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# VIII IMMUNOLOGY OF THE CAT

## 1. Introduction

During the past 20 to 30 years, there has been a growing interest in the cat as a companion animal. In many countries, the number of cats has exceeded that of dogs. For instance Switzerland with 1.3 million cats has about three times the number of dogs. As a consequence, veterinary immunologists in industry and academia have devoted increasing efforts in the study of the feline immune system, pursuing both applied and basic research. The applied research is devoted mainly to improving the cat's health by studying pathogenesis, immune reactions and diagnostic possibilities of various diseases and to the development and improvement of vaccines. Indeed, the numbers of vaccines for use in cats has grown tremendously during the last 10 years. In addition to the applied research from which cats profit directly, the cat has attracted much interest from basic research as a model for various human diseases. There were two events which triggered increased study of the feline immunology: the first was the discovery of the feline leukemia virus (FeLV) in Scotland by Jarrett's group (Jarrett *et al.*, 1964) and the second was the isolation of the feline immunodeficiency virus (FIV) in California by Pedersen and coworkers (Pedersen *et al.*, 1987).

The discovery that FeLV caused transmissible lymphosarcomas in an outbred species was considered important for tumor genesis also in man. The event triggered a great number of studies relating to mechanisms of tumor development and epidemiology of FeLV. As a consequence of the hypothesis that the FeLV-induced feline oncornavirus associated cell membrane antigen (FOCMA) supposedly a nonvirus coded tumor specific antigen, (Essex *et al.*, 1975) might protect cats against FeLV-induced tumors, many studies were initiated relating to the immune mechanisms that could be involved in protection against these tumors. When it became clear that cats are capable of spontaneously overcoming FeLV viremia, many groups started on the development of a vaccine against FeLV infection. Several FeLV vaccines, including a highly efficacious recombinant one, are now available to veterinarians.

The discovery of FIV attracted much interest from basic researchers as FIV was immediately recognized as an important model for the study of HIV and AIDS (Gardner *et al.*, 1991, Bendinelli *et al.*, 1991). Many research groups switched their interest to the study of FIV in cats. In the early days of FIV research, few reagents existed to investigate the feline immune system during FIV infection. This soon changed in Europe with the support from the European Concerted Action on FIV Vaccination and after the first description of antifeline $CD4^+$ antibodies (Ackley *et al.*, 1990), a number of different antifeline leukocyte differentiation antigens are now available and the methods for their use have been established. To date, few assays exist that allow the quantification of feline cytokines on the protein level. However, sequences of most cytokines described in mice and other species have also been obtained in the feline system.

It is the goal of this chapter to provide an overview on current state of knowledge of the cat's immune system and on the source of reagents available. The topics of the chapter on the immunology of the cat are listed in analogy to those of the other chapters in this handbook. Because little information is available on the T-cell receptor and on leukocyte traffic, no specific sections can be listed on these topics. Diseases of the immune system are important in the cat; therefore, a separate section on immunological diseases is included. The editor and authors of this chapter hope that the information may help researchers with previous experience or new interest in the immunology of the cat to gain a rapid overview on what has been done and can be done in feline immunology.

## 2. Lymphoid Organs and their Anatomical Position

The lymphoid organs can be classified as either central or peripheral. The central lymphoid organs of the cat are the thymus and the bone marrow which are identified as areas of lymphocyte development and differentiation.

Lymph nodes, tonsils, spleen and Peyer's patches of the gut are classified as peripheral lymphoid organs (Vollmerhaus *et al.*, 1981, 1994; Gershwin *et al.*, 1995). They are used as a platform for lymphoid cells which have achieved immune capability to come into contact with antigens. Thus, the peripheral lymphoid organs serve as sites of development for specific features of immunity.

As far as known, the lymphoid organs of the cat show characteristics in development, structure and function

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similar to that of other mammals. Knowledge of topography and function of lymphatic tissue has become important in connection with feline retrovirus infections as model for HIV and AIDS in humans. Therefore, the information presented in this section should help identify lymphatic tissue which can be surgically removed without sacrificing the animal.

## Central lymphoid organs

### Thymus

The feline thymus is an elongated multilobed structure located in the thoracic mediastinum. Each thymic lobule is clearly divided into an outer cortex region and an inner medulla. The cortex is composed of dense aggregates (no follicles) of small lymphocytes (thymocytes) which are surrounded by reticular connective tissue. It can be seen by FACS analysis that 93% of thymic cells are stained by a feline pan-T cell marker (Tompkins *et al.*, 1990b). The medulla shows a relatively low cellular density and consists of epitheloid reticulum cells, sparse lymphocyte populations and Hassall's corpuscles. The thymus weighs about 0.32–0.39 g (0.4% of bodyweight) in neonates; it increases in weight and size, and undergoes progressive involution in which the thymic tissue is successively replaced by connective and adipose tissue, starting in the fourth month post partum. Only efferent lymphatics are present in the thymus, which lead to the nodi lymphatici (nll.) mediastinales. The thymus is innervated by the vagus nerve and the truncus sympathicus (Vollmerhaus *et al.*, 1981, 1994; König, 1992; Gershwin *et al.*, 1995).

### Bone marrow

The bone marrow in the central cavities of developing long bones is the source of hematopoietic cells-including immature lymphocytes (which are derived mainly from mitotic divisions of lymphoblasts or prolymphocytes) (Gershwin *et al.*, 1995). Islands of hematopoietic cells and their stem cell precursors are surrounded by a supporting network of primitive reticulin-producing cells, fibroblasts, collagen and adipose tissues.

Lymphocytes leaving the bone marrow by efferent lymphatic vessels either migrate to the spleen and lymph nodes directly if they participate in antibody production, or they pass through the thymus, and then to the lymph nodes if they are destined for cellular immunity. This cellular migration mostly occurs before birth, indicating that lymphoid tissues of the neonate are well developed (Friess *et al.*, 1990; Gershwin *et al.*, 1995).

## Peripheral lymphoid organs

### Lymph node

The main function of the lymph node is to filtrate the lymph and to sift out foreign antigenic material.

The node consists of a capsule, a cortex region and a medulla. The cortex contains a dense mass of lymphocytes in which germinal centers are embedded. In these germinal centers bone marrow-derived B lymphocytes undergo differentiation into antibody-producing plasma cells. Mature B cells then move to the peripheral area (mantle) of the germinal center and then to the sinus areas in the medulla. The medulla contains large blood vessels that branch and form lymph cords. These are supported by a loose network of reticular tissue. Between these cords there is a loose network of lymphatic sinuses which contain macrophages, lymphocytes, and plasma cells. The paracortical, or T-cell-dependent area located between the medulla and the cortex area is involved in cellular immunity (Friess *et al.*, 1990; König, 1992; Gershwin *et al.*, 1995). FACS-analysis of single cell suspensions obtained from feline popliteal lymph nodes reveals that up to 49% of the cells belong to the T-cell population (Tompkins *et al.*, 1990).

Size, cellular density and composition of the lymph node change during the lifespan. This is mostly due to antigen-stimulation and consecutive enhanced 'trapping' of circulating lymphocytes followed by a transient shutdown in the exit rate of lymphocytes. Later, most of the cells in the node are derived from clones of these antigen-stimulated lymphocytes (Gershwin *et al.*, 1995).

The lymph enters the node via the afferent route at the capsular surface and exits via the efferent route at the hilus neighbored by venoles and arterioles.

### Spleen

The main function of the spleen is vascular filtration of foreign material and removal of damaged or aged erythrocytes. In addition, the feline spleen is able to hold up to 16% of the total blood in order to support the general circulation. This can be actively done by contraction of smooth muscles infiltrating the capsule and the trabeculae of the organ. The feline spleen is classified as a reticular spleen as opposed to the sinusoid spleen of the dog (Friess *et al.*, 1990; Vollmerhaus *et al.*, 1994).

The spleen is a large vascular organ located in the gastrosplenic ligament along the greater curvature of the stomach, 114–185 × 14–31 mm in size, in the adult cat, depending on the amount of blood contained (König, 1992; Vollmerhaus *et al.*, 1994). The organ is surrounded by a capsule and divided by a trabecular framework into lobules. Central arterioles entering at the hilus continue in the neighborhood of the trabeculae as smaller straight arterioles and end as capillaries. These capillaries are embedded in a reticular network; this is the red pulp of

**Table VIII.2.1** Palpable lymph nodes of the cat[a]

| *Lymph node* | *Topography* | *Number[b]/Size* | *Lymphatic drainage* |
|---|---|---|---|
| nll. Mandibulares | Postero-lateral to the proc. angularis of the mandibula, medial and lateral to the vena linguofacialis | 2/12–15 × 8–10 × 6–8 mm | Lips, chin region, mouth cavity, cheek glands, eyelids |
| nll. Retro-pharyngei laterales | Behind the ear and caudal of the parotid gland along the vena auricularis caudalis | 1–7/0.5–15 × 1–3 × 0.5–3 mm | Region of the ear, eye, forehead, neck and parotid gland |
| nll. Cervicales superficiales dorsales[c] | In front of and below the cervical part of the musculus trapezius | 1–3/8–20 × 5–6 × 3–4 mm | Dorsal area of the neck, shoulder and the forelimb |
| nl. Axillaris propius | In the fork between vena thoracica lateralis and vena axillaris | 1–2/3–6 × 4–5 × 2–4 mm | Skin and subcutaneous region of the medial aspect of the upper and lower arm, lateral thoracic wall |
| nll. Axillares accessorii[c] | Along the vena thoracica lateralis from the level of the 3rd to 6th intercostal spaces | 2–5/5–10 × 2–2 × 2–3 mm | Skin of the inside of the upper and lower forelimbs, lumbal region, lateral and dorsal chest wall |
| nl. Inguinalis superficialis[c] | Embedded in adipose tissue between arteria and vena pudenda externa in the femoral canal | 1/5–15 × 3–5 × 2–3 mm | Inguinal and gluteal regions, in female cats the posterior half of the mamma |
| nll. Epigastrici caudales[c] | Embedded in adipose tissue along arteria and vena epigastrica caudalis superficialis | 1–5/3–8 × 2–5 × 1–3 mm | Caudal part of the ventral abdominal wall and subcutis of the thigh |
| nl. Popliteus superficialis | Subcutaneously in the flexor region of the knee beneath vena saphena lateralis | 1/6–7 × 4–5 × 4 mm | Cutis, subcutis and muscles of the shank and hind foot |

nl., nodus lymphaticus; nll., nodi lymphatici.
[a]References: Vollmerhaus *et al.*, 1981, 1994; Meier, 1989.
[b]On each side of the cat.
[c]Palpable only in young and lean cats.

the spleen which has no afferent lymphatics. The white pulp is also embedded in a reticular network and is the actual lymphatic tissue of the spleen. It is composed of Malpighian bodies, representing the sites of B-lymphocyte differentiation which are present at or near the divisions of central arterioles. The areas surrounding the central arterioles consist of T lymphocytes and also belong to the white pulp as well. In the feline spleen, up to 65% of the cells are T-cells (Tompkins *et al.*, 1990).

### Tonsils

The tonsils located in the pharynx consist both of T lymphocytes and lymph follicles containing B lymphocytes, but lack afferent lymphatics and unlike the lymph node, a definite capsule (Friess *et al.*, 1990; Vollmerhaus *et al.*, 1994; Gershwin *et al.*, 1995).

### Peyer's patches

The Peyer's patches are situated in the gut. With respect to function and morphology, they are analogous to the tonsils. However, there are, in addition, numerous solitary lymphoid nodules spread throughout the lamina of the gut. Their main function is the production of immunoglobulin A (IgA) along with other types of immunoglobulins. The epithelial cells covering areas of Peyer's patches can be identified as 'M' cells with micropinocytic properties, allowing the cells to sample antigens. Beneath this area of specialized epithelium there are aggregates of T cells, B cells and plasma cells. Lymphocytes that home to Peyer's patches show an adhesion molecule called VLA-4 (very late antigen) (Gershwin *et al.*, 1995).

## Lymph nodes and lymph collecting ducts of the cat

The anatomically most accessible lymphatic tissue are the lymph nodes. Therefore, many experimental studies involving the cat as an animal model for various diseases focus mainly on the immunological and pathological changes in lymph nodes.

The lymphatic system of the cat was first described in particular by Sugimura *et al.* (1955, 1956, 1958, 1959). These first descriptions of size, number and weight of

feline lymph nodes were followed by adaptations and variations such as number of nodes and nomenclature (Vollmerhaus *et al.*, 1981, 1994; Meier, 1989).

The size of a lymph node depends on age and weight of the animal and presence or absence of pathological conditions. The largest sizes are seen under pathological conditions (Meier, 1989).

A vital condition for the immune functions of the lymphoid organs is the anatomical connection of the circulating blood system and the lymph vessels. The peripheral lymph nodes are connected with the blood stream via postcapillary venules in the cortex of the node. Lymphocytes exit into the lymph node by receptor-ligand-binding between their cell adhesion molecules, called integrins and selectins and high endothelial cell venules. L-selectin was found to be such a homing receptor on lymphocytes for peripheral nodes (Gershwin *et al.*, 1995).

While T cells circulate through the paracortical area, B cells circulate through the germinal centers of the cortex. They leave the lymph node through efferent lymph vessels leading to the next lymph node or eventually into lymph collecting ducts. These ducts finally empty into the venous blood system to complete the lymphocyte circulation (Vollmerhaus *et al.*, 1981, 1994; Gershwin *et al.*, 1995).

## Palpable lymph nodes of the cat

The feline lymph nodes which are palpable and constant in occurrence under physiologic conditions are listed in Table VIII.2.1. In addition, lymph nodes are indicated which are palpable only in young and lean cats.

The diagrammatic outline of the lymph nodes listed in Table VIII.2.1 are indicated in Figure VIII.2.1.

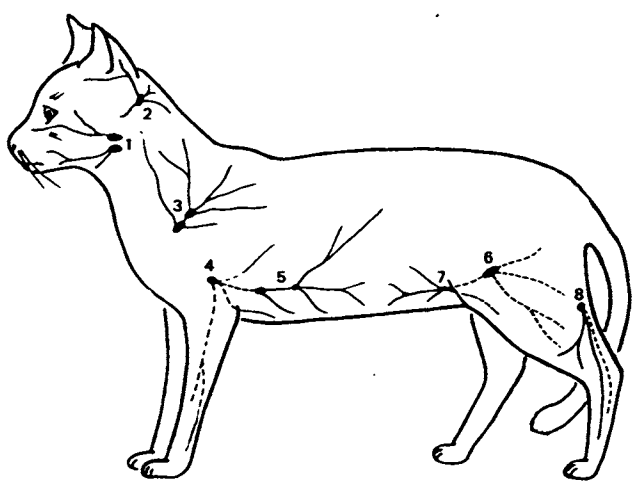


**Figure VIII.2.1** Diagram of the palpable lymph nodes of the cat (1) nodi lymphatici (nll.) mandibulares; (2) nll. retropharyngei laterales; (3) nll. cervicales superficiales dorsales[a]; (4) nodus lymphaticus (nl.) axillaris propius; (5) nll. axillares accessorii[a]; (6) nl. inguinalis superficialis[a]; (7) nll. epigastrici caudales[a]; (8) nl. popliteus superficialis. [a]Palpable only in young and lean cats (reproduced from J. Frewein and Vollmerhaus, 1994, with kind permission of Prof. Frewein and the publisher).

## Physiologically nonpalpable lymph nodes of the cat

The various lymph centers, their most important lymph nodes and the corresponding drainage areas of the nonpalpable feline lymph nodes are listed in Table VIII.2.2.

Figure VIII.2.2 gives a schematic survey of most of the lymph nodes listed in Tables VIII.2.1 and VIII.2.2.

## Lymph collecting ducts of the cat

The lymph collection ducts carry the lymph from the superficial and visceral lymph centers to the venous blood stream.

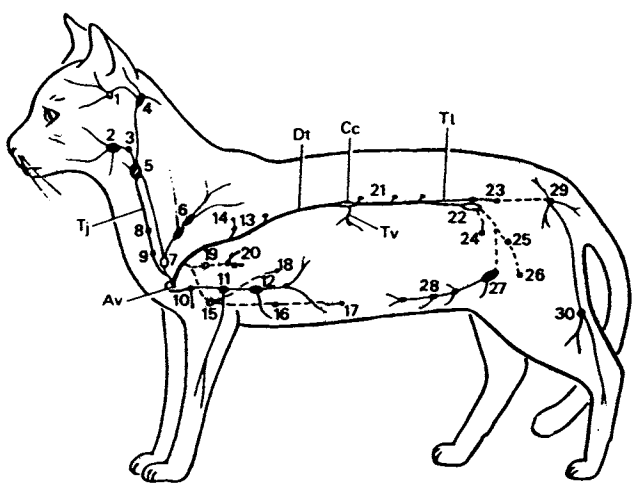


**Figure VIII.2.2** Diagram of the lymph nodes and lymph collecting ducts of the cat without the intestinale nodes (Av) angulus venosus; (Cc) cysterna chyli; (Dt) ductus thoracicus; (Tj) Truncus jugularis; (Tl) Truncus lumbalis; (Tv) truncus visceralis. (1) nodus lymphaticus (nl.) parotideus[c]; (2) nodi lymphatici (nll.) mandibulares[e]; (3) nll. mandibulares accessorii[b]; (4) nll. retropharyngei laterales[e]; (5) nl. retropharyngeus medialis; (6) nll. cervicales superficiales dorsales[d]; (7) nl. cervicales superficialis ventralis[b,c]; (8) nl. cervicalis profundus medius[a]; (9) nl. cervicalis profundus caudalis[b]; (10) nl. axillaris primae costae[a]; (11) nl. axillaris proprius[e]; (12); nll. axillares accessorii[d]; (13) nll. thoracici aortici[a]; nl. intercostalis[a]; (15) nl. sternalis craniales; (16) nl. sternalis caudalis[a]; (17) nl. epigastricus cranialis[a]; (18) nl. phrenicus[a]; (19) nll. mediastinales craniales; (20) nll. bifurcationis and pulmonales[a]; (21) nll. lumbales aortici; (22) nll. iliaci mediales; (23) nll. sacrales; (24) nl. subiliacus[a]; (25) nl. iliofemoralis[a]; (26) nl. femoralis[a]; (27) nl. inguinalis superficialis[d]; (28) nll. epigastrici caudales[d]; (29) nl. ischiadicus[c]; (30) nl. popliteus superficialis[e]. [a]Inconsistent in occurrence; [b]Almost constant in occurrence; [c]Palpable when pathologically enlarged; [d]Palpable only in young and lean cats; [e]Palpable under physiologic conditions (reproduced from J. Frewein and Vollmerhaus, 1994, with kind permission of Prof. Frewein and the publisher).

**Table VIII.2.2** Main lymph centers of the cat and their lymph nodes[a]

| *Lymphocentrum* | *Lymph node* | *Number* | *Lymphatic drainage* |
|---|---|---|---|
| Lc. Parotideum | nl. Parotideus[d] | 1–2 | Upper eyelid, parotid gland, parts of the upper half of the head |
| Lc. Mandibulare | nll. Mandibulares | 2 | Upper and lower lips, chin region, mouth cavity, cheek glands, eyelids |
| | nll. Mandibulares access.[b] | 1–4 | |
| Lc. Retro-pharyngeum | nll. Retropharyngei laterales[f] | 1–7 | See Table VIII.2.1; oral cavity and tongue, cervical section of the oesophagus and trachea, thyroid and mandibular gland, parotid gland |
| | nl. Retropharyngeus medialis | 1 | |
| Lc. Cervicale superficiale | nll. Cervicales superficiales dorsales[e] | 1–3 | See Table VIII.2.1 |
| | nl. Cervicales superficialis ventralis[c,d] | 1–2 | Ventral part of the neck, thoracic inlet |
| Lc. Cervicale profundum | nl. Cervicalis profundus medius[b] | 1 (unilateral) | Thyroid gland, cervical parts of trachea and oesophagus |
| | nl. Cervicalis profundus caudalis[c] | 1–6 | Trachea, oesophagus, thyroid gland |
| Lc. Axillare | nl. Axillaris proprius[f] | 1–2 | See Table VIII.2.1 |
| | nl. Axillaris primae costae[b] | 1 | Lateral wall of the thorax |
| | nll. Axillares accessorii[e] | 3–7 | See Table VIII.2.1 |
| Lc. Thoracicum dorsale | nll. Thoracici aortici[b] | 1–5 | Peritoneum |
| | nl. Intercostalis[b] | 1–2 | Pleura |
| Lc. Thoracicum ventrale | nl. Sternalis craniales | 1–5 | Ventral thoracic and abdominal wall, diaphragm, pericardium |
| | nl. Sternalis caudalis[b] | 1 | |
| | nl. Epigastricus cranialis[b] | 1 | |
| Lc. Mediastinale | nl. Phrenicus[b] | 1 | Diaphragm; heart, trachea, thymus, oesophagus, pleura, pericardium |
| | nll. Mediastinales craniales | 2–8 | |
| Lc. Bronchale | nl. Bifurcationis seu tracheobronchialis dexter | 1–2 | Mainly the lung, but also heart, pericardium, mediastinum and diaphragm |
| | nl. Bifurcationis seu tracheobronchialis sinister | 1–2 | |
| | nl. Bifurcationis seu tracheobronchialis medius | 1–2 | |
| | nl. Pulmonalis[b] | 1 | Lungs |
| Lc. Lumbale | nll. Lumbales aortici | 2–7 | Diaphragm, kidneys, adrenals, dorsal abd. wall, ovary, oviduct, uterus, testes |
| Lc. Coeliacum | nll. Lienales | 1–3 | Spleen, greater curvature of the stomach, left lobe of the pancreas; stomach, liver oesophagus, diaphragm, duodenum, pancreas |
| | nll. Gastrici | 1–4 | |
| | nll. Hepatici | 2–4 | |
| | nl. Pancreaticoduodenalis | 1–2 | |
| Lc. Mesentericum craniale | nll. Jejunales | 2–20 | Small intestine and body of pancreas |
| | nll. Caecales | 1–3 | Caecum, ileum |
| | nll. Colici | 3–9 | Ileum, ascending and transverse colon, descending colon and caecum |
| Lc. Mesentericum caudale | nll. Mesenterici caudales | 1–3 | Descending colon and rectum |
| Lc. Iliosacrale | nll. Iliaci mediales | 2–4 | Pelvic wall, pelvic limb, uterus, urinary bladder, sometimes oviduct or testis |
| | | 1–6 | |
| | nll. Sacrales | | Rectum, uterus, vagina, urinary bladder, ureter, wall of the pelvis, pelvic outlet, tail and hind limb |
| Lc. Inguinale profundum | nl. Iliofemoralis[b] | 1 | Parts of the neighboring ventral abdominal wall, gluteal region, thigh |
| | nl. Femoralis[b] | 1 | |
| Lc. Inguinale superficiale | nl. Inguinalis superficialis[e] | 1 | Inguinal and gluteal regions, posterior half of the mamma; caudal part of the ventral abd. wall and subcutis of the thigh |
| | nll. Epigastrici caudales | 1–5 | |
| | nl. Subiliacus[b] | 1 | |
| Lc. Ischiadicum | nl. Ischiadicus[d] | 1 | Skin, subcutis, fasciae of the thigh, anal region, hind limb |
| Lc. Popliteum | nl. Popliteus superficialis[f] | 1 | See Table VIII.2.1. |

[a]References: Vollmerhaus *et al.* (1981, 1994); Meier (1989).
[b]Inconsistent in occurrence.
[c]Almost constant in occurrence.
[d]Palpable when pathologically enlarged.
[e]Palpable only in young and lean cats.
[f]Palpable under physiologic conditions.
Lc., lymphocentrum; nl., nodus lymphaticus; nll., nodi lymphatici.

