CHAPTER

5

DEFENSE MECHANISMS OF THE AVIAN HOST

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The defense mechanisms of avian species are generally comparable to those of mammals despite fundamental differences in the structure of the system. Detailed information is available only for the chicken, which serves as the model for studying the development of bursa- and thymus-derived lymphocytes. Conclusions concerning the immune system of other avian species from information derived from the chicken may or may not be valid. Initial comparisons among the immune systems of chickens, ducks and geese indicate substantial similarities. The strength and functionality of the defense system is genetically determined, and in free-ranging birds is based on natural selection. Likewise, birds from arid or arctic environments are not exposed to the same pathogens as birds from temperate or tropical areas, and consequently have developed adaptations to a different group of pathogens. Birds indigenous to one environment that are moved to a different environment may have no immediate protection against the new group of microorganisms they would encouter. Many captive birds have been inbred for color mutations. This inbreeding may weaken the immune system and cause these birds to be more susceptible to disease than their free-ranging relatives (Figure 5.1).

The purpose of the defense system is not only to protect the individual against invasive organisms, but also to eliminate abnormal body cells. These include cells with minor structural or antigenic deviations, such as old cells, virus-infected cells and transformed (cancer) cells. The defense system also functions in the recognition of foreign cells, as is observed in graft rejection phenomena. For this system to function properly, it is mandatory that the body be able to distinguish between normal body cells (self antigens) and those antigens that are unlike self (foreign antigens). If the body becomes intolerant of its own cells, then an autoimmune disease occurs.

The defense system consists of several integrated components: nonspecific defense, and specific defense, which includes the humoral immune system, cell-mediated immune system and tolerance.

FIG 5.1 In general, inbreeding only to obtain specific color mutations can be expected to weaken a bird's immune system compared to its wild-type relatives. Cockatiels with color mutations have a reduced life-span and increased infectious disease problems. Few color mutation cockatiels approach the 15- to 20-year longevity that their wild-type relatives enjoy. For the health of the individual animal, inbreeding should be discouraged.

Each component of the defense system is intricately connected to the other components through the interaction of cells and hormone-like mediators or secretions. These mediators are responsible for activating or suppressing other components of the system, keeping the defenses in proper balance. It is essential for the avian clinician to have an understanding of the importance and interaction of the important components of the defense system.

Nonspecific Defense

Epithelial Surfaces

The primary barriers that any animal has in preventing pathogen access to the body are the skin and the mucosal linings of the intestinal, respiratory, urinary and reproductive tracts. In the normal host, this is achieved by establishing environments that are suitable exclusively for the best-adapted microorganisms with a low pathogenicity or none at all, which effectively inhibit colonization by other, less well adapted and frequently more pathogenic organisms. This is achieved by adhesion of bacteria to the epithelial cell, eg, by pili or fimbria, by production of bacteriocins and by tolerance of environmental conditions (Figure 5.2). The skin serves, among other things, as a physical barrier to potentially invasive microorganisms.

