, *its innate immunity has a more complex organization:* constituents are soluble cells and factors with different structure and function, sometimes acting on different targets, but on the whole often become in turn integrated [148]. Recent studies have individuated the TcRγδ, which also in natural immunity precedes an αβ in antigen responses, thus influencing αβ-expressed IL pattern, discriminating between pathogens [152]. In this way, γδ could begin to supervise αβ responses to infectious agents due to their much earlier activation and conversion to memory [210]. A similar control could be extended to NK cells [287].

The natural defenses, the skin and mucosal barriers, are strengthened by mucosal secretions containing sIgA, able to complex with antigens and to identify antibodies capable of their recognition, and to complex in turn with antigens. Activation of the complement cascade can lead to directly destroying undesired hosts or to facilitating their phagocytosis. Complement and phagocytes are the first to activate when an infection approaches, supplying host defenses with notable contributions by means of a nonspecific protection against invasive pathogens, also without antibodies and/or T CTLs intervening [531]. Therefore specific immune mechanisms act in concert with those of innate immunity. We will examine:

1. Anatomic barriers: skin, mucus, and secretions
2. Proteins with anti-infectious activity (PAA):

a) Lysozyme
b) IFN
c) Complement
d) APP or pentraxins

3. Phagocytes: neutrophils and macrophages

1. *Anatomic barriers,* or physicochemical barriers, offer not only a mechanical, but also chemical protection, performed via production of biological molecules with antibacterial activity, including lactoperoxidase, lysozyme, lipase, spermine and fatty acids with bactericidal power. *Skin* is provided with the *stratum corneum,* which by means of its physiological desquamation resists penetration of a great number of microbes. In addition, it is normally impermeable to the greater part of infectious agents, because of bacteriostatic, bactericidal and fungicidal effects of triglycerides, free fatty acids and lactic acid, present in sebaceous secretions and sweat, which also contains lysozyme. As a compensation of the thinner epithelial stratum, mucus-capturing microorganisms are present on *mucosal surfaces,* subsequently removed from airways by the mucociliary apparatus. The secreted mucus layer overlays the epithelium in the respiratory, gastrointestinal, and genitourinary tracts, and the epithelial cilia sweep away this mucus layer, permitting it to be constantly refreshed after it has been contaminated with inhaled or ingested particles. Invaders are transported with a continuous cycle from bronchioles to the pharynx, a protective action that extends to the stomach HCl with bactericidal action. Saliva, swallowing, peristalsis and defecation mechanically expel microorganisms from the gastrointestinal system [353]. Salivation, lacrimation, and coughing, are further mechanisms that are very effective in reducing bacterial assaults: patients presenting with severe changes in lacrimation and salivation (Sjögren syndrome) suffer from severe eye infections and tooth caries. Nasal secretion and saliva contribution are significant, provided with mucopolysaccharides inactivating some viruses of anaerobic germs associated with the normal intestinal bacterial flora, both preventing pathogen attachment via competition for essential nutrients and/or production of inhibiting substances as well as urinary flow ensuring unremitting cleansing. Basic proteins, such as lysine, arginine, spermine, spermidine and particular gps, among others transferrin, make additional contributions [47].

2. *PAA,* important associates of innate immunity, integrate mechanisms that rapidly come into play, with a notably crucial result when the innate system faces the first encounter with infectious agents, since it will take from 4 to 15 days before elaborating antibodies and cytotoxic cells. Not all germs are assaulted with identical means and proportions: in biological fluids and interstitial spaces, the intervention of factors bound to antibodies and complement is more germane, unlike parenchyma where phenomena of cell immunity prevail (ILs, cytotoxic cells).

2a. *Lysozyme,* produced by macrophages and neutrophils, is found in salivary, lacrimal, nasal secretions, intestinal and respiratory mucus, lymph nodes and spleen. It is able to split β-glycoside bindings, in particular β 1–4 links between *N*-acetylglucosamine and

Table 1.59. Anti-infectious and anti-inflammatory activities of complement

Component(s)	Function in host defense
C1, C4, C4a, C4b	Neutralization of viruses ↑
C1q	Opsonization and phagocytosis ↑, antibody formation ↑, binding to CICs, cytotoxicity mediation
C2 kinin	Vascular permeability ↓
C3 fragment (C3e)	Release of granulocytes from bone marrow ↑
C3a	Antibody formation, antigen-induced T-cell proliferation, cytotoxicity mediated by T and NK cells ↑
C3a, C4a, C5a	Release of histamine and other mediators from mast cells ↑
C3b	Opsonization, IC phagocytosis, B-cell growth, IL_2-dependent T-cell growth, killing mediated by T and NK cells ↓, antigen presentation, clearance of CICs, antigen localization in lymphoid tissues
C3b soluble	Antigen-induced T-cell proliferation, cytokine production ↑
iC3b	Opsonization, phagocytosis, ADCC ↑
C3d, C3dg	B-cell growth↑
C3d, C3dg soluble	B-cell growth, IL_2-dependent T-cell growth ↓
C5a	Chemotaxis of PMNs inducing the influx into inflammatory sites, stimulation of phagocytes to release cytokines (TNF, IL_1),granule enzymes and O_2 metabolites, antigen-induced T-cell proliferation, antibody formation ↑
C5b6789 (MAC)	Lysis of bacteria, fungi, protozoa, viruses and virus-infected cells
Factor Ba	B-cell proliferation
Factor Bb	B-cell growth and differentiation ↑
Factor H	Growth of murine lymphocytes

Modified from [241].
IC immune complexes, ↑ increase/up-regulation, ↓ decrease/down-regulation, *PMN* polymorphonucleates.

N-acetylmuramic acid, a normal constituent of several bacterial cell walls.

2b. *IFN α, β, γ,* have several effects, including a timely antiviral activity, especially IFN-λ1–3 (*IL_{28a}*, *IL_{28b}*, *IL_{29}*) [377]. All IFN-α, -β, -ω, and -λ subtypes are expressed in influenza-virus-infected monocyte-derived DCs and PDCs [88]. It is significant that IFN-γ induce in murine B cells the IgG_1 isotype switch that increases phagocytosis, activating the complement classic pathway and linking macrophage Fc receptors [148].

2c. *Complement* [241, 633] represents, together with antibodies, the main component of the humoral defense system against microorganisms. *If activated it participates in host protection* in a specific and nonspecific way, intervening, besides phagocytosis, with functions divided into lytic and nonlytic, such as chemotaxis, opsonization and anaphylactogen activity (Table 1.59) [241]. In addition, with its receptors, it acts on B-cell antibody synthesis, immune memory, and CIC solubilization and clearance [159] (Tables 1.60, 1.61) [241, 260, 474]. From a simplistic viewpoint among immune reactions, complement is comparable to a motor vehicle, while antibody is an ignition key: indeed, once antibody has recognized the non-self molecule, it has specific functions, including complement activation and its fixation on the cell surface [633]. Complement components, provided with defensive and immunoregulatory properties, are normally present in the bloodstream in an inactive state and all act in concert, but each must be sequentially activated and in suitable conditions (a relatively small starting signal is sufficient), so that the typical mechanism of cascade reaction is triggered [633]. The primary source of such proteins is the liver, with smaller contributions from tissue macrophages, epithelial cells of the gastroenteric tract, and PBMCs. Like Igs, it is hypothesized that they arose late in evolution and are found only in vertebrates [36]. Complement is formed by >25 serum proteins interacting with nine functional components, designated C1–C9, reflecting the orderly sequence of their activation, with the exception of C4, which is activated after C1 and before C2. As regards nomenclature, a horizontal bar over a component denotes active enzyme activity of either a protein or a protein complex; proteins that become inactivated either by enzymatic cleavage or internal rearrangement are prefixed a small i (iC3); small postscripts a and b (C3a, C3b) indicate the biologically active fragments of a component; C1 subunits are designated q, r, s. The C1 complex consists of C1q, two molecules of C1r, and two molecules of C1s which bind to antibodies bound to an anti-

Table 1.60. Receptors binding complement components

Receptor(s)	Ligands	Major functional results of binding	Cell distribution
CR1 (CD35)	C3b, C4b, iC3b	Phagocytosis ↑ IC clearance, BC activation, antigen presentation, cofactor for cleavage of C3b or C4b	M, N, B, E, BC, CD4, ER, CD
CR3 (CD11b/18)	iC3b	Phagocytosis ↑ cell adhesion ↑	M, N, NK/K cells, CD
CR2 (CD21)	C3d, C3dg iC3b, C3b, EBV	Primary antibody response ↑ BC activation, also of BC memory, receptor for EBV infection	BC, CD, immature Epithelial cells
CR4 (CD11c/18)	C3dg, C3d	Phagocytosis mediated or not by FcR ↑	M, N, NK/K cells, CD
C4a/C3aR	C4α, C3α	Anaphylotoxin (see text)	M, B
C5aR	C5a, C5a des arg	Chemotaxis muscle and endothelial cells	MC, B, N, E, M, smooth
C1qR	C1q	Anaphylotoxin (see text) phagocytosis ↑ chemotaxis ↑	M, B, N, E, BC, endothelial cells, fibroblasts

Modified from [241].
R receptor, *M* mononucleates, *N* neutrophils, *B* basophils, *E* eosinophils, *BC* B cells, *ER* erythrocytes, *CD* follicular dendritic cells, *MC* mast cells, *IC* immune complexes, ↑ increase, *ND* not defined.

Table 1.61. Regulatory proteins of the complement system

Proteins	Target(s)	Biological functions
Soluble proteins		
C1 INH	C1r, C1s	Inhibits the serine proteases, binds to C1r, C1s inhibiting their participation in the classic pathway, binds to C1 inactive preventing its activation, inhibits kallikrein, plasmin and factors XIa and XIIa
C4bp	C4b	Increases decay of C3 classic convertase, cofactor of C4b cleavage mediated by factor I
Factor H	C3b	Up-regulates decay of C3 alternative convertase, cofactor of C3b cleavage mediated by factor I
Factor I	C4b, C3b	Cleaves and inactivates C4b, C3b using as cofactors C4bp, factor H, MCP
Properdin (P)		
Protein S or vitronectin	C5b-7	Binds to C5b-7 complex and prevents MAC insertion into cell membranes
SP40/40	C5b-9	Modulates MAC formation
Membrane proteins		
CR1 (CD35)	C3b, C4b, iC3b	Up-regulates decay of C3 classic and alternative convertase
DAF (CD55)	C4b2B, CebBb	Up-regulates decay of C3 classic and alternative convertase
HRF o C8bp	C8	Inhibits complement lysis
	C9	Blocks the binding of C9 to C8, preventing both MAC insertion into lipid membranes of autologous cells and complement lysis
MACIF (CD59)	C8	Blocks the binding of C7, C8 to C5b, C6, preventing MAC development and complement lysis
MCP (CD46)	C3b, C4b	Assembly and decay of C3b and C4b mediated by factor I

Modified from [260, 474].
C1 Inh C1 Inhibitor, *C4bp* protein binding C4, *C8bp* protein binding C8, *HRF* homologous restriction factor, *MAC* membrane attack complex, *MACIF* membrane attack complex inhibitory factor.

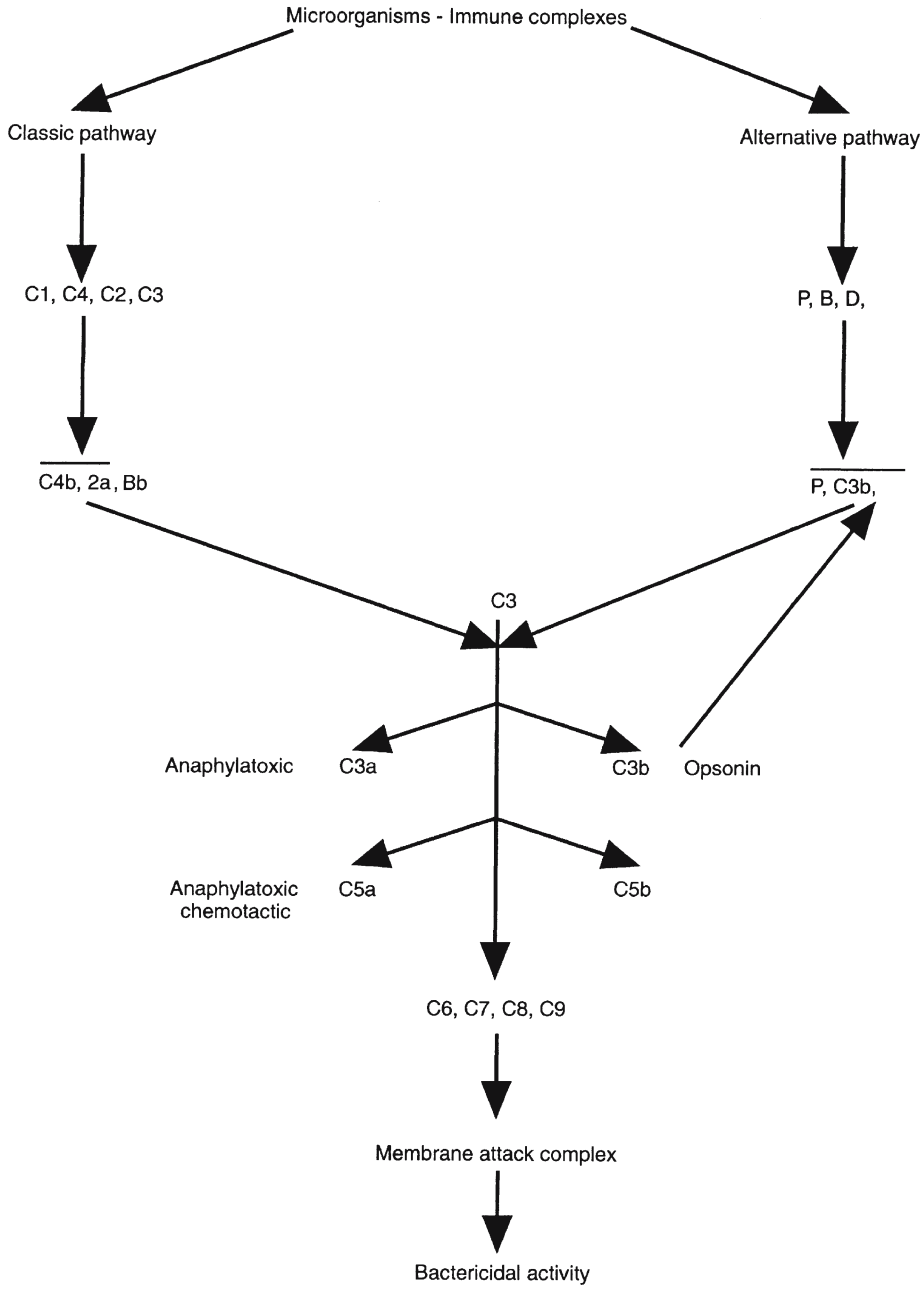


Fig. 1.63. A schematic representation of the complement system. *B* B fragment, *Bb* larger fragment of B, *D* D factor, *P* properdin. (Modified from [648])

gen on the surface of a bacterial cell [345]. Letters of the Latin alphabet, P, B, D (initials of properdin, factor B, factor D), designate the alternative pathway; factor B is divided into a small fragment (Ba) and a larger one (Bb) [648]. Proteins are activated by two pathways: the classic and alternative pathways (Fig. 1.63) [648].

The *classic pathway* is activated by antigen–antibody complexes, the alternative pathway by microbial-cell walls, and the lectin pathway by the interaction of microbial carbohydrates with MBP in the plasma [618]. This pathway is so called because it was described first, it is more effective, but to be activated requires the presence of acquired immunity. The initial seed is C1q, the first component, with a MW of 400 kD, which interacts with antigen–antibody complexes, or with IgM or IgG_{1-3}. To activate IgM, with a much higher MW, a single pentameric IgM is sufficient, with IgG_3, IgG_1 and IgG_2 following in an orderly fashion. IgA, IgD, IgE, and IgG_4 cannot bind to C1q; consequently no such antibody is able to activate this pathway. Complement interactions with natural antibodies are of crucial significance for the host, being inhibited complement-mediated autoimmune reactions [350]. More rarely, activation may be mounted by various substances, including bacterial LPS, CRP, certain viruses, etc. Activated C1q activates C1r, which, in turn, activates C1s, with MWs of 95 and 85 kD, respectively, to form a C1 complex. C1s, if activated, is able to act on its natural substrates, namely C4 and C2 [633].

C4 is a 180-kD gp synthesized by macrophages; activated C1s results in C4 cleavage into anaphylotoxin, C4a, and a larger fragment, C4b. C4b possesses several functions, especially that of binding both to molecules adjacent to the antigen–antibody complex that has initiated the cascade and the next component, C2.

C2 is a 115-kD gp; more than by C4b, it is activated by molecules next to C1s, but remains bound to a complex with C4b to form the C3/5 convertase (C4b2a), which in turn splits and activates both C3 and C5 [345].

C3 (1.2 g/l) has a central role in the complement cascade: it consists of two S-S-linked α and β chains. When C3 is activated by the convertase, two highly active biological forms produce a small peptide, C3a cleaved from the α chain, and a larger C3b fragment [159]. C3 splitting is due to C3/5 convertase, secreted through both pathways [648].

The *alternative pathway* was discovered more recently, but is phylogenetically the earlier pathway. It includes C3 and factors B and D. Activation results also from non-immunological mechanisms and yields physiologically active substances, thus achieving the complement bactericidal and opsonic effects in the absence of bound antibodies for initiation. The pathway is triggered by contact of complement proteins with LPS from cell walls of bacteria, virus, yeasts, parasites, a factor present in cobra venom, and most likely by aggregated IgA not activating the classic pathway [241].

The antibody-independent activation calls for an extreme instability of internal bonds of the native C3 molecule. Based on such potentialities, C3b binds factor B, thereby forming the C3bB complex, further activated by factor D, which cleaves factor B while bound to C3b to generate the enzymatic complex C3bBb. This complex acts as a C3 convertase and, similarly to the classic pathway, releases C3a and C3b from C3, allowing C3b to resume its properties, increasing C3 convertase and C3 activated levels. A closed circuit is established, where the alternative pathway acts as a positive feedback loop with active amplification; therefore more substrate is cleft, more C3b results. If this mechanism remains uncontrolled, it could rapidly consume the entire C3 and the subsequent components of the cascade. Consequently, this amplification is balanced by a rapid C3bBb complex dissociation. The P binding stabilizes this enzyme. C3b is also largely inactivated by factor H, which competes with factor B to bind to C3b, practically preventing C3bBb formation, and by C3b inactivator eliciting a further C3b degradation to C3c and C3dg fragments. Certainly, factor H and the proteins linked to its binding site contribute to the protection of healthy host cells, regulating C3 activity [689].

Notably, C3b is present in trace amounts in normal serum, probably because there are low concentrations of factors B and D. It is also postulated that LPS of Gram+ bacteria and other substances that trigger the alternative pathway amplification loop somehow protect the small C3b amounts from total inactivation, so that the above substances initiate the alternative pathway. There is evidence that thioesters are present in the native forms of complement proteins C3 and C4 and that their molecular conformational changes dramatically on activation [122]. C3b is a 77 amino acid residue polypeptide and following the above-mentioned changes exposes the thioester bond, which is very reactive, and interacts with amine ($-NH_2$) and hydroxyl (–OH) on proteins and carbohydrates, allowing C3b a rapid covalent link to other biomolecules, since the thioester half-life is 60 µs while that of native C3 is >200 h [470]. Therefore, thioester hydrolysis ensures that activated molecules do not diffuse away from the activation site to bystander cells of the host [122]. As is seen in Tables 1.60, 1.61, C3 is distributed to different receptors (CR1, CR3, CR2) through its ligands (C3b, iC3b, C3dg), thus being capable of interacting with different cell types and bringing about a large spectrum of biological functions (Table 1.59), considering that C3b also has opsonizing properties [18].

When the *common final pathway* is activated, C5–C9 assembly and activation constitute the lytic activity of complement on target cells. C5–C7 are globular proteins with a MW of 180, 130 and 120 kD, respectively. C3b acts as an acceptor site for C5, which is cleft to form two anaphylotoxins, a small fragment, C5a, and a larger, C5b, which binds C6 and C7 to form a C5b67 or C567 complex on the cell membrane, which in turn, through conformational changes, modulates C8 and C9 activation, two 160- and 80-kD proteins, respectively [633].

The final act is the polymerization of perforin-like C9, around the C5b678 complex, which links six C9 molecules involving the assembly of MAC (*membrane attack complex*) (Fig. 1.63) with a MW of $\approx 10^6$ D resembling perforins, since it is a molecule forming pores on membranes with walls constituted by C9; in this way there is Na influx and K outflow, with a consequent increase in membrane permeability and subsequent target cell lysis [648].

The finding that MBL (mannose-binding lectin) residues binding to mannose can initiate complement actiation was followed by the discovery of the MBL-associated serine protease (MASP) enzymes. MBL activates complement by interacting with two serine proteases 1 and 2 (MASP1 and MASP2). MBL binding to its microbial ligands activates MASP1 and MASP2. MASP2 cleaves and activates the complement components C2 and C4 and MASP1 may cleave C3 directly [338]. The cleavage products C2a and C4b then form a C3 convertase, which initiates the complement cascade by cleaving the C3 protein. The MBL complex and its proteases functions similarly to the C1 complex of the classic complement cascade [345]. These components of the complement system have been named the MBL pathway [633].