The truncus jugularis collects the lymph from the lymph nodes of the head and throat and joins the venous angle. The truncus viscerales is formed by the efferent vessels of the lymph nodes of the coeliac and cranial mesenteric lymph centers. The truncus lumbalis collects the lymph from the pelvis, the nll. sacrales and the nll. iliaci mediales. The trunci viscerales and lumbales form the cysterna chyli which is 7–30 mm long and lies between the renal vessels and the crura of the diaphragm. The ductus thoracicus developed from the cysterna chyli is formed like a rope-ladder and end as a single trunk into the venous angle. The diagrammatic outline of these ducts are seen in Figure VIII.2.2.

## 3. Leukocyte Differentiation Antigens

### Introduction

The development of antibodies recognizing feline leukocyte differentiation antigens is central to the study of the feline immune system in health and disease. However, the production of monoclonal antibodies can be a costly and time-consuming process and researchers studying the feline immune system seldom undertake monoclonal antibody production unless there is a pressing need for a reagent with a defined specificity. Thus, the identification of antibodies that recognize feline leukocyte differentiation antigens has tended to reflect areas of most active investigation, for example the discovery of feline immunodeficiency virus (FIV) fuelled the production of antibodies against the feline homologues of CD4 and CD8 in order that T lymphocyte subsets could be monitored in FIV-infected cats, and that the question of whether feline CD4 acts as a receptor for FIV could be addressed.

The expression of leukocyte differentiation antigens in the feline immune system has been reviewed by Willet and Callanan (1995). This section will focus on recent progress in the study of feline leukocyte differentiation antigens, highlighting areas of similarity and difference from the human immune system.

### T-cell antigens

The feline homologue of CD4 is perhaps the best studied of the feline leukocyte differentiation antigens, primarily because CD4 was identified as the primary binding receptor for human immunodeficiency virus and researchers sought to define the role of the feline homologue of CD4 in FIV infection. Expression of CD4 in the feline immune system resembles that of murine CD4 in that expression is restricted solely to helper T lymphocytes and their thymic precursors and is notably absent from monocytes and macrophages (Ackley and Cooper, 1992). In contrast, CD4 expression in the human and rat immune systems extends to monocytes and macrophages. Indeed, CD4 expression in the human immune system has been studied extensively in relation to the cell tropism of human immunodeficiency virus (HIV) and it would appear to be widespread, with levels of CD4 being detected on eosinophils, follicular dendritic cells, megakaryocytes and microglia. The sole example of a non-T-lineage cell that expresses CD4 in the cat is the Langerhans cell (Tompkins *et al.*, 1990b) although only a single anti-CD4 antibody, vpg39, has been demonstrated to recognize these cells. cDNAs encoding the feline homologue of CD4 have been cloned (Dumont-Drieux *et al.*, 1992; Norimine *et al.*, 1992) and have revealed that the feline CD4 molecule contains a 17-amino-acid insertion in D2. Structural studies predict that this insertion may affect the flexibility of the hinge region between D2 and D3. Feline CD4 is also unusual in that the first cysteine involved in intrasheet disulphide bridge in D2 of human CD4 (residues 130 and 159) has been replaced by a tryptophan residue, ruling out the possibility of a similar disulphide bridge forming in feline CD4. Several antibodies have been described which recognize feline CD4, including Fel7, vpg30–39 and cat 30A. The Fel7 antibody and the antibodies of the vpg30–39 series have been epitope mapped using soluble feline CD4 produced in CHO cells and recognize five distinct epitopes on the molecules. Whether the epitopes can be related to functional properties of CD4 such as MHC class II binding or a putative interaction with interleukin 16 has yet to be established, although the epitope recognized by vpg39 is polymorphic in nature and absent from PBMC of some cats (Willet *et al.*, 1994a).

Several antibodies have been described which recognize the feline homologue of CD8 (Klotz and Cooper, 1986; Tompkins *et al.*, 1990b). As in other species, feline CD8 appears to exist as a heterodimer of $\alpha$ *and* $\beta$ chains; immunoprecipitations with the FT2 and 3.357 antibodies yielded two distinct protein species and northern blotting analysis of mRNA from feline PBMC with a murine Ly-3 probe suggested the existence of CD8$\beta$ in PBMC (Pecoraro *et al.*, 1994b). A cDNA has been cloned encoding the feline CD8$\alpha$ chain (Pecoraro *et al.*, 1994a) and the expression product is recognized by the cross-species reactive antihuman CD8-antibody OKT8. Interestingly, the feline CD8 specific antibodies FT2, 3.357 and vpg9 do not recognize the $\alpha$-chain cDNA clone when expressed on either CrFK or CHO cells suggesting that either they recognize the $\alpha/\beta$ heterodimer or are $\beta$-chain specific. Molecular cloning of the feline CD8$\beta$-chain should confirm the reactivity of antifeline CD8 antibodies and establish whether feline CD8 exists solely as an $\alpha/\beta$ heterodimer or whether $\alpha/\alpha$ homodimers/multimers exist.

CD4 and CD8 positive lymphocytes together comprise the majority of T lymphocytes in the peripheral circulation. All CD4 and CD8 positive lymphocytes are recognized by the 43-Pan T antibody which recognizes a CD5-

like molecule (Ackley and Cooper, 1992). The 43-Pan T precipitates a single 72 kDa glycoprotein which is phosphorylated in both resting and activated T cells and is upregulated by stimulation of T cells with phorbol ester or phytohaemagglutinin. Moreover, addition of the 43 Pan-T antibody to cultures of Con A stimulated PBMC augments the proliferative response. The 43-Pan T antibody therefore displays many of the characteristics of the feline homologue of CD5. Although other Pan T antibodies have been described, these antibodies have still to be characterized further. In combination with the anti-CD4 and CD8 antibodies the 43-Pan T antibody can be utilized to enumerate T lymphocyte numbers in the peripheral blood or to define the T-cell compartment in lymphoid tissues. There is no evidence to suggest that the 43-Pan T ligand is expressed on a minority of B cells, as is observed with human CD5. Thymocytes express the ligand recognized by the Fel 5F4 antibody (P. F. Moore, unpublished data), a prospective antifeline CD1a antibody. As with human CD1a, the Fel 5F4 antibody also stains Langerhans cells, intra-epithelial dendritic cells and interdigitating dendritic cells in the lymph node (Tompkins *et al.*, 1990b).

Immunohistochemical studies of feline T lymphocyte subpopulations can be achieved using antipeptide reagents such as anti-CD3 (Dako), an example of a polyclonal serum raised against a conserved intercellular domain of a cell surface molecule that shows broad cross-species reactivity.

## B-cell antigens

The enumeration of feline B cells in peripheral blood has previously only been possible using reagents which recognize surface immunoglobulins. While cross-species reactive antibodies such as BE-5 (CD21), RFB4 (CD22) and B-B20 (CD40) can be utilized for the enumeration of feline B cells the reliability of these antibodies is somewhat erratic. While true Pan-B-cell reagents for use in the cat have yet to be identified there are now good cross-species reactive antibodies in RA3.6B2 (CD45R) and CA2.1D6 (CD21). The anti-CD45R antibody is a rat antibody raised against murine B cells but cross-reacts with both human and feline cells. Although the principal reactivity of this antibody is with B cells, the ligand for the antibody (the high molecular weight form of leukocyte common antigen) is also present on murine NK cells and non-MHC restricted CTLs. The antibody has been used immunohistochemically to enumerate B cells in both frozen and paraffin-embedded lymph node sections, and by flow cytometry to enumerate B cells in peripheral blood where less than 1.0% of RA3.6B2-positive cells were co-stained with a Pan-T antibody CF255 (Monteith *et al.*, 1996). The data suggest that greater than 99% of the cells recognized in peripheral blood by RA3.6B2 were B lymphocytes. Whether this antibody will prove useful for the enumeration of B cells in diseased cats where subpopulations of leukocytes may have undergone expansion remains to be seen. However, it has been used very successfully to immunophenotype feline lymphosarcomas (Jackson *et al.*, 1996).

The anticanine CD21 antibody CA2.1D6 cross-reacts well with feline B lymphocytes. B cells are also the principal CD21-expressing cell type, although follicular dendritic cells and some epithelial cells have been demonstrated to be CD21-positive in humans. The RFB9 antibody, an antihuman CD21 antibody, can be used to stain the dendritic cell network in the germinal centres of the feline lymph node; however, this antibody fails to recognize B cells in peripheral blood. Thus, the CA2.1D6 appears to be a true anti-B cell antibody and for flow cytometric analyses would be the reagent of choice. The utility of this antibody in the study of the feline immune system was illustrated by the studies of Dean *et al.* (1996b) where CD21 expression was shown to be mutually exclusive of expression of CD4 and CD8, and in those of Quackenbush *et al.* (1996) where CA2.1D6 was used to investigate the replication of feline leukaemia virus in B cells.

## Adhesion molecules

There has been relatively little research on adhesion molecules in the feline immune system, however some notable advances have been made. Cross-species reactive monoclonal antibodies recognizing antigens on the surface of endothelial cells have been described (Weyrich *et al.*, 1995; Buerke *et al.*, 1996). The antibodies recognizing P-selectin (PB1.3), ICAM-1 (RR1/1), E-selectin (Cy1787) will be of value in the study of neutrophil trafficking and adherence. The cross-species reactivity of several antibodies recognizing the $\beta$1-integrins has been evaluated and antibodies recognizing the feline homologues of VLA2 (CD49b), VLA4 (CD49d) and VLA6 (CD49f) identified (B. Willett, unpublished observations). These reagents will be of value in the study of lymphocyte migration and adhesion.

## Nonlineage

The search for the cellular receptor for FIV identified a monoclonal antibody, vpg15, which recognized the feline homologue of CD9 (Hosie *et al.*, 1993; Willett *et al.*, 1994a). Subsequently a cDNA encoding feline CD9 was cloned and characterized (Willett and Neil, 1995). Feline CD9 resembles CD9 from other species except for a unique sequence at the crown of the first predicted extracellular loop. The first extracellular loop of human, bovine and murine CD9 contains a putative site for *N*-linked glycosylation; this is absent from feline CD9 (Willett and Neil, 1995). Despite this divergence of amino acid sequence, the feline CD9 clone proved to be functional in assays of B-cell migration (Shaw *et al.*, 1995). CD9 is expressed on

activated but not resting T cells and thus may be used as a marker for activation. T cell activation can also be evaluated using the 9F23 antibody which recognizes the interleukin-2 receptor alpha (IL2-Rα) chain. This antibody recognizes a binding site on the IL-2Rα distinct from the IL-2-binding site and has been used to evaluate the responsiveness of feline PBMC to mitogens in FIV-infected cats. The authors concluded that induction of IL2-Rα expression on Con A-stimulated PBMC was significantly depressed in FIV-infected cats (Ohno *et al.*, 1992a). A cDNA encoding feline IL-2Rα has been cloned and expressed in CrFK cells. The expression product binds recombinant human IL-2 and is recognized by the 9F23 antibody (Goitsuka and Hasegawa, 1995).

Monoclonal antibodies have been generated which define feline bone marrow erythroid (FeEr1), myeloid (FeMy) and lymphoid (FeLy) cell lineages (Groshek *et al.*, 1994). Although the identity of the antigens recognized by these antibodies remains to be established, they should prove useful reagents in the immunophenotyping of feline haemopoietic neoplasia.

## NK cells

There has been a single report of the use of an antibody specific for feline NK cells. The study by Zhao *et al.* (1995) utilized a cross-species reactive antihuman CD57 monoclonal antibody to evaluate LAK cells in FIV-infected cats. CD57 is expressed by approximately 50% of resting NK cells in man, but is rapidly lost after activation. It is also expressed on a subset of T cells and it is this $CD57^+$ T cell subset that appears to be elevated in HIV-infected individuals. A list of some of the feline leukocyte differentiation antigens identified to date is shown in Table VIII.3.1.

## Conclusions

The identification of antibodies that can be used for the enumeration of feline lymphocytes has provided researchers in feline immunology with the opportunity to study the major lymphocyte subsets in the feline immune system in health and disease. However, there is clearly a pressing need for reagents specific for NK cells and monocytes. So far, screening of antibodies against leukocyte differentiation antigens of other species has failed to identify cross-species reactive antibodies. Molecular cloning of feline leukocyte differentiation antigens may provide a source of pure antigen for immunization; specificities that have proven intractable may then be obtained by either expressing the cDNA in murine cells and using the transfected cells to immunize mice, or mice may be inoculated directly with the eukaryotic expression vector DNA itself, i.e. DNA immunization. Previous studies have illustrated the similarities and differences between the immune systems of the cat and man, the species-specific patterns of CD4 expression should remind

**Table VIII.3.1** Feline leukocyte differentiation antigens for which specific monoclonal antibodies have been identified

| *Specificity* | *Antibody* | *Accession number* | *References* |
|---|---|---|---|
| CD4 | Fel7, vpg30–39, cat 30A | | Ackley *et al.* (1990); Tompkins *et al.* (1990b); Dumont-Drieux *et al.* (1992); Norime *et al.* (1992); Willett *et al.* (1994a) |
| CD8 | FT2, vpg9, 3.357, OKT8 | CD8a-D16536 | Klotz and Cooper (1986); Tompkins *et al.* (1990b); Willett *et al.* (1993); Pecoraro *et al.* (1994a) |
| CD5 | 43 Pan-T | | Ackley and Cooper (1992) |
| CD9 | vpg15, FMC56 | L35275, D30786 | Hosie *et al.* (1993); Willett *et al.* (1994a) |
| CD10 | J5 | | Horton *et al.* (1988) |
| CD18 | MHM23 | | Dakopatts Ltd[a] |
| CD21 | CA2.1D6 | | Dean *et al.* (1996b); Quackenbush *et al.* (1996) |
| CD25 | 9F23 | D16143 | Ohno *et al.* (1992b) |
| CD29 | 4B4 | U27351 | Coulter Immunology Ltd[b] |
| CD35 | To5 | | Aasted *et al.* (1988) |
| CD49b | 16B4 | | Serotec Ltd[c] |
| CD49d | 44H6 | | Serotec Ltd |
| CD49f | 4F10 | | Serotec Ltd |
| CD45R | RA3.6B2 | | Pharmingen[d] |
| CD54 | RR1/1 | | Weyrich *et al.* (1995) |
| CD62E | Cy1787 | | Weyrich *et al.* (1995) |
| CD62L | huDREG-200 | | Buerke *et al.* (1996) |
| CD62P | PB1.3 | | Weyrich *et al.* (1995) |

[a]Dako Ltd, 16 Manor Courtyard, Hughenden Avenue, High Wycombe, Bucks HP13 5RE, UK.
[b]Coulter Electronics Ltd, Northwell Drive, Luton, Beds LU3 3RH, UK.
[c]Serotec, 22 Bankside, Station Approach, Kidlington, Oxford OX5 1JE, UK.
[d]Pharmingen Deutschland GmbH, Flughafenstrasse 54, Haus A, 22335 Hamburg, Germany.

researchers in immunology that we cannot simply extrapolate from the human or murine immune systems and expect the same rules to apply in other species. Even when the feline homologue of a human leukocyte differentiation antigen has been cloned and sequenced, functional studies should follow to confirm that the molecule has the same function in the cat as in man. The knowledge gained by such studies will benefit both the study of feline immunology, and the understanding of the function of the immune system in general.

## 4. Cytokines

Cytokines are molecules which are produced and secreted by leukocytes and several other cells. They act as intercellular mediators similar to hormones. The many lymphokines, monokines, interleukins, interkrines, chemokines and hormones, activating or inhibiting factors, or even growth factors are now generally and collectively known and described as cytokines. These factors are important for the differentiation and division of hematopoietic stem cells, for the activation or inhibition of lymphocyte functions and lymphocyte proliferation, and for the activation of phagocytes. In addition, they can function as chemokines (chemoattractants) and as cytotoxins. They act mostly in paracrine action on cells other than those from which they originate. However, they may also regulate the cell that produces them (autocrine action). Communication between immune regulator and effector cells, between antigen-presenting cells, lymphocytes and other cells occurs not only through direct cell to cell contact but also by secreting a plethora of soluble factors, leading to an intricate network between these cells.

During the last 10 years it has become clear that the activation of the cellular (T helper 1 pathway TH1) and humoral (T helper 2 pathway TH2) arms of the immune system are driven by different sets of cytokines (Mosmann and Coffman, 1989). It is mainly in this context that interest in feline cytokines, especially in connection with retrovirus and coronavirus infections, has arisen during the early 1990s.

Several feline cytokines have been cloned and sequenced and some have also been expressed. However, for most feline cytokines no specific antibodies are available that can be used for the detection of the cytokines in organ sections by immunological methods or for their quantification in cell culture supernatants or in body fluids. In some cases, antibodies specific for cytokines of other species show immunological cross-reactivity that allows application in the feline system. In this section, the known feline cytokines are listed together with information on their sequence, cross-reactivity with those of other species, cell source, function and methods of detection and quantification.

### IL-1

Goitsuka *et al.* (1987) were the first to describe IL-1 at the protein level in cats. They showed that IL-1 is released by LPS-stimulated alveolar macrophages and by peritoneal exudate cells collected from cats with feline infectious peritonitis (FIP). Three isoforms were described, with isoelectric points of 4.1, 4.8 and 5.3, respectively. It is unclear which of these isoforms correspond to the human IL-1$\alpha$ and IL-1$\beta$. Hasegawa and Hasegawa (1991) demonstrated by *in situ* hybridization IL-1$\alpha$ mRNA to be produced by macrophages present in the inflammatory lesions in cats with FIP. Induction of IL-1 by LPS-stimulated macrophages was also confirmed by Daniel *et al.* (1993). The sequence of feline IL-1 has not been published.

### IL-2

IL-2 which was originally designated T-cell growth factor (TCGF) is produced by lectin- or antigen-stimulated mature T-lymphocytes; the gene coding for IL-2 is located on the cat's chromosome B 1 (Seigel *et al.*, 1984). Tompkins *et al.* (1987) described a cell culture assay that allowed quantification of feline IL-2. Recombinant human IL-2 is highly capable of stimulating feline T-lymphocytes, an observation made by many research groups but never specifically investigated and published. After systemic application, human IL-2 induced eosinophilia in cats (Tompkins *et al.*, 1990a). Feline IL-2 has been sequenced and expressed in COS cells; it consists of 154 amino acids, including a putative signal sequence, and has 81%, 69%, 60% and 64% identity to human, bovine, murine and rat IL-2, respectively (Cozzi *et al.*, 1993; for Accession number see Table VIII.4.1). Feline IL-2 shows a similarity of 90% with canine IL-2 (Dunham *et al.*, 1995). Interestingly, while recombinant human IL-2 promotes proliferation of both, human and feline leukocytes, recombinant feline IL-2 only promotes proliferation of feline cells, but not human cells (Cozzi *et al.*, 1995). Around 20% of feline lymphocytes in the peripheral blood stream are positive for the IL-2 receptor (Iwamato *et al.*, 1989); in that study, the IL2 receptor was detected by a monoclonal antibody to human IL-2 receptor cross-reacting with the feline counterpart. In feline immunodeficiency virus (FIV) infection, expression of IL-2 is markedly inhibited (Tompkins *et al.*, 1989a; Bishop *et al.*, 1992; Lawrence *et al.*, 1992, 1995). In conjunction with nucleoside analogues and other cytokines, IL-2 has been used with some success to treat feline leukemia virus (FeLV) infections (Zeidner *et al.*, 1990, 1993).

### IL-4

Feline interleukin-4 has been cloned and sequenced by Schijns *et al.* (1995a; for EMBL Accession number see

**Table VIII.4.1** EMBL GenBank Accession numbers, characteristics and references of characterized feline cytokines

| *Gene product* | *Accession no.* | *Length (bp)* | *Last update (m/y)* | *Reference* |
|---|---|---|---|---|
| IL-1$\beta$ | M92060 | 804 | 9/93 | Daniel *et al.* (unpublished data) |
| IL-2 | L25408 | 462 (partial) | 11/94 | |
| | L19402 | 779 | 10/93 | Cozzi *et al.* (1993) |
| IL-4 | U39634 | 561 | 2/97 | Lerner and Elder (unpublished |
| | U82193 | 284 (partial) | 1/97 | data) |
| | X87408 | 521 | 10/95 | Schijns *et al.* (1995a) |
| IL-6 | D 13227 | | | Ohashi *et al.* (1989) |
| | L16914 | 724 | 3/94 | Bradley *et al.* (1993) |
| IL-10 | U39569 | 737 | 11/95 | Scott and O'Reilly (unpublished data) |
| IL-12 (p35) | U83184 | | 12/96 | Fehr *et al.* (1997) |
| | Y07761 | | | Schijns *et al.* (1997) |
| IL-12 (p40) | U83185 | | 12/96 | Fehr *et al.* (1997) |
| | Y07762 | | | Schijns *et al.* (1997) |
| IL-16 | AF003701 | 390 | | Leutenegger *et al.* (1998) |
| IFN-$\beta$ | U81267 | 561 | 2/97 | Lyons *et al.* (1997) |
| IFN-$\gamma$ | X86972 | 543 | 3/96 | Schijns *et al.* (1995b) |
| | D30619 | 567 | 6/96 | Argyle *et al.* (1995) |
| TNF-$\alpha$ | M92061 | 705 | 9/93 | Daniel *et al.* (1993) |
| | U82193 | 284 (partial) | 1/97 | Lyons *et al.* (1997) |
| | X5400 | 1722 | 3/93 | McGraw *et al.* (1990) |
| TNF-R (p80) | U51429 | 247 (partial) | 6/96 | Duthie *et al.* (1996) |
| TNF-R (p60) | U72344 | 542 (partial) | 10/96 | Duthie *et al.* (1996) |
| SCF (stem cell factor) | D50833 | 953 | 2/97 | Dunham and Onions (1996b) |
| MGF (mast cell growth factor) | U82188 | 430 | 1/97 | Lyons *et al.* (1997) |
| NGF-$\beta$ (nerve growth factor-$\beta$) | U82190 | 136 | 1/97 | Lyons *et al.* (1997) |

Table VIII.4.1). The IL-4 nucleotide sequence was found to be 83%, 82%, 80%, 79%, and 61% homologous to that of IL-4 from killer whale, pig, human, manatee, and rat, respectively. Dean *et al.* (1996a) measured the level of mRNA expression in peripheral and intestinal lymph nodes during the early phase of FIV infection and found that increased levels of IL-4 mRNA levels were paralleled by increased IL-10 mRNA expression.

