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TIMES AND TRENDS

Inflammation: patterns and new concepts

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The entry of microorganisms into the body induces an inflammatory reaction. The strength of this reaction is a function of the pathogenicity (and/or virulence) of the organisms and the reactive ability of the host. The histological expression of the host as well as immunological and biochemical responses depend on non-specific barriers which fail to prevent entry and the control, by different systems and in different tissues, of the inflammatory reaction. Inflammation can be classified into acute and chronic inflammation. Obviously, the objective of the process includes repair and destruction of the infectious agent. Histological expression of inflammation reflects the protective response. Animal models have been used in experimental pathology for kinetic studies to describe and analyse the different steps of the inflammatory process. This review summarizes the sequence of events in acute and chronic inflammation and attempts to classify patterns of the inflammatory process according to the infectious agent.

Acute inflammation

Haemodynamic changes, vascular permeability and oedema

Various patterns are possible.

1) The initial variable vasoconstriction of arterioles is followed by vasodilatation inducing increased vascular permeability. The heat and redness are caused by the increased blood flow. These details are different according to the severity of the injury and the site involved. Leakage of blood appears immediately or within minutes after injury, peaks 5-10 min later and decreases within 30 min. This leakage can be induced by bradykinin, histamine, leukotriene and serotonin, and is inhibited by cytochalasin B and EDTA. Histamine and bradykinin from mastocytes and platelets are the two major components of this reaction. Prostaglandins are also involved, particularly PG2 which potentializes the action of histamine and bradykinin. The contraction of endothelial cells (and other mechanisms) results in gap formation facilitating entry of various substances, especially proteins, into the tissues (Joris *et al.*, 1987).

2) Leakage begins immediately and continues for several hours or days, affecting the entire circulatory system, i.e. capillaries, arterioles and venules.

3) Occasionally, the leakage continues for several days or more, also occurring via intercellular gaps.This phenomenon may be caused by thermal injury or bacterial toxins, but the mechanism is unknown. The relationship between endothelial lesions and leakage is complicated, and at least 4 mechanisms have been suggested: i) endothelial cell contraction, opening intercellular gaps; ii) endothelial retraction, with reorganization of cytoskeletal components possibly induced by cytokines including interleukin 1, TNF and IFNγ;

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iii) leukocyte-mediated endothelial injury: proteolytic enzymes may cause endothelial injury resulting in increased permeability; iv) necrosis of endothelial cells in burns or bacterial infections. This reaction corresponds to an immediate and sustained response affecting arterioles, venules and capillaries. Then there may be repair or thrombosis of vessels (Majno *et al.*, 1961).

Leakage provides albumin, fibrinogen, coagulation factors, immunoglobulin, complement components and other proteins to the injured tissue site.

The histological lesions corresponding to these steps of acute inflammation are observed in anthrax caused by *Bacillus anthracis*. Major oedema occurs a few hours after contamination and tissues are invaded by *B. anthracis*. Oedema may be lethal if it forms quickly in the lung, larynx or brain. In the lung, oedema and congestion are generally the first morphologically visible events. Another example is *Legionella pneumophila pneumonia*, an acute infectious disease which may cause death. Bronchus, bronchiolae and alveoli are completely invaded by oedema, mucus secretion and macrophage infiltrates.

Exsudation

Exsudation of leukocytes is the second major feature of inflammation beginning in the first few hours of the inflammatory reaction. This phenomenon is essential for phagocytosis and release of chemical factors. The process is controlled by chemical attractants and involves two steps: margination and emigration.

Margination and adhesion are controlled by adhesion molecules i.e. integrins, selectins and members of the immunoglobulin (Ig) superfamily. Margination of leukocytes is regulated by adhesion between receptors of both endothelial cells and leukocytes.

Selectins E (ELAM-1) and P (GMP 140) are specific for endothelial cells and selectin L for leukocytes.

Molecules of the Ig superfamily include intercellular adhesion molecule (ICAM-1) present at the surface of endothelial cells. It interacts with β2 integrins and lymphocyte-function-associated antigen (LFA-1) of white blood cells (WBC). The vascular cell adhesion molecule (VCAM-1) present on endothelial cells is another member of the Ig superfamily which interacts with the integrin β1, VLA4 of WBC.

The integrins (Hynes, 1987; Kishimoto and Anderson, 1992) include three types of molecules: β1, β2 and β3.The ligands of β1 integrins (VLA1 are present on activated T and B lymphocytes and monocytes, VLA2 on T lymphocytes, VLA3 on CTL and monocytes, VLA4 and LPAM 2 on resting lymphocytes, monocytes and thymocytes, VLA-6 on T lymphocytes and monocytes and CD 51/CD29 absent on leukocytes) include collagen, fibronectin and laminin. The β2 integrins (LFA-1 on T or B lymphocytes, macrophages and NK cells; MAC-1 and p150, 95 on monocytes-macrophages, granulocytes and NK cells) may interact with ICAM 1, ICAM 2, ICAM 3 or various infectious agents *(Leishmania, Bordetella)*.

The β3 integrins (gpIIb/IIIa, vitronectin receptor on B lymphocytes and monocytes and the leu-

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CMV = cytomegalovirus.
EBV = Epstein Barr virus.
ECM = extracellular matrix.
EGF = epidermal growth factor.
ELAM = endothelial adhesion molecule.
GF = growth factor.
FGF = fibroblast growth factor.
HNP = human neutrophil peptide.
ICAM = intercellular adhesion molecule.
IFN = interferon.
Ig = immunoglobulin.
LFA = lymphocyte function associated antigen.
MBP = major basic protein.
MHC = major histocompatibility complex.
NK = natural killer.
NO = nitric oxide.
PDGF = platelet-derived growth factor.
PG = prostaglandin.
TCR = T-cell receptor.
TGF = transforming growth factor.
TNF = tumour necrosis factor.
VCAM = vascular cell adhesion molecule.
VEGF = vascular endothelial growth factor.
VLA = very late antigen.
VPF = vascular permeability factor.
WBC = white blood cell.

kocyte response integrin on monocytes and granulocytes) also have specific ligands which include fibronectin, vitronectin and the von Willebrand factor. These adhesion molecules may be complementary molecules enabling the attachment of WBCs to endothelium or to filamentous proteins of the cytoskeleton (collagen, laminin). Then, the WBCs cross the endothelium barrier (gaps). Several mechanisms are implicated: the distribution of adhesion molecules to the cell surface (P-selectin is redistributed after stimulation by histamine and thrombin), the induction of adhesion molecules by cytokines (IL1 and TNF) and the avidity of binding.

Migration of WBCs

WBCs migrate when their chemical attractants contact their cell surfaces (C5a, leukotriene B4, fibrinopeptides, neutrophil proteins, bacterial factors, products of collagen or fibronectin and cytokines belonging to the IL8 family). Polymorphonuclear cells are induced to insert pseudopods into endothelial junctions by numerous molecules. The Bc protein induces activation of phospholipase C. Phospholipase A2 may be activated by contractile elements assembled by Ca^{++} triggers and Ca^{++}, and is involved with actin-binding protein, acumentin, calmodulin and gelsolin in transforming actin into fibrillar forms allowing expansion or contraction. There also exist other mechanisms, such as hydrolysation of PIP2 into PIP3.

Neutrophils are present at the beginning of the process up until the first 24 h and represent the first cell population at the site of the lesion. They die at between 24 and 48 h. Monocytes emigrate later and continously and are present after the death of neutrophils.The exact composition of the cell population present at the site of inflammation depends on the pathogen (virulence, dose effect) chemoattractants, adhesion cells present in the tissue, cytokines and other biochemical factors.