Activation of the whole cascade elicits the formation of several products with different biological activity: some adhere to external cells, altering their properties

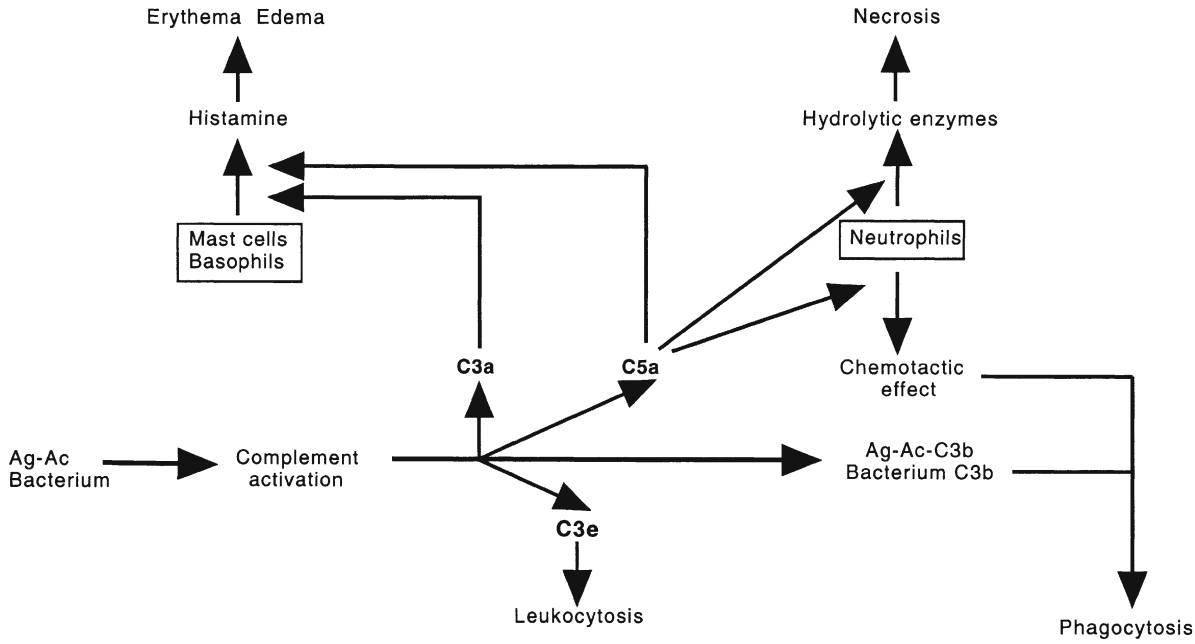


Fig. 1.64. Role of complement in inflammation. (Modified from [18])

and determining the lysis, for example, of infecting microorganisms; others provoke a local inflammatory reaction [260]. Complement thereby plays an essential role in each humoral defense system against external aggressions. A particular characteristic of this system is the lack of action specificity and intervention against harmful agents of varying nature, although antibody responses form a substantial way of activation; however, such aspecificity, if on the one hand it allows notable savings of selective defense systems, on the other hand it can foster an aggressive response against host components [159]. Table 1.59 and Fig.1.64 [18] document how complement actively participates in host defense and inflammation, and Tables 1.60 and 1.61 summarize both features and functions of complement receptors and regulatory proteins. Several cells are expressed, namely, known integrins including CR3 (CD11b/18) and CR4 (CD11c/18) [53], CR2 (CD21), able to activate B lymphocytes, and CR1 (CD35) distributed to human red cells. Some proteins act as regulators, just to control undesired activities: CR1, DAF (decay accelerating factor), CD55 and MCP (membrane cofactor protein), or CD46, prevent the formation of a full C3-splitting enzyme and related biological effects; CD59 or MACIF (membrane attack complex inhibitory factor) and C8 bp (protein binding C8) or HRF (homologous restriction factor) preclude a full MAC development [241]. During the activation C3a, C4a, C5 anaphylotoxins are up-regulated, genetically correlated, but also efficient in different ways (C5a≫C3a≫C4a), for which phagocytes, endothelial cells and smooth muscle cells express receptors, also mediating infectious germ contact with antibodies, complement and phagocytes. C5a, generated by activation of both complement pathways, is the most chemotactic for PMNs, also inducing their microbicidal activity [648]. Anaphylotoxins are active but with a negative role in inflammation, causing aspecific release of histamine and other mediators from metachromatic cells and their consequent degranulation, increasing vascular permeability. C5a is much more active in provoking bronchoconstriction and smooth muscle contraction, it binds to specific receptors on bronchial and alveolar epithelial cells, vascular smooth cells, endothelium, etc., delivering an unexpected up-regulation to various target cells. C5a also couples with a G protein transducing signals to the cell [637]. Additional active complement fragments deliver biological activity: macrophage distension by Bb fragment, Ba fragment is chemotactic, C2 kinin (C2 fragment) increases vascular permeability, and C3e (Table 1.59) increases circulating leukocyte titers mobilizing medullary reserves [18].

To confirm such collaboration of two levels of immunity, we mention that CD19, a component of acquired immunity, is associated with CD21 receptor of C3d and thus is part of innate immunity [571]. CD19 is necessary for normal antibody responses to antigens, being dependent on B–T interactions, and hence it amplifies signal transduction [571]: for this purpose a cross-linking with mIgs and a complement covalently linking carbohydrates to C3d fragment and PSAs are necessary [122]. Regarding infectious diseases, recent studies have demonstrated the substantial defense capabilities of C3 and C4. Fixation of PSAs, with C3d support, to FDCs and B cells of the marginal zone equipped with CD21 on their surface shows that an important anti-infective function is played by the spleen [423], scarce in babies

aged <2 years and absent in C3-deficient individuals [633]. C3d deposition on external membranes of noxious cells is able to perforate such cells, followed by signaling to immune cells and increasing immune stimulation; therefore a molecular adjuvant of innate immunity may select antigens for recognition by acquired immunity [116]. These results should be complemented, in this context, with pathogenetic implications, such as HIV part opsonization with C3, which widens virus diffusion, and usage of CD55 as a receptor for *Escherichia coli*, CD35 and CD21 (CR1 and 2) from virus and mycobacteria, CD46 from measles virus and M protein CD46 and/or H factor from *Staphylococcal pyogenes* for adhesion to keratinocytes [415].

Another machinery put forward by bacteria is a mimicry to hold the products of complement activation off the cell walls to resist their attack. Further capsules containing sialic acid are very poor activators of the alternative pathway in the absence of antibody, in addition to representing an effective defense barrier [159]. *Endotoxin* (an LPS) and its receptor CD14 [537] is a cell-wall component of the outer membrane of Gram– bacteria, which can thus be protected, either because they activate complement at a safe distance from their outer walls, disposing of long lateral polysaccharide chains, or because they are released directly from bacterial walls in a soluble form [97]. Early in life, endotoxin *promotes the development of Th1 CD4⁺ T cells* at the expense of proallergic Th2 $CD4^+$ T cells. Evidence suggests that the lack of endotoxin in the environment might lead to a higher incidence of asthma [537]. A further protective technique employed by bacteria is to release enzymes activating complement proteins adherent to their walls, or borrowing molecules regulatory of their function from host cells, namely, CD55 and CD59, utilized for protection from lysis, as is the case of HIV isolated from cells of patients with current AIDS [484]. There is also a chance that bacteria, virus and parasites are able to inhibit complement activation and elaborate factors with immunoregulatory properties or with a wide spectrum of noxious actions (Table 1.29), proteins with IL-like activity and molecules activating metachromatic cells [168]. Such data could justify a more focused vaccine preparation, thus suggesting that in the future strengthening the antimicrobial therapy inhibiting complement activation may be successful [415]. *Genetic defects of complement components* evaluated on ≈550 patients are marked by recurring infections that are mostly systemic, autoimmune diseases and vasculitis, in addition to pyogenic infections caused by C3 deficiency [632]. *Early in life* severe infections may be complicated with glomerulonephritis [633]. These defects represent 2% of PIDs [544]; the deficiency of the C1-INH control protein is discussed in Chap. 8.

The *liver* increases APP production during the acute phase. These proteins are host protectors since they eliminate reactive O_2 radicals, control serine proteases, activate complement [271], enhance resistance to infections, promote the repair of damaged tissue, and thus participate in the humoral machinery that makes up a second defensive strategy. AAP is a heterogeneous group of proteins such as CRP, α_2M, α_1-antitrypsin, serum amyloid P component (SAP), fibrinogen, ceruloplasmin, C3, etc. (Table 1.62) [148], several depending on NF-κB for an efficient transcription [270]. Inducible by macrophages activated together with platelets, they quickly mobilize as soon as a danger to tissue integrity is announced, including wounds, trauma, and microorganisms, to limit local damage and rapidly enhance host homeostasis, favoring both resolution and repair of damaged tissues [538]. During this process, IL_8 and MCP are synthesized by endothelium, two chemoattractants for neutrophils and monocytes, respectively, while TXA_2 and diverse PGs act on the vascular tone and mediate vasoconstriction and vasodilation, and LTB_4 has the task of attracting phagocytes. APPs are divided into two groups depending on their induction by human hepatocytes [31]:

- By IL_1, IL_6 or TNF, serum amyloid A protein (SAA), α_1 acid gp, C3 and CRP
- By IL_6 fibrinogen, α1-antichymotrypsin, haptoglobin, ceruloplasmin, hemopexin

2d. *Pentraxin* levels substantially increase in response to tissue injury or inflammation (Fig. 1.65) [31].

As is seen in Table 1.5, IL_1 especially regulates CRP, while IL_6 acts on the liver to increase APP synthesis, resulting in a reduction in albuminemia. CRP has the ability to bind to several microorganisms, containing phosphorylcholine in the membrane, the complex having the property of activating complement by the classic pathway. This enhances complement-dependent bacteriolysis, phagocytosis and production of biologically active peptides [538]. ILs raise body temperature (endogenous pyrogens) through PGE_2, also inducing EGF production, and through angiogenic activity. IL_1 and IL_6 in turn act on the adrenocortical axis with resulting ACTH production and thereby cortisol production. Negative repercussions on the acute phase are mediated by IL_4 and IL_{10}, which block the reaction within 24–48 h [31], and by SAA and SAP, which, being IL-induced, allow the adoption of strategies to reduce their levels during chronic inflammations [538].

Structurally and functionally correlated to C1q protein of the classic pathway are the *collectins* [140, 211] (Table 1.62), belonging to the lectin family, and another group of molecules active in first-line defense [140, 490]. Collectins are so named because they consist of a collagenous domain linked to the calcium-dependent lectin domain [345]. According to a fascinating hypothesis, they bind to a wide spectrum of microbes, to interact in perspective with cells or with complement as an active part in the antimicrobial defense [211, 490]. Among them we note the MBL, a plasma protein with many attributes including a crucial role in the above defense, being a member of the Ca-dependent lectin

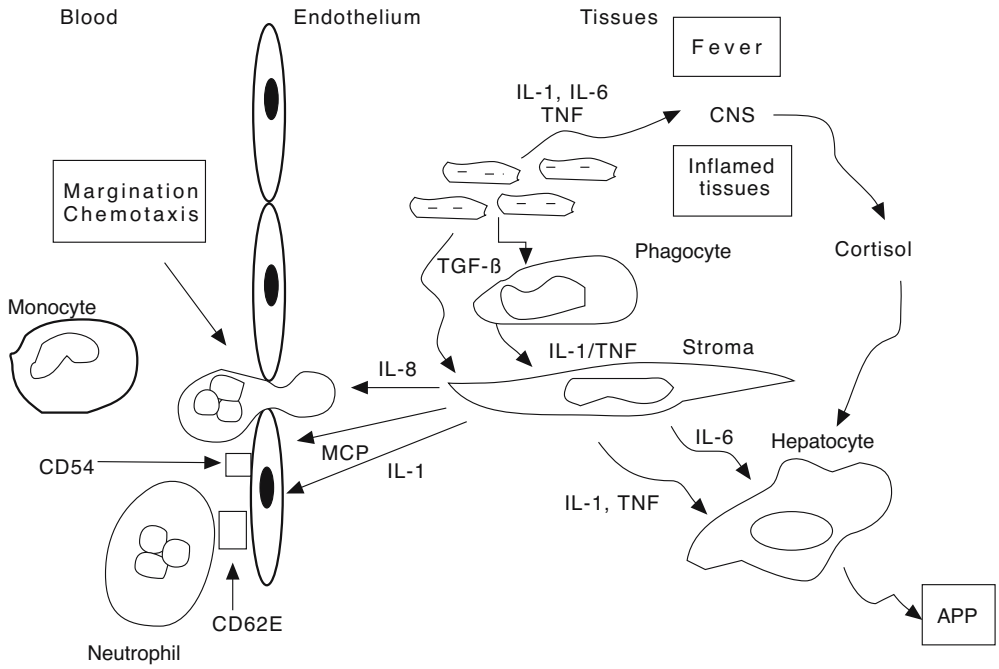


Fig. 1.65. Interactions between cells and ILs in the acute-phase response. Platelets and phagocytes release in the site of tissue lesion the first ILs, activating the adjacent stroma and endothelium, which in turn releases additional ILs. The hepatic response is activated following production of ACTH and cortisol. *MCP* monocyte chemotactic protein. For other abbreviations, see the list. (Modified from [31])

Table 1.62. Main recognition molecules of the innate immunity system

Molecules	Structure	Localization	Ligands	Function
Humoral receptors				
CRP	Pentraxin; Ca^{++}-dependent lectin	Liver synthesis; APR; increases (1 µg/ml to >1 mg/ml) in plasma	Microbial PSs, phosphatidyl-choline	Activates complement and opsonization, enhances phagocytosis
SAP	Pentraxin; Ca^{++}-dependent lectin	Liver synthesis; NL=30 µg/ml	ECM protein; microbial cell wall CHO	Enhances phagocytosis and opsonization, stabilizes ECM proteins
MBL	Collectin; has 18 CRD sites/ molecule on helical collage-nous domains	Liver synthesis; NL up to 10 µg/ml, varies with allelic variants	Microbial cell wall saccharides	Binds C1q collectin receptor; activates complement; enhances phagocytosis; modulates CD14-induced cytokine synthesis
LBP	Lipid transferase	Liver synthesis; NL=<5 µg/ml; increase to 50 µg/ml with APR to serum LPs	Transfers LPSs to CD14 and from CD14 LPSs; has bactericidal power	Enhances sensitivity to LPSs; system for inactivating LPSs; has bactericidal power
sCD14	Leucine-rich protein	Plasma protein; NL=3 µg/ml; origin perhaps from myelo-monocytic cells (unknown)	LPS; several microbial cell wall components	Enhances sensitivity to LPS 100- to 10,000-fold, complex with LPS binds to receptors on endothelium, PMNs and macrophages
C3	S_2-linked dimer	Liver synthesis; NL=1 µg/ml; induced by APR	Forms ester linkage to OH-groups on CHOs and proteins	Attachment of ligand for receptors such as CD21 and CD35

Table 1.62. (Continued)

Molecules	Structure	Localization	Ligands	Function
Cellular receptors				
Mannose receptors				
Macrophage MR	8 CRDs	Tissue macrophages; endothelial hepatic cells	Multiple CHO	Potentially targets antigens bound to class II molecules
DEC 205	10 CRDs; mannose-type receptor	Dendritic cells; thymic epithelium	Multiple CHO	Potentially targets antigens bound to class II molecules
Scavenger receptors				
Type I	Type II trimeric transmembrane protein with endothelial helical collagenous and terminal SRCR domains	Tissue macrophages; hepatic cells	Bacterial and yeast cell walls	Clearance of LPS and microbes; adhesion
Type II	Alternatively form missing terminal SRCR domain			
MARCO	Extended form similar to type I	Marginal zone of spleen; medullary lymph nodes; macrophages	Bacterial cell wall	Bacterial clearance
LPSR CD14	Leucine-rich protein; lipid-anchored glycoprotein	Monocyte-macrophages; PMNs	LPS; several microbial cell wall components	LPS sensitivity; microbial clearance; pro-inflammatory cytokine induction
Complement receptors				
CD35 (CR1)	30 SCR	Monocyte-macrophages; PMNs; lymphocytes	C3b, C4b	Enhances cleavage of C3b and C4b
CD21 (CR2)	15 SCR	B lymphocytes; FDC	iC3b, C3dg, C3d	Increases B-cell activation by antigens
CD11b, CD18 (CR3)	Integrin	Monocyte-macrophages; PMNs; NK cells	iC3b, fibrinogen, LPS	Adhesion; LPS clearance

Modified from [148].
APR acute phase response, *CHO* carbohydrates, *CRD* Ca^{++}-dependent carbohydrate recognition domain, *CRP* C-reactive protein, *ECM* extracellular matrix, *LPS* lipopolysaccharide, *LBP* lipopolysaccharide binding protein, *MBL* mannose binding lectin, *MR* mannose receptor, *NL* normal levels, *PSs* polysaccharides, *SAP* serum amyloid protein, *SCR* short consensus repeat, *SRCR* scavenger receptor cysteine-rich domain.

family that binds to microbial carbohydrates to initiate the lectin pathway of complement activation [345]. MBL is formed by a head region that binds to mannose residues and a tail with a triple helix mimicking C1q and thus activates several complement fractions, eventually killing and opsonizing the invader [490]. In particular, MBL appears to be a potent mediator of innate immunity in infants aged 6–18 months, in whom passively transferred maternal antibodies have disappeared and the immune system has not yet matured [575]. MBL levels are comparable to adult levels at 3 months of life, with a wide range (from 10 ng/ml to 10 mg/ml) that increases during APR [575]. Chapter 22 reports a susceptibility to infections caused by MBL defect; however, this phenomenon is diversely interpreted related to large interindividual variation in MBL levels [313]. Additional collectins have sparked interest, such as bovine conglutinin, another opsonin inducing Gram– bacteria eradication by coating them with C3b, which can activate opsonization [648]. The family has further expanded to include SP-A and -D (surfactant protein A and D), able to stimulate phagocytosis and agglutination of Gram–

Table 1.63. Phagocytic cells

Circulating phagocytes	
Granulocytes	Neutrophils (polymorphonuclear)
	Eosinophils
	Basophils
Mononucleates	Monocytes
Mononuclear phagocytes	
Macrophages	Tissue macrophages
	Alveolar macrophages
	Serosal macrophages
	Spleen and lymph node sinus macrophages
	Mesangial macrophages
Kupffer's cells	
Astroglial cells	

Modified from [352].

bacteria and virus, indicating a role in the removal of particulate material from the airways [211, 490], bovine collectin-43 and two novel proteases associated with MBL (MASP) [140].

3. Phagocytes fulfill basilar functions of nonspecific defense: one of their main assignments is to present selected antigens to T and B lymphocytes. The cells are divided into circulating, including granulocytes, macrophages and eosinophils, and nondividing, the macrophages (Fig.1.32 c, d, e) distributed throughout body tissues and assuming specialized structures and functions related to the different locations where they operate such as Kupffer's cells and splenic macrophages in the red pulp (clearance of corpuscular elements and soluble factors), LCs and splenic macrophages in the white pulp and lymph nodes (processing and presentation of the antigen to T cells), and finally bone marrow macrophages (growth and differentiation of hemopoietic cells) (Table 1.63) [352]. IFN-α sensitizes macrophages to microbial recognition by up-regulating the TLR3, TLR4 expression also enhancing the MyD88 (myeloid differentiation protein gene), TIR (Toll/IL_1R), TIR domain-containing adaptor inducing IFN-β (TRIF), IKKη, RIP-1, IL_{28} and IL_{29} genes expression [529]. Mast cells are provided with phagocytic activity [348] and therefore able to orchestrate, for host protection, inflammatory responses against invading microbes, in addition to tissue damage by antibody-independent activation [168]. Mast cells are to-day seen as a sentinel in host defense against bacterial infections, and evidence for their involvement in early responses to viral and fungal pathogens is growing. Mast cells are activated during innate immune responses by multiple mechanisms, including well-established responses to complement components. Changes in vascular permeability induced by *early release of preformed mediators* and lipid mediators enhance the availability of complement components and some initial inflammatory cells to the site of infection [332, 347]. Mast cell proteases have also been demonstrated to play critical roles in vivo in the recruitment of both neutrophils and eosinophils to sites of inflammation [502] and an *immune complex hypersensitivity reaction in the synovium* [347].