## IL-6

Feline IL-6, which was originally found to have a molecular weight of 30 000–40 000, has biological properties similar to IL-6 of human and murine origin but is not neutralized by antihuman IL-6 antiserum (Ohashi *et al.*, 1989; Goitsuka *et al.*, 1990).

Feline IL-6 was sequenced (Bradley *et al.*, 1993; Ohashi *et al.*, 1993; for the EMBL Accession number see Table VIII.4.1) and was found to be 752 bp long. It shows homology of 81%, 76%, 63%, and 61% with pig, human, rat, and mouse IL-6, respectively. The predicted amino acid sequence exhibits 66%, 53%, 37%, and 30% homology with pig, human, rat, and mouse IL-6, respectively. After transfection with IL-6 cDNA, Crandell feline kidney cells (CFK) produced biologically active proteins that showed hybridoma growth-promoting activity (Ohashi *et al.*, 1993). It is not clear how the difference between the apparent molecular weight of 30 000–40 000 described (Ohashi *et al.*, 1989; Goitsuka *et al.*, 1990) and the predicted molecular weight is explained. In humans, IL-6 is known to be important in the differentiation of activated B cells into Ig-secreting cells (Chen-Kiang, 1995). FIP and FIV infections are diseases in which B cells are greatly stimulated. The observation by Goitsuka *et al.* (1990) and Ohashi *et al.* (1992) that in cats with FCoV and FIV infection plasma IL-6 activity was significantly higher than in healthy controls, suggests that in the cat also, IL-6 plays an important role in B-cell activation. IL-6 was produced by peritoneal exudate cells collected from cats with FIP (Goitsuka *et al.*, 1990) which suggests that macrophages might be an important origin of IL-6.

## IL-10

Feline IL-10 was recently cloned and sequenced by Scott and O'Reilly (1993; for EMBL Accession number, see Table VIII.4.1) and expressed in *Escherichia coli* (Leutenegger *et al.*, 1998b). In mice, IL-10 is known to inhibit antigen-specific T-cell activation by suppressing IL-12 synthesis (D'Andrea *et al.*, 1993). This leads to down-regulation of antigen presentation and accessory cell functions of monocytes, macrophages, Langerhans cells and

**Table VIII.4.2** Homologies between known IL-12 sequences of different species: percentages of nucleotide (NA) and amino acids (AA) identity between the feline, canine, human, bovine, porcine and murine IL-12-p35 (top) and p40 (bottom) sequences

| | *NA (%)* | | | | | |
|---|---|---|---|---|---|---|
| *AA (%)* | *Feline IL-12* | *Canine IL-12* | *Human IL-12* | *Bovine IL-12* | *Porcine IL-12* | *Murine IL-12* |
| *p35* | | | | | | |
| Feline IL-12 | | 93.3 | 89.9 | 87.4 | 85.8 | 66.8 |
| Canine IL-12 | 91.5 | | 88.0 | nd | 87.4 | 69.4 |
| Human IL-12 | 87.8 | 85.0 | | 86.9 | 85.8 | 73.1 |
| Bovine IL-12 | 82.0 | 81.1 | 83.1 | | nd | nd |
| Porcine IL-12 | 85.3 | 83.4 | 84.5 | 89.2 | | 67.5 |
| Murine IL-12 | 57.9 | 55.6 | 60.2 | 59.3 | 59.7 | |
| *p40* | | | | | | |
| Feline IL-12 | | 93.3 | 89.9 | 87.4 | 85.8 | 66.8 |
| Canine IL-12 | 91.5 | | 88.0 | nd | 87.4 | 69.4 |
| Human IL-12 | 87.8 | 85.0 | | 86.9 | 85.8 | 73.1 |
| Bovine IL-12 | 82.0 | 81.1 | 83.1 | | nd | nd |
| Porcine IL-12 | 85.3 | 83.4 | 84.5 | 89.2 | | 67.5 |
| Murine IL-12 | 57.9 | 55.6 | 60.2 | 59.3 | 59.7 | |

dendritic cells. IL-10 is considered to be a potent immunosuppressant, both *in vitro* and *in vivo* and it attracts much interest as a potentially important immunoregulatory protein in the control of inflammatory, autoimmune and other immune-mediated diseases (de Vries, 1995). No *in vitro* assays are available to quantify feline IL-10. When IL-10 mRNA was determined by reverse transcription-polymerase chain reaction (RT-PCR) in LPS stimulated alveolar macrophages, it was reported to be significantly increased in FIV infected cats (Ritchey and Tompkins, 1996). In FIV infection, production of IL-10 mRNA in different lymphnodes was found to be increased after onset of viremia (Dean *et al.*, 1996a).

## IL-12

As with other species, feline IL-12 is a heterodimeric protein consisting of 2 chains (p35 and p40) which are linked through disulfide bonds. While p35 was sequenced by Bush *et al.* (1994), both chains were sequenced and the sequences deposited (Fehr *et al.*, 1998; Schijns *et al.*, 1996; for EMBL Accession number see Table VIII.4.1). IL-12, originally known as natural killer cell stimulatory factor (NKCSF, Kobayashi *et al.*, 1989), is a key cytokine of the $T_H1$ pathway inducing IFN-$\gamma$ (Trinchieri, 1995). Feline IL-12 is closely related to the IL-12 of other species (Table VIII.4.2). Using RT-PCR, Rottmann et al. (1996) measured IL-12 and IL-10 expression in bronchial lymph nodes of FIV positive cats with experimental *Toxoplasma gondii* infection. After *T. gondii* infection, levels of IL-12 and IL-10 mRNA were decreased in FIV-negative cats while FIV infected cats did not show this reduction. These results suggest that in early asymptomatic FIV infection cytokine regulation is impaired.

## IL-16

IL-16, formerly known as lymphocyte chemoattractant factor (LCF) is a chemoattractant factor produced by $CD8^+$ T cells with predominant chemotactic effects for $CD4^+$ T cells (Cruikshank and Center, 1982). Although LCF does not induce T cell proliferation in lymphocytes, the factor induces IL-2 receptor and MHC II (HLA-DR) upregulation. *In vitro*, IL-16 is secreted from lymphocytes after Con A, histamine or serotonin stimulation (Center *et al.*, 1983; Laberge *et al.*, 1995, 1996). Although mRNA for IL-16 is also detectable in $CD4^+$ cells, the protein is translated and stored in biologically active form mostly in $CD8^+$ cells (Theodore *et al.*, 1986). After stimulation by histamine, IL-16 is secreted within 4 h.

The T-cell specific chemoattractant factor was described as a 14 kDa protein which appears to be linked noncovalently to form a tetrameric bioactive molecule with a mass of 56 kDa (Cruikshank and Center, 1982). At that time, only IL-1 was known to exist in multimeric forms with various degrees of bioactivity (Furutani *et al.*, 1986). The sequencing of the feline IL-16 cDNA revealed that codon 26 which is deleted in some of the human and also in some of the African green monkey genes (Bayer *et al.*, 1995) appears to be deleted also in the feline gene (Table VIII.4.3; Leutenegger *et al.*, 1998a).

**Table VIII.4.3** Homologies of nucleotide (N) and amino acid (AA) sequences of recombinant IL-16 (Needleman and Wunsch Algorithm)

| | *N (%)* | | |
|---|---|---|---|
| *AA (%)* | *Human* | *AGM*[1] | Feline |
| Human | — | 96.7% | 85.6% |
| Africa green monkey (AGM) | 95.4% | — | 88.9% |
| Feline | 84.6% | 86.9% | — |

[1]African green monkey.

## IL-18

A novel cytokine designated interferon-γ-inducing factor (IGIF) was described in the human and murine system (Okamura *et al.*, 1995; Micallef *et al.*, 1996; Ushio *et al.*, 1996). This cytokine, which has also been designated IL-18, has been cloned, sequenced and expressed from a human cDNA library (Ushio *et al.*, 1996). In synergy with IL-12, IL-18 was found to induce IFN-γ and to decrease IL-10 expression. The feline counterpart of IL-18 was amplified from alveolar macrophages by RT-PCR (Argyle *et al.*, 1996).

## IFN-α

IFN-α has not yet been sequenced. However, based on its cross-species activity, recombinant human IFN-α has been reported to be successful for the treatment of FeLV infection (Weiss *et al.*, 1991).

## IFN-β

The feline analogue to IFN-β was sequenced by Lyons *et al.* (1993; for EMBL Accession number see Table VIII.4.1). In contrast to IFN-α, IFN-β is strictly species specific. No information is available on the effect of feline IFN-β.

## IFN-γ

Feline IFN-γ has recently been sequenced after RT-PCR amplification (Argyle *et al.*, 1995; Schijns *et al.*, 1995b; for EMBL Accession number see Table VIII.4.1). At the nucleotide level, it shares 78% and 63% homology with the cDNA of human and murine IFN-γ. At the amino acid level, the feline IFN-γ shares 63% and 43% homology with human and murine homologs, respectively. For many years, IFN-γ has been known to have profound effects on the different functions of the immune system, especially on the $T_H1$ pathway (Young and Hardy, 1995). In cats, IFN-γ activity has been studied only in FIV infection. During the acute phase of FIV infection, IFN-γ and IL-12 mRNAs rise transiently to very high levels; thereafter these cytokines return to baseline concentrations (Dean *et al.*, 1996a). In contrast, while the mRNA levels of IFN-γ and IL-12 return to the original concentrations, IL-4 and IL-10 mRNAs remain elevated. These results suggest that during early FIV infection a $T_H1$ immune response is triggered which later switches to a $T_H2$ type of immune response. It has to be stressed that studies dealing with expression of IL-12 and IFN-γ are based on quantification of mRNA expression: it is important that these findings are confirmed by quantification of the respective proteins.

## TNF-α

TNF-α has been sequenced (McGraw *et al.*, 1990; Daniel *et al.*, 1993; Lyons *et al.*, 1993; for EMBL Accession number see Table VIII.4.1) and expressed in *E. coli* as functional protein (Rimstad *et al.*, 1995). The biologically active protein had a molecular mass of 17 kDa. Feline TNF-α immunologically cross-reacts with antibodies specific for human TNF-α (Lehmann *et al.*, 1992; Rimstad *et al.*, 1995). TNF-α production is increased in FIV infected cats (Lehmann *et al.*, 1992) probably reflecting increased production by macrophages infected by FIV (Lin and Bowman, 1993). FeLV infection of macrophages also has been reported to trigger increased TNF-α production (Khan *et al.*, 1993). In a L929 cytotoxic assay, TNF-α was found to display CD50 activity at 15 ng/ml (Rimstad *et al.*, 1995). Cats given recombinant feline (rf)TNF-α intravenously manifested the typical biological effects of TNF-α, including fever, depression, and piloerection. The rfTNF-α upregulated IL-2 receptor and MHC-II antigen expression on peripheral blood mononuclear cells stimulated *in vitro*, but had no effect on TNF-α receptor and MHC I antigen expression (Rimstad *et al.*, 1995). When BAL cells collected from cats with long-term FIV infection were stimulated by LPS or Con A and phorbol myristate acetate, less TNF-α was produced than by cells from cats not infected by FIV (Ma *et al.*, 1995). However, in the acute phase of FIV infection, significantly more TNF-α was produced than in noninfected cats. According to Kraus *et al.* (1996) expression of FIV p24 during development of viremia during the acute phase of FIV infection is closely associated with expression of TNF-α, which suggests a close interrelationship between FIV and TNF-α expression. TNF-α expression was detected in feline hearts shortly after endotoxin application (Kapadia *et al.*, 1995). Ohno *et al.* (1993) demonstrated that TNF-α induces apoptosis in CmFK cells chronically infected by FIV while apoptosis was not found in noninfected cells. These findings may be important in the understanding of the pathogenesis of FIV induced immunosuppression. The feline TNF-receptor was recently sequenced (Duthie *et al.*, 1996; for EMBL Accession number see Table VIII.4.1)

### Stem cell factor

Stem cell factor (SCF) has been found to be an essential hematopoietic cytokine that interacts with other cytokines to facilitate survival of hematopoietic stem and progenitor cells, to influence their entry into the cell cycle and to stimulate their proliferation and differentiation (Hassan and Zander, 1996). Two isoforms of feline stem cell factor (fSCF) have been sequenced by RT-PCR and expressed in *E. coli* (Dunham and Onions, 1996a,b; for EMBL Accession number see Table VIII.4.1), The two isoforms had 274 amino acids and 246 amino acids. Feline SCF shows a high degree of homology to the SCFs of other species at both the nucleic acid and protein level. *In vitro*, the recombinant protein was found to induce cell proliferation in different cell lines (EC50 4–40 ng/ml). When injected into specific pathogen-free (SPF) cats, SCF was found to induce neutrophilia and to increase the number of peripheral blood colony forming units (Dunham and Onions, 1996b). Feline SCF may prove to be a useful cytokine for therapy of retrovirus induced anemias and neutropenias.

### Mast cell growth factor

The mast cell growth factor (MGF) stimulates growth of mast cells. The sequence of feline MGF (Lyons *et al.*, 1993; for EMBL Accession number see Table VIII.4.1) shows a 37% similarity with that of feline SCM sequenced by Dunham and Onions (1996b; for EMBL Accession number see Table VIII.4.1).

### Nerve growth factor

The human nerve growth factor (NGF) consists of a family of factors responsible for the survival, differentiation and functional activities of sensory and sympathetic neurons in the peripheral nervous system (Barde, 1990). It also supports the development and functional activities of cholinergic neurons in the central nervous system. The 7S form of NGF is a complex of three proteins ($\alpha$, $\beta$ and $\gamma$), The 26 kD $\beta$ subunit is a homodimer of two disulfide-bonded proteins with a length of 118 amino acids and displays the biological activity of NGF. Feline NGF-$\beta$ has been sequenced (Lyons *et al.*, 1993; for EMBL Accession number see Table VIII.4.1). However, the biological effects of the feline NGF-$\beta$ have not been investigated thoroughly.

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## 5. The Major Histocompatibility Complex

### Introduction

Early studies on the feline major histocompatibility complex (MHC) by Pollack *et al.* (1982) using *in vitro* lymphocytotoxicity assays suggested that cats fail to develop lymphocytotoxic antibodies in response to pregnancy or transfusion. Moreover, while immunization with foreign cells induced lymphocytotoxic antibodies, the response was not allospecific, as observed in other species. Similarly, only weak mixed lymphocyte reaction (MLR) responses were detected between unrelated cats of different breeds. The authors concluded that there may be limited polymorphism in the feline MHC. Subsequent studies by Winkler *et al.* (1989) investigated the development of cytotoxic antibodies in the cat following skin grafting between unrelated cats. The alloantisera generated displayed lymphocytotoxicity and permitted the identification of six clusters of overlapping feline MHC (termed FLA) specificities. Using these sera the authors were able to define FLA haplotypes and show that the specificities segregated as a single Mendelian complex.

### Genetic characterization of the feline major histocompatibility complex

The genetic characterization of the feline major histocompatibility complex MHC has been reviewed in detail by Yuhki (1995). The feline MHC class I and II (FLA I and FLA II) loci map to the centromeric region of chromosome B2. The FLA I molecule is encoded from a single open reading frame and gives rise to a 363 amino acid (a.a.) protein similar in structure to human HLA I. Following a 24 a.a. leader sequence there are three extracellular domains of 90 a.a. (a1), 92 a.a. (a2) and 92 a.a. (a3), a 31 a.a. transmembrane domain and a 34 a.a. cytoplasmic domain. Cysteine residues at positions 101, 164, 203 and 259, and the predicted site for *N*-linked glycosylation (amino acids 86–88) are conserved between feline, human and murine class I molecules (Yuhki and O'Brien, 1988). Interestingly, Winkler *et al.* (1989) were able to identify only one transcriptionally active MHC class I locus (with two alleles), perhaps explaining previous reports suggesting limited diversity in the feline MHC. However, abundant polymorphisms were observed at both the MHC class I and class II loci.

Screening of feline cDNA libraries using human MHC class II specific (DRA and DRB) probes identified three DRA and DRB equivalents and suggested the existence of at least two DRA and two DRB loci. Screening of the libraries with human MHC class II specific (DQA and DQB) probes failed to identify cross-hybridizing clones,

suggesting divergence at this locus. Feline MHC class II DRA molecule consists of a 25 a.a. leader sequence followed by an 84 a.a. a1 domain, a 107 a.a. a2 domain, a 23 a.a. transmembrane domain and a 15 a.a. cytoplasmic domain. The DRB molecule consists of a 29 a.a. leader sequence followed by a 95 a.a. b1 domain, 104 a.a. b2 domain, 23 a.a. transmembrane and 16 a.a. cytoplasmic domain. Interestingly, although polymorphic residues were observed between feline MHC DRA chains, none of the mutations resided in either of the a1 domains, the domains of the molecule containing the antigen recognition site. In contrast, polymorphisms were observed in the b1 domain of feline MHC DRB, in the region comprising the antigen recognition site (Yuhki, 1995).

## Biochemical characterization of feline MHC antigens

Early studies by Neefjes *et al.* (1986) analysed the biochemical characteristics of feline MHC molecules. Detergent extraction followed by immunoprecipitation demonstrated abundant MHC class I molecules, and low levels of MHC class II antigens in unstimulated human PBLs. In contrast, similar analyses of feline PBLs demonstrated high levels of both MHC class I and MHC class II on unstimulated PBLs. In the murine and human immune systems MHC class II expression is restricted, with predominant expression on B cells, macrophages, dendritic cells and activated T cells. In contrast, both resting and activated T cells express high levels of MHC class II molecules in the cat (see below). The $\alpha$-chain of feline MHC class II has an acidic pI and apparent $M_r$ of $35 \times 10^3$ whereas the $\beta$-chain has a basic pI and an apparent $M_r$ of $30 \times 10^3$ (Neefjes *et al.*, 1986). The feline MHC class I $\alpha$-chain has an apparent $M_r$ of approximately $45 \times 10^3$. Interestingly immunoprecipitation of feline MHC class I from cultured feline lymphocytes with the cross-species reactive antibody W6/32 did not yield $\beta_2$-microglobulin (Neefjes *et al.*, 1986). While this may reflect exchange of $\beta_2$-microglobulin with bovine $\beta_2$-microglobulin from the culture medium, it is possible that W6/32 recognizes feline MHC class I in the absence of $\beta_2$-microglobulin, as has been reported with other species.

## Expression of MHC molecules in the feline immune system

Several reagents have been identified with which the expression of MHC antigens in the feline immune system can be studied. Early studies on the feline MHC utilized cross-species-reactive antibodies such as W6/32 (anti-human MHC class I) and ISCR3 (antimurine IE). Recently, reagents specific for feline MHC class II have been developed including 42-3H2 (Rideout *et al.*, 1990) and vpg3 (Willett *et al.*, 1995). These antibodies were generated as a fortuitous byproduct of feline immunodeficiency virus research, both antibodies resulting from fusions of splenocytes from mice immunized with purified FIV. It has been shown that as lentiviruses bud from cells they incorporate a range of cellular proteins into the viral envelope (Arthur *et al.*, 1992). FIV appears to incorporate a significant quantity of MHC class II molecules and several groups have reported anti-MHC class II antibodies predominating during immunization of mice with FIV.

Flow cytometric analysis of feline peripheral blood mononuclear cells using the 42-3H2 antibody revealed that MHC class II molecules are expressed on the majority of T and B lymphocytes (Rideout *et al.*, 1990). Importantly, both resting and activated T cells express MHC class II molecules, giving rise to a characteristic bimodal histogram when MHC class II expression is analysed on lymphocytes. The high-intensity peak consists predominately of B lymphocytes whereas the low-intensity peak consists predominately of T lymphocytes (Rideout *et al.*, 1990; Willett and Callanan, 1995). In the FIV-infected cat higher levels of MHC class II expression are detected on a subset of $CD8^+$ lymphocytes which usually express reduced levels of MHC class II ($CD8^{low}$), suggesting that this subpopulation may represent activated $CD8^+$ lymphocytes (Willett and Callanan, 1995). While Ohno *et al.* (1992a) reported that higher levels of MHC class II could be detected on PBMC from FIV-infected cats compared with specific pathogen-free (SPF) cats, the authors did not establish whether this was due to an increase in the number of B lymphocytes or upregulation of T cell MHC class II expression. In contrast, Rideout *et al.* (1992) demonstrated that there was a persistent elevation in the percentage of $CD4^+$ and $CD8^+$ T lymphocytes expressing MHC class II antigens shortly after FIV infection but that a similar elevation was present in cats chronically infected with FeLV. The data suggest that despite a basal level of MHC class II expression on feline T cells, expression can be upregulated following T cell activation.

An early study of the feline MHC by Pollack *et al.* (1982) using a cross-reactive antimurine IE antibody suggested that activated and resting feline T lymphocytes could be differentiated on the basis of expression of an IE-like molecule. Therefore, it would appear that while some feline MHC class II molecules are expressed on all T cells (42-3H2 and vpg3 reactive), additional MHC class II molecules are expressed only upon activation (IE-like). The expression of MHC class II molecules on resting T cells is not unique to the cat, similar phenomena have been described in the dog, pig and horse (Thistlewaite *et al.*, 1983; Doveren *et al.*, 1985; Crepaldi *et al.*, 1986). The differentiation of activated T lymphocytes on the basis of MHC class II expression has been observed in the horse where IA-like molecules are constitutively expressed on all T cells while IE-like molecules are restricted to activated T cells.

**Table VIII.6.1** The feline AB blood group system

| Type | *Disialoganglioside* $(NeuNAc)_2GD_3$ | *Intermediate* | | $(NeuNGc)_2GD_3$ | *Alloantibodies* | *Frequency* |
|---|---|---|---|---|---|---|
| | | *x* | *y* | | | |
| A | + | + | + | +++ | + | Most common blood type |
| B | ++++ | – | – | – | +++ | Common in certain breeds and geographic locations |
| AB | ++ | ++ | ++ | ++ | – | Extremely rare |

## 6. Red Blood Cell Antigens

Although naturally occurring alloantibodies were recognized in cats in 1915, it was not until the second half of the twentieth century that two major feline blood types, known as type A and B, were discovered. In 1981, Auer and Bell (1981) also identified an extremely rare blood type AB that reacted with anti-A and anti-B reagents. These three blood types form the only known blood group system in cats designated as the AB system, although it is not related to the human ABO system (Bell, 1983) (Table VIII.6.1).

In contrast to most blood types in other species, type-A and type-B antigens are not codominantly inherited (Giger *et al.*, 1991a). The A allele is dominant over the B allele. Thus, all type-B cats are homozygous for the B allele (genotype B/B), whereas type-A cats can be homozygous or heterozygous for the A allele (genotype A/A or A/B). The AB allele is recessive to the A allele, but dominant over the B allele. There may be an additional genetic mechanism responsible for the inheritance of the AB blood type in cats, but they are not generally produced by breeding type-A cats to type-B cats (Griot-Wenk *et al.*, 1996).