The tissue reacts by acute suppurative diffuse inflammation: *Clostridium* cellulitis, anthrax, septicaemia caused by pyogenic bacteria or localized (abscess induced by pyogenic bacteria) with a majority of neutrophils. As the inflammatory process continues, neutrophils may be present for a prolonged period, particularly in pyogenic bacterial infections.

Phagocytosis can be defined as the killing of microorganisms. Two cell types are able to phagocytose: neutrophils and macrophages. Phagocytosis involves three steps: recognition and attachment, engulfment and degradation.

Macrophages recognize most of the microorganisms which are coated by opsonins. The Fc fragments of immunoglobulin G and C3b are the two major opsonins coating the Fcγ R(Fc) and complement receptors 1, 2 and 3 (C3b and C3bi) on the macrophages (Unanue, 1987).

Engulfment is promoted by activation of receptors (C3 receptor) resulting from binding fibronectin, laminin or cytokines. The cytoplasm becomes enlarged, with a pseudopod form creating a phagocytic vacuole fusing with the membrane of a lysosomal granule. The discharge of the granule's contents into the phagolysosome corresponds to the degranulation of neutrophils or monocytes/macrophages. This process is associated with a higher concentration of cytosolic calcium, activation of phospholipase C and protein kinase, and increased DAG and IP3 production.

The last phase is that of killing and degradation. This process is closely associated with oxygen-dependent mechanisms. Phagocytosis induces a burst (activation of NADPH oxidase) of production of reactive oxygen metabolites, reducing oxygen to superoxide ion (O_2^-). This biochemical process (not detailed here) provides large amounts of H_2O_2 in the phagolysosome vacuole. H_2O_2 is converted by myeloperoxidase granules into HOCl which destroys bacteria (halogenation or lipid peroxidation) and also viruses, protozoa *(Trypanosoma, Toxoplasma, Leishmania),* fungi (yeasts) and other parasites *(Filariae* and *Schistosoma).* This mechanism is involved in most cases of infectious diseases. However, bacterial killing may occur independently of the oxidative burst, by way of NO (NO synthetase), lysozyme and defensins from leukocytes, major basic protein of eosinophils and also

a bactericidal permeability-increasing protein. Defensins, for example, represent 50% of granule proteins in leukocytes and four molecules are known: human neutrophil peptides (HNP) 1 to 4. They are able to destroy *Herpes simplex, Cryptococcus neoformans,* some Gram-positive and Gram-negative bacteria and *Treponema.*

In schistosomiasis, the degranulation of eosinophils contributes to the destruction of the parasite and in allergic diseases the liberation of aryl sulphatase and histaminase induces inactivation of leukotrienes and histamine.

These steps in the acute inflammatory response are orchestrated by interactions between chemical mediators (arachidonic acid metabolites), vasoactive amines (histamine from mast cells, serotonin from platelets and mast cells), plasma proteases especially complement, kinin and the clotting system, and cytokines.

This review cannot address all these factors, which have been described elsewhere. Two cytokines are present in inflammatory foci: interleukin 1 (IL1) and TNF. They coordinate mechanisms playing a major role in the inflammatory process. Indeed, IL1 was first recognized as a stimulator of T cells; IL1 and TNF have cytotoxic effects on vascular endothelium, bone, cartilage and muscle. IL1 (α and β) also mediates reversible and dose/time-dependent increases in: i) synthesis of complement proteins; ii) synthesis of alpha-1 antitrypsin and acute phase proteins; iii) production of interferon-beta 2; iv) activation of neutrophils and macrophages; v) induction of prostaglandins.

Immunological reactions and outcomes

The host-bacteria (or virus or parasite) interaction may have any of several outcomes which can be classified into 3 groups: complete resolution and return to normal, healing characterized by fibrosis or abscess formation, frequent with pyogenic organisms.

These immunological reactions contribute to determining outcome. If the innate system (lysozyme, arachidonic acid metabolites, acute phase reactants, interferon α and β, neutrophils and NK cells) predominates during the early period, the adaptative system can be activated for a prolonged time and establish immunological memory to prevent reinfection (antibodies, soluble factors induced by macrophages, lymphocytes and plasmocytes).

Complement has an ambiguous role, since the alternative pathway belongs to the innate immune system, although the classical pathway is part of the adaptative immune system. Macrophages also play a pivotal role: they belong to the phagocytic system (innate) and act as antigen-presenting cells in the adaptative immune system. Macrophages perform their immune function by displaying all foreign antigens on their surface in close association with MHC molecules. T suppressor cells are combined with the class I MHC, and T helper cells recognize class II MHC. This phenomenon reflects genetic differences regulating susceptibility to infectious diseases, although numerous non-HLA factors are now implicated in genetic susceptibility to infectious diseases. Individual MHC molecules determine which antigens will be recognized by T cells. Other cells belonging to the mononuclear phagocyte system are involved in inflammation: migrating Langerhans cells, interdigitating Langerhans cells and follicular dendritic cells have also been implicated in the recognition of foreign particles.

Chronic inflammation

Progression of the tissue response and failure to resolve an infection may result in chronic inflammation. The definition is "inflammation for prolonged periods (weeks or months), in which tissue destruction, inflammatory cell recruitment and a healing process proceed simultaneously". In fact, this term covers several phenomena.

1) Persistent infection by certain microorganisms (*Treponema pallidum, M. tuberculosis,* yeasts and intracellular parasites), inducing repeated bouts of acute inflammation. Generally, these infectious agents are intracellular, and the infiltrate displays a "specific" pattern considered as a "granulomatous reaction", although the term "specific" is incorrect. The infectious agent may

multiply in the phagocytic vacuole of macrophages (persistence or multiplication), or extracellularly (multiplication).

2) Autoimmune responses induced by autoantigens or other mechanisms, inducing prolonged recruitment of T cells.

Chronic inflammation appears as infiltration by macrophages, lymphocytes and plasma cells for a prolonged period following the persistence of a pathogen (for example, *M. tuberculosis*). Chronic macrophage accumulation occurs during continued recruitment of macrophages (chemotactic substances), local survival of macrophages and proliferation. Morphologically, recruitment of inflammatory cells (granuloma and granulomatous inflammation) is correlated with fibrosis and sometimes an eosinophilic reaction.

Granulomatous inflammation

Various approaches have been used to study and classify granulomas. They may be classified as immune and non-immune granulomas, or high-turnover and low-turnover granulomas. The first cell to encounter the foreign agent is the macrophage. The exogenous antigen may be degraded (totally or partially, slowly or rapidly) within the lysosomal vacuoles of the macrophages. Substances are internalized and then coexpressed on its surface with class II major histocompatibility complex molecules.

Pathologists also describe granulomas as necrotic, fibronecrotic or tuberculoid, with or without giant cells. Granulomas consist of aggregations of macrophages with an epithelioid pattern, surrounded by lymphocytes and sometimes plasma cells. Giant cells with a large cytoplasm and containing 5 to 20 nuclei may also be observed. Macrophages are encountered in numerous infections caused by intracellular bacteria, of which tuberculosis is the best known (but also cat scratch disease, lymphogranuloma inguinale, leprosy, brucellosis, listeriosis, etc) and numerous intracellular parasites *(Leishmania, Trypanosoma, Toxoplasma)* and/or fungi *(Histoplasma blastomyces, Coccidioides).*

Necrosis is a fickle feature, frequent in tuberculosis (TB), with a caseous pattern, cat scratch disease, and yeasts (histoplasmosis), but is rarely observed in leprosy and sarcoidosis. Necrosis was attributed to TNF but, in fact, numerous factors are involved. Pathologists distinguish between foreign body granuloma and immune granuloma related to T-cell-mediated immunity. Most cells belong to the mononuclear phagocyte system or the lymphocyte lineage, and generally a few polymorphonuclear cells are present.