Circulating and nondividing cells, *PMNs* (Fig. 1.32) have CR1, CR3 receptors, for IgG Fc fragments and several others (Table 1.64) [18]. PMNs recruited to the site of injury ingest the intruders, bacteria, foreign materials or damaged tissues: IgGs and C3b are dedicated to coating infecting particles, promoting both opsonization and subsequent phagocytosis. Prominent is the role played by monocytes, numerically inferior to PMNs, but with the advantage of not being terminal cells and changing into tissue macrophages, two to three times larger than PMNs, readily recruited during local inflammatory or degenerative processes [345]. When the inflammatory process initiates, macrophages instigate a bit of a delayed maneuver, whereas PMNs, more mobile, are the first to accrue, perhaps facilitated by IL_5 augmenting IgA production (Table 1.5), but above all by IAPs (integrin associated proteins, CD47), which activate PMNs, even in extravascular sites [302]. Such cells are also termed *professional phagocytes,* because they are provided with membrane receptors for IgG_1 and IgG_3 Fc fragments and C3b fragment. Nonspecific inflammatory reactions are the first components of innate immunity to appear in the phylogenesis, and it seems likely that such primitive responses have influenced the immune responses' evolution. In this sense, Th response polarization could have been an evolutive consequence of nonspecific responses of a divergent sign directed to counteract different types of infection [470]. *Macrophages* and NK cells exemplify the primitive limb of IFN-γ-dependent immune responses required for defense against infection with virus and other intracellular invaders, whereas metachromatic cells could have evolved to combat extracellular parasites [470]. Macrophages, perhaps the phylogenetically more ancient immune cells, can secrete IFN-γ due to their IL_{18} synthesis [669]. Stimulated, moreover, by bacterial and viral contact and primed by CD14 a receptor that functions with TLR4 in LPS responses [194] and γδ T cells [111], in the first stages, macrophages release TNF-α and IL_{12}, which synergize with T-cell generated IL_2 and macrophage-generated IL_{15} [68], and both prime *NK cells* [583] *to express IFN-γ* [146]. IFN-γ and IL_{18} form two potent stimuli to suppress IgE responses [421, 669], enhancing macrophages' microbicidal power. This power is necessary for macrophages to combat invasions by endocellular facultative microorganisms, particularly resistant to the phagocyte microbicidal action despite Igs opsonization. Microorganisms duplicate within the macrophages; however, the trapped intruders are not killed:

Table 1.64. Principal ligands of phagocyte membrane receptors

Opsonins
Fc IgG (diverse), IgA, IgE
iC3b
C3d
C1q
Coagulation factors
Fibrinogen
Thrombospondin
Thrombin
Factors VII and VIIa
Urokinase
Molecules of cellular matrix
Fibronectin
Laminin
Sulfate polysaccharides
Hyaluronic acid
Adhesion and recognition molecules
Sheep erythrocytes
Erythroblasts
CD54
CD62E
CD11a/CD18
Endocytosis and transport additional molecules
Fucosylated glycoconjugate
Galactosylated particles
β-Glucan
Native and modified LDL lipoproteins
Modified aldehydes
Transferrin
Lactoferrin
Ceruloplasmin
α_2-Macroglobulin
Hemopexin
Glycoprotein mannose-6-phosphate

Growth factors, ILs, polypeptides
CSF-1
GM-CSF
IL_1
IL_2
IL_3
IL_4
TNF
IFN-γ
IFN-α, IFN-β
Insulin
Glucagon
Somatomedin
Vasopressin
Parathormone
TGF-β
C5a
Peptides and mediators of inflammation
f-Met-Leu-Phe
Angiotensin
Bradykinin
VIP
Tuftsin
LTB_4
Neurotensin
Drug substances
β_2-Adrenergics
Nicotinics
Cholinergics
Histamine
Serotonin
Benzodiazepine
Phorbol esters
Adenosine

Modified from [18].

their intracellular growth can be blocked only by an IL intervention [251].

During the direct antibacterial activity supplemented by macrophages, NK cells and, while the response progresses, Th1-like ILs prime CSMs and HLA class II molecule expression on macrophages, thus preferentially triggering Th1 T cell expansion in concert with IL_{12}, whereas Th2 T cell response is limited by IFN-γ counterregulation [251]. However, during a chronic infection intracellular bacteria may prime HLA class II molecule expression on macrophages when CSMs are absent, thus provoking Th1 T cell anergy [251]. Th2 T cells dominate only because counterbalancing counterparts are missing and consequently play a large part in the Th1 T cell reduction in numbers [251]. Therefore NK cells and macrophages cooperate to eliminate microbial infections [25]; on the contrary, Th2-like ILs sanction their progression [365]. However, it is possible that endogenous IFN-α/β reduce IL_{12} expression, also stimulating CD8 T cells, as if NK-cell and IFN-γ protective activity should be hampered to avoid unwelcome effects for the host [43].

To adhere to microbes, *neutrophils* engage CD11b/CD18, potentially playing a vital role in cell activation and triggering the oxidative and nonoxidative apparatus in an attempt to destroy the invader (or the host tissues), while adhesion mechanisms provide an environment where killing can progress protected by tissue fluids [154]. From this viewpoint, the degranulation has three important roles in host defense with release of granules (Table 1.23):

- Azurophil is able to potentiate the digestive and microbicidal activities of phagocytes
- Secondary or specific granules enable the cells to regulate inflammation
- Tertiary, associated with translocation of receptors for CR1, CR3, FMLP (formylmethionyl leucylphenylalanin), laminin, NADPH oxidase and cyto-

chrome c and subsequent interaction with these receptors, helping neutrophils in their phagocytic activity [315, 663].

The process of phagocytosis schematically includes six different stages:

1. Adhesion to foreign particles
2. Chemotaxis leading to contact with particles
3. Opsonization mediated by IgGs interacting with peptides and enhancing their digestion
4. Ingestion
5. Metabolic activation
6. Lysis

1. Adhesion. Adhesion is mediated by cytophilic IgGs present on the surface of particles to be phagocytosed, and by their Fc receptors on phagocyte membranes. Part of the cells remain in the bone marrow as part of a large reserve pool (*fixed cells*). Two PMN pools are included in the circulation, the circulating PMN pool and marginating pool along the postcapillary of vascular endothelium. As soon as the first signs of injury or infection are manifested, fixed and circulating PMNs are mobilized and increase their adhesion to the epithelial lining of local blood vessels, supported by endothelial cells and mediated by IL_1 and other mediators of inflammation. Even if clear-cut mechanisms are not fully understood, it is likely that PMN adhesion to cells is mediated by surface glycoproteins such as iC3b, β integrins and interactions between CD11b/CD18 and iC3b for complement-coated bacteria or endothelial cells [663], which in turn synthesize CD62E. In addition, PMNs possess receptors for ECM components, laminin and FN, facilitating their adhesion to host tissues or bacteria walls [311].

2. Chemotaxis. Chemotaxis is the start of directional migration of organisms or other cells provided with motility, able to travel up to a concentration gradient of *chemotactic factors* or *chemotaxins* (chemo + "ταξις," distribution). Cell response is directed to a large extent by signals released from chemotactic factors, or cell locomotion is piloted toward sites where signals are more expanded. Differently from tissue eosinophils, present at the level of epithelial surfaces and in contact with the environment, fixed and circulating PMNs are absent from tissues until they are recruited from the bloodstream as a result of tissue damage. To meet this demand, a variety of chemotactic factors can potentially regulate PMN recruitment and migration to the site of inflammation: these stimuli yield chemotaxin production and, in ever greater concentrations, attract circulating PMNs to capillaries around the site of injury, where they actively insinuate themselves between endothelial cells. Studies have verified that if PMN migration is delayed as little as 2 h, their capacity to localize the inflammatory process is severely jeopardized [311]. Among the more known chemotactic factors, we enlist FMLP, LTB_4 and C5a, which certainly play the most important role

Table 1.65. Chemotaxic defects

Chemotaxic defects due to insufficient production of chemoattractant substances
Complement components
C1r C2 C3 C5
Agammaglobulinemia
Coagulation/fibrinolytic pathway
Prekallikrein Hageman factor
Cellular
Mucocutaneous chronic candidosis Wiskott-Aldrich syndrome
Chemotactic defects due to defects of cellular functions
Primary immunodeficiencies
Chédiak-Higashi syndrome Chronic granulomatous disease (CGD) Hyper-IgE syndrome Leukocyte adhesion disorder (LAD) Shwachman syndrome Specific granule deficiency
Additional affections or physiological states
α-Mannosidase deficit Bone marrow graft Congenital ichthyosis Diabetes mellitus Drugs Hypophosphatemia Neonatal neutrophils Neutrophil actin dysfunction Tuftsin deficit

Modified from [663].

[345]. Others derive from cell debris, the bloodstream (C567, fibrin or collagen fragments), lipids (LTB_4, PAF), peptides containing *N*-formyl-methionine (from bacterial or mitochondrial protein breakdown), including N-FMLP with specific receptors on PMN membrane, and ILs such as GM-CSF and IL_5 [663]. Apart from bacteria, macrophages, lymphocytes, platelets, and mast cells can also attract PMNs; certain chemotaxins such as C5a, LTB_4 and PAF are specific for varying types of leukocytes and some *chemokines* for PMNs (Table 1.57). PMNs with nanomolar amounts of chemotactic factors respond by increasing both adhesion and the number of receptors specific for such factors. Binding of chemoattractants and activation of G proteins and their effector enzymes result in the cell orientation toward the higher concentration of the stimulus. Cell shape changes from a round to a triangular structure with the cell front at the triangle base. Accordingly,

rugosities are formed on the cell surface and following AA metabolism activation, membrane polarity is altered because rugosities are rapidly concentrated on a pole: here contraction waves begin and the cells utilize microactin filaments, which provide the contractile forces required for cell movements. Microtubules composed of tubulin allow the locomotion, and actin alternation of polymerization and depolymerization may be a critical regulator of microfilament contractility [311]. The HIgES shows the close correlation between efficient chemotaxis and a normal response to infection, as well as the possible influence of histamine and additional amines on PMN mobility. Table 1.65 [663] outlines the defects of chemotaxis depending on their origin: chemoattractants or cell substance deficiency (the phagocyte defects may have an 18% prevalence within PIDs) [544].

3. Opsonization. Bacteria expressing capsular polysaccharides pose a unique problem to phagocytosis inhibited by the charge and hydrophilia typical of such bacteria, especially the polysaccharide capsule of pneumococci, a virulence factor expressing a strong negative chemotactic activity. *Opsonization* (from the Greek "οφωνειν," acquire foods and therefore prepare for eating) is a not immunological contact between cells and bacteria, mediated by the *opsonin* family comprising Igs, CR3, CR4, and FN. Igs contribute chiefly via IgM, IgG_1 and IgG_3 with CD23 or CD64, interacting with epitopes on bacteria via its Fab portions, while C3b and iC3b – induced by both classic and alternative pathways – adhere to the bacterial (or other intruder) surface to approach neutrophils. Antibody fixation also allows activation of the complement classic pathway so that the Fc portion, Clq and C3 act synergically to facilitate the capture of bacteria from phagocytes. Complement proteins exercise an important conclusion of Ig-mediated opsonization, because CR and FcR concurrent involvement often fulfills a marked synergic action in activating phagocytes and stimulating their functions. In this context, addition of complement to Ig-opsonized bacteria promotes phagocytosis, in parallel reducing the Ig number necessary for an efficient ingestion of bacteria [345]. An additional, potent opsonizing stimulus is supplied by *tuftsin,* a tetrapeptide (Thr-Lys-Pro-Arg) produced by the spleen. Its action appears to be indispensable, as demonstrated in patients with familial deficiency of this peptide and in splenectomized patients, who suffer from severe infections occurring with *Candida, S. aureus,* and *Staphylococcal pneumoniae* [311]. Macrophages, IgA and IgE receptors also play a striking role in opsonization. Lectins have been identified on different cell membranes, mainly macrophages, as receptors of mannose-fucose, galactose binding laminin, sialic acid or sialoadhesine, and β-glucan with specificity for CD11b/CD18. However, there are bacteria blocking opsonization or deviating C3b covalent binding and MAC adhesion to a position on the cell surface distant from the cell membrane where no defense mechanism can be exploited, or resisting opsonization by expressing capsular polysaccharides, as mentioned above: C3b causes their breakdown by binding Factor H instead of Factor B [159].

4. Ingestion. The concerted action of two receptors multiplies hundreds of times the ingestion of phagocytosed particles previously trapped within vacuoles. The cell membrane binds and invaginates engulfed particles adhering to its surface, thus forming a *phagosome,* subsequently approximating cytoplasm and fusing with lysosomes to create the structure called *phagolysosome,* with reduced pH. The final transformation therefore takes place in phagolysosomes, membrane-bound intracellular vesicles containing a rich supply of proteolytic enzymes operating degradation, thus completing the reduction to constituents of amino acid fragments: a portion of antigen fragments thus produced is expelled and transported to the cell surface where it is expressed, but the major portion is stored internally in endosome [540].

5. Metabolic Activation. Phagocyte exposure to varying stimuli increases cell respiration and, consequently, O_2 generates reactive metabolites within the phagosome to clear target cells from the body, that is, $O_2^{\cdot -}$ then reduced to H_2O_2, as described below. Such O_2 products are highly toxic since they oxidize cell components of parasites, tumor cells and other tissues [159].

6. Lysis. After a preventive opsonization with formation of pseudopods, by virtue of FcεRII and CR3, the cells can bind more tightly to antigens, facilitating antigen uptake and internalization (endocytosis). Following lysis carried on by the C5b-9 terminal complex, the final disruption of imprisoned bacteria ensues, also mediated by MPO catalyzing H_2O_2 transformation into HOCl with bactericidal activity. Phagocytosis is the end point, therefore any interruption of the process compromises host defenses [540].

Microbicidal Activity

O_2-Dependent Mechanisms

The anti-microbial PMN activity depends on O_2-dependent or O_2-independent microbicidal mechanisms. A paradigmatic example of the lacking activation of O_2-dependent microbicidal mechanisms is *chronic granulomatous disease* (CGD) resulting from a genetically determined enzyme deficiency of leukocyte intracellular bactericidal function, thus being unable to kill ingested bacteria or fungi, particularly catalase-producing bacteria. CGD especially results from genetic defects in the various components of NADPH-oxidase, a potent enzyme that is central in the respiratory burst. This process reduces O_2 to superoxide and then to H_2O_2, thus leading to NADP formation. Activation of glucose oxidation during phagocytosis starts trouble for each

invader. A roughly tenfold increase in the activity of the hexose monophosphate shunt is provided by an increase in the NADP/NADPH ratio, determined in turn by activity of the same oxidase and a chain of redox reactions involving glutathione. This enzyme system has the goal of transforming into H_2O and O_2 the H_2O_2 produced in excess and potentially toxic for cells. Catalase-positive bacteria cannot be killed by the phagocyte MPO/halide system, since they synthesize catalase destroying any excess H_2O_2 they produce, whereas catalase-negative bacteria are killed, since they fail to stop H_2O_2 production and trigger the MPO/halide system: for this reason in CGD patients they play the role of scavenger bacteria. Two oxidoreductive groups involved in the electron transport chain, namely flavine and cytochrome b558, are defective in the X-linked from (≈ 60% of cases), but not in autosomal recessive CGD (≈ 30%). *Glucose-6-phosphate-dehydrogenase deficiency* (G6PD), inherited as an X-linked disorder, also leads to impaired intracellular killing via the reduced glucose oxidation. *MPO deficiency,* inherited with an autosomal recessive pattern, is associated with susceptibility to systemic candidiasis, since intracellular killing is reduced and/or delayed (also greatly) as a result of impaired halide system function. MPO deficiency has a greater incidence than in the past, frequently as a casual laboratory finding in healthy subjects. The underlying basis of such defects is that the oxidative metabolism requires a notable O_2 consumption: $O_2^{\bullet -}$ is generated, reacts with two NADPH molecules and acquires an electron; the reaction takes a few seconds and is followed by bacteria adhesion and phagocytosis via PMNs. The speed highlighting this reaction shows that the oxidase could be localized on PMN membrane [663].

Therefore, an unremitting influx of O_2 radicals is also achieved around and beyond the plasma membrane of phagocytosed intruders, thus intensifying the toxic effects at their expense. One product of this metabolic process, H_2O_2, carries on microbicidal activities, and because of its oxidant properties reacts with MPO stored in azurophil granules and released into phagosomes during PMN degranulation. This highly oxidant enzyme reacts in turn with halide (predominantly iodine and chloride) ions present in phagocytic vacuoles, catalyzing the halogenate reaction (iodination or chloruration), which is as toxic for microbial wall proteins. It should be noted that PNMs undergo apoptosis following an excessive *necrotizing activity* [630] (Fig. 1.34). During oxidoreductive metabolism, additional O_2 radicals are generated (normally imprisoned within phagolysosomes), including $^1O_2$ and OH radicals. *$O_2^{\bullet -}$ toxicity* for bacteria is demonstrated by the high concentrations of superoxide dismutase (SOD) (converting $O_2^{\bullet -}$ into O_2 and H_2O_2), possessed by several O_2-resistant aerobic microorganisms, whereas O_2-sensitive anaerobic organisms are devoid of SOD. Human beings are provided with low SOD levels localized in the cytosol, whose activity does not interfere with $O_2^{\bullet -}$ microbicidal activity within phagosomes, thus protecting cytoplasm from highly diffusible toxic radicals [663].

The following reaction is produced:

1) $2\ \mathrm{NADPH} \xrightarrow[\mathrm{SOD}]{\text{NADPH oxidase}} H_2O_2 + 2\ \mathrm{NADP};$

2) $2\ O_2^{\bullet -} + 2\ H_2 \rightarrow O_2 + H_2O_2;$

3) $O_2^{\bullet -} + 2\ H_2\ O_2 \rightarrow {}^1O_2 + \mathrm{OH} + \mathrm{OH}^-$

$^1O_2$ is an O_2 molecule produced by neutrophils during phagocytosis; it is highly reactive and unstable and emits luminosity (chemiluminescence) as it returns to the ground state. It is formed by spontaneous $O_2^{\bullet -}$ dismutation, or by MPO, hypochlorite and H_2O_2 reactions. This microbicidal action could depend on the ability of splitting double C atom bonds of bacterial membranes. The role played by *hydroxyl radicals* in microbicidal actions is demonstrated by their inhibition of bactericidal killing by means of hydroxyl scavengers, benzoate, ethanol and mannitol [420].

O_2-Independent Mechanisms

In addition to producing *toxic O_2 compounds,* phagocyte cells express bactericidal mechanisms, assuring a relevant defense function, eminently via bactericidal proteins of azurophil granules (Table 1.23), especially considering Chédiak-Higashi syndrome. These proteins bind lipid A and the core oligosaccharides of LPS, thus altering bacterial cell wall permeability and amino acid uptake. Such mechanisms can be particularly important in patients with CGD or MPO deficiency. For example, NO is credited with a powerful antimicrobial action. In a potential biodefense strategy *cathelicidins and defensins* are major families of antimicrobial peptides in mammals with a broad spectrum of antimicrobial activity [169]. Both peptides disrupt the integrity of the microbial membrane, which is mediated by their cationic and amphipathic properties, which enable them to bind to negatively charged microbes and insert into their membranes [342]. An emphasis was given to azurophil granules, delivering *defensins,* which cleave an array of substances, including peptidoglycans of Gram+ bacteria, cationic proteins acting at an alkaline pH, BPI fixing LPS of Gram+ bacteria perforating their membrane, proteins binding the B_{12} vitamin, lysozyme, lactoferrin, an iron-binding cationic protein that blocks the activity of Fe-dependent bacterial enzymes, neutral hydrolases (proteases, nucleases) acting at an acid pH within phagosomes, neutral proteases, etc.

Defensins (α and β) are activated by MMP-7 [118]. To date, 6 α-defensins have been identified; 4 of them are known as α-defensins 1, 2, 3, and 4, the other 2 α-defensins are known as human defensins 5 and 6. There are 14 β-defensins, however, in human beings, 4 types of β-defensins have been identified as yet, plus the ϑ defensins [169]. In keratinocytes IL_{22} activated STAT3 and directly and transcriptionally increased the expression of β-defensin 2 and β-defensin 3 [650]. Defensins form 5%–10% of neutrophil total protein content, have antibacterial or

cytotoxic properties, and are more effective at an alkaline pH, therefore before phagolysosome acidification. These disinfectants against a wide spectrum of Gram+ and Gram– bacteria and *fungi* apparently insert themselves into microbial membranes and inhibit their growth, thus activating host cell processes involved in immune defense and repair [169]. By using chemokine receptors on DCs and T cells, defensins are believed to contribute to the regulation of host adaptive immunity against microbial and viral (HIV) invasion: their killing is consequent to disruption of microbial membrane [660]. Human defensins stimulate IL_1, IL_8, TNF-α release and decrease IL_{10} release [489].

Thus, like the cathelicidins, defensins can participate in immune defense in at least 2 ways, both killing bacteria and influencing the cellular innate and adaptive immune response [169].

Cathelicidins are a class of small cationic peptides that are an active component of mammalian innate immunity and are expressed at high levels in neutrophils, in skin and in other epithelial cells and can act as natural antibiotics by directly killing a wide range of microorganisms [118, 676]. About 30 different cathelicidins have been described in mammals, but so for only one has been identified in humans and one in mice. The human cathelicidin (LL-37) and the murine cathelicidin-related antimicrobial peptide (CRAMP) are both expressed by mast cells [118]. Recent findings suggest that their function is to disrupt the integrity of the microbial membrane, which is mediated by their cationic and amphipathic properties, which enable them to bind to negatively charged microbes and insert into their membranes [118]. Thus, after proteolytic cleavage the cathelicidin-like domain can contribute to innate host defense via inhibition of bacterial growth and limitation of cysteine-proteinase-mediated tissue damage, both functions being complementary to LL-37. LL-37 also represents a multifunctional effector molecule for innate immune defense of the skin [676] and for the local treatment of pulmonary infections [622]. In addition to the antimicrobial effects, LL-37 has been shown to have chemotactic effects on mast cells, which might aid in the migration and accumulation of mast cells at the site of inflammation in several diseases [382].

A defensive role could be assigned to *scavenger receptors,* defined by their ability to carry on the following functions:

- *Macrophage* activation and homing
- *Clearance* of LPS, microbes and toxic substances
- *Phagocytosis* of cells undergoing apoptosis
- *Antigen uptake and/or processing* in the acquired immunity [420].