Specific neuraminic acids on gangliosides, containing ceramide dihexoside (Galb1-4Glc-cer) as a backbone, correlate with the feline AB blood group antigens (Andrews *et al.*, 1992; Griot-Wenk *et al.*, 1993). Although disialogangliosides predominated, mono- and tri-sialogangliosides were also isolated. Type B cats express only *N*-acetyl-neuraminic acid on these gangliosides. A-type red cells express predominantly *N*-glycol-neuraminic acid containing gangliosides, but also some *N*-acetyl neuraminic acids. Equal amounts of the above two neuraminic acids containing disialogangliosides and two intermediary forms were found on type AB erythrocytes. In addition to these glycolipid patterns, differences in the glycoprotein patterns were also identified.

Naturally-occurring alloantibodies have been used for blood typing cats (Bücheler and Giger, 1993). All type-B cats have very strong anti-A alloantibodies which act as strong ($>1:64$) agglutinins and hemolysins. Type-A cats have generally weak anti-B alloantibodies (1:2). Therefore, the anti-B serum has been replaced with *Triticum vulgaris* lectin which strongly agglutinates type B cells (Butler *et al.*, 1991). A simple card test is now available to differentiate between type A, B, and AB cats (DMS Laboratories, 2 Darts Mill Rd, Flemington, NJ 08822; 1-800-567-4367).

The frequency of blood type A and B among domestic shorthair and longhair cats differs markedly between parts of the United States and other parts of the world (Giger *et al.*, 1991a,b, 1992). The distribution of type A and B among purebred cats varies even more, although no geographic variation has been noted. Knowledge of the blood type frequency in each breed is important when estimating the risk of AB incompatibility reactions. The AB blood type has been recognized in domestic shorthair and longhair cats and purebred cats with blood type B, but occurs extremely rarely (Griot-Wenk *et al.*, 1996). With less than 1%, type AB is not listed in Tables VIII.6.2 and VIII.6.3.

Although the function of these red blood cell antigens remains unknown, they are responsible for serious blood incompatibility reactions. Owing to the fact that cats have naturally occurring alloantibodies, even a first mismatched

**Table VIII.6.2** Frequency of blood type A in domestic shorthair and longhair cats

| | *Type A (%)* |
|---|---|
| Asia, Tokyo, Japan | 90.0 |
| Australia, Brisbane | 73.7 |
| Europe | |
| Austria | 97.0 |
| England | 97.1 |
| Finland | 100 |
| France | 85.1 |
| Germany | 94.0 |
| Italy | 88.8 |
| Netherlands | 96.1 |
| Scotland | 97.1 |
| Switzerland | 99.6 |
| North America, United States | |
| Northeast | 99.7 |
| North Central/Rocky Mountains | 99.6 |
| Southeast | 98.5 |
| Southwest | 97.5 |
| West Coast | 95.3 |
| South America, Argentina | 97.0 |

Type B = 100-type A; type AB is less than 1%.

**Table VIII.6.3** Frequency of blood type A in purebred cats in the United States

| | *Type A (%)* |
|---|---|
| Abyssinian | 86 |
| Birman | 84 |
| British shorthair | 60 |
| Burmese | 100 |
| Cornish rex | 66 |
| Devon rex | 59 |
| Exotic shorthair | 75 |
| Himalayan | 93 |
| Japanese Bobtail | 84 |
| Maine Coon | 98 |
| Norwegian Forest | 93 |
| Persian | 84 |
| Russian blue | 100 |
| Scottish fold | 82 |
| Siamese | 100 |
| Somali | 83 |
| Sphinx | 82 |
| Tonkinese | 100 |

Type B = 100-type A; type AB frequency is less than 1%.

blood transfusion can result in life-threatening hemolytic transfusion reactions particularly when a type-B cat receives type A blood (Giger and Bücheler, 1991). Furthermore, type A and AB kittens born to queens, even when primiparous, with blood type B may develop neonatal isoerythrolysis when receiving colostrum during the first day of life (Giger, 1991; Casal *et al.*, 1996). Kittens at risk may be blood typed at birth with cord blood and type A and AB kittens born to type B queens may be successfully foster-nursed for 24 h.

# 7. Immunoglobulins

## Introduction

As with any mammalian species analyzed to date, cats have different immunoglobulin (Ig) isotypes designated IgA, IgG and IgM (Aitken, *et al.*, 1967; Okoshi *et al.*, 1968; Klotz *et al.*, 1985; Grant, 1995; Paul, 1995). However, the number of subclasses identified for different Ig isotypes varies considerably from species to species. The mouse has one IgA class, human has two but the rabbit has as many as 13 different subclasses of IgA (Spieker-Polet *et al.*, 1993). In contrast, rabbits have only one class of IgG, mice and humans have four but pigs have at least five different subclasses of IgG (Butler and Brown, 1994). The analysis of the Ig isotypes summarized above was made by analyzing the genes encoding the different Ig molecules. This has provided conclusive evidence for the structure of Igs which was not possible to elucidate otherwise because only a few amino acid differences may represent different isotypes, allotypes or Ig subclasses.

To date, no data have been published analyzing genes encoding cat Ig. Therefore, the information available has been obtained by using monoclonal antibodies (mAb) specific to cat Ig and biochemical methods to analyze Ig. This information forms the basis for the discussion of cat Ig.

## Cat Ig light chains

Cats have two different classes of Ig light chains: $\kappa$ and $\lambda$ each with a molecular mass of 25 kDa. The ratio between $\kappa$ and $\lambda$ light chains expressed on Ig in various tissues and fluid has been estimated to be 1 : 3 (Hood *et al.*, 1967; Klotz *et al.*, 1985). mAb specific for $\kappa$ and $\lambda$ light chains have been described (Klotz *et al.*, 1985; Grant, 1995).

## Cat Ig heavy chains

### IgM

Cat IgM was first analyzed by Aitken *et al.* (1967) by agar gel diffusion techniques. As in other mammals, there appears to be only one IgM class. Some cat IgM binds to *Staphylococcus aureus* Protein A (SpA; Goudswaard *et al.*, 1978; Lindmark *et al.*, 1983). However, it cannot be excluded that variable regions representing $V_H$III like families (Paul, 1995) from cat bind to SpA rather than the Ig heavy chains from IgM (Sasso *et al.*, 1991). There are mAbs specific to cat IgM (Klotz *et al.*, 1985; Grant, 1995). The serum concentration of IgM in specific pathogen-free cats is 0.32 mg/ml ($\pm$SD 0.27; $n$ = 22; Grant, 1995).

### IgA

At least one IgA isotype is present in cats but some workers have found evidence for two subclasses of IgA based on differential binding to SpA or binding to a mAb (Grant, 1995). However, different glycosylation of IgA, dimeric IgA or allotypes of IgA (Brown *et al.*, 1995) cannot be excluded. The serum concentration of IgA in specific pathogen-free cats is 0.31 mg/ml ($\pm$SD 0.24; $n$ = 22; Grant, 1995).

### IgG

There is evidence for at least two (Schultz *et al.*, 1974) and possibly up to four IgG subclasses in cat named IgG1 to IgG4 based on anionic exchange chromatography (Grant, 1995), the analysis of differential binding of cat IgG to SpA using the methods of Seppala *et al.* (1981) and mAb to cat IgG (Klotz *et al.*, 1985; Grant, 1995). The heavy chain of IgG has been estimated to be 50–55 kDa. There is no mAb which exclusively recognizes one of the postulated four

IgG subclasses (Klotz *et al.*, 1985; Grant, 1995). mAb to cat Ig, which have been described in some detail by Grant (1995) have been evaluated for cross-reactivty to human, cow, dog, goat and horse Ig. Some cross-reactivity was found among the same isotypes present in different species.

### IgE

Clinical evidence of type I allergies in cats associated with IgE implies that IgE is present in cats. However, although all mammalian species analyzed in any detail have IgE, no cat IgE has yet been described.

### Conclusion

mAb against cat $\kappa$ and $\lambda$ light chains and mAb against IgM, IgA and IgG exist (Klotz *et al.*, 1985; Grant, 1995). There is no specific mAb against the putative subclasses of cat IgA or IgG. Ongoing analysis of Ig genes in some laboratories will improve understanding of the structure of Ig in cats.

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## 8. Passive Transfer of Maternal Immunity

### Introduction

At birth, kittens are highly susceptible to microbial infections because of their immature immune system. During the course of maturation, kittens are protected from microbial infection mostly by humoral immunity transferred from their mothers.

### Transplacental immunity

The structure of the feline placenta is different from those of most domestic animals. The fetal chorionic epithelium in feline placenta is in close contact with the endothelium of maternal capillaries, and this type of placenta is called endotheliochorial placenta (Leiser and Kaufmann, 1994). This type of placentation allows a small amount (5–10%) of maternal immunoglobulins (primarily IgG) to transfer to the fetus (Scott *et al.*, 1970; Schultz *et al.*, 1974). The amount of immunoglobulin transferred to the fetus is very difficult to determine by standard quantitative assays, such as radial immunodiffusion and immunoelectrophoresis (Schulz *et al.*, 1974). Consequently, detection of transplacental immunoglobulins in the fetus has been demonstrated more readily by assays which detect antigen-specific antibodies in the serum of presuckling kittens, such as neutralizing antibody assay, enzyme-linked immunosorbent assay, and immunoblot analysis (Harding *et al.*, 1961; Scott *et al.*, 1970; Pu *et al.*, 1995). There is no information available about transplacental cell-mediated immunity in cats.

### Immunity transferred by colostrum and milk

Newborn kittens receive a majority of maternal antibodies via ingestion of colostrum. During late pregnancy and under the influence of hormones, the mammary glands of queens secrete milk into mammary alveoli. The milk preformed before parturition is called colostrum. Colostrum is rich in immunoglobulins and the immunoglobulin fraction consists predominantly of IgG; IgA and IgM are present in lower concentration (Table VIII.8.1). The concentration of IgG in colostrum is two- to four-fold higher than that found in serum. A majority of IgG and a considerable portion of IgA in the colostrum are actively transported from the circulation into mammary alveoli by specific receptors on the acinar epithelial cells of the mammary glands (Gorman and Halliwell, 1989). Colostrum contains trypsin inhibitors, as well as antimicrobial factors such as lysozyme, lactoferin, and lactoperoxidase (Stabenfeldt, 1992). In addition, colostrum has high concentrations of lipids and lipid-soluble vitamins (particularly vitamin A), proteins (such as caseins and albumin), and minerals, and also low levels of carbohydrates (Reece, 1991; Stabenfeldt, 1992). All of these components are nutritionally important to newborn kittens.

Milk is produced by queens immediately after the consumption of colostrum by kittens. The composition of milk is considerably different from that of colostrum. Milk has low levels of IgG and IgA and lacks IgM (Table VIII.8.1). IgG is the predominant immunoglobulin class in the milk of cats, unlike other nonruminant animals such as dogs, pigs and horses, in which IgA is the predominant class (Tizard, 1996). A majority of IgG and IgA is synthesized locally in the mammary glands, unlike colostrum which consists of immunoglobulins transported from the serum. Milk also differs from colostrum in that it contains less lipids, proteins, and minerals, and has more carbohydrates (Reece, 1991; Stabenfeldt, 1992).

Within the first 24–36 h after birth, colostral immunoglobulins (IgG, IgA, and IgM) are absorbed by the intestinal epithelial cells of newborn kittens (Figures VIII.8.1 and VIII.8.2). The trypsin inhibitors present in colostrum decrease the proteolytic activity in the gastrointestinal tract of newborn kittens, thereby enabling the immunoglobulins to retain their structure and function

**Table VIII.8.1** Immunoglobulin levels (mg/dl) in serum and milk of nursing queens[a]

| | | *Average immunoglobulin concentration (range) at postpartum (weeks)* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Sample* | *Ig Isotypes*[b] | *0* | *1* | *2* | *3* | *4* | *6* | *References* |
| Serum | IgG | 764 (420–1550) | 594 (350–840) | 611 (340–840) | 434 (385–495) | 906 (660–1450) | 742 (480–920) | Pu *et al.* (unpublished data) |
| | IgA | 138 (66–310) | 77 (26–175) | 66 (0–210) | 88 (41–135) | 175 (26–500) | 179 (41–260) | |
| | IgM | 444 (230–700) | 402 (245–560) | 420 (0–800) | 405 (200–690) | 576 (208–800) | 525 (265–750) | |
| Milk | IgG | 1572 (685–3150) | 189 (60–400) | 99 (0–230) | 90 (80–120) | 110 (60–220) | 117 (60–200) | |
| | IgA | 57 (26–127) | 14 (0–35) | 7 (0–23) | 15 (0–26) | 5 (0–21) | 7 (0–21) | |
| | IgM | 47 (0–280) | 0 | 0 | 0 | 0 | 0 | |
| Serum | IgG | 1375 | | | | | | Pedersen (1987) |
| | IgA | 215 | | | | | | |
| | IgM | 116 | | | | | | |
| Milk | IgG | 4400 | 440 | 360 | 300 | 315 | 100 | |
| | IgA | 340 | 44 | 20 | 20 | 24 | 24 | |
| | IgM | 58 | 2 | 0 | 0 | 0 | 0 | |
| Serum | IgG | 1894 (1171–2258) | | | | | | Gorman and Halliwell (1989) |
| | IgA | 285 (102–582) | | | | | | |
| | IgM | 247 (60–390) | | | | | | |
| Milk | IgG | 3570 (2750–4674) | | | 189 (94–255) | | | |
| | IgA | 254 (50–488) | | | 13 (9–20) | | | |
| | IgM | 110 (31–300) | | | 20 (10–40) | | | |

[a]Specific pathogen-free cats were used in all studies except for the study by Gorman and Halliwell (1989) which did not specify the cat source.
[b]Immunoglobulin (Ig) isotypes were quantified using radial immunodiffusion assay plates (Bethyl Laboratories, Inc., Montgomery, Texas, USA) by Pu *et al.*, and unspecified methods by Pedersen (1987) and Gorman and Halliwell (1989).

(Tizard, 1996). The ingested immunoglobulins bind first to specific Fc receptors on the surface of intestinal epithelial cells. Subsequently, immunoglobulins are taken up by the epithelial cells via pinocytosis and transferred into the lacteals and then the circulation (Tizard, 1996). Once maternal immunoglobulins are absorbed into the circulation, a small portion is released back onto the mucosal surface, thereby providing the local immunity. However, a majority of maternal immunoglobulins remain in the circulation and confer systemic immunity (Tizard, 1996). In contrast, immunoglobulins present in milk remain mainly in the intestine, and consequently, provide local immunity for the gastrointestinal tract (Tizard, 1996).

Upon passive transfer, the levels of serum immunoglobulins in newborn kittens approach those found in adults (Scott *et al.*, 1970) (Figure VIII.8.1). The transferred immunoglobulins are important in protecting newborn kittens against infections, such as feline leukemia virus (FeLV) (Jarrett *et al.*, 1977), feline panleukopenia virus (FPV) (Scott *et al.*, 1970) and feline immunodeficiency virus (FIV) (Pu *et al.*, 1995). The lack of protection in some kittens may result from failure of queens to produce or transfer sufficient amounts of protective antibodies specific for pathogens encountered during this critical period (Tizard, 1996).

It is still unclear whether maternal immune cells that are present in the colostrum and milk can be transferred from queens to kittens. However, studies with other domestic animals indicate that maternal immune cells can be transferred via colostrum and milk to the newborns and provide selected cell-mediated immunity (Tizard, 1996).

## Vaccination of kittens

Vaccination of kittens should be scheduled according to the level and nature of the passive immunity received

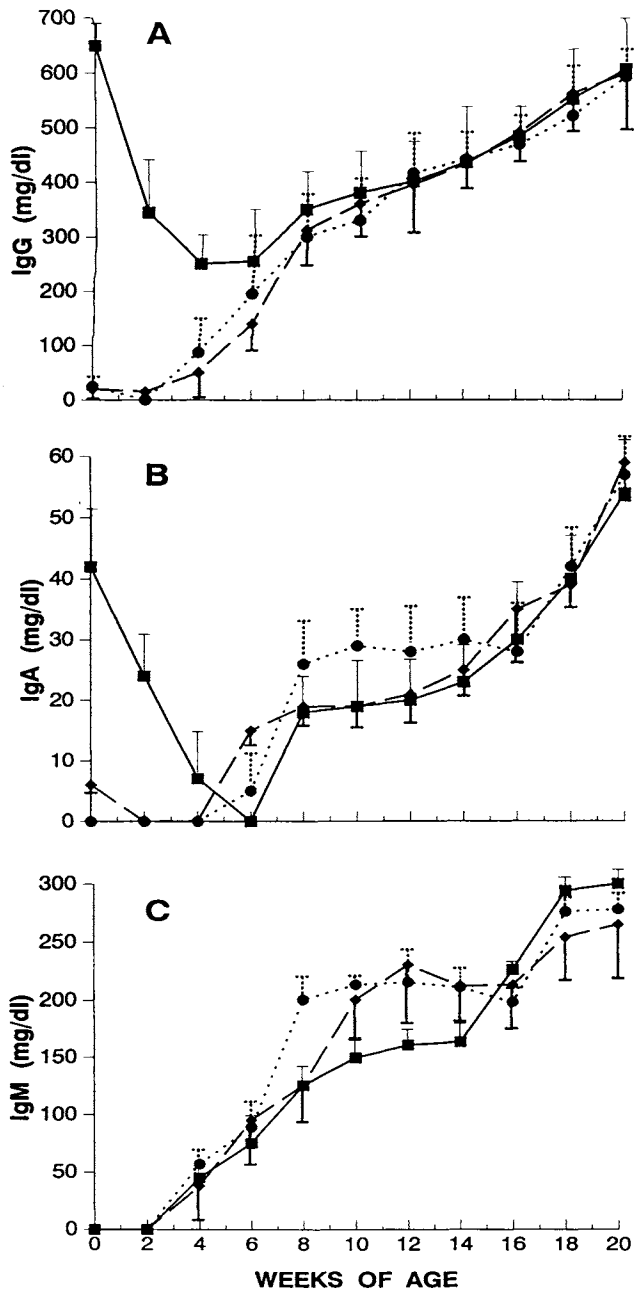


**Figure VIII.8.1** Serum levels of IgG (A), IgA (B), and IgM (C) in kittens ($n$ = 6) receiving either colostrum plus milk (——), milk alone (– – –), or milk replacement (.....). The immunoglobulin levels were determined by radial immunodifusion assays (Bethyl Laboratories, Inc., Montgomery, Texas, USA).

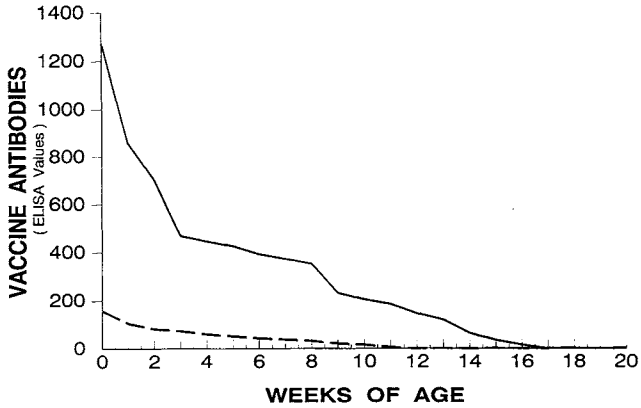


**Figure VIII.8.2** FIV-specific antibodies detected in serum samples of kittens born to FIV vaccinated queens. Immediately after birth, kittens ($n$ = 4) were either nursed by their mothers (——) or FIV antibody-free surrogate queens (– – –). FIV-specific antibodies were detected by FIV transmembrane peptide ELISA.

by the kittens. This is based on the concept that maternal antibodies reactive to vaccine antigens can interfere with the development of the immunity elicited by the vaccination (Scott *et al.*, 1970; Scott, 1971). Vaccine interference can result in delay and/or failure in the development of protective levels of vaccine-induced immunity. In general, the level of vaccine interference correlates directly with the titer of specific antibodies present in the serum of kittens. Thus, the vaccination schedule of kittens should be designed in a manner which avoids or overcomes potential vaccine interference. Ideally, kittens should receive the first vaccination at a time when pathogen-specific maternal antibodies are depleted. It is possible to predict this time-point based on the titer of specific antibodies in queens and the half-life of specific maternal antibodies in kittens. The half-life of maternally derived antibodies varies with their isotypes; for example 15 days for FeLV antibodies (Jarrett *et al.*, 1977), 9.5 days for FPV antibodies (Scott *et al.*, 1970), 7 days for feline enteric coronavirus antibodies (Pedersen *et al.*, 1981), and 18.5 days for feline rhinotracheitis virus antibodies (Gaskell, 1975). The half-life of antibodies may also vary between kittens from different litters (Scott *et al.*, 1970). Generally, specific antibody titers in kittens and queens are not usually determined before vaccination, thus, vaccination schedules are designed according to the known half-life of specific antibodies. For example, if the half-life of antibodies for a specific pathogen is 15 days, the maternal antibodies remaining in the serum of kittens will drop to insignificant levels (approximately 1.5%) by 3 months old. Because the half-life of antibodies against a majority of pathogens is equal to or less than 15 days, the primary vaccination should be administered to kittens at about 3 months old. However, it is important to note that low titers of residual maternal antibodies to FPV (Scott *et al.*, 1970) and FIV (Figure VIII.8.2) have been detected in the serum of kittens at 4 months old. Thus, to overcome the potential vaccine interference, vaccine boost at 4 months old is important in ensuring successful vaccination, as has been suggested by others (Scott *et al.*, 1970; Scott, 1971). This view is further supported by results from experimental vaccine studies which demonstrate the ability of vaccine boosts to overcome maternal antibody interference (Scott, 1971) (Figure VIII.8.3).

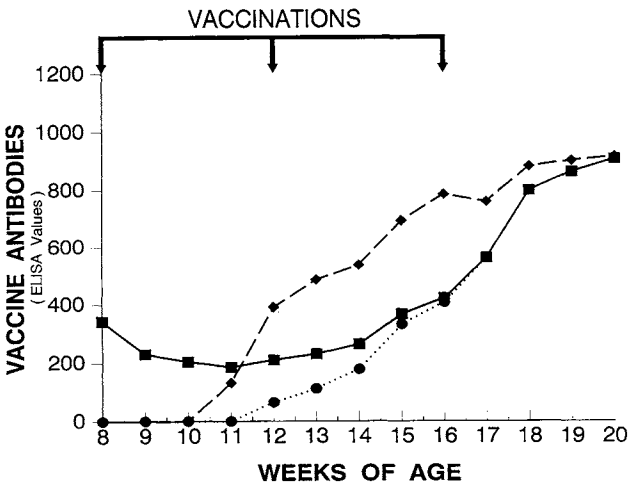


**Figure VIII.8.3** Vaccine antibody titers of kittens ($n = 6$) that received colostrum and milk from either vaccinated (——) or unvaccinated (– – –) mothers prior to vaccinations at 8, 12, and 16 weeks old. The actual antibody titers produced by kittens (.....) after vaccination were calculated using the following formula: antibody titer in vaccinated kittens subtracted by antibody titer in unvaccinated littermate.