The concept of immune granuloma suggests a specific T-cell-mediated response, with $CD4^+$ T cells present within the granuloma in epithelioid forms (tuberculoid leprosy, benign histoplasmosis, leishmaniasis), where immunity is considered normal. In TB affecting HIV patients, epithelioid lesions and giant cells form only in patients with a CD4 T-cell density above 200 µl.

CD4 T cells are present in foamy lesions, and macrophages contain numerous bacilli but do not express the interleukin 2 receptor (IL2R/CD25).

The role of T-cell recruitment has been studied in leprosy. The CD4/CD8 ratio is 1.2-5/1 in tuberculoid and 0.2-1/1 in lepromatous lesions where bacilli are numerous within macrophages. In lepromatous lesions, the levels of messenger RNA coding for IL4, IL5 and IL10 are high. Messengers for IL2 and IFNγ were abundant in tuberculoid lesions (Yamamura, 1991).

Macrophage activation (table I) was defined as competence for phagocytosis, including destruction of bacteria or tumour cells associated with presentation of antigens and production of cytokines (IL1, TNF). All these functions destroy exogenous antigens. This is a complex phenomenon, as evidenced by the membrane receptors. There are at least 50 different receptors on the membrane of mononuclear phagocytes and more than 100 secreted products (Adams and Hamilton, 1984). The different steps in macrophage activation are summarized in table I. TNFα and IL1 are the two major molecules implicated in the formation of granulomas. A vigorous granulomatous response is induced, for example, when agarose beads coated by IL1 or TNFα are instilled in the trachea of mice, although no signifiant cellular response appears with uncoated beads. Other

Table I. Activated macrophage and granulomatous inflammation.

Macrophage	Non-primed	Primed	Activated
Morphology			
Size	=	↗	↗ epithelioid
Adhesion	weak	↗	↗
Phenotypic characteristics			
Class II MHC(Ia)	+/–	+++	+
MTB	++++	+	+
LFA; CR3, FccR	–	+	+
Metabolic characteristics			
H_2O_2	+/–	+	+++
NO_2^-, NO_3^-	–	–	++
PGE2	+/–	+	+
Secreted TNF	–	+/–	++++
Secreted IL1	–	+/–	++++
CP/secreted protease	–	–	++++
Functions			
Proliferative	+/–	+/–	–
Bactericidal	+/–	+	++++
Kill tumour cells	–	–	++++
Present antigen	–	++++	++
Induction	–	IFNγ,α,β	LPS, TNFα, MDP, GM-CSF1 BSF1, calcitriol, retinoic acid

factors and macrophage mediators implicated include arachidonic acid metabolites, prostaglandin E (which downregulates class II molecule expression) and prostaglandin F2a (an upregulator). The presentation of antigens stimulates T cells to release cytokines (IL2 and IFNγ).

Fibrosis

Fibrosis and scarring show similarities with healing and involve four components: i) formation of blood vessels; ii) proliferation of fibroblasts; iii) maturation and organization of the fibrous tissue; and iv) deposition of extracellular matrix (ECM).

This process begins 24 h after injury and involves fibroblasts and endothelial cells proliferating for several days. The process is stimulated by fibroblast growth factor, vascular endothelial growth factor and vascular permeability factor. These molecules mediate angiogenesis and are often produced by activated macrophages. Migration and proliferation of fibroblasts are also triggered by PDGF, EGF, TGFβ and other fibrogenic cytokines and activating metalloproteases (enzymes that degrade the ECM). It has been postulated that TGFβ is essential for the development of fibrosis Of the three isoforms TGFβ1, β2 and β3, isoform β1 is probably the most active stimulator of fibroblast migration, synthesis of

collagen and fibronectin. Simultaneously, collagen is synthesized and degraded, permanently remodeling the connective tissue network.

Fibrosis occurs in numerous infectious diseases, for example, late syphilis, schistosomiasis, chromomycosis and lobomycosis. Fibrosis may be considered to be a host factor restricting the spread of the disease.

Eosinophil function

Eosinophilia is induced by parasitic antigens (*Schistosoma, filariae,* etc.) or allergens. Eosinophilic maturation takes 5-6 days and surface receptors appear for immunoglobulins and complement.

The cytoplasmic granules contain large amounts of histaminase, peroxidases, aminopeptidases, cationic proteins, phospholipase D and lysophospholipases which all contribute to the destruction of foreign substances. Other granules contain the major basic protein suspected of being involved in the Splendore Hoeppli phenomenon described in zygomycosis, sporotrichosis and some parasites, including schistosoma and filariae.

Eosinophilia is dependent on lymphokines (IL5, IL10) released by sensitized T lymphocytes. Other factors involved are C5a, chemotactic for eosinophils, histamine, leukotriene B4 and hydroxy-eicosatetranoic acids. Eosinophils bear C3b and Fc receptors and may kill schistosomes and other parasites via degranulation and release of major basic protein. Eosinophils may also function as phagocytes (parasites and immune complexes).

Morphological patterns in acute and chronic inflammation (figs. 1 and 2)

These patterns are generally well known, although they are not always easily explained. The morphological, biological and molecular features of new or emerging diseases can be used for classification. These histological patterns, in turn, can be used to make reasonable predictions about the causative agents (Robbins, 1994; Rubin, 1990). The study of kinetics, membrane receptors, identification of target cells, various biological approaches (complement, cytokines, animal knockout) and integration of genetic material in cases of chronic inflammation caused by viruses are generally fertile ground in physiopathological research.

The following classification is an attempt to separate morphological patterns of inflammation.

No or superficial (non-penetrating) cell destruction

Generally, there are no lesions in such cases. Sometimes, a mild lymphoplascytic response may be observed, as is the case for non-pathogenic agents and others, i.e. coronaviruses, rhinoviruses, rotaviruses, *Bacillus anthracis* (toxin), *Vibrio cholerae* (toxin), CMV and herpes virus during the latency period. Parasites *(Toxoplasma* or *Leishmania)* are responsible for latent infection which may disseminate when the $CD4^+$ T-cell count decreases in immunocompromised patients.

Necrosis: extensive cell death (necrosis or apoptosis)

A lymphocytic response may be observed, for example, with *Rickettsias, CMV, herpes,* hepatitis virus including the yellow fever virus and all flaviviruses, as well as chlamydia infections; with a host response: prions (Creutzfeld Jacob disease, kuru), fungal infections in immunocompromised patients, including *Cryptococcus neoformans,* and *Fusarium;* or in cytopathic inflammation: viruses inducing a nuclear inclusion (*CMV* and *herpes*) or a cytoplasmic inclusion or characteristic vacuole: rabiesvirus, poxviruses, Ebola virus. Among bacteria, the most striking example is *Chlamydia trachomatis* (urethritis or cervicitis).