Macrophages use CD36, vitronectin and thrombospondin receptors to engulf cells undergoing apoptosis [165]. *Eosinophils* are additional cells active in innate immunity, since they arm against parasites and some forms of tumor cells the cytolytic activities of MBP and ECP their cationic proteins. ECP is able to perforate the membrane of a target cell, similarly to the perforins of both CTLs and NK cells.

For completeness or an alternative to antimicrobial defense, we should mention another potent defense establishing a link between innate and acquired immune systems, represented from *DCs* [352], which assure a system of sentinel receptors activated by microenvironmental cellular and tissue damage [221]. A key element supporting this hypothesis is that the innate immune system has evolved an evolutionary strategy, developing several parallel mechanisms, oriented to an immunoregulatory direction, but *sensitive to local injury.* In this context, PLA_2 which in patients with rheumatoid arthritis (RA) is associated with propagation of inflammation, also favored DC maturation and PLA_2-generated DCs stimulated IFN-γ secretion by allogeneic T cells. These effects were correlated with the activation of NF-κB, AP-1 and NFAT. Thus a transient increase in PLA_2 activity generates signals that promote transition of innate to adaptive immunity during the APR [422]. We have discussed complement, NK cells, T-cell TcR and phagocytes. DCs exemplify the pathway joining these mechanisms: activation of any of these mechanisms may be associated with a switch from DC precursors to the mature phenotype [251]. At this level, DCs act as APCs, activated by GM-CSF and TNF-α in synergy with IL_1, IL_6 and IL_{12}: their role is focused on local tissue repair and therefore amplifies a self-limiting control mechanism, to shift the balance of the response back to tolerance. When tissue injury is present, NK cells could provide a negative-feedback effect on antigen presentation [221]. Furthermore, DCs could play a triple role:

- *In viral infections* as potent inducers of CTL cells, also presenting antigens on HLA class I molecules.
- *Immune responses* triggered by the association of allergenic and toxic molecules would prime pollution-activated APC DCs to provide the necessary costimulatory signals to activate T cells specific for those allergens.
- Action *as sensitive sensory receptors,* since DC peripheral sentinels might be regarded as a kind of sensory nerve ending, susceptible to local chemical signals, and designed to monitor tissue damage [221].

The DC defensive effect is probably amplified during acute inflammation, in particular presenting viral antigens to T cells, which may accordingly protect the host against concomitant viral infection [352], or inducing T-cell-mediated tolerance to inhaled antigens, since DCs are the only cells present in airway epithelial cells expressing surface HLA antigens, a prerequisite for antigen presentation [467].

Recent studies have shed light on the pivotal role played by memory B and T lymphocytes, critical for host protection from secondary viral infections (Table 1.42) and ILs within innate immunity, which is entitled to the regulation of several of the nonspecific mechanisms discussed so far, with the objective of clearing infections [688]. Purposely we point out the inhibi-

tion of apoptosis mediated by viral genes (Table 1.19), to guarantee the species survival until genome replication and to counteract host responses to infection [192]. Several viral genes have binding sites either for NF-κB, which can assure their replication, as demonstrated for HTLV-I (human T-cell leukemia virus), or for IL and chemokine receptors (Table 1.57) [688], interfering with their activity and modulating virus evasion strategies [11]. Poxvirus proteins are able to block different arms of the host response against infection (complement activation, IL function, antigen presentation to T cells) or mimic host growth factors [11]. Thus, members of the family activating NF-κB have a key role in the induction of immune responses in mammals, including TLRs [353]. More positively, pathogen recognition, whether mediated via the TLRs or via the antigen-specific TcR and BcR, initiates the activation of distinct signal transduction pathways that activate NF-κB. Activation of NF-κB by these pathways is necessary for lymphocyte activation, expansion, and effector function in response to infection. However, IL_{10} and CSs inhibit NF-κB [470]. *Chemokines* play a versatile role in the defense against infections, even viral: a rapid inflammatory response is expected to counteract bacterial and fungal pathogens, and in this context the fast chemokine induction was observed in several animal models and in clinical studies. Deletion of endogenous genes for MIP-1α in knockout mice provoke a great delay in influenza episode resolution, as well as a reduced CD8 T cell recruitment in infected airways [95]. Above all, CCL3, CCL4 and CCL5 are in the first line against AIDS, highly expressed in the lymph nodes of these patients [9], and when some receptors (CXCR = α and CCR of β chemokine receptor) act as cofactors for HIV entry to macrophages (CCR3 and CCR5) and T cells (CXCR-4) [9, 83, 150], chemokines have an anti-HIV effect primarily competing for receptor binding; thus MIP-1α, MIP-1β and RANTES bind to CCR5 and SDF-1 CXCR-4 [405].

Human TLRs belong to a family of pattern recognition receptors (PRRs) that aid to recognize microbial products derived from several classes of microbes, as well as endogenous ligands that represent a danger signal [448]. Transcripts for TLR4 and TLR2 were expressed in whole tissue extracts of fetal gut and skin [243]. The TLR7-, TLR8-, and TLR9-dependent induction of IFN-α/β and -λ is strictly IRAK-4 dependent and plays an important role in protective immunity to most viruses in humans. IRAK-4-deficient patients may control viral infections by TLR3- and TLR4-dependent and/or TLR-independent production of IFNs. Thus 5 TLRs seem to play a crucial role in this innate immune response [662]. However, *children who had defective signaling* in the molecule IRAK-4 had a greatly increased risk for pyogenic infections [431]. TLRs are structurally characterized by a cytoplasmic TIR domain that, via the signal transduction factor essential for several TLR-mediated responses MyD88, connects the receptor to the intracellular signaling machinery shared by IL_1 and IL_{18} [448]. *LPS are associated with TLRs.* TLR4 and TLR3 recognize LPS from Gram– bacteria [345]. The recognition of microbial components by TLRs leads to activation of innate immunity: progress in elucidating the molecular mechanisms for LPS tolerance has been made through the analysis of TLR-mediated signaling pathways [491, 561]. *So far the TLR family includes* 11 *members that have been identified* [677], *which bind to microbial products, activating host defense responses.* These TLRs and their signaling pathways are represented in such diverse creatures as mammals, fruit flies, and plants, and several of them appear to recognize specific microbial products, including LPS, bacterial DNA. TLR signaling represents a key component of the innate immune response to microbial infection [299]. The analysis of DC and PBMC activation has shown that TLR2 agonists are able to block the induction of IP-10, $IL_{12}p35$, and IFN-γ, but not IL_{15} and IFN-β by TLR3 and TLR4. TLR2 stimulation led to rapid release of IL_{10} that is responsible for inhibition of IP-10 and $IL_{12}p35$ induction [448]. TLR4 agonist specifically promoted the production of the Th1-inducing $IL_{12}p70$ and IP10, which is also associated to Th1 responses. Instead, TLR2 stimulation failed to induce $IL_{12}p70$ and IP-10 but resulted in the release of the IL_{12} inhibitory p40 homodimer, producing conditions that are predicted to favor Th2 development. TLR2 stimulation also resulted in preferential induction of IL_8 and p_{19}/IL_{23} [449]. Thus TLR primary responses to their agonist may be modified by cross-talk between different TLRs [448]. The receptor superfamily also includes $IL_{18}R$ [131]. *Ligands* and chromosomes [537] *for* 10 TLRs, such as TLR1 (4p14), TLR2 (4q32), TLR3 (4q35), TLR4 (9q32-33), TLR5 (1q32-33), TLR6 (4p14), TLR7 (Xp22.3), TLR8 (Xp22) TLR9 (3p21.3) and TLR10 (4p14), *have been characterized* [50, 191, 333, 448]. Stimulation of resting B cells with anti-μ and anti-CD40 antibodies increased expression of TLR9 and TLR10 [50].

The newest member of the TLR family to be identified is TLR11; however, it is not clear whether humans express TLR11, as the murine Ser119 residue appears to be replaced by a stop codon in humans [187]. TLR10 is notably expressed in lung tissue, where involvement in the innate immune responses of the lung to common respirable exposures such as allergens may be a *potential asthma candidate gene* [285], thus suppression of TLR responses may reduce excessive inflammation in chronic diseases [185]. All TLRs with the exception of TLR3 can signal via MyD88, TLRs 2 and 4 utilize MyD88, and TLR3 and 4 can engage TRIF and TRAM (TRIF-related adaptor molecule) under certain circumstances [188, 658]. TLR4 recognizes LPSs and lipoteichoic acids from Gram– and Gram+ bacteria, respectively, and TLR9 is critical for recognizing bacterial DNAs [345]. A CpG oligonucleotide, a TLR9 agonist, also stimulated TLR9 expression in B cells [50]. TLR4 and TRAF6 [374] were shown to induce the activation of the NF-κB signaling pathway: by this pathway, TLR4 activation

induces the expression of a variety of ILs and CSMs [345]. IFN-γ up-regulates TLR4 expression, and was shown to counteract the LPS-induced down-regulation of TLR4 [50]. Recently the TIR domain-containing adaptors have increased in number: they include MyD88, TIRAP (TIR domain-containing adaptor protein), TRIF, and TRAM [491, 560, 658]. These TIR play essential roles in TLR signaling. MyD88 is essential for inflammatory IL production via all TLRs, whereas TRIF is involved in TLR3- and TLR4-mediated MyD88-independent induction of IFN-β [560]. These findings confirmed that TLRs may function as receptors of the innate immune system [345, 560]. Moreover, the activated TLR4 recruits the *MYD88/IRAK signaling* pathway. Further downstream, IRAK and IRAK-2 interact with the adapter molecule TNRF6 that bridges them to the protein kinases TAK1 (transforming growth factor-β-activated kinase) and NIK [261, 372]. Once activated, the MyD88/IRAK signaling cascade bifurcates and leads to the activation of a TF NF-κB and c-jun N-terminal kinase (JNK), which initiate the transcription of proinflammatory IL genes that subsequently drive the transcriptional induction of several IL genes (see above) [374, 563]. However, a dominant-negative version of MyD88 specifically inhibited TLR4-induced NF-κB activation, lending functional credence to the interaction occurring between TLR4 and MyD88 [373, 374]. Genetic, gene transfer, and dominant-negative approaches have involved TLRs 2 and 4 in LPS recognition and signaling [374]. The new IFN, FLN29, is a negative feedback regulator of TLR signaling in innate immunity; FLN29 may thus play an important role in endotoxin tolerance [335]. Two members of the NOD family, NOD1 and NOD2, together with TLRs, have also been shown to be involved in the innate immune response as sensors of specific bacterial components [429]. Genetic variation in the genes encoding the NOD-LRR proteins, and NOD2 in humans and Naip5 (neuronal apoptosis inhibitory protein 5) in mice, is associated with inflammatory disease or increased susceptibility to bacterial infections [79]. NALPs [NACHT (neuronal apoptosis inhibitor protein)], LRR- and pyrin domain (PYD)-containing proteins 1–14 have been defined in the human genome. NALP1 protein forms a large, signal-induced molecular platform, the inflammasome, *resulting in the activation of proinflammatory caspases* [582]. Instead, IL_{32} synergized with the NOD1- and NOD2-specific muropeptides of peptidoglycans for the release of $IL_1\beta$ and IL_6 via a caspase 1-dependent mechanism; the marked expression of IL_{32} in colon mucosa suggests a role of IL_{32} in the pathogenesis of Crohn's disease (CD) [379]. NOD2-S is preferentially expressed in the human colon and is up-regulated by the antiinflammatory IL_{10}. Overexpression of NOD2-S down-regulates NOD2-induced NF-κB activation and IL_8 release. NOD2-S also interferes with the maturation and secretion of pro-$IL_1\beta$ downstream of NOD2 and its adaptor molecule receptor-interacting protein kinase 2. Increased levels of NOD2-S can abolish residual NOD2 signaling by the CD-associated R702W variant. However, interfering with the delicate balance of NOD2 signaling in inflammatory bowel disease, e.g., by changing the ratio of NOD2 splice variants, may have detrimental effects on the CD course [475]. Until recently, most TLR/ligand interactions had been shown to favor Th1-like responses rather than promoting the Th2 responses commonly associated with allergic disease. However, this concept was recently challenged in rodent models, in which both TLR ligand concentration and the route of immunization were shown to induce *a Th2 immune response* [450]. How might this shift be achieved? [537]. Depending on the early environment of the individual, this might lead to Th cells that are dominated by allergy-prone Th2 cells, which support the production of antigen-specific B cells. The increase in the Th2 cells comes at the expense of Th1 lymphocytes [450]. The synergistic interactions of TLR ligands and antigen might have relevance to the exacerbation of IgE-mediated allergic diseases by infectious agents [438]. In addition to host defense against pathogens, the TLR-dependent pathways are involved in a variety of immune responses [560]. Therefore, an important challenge for the future will be to develop suitable suppression of TLR responses to reduce excessive inflammation in chronic diseases [187].

A prominent role in innate immunity is played by *keratinocytes* expressing both TLR2 and TLR4 at the mRNA and protein levels; TLR2 and TLR4 are present in the normal human epidermis in vivo and their expression is regulated by microbial components [432]. The expression of MyD88 has been demonstrated in keratinocytes. LPS and IFN-γ increased the expression of TLR2 and TLR4 50-fold and fivefold, respectively [432].

A prevalent dogma is that a key defense mechanism belongs to Th1 and Th2 T cells mediating killing, Th1 T cells of intracellular and Th2 of extracellular parasites. However, everything can be modulated by ILs, their number [31] and chronology of intervention. Th1 and Th2 T cells are potentially activated by signals deriving from phagocytes and NK-CMI. Moreover, we speculate that Th2 T cells act only in immune reactions independent of phagocyte cells: several ILs generated by both T cells inhibit macrophage functions potentially provoking severe damage to the host [471]. IL_{12} establishes a bridge with acquired immunity and provides defense against infections at different levels: it influences rapid IFN-γ production from T and NK cells, modulates virgin T-cell differentiation into Th1 lymphocytes due to their capacity for *up-regulating IFN-γ titers and reducing IL_4 production* from virgin T cells, and is also necessary for the optimal proliferation of IFN-γ producing Th1 cells in response to antigens and APCs [181]. Thus, IFN-γ and IL_{12} form a positive feedback system amplifying IFN-γ levels to activate macrophages and IL_{12} to promote proliferation and activation of NK and Th1 cells [181]. Recently, the primary function of IL_{12} has been shown to up-regulate adhesion molecules, and IL_{28} and IL_{29} medi-

Table 1.66. Primary antibody-mediated defenses against pathogen microorganisms

Immunologic function	Pathogens affected	Main Ig involved	Nonspecific cofactors required
Opsonization	V, B, M	IgG, IgM	Phagocytes and complement (in some cases)
Neutralization	V	IgG, IgM, IgA	Complement (in some cases)
Neutralization	B	IgG	–
Inhibition of binding	B, M (?)	IgA	–
Cytolysis	V[a], B, P	IgG, IgM	Complement
Enzyme inhibition	V, B (?)	IgG	–
ADCC	V, B (?), M (?)	IgG	–
Growth inhibition	Mycoplasma B	IgG, IgA IgA	– Lactoferrin

Modified from [360].
V Viruses, *B* Bacteria, *M* Mycetes, – none.
[a] Lysis of virus-infected cells.

Table 1.67. Critical immunological effector mechanisms for protection against secondary virus infections

Viruses		CTL (perforin)		T (cytokines)		Antibodies	Neonatal antibody protection
		Resting	Activated	Resting	Activated		
Cytopathics	IV	–	–	–	–	++	+
	Periphery	–	–	–	++	+	±
Noncytopathics	IV	+	+	–	–	+	+ (NN)
	Periphery (antiviral)	–	++	–	–	–	– (NN)
	Periphery (immunopathology)	–	++	–	–	–[a]	–

The combinations shown with "+" or "++" mark the critical memory effector mechanisms for protection.
Modified from [688].
IV intravenously, *NN* not necessary.
[a] Can enhance immunopathology.

ate antiviral activity in cells in response to viral infection [519]. In addition, activated T cells generate additional ILs with a multitude of functions, including the widening of antimicrobial activities of immune cells, macrophages, neutrophils and NK cells [299]. Most CTLs are CD8 and also a CD4 T cell subset and HLA class I or II restricted. Similarly, T cells can directly kill microorganisms not expressing HLA molecules: this direct antimicrobial activity can also occur when HLA restrictions are absent [299]. CD8 T cells, unlike NK cells, also attack *Candida albicans* and show a direct antiparasite activity, although several of their activities are played together with NK cells [299]. Loss of direct T-cell activity could contribute to AIDS and other severe PID infections [299]. *Mice lacking T-bet* fail to control a Th1-dependent infection, yet interesting evidence suggests a pathogenic role of Th1 cells in autoimmunity [556]. Also suggestive is an antibacterial defense achieved via the innate immune system, such as innate opsonins, complement components, and certain antimicrobial peptides, leading to a polyvalent machinery formation presumably yielding a lymphocyte pathway of recognition and activation [194]. For example, opsonins, by binding to microbial cell walls and flagging them for recognition by the complement system and phagocytes, could be effective antibacterial agents [345]. As a corollary, pathogens with components capable of inducing phagocytes to produce IL_{12} already in the early events of infection elicit Th1-like responses [106]. Instead, the optimal responses against organisms with a complex structure, for example, intestinal nematodes cannot be rapidly killed because they are so large, are thus susceptible to active attack by Th2 T cells, which directly inhibit parasite growth without killing them

[592], also trying to prevent Th1 responses and bypassing macrophage activation. Tables 1.66 and 1.67 [360, 688] include antibody-mediated defenses against pathogen microorganisms related to memory effector mechanisms critical for newborn protection (Table 1.67).

Because innate immune stimulation is thought of as a broadly applicable strategy for biodefense and potentially a boon for vulnerable special populations, such as the *very young*, as well as *children* and *adolescents* whose adaptive immune systems are impaired by chemotherapy or HIV, innate immune function in different age groups and disease conditions requires that the exciting possibility that powerful inborn defenses against infection is manipulated to provide a counterweapon against broad classes of bioterror agents and newly emerging infectious diseases [194]. Such defenses are more realistic because of a growing understanding of the *pathogen genomes* [183].

In *human neonates,* despite normal basal expression of TLRs and membrane CD14, innate immune responses of mononuclear cells to LPS are characterized by a strikingly reduced release of the pro-inflammatory Th1-like TNF-α and IFN-γ with relative preservation of antiinflammatory Th2-polarizing ILs. These differences extend to a range of TLR agonists, including bacterial lipopeptides (BLPs), and are due to differences in soluble factors present in blood plasma. The suppressed TLR-induced TNF-α release from monocytes by soluble factors in neonatal blood plasma is likely to alter both innate and adaptive immune responses in neonates profoundly. Thus, neonates are at increased risk of overwhelming infection, yet the mechanisms underlying this susceptibility are incompletely defined [297]. Moreover, exposure to reduced levels of sCD14 in the fetal and neonatal gut is associated with the development of atopy, eczema, or both. A supply of sCD14 [of which breast milk is rich (Chap. 2)] could affect disease outcome, although the IgE- and non-IgE-dependent consequences of this require elucidation [243]. In contrast to the protective effects of TLR9, immunization with Ag in the context of TLR2 ligands, can result in experimental asthma [450]. Furthermore, TLR10 may be a potential asthma candidate gene: TLR10 is notably expressed in lung tissue, where a *potential asthma candidate gene* my be involved in the innate immune responses [289].

Neonatal deficiency of innate cellular immunity includes a reduced IFN-γ production by neonatal lymphocytes, hyporesponsiveness of neonatal macrophages to IFN-γ activation, a reduced production of IL_{12} by CB mononuclear cells, defective STAT-1 phosphorylation in CB monocytes, a high IL_{13} production by neonatal T cells, a hyper-methylation at CpG sites within the IFN-γ promoter region, a defective production of IL_{18} by CB mononuclear cells, a reduced Myd88 expression in newborn monocytes, and an impaired response by neonatal monocytes to multiple TLR ligands [329].

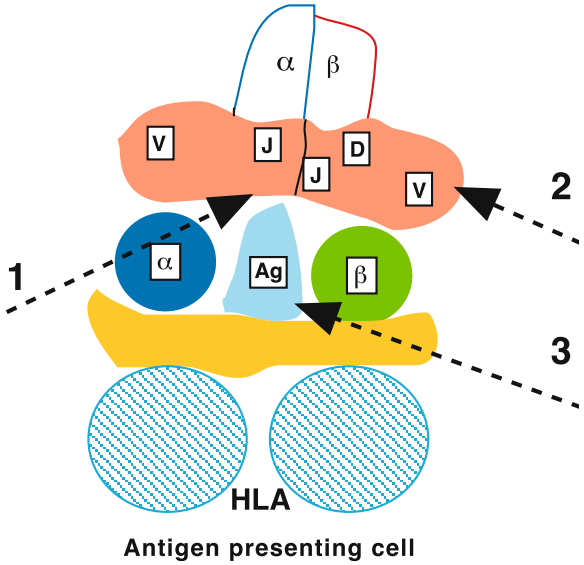


Fig. 1.66. Sites of potential peptide intervention. For details see text. (Modified from [404])

Therapeutic Perspectives

Following the leitmotif of the unabated flood of data that has taken us from the early studies on immune cells to some of the most complex areas of research in today's basic immunology, the important advances in the range of strategies heralding possible and interesting developments, which have been or will be focussed to hit specific targets, in particular allergic inflammation. We note that the task appears to be ever more challenging as the intricacy of immune mechanisms unravels.