### Failure of passive transfer of immunity

Compared with large newborn domestic animals (i.e. calves, foals, and lambs), failure of passive transfer of maternal immunity to kittens may not play a key role in neonatal mortality. Newborn kittens can survive without ingestion of colostrum providing that they receive an appropriate milk replacement containing the adequate nutritional content (Figure VIII.8.1). In addition, such newborn kittens require special care, such as maintenance of proper hygiene and keeping them in sanitary containers under proper temperature and humidity conditions.

## 9. Neonatal Immune Response

Although little is known about the development of the immune system in cats, it most likely follows a pattern similar to other mammals in that the thymus is the first lymphoid organ to develop, followed by the secondary lymphoid tissue. The feline thymus first appears during the fifth week of gestation and occupies the cranioventral mediastinal space. Lymphocytes from the bone marrow seed the thymus by 40 days of gestation. The secondary lymphoid tissue such as spleen and lymph nodes are then seeded with mature T cells (from the thymus) and B cells (from the bone marrow). In the cat, population of the secondary lymphoid tissue is largely complete at the time of birth.

Thymectomy performed at 5 weeks old had no direct effect on numbers of circulating B and T lymphocytes or on the responsiveness of peripheral lymphocytes to concanavalin A or pokeweed mitogen. Survival of skin allografts performed 3 weeks after thymectomy was slightly prolonged compared with nonthymectomized littermates. Kittens appear to be similar to the cow, sheep, and pig in that neonatal thymectomy does not have important immunologic or physiologic consequences (Hoover *et al.*, 1978). This contrasts with mice and puppies which develop a severe wasting and immunodeficiency syndrome following early thymectomy (Roth, 1967).

Thymic aplasia and hairlessness has been reported in Birman kittens. Histological examination of 2-day-old kittens revealed reduced germinal centers and paracortical depletion of lymphocytes in the lymph nodes, spleen, and Peyer's patches. Thymic tissue could not be identified. Pedigree analysis of five litters containing both normal and affected kittens suggested an autosomal recessive mode of inheritance. Although the clinical and histologic appearance was similar to congenital hypotrichosis with thymic aplasia in other species, further immunological studies have not been performed (Casal *et al.*, 1993).

In contrast to surgical thymectomy, neonatal infections with lymphotropic viruses can lead to premature thymic atrophy and a wasting syndrome associated with immune deficiency. The immune deficiency occurs because lymphocytes in the secondary lymphoid tissue, as well as those in the thymus, are depleted. The most common cause of thymic atrophy in kittens is infection with feline leukemia virus (FeLV), and affected kittens have increased survival of skin allografts, indicating defective T-cell immunity (Perryman *et al.*, 1972). Feline panleukopenia virus (FPV) also induces severe thymic atrophy along with lymphocyte depletion of the spleen, lymph nodes, and Peyer's patches, and destruction of bone marrow myeloid cells, leading to marked panleukopenia (Rohovsky and Griesemer, 1967; Larson *et al.*, 1976). FPV causes a lytic infection of rapidly dividing cells. Thus, infection of feline fetuses with FPV at 35 days gestation, during early thymic development, results in immune dysfunction, as demonstrated by delayed skin allograft rejection, while infection at 45 or 55 days of gestation, after the thymus is well developed, has no effect. Postnatally infected cats of various ages had no alteration in graft rejection times or humoral immune responses and only transient depression in lymphocyte responses to the T-cell mitogens Con A and PHA. Thus, the immunosuppression of FPV is due to transient panleukopenia and depressed T-cell responses, and is not as profound as that induced by FeLV infection (Schultz *et al.*, 1976).

Although found in secondary lymphoid tissue prior to birth, there is evidence that lymphocytes in the neonatal cat continue to mature both phenotypically and functionally for a period of time after birth. Analysis of lymphocyte subsets in perinatal kittens demonstrated a steady decrease in null lymphocytes, with a concomitant increase in T and B lymphocytes from 56 days of gestation through to 8 weeks old (Sellon *et al.*, 1996). In addition, the $CD4^+$:$CD8^+$ ratios of kittens are much higher than in

adult cats due to high numbers of $CD4^+$ cells and very low numbers of $CD8^+$ cells. As kittens mature from birth to nearly 1 year old their $CD4^+$:$CD8^+$ ratios slowly decline as $CD8^+$ cells increase (English *et al.*, 1994; Sellon *et al.*, 1996). This gradual increase in $CD8^+$ cell numbers is most likely a reflection of a $CD8^+$ response to increasing antigen exposure.

It is generally believed that most mammals are able to mount an immune response at the time of birth but that the magnitude of the response is much less than that of an adult. In support of this, IL-2 production in response to Con A stimulation is much lower in kittens less than 10 weeks of age compared to adults (M. B. Tompkins, unpublished data). This suggests that, although neonatal kittens have high numbers of $CD4^+$ cells, these cells are not able to function at full capacity. Resistance to FeLV infection is also a reflection of functional maturation of the immune response. One-hundred per cent of neonates and 85% of weanling kittens become persistently infected with FeLV upon challenge, while only 15% of cats older than 4 months develop a persistent infection (Hoover *et al.*, 1976). This age-related resistance has been shown to be associated with maturation of macrophage function. Macrophages from neonatal kittens are highly permissive for FeLV infection and replication, while macrophages from adult cats are not (Hoover *et al.*, 1981).

Immunological tolerance may be induced in neonatal kittens up to 25 days old. Unresponsiveness may be induced following exposure to antigens administered either orally or parenterally. As in other species, tolerance is more likely to develop to simple soluble antigens than to complex antigens (Gorham *et al.*, 1971)

The neonatal kitten depends primarily upon maternal antibody for protection from infectious diseases. Newborn kittens obtain nearly all of their maternal antibody from colostrum, although a very small amount of IgG also may be transferred across the placenta. The colostral phase of lactation lasts approximately 5 days, during which time the concentration of IgG and IgA in milk is several-fold greater than that in the dam's serum (Yamada *et al.*, 1991) The neonate is able to absorb the antibodies most efficiently during the first 16 h of life (Casal *et al.*, 1994), although this period may be extended somewhat if feeding is delayed. The transfer of colostral antibodies, especially IgG, is so efficient that immunoglobulin levels in the neonate may exceed those of the dam shortly after birth (Yamada *et al.*, 1991).

The half-life of maternally derived immunoglobulin is variable and depends, in part, on its isotype. As in other species, the half-life of transferred IgG ($4.14 \pm 1.29$ days), IgA ($2.03 \pm 0.33$ days), and IgM ($2.2 \pm 1.2$ days) is shorter than that of endogenously synthesized immunoglobulin. Transferred maternal antibody wanes to a nadir at 4–5 weeks old, leading to a period of increased susceptibility to pathogens before endogenous antibody production reaches adult levels at about 12 weeks old (Yamada *et al.*, 1991). While passive immunity is vital to protection from infection of the newborn kitten, immune responses to both live and inactivated antigens are suppressed by high levels of maternal antibodies. Interference with oronasal (herpes virus, calicivirus) and parenteral (herpesvirus, panleukopenia virus) vaccination is a well-documented phenomenon of cats that nurse immune queens (Pedersen, 1987).

Failure of passive transfer occurs in kittens that fail to nurse vigorously in the first 24 h after birth and often leads to death from bacterial sepsis during the first and second weeks of life. Hypoimmunoglobulinemia in these kittens may be corrected by the parenteral administration of 3–5 $cm^3$ of serum from a healthy, immunized cat, preferably one from the same environment in which the kitten is reared.

In addition to systemic passive transfer, maternal antibodies in the milk (lactogenic immunity) provide nursing kittens with continuous local protection against pathogens in the oral cavity and gastrointestinal tract during the first weeks of life prior to the development of local mucosal immunity (Pedersen, 1987).

## 10. Nonspecific Immunity in the Cat

### Introduction

In mammalian species, the immune system can be categorized into two distinct but overlapping components. The 'innate' or nonspecific immune system consists of natural anatomical barriers, macrophages, natural killer cells, neutrophils, and eosinophils. The nonspecific immune system is the first to make contact with pathogens and is characterized by an immediate response, lack of antigen specificity, and lack of immunological memory. The cells of the innate immune system interact directly with pathogens and subsequently secrete cytokines which enhance their microbicidal activity and serve as cofactors for initiating the second component of the immune response, the acquired antigen-specific, T-cell immune response.

The specific interactions which provide innate immunity in the cat have not been studied extensively. Innate immunity in the cat is presumed to be similar to that in other species. This brief review focuses on the individual components of the feline innate immune system.

### Natural barriers

The natural barriers, such as the skin or mucosal surfaces which line the respiratory or gastrointestinal tract, form the first line of defense against invading pathogens. Specific information which evaluates nonspecific immunity in the context of natural barriers in the cat is currently not available. It is assumed that the components of these are

similar to other mammals. The anatomical location of mucosa-associated lymphoid tissue (tonsils, Peyer's patches) in the cat has been described previously (Pedersen, 1987; Tompkins, 1993).

## Neutrophils

The role of the feline neutrophil in nonspecific immunity is the phagocytosis and killing of microbes. In other species, the neutrophils have been shown to secrete cytokines (Lloyd and Oppenheim, 1992), but this has not been investigated in the cat. Feline peripheral blood polymorphonuclear cells can be separated from mononuclear cells by utilizing a double-density gradient technique (Toth *et al.*, 1992). Feline neutrophils are morphologically similar to those in other species. The morphometry of granule genesis which accompanies feline neutrophil maturation has been studied (Fittschen, 1988a). The complex carbohydrate staining of feline neutrophil primary and secondary granules resembles that in humans and rabbits; however, cats lack tertiary granules analogous to those found in other species (Fittschen, 1988b). Cat neutrophils also have a third (late-forming) type of granule which has not been described in other species (Fittschen *et al.*, 1988b). Recombinant canine granulocyte colony-stimulating factor (rcG-CSF) and glucocorticoids have been shown to cause significant elevation in peripheral neutrophil counts in cats (Obradovich *et al.*, 1993; Duncan *et al.*, 1994).

Studies of feline neutrophil function have evaluated chemotaxis, phagocytosis, and oxidative burst activity during disease states. Feline neutrophils, similar to equine, porcine, bovine, and canine neutrophils, do not respond chemotactically to *N*-formyl-methionylleucyl-phenylalanine (FMLP) (Gray *et al.*, 1986). Neutrophil and endothelial cell interactions involving integrins and selectins have been well studied in the cat and serve as a model for neutrophil-induced endothelial damage in myocardial ischemia (Ma *et al.*, 1991; Lefer and Ma, 1994; Murohara *et al.*, 1994). Supernatant from cultured peritoneal exudate cells (PEC) from cats with effusive feline infectious peritonitis (FIP) was chemotactic for peripheral blood neutrophils from healthy cats (Tsuji *et al.*, 1989). Peripheral blood neutrophils collected from FIP-infected cats also showed reduced chemotaxis to zymosan-activated serum while showing similar chemotactic responses to control neutrophils when exposed to PEC supernatants (Tsuji *et al.*, 1989). Neutrophils from FeLV-viremic clinically affected cats had significantly lower chemotactic responses than did those from subclinically affected FeLV-viremic cats (Kiehl *et al.*, 1987). The chemiluminescence response as an indicator of oxidative burst activity in feline neutrophils has been found deficient in cats infected with feline leukemia virus (FeLV) and feline immunodeficiency virus (Lafrado *et al.*, 1987; Hanlon *et al.*, 1993).

## Eosinophils

The role of the eosinophil in the cat is thought to be anthelmintic immunity and allergy. Specific information on characterization of feline eosinophil granule components, surface receptor expression, chemotactic stimuli, and effector functions is not available.

A technique for isolation of feline eosinophils via peritoneal lavage has been previously described (Moriello *et al.*, 1993). In the lung, eosinophils have been found to be a significant component of the cell population obtained by bronchoalveolar lavage in specific pathogen free and conventional cats (Hawkins and DeNicola, 1989; McCarthy and Quin, 1989). Human recombinant interleukin-2 (rHuIL-2) induces a peripheral eosinophilia in cats secondary to an enhanced maturation response in bone marrow precursor cells (Tompkins *et al.*, 1990a). In addition to this maturation signal, rHuIL-2 induces a potent activation signal for eosinophils, as measured by a decrease in density and an increase in longevity in culture (Tompkins *et al.*, 1990).

## Globule leukocyte

Although not a polymorphonuclear cell, the globule leukocyte is mentioned here because of its morphologic similarity to the eosinophil. The globule leukocyte is slightly larger than an eosinophil and has eosinophilic cytoplasmic granules on hematoxylin and eosin staining. Unlike eosinophils, the globule leukocyte has a single, excentric nucleus and not a bilobate nucleus as seen in feline eosinophils. Globule leukocytes are found in the mucosa of the intestinal, respiratory, and urogenital systems of many species including the cat. Although their function is poorly understood, their mucosal location indicates a possible role in mucosal innate immunity. The origin of the globule leukocyte is also a matter of debate, with current opinions focusing on mast cell or lymphocytic lineage. A neoplasm of globule leukocytes in the cat has been reported (Honor *et al.*, 1986).

## Natural killer cells

Classically, the NK cell's role in nonspecific immunity is the non-MHC restricted recognition and removal of viral-infected and neoplastic transformed cells. These functions have been described in the cat. Morphologically, natural killer cells, along with lymphokine-activated killer (LAK) cells and cytotoxic T lymphocytes (CTL), have large, azurophilic cytoplasmic granules and are collectively known as 'large granular lymphocytes'. Phenotypically, NK cells express CD16, CD56, and CD57 but do not express CD3, CD4, or the T-cell receptor. A unique subpopulation of NK cells that express CD8 and exhibit non-MHC-restricted cytotoxic activity has been reported

in cats (Zhao *et al.*, 1995). A method of induction of cytotoxic large granular lymphocytes from peripheral blood mononuclear cells has been described in cats by Tompkins *et al.* (1989b).

Most studies on NK cell function in cats have concentrated on NK cell cytotoxicity to sensitive tumor cell lines or virally infected targets. For example, a reduction of NK cell-mediated cytotoxicity against baby hamster kidney cells was demonstrated in cats infected with feline immunodeficiency virus (FIV) (Hanlon *et al.*, 1993). Other studies have shown that NK cells from FIV-infected cats are still able to bind to target cells but have reduced ability to kill them, and the defect could not be overcome by *in vitro* treatment of effector cells with IL-2 (Zaccaro *et al.*, 1995). A population of $CD57^+CD8^+$ LAK cells obtained from FIV-infected cats had cytotoxic activity against FIV-infected target cells which exceeded the cytotoxic activity of LAK cells derived from non FIV-infected cats (Zhao, 1995). The same population of cells also exhibited cytotoxic activity against FeLV-infected cells, indicating the cytotoxic activity was not antigen specific.

### The mononuclear phagocytic system

The mononuclear phagocytic system of cats is similar to other species and consists of blood monocytes and tissue or resident macrophages. The resident macrophages are the first to contact pathogens and, through phagocytosis and cytokine production, regulate activation of NK cells (IL-12) and recruitment of neutrophils (IL-1, IL-8). Activation of NK cells results in NK cell-derived IFN-$\gamma$, which activates the macrophage, resulting in augmented effector functions. In secondary lymphoid organs, the macrophage, via upregulation of MHC II molecules and elaboration of cytokines, presents antigen to T cells, thus completing the bridge between nonspecific and antigen-specific immunity.

Methods have been published for the isolation of feline macrophages from the lung (BAL), peritoneum, and bone marrow (Stoddart and Scott, 1988; Hawkins and DeNicola, 1989; Daniel *et al.*, 1993). Pulmonary intravascular macrophages have been demonstrated in cats and removal of blood-borne pathogens/particulates is performed by pulmonary intravascular macrophages and not by spleen or liver macrophages as in humans, mice, and dogs (Winkler, 1988).

Feline macrophages have also been observed as secretory cells. Bioactive TNF has been detected from feline alveolar macrophages stimulated with LPS (J. W. Ritchey, unpublished data) and thioglycolate-elicited peritoneal macrophages (Lin and Bowman, 1993). TNF mRNA and IL-1 mRNA and protein have been demonstrated in bone marrow-derived macrophages stimulated with LPS (Daniel *et al.*, 1993). Constitutive cultures of feline alveolar macrophages express mRNA for TNF, IL-6, IL-10, and IL-12, but do not have detectable levels of IL6 or TNF as measured by bioassay (J. W. Ritchey, unpublished data). In addition to TNF, feline alveolar macrophages stimulated with LPS produce bioactive IL-6 (J. W. Ritchey, unpublished data). Macrophage function has also been studied in disease states. Alveolar macrophages from FIV-infected cats constitutively produce TNF and IL-6 as early as 4 weeks post infection (J. W. Ritchey, unpublished data). Peritoneal macrophages from cats infected with FIV had decreased IL-1 secretion, although the macrophages had enhanced antimicrobial activity compared to controls (Lin and Bowman, 1992). This may be related to macrophage activation by *in vivo* conditioning from IFN$\gamma$, which our laboratory has demonstrated to be upregulated in the lymph nodes of FIV-infected cats (J. Levie, unpublished data). Differences also exist between different populations of resident macrophages because feline peritoneal macrophages had higher microbicidal activity and released more IL-1 in response to LPS stimulation than feline alveolar macrophages (Lin and Bowman, 1991). Lastly, the intrinsic resistance to infection and replication of coronaviruses in feline peritoneal macrophages correlates with *in vivo* virulence and the development of feline infectious peritonitis (Stoddart and Scott, 1989).

### Conclusions

Although much remains to be examined of the feline nonspecific immune system, what is known suggests that this system in the cat is very similar to other mammalian species. With the continued development of reagents for use in feline immunological research and the continued application of the cat as models for disease (AIDS, myocardial ischemia), the future holds promise for further, indepth examination of feline nonspecific defense mechanisms.

## 11. The Complement System

The first documented quantitation of cat complement activity appeared in 1938 (Dingle *et al.*, 1938). Apparently typical classical and alternative complement pathways have been identified in cat serum (Grant, 1977; Grant *et al.*, 1979; Day *et al.*, 1980; Kobilinsky *et al.*, 1980; Grant and Michalek, 1981; Goddard *et al.*, 1987; Hosoi *et al.*, 1990; Fevereiro *et al.*, 1993; Buerke *et al.*, 1995). Cat C1 has been partially purified (Olsen *et al.*, 1974), and cat C3 was purified and identified as a dimer of two polypeptide chains of 128 and 71 kDa (Jacobse-Geels *et al.*, 1980). Cat complement (C′) components appear to be interchangeable with those of other species, e.g. cat C1 will initiate the classical pathway when subsequent components are derived from guinea-pig (Olsen *et al.*, 1974); guinea-pig C2 will activate purified cat C3, and the resulting C5 convertase will initiate formation of the membrane attack complex with purified human C′ components C5 through

C9 (J. M. McCarty and C. K. Grant, unpublished data). As in other species, spontaneous activation of feline C1 is prevented by C1 esterase inhibitor C1 INH (Buerke *et al.*, 1995), and cat C3 is inhibited by cobra venom factor (CVF). Like humans, mice, rabbits and cows, the cat C2 gene is very closely spaced to the related Factor B gene, and the upstream sequence element of all five mammalian species is highly conserved (Moreira *et al.*, 1995).

Human serum C′ has been proven to inactivate some retroviruses by an antibody independent mechanism whereby C1 binds directly to a receptor on the transmembrane region, and subsequent virolysis occurs via the classical pathway (Bartholomew *et al.*, 1978). Cat serum possesses the same property and will effectively inactivate human immunodeficiency virus type-1 (HIV-1) (Hosoi *et al.*, 1990). Cat retroviruses – feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV) – do not appear to be efficiently inactivated by cat C′ alone. In the presence of cat antibodies to retrovirus envelope proteins, however, virolysis is mediated by cat C′ via the classical activation pathway.

Cat C′ appears to be relatively weak when compared with other species in hemolytic or C′ fixation tests employing rabbit antisheep red blood cell (RBC) serum and sheep RBC targets. If bovine or human RBC are substituted (with the appropriate rabbit antibovine RBC or rabbit antihuman RBC immune serum), then cat serum exhibits average or strong hemolytic functions respectively (Dingle *et al.*, 1938; Grant, 1977).

To summarize, Cat C′ factors appear to be similar to the C′ components of other mammalian species, and *in vitro* interchangeability with human or guinea-pig components has been demonstrated. As such, valid inferences can probably be drawn from studies of C′ metabolism in health or disease of other species; this is important because a paucity of information exists on the interactions of feline C′ and pathogenesis. Complement level fluctuations, and in particular hypocomplementemia, have been regularly observed in FeLV infections, FeLV-related tumors and nonregenerative anemias, but C′ levels and C′ consumption are factors in the pathogenesis of other viral infections such as feline infectious peritonitis (FIP), feline herpesvirus type-1 (FHV), and probably FIV and parasitic infections. Complement-dependent antibodies lyse viruses and other pathogenic organisms, but they are less effective if C′ levels are severely reduced or not available, hence further studies of cat C′ levels during the courses of pathogenesis are required.

**Table VIII.11.1** Roles for cat complement in feline immune responses to infectious disease agents: summary of citations

| *Infectious agent* | *C′-related observations* | *References* |
|---|---|---|
| FeLV | (a) C′ depletion by cobra venom factor did not promote viremia in early stage experimental infection, but the CVF was administered before cytotoxic anti-FeLV gp70 antibodies might appear. | Kraut *et al.* (1985); Johnson *et al.* (1988) |
| | (b) Classical pathway of the cat C′ system is activated *in vitro* by incubation of FeLV with normal cat serum. | Kraut *et al.* (1987) |
| | (c) C′ levels in naturally infected, healthy, cats are relatively stable. Hypocomplementemia observed in 55% cats with leukemias or lymphomas and 45% of cats with nonregenerative anemias. | Grant *et al.* (1979) |
| | (d) Hypocomplementemia, and increased circulating immune complexes (CIC), noted in infected cats, and cats bearing virus-related tumors. | Day *et al.* (1980) |
| | (e) Virus infected erythroid, and granulocyte-macrophage bone-marrow progenitors, lysed by anti-gp70 antibodies and C′. | Kobilinsky *et al.* (1980) |
| | (f) Virus-producing cat leukemias and lymphomas are lysed by cat anti-gp70 antibodies and cat C′. | Grant *et al.* (1977, 1979); Grant and Michalek (1981) |
| | (g) Deposition of IgM and C3 detected in the kidneys of the majority of naturally infected cats. Kidney functions were severely disrupted in 40% of these cats. | Olsen *et al.* (1974) |
| FIV | No evidence found for Ig and C′ mediated lysis of bone marrow white cell progenitors in FIV infections (cf. Kobilinsky *et al.*, 1980) | Linenberger and Abkowitz (1992) |
| FIP | (a) Hypocomplementemia detected in 45% of natural infected symptomatic cases. | Grant *et al.* (1979) |
| | (b) Experimental infection resulted in CIC formation, initial C′ concentration increases, then hypocomplementemia and death. | Horimoto *et al.* (1989); Hosoi *et al.* (1990) |
| FHV | (a) Cat anti-FHV antibodies plus C′ lyse FHV-infected cells via the alternative pathway of C′ activation. | Goddard *et al.* (1987) |
| | (b) Neutralization of FHV increased by addition of C′. | Jacobse-Geels *et al.* (1982) |
| Brugia pahangia | (a) C′ mediates cat granulocytes to adhere to and then kill microfilariae. | Jacobse-Geels *et al.* (1980) |

## 12. Ontogeny of the Immune System

The cat has an endotheliochorial type of placenta and receives its passively acquired immunoglobulin both pre- and post-natally. The transmission of immunoglobulin across the small intestine is quantitatively the most important (Harding *et al.*, 1961), and whilst in pre-suckled kitten serum IgG can be quantified, little or no IgA or IgM can be detected (Schultz *et al.*, 1974). To date, there have been relatively few age-related studies to the development of cat serum immunoglobulins (Barlough *et al.*, 1981). Early studies would indicate that by 50 days of gestation there are significant serum immunoglobulin levels (Okoshi *et al.*, 1968), which are comparable to that found in presuckled neonates. Following birth serum IgG levels fall over the first month of life, presumably reflecting the half-life of the passively transferred immunoglobulin. Subsequently, they rise but adult levels are not achieved until more than 1 year old (Yamada *et al.*, 1991).