Serous inflammation (outpouring of a thin fluid) in the peritoneal, pleural or pericardial cavities

All RNA viruses (rubella and parvovirus) are included in this group. Pneumococcal

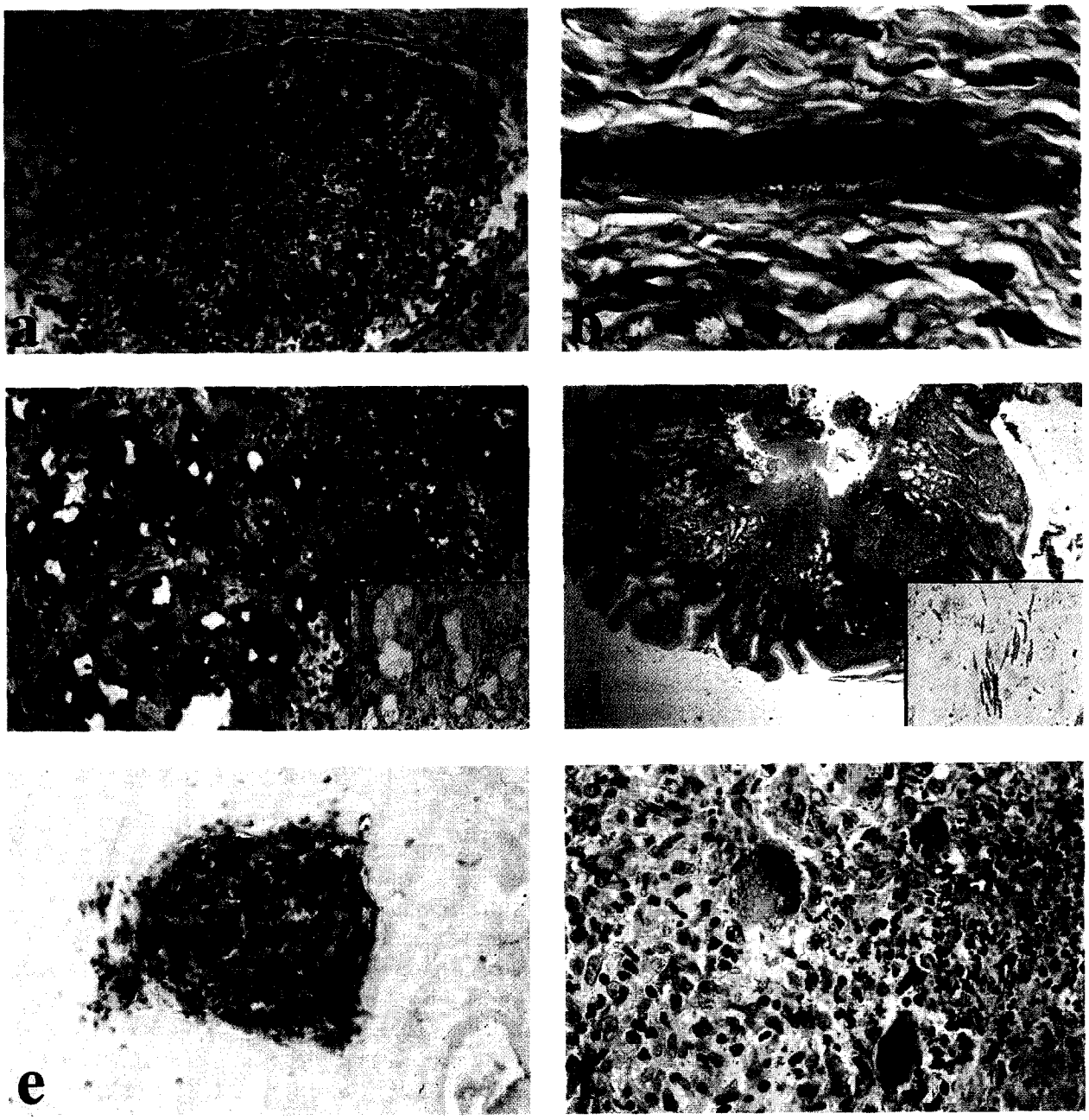

Fig. 1. Histopathological patterns induced by bacteria, fungi and parasites (light microscopy).

a) Invasive pulmonary aspergillosis in a leukaemic patient. Infarct and destruction of lungs by *Aspergillus hyphae*. HE ×250. b) Leprosy. Accumulation of *M. leprae* in a Schwann cell. Fite Cambre Turner method (Ziehl) ×1000. c) Bacillary angiomatosis in an HIV-positive patient. Proliferation of vessels and polymorphonuclear cell infiltrate. HE ×250. *Inset:* Clusters of *Bartonella* (ex-*Rochalimea*) adhering to the basal pole of endothelia. Whartin Starry stain ×400. d) Chronic lymphocytic gastritis due to *Helicobacter heilmanii*. HE ×100. Insert: *H. heilmanii* in a crypt. Whartin Starry ×1000. e) Chronic gastritis with numerous lymphocytes in the chorium. Follicular pattern. Combined double immunohistochemistry. APAAP and avidin biotin method. High density of B cells (blue) compared to the T-cell population (red). (Collaboration A. Labigne and R. Ferrero). f) A model of chronic granulomatous lesion in human pathology: leishmaniasis. Combination of abscess and giant epithelioid cells. Very few parasites. HE ×250.

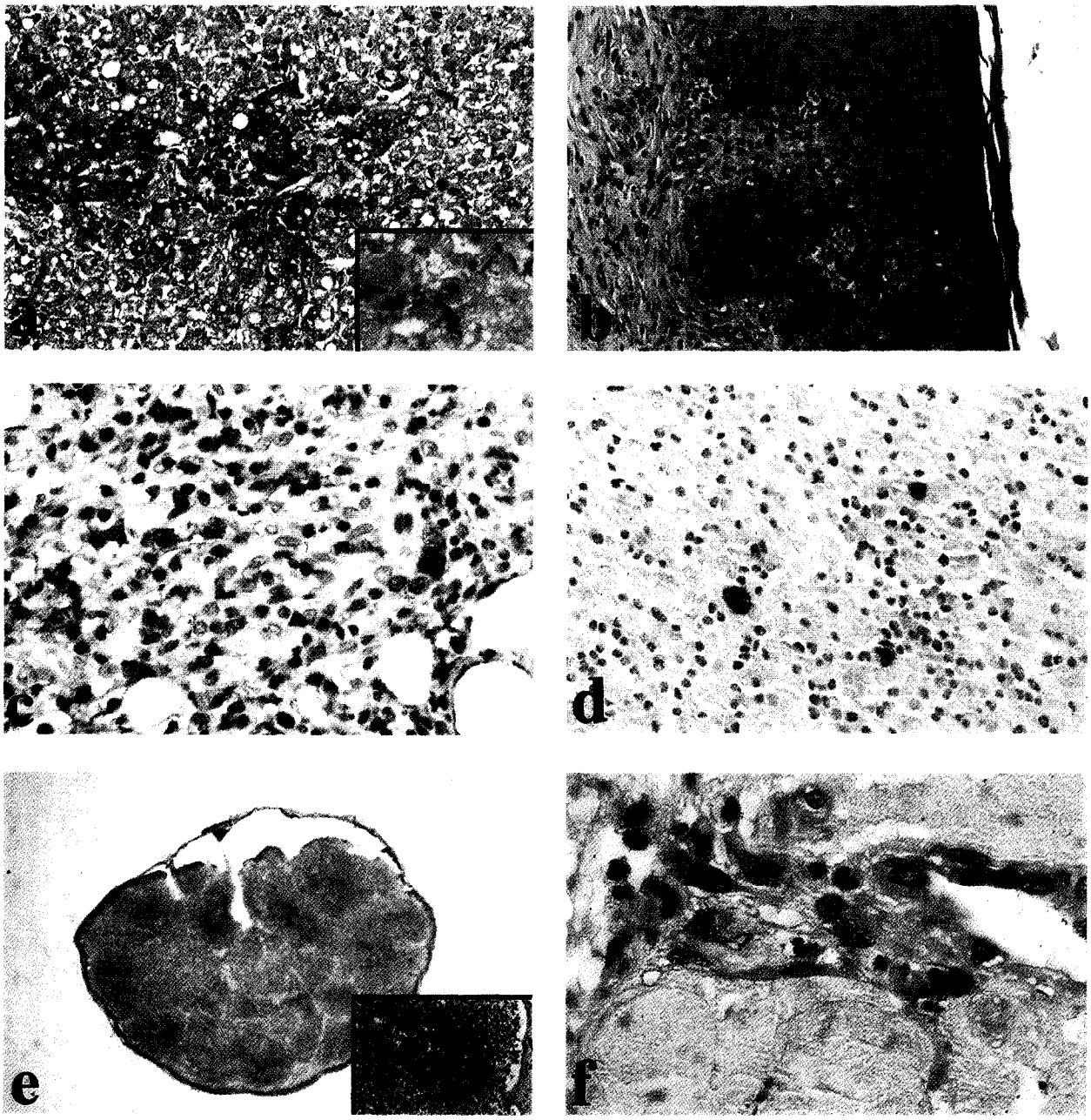

Fig. 2. Histopathological patterns induced by viral infections (light microscopy).