Substantial evidence shows that *synthetic peptides* (Chap. 13) are able to disarm potentially harmful allergen-reactive T cells and/or inhibit IgE binding to allergens and/or compete with IgE antibodies for receptor binding. Logically a census of all relevant epitopes present on allergenic molecules is presently inconceivable, and certainly cross-reactivity imposes a further but not indifferent obstacle; however, this arm of immunology is making rapid progresses, as is shown in Fig.1.66 [404]. The functional inactivation can be selectively referred to as specific clones of T lymphocytes (*arrow 1*) or to a subset of T cells with different specificities, and TcRs with particular features but reciprocally common (*arrow 2*), or lastly to the substitution of epitopes bound to the disease with inert peptides at the level of the peptide–HLA complex combination site (*arrow 3*). Another option, devoid of toxic effects for human beings, is that of employing synthetic peptides corresponding to linear sequences of HLA class I molecules (residues 56–69, 60–84, 99–113 and 222–235) to cause T-cell anergy, increasing the influx of intracellular Ca and interrupting the normal pathway of signal transduction [277]. To these strategies down-regulating T cell modulation to

promote apoptosis or, on the contrary, to render T cells unable to respond to IgE synthesis, experiments have offered new insights into the inhibition of IgE binding to metachromatic cells or, alternatively, IgE conjugated with toxins able to dampen mast cells, or synthetic IgE fragments with deletion of specific responses.

Characterization of IgE receptors can provide specific clues to potentially blocking IgE binding to other cells: the use of anti-IgE IgGs with a double epitope specificity could modulate IgE-producing B lymphocytes, either bridging IgE bound to FcεRI or blocking IgE binding to its receptor, increasing or, respectively, abolishing their activity [358, 514].

Atopic individuals have *natural anti-IgE antibodies* directed against the IgE Fc region, different from anti-idiotype antibodies, which are not directed against allergen-specific IgE and therefore possess an immunoregulatory effect on IgE antibodies. Furthermore, anti-idiotype antibodies cannot establish a cross-linking with basophil IgE inducing their degranulation, thus blocking allergen binding to cells expressing IgE receptors [638]. However, to accomplish a therapeutic intervention, the degranulation impact should be very limited, but in view of the scarce number of metachromatic cells expressing the corresponding idiotypes, several authors deem this goal feasible [638]. Moreover, taking into account the particular convex structure of IgE antibodies [553], a strategy blocking IgE bound to FcεRI without triggering mast cell degranulation should be based on natural autoantibodies directed toward an IgE region (residues 305–313) across the FcεRI *locus* (residues 330–345) [513].

As an alternative, *IgE synthesis could be directly inhibited* (as well as synthesis of other Igs); incrementing CD14 engagement on monocytes increases monocyte activation, since the exerted block is at the B cell level, which is conceivable in either T-dependent or T-independent systems. CD14 delivers a negative signal that terminates T-cell proliferation, the repercussions of an event about which too little is as yet known [608].

What is most likely to be successful is the attempt to inhabit IL_4, the factor triggering IgE synthesis:

- In a *mutant IL_4*, substituting the aspartic acid of a protein with a tyrosine residue in position 124 (IL-4.Y124D) blocks IL_4R activation while leaving the binding capacity, and a IL_4- and IL_{13}-induced dose-dependent inhibition of IgE synthesis. By increasing the dose 100-fold, the inhibition increases up to 95% [437]. Moreover, the addition of an IL_4 binding protein, a sIL_4R, blocked the IgE enhancement by $CD8^+$ T cells [346]. However, if the peripheral blood of atopic patients contains long-lived B cells with a previous isotype switch to IgE, these antibodies cannot be directly influenced by ILs [125].
- The intervention of a *potential suppressive factor* of transcriptional events, inversely regulating IL_4 transcription of a not yet identified protein, determines a high capacity of binding to Th1 T cells at the moment of T-cell activation, coupled with a virtual absence of IL_4 after 16–24 h; instead, the high binding capacity of Th2 T cells is expressed with not yet stimulated cells. However, it is decreased when Th2 are activated. Such events are correlated with high inductive signals for IL_4 after the same time span [206].
- Increasingly, soluble *sIL_4R* may be able to block in vitro IL_4-mediated B cell activation, proliferation and differentiation in human PBMCs and therefore both IL_4-dependent IgE synthesis and CD23 induction on B cells. The clinical basis of using sIL_4R is the location in the blood, rendering sIL_4R a potentially stimulating candidate. Moreover, IL_4-deficient mice obtained via gene targeting do not display immune abnormalities, except for their inability to produce IgE [172]. A contrary effect has been assessed [492].
- An interesting subject is the demonstration that *anti-CD23 antibodies* block IgE synthesis in an isotype-restricted fashion, that is, inhibiting B lymphocyte differentiation in IgE-producing cells under the IL_4 influx [16].
- STAT6 is phosphorylated by IL_4, so any agents that *block STAT6 function* may be useful for treating atopic disease [562].
- Even more interesting is the likelihood of inducing a *peptide-mediated IL_4 anergy*. Using a clone of T cells incubated in a culture medium or with a specific peptide in either an immunogenic or nonimmunogenic dose, and stimulating again with a nonimmunogenic specific peptide and the related APCs, it was elucidated that T cells becoming unresponsive completely lose the ability of secreting IL_4, but fully maintain the ability of producing IFN-γ [138].
- In the animal model, the concerted action of IL_{12} together with IL_{18} and anti-CD40 *inhibits IL_4 and IgE synthesis* by induction of IFN-γ production from activated B cells, however, without inhibiting B cell proliferative response [669].
- A significant series of experiments in the animal model with synthetic peptides assure relevant implications for SIT. Administering a peptide along with an adjuvant in APCs induces T-cell proliferation and development of immunity. If a peptide able to activate T cells is administered, but disregarding the adjuvant (or CD28/CD80 interaction is missing), the *2nd signal is abrogated*. T-cell *clonal anergy* ensues as does the eventual apoptosis that drives to tolerance, as discussed in "Apoptosis" [138] (Fig. 1.22c). A similar option could prove successful in atopic patients [276].
- Ongoing studies on single amino acid substitutions have paved the way to assemble a peptide with a T epitope possibly able to *interrupt T/B interactions* and the consequent isotype switch to IgE, exciting IFN-γ production or inhibiting CD4 proliferation. However, skin prick tests (SPTs) with a modified allergen failed to drive immediate reactions [223].
- A potentially promising candidate is *clonal deletion* [138], a process of negative selection following, for

Table 1.68. Partial list of the agents inhibiting apoptosis

Inhibitors		
Physiological inhibitors	**Viral genes**	**Pharmacological agents**
Androgens	Virus LMW5-HL Herpesvirus γ1 34.5	
CD154	Baculovirus IAP	Tumor promoters
Estrogens	African swine fever	α-Hexachlorocyclohexane
Extracellular matrix (EC)	Baculovirus p35	Cysteine protease inhibitors
Growth factors (GF)	Adenovirus E1B	Calpain inhibitors
Neutral amino acids	Cowpox virus crmA	PMA
Zn	EBV BHRF1, LMP-1	Phenobarbital

Data from [576].

example, a self-HLA–antigen complex interaction, an event leading to apoptosis and thereby a powerful mechanism aimed at inducing tolerance to self-antigens (Fig. 1.22).

- The armamentarium of T epitopes has intriguing repercussions for SIT, based on the *switching of immune responses* from Th2 to Th0 T cells. A prominent feature, however, is a feasible switch to Th1 and DTH (Chap. 13).
- Administration of peptides corresponding to T epitopes (at least 10–12 amino acids) elicits a *T cell tolerance* to a complete antigen in humans [404], hence immunodominant T cell epitopes of an allergen actually also function as high inducers of T-cell nonresponsiveness [619].

What was summarized above on sCD40 makes it feasible to consider an isotype switching inhibition after the second signal expressed by ILs, to stop T–B interactions via CD40 and CD154 thus far achievable in vitro [132]. In addition, CsA abrogates CD154 stimulation in mast cells [174]: such data explain why CD154 regulatory effects are inhibited on APCs and IL_{12} as regards the stimulation of adhesion molecules useful for T cells and macrophage production of inflammatory ILs [187].

- A not yet explored and totally different strategy has the objective of suppressing B_{IgE} cells in patients with IgE-mediated atopy using mABs activated by a procedure tested on B cells expressing membrane-bound IgE, more precisely including amino acid residues and Ig membrane-anchoring segments extending from the C-termini of H chains. One potential application could be a methodology that can modulate isotype-specific antibody production to suppress B cells undergoing switch recombination, but not circulating IgE or IgE bound to mast cells [108].
- A fusion protein of 2 major allergens bypasses IgE binding and mast cell/basophil IgE FcεRI cross-linking and protects from IgE development [282].
- A recent study that certainly has striking consequences on AD treatment has shown that FK-506 and CSs inhibit both NFAT and AP-1, and hence IL_5 transcription [394]: since TFs are alike for diverse ILs, it is probable that further studies will report the inhibition of additional ILs.
- As outlined in Chap. 22, atopic children, some with HIgES, have been successfully cured with intravenous IgGs (IVIgs), suggesting that *IVIgs inhibited IgE production* of PBMCs (T cells) cultured in vitro and stimulated by IL_4, without influencing IgA and IgM levels, and also of normal B cells stimulated by IL_4 + anti-CD40.
- The significance of CSMs results from their ability to block antibody production following in vivo administration of CD152-Ig and anti-CD86 [115]. CD152-Ig specifically abates IL_4 production and if administered early after the start of an infection *blocks its progression* [242, 685].
- A new strategy *to inhibit or,* depending on the case, to *cause apoptosis* (Tables 1.68, 1.69) [576] acts on NF-κB as well as TNF-α [599], or modulates bcl-2/ced-9, or alternatively ICE/ced-3 [267, 661], or ced-4: if a mammal homolog is identified the issue could encompass all changes deriving from apoptosis dysregulation.
- A recent approach could be aimed at *eliminating eosinophil influx* into airways, which has been achieved treating animals with anti-MIP-1α, anti-RANTES and anti-MCP-3. So the rationale to focus CCR3 antagonists is available, a potential mediator of CCL chemokine effects on eosinophils [9].
- Another strategy is *the blockade of chemokine receptors:* CCR3, CCR4 and CCR8 are the most obvious targets for therapeutic investigation.
- Attention is also turning to small molecular weight (SMW). SMW IL_4 inhibition seems to *decrease IgE synthesis,* lung eosinophilia and mucous production and could be used to treat asthma and other atopic disorders.
- The development of new therapies for atopic allergy is now focusing on local Th1-driving IL_{12}-promoting substances to target both the development of new Th2 cells and the persistent population of established allergen-specific Th2 cells to *revert established effector*

Table 1.69. Partial list of the agents inducing apoptosis

Inducers			
Physiological activators	**Damage-related inducers**	**Therapy-associated agents**	**Toxins**
Ca	Antimetabolites		
CSs			
GF withdrawal	Free radicals		
Loss of EC attachment	Nutrient deprivation -		
Neurotransmitters			
Glutamate	Tumor suppressors p53	Methotrexate	
Dopamine	CTL	γ Radiations	
N-m-*d*-aspartate	Oxidants	UV radiations	
TGF-β	Oncogenes myc, rel, E1A	Arabinoside, vincristine, nitrogen mustard	
TNF family	Heat shock	Chemotherapeutics:	Ethanol
FasL	Viral infections	Cisplatin, bleomycin	β-Amyloid peptide
TNF	Bacterial toxins	Doxorubicin, cytosine	

Modified from [576].

Th2 cells in humans into predominant Th1 phenotypes [530].

The newly described IFN-λ and $IL_{27–29}$ that mediate antiviral activity in cells in response to viral infection are the major initial weaponry against most viruses (Table 1.5).

The development of new therapies for atopic allergy is now focusing on local IL_{12}-promoting substances to target both the development of new Th1 cells and the persistent population of established allergen-specific Th1 cells:

- IL_{27}/WSX-1 plays an important role in the down-regulation of BHR and lung inflammation during the development of allergic asthma via its suppressive effect on IL production [357].
- Since IFN-γ induces T-bet expression, T-bet would be an attractive target to identify anti-asthmatic drugs [155].
- Anti-human IL_{13} antibody also looks promising for lowering tissue eosinophil levels and is in preclinical trials [44].
- Antagonists of proteinase-activated receptor or inhibitors of proteases that activate this receptor may be worthy therapies for asthma [502]. Tryptase is a therapeutic target in asthma and selective tryptase inhibitors can reduce allergic airway inflammation [392].
- CD300a was shown as a future potential target for the treatment of allergic and eosinophil-associated diseases. By suppressing the activity of human eosinophils, cross-linking of CD300a on the eosinophils inhibited the IL_5/GM-CSF antiapoptotic effects and blocked the release of TNF-α, $IL_1β$, IL_4, 3T3 fibroblast proliferation, IL_5-mediated JAK2 phosphorylation, eotaxin- and IL_5/GM-CSF-mediated ERK1/2 and p38 phosphorylation, but also blocked IFN-γ release [366].
- Selected defensins and/or chemokines may be good candidates for the development of vaccine adjuvants, since they are believed to enhance adaptive immunity markedly [660].
- The effect of dietary vitamin E on atopic disorders is beneficial since it blocks TF binding to two pivotal IL_4 promoter binding sites for NF-κB and AP-1 and interferes with promoter activity upon T cell activation [311]. Moreover, vitamin E suppresses CD95L (APO-1/Fas) mRNA expression and protects T cells of HIV-1-infected individuals from CD95-mediated apoptosis, evidence that vitamin E can affect T cell survival [312].

Allergens

Following the increased prevalence of atopic disease, it has become even more necessary to have at hand purified allergens to obtain more characterized diagnostic and therapeutic tools [94, 127, 433, 639, 675]. Allergens responsible for immune reactions are mostly proteins and are classified into major, intermediate and minor allergens based on the frequency with which an IgE-mediated allergy occurs. This characteristic can be analyzed with laboratory methods; CRIE (crossed radioimmunoelectrophoresis) is the most frequently used technique in two phases. In the first phase, all proteins of a given allergenic substance are separated electrophoretically and recognized as precipitates in specific rabbit antisera. In the second phase, a pool of RAST sera positive for the substance under scrutiny is applied to the procedure:

sIgE will only fix to allergenic proteins, subsequently read by autoradiography utilizing anti-IgE antisera marked by radioactive tracers. Thus *major allergens* are defined as components that bind IgE in 50% of the sera from a group of patients with the same allergy, *minor allergens* when components react with 10% of the sera, and *intermediate allergens* when components share halfway properties. Most commonly, an allergenic source may enclose allergens belonging to the three above types: for instance, egg white contains at least 20 different proteins, but only four or five of these proteins are allergenic. Actually only major and minor allergens are taken into account. However, not all patients recognize all major allergens and some patients only recognize allergens that are not recognized by the majority of allergic patient sera (Chap. 13). From a clinical point of view, allergen sources can be classified as inhalant allergens: pollens, mites, animal danders, molds, chemical/pollutant substances, drugs; ingested allergens: foods, chemical substances, drugs, etc.; insect allergens: insect venoms; and contact allergens: foods, chemical substances, topical drugs, cosmetics, etc. [94, 126, 433, 639, 675].

Table 1.70. Completely cloned allergens (recombinant DNA) with the pertinent amino acid sequence

Foods	Ara h 1, Mal d 1, Met e 1, Sin a 1
Animals	Bos d 1, Can f 3, Chi t 1, Fel d 1
Mites	Blo t 5, Der p 1, Der p 2, Der p 3, Der p 5, Der p 6, Der p 7, Der f 1, Der f 2, Der f 3, Der f 9, Der f ?, Eur m 1, Lep d 1
Molds	Alt a 6, Alt a 7, Alt a 10, Asp f 1, Asp o 2, Cla h 3, Cla h 4, Cla h 5, Cla h 6, Pen n 1
Pollens (plants and trees)	Aln g 1, Amb a 1, Amb a 2, Amb a 5, Amb p 5, Amb t 5, Bet v 1, Bet v 2, Bet v 3, Car b 1, Cor a 1, Cry j 1, Cry j 2, Cyn d 1, Dac g 2, Hol l 1, Hor v 1, Lol p 1, Lol p 2, Lol p 5, Ole e 1, Par j 1, Pha a 1, Pha a 5, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 11, Poa p 9, Tri s 11, Zea m 11
Insects	Api m 1, Api m 2, Bla g 2, Bla g 4, Dol m 1, Dol m 2, Dol m 5, Myr p 1, Ves v 5

Data from [112, 496, 604].

Allergen Standardization

Standardization regards commercial allergens, provided that they correspond to the main rules established with respect to their content of major and minor allergens, safety and potency or biological activity. It is highly necessary because crude allergen extracts incorporate all components of original materials, hence not only all potentially sensitizing allergens, but also irrelevant material with potential primary irritant activity. We define allergen extracts as complex mixtures of substances with a known composition, reflecting that of a relevant allergen source material that could include even 100 proteins, but with a known total allergenic potency that is constant between batches [126]. However, the extracts may vary according to the quality of the initial material and be insufficiently purified and also unstable. Therefore, allergen extracts contain major allergens in inadequate concentrations, and are contaminated by irrelevant components to which the patients are not sensitized, or important allergens are lost during extractive procedures due to proteases purified together with allergens [126]. In addition, measurement of allergen levels present in planned extracts is often difficult because of a mixture complexity, or because allergen components vary from one supplier to another, either for allergen potency or quality, or from one year to another or one production lot to another of a single supplier [112]. *It is not possible to compare the potency of allergen extracts produced by different manufacturers, even if quantified with the same techniques* and if done, it might lack the indication of major and minor allergens [112]. To ensure uniformity among future batches, units of measure have been introduced. As a consequence, manufacturers can employ appropriate, although of varying calibration, methods for allergen extracts, provided that such methods are clearly documented and constant. The WHO/IUIS has established International Units (IU), the Biologic Units (BU) are widely used in northern countries and in several European countries, and in the US are expressed as Bioequivalent Allergy Units (BAU). Such methods are expensive and demanding; therefore promising improvements in the procedure are currently *recombinant allergens* (RA) (Table 1.70) [112, 496, 604, and Internet data, August 2006].

Standardization Techniques

Allergen standardization consists in the adoption of methodologies ensuring both uniformity and reproducibility between different batches of the same allergen extract, that is, the procedures to select the raw materials, assemble and control allergen extracts, which allow suppliers to produce different batches with the same allergen provided with wholly matching characteristics. The system based on SQ units (standardized quality unit), crossed immunoelectrophoresis (CIE) and CRIE makes it possible to demonstrate the single epitopes of standard extracts. For each new extract, the existence and amount of all allergens is controlled by autoradiography; subsequently the batches presenting quantitative differences are equilibrated to standard concentrations using quantitative immunoelectrophoresis [126]. Reference internal extract (in-house standard) is prepared to guarantee the protein composition of allergen ex-

tracts via immunochemical analyses, such as CIE, CRIE, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), high-performance liquid chromatography (HPLC), isoelectrofocalization (IEF) and other molecular biology techniques. Currently, it is necessary to employ in vitro and in vivo standardization methods, both based on interaction between allergens and sIgE produced by sensitized patients [675].

In Vitro Standardization Methods

The most important in vitro standardization techniques are based on binding inhibition of pooled sera containing sIgE to allergens and on gel precipitation.

RAST inhibition quantifies single allergens or total allergenic potency of raw extracts: soluble antigens, identical or cross-reacting with antigens of solid phase, are combined with the serum pool. The resulting binding inhibition of anti-IgE antibodies labeled with I^{125} to solid phase antigens shows the identity of the soluble antigen with that bound to the solid phase. Consequently, varying amounts of soluble allergen extracts are added to the RAST first phase as inhibitors. Dose-response inhibition curves may determine activity relative to reference extracts: parallel inhibition curves indicate similar composition, while nonparallel curves do not. The necessary reagents require:

- *Allergens* derived from high-quality materials where all major and minor allergens are represented.
- *Pooled sera* containing IgE from a panel of patients characterized for their sensitivity to the allergen in question.
- *Reference extract* of high quality.

Similarly, ELISA inhibition, rocket electrophoresis, isoelectric focusing, gel electrophoresis, etc. are used [94].

Analysis of single allergens is accessible only when major allergens have been identified, thereby anti-allergen-specific antibodies allow subsequent allergen quantification with an immunological essay. For this purpose, several techniques are utilized, for example, an enzymatic essay to characterize the extracts as regards *Hymenoptera* venoms equipped with several enzymes [433].

Gel precipitation techniques include CIE, CRIE, immunoblotting and histamine release by basophils.