### B lymphocyte system

To date only one detailed study of the ontogeny of the feline B lymphocyte system has been reported (Klotz *et al.*, 1985). Lymphocytes first appear in fetal circulation at about 25 days of gestation. Studies in other species have shown that pre-B cells (cytoplasmic $\mu^+$, surface $Ig^-$) are first detectable in fetal liver and later in bone marrow, where they are generated throughout life. Pre-B cells have been detected in the fetal liver of cats of 42 days of domestic gestation, but earlier fetuses have not been examined. Surface $IgM^+$ B cells were also detectable in 42-day-old fetal liver, and it would appear that, as in other species, feline liver and bone marrow are the sites of origin and differentiation of cells of the B-lineage. Following birth the frequency of splenic B cells increases rapidly, with no further increase observed between 12 weeks old and adulthood. The majority of splenic, blood and bone marrow B-cells express surface IgM, with a smaller number expressing surface IgG. In cats, plasma cells secreting IgM are most frequent in the bone marrow from 1-week-old animals, but as animals mature the frequency of IgG plasma cells increases, reflecting isotype switching and clonal expansion in response to environmental antigens. In contrast to humans, rodents and rabbits, feline B cells preferentially express $\lambda$ over $\kappa$ light chains ($\approx 4:1$), but this would appear to be independent of age and environmental exposure (Klotz *et al.*, 1985).

### T lymphocyte system

Following birth the absolute numbers of lymphocytes increase until adult numbers are achieved by 13–15 weeks old. A recently published study (Sellon *et al.*, 1996) has extended these findings and shown that during the last few weeks of gestation there were significant changes in the numbers and proportion of lymphocyte subsets. The most dramatic was a threefold increase in the proportion of cells staining for a pan-T cell marker, and it has been speculated that this may reflect hormonal influences associated with parturition. During the first 4 weeks after birth there is a dramatic increase in the number of $Ig^+$ B cells, probably reflecting a response to environmental antigens. Interestingly, at this time there is also an increase in the absolute number and proportion of null cells ($sIg^-$, pan $T^-$). Kittens are born with greater CD4 : CD8 ratios ($\approx 3.5:1$) than adults ($\approx 1.5:1$). Changes in the ratio towards an adult phenotype would appear to be primarily due to an increase in $CD8^+$ T cells, but this may not be reached until about 1 year old, and this would appear to be sensitive to antigenic exposure.

As a consequence of the paucity of data upon the feline immune system it is not beneficial to compare and contrast features of the ontogeny of its immune system with that found in other species. It is sufficient to say that it would not appear to be significantly different from other species with an endotheliochorial placenta.

## 13. Mucosal Immunity

### Introduction

Compared with rodents and man and even some other domestic species such as the pig and cow, the feline mucosal immune system has received scant attention. The current interest in the cat as an experimental model for *Helicobacter pylori* infection, feline immunodeficiency virus (FIV) mucosal vaccine studies and small bowel transplantation, would suggest that this neglect may be rapidly rectified.

### The gastrointestinal immune system

The gastrointestinal tract is one of the largest immunological organs of the body, containing more lymphocytes and plasma cells than the spleen, bone marrow and lymph nodes combined. The gut-associated lymphoid tissue comprises cells organized within the lymphoid follicles of the Peyer's patches as well as those distributed throughout the lamina propria and intestinal epithelium.

#### Secretory immunoglobulins

As with other mammals, IgA in the cat is the predominant immunoglobulin in mucosal secretions (Vaerman *et al.*, 1969). It is found in large amounts in saliva, tears, respiratory and intestinal secretions, milk and bile

(Schultz *et al.*, 1974). In serum cat IgA exists mainly as a dimer with only trace amounts in the monomeric form. In the small intestinal lamina propria IgA-producing cells predominate, accounting for between 40% and 80% of the total plasma cell pool. In contrast, IgG-positive cells (51%) are more frequent in colonic tissues, with smaller numbers of IgA- and IgM-producing cells (Klotz *et al.*, 1985).

## The cells of the gastrointestinal immune system

The general anatomy and morphology of the feline intestinal tract have not been comprehensively described. The intestinal lamina propria is heavily populated with 'immune cells' and, in the colon, it has been shown that greater than 90% of the cells stain with an anti-fCD45 monoclonal antibody (Sturgess, 1997). In the majority of species studied to date there is a preponderance of $CD4^+$ over $CD8^+$ T cells in the lamina propria. In the cat small intestine this distribution is less marked and approximately equal numbers of fCD4 and fCD8 cells have been enumerated by both immunohistology on tissue sections and flow cytometry of cells isolated by collagenase digestion (Durgut, 1996). In the feline colonic lamina propria the fCD4:fCD8 ratio is always less than 1 (Sturgess, unpublished data). Furthermore it may be of significance that whereas in the colon the sum of the $fCD4^+$ and $fCD8^+$ cells approximate to the number of cells expressing the fCD5 marker, in the small intestine there is a greater number of $fCD4^+$ and $fCD8^+$ cells.

Intraepithelial cells (IELs) may be enumerated on a morphological basis by identifying those cells which are located towards the lumen from the basement membrane. As in other species IELs are more numerous in the feline small intestine ($\approx$23 IELs/100 epithelial cells, EC) than colon ($\approx$4 IELs/100 EC). At both sites the majority of feline IELs are $fCD8^+$, $fCD5^+$. The intestinal epithelium of rodents, man and guinea pigs express MHC class II antigens, whereas that of pigs, sheep and cattle do not. In species expressing epithelial class II it is restricted to the small intestine, the large bowel epithelium being negative or only very weakly positive in healthy animals (Bland, 1988). The tissue distribution is restricted to the fully differentiated absorptive enterocytes on the upper two-thirds of the villus. In the cat, the colonic epithelial cells are uniformly negative for MHC class II antigens, while a small proportion of the small intestinal epithelial cells contain MHC class II antigens intracellularly, within vesicular like structures in the supranuclear region. The biological role of epithelial class II antigens is unclear but it has been shown in species with constitutive expression of class II that it can be further enhanced in response to intestinal inflammation. Under these conditions not only is there increased expression upon mature small intestinal enterocytes, but expression may also be induced upon immature crypt cells and, in inflammatory bowel disease, upon colonic epithelium. No results of similar studies in the cat have been reported.

## Functional studies of the gut immune system

It is generally accepted that the Peyer's patches are the major site of induction of mucosal responses. In contrast, it is suggested that the lamina propria is essentially an effector organ involved in surveillance and in the provision of help during the rapid responses to recall antigens. Such mechanisms may include both active protective responses against potential pathogens as well as the prevention of damaging allergic responses to dietary and environmental antigens. Difficulties associated with the isolation of viable cells from the lamina propria and Peyer's patches have delayed functional analysis of the cellular and molecular basis of these mechanisms. However, it has been shown that whereas Peyer's patch cells produce relatively more IL-2 and IFN-$\gamma$, lamina propria cells synthesized relatively more IL-4, IL-5 and IL-6. Recent studies in cats have focused upon reverse transcription-polymerase chain reaction (RT-PCR) analysis of cells isolated from the colon and have detected mRNA encoding for a number of cytokines. While the detailed analysis of local cytokine production and receptor expression in this species has yet to be completed, it is noteworthy that in asymptomatic feline immunodeficiency virus (FIV)-infected cats there was enhanced expression of IL-2, IL-6 and IL-10 (Sturgess, unpublished observations).

## Infection via the intestinal tract

*Helicobacter pylori* infection has recently been described in domestic cats and in view of the possible zoonotic implications this finding has promoted considerable attention. *Helicobacter pylori* infection is associated with a lymphofollicular gastritis, consisting of $IgM^+$ B cells assembled into multiple lymphoid follicles surrounded by clusters of $fCD4^+$ and $fCD8^+$ T cells (Fox *et al.*, 1996).

Although biting probably provides the major route of transmission of FIV it has been shown that it is possible to infect cats across the intact vaginal and rectal surfaces. Moreover, following infection it has been possible to detect proviral DNA. Replicating virus and viral proteins can be detected in the colonic follicle associated epithelial cells and occasional cells in the lamina propria (Bishop *et al.*, 1996). Cats infected with FIV via the rectal route remain asymptomatic for more than 1 year and this is associated with an increase in the number of colonic lamina propria $fCD8^+$ T cells and increased expression of IL-2, IL-6 and IL-10 mRNA. These results might suggest that lamina propria cytotoxic cells (CTLs) may play a role in the control of infection during the pre-AIDS period but this remains to be determined.

# 14. Immunological Diseases

## Introduction

Diseases of an immunological nature can be classified into six categories: (1) disorders of immediate hypersensitivity or allergies, (2) diseases resulting from the reaction of autoantibodies and alloantibodies, (3) conditions resulting from the deposition of immune complexes, (4) diseases mediated by cellular immunity, (5) gammopathies, and (6) acquired and congenital immunodeficiencies. Each of these categories of immune disease is a result of normal immune reactions that have been subverted or perturbed in some manner. This section deals with the first five categories of immune diseases. Immunodeficiencies, because of their great current interest, will be covered in a separate chapter.

The types of immune diseases that occur in cats parallel those of dogs, humans, and other species of animals. As in humans and dogs, a common group of cofactors link all immunological diseases of cats, regardless of category. These cofactors include: (1) genetic predisposition, (2) drug therapy for other disorders, (3) infectious diseases, particularly those of a persistent nature, (4) cancer, (5) diet, (6) age, particularly the very young and old, and (7) gender, with sexually intact females suffering more than intact males and with male/female castrates having similar and intermediate disease incidence.

## Mechanisms of allergy

Allergy is defined as a disease or reaction caused by an immune response to one or more environmental antigens, resulting in tissue inflammation and organ dysfunction. Allergic diseases are mediated by the IgE system. This system is an integral part of the skin and mucosal defenses to parasite invasion and migration. Parasites shed surface proteins into the surrounding tissues during invasion and migration, especially at key stages in their growth, such as molting. These antigenic substances stimulate the production of specific IgE antibodies by plasma cells in local diffuse lymphoid aggregates. Once produced, IgE antibodies circulate only briefly before becoming firmly bound to the surface of tissue mast cells and basophils. Basophils and mast cells that are coated with specific IgE antibodies are said to be 'sensitized'. Sensitized basophils and mast cells are potent deterrents to further parasite migration or invasion. Antigens released by the parasites during invasion or migration will bind to specifically sensitized mast cells in the immediate area and, if sufficient amounts of specific IgE are complexed, the mast cells (or basophils in the case of blood-borne parasites) will release their granules into the surrounding milieu. These granules contain factors such as prostaglandins, leukotrienes, histamine, eosinophil chemotactic factor, slow-reacting substance of anaphylaxis, and platelet-activating factor. The resultant reaction is characterized by increased vascular permeability at the site, local edema, fibrin meshing, smooth muscle contraction and the influx of large numbers of eosinophils. This inflammatory reaction slows the migration of the parasites and provides a milieu in which the immune attack can be launched. The primary effector cell is the eosinophil, which, when activated, has the ability to contact directly and kill parasites through potent oxidants and cationic substances present in its granules.

Allergic diseases involve the same mechanisms as parasite immunity, except that IgE antibodies are inappropriate, i.e. usually nonparasitic, antigens. These inappropriate antigens are referred to as 'allergens'. Allergens are most likely to be taken into the body by the same routes as parasites – through oral and respiratory mucous membranes and the skin.

## Specific allergic diseases

### Systemic allergies

Systemic allergies result from the entry of allergens into the bloodstream, either via injection or absorption across the mucous membranes. The most frequent cause of systemic allergic reactions in cats are vaccinations, followed by injectable medications. Oral medications, certain ingested foods and insect bites are less common offenders. The mildest form of systemic allergy is urticaria. Urticaria, or hives, are small, raised, circular areas of edema, hyperemia and pruritus that appear on the skin within minutes of systemic allergenic exposure. Angioneurotic edema (facial-conjunctival edema) is the next most severe form of systemic allergy. This reaction is characterized by the rapid appearance of edema around the eyes, face and lips, and mild signs of anaphylactic shock. Anaphylactic shock is the most severe form of systemic allergy. The target organ for anaphylactic shock in the cat is the portal vasculature; affected cats rapidly exhibit nausea, incoordination, pallor and discoloration of the mucous membranes of the mouth, rapid thready pulse, vomiting or diarrhea and, in severe cases, death. The cause of death is shock brought about by the rapid pooling of venous blood in the intestines, spleen and liver.

### Allergies of the skin

Nonseasonal allergic reactions of the skin of cats are usually associated with dietary allergens. Two types of lesions are seen. The first consists of highly pruritic plaque-like lesions about the head and neck, which are frequently subject to self-mutilation by scratching and further complicated by secondary bacterial infection. Miliary dermatitis is the second and more common manifestation of skin allergies of dietary origin. It is character-

ized by numerous small scabs, usually along the dorsum, that are sloughed off with small tufts of hair.

Seasonal skin allergies, most often from pollens, are uncommon in cats. They are usually characterized by pruritus, erythema and hair loss on the ears and about the eyes and face. Allergies to topical ocular and otic medications are common in young cats being treated for infections of the ears or eyes such as ear mites or chlamydiosis. This phenomenon is akin to 'allergic breakthrough' in humans, which is often associated with common infections. The infections appear to induce a dysregulation of normal T-cell regulation and a heightened IgE response to potential allergens. The resulting allergic otitis or conjunctivitis can closely resemble the infectious diseases that initiated the treatment, making it difficult to determine when the infection ends and the allergy starts. Once medication is stopped, however, the otitis or conjunctivitis rapidly resolve.

Flea allergic dermatitis is much less common in cats than in dogs, probably because the cat is the natural host for the common flea. The cat, therefore, has evolved a more benign host–parasite relationship than the dog. Flea allergic dermatitis, when it occurs in cats, is often a mixture of immediate (IgE-mediated), intermediate (immune complex or Arthus-mediated) and delayed (T-cell mediated) hypersensitivities.

### Eosinophilic granuloma complex

Eosinophilic granulomas occur as ulcerative lesions on the margins of the upper lips (rodent ulcers), as tumor-like proliferations on the dorsum of the tongue or on the hard palate, or linear encrustations on the backs of the legs and paws. The central lesion is a peculiar necrosis of underlying collagen; bundles of necrotic collagen are surrounded by dense infiltrates of closely adherent eosinophils, reminiscent of the IgE-mediated attack on parasites. Although eosinophils are prominent in the lesions, the role of allergy, if any, is uncertain. The author has found no response to strict dietary control and to confinement away from common biting insects. The disease has a strong genetic component and, like many other immune diseases, intact females have the most severe lesions and intact males the least severe. Ovariohysterectomy and castration tends to equalize the incidence and severity at an intermediate level. It also responds to glucocorticoid therapy.

### Allergies of the respiratory tract

Allergic conjunctivitis, associated with mild to moderate reddening of the conjunctiva and excessive tearing, has been observed as a seasonal condition in cats. The probable cause is pollen. A chronic mild to moderately severe conjunctivitis has been linked to continuous household exposure to cosmetics and cigarette smoke. A chronic hyperplastic conjunctivitis, characterized by intense inflammation and granular hyperplasia of the conjunctival membranes, is an uncommon condition of cats. Allergic rhinitis, a common condition in humans, is uncommon in cats but has been seen under similar conditions as allergic conjunctivitis. Seasonal allergies of the nasal passages and conjunctiva require only temporary treatment, while chronic allergies are best treated by changes in environment.

Allergic bronchitis, a common disease of dogs, is rarely seen in cats. Allergic bronchiolitis, however, is frequent in cats but uncommon in dogs. The disease is triggered by environmental allergens that are minute enough in size to be inhaled into the smaller airways. Allergic bronchiolitis is characterized by intermittent coughing or retching, mild to moderately severe diffuse peribronchiolar infiltrates on chest radiographs, and eosinophilia in tracheal wash or bronchiolar brushes. Severe cases may be accompanied by weight loss and fatigue. An important differential diagnosis is chronic lung-worm infestation.

Cats are the only animal species that suffer from a true allergic asthma. However, true asthma in cats is often grouped together with the aforementioned chronic allergic bronchiolitis under the term 'feline asthma'. True asthma in cats, as in man, is characterized by the sudden and transient attacks of severe bronchiolar constriction, the production of a thick tenacious mucus, and expiratory dyspnea. Asthmatic attacks in cats occur more often in summer, especially after they have been put outdoors. Status asthmaticus is not unusual in asthmatic cats and death can ensue if untreated. For this reason, owners of asthmatic cats will often keep injectable epinephrine (adrenaline) at hand.

### Allergic bowel disease

Allergic bowel disease is extremely common in cats, probably because modern commercial cat foods are alien to cats' ancestral diets. Wild cats are entirely carnivorous, while modern domestic cats are usually fed foods rich in cereals, egg and milk by-products, and soy protein. Meats such as beef and fish, common in commercial cat foods, are also unnatural in wild cat's diets. Allergies of the stomach and upper small intestine are very common in cats and manifested by vomiting of food within 30 min or less of eating. Allergies centered in the jejunum and ileum produce loose stools of normal frequency and volume. Because a cat's stool is often buried in the litter pan, owners will frequently not notice that it is abnormal. The stool may be extremely odorous, however, in which case the owner will be alerted to a problem every time the cat has a bowel movement. Allergic colitis is most frequently accompanied by fresh red blood in the stool and mucus. Frequent, high volume, loose stools are seen in only severe cases of colitis. Eosinophilia is often mild to nonexistent in allergic enteritis and colitis, although eosinophilic infiltrates are common in biopsies of inflamed tissues. In addition to changes in the consistency or odor of the stool, many cats with allergic bowel disease will have hair

coats that are dry, thin from excessive shedding, and lackluster. Plaque-like pruritic lesions about the head and neck, or miliary dermatitis, are accompanying features in some cats.

Eosinophilic enteritis is a term used for allergic bowel disease that is accompanied by a significant blood eosinophilia. Eosinophilic enteritis is much more severe than allergic enteritis and is frequently accompanied by significant diarrhea and weight loss. Blood and mucus is observed in the stool when the colon is also involved.

Bowel allergies, regardless of their site, are diagnosed and treated in the same manner, by feeding a hypoallergenic diet composed of a single animal meat, such as lamb, rabbit, or turkey. Severe cases, especially when eosinophilia is present, benefit from added corticosteroid treatment. When the bowel disease has subsided, a satisfactory long-term diet is found by trial and error with introduction of one new food at a time.

Allergic breakthrough leading to allergic enteritis is also frequent in young cats undergoing common intestinal infections. Typically, a group of cats develop an infectious type of enteritis, which clears up in most but becomes chronic in a small proportion. The tendency is to continue to search for an infectious cause in these latter animals, while the true cause is food allergy. The cats will respond well to hypoallergenic diet and after several months can be reintroduced to normal food.

## Autoantibody and alloantibody diseases

### Diseases involving alloantibodies

Only two major blood types have been recognized in cats, type A and type B (rarely AB); type A is dominant to B. Up to 98% of random outbred cats are blood type A, depending on the area of the world (highest in USA and Europe, and somewhat lower in Australia). Cats with blood type A often have preformed alloantibodies to type-B red cells, as do type-B cats to type-A cells. Alloantibodies against type-A cells are usually at higher titer and much more lytic than antibodies to type-B red cells. As a result, the most severe alloantibody reactions occur when type-A red cells (type-A donor) are exposed to type-A antibodies (type-B recipient), a situation occurring in neonatal isoerythrolysis and mismatched blood transfusions.

Neonatal isoerythrolysis is a common problem in certain pedigree breeds of cats that have inadvertently accumulated a high incidence of the type-B gene. Queens homozygous for type-B red cells, when bred to a homozygous blood type-A tom, will produce mostly type-A kittens. Following nursing, highly lytic type-A alloantibodies are passed in the colostrum, triggering an acute hemolytic episode in the newborns following nursing. Kittens usually die within the first day or two of life, and show signs of depression, hemoglobinuria and pallor.

Blood transfusion reactions are, fortunately, rare in outbred cats, mainly because of the extremely high incidence of blood type A, making it unlikely for randomly selected donors and recipients to have different blood types. The situation may be quite different among certain pedigree breeds.

### Diseases involving autoantibodies

Autoantibodies are produced in a number of different situations. Some cats, especially pedigreed animals, have a genetic predisposition towards autoantibody formation. This predisposition, acting with unknown environmental triggers, leads to a breakdown in normal self/nonself recognition. Certain chronic infectious diseases, such as feline immunodeficiency virus (FIV), feline leukemia virus (FeLV) and haemobartonellosis are frequently associated with autoantibody diseases. Drugs administered chronically for other disease conditions, such as propylthiouracil for hyperthyroidism, can cause autoantibodies to be produced. Certain types of neoplasms, especially lymphoid and myeloid tumors, have also been associated with autoantibodies.

Autoimmune hemolytic anemia (AIHA) in cats, which is similar to its human counterpart but unlike the canine disease, is more likely to be secondary than primary (idiopathic). The most common secondary causes are FeLV infection and haemobartonellosis. Lymphoid and myeloid neoplasia, cancer of other types, systemic lupus erythematosus (SLE) and drugs (e.g. propylthiouracil) are less common predisposing conditions.

AIHA in cats is more likely to be chronic than peracute or acute in nature. Except for cats with underlying infections such as *Haemobartonella* or FeLV, most AIHA cases in cats are Coombs' antibody and in-saline agglutination negative. A chronic, nonresponsive, Coombs' antibody-negative anemia suggests that the immune attack is directed against immature stages of red blood cells in the marrow and not against mature cells in the circulation. Coombs'-positive AIHA is more likely to be acute, responsive, and associated with autoantibodies against mature circulating red blood cells. The frequent secondary nature of AIHA in cats makes treatment more difficult and the prognosis worse. Secondary conditions must be searched for and eliminated if possible. Treatment, when indicated, usually involves corticosteroids or a combination of corticosteroids and other immunosuppressive drugs.