a) Acute viral inflammation: yellow fever in a non-vaccinated patient. Midzonal necrosis, steatosis and accumulation of virus in the cytoplasm of hepatocytes. Immunohistochemistry using anti-yellow fever virus. APAAP method ×400. *Inset:* Fragmentation of a nucleus of a hepatocyte suggesting apoptosis. ×1000. b) T-cell lymphoma, mycosis fungoid related to chronic HTLV-I infection in man. Cluster of atypical cells in the epidermis. HE ×250. c) Same patient. T-cell lymphoma. Immunohistochemistry with CD30 (Dako). APAAP method ×400. d) Hodgkin's disease in an HIV-positive patient. Sternberg cell (CD15- and CD30-positive cells: data not shown). Epstein-Barr virus. *In situ* hybridization using the EBER probe ×400. (ARN EBER-1-biotinylated oligonucleotide probe, Dako). e) Lymph node of a green monkey experimentally infected with SIV. Network of follicular dendritic cells (FDC) in follicles. Frozen section. APAAP method ×100. *Inset:* follicular dendritic cell network APAAP ×400. Collaboration with the Unité de Biologie des Retrovirus (F. Barré-Sinoussi) and the Institut Pasteur, Bangui (J. Morvan). f) Liver of an HIV-positive patient with disseminated histoplasmosis. Macrophage infiltrate. TNFα accumulation in infected macrophages. Paraffin section. APAAP method ×400.

infection and *M. tuberculosis* also cause serous inflammation.

Fibrinous inflammation following vascular leakage (pericardium and pleura)

Fibrinous inflammation may be associated with serofibrinous or fibrinopurulent patterns. These lesions are observed in cases of plague *(Yersinia pestis),* diphtheria, bacillary dysentery *(Shigella),* pseudomembranous colitis *(Clostridium difficile),* amoebic meningoencephalitis caused by *Acanthamoeba* and acute pneumonia due to *Streptococcus pneumoniae.*

Suppurative or purulent inflammation, diffuse or localized

This may be diffuse or localized with numerous neutrophils: all pyogenic bacteria (Gram$^+$ or $^-$), *Nocardia, Actinomyces* and *Clostridium* characterized by a deficient neutrophilic response inducing a suppurative inflammation.

It may involve an abscess with eosinophils: migration of metazoas as *Fasciola* in the biliary duct, *Paragonimus* in the lung; microfilariae in lymph nodes and skin, mycosis, including zygomycosis.

Ulcers of organs or tissue

Necrosis is common in the mucosa of the mouth, stomach, small intestine and colon, and also in the urogenital tract. The best examples are peptic ulcers of the stomach and duodenum, amoebic ulcers, *Yersinia* infections and all pyogenic bacteria and genital infections including granuloma inguinale, syphilis (plasma cells and perivascular lymphocytes) and chancroid (polymorphonuclear cells and monocytes).

Ulcers develop from acute inflammation (polymorphonuclear infiltration, vascular dilatation, then necrosis) to become chronic lesions (scarring, accumulation of lymphocytes and plasma cells) which can subsequently heal.

Association of acute and chronic, diffuse or localized inflammation

a) Diffuse, with neutrophils: *Legionella pneumophila* or *Mycoplasma pneumoniae* infection, *Campylobacter, Listeria, Nocardia* and *Actinomyces* infection, the latter with sulphur granules.

b) Diffuse, with eosinophils or *Splendore hoeppli* phenomena: fasciolasis, trichomoniasis and sporotrichosis (very rare yeasts and eosinophil material surrounding the yeast); filariae and numerous nematodes.

c) With abscesses, such as yaws and various mycotic infections (candidiasis, cryptococcosis, blastomycosis).

d) With extensive necrosis and vascular destruction: aspergillosis, mucormycosis and *Fusarium* infection.

Chronic inflammation

a) Diffuse with lymphocytes and:

— macrophages: *Leishmania,* atypical mycobacteriosis, *Klebsiella, Staphylococcus,* lepromatous leprosy, histoplasmosis and other intracellular yeasts;

— plasma cells: *Klebsiella* (Rhinoscleroma) and Treponema (Pinta); African trypanosomiasis and chagasic myocarditis, malakoplakia;

— epitheloid and giant cells: leishmaniasis, *Histoplasma capsulatum* and *Histoplasma var duboisii,* yeasts such as lobomycosis and spherules, for example, coccidioidomycosis and rhinosporidiosis.

b) Nodular patterns of mononuclear and plasma cells:

— all the *Borrelia (Borrelia recurrentis, burgdorferi)* and *Trypanosoma* with a perivascular distribution;

— with eosinophils and epithelioid cells surrounding the infectious agent: nematode adults or larvae, cysticercosis and echinococcosis, schistosomiasis eggs.

c) Granulomatous:

— with suppurative or caseating necrosis: cat scratch disease, *Yersinia*, tuberculosis, yeasts including *Blastomyces, Fonsecaa, Cladosporium* and *Sporothrix;*

— fibrocaseous: *Mycobacterium, Histoplasma capsulatum, Coccidioides immitis, Cryptococcus;*

— with eosinophils: all nematodes, larvae, trematodes such as schistosomiasis and fasciolopsis;

— with fibrinoid: rickettsial disease (Q fever);

— without central necrosis: tuberculoid leprosy, leishmaniasis, sarcoid, cat scratch disease, toxoplasma.

These histological expressions of inflammation are generally associated with particular pathogens in tissues. These pathogens can be identified by standard staining in tissue sections and also by standard microbiological or virological methods.

Immunohistochemistry on tissue sections has been widely used, and new molecular biological techniques, particularly *in situ* hybridization and consensus sequence-based polymerase chain reaction, have led to the identification of conventional infectious agents and also unculturable infectious agents. For emerging diseases, the identification of the infectious agent is the first step in epidemical control and pathophysiological study of the disease.

Novel aims in pathophysiology

New pathogens and molecular approaches

Numerous agents have been identified since the end of World War II. They are summarized in table II. This list is not exhaustive and numerous varieties or atypical strains can be added (Chabasse, 1994; Fenelon, 1996; Lederberg *et al.*, 1992; Walker *et al.*, 1996). Pathogenicity is a function of immunity and host cell resistance and numerous human opportunistic infections appear to be associated with AIDS infection or granulocytopenia. Modern techniques of molecular biology enable identification of pathogens and have revolutionized our knowledge of diseases (Gao and Moore, 1996). Examples are the demonstration of the link between AIDS, Kaposi sarcoma and herpesvirus 8 (HH8), AIDS lymphoproliferative disorders, particularly lymphoma and Epstein-Barr virus (EBV) (Audouin *et al.*, 1992), the discovery of new viruses such as HCV, Ebola and Hantaan virus, and insight into HTLV-I-related pathology.