In Vivo Standardization Methods

The standardization method established in Scandinavia expresses the results as HEP (histamine equivalent potency), the allergenic activity of an extract is estimated measuring the skin response to the extract in a pool of subjects with known allergy to the tested allergen. SPTs test the quantity of the extract that produces a wheal the same size as the wheal size produced by histamine hydrochloride at the concentration of 1 mg/ml: this result corresponds to one HEP unit. However, the results are now usually expressed as BUs, which is defined as the wheal equivalent to histamine 1 mg/ml = 1,000 BU/ml. The BU gives valid indications on total biological activity, but not on the presence in the extract of single allergens; BUs are referred to as the in-house standards of each manufacturer, and therefore depend on patient selection, reactivity and compliance [112]. An analogous technique is skin activity reference allergen/histamine (SARAH) [433]. The approach established in the US based on AUs (allergy units) is valid for mites and other allergens: reference controls regarding the extracts are Der p-sensitive individuals intradermally tested with increasing doses, with subsequent evaluation of the flare rather than the wheal size as with HEP units. The reference standards for subsequent allergen dilution is the sum of erythema diameters. For other allergens, the AU provoking reactions of the same diameter in patients with multiple sensitizations is defined as BAUs. However, extracts standardized in BAUs are not comparable with corresponding BU extracts, since the techniques do not reduce the likelihood of detecting a different potency in lots of the same allergen extract produced by different manufacturers, nor the variability among lots of the same manufacturer, nor the problems of related extract potency between BUs and two different societies [112]. There is variability due to isoallergens, different isoforms of the same allergen, contained only in varying levels in the extracts [551]. Finally, besides the variations between the techniques used by laboratories, we must also take into account different subjects in patient pools [94].

Units for SPTs and SIT

Both the US FDA (Food and Drug Administration) and Nordic guidelines prevailing in Europe can be followed. In the first case, an allergen concentration is defined that can be recommended for SIT and is based on intradermal skin testing of the most sensitive patient: 100,000 AU/ml. In the second case, the unit is based on SPTs done on patients with average sensitivity and corresponds to the concentration eliciting a wheal that is the same size as the wheal produced by 10 mg/ml histamine and equals 10,000 BU/ml [126]. The ideal extract should comprise definite quantities of all major and minor allergens of each biologically potent allergen: such analyses obviously play a highly relevant role in assembling SIT extracts, which should include only allergens (or allergen epitopes) pertinent to the single patient [112]. According to Scheiner et al, SPTs have a high sensitivity (82.1 %) and specificity (66.3 %); to their detriment are the rapid denaturation of some natural allergens (such as the apple) during extraction processes. Furthermore, in the extracts the allergen levels can be naturally reduced (such as the cypress), or following destruction via enzymes present there [496].

Advantages of Standardization

The principal advantage is the testing of standardized extract biological effects, and the variability in relative titers of major allergens is much lower than extracts that have not been standardized (two- to threefold vs ten- to >100-fold). Another point in favor is clarified by the therapeutic effectiveness: consistent data show that when SIT maintenance doses correlated with major allergen levels are reached, evident improvements in patient symptom scores are noted [126].

Among the more recently achieved results in above-mentioned studies, we include the delineation of antigenic or allergenic epitopes, either major or minor and/or dominant in individual antibody repertoire, using techniques based on recombinant DNA. In addition, cloning single-helix DNA cDNA to an RNA chain, which was synthesized by inverse transcription, cDNA-coded protein amino acid sequences drawn from cDNA libraries were established, a complex of DNA cloned fragments representing the whole genome [443]. Hitherto, several allergen amino acid sequences have been determined, above all arthropods and pollens, employing cDNA-based techniques and it is auspicious that the list is so extensive that it includes all allergens [360] and Table 1.70. We stress that with knowledge of primary tools, it is reasonable to predict spatial conformation, and that computerized programs can help disclose both biochemical properties and biological functions. These results will bring out and make it possible to evaluate protein allergenicity [678]. Moreover, using synthetic peptides based on known sequences, it is practical to determine T and B cross-reactive epitopes, as well as the regions of molecules containing them [551]. The dominant epitopes referred to as Amb a 1, Bet v 1, Lol p 1, Poa p 1 and Sin a 1 were identified in parallel, even if studies done on mice are not always applicable to human beings [501, 511].

Several RAs are now available (Table 1.71) [319, 498, 604]. RAs should be preferred without exception: RAs can be precisely manipulated, targeted, engineered and formulated at defined concentration and potency. They may be produced in suitable purity and batch consistency and hence might offer a perfectly standardized diagnostic material. A WHO and IUIS international committee has fixed in IUs some standardized allergenic extracts, to which laboratories should refer, thus providing more quantitative and meaningful extracts than methods that are by now obsolete [675]. However, the majority of foods and molds have not been even partially characterized. In the US, the assortment is wider: the FDA (last updated: 2, 26, 2006) has also approved cat epithelium, Can d, Der p, Der f, several *Hymenoptera* venoms Api m, Dol a, Dol m, Pol a, Ves g, Ves p spp, pollens (Agr a, Ant o, Cyn d, Dac g, Fes e, Hev b, Lol p, Ole e), Phl p, Poa p, Amb a and Amb e (ragweed), although for Lol p 1, Lol p 3 and Ole e the complete amino acid sequence is available [94, 496, 501]. Standardized allergen extracts are commercially available. The FDA has established that all missing allergens should be standardized [94]. In several countries of the European Union, allergen extracts are subjected to registration and/or a strict quality control. For example, with RAST inhibition, it is controlled that the levels of biological potency of produced lots remain constant. Since ≈50 major allergens might cover up to 90% of all IgE specificities, commercialized but not standardized extracts will be excluded from such regulations regarding only small groups of patients residing in specific geographical areas [94].

Table 1.71. Recombinant allergens

Animals	Fel d 1, Mus m 1
Food	Api g 1, Api g 4, Dau c 1, Mal d 1
Grass pollen	Par j 1, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Phl p11, Phl p12
Insects	Bla g 1, Bla g 2, Bla g 4, Bla g 5
Mites	Der p 1, Der f 2, Eur m 1
Molds	Alt a 1, Alt a 2, Asp f 1 to Asp f 18
Tree pollen	Aln g 1, Bet v 1, Bet v 2, Cor a 1, Hev b 3, Hev b 7, Hev b 8, Hev b 9, Hev b 10, Hev b 11
Weeds	Art v 1

References [319, 498, 604] and Internet data, August 2006.

From this point of view, particular observations refer to *profilins,* vegetal panallergens present in many organisms (Table 1.72) [468, 594, 596–598, 602], prominent allergens in the *pollens of trees, grasses and weeds,* all involved in cross-reactivity observed in pollinosis patients between foods and pollens of only distant phylogenetically correlated plants, even in latex [597, 603].

Cross-reactions among allergens are outlined in Table 1.73 [17, 52, 128, 134, 406, 417, 434, 518, 598, 603, 604]. Above all, pollinosis patients suffer from cross-reactions and type I reactions between isoallergens of group I pollens with a MW of 26–32 kD, especially of Phl p and Lol p, with a marked degree of analogy in amino acid sequences. Furthermore, Phl p 1 has several T epitopes [497]. Mal d 1 has homology with *Fagales* group I [543]; Mal d 1, Api g 1 and Cor a 1 belong to the group of proteins related to pathogenesis, expressed by vegetables in stress conditions.

We now examine the allergens thus far identified and characterized, underlining the most recent updating, based on recent revision [639]. Allergens have the C or P letters depending on whether the related data on amino acid sequences are complete or partial [667]. In addition, we found Lol p 11 as the second allergen after Bet v 2 present in the pollens of vegetables and trees [134], and others [348, 543] (Table 1.74) [8, 15, 17, 60, 89, 98, 112, 121, 128, 134, 149, 199, 204, 265, 295, 310, 319, 348, 355, 365, 397, 384, 416, 405, 434, 471–473, 478,

Table 1.72. Profilins purified and characterized by allergenic raw materials

Provenance	MW (kD)
Pollens	
Ambrosia artemisiifolia	10–38
Artemisia vulgaris	14
Betula verrucosa (Bet v 2)	15
Betula verrucosa (Bet v 3)	30
Cynodon dactylon (Cyn d 12)	≈14
Helianthus annuus	≈16
Hevea brasiliensis	14
Lolium perenne	≈12
Mercurialis annua (Mer a)	14–15
Olive (Ole e 2)	15–18
Phleum pratense (Phl p 12)	44
Phoenix dactylifera	≈14
Zea mays	≈14
Foods	
Apple (Mal d 1)	18
Banana (Mus xp 1)	15
Carrot (Dau c 1)	16
Celery (Api g 4)	≈15
Cherry (Pru av 4)	15
Fennel	
Hazelnut (Cor a 1)	17
Kiwi (Act c 1)	30
Litchi	15
Muskmelon	13
Peach (Pru p 4)	14
Peanut (Ara h 5)	15
Pepper (Cap a 2)	14
Pineapple (Ana c 1)	15
Soybean (Gly m 3)	14
Sunflower (Hel a 2)	15.7
Tomato (Lyc e 1)	14
Watermelon	13
Zucchini	13

Data from [468, 594, 596–598, 602].

497–499, 511, 543, 549, 551, 577, 604, 639, 652, 674]: see Figs. 1.67–1.80 for examples. Table 1.74 is completed with the structural and antigenic homologies of Fel d 1 with other superior felines (jaguar, lion, leopard and tiger), of Can d 1 with other Canidae (wolf, jackal, etc.) and of Equ c with other Equidae (donkey, mule, zebra, etc.). CM allergens are shown in Table 1.75 [24, 121]: five different casein molecular species were identified in a purified form, synthesized by structural genes localized on the same chromosome. Regarding animal panallergens, *tropomyosin*, present in Pen a 1, Met and 1 and Der f 10, homologous to Mag44, is a band I muscular protein inhibiting contractions, unless its position is not blocked by troponin present, for example, in Bla g 5, etc. [107, 297, 649]. It is notable that tropomyosin is shared by Met e 1 and Pen a 1 with Tod p 1, Der f 10, Der p 10, Lep d 10 and Ani s 2 [8]. Several allergens are included in the lipid transfer protein (LTP) family: a 50-kD salt-unextractable protein not affected by heat treatment be-

Table 1.73. Main cross-reactions among allergens

Foods
Act c 1 (kiwi) with Phl p (Timothy) and Bet v (birch)
Gad c 1 (cod fish) with several other fishes
Mal d 1 (apple) with Bet v 1, Bet v 2, Api g 1 (celery) and Pru p l (peach)
Met e 1, Pen a 1, Pen i and Tod p 1 = squid (tropomyosins) along with other mites and insects (see below)
Egg and chicken (bird-egg syndrome, Chap. 9)
Limpet with Der p (Chap. 20)
Animals
Can f 3 (Dog) with albumin of Fel d 1 (Cat)
Fel d 1 with dander antigens and of other felines (partial) and pork meat (pork-cat syndrome)
Can f, Fel d, Equ c (horse) and sheep have interspecies cross-reacting epitopes
Rat n 1 (Rat) with Rat n 2
Insects
Chi t 1 (*Chironomus thummi*) with Hb of other chironomids
Mites
Der m 1 cross-reacts with other mites
Between Der p 1 (Mite) and Der f 1 and Hel a 1 (snail)
Der p 1 has 85% homology with Eur m 1
Between Der p 2 and Der f 2
Der f 7 has 86% cross-reactivity with Der p 7
Der p 10, Der f 10, Lep d 10, Anis 3 (nematode) and Per a 7 (American cockroach) (tropomyosins)
Per a 1 with Bla g 1
Plants and trees
Amb a 1 (ragweed) with Amb a 2 and vice versa, with Cry j 1 (Sugi), tomato and corn
Bet v 1 and Bet v 2 with Pru av 1, Pru av 4 and Api g 4
Bra j 1 (mustard) with Sin a 1 and vice versa
Car b 1 (hornbeam), Cor a 1 (hazel), Aln g 1 (alder) and Que a 1 (white oak) with Fagaceae
Hev b 5 (latex) with Act c 1
Mer a (Mercurialis) with Art v (Artemisia), Fra e (*Fraxinus*), Ole e (*Olea*), Par j (*Parietaria*), Ric c (*Ricinus*)
Between each Phl and its group and between Par j 1, and Par o 1
Stressed vegetables express some PR, Protein related to pathogenesis, including Mal d 1, Api g 1 and Cor a 1

See in Table 8.14 the latex cross-reactions and in Table 9.48 the cross-reactions between foods and vegetables. Can f can also be named Can d. Additional cross-reactions may occur between two profilins combined (Table 1.72), for example, Gly m 3 and Bet v 1, which may trigger severe clinical reactions. Data from [17, 134, 299, 406, 417, 434, 518, 598, 603, 604].

longing to corn has been reported [416]. This is a major allergen that has not come from the WHO IUIS Allergen Nomenclature Subcommittee[639].

Fig. 1.67. *Ambrosia tenuifolia* (short ragweed)

Fig. 1.68. *Cynodon dactylon* (Bermuda grass)

Fig. 1.69. *Festuca elatior*

Fig. 1.70. *Phleum pratense* (timothy)

Fig. 1.71. *Betula* (birch)

Fig. 1.72. *Olea europea* (olive).

Fig. 1.73. *Lolium perenne* (rye grass)

Fig. 1.74. *Artemisia* (mugwort)

Technically, the allergens are classified according to linnaean nomenclature, where any species is indicated using a binomial composed of two Latin names: the first three letters of the abbreviations indicate the genus and the first letter of the species name, followed by a number, progressive, referring to epitope historic or temporal identifications. Instead of Roman letters, Arabic numerals are used to show the identification order [639]. For some allergens not included in the above-mentioned revision, we kept the previous specifications [397] with the Roman numerals already attributed.

Table 1.76 [601] summarizes the T epitopes of many allergens and Table 1.77 [601] the association of single allergens with HLA molecules and IgE responses, with several factors of relative risk.

Fig. 1.75. *Parietaria officinalis*

We show the foods derived from genetically modified organisms (GMO) or crop plants in Tables 1.78 and 1.79 [110, 348]. In GMOs, insect protection is achieved by means of plants producing insecticide proteins not toxic for human beings, while herbicide tolerance is mediated by plants provided with enzymes disarming herbicides. A prerequisite is that such products be subjected to extensive assessments to ensure food safety and digestibility [348]: however, it is feasible that genes of a given plant are transferred to a nearby one and that other genes damage the so-called useful insects, also including fish deriving from monosexualization or maternal DNA doubling. Therefore, verification of potential allergenicity of transgenic food modified with genetic engineering now appears to be necessary, such as in the case of soy (Fig. 1.81) [42]. The challenges posed by GMOs and bovine spongiform encephalopathy (BSE) will be discussed in Chaps. 9 and 24.

Fig. 1.76. *Parietaria judaica*

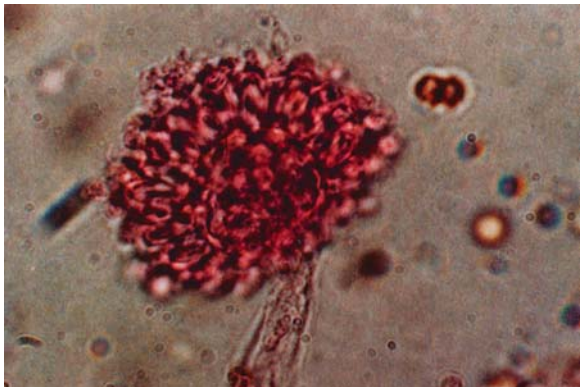


Fig. 1.77. Fungi. *Aspergillus fumigatus*

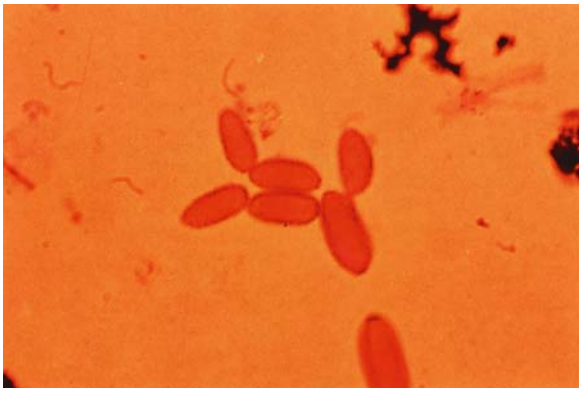


Fig. 1.78. Fungi. *Cladosporium*

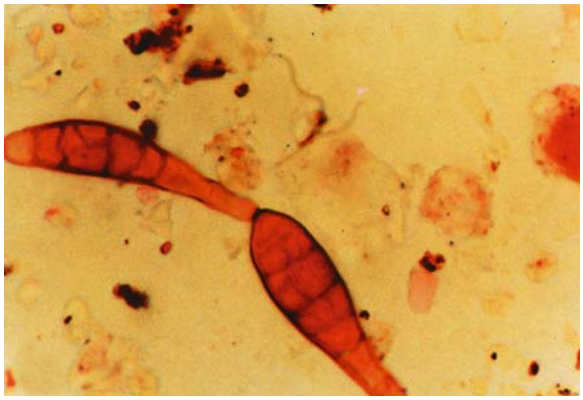


Fig. 1.79. Fungi. *Alternaria*

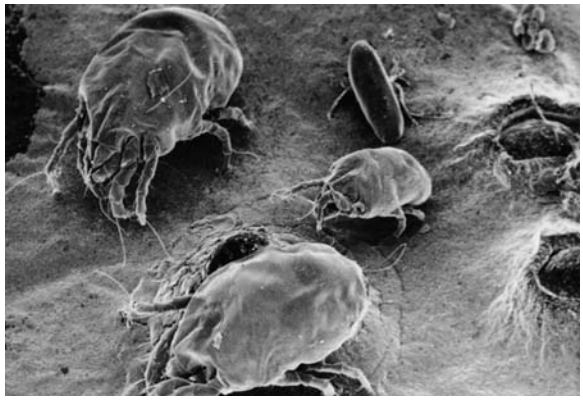


Fig. 1.80. EM view of mite family: egg, larva and adult

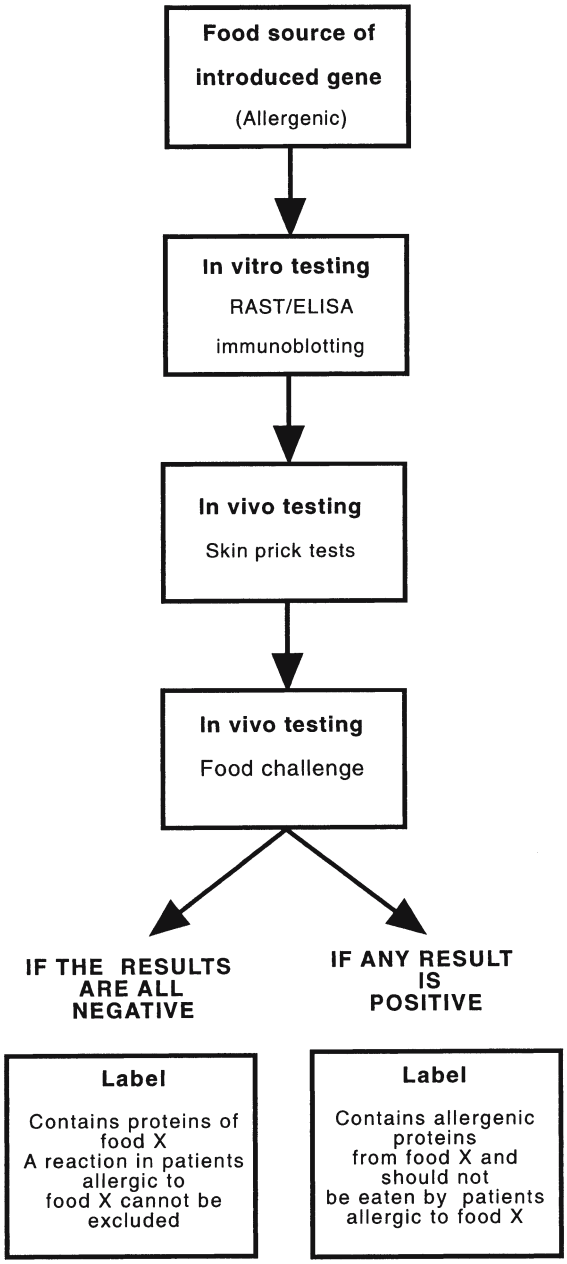


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Fig. 1.81. Allergy risks of transgenic foods. Flow chart for investigation of genetically modified foods for potential allergenicity before their release on the market, with suggestions on labeling of the pertinent foods. (Modified from [42])

Table 1.74. Allergens characteristics

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
1. *Foods* (listed independently of the family)					
Abalone (*Haliotis midae*)	Hal m 1	49			
Apple (*Malus domestica*)	Mal d 1	18	C		Profilin, hom: Bet v 1 [348, 604]
	Mal d 2		C		Hom: thaumatin
	Mal d 3	9	C		LTP
Apricot (*Prunus armeniaca*)	Pru ar 1		C		Hom: Bet v 1
	Pru ar 3	10	C		LTP
	Pru av 4				LTP [499]
Asparagus (*Asparagus officinalis*)	Aspa 01	9	P		LTP
Atlantic salmon (*Salmo salar*)	Sal s 1	12	C		Parvalbumin
Avocado (*Persea americana*)	Pers a 1	32	C		Endochitinase
Banana (*Musa paradisiaca*)	Mus xp 1	16	C		Profilin
Barley	Hor v 1	15	C	52	α-Amylase/trypsin inhibitor [348]
(*Hordeum vulgare*)	Hor v 9	30	C		
	Hor v 15	15	C		
	Hor v 16				α-Amylase
	Hor v 17				β-Amylase
	Hor v 21	34	C		Hordein
Black walnut (*Juglans nigra*)	Jug n 1	19	C		2S albumin
	Jug n 2	56	C		Vicilin-like protein
Brazil nut (*Bertholletia excelsa*)	Ber e 1 Bet e 2	9 29	C C		High-methionine protein, composed of two subunits
Carrot	Dau c 1	16	C		Hom: Bet v 1
	Dau c 4		C		Profilin
Celery (*Apium graveolens*)	Api g 1	16	C		Hom: Bet v 1, ribonuclease
	Api g 4				Profilin sharing IgE-binding epitopes with Bet v 2 [498]
	Api g 5	55/58	P		
Cherry (*Prunus avium*)	Pru av 1	18	C		Hom: Bet v 1, ribonuclease
	Pru av 2		C		Hom: thaumatin C
	Pru av 3	10	C		LTP
	Pru av 4	15	C		Profilin
Chicken (*Gallus domesticus*)	Gal d 1	28	C	34	Ovomucoid, protease inhibitor
	Gal d 2	44	C	32	Ovalbumin, hom: serine protease inhibitors
	Gal d 3	78	C	47	Ovotransferrin or conalbumin, iron transport protein
	Gal d 4	14	C	50	Lysozyme
	Gal d 5	69	C		Serum albumin
Cod fish (*Gadus callarius*)	Gad c 1	12	C		β-Parvalbumin, diffused cross-reactivity with other fish