Autoimmune thrombocytopenia (AITP) is uncommon in cats, and like AIHA, it is often secondary. The most common predisposing disorders are FeLV or FIV infections. Propylthiouracil, used to treat hyperthyroidism, has also been linked to both AITP and AIHA. Cats are much more resistant to clinical disease when thrombocytopenic than dogs, so many cases may go undiagnosed or are picked up coincidentally during diagnostic work-ups for other complaints. Echymotic and petechial hemorrhages of the skin, epistaxis, melaena, and hematuria have all

been associated with AITP in cats. Prognosis and treatment are the same as for AIHA.

Diseases caused by autoantibodies to structures other than blood cell membranes have been described in cats, but are much less common in this species than in dogs. Pemphigus foliaceous is a chronic exfoliating dermatitis that commonly affects the ears, nasal planum, orbital ridges and feet. The condition is caused by autoantibodies against the intracellular cement substance that binds the uppermost layer of noncornified epidermal cells to each other and to the overlying cornified cells. It is usually idiopathic in nature, although antibiotic treatment has apparently triggered one case and purebreds are more likely to be affected than outbred cats. The resultant autoantibody binding causes a subcorneal blister that rapidly ruptures. Pemphigus erythematosus is a more virulent version of pemphigus foliaceous. Lesions are more widespread and severe, with a tendency to involve mucocutaneous junctions. Cats with pemphigus erythematosus are positive for antinuclear antibodies as well as having typical pemphigus-type bullous disease. Pemphigus vulgaris and bullous pemphigoid, two common disorders of dogs, are extremely rare in cats. The former is caused by autoantibodies to the intracellular cement binding the basal cells to their overlying epidermal cells, while the latter is caused by antibodies to the basal lamina binding the entire epidermis to the subcutis.

Myasthenia gravis is caused by autoantibodies against the acetylcholine receptors of skeletal muscle. The condition is very rare in cats, although common in dogs. In one feline case, the disease was associated with cysts in the thymus. Myasthenia gravis in people is often associated with thymoma. The disease is associated with rapid muscle weakness upon exercise with improvement of muscle strength following rest.

## Immune complex diseases

Immune complex diseases are caused by the chronic deposition of antigen–antibody–complement complexes within the basement membranes of blood vessels. The requirements for immune complex disease are a chronic source of antigen, a concomitant antibody response to the antigen, complement binding, and intravascular or intrabasement membrane deposition of the antigen–antibody–complement complex. The hallmark lesion of immune complex disease is vasculitis of either acute or chronic nature.

Like other immune diseases, immune complex disorders are either idiopathic or secondary. The most common secondary causes are chronic infectious diseases such as FIV or FeLV infections.

### Systemic lupus erythematosus (SLE)

SLE has been referred to as the penultimate immunological disease, both for the high incidence in man and animals and the wide range of immunologic and immunopathologic manifestations. SLE has three immunological components: (1) immune complex disease (vasculitis) involving one or more organ systems, (2) heightened B-cell responses, and (3) autoantibody production, especially to various nuclear proteins (i.e., antinuclear antibody or ANA) and to cell surface antigens. Like its counterparts in dogs and humans, SLE in cats has a strong genetic component, and as with dogs, it is more likely to be seen in pedigreed than outbred animals (in particular Persians, Siamese and related breeds).

Cats with SLE are usually from $1\frac{1}{2}$ to 6 years old at presentation. The most frequent presenting signs are bizarre psychotic behavior (hiding, fear, apprehension), twitching of facial muscles, and intermittant fever/weight loss. Although most cats with SLE have significant polyarthritis, lameness is only observed in one-third of animals. A chronic crusty skin disease, especially on the face and head, is the third most common presenting complaint, and renal disease the fourth. AIHA and AITP are less frequent presenting disorders. Physical and laboratory findings include fever, generalized lymphadenopathy, leukopenia and suppurative synovial fluid taps from tarsal and carpal joints. Conjunctivitis and palatine/glossal ulcers are occasionally observed. The ANA test is usually positive at titers ranging from 1:10 to 1:400, with either a speckled or homogenous pattern. Care must be taken not to over-interpret positive ANAs in normal cats or cats with disease signs incompatible with SLE. Many normal cats have low titers of ANA (less than 1:10) and ANAs at even high titer develop in a number of infectious conditions such as acute FeLV and FIV.

A significant proportion of cats presenting with signs of SLE go on to develop progressive glomerulonephritis and renal failure. Indeed, about 5% of younger pedigree cats that present with signs of chronic renal failure are probably suffering from SLE. This aspect of SLE of cats more closely resembles lupus in humans than in dogs. Cats with SLE should be treated chronically with a combination of corticosteroids and chlorambucil or cyclophosphamide. Remission is achieved once the ANA titer declines to zero. Kidney function should be periodically monitored and the disease should be considered lifelong in most animals.

### Discoid lupus erythematosus

This is a common disorder of dogs, but is rare in cats. The lesion is usually localized to the nasal planum and is worsened by exposure to sunlight. Histopathological changes in the affected skin, characterized by liquefaction of the basal cell layer, subdermal inflammation and immune complex deposition in the basal lamina are

identical to those seen in SLE. Affected cats are usually ANA-negative, however, and lesions are not present in other organs.

### Chronic progressive polyarthritis

Two forms of the disease are recognized, a periarticular osteoproliferative arthritis similar to human Reiter's disease and a chronic erosive polyarthritis similar to human rheumatoid arthritis. The Reiter's form of the disease is the more common and occurs exclusively in younger male cats from $1\frac{1}{2}$ to 4 years old. Affected cats present with acute polyarthritis, high fever, lymphadenopathy, pronounced reluctance to move and pain on joint palpation. Affected joints, especially distal limb joints, are often swollen and reddened. The high fever lessens somewhat after several weeks and the condition becomes lower grade with progressive periarticular new bone formation, weight loss and malaise. Synovial fluid taps demonstrate high numbers of nondegenerative neutrophils.

The rheumatoid form of chronic progressive polyarthritis tends to affect older male cats and is much more chronic and insidious in its course, and fever and other constitutional signs are absent or mild. Instead of periarticular new bone formation, there is a destruction of the joint surfaces of more distal joints with associated subluxations, luxations and deformities. Affected cats are often not febrile and, except for the progressive joint disease, may even ambulate without much apparent pain.

Chronic progressive polyarthritis appears to be caused by immune reactions against a common retrovirus of cats, feline syncytium-forming virus. Because up to 60% of outdoor cats in the United States will ultimately acquire this lifelong infection without obvious clinical signs, cats which develop chronic progressive polyarthritis must be predisposed by both gender and genetics. In these respects, chronic progressive polyarthritis of cats resembles Reiter's disease of humans.

Treatment with combination immunosuppressive drug therapy (glucocorticoids and cyclophosphamide) has been only partly rewarding. Remission is difficult to achieve and, even if obtained, it is hard to sustain.

### Arthritis associated with FIV infection

Lameness associated with one or several limb joints has been observed in cats with FIV infection. Synovial fluid demonstrates an increased number of nondegenerative neutrophils. The condition responds to corticosteroid treatment. The condition is either of immune complex origin, or results from inflammation evoked by the presence of virus infected macrophages in the synovium.

### Idiopathic polyarthritis

A nonerosive polyarthritis has been observed on occasion, mainly in younger female cats. Because of cat's propensity to mask clinical signs of joint pain, many more cases probably go unrecognized. Affected cats show minimal signs of lameness and stiffness and fever, when present, is often mild. Lymphadenopathy is seen in a proportion of cases. The diagnosis is by joint taps of both tarsal and carpal joints and the demonstration of large numbers of nondegenerative neutrophils. Affected cats should be tested for predisposing conditions that may present in a similar manner, such as FeLV, FIV or SLE. The condition is often self-limiting after several weeks or months, but can be hastened to resolution by corticosteroid treatment.

### Idiopathic systemic vasculitis

Cats occasionally present with severe vasculitis of unknown origin. In one form, usually seen in young cats, ulcers appear on the hard palate, tongue, cornea, foot pads, and skin. Ulcers appear in waves, heal slowly, then reappear. Deep biopsies will usually demonstrate a leukocytoclastic vasculitis. A second form of vasculitis is associated with severe edema and subcutaneous bruising that is widespread on the trunk or, in rare cases, within the abdomen. These cats are more likely to have associated clotting disorders, probably owing to concomitant disseminated intravascular coagulopathy. Affected cats are prone to develop thrombosis of pulmonary or coronary arteries and sudden death. The former condition responds to a combination of corticosteroids and cyclophosphamide or chlorambucil, but treatment is often indefinite. The latter condition responds poorly to treatment and has a much more grave prognosis.

A condition analagous to polyarteritis nodosa of humans has been described in cats, but is now considered to be a manifestation of feline infectious peritonitis (FIP). Indeed, the classical pyogranulomatous peri-venular lesions of effusive FIP are typical of an Arthus-type vasculitis.

Toxic epidermal necrolysis (TEN) is an acute arteritis characterized by diamond-shaped full-thickness necrosis of segments of skin; necrotic areas correspond to the area perfused by the effected vessels. The condition in cats is usually associated with injections of steroids and/or antibiotics several days earlier and, in rare circumstances, it is either idiopathic or associated with infections such as FIP. Areas of affected skin are pruritic at the onset, due to hypoxia of the nerves. This pain disappears within 24–48 h as the affected skin and its nerves die. The affected skin then becomes discolored and undergoes dry gangrene. If enough of the skin is affected, the condition can be fatal. However, this is seldom the case and the condition is managed by excising dead skin and allowing the wounds to heal by secondary intention or with grafting.

### Glomerulonephritis

Glomerulonephritis is also either idiopathic or secondary. Cats with idiopathic disease are more apt to present

mainly with signs of renal disease, whereas cats with secondary disease usually present for the underlying disease and glomerular disease is detected only during the clinical work-up. Common secondary causes include SLE, FIV and FeLV infections.

Glomerulonephritis in cats differs greatly from the canine disease in one important aspect; proteinuria is much less severe than in dogs, even given similar degrees of histopathology. Cats with glomerulonephritis are therefore much less likely to present with classical nephrotic syndrome (hyperproteinuria, hypoproteinemia, edema, hypercholesterolemia, hyperfibrinogenemia and hypercoagulability) than dogs. Rather, glomerulonephritis in cats is more likely to end in renal insufficiency with elevated blood urea nitrogen and creatinine and reduced urine concentrating ability. Because of the tendency of cats to be less proteinuric, the diagnosis of glomerulonephritis often rests on kidney biopsy or post-mortem examination.

Idiopathic glomerulonephritis is very uncommon in cats compared with dogs. As discussed above, affected cats usually present with renal insufficiency and, infrequently, with nephrotic syndrome.

## Immunological diseases caused by cellular immunity

Cell-mediated immunity is the major host defense mechanism against altered host or foreign (allogenic or xenogenic) cells. Alterations to host cells can occur through intracellular microbial infection, chemical alterations of cell surface proteins, or malignant transformation. Cellular immunity can be innate (natural killer or NK cells, lymphokine-activated killer (LAK) cells) or specific (cytotoxic T cells, CTLs). Specific T cell-mediated killing can be further augmented by the activation of macrophages. The hallmark lesion of cell mediated immune injury is the infiltration of lymphocytes, plasma cells and macrophages.

### Delayed-type hypersensitivity disease of the skin

Cats can develop chronic delayed-type hypersensitivity disease of the skin underlying plastic flea collars and in the areas of flea bites. These reactions are often very pruritic and persist for many days after the initiating stimuli are eliminated.

### Chronic plasmacytic/lymphocytic enteritis

Cats commonly develop a chronic inflammation of the small bowel characterized by villus atrophy, thickening of the bowel wall, and moderate to dense plasmacytic/lymphocytic cell infiltrates. The condition manifests as loose stools and weight loss in the face of a good or even excessive appetite. This type of bowel disease is different in immunopathogenesis from allergic and eosinophilic enteritis, which involve IgE and immediate hypersensitivity. However, both allergic enteritis and chronic plasmacytic/lymphocytic enteritis are caused by antigenic material in the diet. The treatment of chronic plasmacytic/lymphocytic enteritis also involves the feeding of a hypoallergenic diet and corticosteroid treatment.

### Cell-mediated diseases of unknown (autoimmune?) etiology

Over 50% of cats more than 10 years old suffer from a characteristic and progressive chronic interstitial nephritis. The disease is characterized by intratubular infiltrates of lymphocytes and plasma cells, wedge-shaped infarcts extending from the pelvis to cortex that result in scarring, decrease in kidney mass, and irregularity in shape and feel. Affected cats slowly lose renal function over a period of several years, eventually becoming polyuric, thin, hypertensive and anemic. This disorder is a major cause of death among older cats, along with cancer. There is no known treatment to halt the relentless destruction of the kidney.

'Big pad' disease is another disorder peculiar to cats and of unknown etiology. The pads of the feet, in particular the metacarpal and metatarsal pads become grossly enlarged and pillow-like to palpation. As they become larger, the pads often crack, causing the cat to become sore-footed. Biopsies show a dense plasmacytic/lymphocytic infiltrate. Corticosteroid treatment is used in cats showing clinical signs.

Polychondritis of the cartilage of the ears has been observed in cats. The ear pinnae become progressively thickened and contorted, much like the proverbial 'boxer's ear'. Biopsies show islands of ear cartilage that are isolated and surrounded by a predominantly lymphocytic infiltrate. The condition will only rarely involve other cartilage in the body and is self-limiting as the ear cartilage is destroyed.

Cholangiohepatitis is the feline equivalent of chronic active hepatitis in dogs and humans. The disease is characterized by a lymphocytic/plasmacytic infiltration around cholangioles, cholangiolar proliferation, biliary stasis, fibroplasia and parenchymal scarring. Cats are markedly icteric, but disproportionately healthy. Many of the cats with this disease have high titers of ANA, but it is uncertain whether this is part of the etiology of the disease (i.e., a form of lupoid hepatitis) or whether the ANA is merely a byproduct of slow hepatocyte death, nucleic acid release, and enhanced state of immune reactivity. Treatment is with glucocorticoids or combination immunosuppressive drug therapy.

### Granulomatous diseases of infectious etiology

Granulomatous diseases result from inadequate cellular immunity, usually against intracellular microbial pathogens. The production of strong cellular immunity will lead to rapid containment of the invading microbes and mild or negligible disease signs. At the opposite extreme, a com-

plete lack of cellular immunity will lead to overwhelming systemic disease. Individuals that mount partial cellular immunity, however, develop granulomatous inflammation. The granuloma is a partially successful attempt to limit the spread of an intracellular pathogen. The offending microbes are found in the center of the granuloma within macrophages, while radiating outward are areas of neutrophilic infiltrate, lymphocytic/plasmacytic inflammation and, in chronic granulomas, fibrous tissue. Because the microbes are only weakly contained, living organisms frequently break out of the lesion, often within macrophages, and initiate new granulomas in adjacent or distant sites. The course of disease, i.e. rapid recovery, disseminated illness or granulomatous disease, is a function of the immune system and modulating genetic and environmental factors.

Two classic granulomatous diseases in cats include the dry or noneffusive form of FIP and chronic ulceroproliferative faucitis/periodontitis associated with chronic feline calicivirus infection. The former condition is untreatable and inevitably fatal, while the latter may be slowed with corticosteroid treatment and judicious removal of teeth as periodontal tissue becomes involved. Granulomatous inflammation is also associated with a number of superficial skin infections of cats, including focal mycobacteriosis (*Mycobacterium fortuitum*, *M. cheloni*, *M. smegmatis*, *M. lepraemurium*), acintomycosis/nocaridosis, bacterial L-forms, sporothricosis, miscellaneous saprophytic soil fungi, protothecosis, and leishmaniasis, to name but a few.

### Gammopathies (dysproteinemias or paraproteinemias)

The term gammopathy refers to excessive levels of antibody globulins in the blood. Two general types of gammopathies are recognized: (1) polyclonal, and (2) monoclonal. Polyclonal gammopathies involve increases in all major immunoglobulin classes (IgG, IgA and IgM), while monoclonal gammopathies involve only a single immunoglobulin class.

#### Polyclonal gammopathy

A polyclonal gammopathy is seen in a number of chronic infectious diseases of cats, but the most noteworthy is FIP. Most cats with FIP, especially those with the dry form, will demonstrate progressive rises in all immunoglobulin classes. The antibodies produced are not just against viral antigens, because actual FIP virus antibody titers often bear no direct relationship with the actual level of immunoglobulins in the blood.

A benign polyclonal gammopathy is seen in many aged cats. This is probably associated with a asynchronous aging of the T-cell compared with the B-cell system. Although aged cats with a polyclonal gammopathy should be checked for underlying diseases, care must be taken not to over-react to this finding in otherwise healthy animals.

#### Monoclonal gammopathy

Monoclonal gammopathies are usually caused by plasma cell tumors, i.e., multiple myeloma. Less commonly, they are associated with chronic lymphocytic leukemias, infectious diseases, or benign (idiopathic) causes. The term multiple myeloma comes from human medicine, where this particular tumor has a predilection to spread widely in the marrow cavities of flat bones. This is a misnomer for the cat, however, because most plasma cell tumors of cats arise from the viscera (liver, spleen, intestine, chest cavity), and a smaller proportion infiltrate bone. The most common immunoglobulin type seen in feline plasma cell tumors is IgG, followed distantly by IgM and then IgA.

Plasma cell tumors can present in a number of manners. If the tumor infiltrates and destroys tissues locally, signs will be referable to the organ involved just as any other type of tumors. Plasma cell tumors can also cause disease through the proteins that they produce. High levels of monoclonal antibody globulin, especially IgM or IgA, can cause the blood to become highly viscous, leading to heart disease and hemorrhage in organs such as the eyes and brain. Some myeloma proteins can agglutinate or congeal in the cold (cryoglobulins), leading to thrombosis of small vessels in the cooler extremities such as the ears. Pathological accumulations of a monoclonal antibody are almost always associated with a significant decrease in normal immunoglobulins (probably by a negative-feedback mechanism). If normal antibody levels are decreased too much, immunodeficiency can occur.

## 15. Immunodeficiency Diseases

### Introduction

Immunodeficiency diseases were at one time considered rare and personified by the famous 'boy in the glass bubble', who suffered from a severe combined immunodeficiency disorder of genetic origin and lived his short life in strict isolation. With the world-wide human immunodeficiency virus (HIV) pandemic, however, immunodeficiency has become the byword of the age. It is clear, however, that immunodeficiency results from a myriad of causes, ranging from subtle to catastrophic in its clinical appearance. Animals are also not unique in suffering from various immunodeficiencies and all forms of the disorder described in humans have been reported in one or another species of animals, including the cat.

The hallmark of immunodeficiency is inappropriate infections: infections caused by microbes that are normally not pathogenic, infections that are unusually severe or

persistent, multiple infections in the same individual, the recurrence of infections that occurred in a subclinical form earlier in life, and infections that are unexplainably difficult to treat.

## Innate versus adaptive immunity

Immunodeficiency results from inadequacies in either innate (natural) or adaptive (acquired) immunity. Adaptive immunity can be subdivided into two major processes, i.e., humoral immunity and cell-mediated immunity. Humoral immunity involves the production of antigen-specific antibodies, while cell-mediated immunity is mediated by antigen specific cytotoxic T lymphocytes (CTLs). A second characteristic feature, in addition to specificity, of adaptive immunity is immunological memory. Immunological memory is the ability to recognize specific foreign substances much more rapidly and vigorously on subsequent compared to primary exposure. Innate immunity involves all host defense mechanisms that are nonspecific in nature and do not engender an immunologic memory. Innate immunity is usually present in more or less the same state before, during, and after an infection.

## Innate immunity

Although many people think of innate immunity mainly in terms of natural killer (NK) cells, lymphokine-activated killer (LAK) cells, the properdin system for alternative complement pathway activation, defensins, mannose-binding proteins, collectins, interferons and numerous other soluble inhibitors, natural defense mechanisms are far broader in scope. Skin and mucous membranes are the most important components of innate immunity. Skin has an outer cornified layer that resists microbial growth and invasion. Glandular secretions onto the skin and mucous membranes also contain nonspecific antimicrobial substances. The cilia of certain mucous membranes, such as those of the upper respiratory and lower urogenital tracts, act as brooms to trap and sweep out foreign material. The ciliary layer also greatly increases the surface area and acts as a trap to hold mucus secretions, the latter being rich in specific and nonspecific immune substances and phagocytic cells. The urine of cats is usually of very high specific gravity, which inhibits bacterial growth.

These innate mucous membrane and skin defenses are highly efficient. Bacteria are present in the mouth, oropharynx and upper esophagus, but have all but disappeared by the stomach. A tremendous bacterial flora exists in the rectum and colon, but is absent from the lower small bowel. Bacteria are present in the oropharynx and the upper trachea, but are absent at all places distal to these. Organisms can also be cultured from the prepuce, vagina and distal urethra, but are absent from the proximal urethra, bladder, ureters and kidneys. Bacteria are present on the surface of the skin but are absent from layers below the stratum corneum. Invasion of microbes from their normal sites to deeper tissues, whether it is gut, skin, respiratory tract or urinary system, can only occur if this normal barrier is in some manner breached or bypassed.

## Adaptive immunity

### Cell-mediated immunity

Cellular immunity occurred earliest in evolution and is absolutely required for host survival. Specific cell-mediated immunity involves CTLs bearing the CD8 cell surface marker. Nonactivated CTLs possess surface immunoglobulin-like receptors that act to recognize foreign antigens; each cell bearing receptors for a single antigen. If a host cell becomes infected or in some other manner antigenically altered, some of the foreign antigens will be arrayed on its surface in conjunction with the proteins of MHC I. The CTLs will then come in intimate contact with the abnormal cell by receptor/ligand interactions. Antigen recognition will cause this specific subset of cells to clonally expand, yielding a larger population of specifically activated CTLs and memory cells. Specifically activated CTLs will then come into intimate contact with the antigenically altered cells through the same recognition mechanism and secrete substances that will cause their death. CTLs also send chemical signals to macrophages, causing them to activate and become cell killers or to destroy any microbes that they may have internalized. CTLs are helped in their activity by $CD4^+$ T cells (T-helper cells). Therefore, deficiencies in specific cellular immunity can arise from deficiencies in number and/or function of CTLs, T-helper cells or macrophages. This system also has inhibitory controls mediated by a subset of $CD8^+$ T-cells known as T-suppressor cells.