Phylogenetic studies of *Bartonella* and the study of rRNA gene sequences showed that *Bartonella* caused bacillary angiomatosis and cat scratch disease. Consensus sequence PCR also enabled linking of *Tropheryma whippelii* to Whipple disease. The presence of *Helicobacter pylori* has been shown to be closely correlated with human ulcers and chronic gastritis; a murine experimental model using *Helicobacter felis* can be considered as a model of human low grade B-cell lymphoma, referred to as MALT syndrome (Enno *et al.*, 1995): double-immunohistochemistry demonstrates that B lymphocytes largely predominated, although a few T cells persisted (fig. 1e). Rearrangement of genes could explain the selection of B cells by specific antigens, but there is no convincing argument in favour of lymphoma.

Cellular microbiology

Recent studies have focussed on the internalization of pathogenic bacteria in cells and the subsequent events, with commonly used *Listeria, Shigella, Salmonella* and *Yersinia* as models (figs. 3 and 4). These studies can be considered to be the first in a new discipline, cellular microbiology (Cossart, 1996) in which pathogens are used to investigate cell biology, host responses and inflammatory processes. One of the main subjects of this new approach is the actin network and, more generally, the cytoskeleton. The control of actin dynamics is poorly understood, but bacteria which can be easily manipulated may provide useful tools to analyse the cascade of events and their control pathways.

Table II. New infectious agents identified since the end of World War II.

Agent	Year	Disease
Bacteria:		
Vibrio vulnificus	1976	Necrotizing fascitis
Clostridium	1977	Pseudomembranous colitis
Legionella pneumophila	1977	Legionnaire's disease
Campylobacter sp.	1977	Enteric pathogens
Borrelia burgdorferi	1982	Lyme disease
Escherichia coli 0157:H7	1982	Haemolytic uraemic syndrome
Helicobacter pylori	1983	Gastric duodenal ulcers
Hemophilus influenzae aegyptius	1984	Brazilian purpuric fever
Chlamydia pneumoniae	1986	Pneumonia
Ehrlichia chaffeensis	1991	Human monocytic Ehrlichiosis
Bartonella henselae and other *Bartonella*	1992	Cat scratch disease and bacillary angiomatosis
Vibrio cholerae 0139	1992	Cholera; new strain
Tropheryma whippelii	1992	Whipple disease
Rickettsia felis	1994	Cat flea-associated typhus fever
Viruses:		
Marburg virus	1967	Marburg haemorragic fever
Lassa virus	1969	Lassa fever
Norwalk agent	1972	Infantile diarrhoea
Rotavirus	1973	Fifth disease
Parvovirus	1975	Chronic haemolytic anaemia
Ebola virus	1976	Ebola haemorrhagic fever
Hantaan virus	1977	Haemorrhagic fever with renal syndrome
Delta virus hepatitis	1977	Hepatitis B coinfection
HTLV-I	1980	T-cell lymphoma-I, leukaemia and tropical spastic paresis
HIV1	1983	AIDS
Herpesvirus 6	1988	Exanthem subitum
Guarnito virus	1989	Venezuelan arenaviral haemorrhagic fever
Hepatitis C virus	1989	Non-A,non-B hepatitis
Sabia virus	1990	Brazilian arenaviral disease
Hepatitis E virus	1990	Enteric non-A,non-B hepatitis
Herpesvirus 7	1990	Exanthem subitum
Barmah Forest virus	1992	Polyarthralgia
Sin Nombre virus	1993	Hantavirus pulmonary syndrome
Herpesvirus 8	1994	Kaposi's sarcoma
Equine morbillivirus	1994	Pneumonia and encephalitis
Parasites:		
Cryptosporidium parvum	1976	Cryptosporidiosis
Enterocytozoon bienseusi	1985	Microsporidiosis
Balamuthia mandrillaris	1990	Leptomyxid amoebic meningo-encephalitis
Encephalitozoon hellem	1991	Microsporidiosis
Cyclospora cayetanensis	1993	Diarrhoeal illness
Encephalitozoon intestinalis	1993	Microsporidiosis
Mycoses:		
Penicillium marneffei	1959	Disseminated forms in AIDS since 1985
Fusarium	1970	Disseminated forms in leukaemia
Scedosporium	1984	Hyalohyphomycosis
Exserohilum rostratum	1995	Disseminated infection in haematology
Microascus cinereus	1995	Disseminated
Chaetomonium globosum	1996	Pneumonia
Aspergillus granulosus	1995	Pneumonia and cutaneous lesions

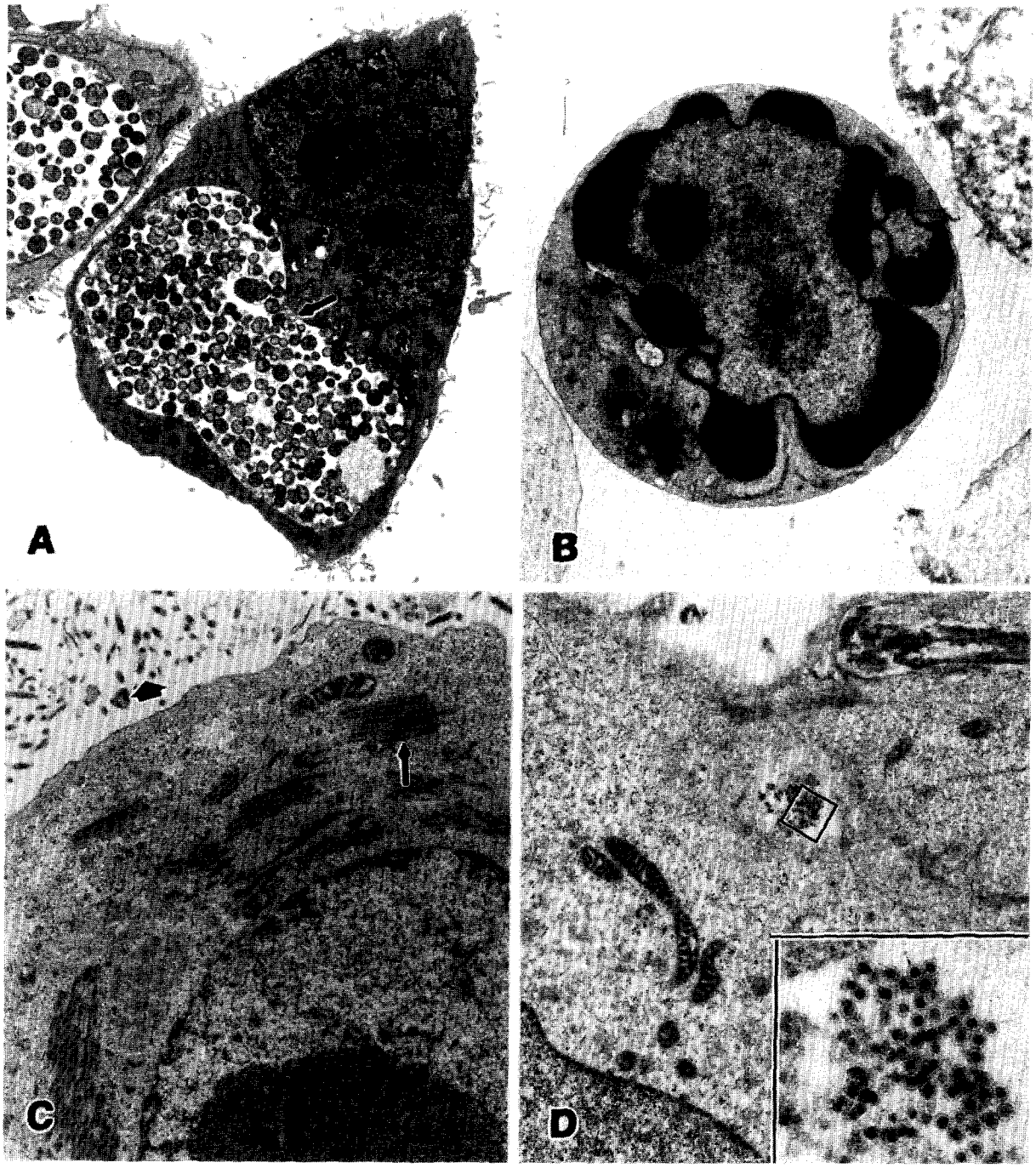

Fig. 3. Ultrastructural features of some parasites and viral infections.