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Corn (*Zea mays*)	Zea m 1	21	P		Lol p1 homolog [348]
	Zea m 11	14	C		The clone C13 is an Ole e 1 homolog [348]
	Zea m 14	9	C		LTP, a 50-kD corn protein that does not correspond to any known corn allergen, has been reported [416]
Cow (*Bos domesticus*)	Bos d 1	25			
	Bos d 2	22			
	Bos d 3	22			Ca-binding S100 hom
	Bos d 4	14.2	C		α-Lactalbumin
	Bos d 5	18.3	C		β-Lactoglobulin
	Bos d 6	67	C		Serum albumin
	Bos d 7	160			Immunoglobulin
	Bos d 8	20–30			Caseins
Cow's milk (Table 1.74)					
Cucumber (*Cucumis sativus*)		13			Profilin [468]
Grape (*Vitis vinifera*)	Vit v 1	9	P		LTP
Hazelnut (*Corylus avelana*)	Cor a 1	17	C	>90	Four variants of Cor a 1, 5, 6, 11, 16, all Bet v 1 hom [348] In 4 other variants IgE reactivity to Cor a 1.0401 was in 95%, to Cor a 1.0402 in 93%, to Cor a 1.0434 in 91%, to Cor a 1.0404. in 74% of sera [319]
	Cor a 2	14	C		Profilin
	Cor a 8	9	C		LTP
	Cor a 9	40	C		11S globulin-like protein
	Cor a 10	70	C		Luminal binding protein
	Cor a 11	48	C		Vicilin-like protein
Kiwi (*Actinidia chinensis*)	Act c 1	30	P		Recognized by IgE in 100% of cases, cross-reacts with Phl p and Bet v [417], cysteine protease
	Act c 2	24	P		Thaumatin-like protein
Lentil (*Lens culinaria*)	Len c 1	16	P		Vicilin
	Len c 2	66	P		Seed biotinylated protein
Lettuce (*Lactuca sativa*)	Lac s 1	9	LTP		
Muskmelon (*Cucumis melo*)	Cuc m 1	66	C		Serine protease
	Cuc m 2	14	C		Profilin
	Cuc m 3	16	P		PR-1 protein 13-kD components of melon, cucumber, watermelon, and zucchini were strongly recognized by the IgE antibodies of patients with melon allergy and were identified as profilins [468]
Mustard (*Sinapsis alba*)	Sin a 1	14	C		2S storage albumin
Mustard, oriental (*Brassica juncea*)	Bra j 1	15	C		Divided into IE-L, 2S albumin large chain, and IE-S2S albumin small chain

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Mustard, rapeseed (*Brassica napus*)	Bra n 1	15	P		2S albumin
Mustard, turnip (*Brassica rapa*)	Bra r 2	25	P		Hom: prohevein
Pea (*Pisum sativum*)	Pis s 1	44	C		Vicilin
	Pis s 2	63	C		Convicilin
Peach (*Prunus persica*)	Pru p 3	10	P		LTP, Pur p I contained in the peel [310]
	Pru p 4	14	C		Profilin
Peanuts (*Arachis hypogea*)	Ara h 1	63.5	C	>90	Vicilin seed storage protein
	Ara h 2	17	P	>90	Conglutin and others with ± concern [60]
	Ara h 3	60	C		Glycinin seed storage protein
	Ara h 4	37	C		Glycinin seed storage protein
	Ara h 5	15	C		Profilin
	Ara h 6	15	C		Hom: conglutin
	Ara h 7	15	C		Hom: conglutin
	Ara h 8	15	C		PR-10 protein
Pear (*Pyrus communis*)	Pyr c 1	18	C		Hom: Bet v 1
	Pyr c 4	14	C		Profilin
	Pyr c 5	33.5	C		Hom: isoflavone reductase
Pepper (*Capsicum annuum*)	Cap a 1w	23	C		Osmotin-like protein
	Cap a 2	14	C		Profilin
Pineapple (*Ananas comosus*)	Ana c 1	15	C		Bromelin, hom: papain and group 1 of mites
Pistachio nut					Four antigenic fractions of 34, 41, 52 and 60 kD; the first one seems to have the highest binding capacity to IgE [384]
Plum (*Prunus domestica*)	Pru d 3	9	P		LTP
Potato (*Solanum tuberosum*)	Sola t 1	43	P		Patatin
	Sola t 2	21	P		Cathepsin D inhibitor
	Sola t 3	21	P		Cysteine protease inhibitor
	Sola t 4	16+4	P		Aspartic protease inhibitor
Rana esculenta	Ran e 1	119	C		α-Parvalbumin
Rice (*Oryza sativa*)	Ory s 1		C		Allergens RAP and RAG 1, 2, 5, 14, 17 [348]
Rye (*Secale cereale*)	Sec c 20				Secalin
Saffron crocus (*Crocus sativus*)	Cro s 1		P		
Sesame (*Sesamum indicum*)	Ses i 1	10	P		2S protein
	Ses i 2	7	C		Albumin
	Ses i 3	45	C		Vicilin-like globulin
	Ses i 4	17	C		Olesin
	Ses i 5	15	C		Olesin

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Shrimp					
Metapenaeus ensis	Met e 1	34	C		Tropomyosin [295]
Penaeus aztecus	Pen a 1	36	P		Tropomyosin, major allergen [543]
Penaeus indicus	Pen i 1	34	C		Tropomyosin, or seralbumin, with diffused cross-reactivity [543]
Penaeus monodon	Pen m 2	40	C		Tropomyosin
Snail (*Helix aspersa*)	Hel a 1	36	P		Tropomyosin
Soybean (*Glycine max*)	Gly m 1	7	P	>90	Glycoprotein, MW of the monomeric form; Gly m is divided into 7 subunits: the best known are Gly m 1A and Gly m 1B [348], then the Kunitz tryptic inhibitor (3 subtypes)
	Gly m 2	8	P	>90	
	Gly m 3	14	C	>90	Profilin, β-conglycin 3 major subunits, α-1, α-2 and β with MW at 76, 72 and 53 kD, respectively, and lectin
	Gli m 4	17	C		SAM22, Pr-10 protein [265]
	Gly m Bd	30			Isolated from the crude 7S-globulin fraction, β-conglycinin, a trimer with MW at 150–200 kD
	Gly m Bd	60			Glycinin, a hexamer with MW at 300–400 kD, A5–B3 subunit
		30			Component from soybean constituted by two polypeptides (A5 and B3) that cross-react with CM caseins [478]
		25			Protein of GMO soybean reacting with IgE of some patients [674]
		50			Allergen pertaining to soy aeroallergen (asthma outbreaks during unloading of soybean from ships with significant hom with chlorophyll A-B binding protein precursors from tomato, spinach, and petunia [89])
Squid (*Todarodes pacificus*)	Tod p 1	38	P		Tropomyosin
Tomato (*Lycopersicon esculatum*)	Lyc e 1	14	C		Profilin, Ole e 1 homolog
	Lyc e 2	50	C		Isoallergen
	Lyc e 2	50	C		β-Fructofuranosidase
Walnut (*Juglans regia*)	Jug r 1	19	C		2S albumin
	Jug r 2	56	C		Vicilin
	Jug r 3	9	P		LTP
Watermelon (*Citrullus lanatus*)		13			Profilin [468]
Wheat					
Triticum aestivum	Tri a 18	17			Wheat germ agglutinins A and D [348]
	Tri a 19	65	P		Gliadin
Triticum durum					Wheat germ agglutinin [388]
Zucchini (*Cucurbita pepo*)		13			Profilin [468]
Other fruits					Strawberry, banana, tangerine, cherry and kiwi (Chap. 9)

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
2. Fungi (molds)					
Alternaria alternata	Alt a 1	28	C	<80	Similar to Alt a-29, Alt a bd 29 and Alt a 31-kD I_{1563}, 70-kD glycoprotein (GP70)
	Alt a 2	25	C	42	Aldehyde dehydrogenase [348]
	Alt a 3	70	C		Heat shock protein
	Alt a 4	57	C		Isomerase
	Alt a 5	45	C		Enolase
	Alt a 6	11	C	8	P_2 acid ribosomal protein [348]
	Alt a 7	22	C	7	
	Alt a 8	29	C		Mannitol dehydrogenase
	Alt a 10	53	C	51	Aldehyde dehydrogenase [543]
	Alt a 11	45	C		Enolase
	Alt a 12	11	C		Acid ribosomal protein
Aspergillus flavus	Asp fl 13				Alkaline serine protease
Aspergillus fumigatus	Asp f 1	18	C		Mitogillin toxin/ribonuclease [348]
	Asp f 2	37	C		
	Asp f 3	19	C		Peroxisomal protein
	Asp f 4	30	C		
	Asp f 5	40	C		Metalloprotease
	Asp f 6	26.5	C		Mn superoxide dismutase
	Asp f 7	12	C		
	Asp f 8	11	C		Ribosomal protein
	Asp f 9	34	C		
	Asp f 10	34	C		Aspartic protease
	Asp f 11	24			Peptidyl-prolyl isomerase
	Asp f 12.	90	C		Heat shock protein
	Asp f 13	34			Alkaline serine protease
	Asp f 15	16	C		
	Asp f 16	43	C		
	Asp f 17		C		
	Asp f 18	34			Vacuolar serine protease
	Asp f 22w	46	C		Enolase
	Asp f 23	44	C		1.3 ribosomal protein
Aspergillus niger	Asp n 14	105	C		β-Xylosidase
	Asp n 18	34	C		Vacuolar serine protease
	Asp N 25	66–100	C		3-Phytase
	Asp n ?	85	C		
Aspergillus oryzae	Asp o 2		C		
	Asp o 13	34	C		Alkaline serine protease
	Asp o 21	53	C		TAKA-amylase A

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Candida albicans	Cand a 1	40	C		Three more allergens at 37, 43, 48 kD [543]
	Cand a 3	20	C		Perixosomal protein
Candida bodinii	Cand b 2	20	C		
Cladosporium herbarum	Cla h 1	13		>60	
	Cla h 2	23	C	43	Enolase [348]
	Cla h 3	53	C	36	Aldehyde dehydrogenase [348]
	Cla h 4	11	C	22	Acid ribosomal protein P2 [348]
	Cla h 5	22	C	22	
	Cla h 6	46	C	20	Enolase [543]
	Cla h 8	28	C		Mannitol dehydrogenase
	Cla h 9	55	C		Vacuolar serine protease
	Cla h 12	11	C		Acid ribosomal protein P1
Coprinus comatus	Cop c 1	11	C		Leucine zipper protein
	Cop c 2				
	Cop c 3				
	Cop c 5				
	Cop c 7				
Fusarium culmorum	Fus c 1	11	C		Ribosomal protein
	Fus c 2	13	C		Thioroedoxin-like protein
Malassezia furfur	Mala f 1				
	Mala f 2	21	C		Peroxisomal membrane protein
	Mala f 3	20	C		Peroxisomal membrane protein
	Mala f 4	35	C		
	Mala f 5	18	C		
	Mala f 6	17	C		
	Mala f 7		C		
	Mala f 8	19	C		
	Mala s 9	37	C		
Malassezia sympodialis	Mala s 1	18	C		
	Mala s 5	17	C		
	Mala s 6	17	C		
	Mala s 7		C		
	Mala s 8	19	C		
	Mala s 9	37	C		
	Mala s 10	86	C		Heat shock protein
	Mala s 11	23	C		Mn superoxide simutase
Penicillum brevicompactum	Pen b 13	33			Alkaline serine protease
Penicillum chrysogenum	Pen ch 1	33		100	Two more allergens at 64 and 62 kD [543]
	Pen ch 13	34			Alkaline serine protease
	Pen ch 18	32			Vacuolar serine protease
	Pen ch 20	68			*N*-acetyl glucosamine

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Penicillum citrinum	Pen c 3	18			Peroxisomal membrane protein
	Pen c 13	33			Alkaline serine protease
	Pen c 19	70	C		Heat shock protein
	Pen c 22w	46	C		Enolase
	Pen c 24		C		Elongation factor 1β
Penicillum oxalicum	Pen o 18	34			Vacuolar serine protease
Psilocybe cubensis	Psi c 1				
	Psi c 2				Cyclophilin
Saccaromyces cerevisiae					Two allergens at 40 and 48 kD [543]
Trichophyton rubrum	Tri r 2		C		
	Tri r 4		C		Serine protease
Trichophyton tonsurans	Tri t 1	30	P		
	Tri t 4	83	C		Serine protease
3. Grass pollens					
Gramineae					Nearly all allergens show hom: groups 1–3 [543]
Agrostis alba (redtop)	Agr a 1	?	P		
Anthoxanthum odoratum (sweet vernal)	Ant o 1	34	P		
Cryptomerica japonica	Cry j 1	38	C		Cry j 1 is divided into 1A and 1B [348]
	Cry j 2	37	C		Of the same allergenicity [199]
Cynodon dactylon (Bermuda grass)	Cyn d 1	32	C	100	Has several isoforms
	Cyn d 7		C		
	Cyn d 12	14			Profilin
	Cyn d 14	9	C		
	Cyn d 15	9	C		
	Cyn d 22w				Enolase
	Cyn d 23	9	C		
	Cyn d 24	21	P		PR-protein
Dactylis glomerata (orchard grass)	Dac g 1	32	P	>95	
	Dac g 2	11	C	75	
	Dac g 3	C			
	Dac g 5	31	P	>90	
Festuca elator	Fes e 1	34	P		Moreover, Fes e 1A and Fes e 2B [348]
Festuca pratensis (meadow fescue)	Fes p 4w	60			
Helianthus annuu (sunflower)	Hel a 1	34			
	Hel a 2	15.7	C		Profilin
	Hel a 5				Expansin
Holcus lanatus (velvet grass)	Hol l 1	11	C		

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Lolium perenne (rye grass)	Lol p 1	27	C	>90	Group 1
	Lol p 2	11	C	60	Group II
	Lol p 3	11	C	70	Group III
	Lol p 4	57	C	74	
	Lol p 5	11	C	80	Lol p IX, Lol p Ib, ribonuclease (unknown)
	Lol p 9				90% of patients allergic to darnel recognize it together with Lol p 1 [43]
	Lol p 10	12			Cytochrome C, hom: group 10
	Lol p 11	18	65		Hom: trypsin inhibitor, 44% of homology with Ole 1 [189], further Lol p IV, 11 kD and 3 allergens at 30, 34 and 50 kD [347]
Phalaris aquatica (canary grass)	Pha a 1	34	P	77	Hom: group 1
	Pha a 5				Has 4 isoforms [476]
Phleum pratense (Timothy)	Phl p 1	27	C	80	Cross-reacts with group 1 allergens [497]
	Phl p 2	10, 12	C	62	
	Phl p 3	10, 12	C		
	Phl p 4	50, 60	P		Significant hom: Amb a 1/2
	Phl p 5	32	C	80	Ribonuclease (unknown) is divided into 5a and 5b [348]
	Phl p 6	11	C		Additional allergens Phl p of 32 kD and Phl p of 38 K [348]
	Phl p 7		C		
	Phl p 11		C		Trypsin inhibitor hom
	Phl p 12		C		Profilin
	Phl p 13	55–60	C		Polygalacturonase
Poa pratensis (blue grass)	Poa p 1	33	P		All with unknown sequence and group 10 homology
	Poa p 5	31/34	P	>95	
	Poa p 9	29, 35	C		In 3 forms: KBG31, KBG41, KBF60 [348]
	Poa p 10	29			Cytochrome C
Sorghum halepense (Johnson grass)	Sor h 1		C		
Zea mays (maize)	Zea m 1	21	P		
	Zea m 11	14	C		
Euphorbiaceae					
Hevea brasiliensis	Hev b 1	58	P	23–>80	Major allergen, rubber elongation factor
	Hev b 2	34/36	C	21	Major allergen, β-1,3-glucanase, microhelix component
	Hev b 3	24	P	36	Prenyltransferase
	Hev b 4	50–57			Component of microhelix complex
	Hev b 5	16	C	56–92	Major allergen (Table 1.73)
	Hev b 6.01	20	C		Hevein precursor
	Hev b 6.02	5	C		Hevein

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Hevea brasiliensis (continued)	Hev b 6.03	14	C		C-terminal fragment
	Hev b 7.01	42	C	83	Hom: patatin from B-serum, cross-reacting with avocado, potato and tomato
	Hev b 7.02	44	C	23	Hom: patatin from C-serum defense-related protein
	Hev b 7.03				Inhibitor of rubber biosynthesis
	Hev b 8	14	C	>90	Latex profilin structural protein
	Hev b 9	51	C		Latex enolase
	Hev b 10	26	C		Mn superoxide dismutase
	Hev b 11	33	C		Class I endochitinase defense-related protein
	Hev b 12	9.3	C		LTP
	Hev b 13	42	P		Esterase
Ricinus communis (Castor bean)	Ric c 1	11	C	96	Small chain, 4 kD, large chain, 7 kD, 2S storage albumin
	Ric c 2	47			Crystalloid protein [543]
4. Weeds compositae					
Ambrosia artemisifolia (short ragweed)	Amb a 1	38	C	>90	Pectate lyase; hom: Amb a 2, Cry j 1, tomato and maize
	Amb a 2	38	C	>90	Pectate lyase; hom: Amb a 1, Cry j 1, tomato and maize
	Amb a 3	11	C	51	Shows homology with electron transport proteins
	Amb a 4	23			
	Amb a 5	5	C	17	Ra 5
	Amb a 6	10	C	21	Ra 6, lipid transferase (?)
	Amb a 7	12	P	20	Ra 7, shows hom with electron transport proteins
	Amb a 10	12			Cytochrome C
Ambrosia psilostachya	Amb p 5				Hom: Amb a 5
Ambrosia trifida (giant ragweed)	Amb t 5	4.4	C		Hom: Amb a 5
Artemisia vulgaris (mugwort)	Art v 1	27–29	C	>70	
	Art v 2	35	P	33	
	Art v 3	12	P		LTP
	Art v 4	14	P		Profilin
Mercurialis annua	Mer a 1	14–15	C		Profilin
	Mer a 2				
Mercurialis perennis, etc.					
Parietaria judaica	Par j 1	12	C	100	[511] or 2 proteins at 8.8 and 9.8 kD, respectively, with allergens homologous to those of Par o 1 and di *P. mauritanica* and with great cross-reactivity [17]
	Par j 2		C		LTP
	Par j 3		C		Profilin

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Parietaria officinalis	Par o 1	15	P	100	[397] or 3 proteins at 8.8 and 2 9.4 kD, respectively, R 100, with several isoallergens with similar MW [98]
	Par o 2	11		82	Phospholipid transfer protein
Parthenium hysterophorus (feverfew)	Par h 1	31			Extensin
5. Tree pollens					
– Betulaceae					
Alnus glutinosa (alder)	Aln g 1	17	C	>90	Hom: Bet v 1 [348]
Betula verrucosa (birch)	Bet v 1	17	C	>95	PR, isoform Bet v 1 N
	Bet v 2	15	C	10	Profilin
	Bet v 3	20	C	<10	Profilin [348]
	Bet v 4	8	C		
	Bet v 6	33.5	C		Hom: isoflavone resuctase
	Bet v 7	18	P		Cyclophilin
Carica papaya (Papaya)	Car p 1	23	C		Papain
Carpinus betulus (Hornbeam)	Car b 1	17	C	>90	Bet v 1 hom [348]
Castanea sativa (Chestnut)	Cas s 1	22	P		Hom: group 1 of Fagales [543] and with Bet v 1 [348]
	Cas s 5				Chitinase
	Cas s 8	13	P		LTP
Cupressus arizonica	Cup a 1	43	C	81	[355]
Cupressus sempervirens	Cup s 1	43	C	81	
	Cup 3 3w	34	C		
Fraxinus excelsior (ash)	Fra e 1	20	P		
Juniperus ashei	Jun a 1	43	P		Pectate lyase
	Jun a 2		C		
	Jun a 3	30	P		Hom: thaumatin, osmotin, amylase/trypsin inhibitor
Juniperus oxycedrus (prickly juniper)	Jun o 4	29	C		Hom: calmomodulin
Juniperus rigida	50	100			[543]
Juniperus sabinoides (mountain cedar)	Jun s 1	50	C		
Juniperus virginiana	Jun v 1	43	C		
Ligustrum vulgare (privet)	Lig v 1	20	P		
Quercus alba (oak)	Que a 1	17	P		Hom: group 1 of Fagales [543] and with Bet v 1 [348]
– Oleaceae					
Olea europaea	Ole e 1	16	C	>90	Allergens present also in Fra e 1, Lig v 1, Syr v 1 [369] hom: soybean trypsin inhibitor and Lol p 11
	Ole e 2	15–18	C	25	Profilin
	Ole e 3	9.2			Ca^{++}-binding protein
	Ole e 4	32	P	80	Hom: Ole e 1
	Ole e 5	16	P	35	Superoxide dismutase