### Humoral immunity

Humoral immunity involves the production of antigen specific antibodies of IgM, IgG, IgA, and IgE subclasses. IgM antibodies are very large, consisting of five basic immunoglobulin molecules connected by a joining protein. IgM antibodies are produced in tonsils, diffuse lymphoid aggregates, spleen and lymph nodes and circulate freely in the bloodstream; they are also secreted into mucous membranes. IgG antibodies are monomeric and are also produced in the same tissues and are found mainly in the bloodstream. IgA antibodies are produced mainly by tonsils and diffuse lymphoid aggregates underlying mucosal surfaces. IgA in the cat is dimeric in the bloodstream; a small secretory protein is added by the epithelial cells as it is transported from the blood side to luminal side of the mucosa. IgA is the principal secretory protein found in mucus. IgE is a monomer and is rapidly bound to basophils and mast cells.

The production of specific immunoglobulin is triggered when antigen-presenting cells (APCs) take up the antigen and array it on their surfaces in association with MHC II. Antibody production is carried out specifically by a subset of lymphocytes called B cells. Most B cells are also under both positive and negative regulatory control by T-helper cells ($CD4^+$ T cells) and T-suppressor cells ($CD8^+$ T cells), respectively. The activity of antibodies are also modulated by other proteins, in particular proteins of the complement cascade. IgM and certain subtypes of IgG are good complement binders, while IgA and IgE are poor. Although it is antibody that targets specific foreign proteins, it is complement factors that modulate the function of the bound antibody, e.g., lysis, opsonization, neutrophil attraction, etc. Because of the close interaction between antibodies and complement proteins, deficiencies in complement may cause similar symptoms as deficiencies in antibodies and vice versa.

### Maternal immunity

The newborn cat is born with negligible levels of immunoglobulin in its blood and with only its IgM system functional. In order to protect the newborn against infections until its immune system is mature, the queen transfers antibodies to the kitten in the colostrum. Colostrum is the first milk; it is rich in IgG and IgA, but contains little IgM. There are two possible explanations for the absence of IgM in colostrum : (1) it is not required because the IgM system is fully functional at birth, and (2) if present, it would inhibit normal IgM production in the same manner as maternal IgG inhibits host IgG. The early maturity of the IgM system is an example of ontogeny recapitulating phylogeny; IgM appears early in chordate evolution, followed by IgG and then IgA. The ability of the kitten to produce IgM antibodies from birth onwards is an important backup to maternal immunity. It is noteworthy that IgM is both systemic and secretory, combining the activities of IgG and IgA.

The intestinal epithelium of the newborn kitten is open for the absorption of IgG and IgA for the first 24 h, after which time no further immunoglobulin is absorbed from the gut lumen to the bloodstream. Therefore, the higher the levels of antibodies in the colostrum, the more likely the kitten will receive adequate levels of antibodies from its mother. This first pulse of maternally derived antibody provides 'passive systemic immunity'. IgG remains in the circulation with a half-life of around 7 days. In a similar manner, the passively acquired IgA will be slowly secreted from the blood into the mucous membranes. Active production of IgM is detectable from birth onward, IgG from about 4–6 weeks of age onward, and IgA from 6–8 weeks onward (following weaning).

In addition to passive systemic immunity, the queen continues to provide considerable amounts of IgG and IgA in the milk. Although the IgG is largely degraded in the stomach, it plays an important role in preventing infection in the oropharynx. Almost all pathogens enter by way of the mouth and the oropharyx and tonsils are often the first tissues to be infected. IgA antibodies consumed in the milk enter the intestine in an intact form and become part of the mucous film of the intestine. Antibodies that are provided in milk from 12–24 h of age to weaning at 6–8 weeks provide what is called 'passive local immunity'.

## Specific immunodeficiencies

### Disorders associated with innate immunity

Deficiencies in classical innate immune defenses (NK cells, LAK cells, properdin, defensins, etc.) have not been described in the cat. There are, however, several anatomical defects in innate defenses that lead to immunodeficiency states. Kittens suffering from severe herpesvirus rhinitis are often left with badly scarred and atrophied turbinates. If the damage is severe enough, the local mucosal barrier is rendered inoperative and these animals suffer for the rest of their life from recurrent bacterial rhinitis/sinusitis. Some kittens are born with small congenital oronasal fistulas; these are often on the midline just behind the upper incisors and are easily overlooked. Similar fistulae can develop following recovery from palate and skull fractures. In addition to fistulae in the hard palate, some kittens are born with a cleft in their soft palate, reminiscent of the normal anatomical appearance of the throat of birds. Food can pass from the oral to the nasal cavity during eating and cause a chronic rhinitis.

Tom cats with urethral obstructions from feline urologic syndrome (FUS) were formerly treated with a perineal urethrostomy. About one-fourth or more of these animals suffered later from chronic urinary tract infections because of the removal of the distal defense mechanisms in the penile urethra. This, along with the development of preventative diets reducing the incidence of FUS, is why perineal urethrostomy is no longer used as a routine treatment for urethral obstruction in cats. Cats that are being treated with fluid and electrolyte solutions while urinary catheters are in place are very prone to urinary tract infections. Normal cat urine, which is of high specific gravity, is inhibitory to bacteria. By lowering the specific gravity of the urine, while bypassing the distal urethral defenses with a catheter, bacteria can both invade into the bladder and replicate. A similar situation occurs in aged cats with chronic kidney disease, polyuria/polydypsia and low specific gravity urine; such cats sometimes develop severe bacterial infections (usually hemolytic strains of *Escherichia coli*).

Secondary bacterial infections of the skin often accompany allergic dermatitis; pruritus induces excessive scratching and abrasion of the skin, thus allowing local bacteria to breach the corneum.

Cats do not cough nearly as well as humans or dogs, and when they develop pneumonia, it is very difficult for them

to clear exudates. This is one reason why pneumonias in cats are more likely to be severe or fatal than in species exhibiting a strong cough reflex.

Innate immune defenses are often overwhelmed in young kittens that are hand-reared and fed high-energy supplements. The overfeeding of such foods, often in bolus form, leads to the dumping of large amounts of undigested nutrients into the lower bowel. This causes an overgrowth of bacteria, especially of nonresident types, in the colon and retrograde bacterial colonization of the small bowel. The result is an intractable enteritis and, if associated with deficiencies in passive maternal immunity (see preceding section), to bacterial sepsis.

### Deficiencies in adaptive immunity

Deficiencies in adaptive immunity are either congenital or acquired and involve cellular or humoral arms, or both. Congenital deficiencies of specific immunoglobulin subclasses, complement components, or severe combined immunodeficiency have not been recognized in cats, but undoubtedly exist. Several acquired immunodeficiencies occur in cats, however. These include undefined immunodeficiencies to specific diseases, age-associated immunodeficiency, stress-induced immunodeficiencies, failure of maternal immunity, and retrovirus-associated immunodeficiencies.

Persian cats as a breed are more susceptible to dermatomycosis (ringworm); they get more lesions, are more difficult to treat, carry and shed the fungi for much longer periods of time, and are much more susceptible to invasive forms of the infection (mycetomas). This immunodeficiency is quite specific because the breed is not more susceptible to other types of infections. Siamese and related breeds of cats are more susceptible to severe feline herpesvirus rhinitis and, as such, they are much more likely to develop chronic rhinitis and sinusitis as a consequence (see preceding discussion on innate immunity).

Cats as a species have very little acquired resistance to the feline infectious peritonitis virus (FIPV) both experimentally and in the field. Over 90% of experimentally and naturally infected cats will die if they are infected with this virus. FIPV is a simple mutant of the much more ubiquitous feline enteric coronavirus (FECV), and in highly FECV endemic environments (pedigreed catteries, shelters and large multiple cat households), about 5% of FECV-infected cats will succumb to FIP. The reason for this susceptibility is probably linked to a lack of extended evolutionary adaptation. FIP was not seen in cats prior to the 1950s, either because the parent FECV virus had not yet evolved as a feline pathogen or because drastic changes in feline husbandry associated with urbanization, pedigreed cat breeding, cat shelters, and the keeping of multiple cats indoors as pets, have greatly enhanced FECV infection.

Age-related immunodeficiency is a common factor in disease of kittens. Kittens infected with feline leukemia virus (FeLV) at 6 weeks old will almost always become persistently viremic, while a majority of kittens exposed to the same dose of FeLV at 16 weeks old will recover. Six-week-old kittens infected with feline enteric coronavirus (FECV) will shed virus at high levels in their feces for many months, whereas cats infected as adolescents will shed much lower levels of virus for only a few weeks. The much higher rate and longer duration of FECV replication in the 6-week-old kittens leads to a much higher incidence of feline infectious peritonitis (FIP). The FIP virus is a mutant of FECV and the greater and longer virus replication, the greater chance that the mutation will occur. Age resistance can also be shown for virtually all common infections of cats, including feline herpesvirus, feline calicivirus, *Chlamydia psittaci*, and *Bordetella bronchiseptica*. Neonatal streptococcal infections also have an interesting age-related pattern in cats. Young primiparous queens have much higher levels of $\beta$-hemolytic streptococci (*Streptococcus canis*) in their genital tract than older queens, and whereas the numbers of streptocci decrease during pregnancy in older multiparous queens, the numbers increase in primiparous queens. As a result, young queens often infect their young during parturition, with considerable neonatal mortality.

Stress-related immunodeficiency occurs in any situation where large numbers of cats are kept indoors, closely confined, and in intimate contact with each other. It is particularly intense in multi-cat environments where kittens are also reared. The mechanism of this immunodeficiency, while assumed to be stress-related, is not precisely known. Cats, especially kittens, raised in such environments handle a number of common infections far worse than well-managed specific pathogen free laboratory cats. Feline herpesvirus (FHV), feline calicivirus (FCV), FECV, feline leukemia virus (FeLV), *C. psittaci*, *Bartonella henselae* (the cat scratch agent), *Microsporum canis*, *Giardia*, *Cyptosporidia*, and *Coccidia* are all infectious agents that are far more severe in catteries, shelters and large multiple cat households than among free-roaming cats or in experimentally infected laboratory cats. Primary infections are clinically more severe, a larger proportion of cats will carry the organisms following recovery and for longer periods of time, and complications are far more common. This factor is the primary reason why most laboratory cats are now maintained in a specific pathogen free state; prior to the use of SPF cats, infectious disease problems among non-SPF laboratory cats precluded their use from many long-term experiments.

Failure of maternal immunity has two components: (1) failure to receive or absorb colostrum, thus causing a deficiency of passive systemic immunity, and (2) a failure to receive mother's milk causing a deficiency in passive local immunity. Kittens orphaned or taken from their mother prior to nursing and fed artificially will be deficient in both passive systemic and passive local immunity and are extremely difficult to raise to maturity. Despite artificial nursing and nurturing, many will develop fatal bacter-

ial sepsis within the first 2 weeks of life, and those that do not frequently suffer from severe and chronic enteritis.

Kittens that receive colostrum, but are weaned shortly thereafter, are much easier to rear artificially but still suffer inordinately from enteric infections. A failure to absorb colostral antibodies has also been described in a proportion of kittens not taken from their mother. It is unclear whether the intestinal tract of these kittens closed prematurely to the absorption of immunoglobulin or if they failed to nurse in a timely manner. Such kittens will still receive passive local immunity from their mothers but are, nonetheless, much more prone to sepsis and early death. Kittens that are known to be deficient in passive systemic immunity should be given injections of serum from older cats. Small amounts of serum can also be given with the artificial milk in orphaned kittens to help provide passive local immunity. The last two steps have been used effectively to rear valuable orphaned and endangered species of wild felids.

Acquired immunodeficiencies are associated with both FeLV and feline immunodeficiency virus (FIV) infections. The half-life of cats with persistent FeLV infection is around 1 year; thus most chronic FeLV carriers will be dead after 3–4 years. The greatest causes of death in FeLV carriers are T-cell lymphomas (mainly thymic, ocular, neurologic or generalized), myeloproliferative diseases, and aplastic anemias.

Immunodeficiency is probably associated with about 1 in 5 or so of FeLV-infected cats. The most noteworthy immunodeficiency is to FIPV infection. When FeLV was still a common infection in cats (in the 1960s and 1970s), about one-third or more of cats with FIP were FeLV positive. It has since been shown that FeLV infection has a highly specific inhibitory effect on FIPV immunity. Cats that are subclinically infected with FIPV will become clinically ill with FIP within several months of FeLV infection. Other types of FeLV immunodeficiency tend to be associated with the profound neutropenias that are often seen in cats with aplastic anemia or myeloproliferative disease. *Haemobartonellosis felis* is commonly seen as an underlying or overlying infection in such cats. Acute necrotizing ulcerative gingivitis is seen almost exclusively in severely neutropenic cats, as is a characteristic multifocal suppurative and necrotizing pneumonia caused by the EF4 bacterium. Other infections seen in leukopenic FeLV-infected cats include tooth root infections, suppurative otitis, and bacterial peritonitis.

The acquired immunodeficiency of FIV infection is highly specific compared with the immunodeficiencies caused by FeLV. One-half or more of FIV-infected cats develop a progressive deficiency in $CD4^+$ T cells, defective macrophage function, follicular atrophy, and loss of follicular dendritic cells. The loss of $CD4^+$ cells appears to be the central lesion in the overall deficiency and tends to impact on cellular immunity more than humoral. Once the $CD4^+$ cell numbers fall below 100–200 cells/$\mu$l blood, affected cats often develop chronic infections of the skin, respiratory tract, and intestines.

## 16. Tumors of the Immune System

Tumors of the immune system of the cat are the most common neoplasms of cats. They are of great scientific importance since for the majority of them a known etiology exists which allows the formulation of hypotheses about the pathogenesis of the tumors. Furthermore, these neoplasms are of high practical importance in veterinary medicine and effective methods for prophylaxis are available.

One of the difficulties of reviewing the tumors of the immune system is that there is no clear-cut border between tumors of immune cells, tumors in primary and secondary immune organs, and tumors of the hematopoietic system in general. These three approaches will therefore be dealt with. The main topic, however, is the discussion of tumors of lymphatic cells whereas tumors of the erythrocytic and megakaryocytic series are mentioned only for completeness in systematic tables. Metastatic tumors in immune organs and tissues as well as tumors in these organs and tissues arising from cells not specific for the immune system (e.g., vascular cells, fibroblasts, epithelium in tonsils) are not dealt with.

As in every tumor classification, a differentiation of tumors is preferable which is based on the origin of the tumor cells. These are the cell lines in bone marrow (granulocytic – neutrophil, eosinophil, basophil – monocytic, megakaryocytic, erythrocytic) and lymphatic organs and tissues (B and T lymphocytes, plasma cell, dendritic cells, macrophages) and distributed cells of the immune system (e.g., Langerhans cells, mast cells).

There are three general methods of classifying the tumors of the immune system in cats. These are: (1) by histological and cytological criteria including, histochemical and immunohistochemical reactions, (2) by surface molecules expressed on these cells (clusters of differentiation, CD), and (3) for lymphomas, also by macroscopical findings. All three approaches have their advantages and their limitations.

The classifications based on the classical pathological differentiation of the hematopoietic tumors of the cat are systematics of human pathology which have been adopted directly or been slightly modified to better fit to the situation in the cat. These classifications are numerous and no one method is the accepted gold standard. The most important systems include the Kiel classification, the French–American–British cooperative group systematic, the National Cancer Institute working formulation, and the Animal Leukemia Study Group classification. These classifications or combinations of them are used mostly by pathologists.

The classification by surface molecules specific for a certain cell type is still limited by several factors. First, not enough monoclonal antibodies against feline immune cell surface molecules have been produced and characterized. Thus, only some of the many CDs known in other animal species and man are in use in the feline system. Second, at least in some tumors the neoplastic cells express unusual combinations of CDs which do not occur on normal cells. Third, the knowledge of the biological importance of the findings obtained in such studies is still not very broad and is equivocal. These classifications are mostly used by immunologists and pathologists with a special interest in this scientific field.

The classification according to the macroscopical appearance of lymphomas in the cat takes account of the presence or absence of a main tumor mass and its location. In leukemias, the tumor cells primarily infiltrate bone marrow, red pulp of the spleen, and medulla of the lymph nodes without forming a solid tumor mass; in lymphomas, such solid tumors do exist. They can occur in a wide variety of organs and their localization bears some implications concerning biological behavior, etiology and pathogenesis of the neoplasms. This classification is used mostly by clinicians and in everyday diagnostic pathology.

An attempt to show the most important and most often used criteria for the classification of tumors of the immune system of the cat is presented in Table VIII.16.1. Tumors of the hemopoietic system account for about 30–50% of the tumors of the cat. About 10–15% of them are myeloproliferative diseases and about another 10–15% are lymphoid leukemias. The remaining 70–80% are lymphomas.

Myeloproliferative disease is usually preceded by a prodromal myelodysplastic phase which is readily recognized in experimental induction of the condition. The most important etiology of myelo- and lympho-proliferative diseases in the cat is the feline leukemia virus (FeLV), a retrovirus of the genus mammalian type C retrovirus group which multiplies in immune cells. About two-thirds of all cats with myelo- or lympho-proliferative diseases are persistently viremic with FeLV. Provirus DNA could be demonstrated in lymphomas in about 20% more cases than virus production. This is interpreted as evidence to suggest FeLV involvement in at least a portion of lymphomas in FeLV-negative cats.

Between the different forms of lymphomas there is a clear difference in the association with FeLV. Mediastinal lymphomas occur in younger animals, are in 90% of the cases T-cell lymphomas (predominantly $CD4^+/CD8^+$ or $CD4^-/CD8^+$) and never B-cell lymphomas, and are in 80–90% of the cases FeLV-producers. The other extreme in conjunction with FeLV infection is the intestinal lymphoma, which occurs predominantly in older animals, has the highest percentage of B-cell lymphomas and only in less than 20% of the cases produces FeLV. FeLV infection was not demonstrated in association with plasma cell tumors, mast cell tumors, and thymomas. The relative risk of developing a tumor is in FeLV-positive cats 29 times higher than in FeLV-negative cats for mediastinal lymphoma, nine times higher for multicentric lymphoma, and six times higher for the miscellaneous lymphoma group. The overall risk of developing the myelo- or lympho-proliferative disease is for a FeLV-positive cat seven times higher than for a FeLV-negative cat.

FeLV does not carry an *onc* gene. Cellular *onc* genes and other cell development regulating genes, however, can be picked up and transduced after infection of a cell and thus foster tumor formation. A transforming effect is also

**Table VIII.16.1** Classification of tumors of the feline immune system

1. Myeloproliferative disease
   - 1.1. Acute leukemia
     - 1.1.1. Myeloblastic/myelocytic leukemia
     - 1.1.2. Monocytic leukemia
     - 1.1.3. Malignant histiocytosis
     - 1.1.4. Erythroleukemia
     - 1.1.5. Megakaryoblastic leukemia
   - 1.2. Chronic leukemia
     - 1.2.1. Chronic granulocytic leukemia
     - 1.2.2. Mast cell leukemia
     - 1.2.3. Megakaryocytic myelosis
     - 1.2.4. Polycythemia vera
2. Myelodysplastic syndromes
   - 2.1. Refractory anemia with excess blast cells
   - 2.2. Chronic myelomonocytic leukemia
   - 2.3. Myeloid metaplasia with myelofibrosis
3. Lymphoproliferative disease
   - 3.1. Lymphoid leukemia
     - 3.1.1. Acute lymphoid leukemia
     - 3.1.2. Chronic lymphoid leukemia
   - 3.2. Lymphoma
     - 3.2.1. Multicentric lymphoma
     - 3.2.2. Mediastinal lymphoma
     - 3.2.3. Intestinal lymphoma
     - 3.2.4. Miscellaneous lymphomas
       - 3.2.4.1. Renal lymphoma
       - 3.2.4.2. Cutaneous lymphoma
         - 3.2.4.2.1. Classical cutaneous lymphoma
         - 3.2.4.2.2. Mycosis fungoides (epitheliotropic T-cell lymphoma)
       - 3.2.4.3. Nasal lymphoma
       - 3.2.4.4. Single lymph node lymphoma
       - 3.2.4.5. Neural lymphoma
       - 3.2.4.6. Ocular lymphoma
       - 3.2.4.7. Other extranodal lymphomas (gingiva, larynx, trachea, muscle)
4. Plasma cell tumors
   - 4.1. Medullary plasmacytoma
   - 4.2. Extramedullary plasmacytoma
5. Peripheral mast cell tumors
   - 5.1. Cutaneous mast cell tumor
   - 5.2. Visceral (multiple) mast cell tumor
6. Thymoma
   - 6.1. Epithelial thymoma
   - 6.2. Lymphoid thymoma

possible via sequences of provirus DNA integrated into the cellular genome which may influence the expression of cellular genes.

The second agent known to enhance the risk for the development of lymphomas is the feline immunodeficiency virus (FIV), a retrovirus from the genus lentivirus. A FIV-positive cat has a five times greater chance of developing lymphomas than a FIV-negative cat. These lymphomas are mostly high-grade B-cell lymphomas of the centroblastic or immunoblastic subtypes as in human and simian immunodeficiency virus infection. Their pathogenesis is thought to be associated with the polyclonal B-cell stimulation occurring after FIV infection. A direct transforming effect of FIV is not to be expected because FIV provirus is not demonstrable in the lymphoid tumor cells of FIV-positive cats.

Most of the tumors mentioned in this chapter lead sooner (within months; e.g., acute leukemia, high-grade lymphomas) or later (within years; e.g., chronic leukemia, low-grade lymphomas) to the death of the cat. The exceptions include local mast cell tumors, cutaneous plasmacytomas, and Mycosis fungoides which can be completely cured by surgery.

## 17. Conclusions

In recent years, much progress has been made in feline immunology because natural retrovirus infections exist in the cat allowing its use as model for retrovirus infections in man. With the increasing availability of reagents and techniques, research will without doubt continue to progress at a rapid pace. Novel approaches in vaccinology based on new adjuvant preparations including cytokines such as IL-12, IFN-$\gamma$ and IL-18, newly produced antigens for mucosal immunization, DNA plasmid injections and others may help to further improve the cat's health. The likelihood that an efficacious feline immunodeficiency virus (FIV) vaccine will be introduced to the field looks promising as many groups could repeat the first successful results published by Yamamoto (1991, 1993). The detection and quantification of cytokines will help characterize the pathogenesis of diseases and assays to measure cytokines may soon become important not only for research but also for veterinary practice. In addition, use of cytokines may eventually become important for immunotherapy of diseases such as FIV infection and feline infectious peritonitis, an almost always fatal disease associated with feline coronavirus infection which affects between 5 and 12% of all cats under 1 year old.

To speed progress, it will be important not only to have additional techniques and reagents available, but also to have exchange between laboratories. The European Concerted Action on FIV Vaccination which was initiated in 1990 and has obtained funding until 1999 has proven ideal for the exchange of knowledge, reagents and personnel between groups at relatively little cost. In addition, it will be important to further improve immunology education not only for researchers but also for veterinary practitioners.

For obvious reasons, the major deficit that currently exists in the feline system, is the difficulty with which cytotoxic T cell studies can be performed. It would be highly desirable if uncomplicated standard reagents could be developed that allow labelling of autologous cells with the target antigen to be studied. To further study the $T_H1/T_H2$ pathways and the cytokines involved, it would be highly desirable if well characterized $T_H1$ and $T_H2$ cell lines were available, as they are in the murine system.

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