A) Electron micrograph of a cell infected with *Chlamydia* sp. The two intracellular forms of the parasite-dense bodies and reticulate bodies can be observed in a single giant phagocytic vacuole. B) Electron micrograph of HIV-infected cell presenting dense and marginalized intranuclear chromatin, which is one of the characteristic features of apoptosis. In this case, the apoptosis of the thymic cell may have been induced by the virus. C) Thin sections of an epithelial cell infected with Ebola virus. Free viruses are seen in the intracellular space (arrowhead) and packed viral capsids are present in the cytoplasm (arrow). D) Cell infected with dengue 2 virus. These small viruses (50 nm) can be observed in intracytoplasmic pockets. *Inset:* magnification of the outlined square zone.

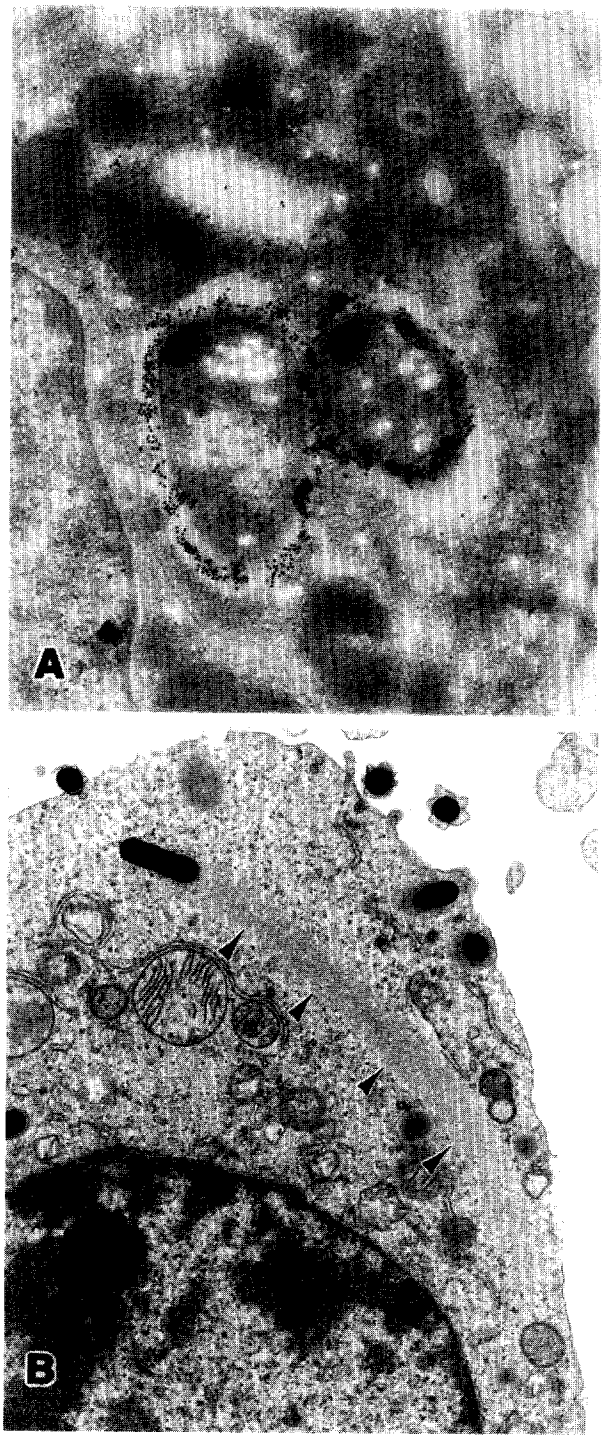

Fig. 4. Intracellular behaviour of bacterial infections.

A) Intracellular *Shigella flexneri* immunogold-stained. LPS is mainly concentrated at the surface of the bacteria, but may be dispersed in the cytoplasm of the cell. B) Intracellular *Listeria* induces actin polymerization (arrowheads) at one pole to generate movement, and thus the spread of the infection from one cell to another.

Genetic resistance to infectious diseases

The effects of intracellular pathogens involve effector cells and molecules recruited by the immune system, especially T lymphocytes. TCRs specifically bind ligands (bacterial or parasitic antigens) which are presented in the context of MHC class I and class II molecules. For example, the presentation of antigenic peptides (of viral origin) to α/β TCRs in the context of MHC class Ia and γ/δ TCRs for MHC class Ib suggests genetic control of intracellular infections. Hypotheses involving MHC-I, for example, have previously been reviewed (Ojcius, 1994).

CTL are considered to be accessory component cells in host defence against intracellular pathogens. The tissue concentration of CTL seems to be increased in long-term survivors of HIV infection and in experimental models. Murine models of leishmaniasis have provided evidence for the genetic control of the disease. C57Bl6, CBA, C3H and 129/Sv/Ev mice are resistant (Th1) and BALB/c mice are susceptible (Th2).The involvement of IFNγ in the Th1 response was one of the first to be demonstrated. Recently, IL12 has been shown to be an activator of macrophages.

IL4, IL10, IL13 and other cytokines are involved in Th2 responses. However, the similarity of the murine and human responses has not been clearly established. Histopathology has failed to find cellular markers which may be used to identify Th1/Th2 lymphocytes in tissue sections.

The genetic control of innate resistance to mycobacterial infections is obvious, since the efficacity of BCG vaccination varies between different populations (Ehlers *et al.*, 1975). In mice, resistance to *Mycobacterium bovis*/BCG is under the control of the *bcg* gene, which also controls the resistance to *M. lepraemurium, M. intracellulare* and *M. avium* via the activation of macrophages. The *bcg* gene may activate macrophages to produce cytokines (IL2 and other cytokines of the Th1 response). IFNγ also enhances the effects of the *bcg* gene, suggesting a link between the *bcg* gene and IFN-responsive sequences.

Rare disseminated human BCG infections have recently been shown to depend on the IFNγ receptor (Levin *et al.*, 1995), suggesting a genetic component control of intracellular mycobacterial multiplication.

Local and mucosal immunity

Remarkable advances have been made in recent years in the study of virulence mechanisms of bacteria. Attachment of bacteria to respiratory or digestive surfaces and mechanisms allowing bacterial entry and adaptation to the environment have been extensively studied. All methods of histopathology may be used in studies of mucosal immunity, particularly the control of cytokines and cell infiltrates in experimental models. The example of complex regulation of macrophage effector function (Krahenbuhl, 1995) by mycobacterial constituents (sulphatides, PGL, cord factor, LPS) confirms that numerous mechanisms are involved (activation of macrophages, T cells and cytokines such as IFNγ and TNFα). The role of immunoglobulins (IgG and IgA) in mucosae during the course of chronic inflammation has not been fully elucidated.