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Olea europaea (continued)	Ole e 6	10	C		Cysteine-rich protein
	Ole e 7		P	47	
	Ole e 8	21	C		$Ca2^{+}$-binding protein
	Ole e 9	46	C		β-1,3-glucanase
	Ole e 10	11	C		Hom: Glycosyl hydrolase
Phoenix dactylifera(date)	Pho d 2	14.3	C		Profilin
Syringa vulgaris	Syr v 1	20	P	16	
– Plantaginaceae					
Plantago lanceolata	Pla l 1	18	P		English plantain
Platanaceae					
Platanus acerifolia	Pla a 1	18	P		Major allergen [15]
	Pla a 2	43	P		Major allergen [15]
	Pla a 3	10	P		LTP
– Taxoidiaceae or Pinales					
Cryptomeria japonica	Cry j 1	41–45	C	>85	Pectate lyase, hom Amb a 1
	Cry j 2	57		76	Polymethylgalacturonase
6. Animals					
Cat (*Felis domesticus*)	Fel d 1	38	C	>80	Allergens in sebaceous glands and saliva; cross-reaction with pig meat [127]
	Fel d 2		C	23	Albumin
	Fel d 3	11	C		Cystatin
	Fel d 4	22	C		Lipocalin
	Fel d 5w	400			IgA
	Fel d 6w	800–1000			IgM
	Fel d 7w	150			IgG
Cavia porcellus	Cav p 1	20	P		Lipocalin hom
	Cav p 2	17	P		Allergens present in hairs, urine, saliva
Oryctolagus cuniculus	Ory c 1	17			Present in saliva
Dog (*Canis domesticus*)	Can f 1	25	C	>70	Allergens present in skin, saliva, parotid gland
	Can f 2	27	C	23	Parotid gland
	Can f 3	69	C	40	Albumin
	Can f 4	18	C		
Horse (*Equus caballus*	Equ c 1	25	C	100	Lipocalin, the allergen is in the horsehair
	Equ c 2	18.5	P		Lipocalin
	Equ c 3	67	C		Albumin
	Equ c 4	17	P		
	Equ c 5	17	C		Two more 14- and 39-kD proteins [149]

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
– Rodents					
Mouse (*Mus musculus*)	Mus m 1	19	C		Prealbumin; allergens present in urine, liver
	Mus m II (Ag3)	16			Reciprocal homology [543]
Rat (*Rattus norvegius*)	Rat n 1	21	C	60	Allergens in urine, saliva
	Rat n 2	16	C	90	
	Rat n III	>200			
7. Worms					
Anisakis simplex	Ani s 1	24	P		
	Ani s 2	97	C		Paramyosin
	Ani s 3	41	C		Tropomyosin
	Ani s 4	9	C		
Ascaris lumbricoides	Asc l 1				[348]
Ascaris suum (worm)	Asc s 1	10	P		
Thaumetopoea pityocampa	Tha p 1	15			Amino acid sequence with no homologies to any other protein described [365]
8. Insects					
American cockroach (*Periplaneta americana*)	Per a 1	20–25	C	50	Cr-PII, Per a 1 reacts with Bla g 1 [434]
	Per a 3	72–78	C	83	In addition, a protein of the allergenic fraction, Cr-PI, perhaps major allergens [616]
	Per a 7	37	C		Tropomyosin
Australian jumper ant (*Myrimecia pilosuls*)	Myr p 1		C		
	Myr p 2		C		
Black fire ant (*Solenopsis richteri*)	Sol r 1		P		
	Sol r 2		C		PL
	Sol r 3		C		
Bumble bee (*Bombus pennsylvanicus*)	Bom p 1	16	P		PL
	Bom p 4		P		Protease
Cat flea (*Ctenocephalides felis*)	Cte f 1				
	Cte f 2	27	C		
	Cte f 3	25	C		
Midge (*Chironomus thummi*)	Chi t 1–9	16	C		Hemoglobin
	Chi t 1.01	16	C		Component III
	Chi t 1.02	16	C		Component IV
	Chi t 2.0101	16	C		Component I
	Chi t 2.0102	16	C		Component IA
	Chi t 3	16	C		Component II-β
	Chi t 4	16	C		Component IIIA
	Chi t 5	16	C		Component VI
	Chi t 6.01	16	C		Component VIIA
	Chi t 6.02	16	C		Component IX

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Midge (*Chironomus thummi*) (continued)	Chi t 7	16	C		Component VIIB
	Chi t 8	16	C		Component VIII
	Chi t 9	16	C		Component X
European hornet (*Vespa crabo*)	Vesp c 1	34	P		PL
	Vesp c 5	23	C		Antigen 5
German cockroach (*Blattella germanica*)	Bla g 1	20–25	C	50	30%–50% prevalence of IgE antibody
	Bla g 2	36	C	58	Aspartic protease
	Bla g 4	21	C	40–60	Lipocalin
	Bla g 5	22	C		Glutathione transferase
	Bla g 6	27	C		Troponin
	Bla g Bd	90			77% of patients with IgE antibodies [204]
Honey bee (*Apis mellifera*)	Api m 1	16	C		PLA_2, in addition Api III, V and VI at 49, 23 and 105 kD, respectively
	Api m 2	41	C		Hyaluronidase
	Api m 4	3	C		Melittin
	Api m 6	7–8	P		
	Api m 7	39	C		Serine protease
Mosquito (*Aedes aegiptii*)	Aed a 1	68	C		Apyrase
	Aed a 2	37	C		
Paper wasp (*Polistes dominulus*)	Pol d 1				
	Pol d 4	32–34	C		Serine protease
	Pol d 5				
Red fire ant (*Solenopsis invicta*)	Sol i 1	37	P		PL
	Sol i 2	13	C		
	Sol i 3	24	C		Hom: vespid group 5 allergens
	Sol i 4	13	C		Hom: Sol i 2
Tropical fire ant (*Solenopsis geminata*)	Sol g 2				
	Sol g 4				
Solenopsis saevissima	Sol s 2				
Giant Asian (*Vespa mandarina*) hornet	Vesp m 1				
	Vesp m 5				
Vespula flavopilosa	Ves f 5	23	C		Antigen 5
– Wasp					
Polistes annularies	Pol a 1	35	P		PLA_1
	Pol a 2	44	P		Hyaluronidase
	Pol a 5	23	C		Antigen 5
Polistes exclamans	Pol e 1	34	P		
	Pol e 5	23	C		Antigen 5
Polistes fuscatus	Pol f 5	23	C		Antigen 5
Polistes metricus	Pol m 5	23	P		Antigen 5
Wasp (*Vespula vidua*)	Ves vi 5	23	C		Antigen 5

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
White face hornet (*Dolichovespula maculata*)	Dol m 1	35	C		PLA_1
	Dol m 2	44	C		Hyaluronidase
	Dol m 5	23	C		Antigen 5
Yellow hornet (*Dolichovespula arenaria*)	Dol a 5	23	C		Antigen 5
Yellow jacket (*Vespula flavopilosa*)	Ves v 1	35	C		PLA_1: has 67% of sequential identity with Dol m
Vespula germanica	Ves g 5	23	C		German yellow jacket, antigen 5
Vespula maculifrons	Ves m 1	35	C		Eastern yellow jacket, PLA_1
	Ves m 2	44	P		Hyaluronidase
	Ves m 5	23	C		Antigen 5
Vespula pennsylvanica	Ves p 5	23	C		Western yellow jacket, antigen 5
Vespula squamosa	Ves s 5	23	C		Southern yellow jacket, antigen 5
Vespula vulgaris	Ves v 1	35	C		PL
	Ves v 2	44	P		Hyaluronidase: has 92% of sequential identity with Dol m
	Ves v 5	23	C		Antigen 5: has 69% of sequential identity with Dol m and 60% with Pol a; it is possible to set an order of cross-reactivity hyaluronidase >antigen 5> PLA_1 [577]
9. Mites					
Acarus siro	Aca s 13	14	C		Fatty acid binding protein
Blomia tropicalis	Blo t 1	11–13		>47	Cysteine protease
	Blo t 3	24	C		
	Blo t 4	56	C		
	Blo t 5	14	C	70	Shows hom: other allergens
	Blo t 6	25	C		Chymotrypsin
	Blo t 10	33	C		Tropomyosin
	Blo t 11	110	C		Paramyosin
	Blo t 12	16	C		
	Blo t 13		C		Fatty acid binding protein
	Blo t 19	7.2	C		Anti-microbial pepsin homology
Dermatophagoides farinae	Der f 1	25	C	79	Cysteine protease, hom: Der p 1, Eur m 1, papain, cathepsins B and H
	Der f 2	14	C	83	Variants 2.1, 2.2 and 2.3
	Der f 3	34	P	42–70	Trypsin, hom: Der p 3, Der p 6, Der f 6, other trypsins and proteases
	Der f 6	30		31	Chymotrypsin, hom: Der p 3, Der p 6, Der f 3, other chymotrypsins and proteases
	Der f 7	22		46	86% homology and cross-reactivity with Der p 7 [469]
	Der f 9				[112]

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Dermatophagoides farinae (continued)	Der f 10	39	C	81	Tropomyosin, hom: Mag44, highly reactive with IgE like Der f 1, 2 [8]
	Der f 11	98	C		Paramyosin
	Der f 14		C		Mag3, apolipophorin
	Der f 15	98	C		Chitinase
	Der f 16	53	C		Gelsolin/villin
	Der f 17	53	C		Ca binding protein
	Der f 18w	60	C		Chitinase
Dermatophagoides microceras	Der m 1	25	P		Cysteine protease
Dermatophagoides pteronyssinus	Der p 1	25	C	>90	Cysteine protease, hom: Der f 1, Eur m 1, papain, cathepsins B and H
	Der p 2	14	C	>90	Lysozyme?
	Der p 3	28/30	C	51 to >90	Trypsin, hom: Der p 6, Der f 3, Der f 6, other trypsins and proteases
	Der p 4	60	P	25–46	Amylase
	Der p 5	14	C	>55	
	Der p 6	25	P	39	Chymotrypsin, hom: Der p 3, Der f 3, Der f 6 and other chymotrypsins and proteases
	Der p 7	22–28	C	53	IgE and monoclonal antibody bind to Der p 7 [518]
	Der p 8	26	C		Glutathione transferase
	Der p 9	28		>90	Serine protease, hom: groups 3 and 6 of mites
	Der p 10	36	C		Tropomyosin
	Der p 14		C		Apolipophorin-like protein
Dermatophagoides siboney	Der s 1	25			
	Der s 2	14			Correlated with Der f
Euroglyphus maynei	Eur m 1	24	C		Cysteine protease, hom: Der p 1, Der f 1, papain, cathepsins B and H [543]
	Eur m 2		C		
	Eur m 14	177	C		Apolipophorin
Glycyphagus domesticus	Gly d 2		C		
Lepidoglyphus destructor	Lep d 1	14–16	P		Hom: group 2 of mites
	Lep d 2				
	Lep d 5		C		
	Lep d 7		C		
	Lep d 10		C		Tropomyosin
	Lep d 13		C		
Tyrophagus putrescentiae	Tyr p 2		C		

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
10. *Homo sapiens* human autoallergens [639]					
	Hom s 1	73	C		
	Hom s 2	10.3	C		
	Hom s 3	20.1	C		
	Hom s 4	36	C		
	Hom s 5	42.6	C		

Allergens are usually ordered according to their common name: those corresponding to foods are listed among foods.
B column shows the percentage of reactivity [543]. All the known homologies are included [543]. The Der (p, f, m) 1 are considered major allergens, similarly to Der (p, f, m) 2; the latter ones, contrary to the first group, are thermostable and pH resistant; 80%–90% of Der p I is contained in the stools and 10% in the body. All insect allergens correspond to the primary antigen 5 and have identical MW; Can d is employed parallel to Can f, *Canis fidelis* [639].
Updated from [639], other data from [8, 15, 17, 60, 89, 98, 112, 121, 128, 134, 149, 199, 204, 265, 295, 310, 319, 348, 355, 365, 397, 384, 416, 405, 434, 471–473, 478, 497–499, 511, 543, 549, 551, 577, 604, 639, 652, 674].
C/P complete or partial availability of data, *hom* homology, *LTP* lipid transfer protein, *PL*, PLA_1, PLA_2 phospholipase, phospholipase A_1, phospholipase A_2, *PR* pathogenesis related protein, *RAP* rice allergenic protein, *RAG* rice allergen, *R* risk.

Table 1.75. Cow milk allergens

Allergens	MW (kD)	g/l	%	Stability at 100 °C	Allergenicity
Caseins (Bos d 8)		24–28	80	+++	++
αs1	23–27	15–19	42		
αs2	23				
β	24	9–11	25		
κ	19	3–4	9		
γ_{1-3}	12–21	1–2	4		
Whey proteins		5–7	20		
β-Lactoglobulin (Bos d 5)	36	2–4	9	++	+++
α-Lactalbumin (Bos d 4)	14.4	1–1.5	4	+	++
Serum albumin (Bos d 6)	69	0.1–1.4	1	±	+
Immunoglobulins (Bos d 7)	0.6–1	2	–	+	
IgG	150–170	0.5–0.8	1.7		
IgM	900–1,000	0.05–0.1	0.2		
IgA	300–500	0.02–0.05	0.1		

Casein is the major antigen and allergen [121].
β-Lactoglobulin has four genetic variants.
Data from [24, 121].

Table 1.76. T-cell epitopes of allergens

Allergen source	Allergen	Size[b]	T-cell epitopes	Individuals tested	T cells
Perennial allergens					
Acarids					
Dermatophogoides pteronyssinus	Der p 1	24 kD, 222 aa	45–67, 94–104, 117–143	2	TCC
	Der p 1		110–119, 110–131	1	TCL and TCC
	Der p 1		1–14, 1–56, 15-94, 57–130, 95–208		
			188–222, 209–222	18	PBMC
	Der p 2	15 kD, 129aa	1–15, 11–24, 20–33, 29–42, 38–51		
			47–60, 56–69, 92–105, 101–114, 116–129	5	TCL and TCC
	Der p 2		1–20, 11–35, 22–50, 36–60, 51–77, 61–86		
			78–104, 81–96, 91–105, 87–112, 105–129	18	PBMC and TCC
	Der p 2		11–25, 16–31, 21–35, 22–40, 71–86		
			81–96, 82–100, 111–129	1	TCL and TCC
	Der p 2		20-33	2	TCC
	Der p 2		1–15, 11–25, 21–35, 31–47, 41–55, 51–65		
			61–75, 71–86, 81–96, 91–105, 101–115		
			111–129	24[a]	PBMC
Mammals					
Felis domesticus	Fel d I	17 kD	39–52, 53–66 (chain 1)		
		70–92 aa	9–21, 22–35, 57–70 (chain 2)	4	TCL and TCC
	Fel d I	(dimer)	1–17, 9–25, 18–32, 29–42, 37–55, 44–60		
			56–70 (chain I)	53[a]	TCL
			1–22, 12–33, 23–48, 34–59, 49–68, 60–82		
			74-92 (chain 2)		
Seasonal allergens					
Trees					
Betula verrucosa	Bet v I	17 kD, 159aa	2–16, 11–22, 61–72, 77–88, 85–96		
			113–124, 145–156, 147–158	6	TCC
	Bet v I		1–16, 27–40, 35–48, 75–92, 77–92		
			93–110, 141–156	2	TCC
	Bet v I		1–16, 11–26, 61–76, 63–78, 65–80, 75–90		
			77–92, 95–110, 97–112, 111–126		
			113–128, 127–140, 141–156	9	TCC
	Bet v I		1–15, 8–23, 19–33, 29–43, 46–63, 58–73		
			65–79, 73–87, 82–96, 90–104, 117–131		
			99–113, 126–140	3	TCL and TCC
Cryptomeria Japonica	Cry j I	41–45 kD, 353 aa	327–346, 337–353	1	TCC

Table 1.76. (Continued)

Allergen source	Allergen	Size[b]	T-cell epitopes	Individuals tested	T cells
Grasses					
Lolium perenne	Lol p I	34 kD, 240 aa	191–210	1[a]	TCC
	Lol p I		Several[c]	6[a]	PBMC
	Lol p I		1–20, 11–30, 21–40, 31–50, 41–60, 50–70		
			71–90, 91–110, 101–120, 111–130		
			121–140, 131–150, 141–160, 151–170		
			171–190, 181–200, 191–210, 221–240	8[a]	TCL and TCC
	Lol p I		Several[c]	6[a]	PBMC
Phleum protense	Phi p I	34 kD, 240 aa	22–36, 25–39, 34–45, 70–84, 73–84		
			91–102, 97–111, 91–102, 100–114		
			109–123, 121–134, 127–138, 130–141		
			142–155, 157–168, 169–183, 211–225,		
			226–240	9	TCC
Poa protense	rKBG60	28 kD, 268 aa	peptide 5. 99–118, 109–128, 149–168		
			159–178, 169–188, 199–218, 219–238		
			229–248, 239–258, 249-268	13[a]	PBMC
Venom allergens					
Insects					
Apis mellifero (honey bee)	Api m I (PLA_2)	19 kD, 134 aa	50–69, 83–97	1	TCL
	Api m I (PLA_2)		45–62, 74–91, 76–93, 81–92, 81–98,		
			107–124, 111–128, 113–124, 114–131	40[a]	PBMC and TCC
Food allergens					
Birds					
Chicken	Ovalbumin	43 kD, 385 aa	1–33, 198–231, 201–213, 261–277	4	TCC

[a] Even though the presence of several T-cell epitopes was described, T-cell epitopes that were recognized by more than 50% of all individuals tested have been identified in these studies.
[b] Sizes are shown as SDS-PAGE mobility of the native protein (in kD) and as number of amino acids (based on the recombinant sequence).
[c] These papers describe reactivity with several peptide pools. Exact amino acid sequences are not clear.
aa, Amino acids, *PBMC* peripheral blood mononuclear cells, *PLA_2* phospholipase A_2, *TCC*, T-cell clone, *TCL* T-cell line.

Table 1.77. Allergen association with HLA molecules and IgE responses

Allergens	HLA-DR	IgE^+ (%)	IgE^- (%)	RR
Amb a 5	DR2/Dw2	100	24	65
Amb a 6	DR5	85	14	35
Amb a 6	DR5	40	6	23
Lol p 2	DR3	47	15	5.3
Lol p 3	DR3	43	18	3.5
Lol p 3	DR3	57	7	18
Alt a 1	DR4	26	16	1.9
Der p 1	DR3	16	17	>1
Der p 2	DR3	19	16	>1
Bet v 1	DRw52a/c	62	33	2.5
Bet v 1	DRB3*0101	51	30	2.5
Fel d 1	DR1	16	9	2.0
Lol p 1	DR3	36	7	7.3
Lol p 1	DR3	33	14	3.1

Some allergens are shown twice since they are reported in different studies.
Data from [601].
RR relative risk.

Table 1.78. Transgenic or genetically modified foods

Introduced proteins	Crop products and targets
ACC deaminase, antisense PG, antisense ACC synthase	Delays without impairing the tomato's natural ripening and softening to obtain a more concentrated juice
Phosphinothricin acetyltransferase	Renders corn tolerant to herbicides
Neomycin phosphotransferase II	Protects potato from insects and delays tomato's natural ripening and softening
Glyphosate oxidoreductase	Renders corn tolerant to herbicides
Btt-HD1 insecticidal protein	Protects corn and tomato from insects
Btt-HD 73 insecticidal protein	Protects potato from insects
CP4 EPSPS synthase	Renders corn, soy and sugar beet tolerant to herbicides
β-D-glucuronidase	Renders soy tolerant to herbicides

There were controversies regarding GMO. Prohibitions and/or restrictions are expressed almost everyday in several countries.
ACC 1-amino- 1-cyclopropane-carboxylic acid, *Btt Bacillus thuringiensis* subspecies *tenebrionis*, *Btk* = *Bacillus thuringiensis* subspecies *kurstaki*, proteins from strains HD-73 and HD-1, *CP4 EPSPS* 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* strain CP4, *PG* polygalacturonase.
Modified from [347].

Table 1.79. Additional GM foods

Apple	Carrot	Kiwi	Rapeseed oil	Rye
Apricot	Cauliflower	Lemon	Orange	Soybean
Asparagus	Celery	Lettuce	Papaya	Spinach
Barley	Chicory	Licorice	Pea	Strawberry
Bilberry	Colza	Lotus	Peach	Sugar beet
Black currant	Eggplant	Maize	Plum	Sweet potato
Broccoli	Fennel	Melon	Potato	Tomato
Buckwheat	Grape	Mustard	Raspberry	Walnut
Cabbage	Horseradish	Oats	Rice	Wheat

Modified from [110].

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