Adhesion molecules

Cell-cell interactions are mediated by several different families of receptors playing a role in acute and chronic inflammation. These families include the integrins, the Ig superfamily, the calcium-dependent cadherins, the LEC-CAM cell adhesion molecules with lectin-like domains (regulating white blood cell/endothelial cell adhesion) and some homing receptors. There exist numerous reviews on integrins and adhesion molecules. A very simplified model of their involvement in inflammation is as follows. The initial inflammatory response to the introduction of a foreign antigen consists of recruitment of macrophages and mast cells that release interleukin-1, TNF, TGF-β and histamine. These cytokines, or bacterial products stimulate the production of adhesion molecules such as ICAM-1, ELAM-1, GMP 140 and VCAM on endothelial cells and also of ICAM-1 and LEU-CAMs on WBCs in vessels. This is followed by the adhesion of WBCs to endothelia and transmigration of polymorphonuclear cells through the endothelium within the infected sites. In an inflammatory immune response, T lymphocytes and macrophages cooperate and the specificity of the reaction is confined to the TCR. Promotion of LFA-1 and ICAM-1 binding may be induced by specific signals. Integrins are central to the pathophysiology of enteric infections. For example, the inactivation of CD18 in a rabbit model of experimental shigellosis suppresses the inflammatory response (Perdomo *et al.*, 1994). In human and experimental malaria, integrins are also implicated in the phenomenon of adherence and sequestration of WBCs.

Cytokines

All steps in the inflammatory reaction are orchestrated by the complex interactions between various cytokines. Cytokines are polypeptides produced by many cells and are numerous: IL1 (Sansonetti *et al.*, 1995), TNF (α and β) the IL8 family and IFNγ play a major role (Cavaillon, 1994) and new molecules are under study.

TNF levels are increased in patients who die of malaria, and there is a balance between pathology and protective effects of TNF. Histological studies show that granuloma formation in mouse liver infected by BCG is associated with local TNF synthesis. Although TNF is released by T lymphocytes, macrophages become a source of TNF working in an autocrine way with epithelioid differentiation. TNF production probably enhances and perpetuates its own synthesis and release (Kindler *et al.*, 1989).

The production of TNF may be under the control of IFNγ released by T lymphocytes. There is also increased expression of IFNγ receptors by TNF-treated macrophages.

Lipoarabinomannan, a major cell wall component of *M. tuberculosis,* induces the transcription of various cytokine genes including those for TNF, granulocytes, macrophages, CSF, IL1a, IL1b, IL6, IL8 and IL10. It does not induce IFNc,

IL2, IL3 or IL4 (Barnes *et al.*, 1992). Other experiments have studied lung granulomas induced by polymer beads coated with purified proteins (derived from *M. tuberculosis* or *S. mansoni* eggs). Lungs and lymph nodes were assessed for Th1-type (IL2, IFNγ) and Th2-type (IL4, IL5, IL10) cytokines. Mycobacterial proteins induced a Th1 response, and schistosoma egg proteins a TH2 response. TNF production by macrophages regulates cytokine production. IL6 was weak and TNF high in granulomas induced with mycobacterial proteins (Chensue *et al.*, 1994).

Other examples of the role of activated macrophages in killing intracellular parasites have been described (Krahenbuhl, 1995). They illustrate the value of methods to detect cytokines in tissues and individual cells. Immunofluorescence and immunohistochemistry staining techniques using polyclonal or monoclonal antibodies have been widely developed in recent years. Numerous cytokines may be identified in frozen or embedded paraffin sections (fig. 2f), revealing a pattern of intracellularly accumulated cytokines, for example TNF, or the presence of IL2 or IL6 in the Golgi apparatus. Hybridization may be used for the detection of mRNA, with increased sensitivity (Litton *et al.*, 1996).

The physiopathology of rheumatoid arthitis (a chronic, intermittent autoimmune disease) has not been elucidated. A murine model has been used (Van de Loo *et al.*, 1995), to show that IL1 is an important mediator in exacerbations of murine arthritis. Improvement in lesions following the administration of anti-IL1 suggests that cytokine inhibitors may be potential therapeutic agents.

Necrosis or apoptosis?

In healthy tissues, cell proliferation and cell death are balanced. Programmed cell death has been intensively investigated in recent years (Majno and Joris, 1995). The morphology of apoptotic cells is known and characteristic molecular features and *in situ* end labelling facilitate the recognition of apoptosis. In viral infections, apoptosis plays a crucial role. Latent Epstein- Barr infection of B cells induces the expression of the LMP-1 gene which upregulates *bcl-2* in the host cell. The depletion of $CD4^+$ T cells in HIV infection is attributed to apoptosis, and another example is apoptosis of the thymocyte.

Cytotoxic T cells recognize virus in combination with MHC class II molecules on the surface of infected cells. The fas ligand at the surface of cytotoxic T cells triggers the fas receptor on the membrane of infected cells and this interaction, via intercellular proteases such as Granzyme B, induces apoptosis. *Shigella* and *Bordetella* also induce macrophage apoptosis. In acute inflammation characterized by the recruitment of neutrophils, most are believed to die, causing necrosis. TNF is able to initiate the process of neutrophil apoptosis. In chronic inflammation, lymphocytes die following fas ligand activation of the fas receptor on the surface of lymphocytes (Bronner *et al.*, 1995). In follicular lymphomas, the *bcl-2* gene is involved in the 14,18 translocation and overexpression of *bcl-2* blocks apoptosis and favours the growth of the lymphoma.

Recent data suggest a fundamental role for the *bcl-2* gene in the maturation of T cells. *bcl-2* expression is correlated with protection of activated T cells against apoptosis. The IL2 cytokine maintains high *bcl-2* levels in activated cells and this may be a general feature of infectious diseases (Akbar *et al.*, 1993).

Virus associated with cancer

The possible pathogenetic role of EBV was first suspected in patients with infectious mononucleosis, Burkitt's lymphoma in Africa and nasopharyngeal carcinoma in Asia. Hodgkin's disease is also on the list of EBV-related neoplasms because of epidemiological and serological evidence. The detection of EBV, using a molecular probe such as EBER in Hodgkin's disease (fig. 2d) scleronodular type is now currently accepted and is associated with the expression of EBV latent membrane protein (LMP) within Reed Sternberg cells.

HTLV-I is associated with T lymphoma and T-cell leukaemia in some geographic areas (Japan

and the Caribbean islands). Immunohistological studies of the phenotype of T cells (CD3, CD4, or CD8) show that markers including CD30 (fig. 2b, c) are associated with poor prognosis. Other viruses such as papillomavirus associated with cervix uteri carcinoma and herpes virus with Kaposi's sarcoma are aetiological and/or cofactors of neoplasms.

Conclusion

The interplays between microbes and their hosts may be studied by investigating morphology. Histopathology and electron microscopy are powerful tools in human and experimental pathology. The study of early or late lesions in human pathology, immunohistochemistry and molecular probes provides insight into these interactions. Numerous pathogens induce an unstable equilibrium with their host (Pincus *et al.*, 1992). Tissue inflammation is generally a direct consequence of this battle, or pacific coexistence in some cases.

Numerous factors may be studied; however, a major difficulty lies in excluding extraneous factors in an experiment. Knockout mice can be useful for this.

The control of this interplay would help improve our understanding of some diseases which are public health problems.

Key-words: Inflammation, Pathology, Electron microscopy, Infection; Bacterial infection, Viral infection, Parasite, Physiopathology; Times and Trends.

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