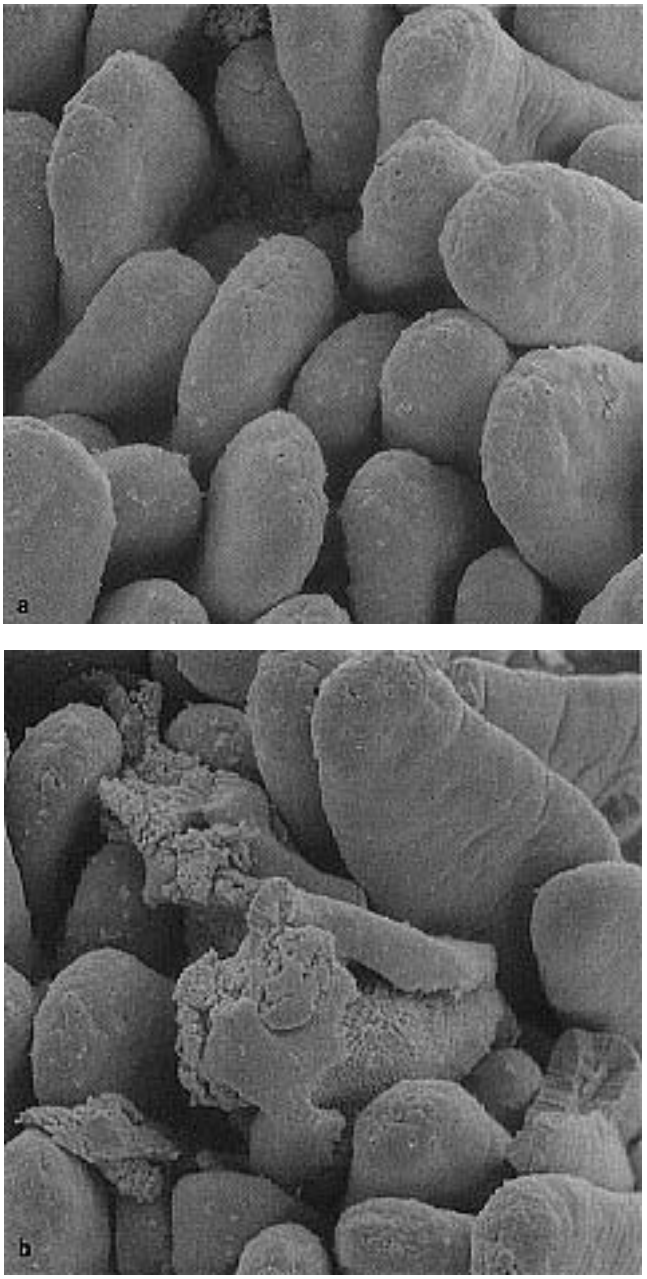

FIG 5.2 **a)** The normal mucosal surface of the intestinal tract is covered with local immune factors (IgA) and autochthonous bacterial flora to provide a barrier that prevents microbes from gaining access to the host. **b)** If the mucosal barrier is damaged by exposure to toxins, malnutrition or primary pathogens, secondary pathogens may colonize the gastrointestinal tract or enter the body and cause systemic disease (courtesy of WS Steffens).

The native flora of the skin is specified and regulated by factors such as desquamation, desiccation and a relatively low pH.[10] Changes in local environmental factors can damage the flora and allow invasion by other microorganisms.

Autochthonous Flora

The normal or autochthonous flora of the intestinal tract is developed in the newly-hatched chick during the first three to four weeks of life. This flora is species-specific, and its composition is governed by the prevailing physical and chemical conditions in the lumen. The acquired flora takes up the available space, occupies receptors and acts competitively against invaders by various mechanisms such as inhibitory metabolic products, bacteriocins and production of a low pH environment. A practical example of the protective nature of resident bacteria in the gastrointestinal tract is the inhibition and expulsion of Enterobacteriaceae by lactobacilli. This inhibition is particularly important in birds in which Enterobacteriaceae are not considered to be normal components of the intestinal flora. The natural development of the immune system also depends on continuous antigenic stimulation by the autochthonous flora. Mucosa-associated lymphatic tissue forms the so-called lymphoepithelial system, which appears to function by capturing and processing antigens from the mucosal surface.

The mucosa of the respiratory, urinary and reproductive tracts of birds is similarly colonized by specific flora whose compositions are relatively unknown; however, thus far it has been shown that none of these mucosal surfaces normally contain Enterobacteriaceae. The mucosal surfaces contain goblet cells that secrete a tenacious mucus. The mucus serves to cover cellular receptors for bacteria or viruses. This mucus also contains lysozyme (which has antibacterial and antiviral activities) and immunoglobulin (Ig) A. In addition, the respiratory mucosa to the level of the secondary bronchi is equipped with cilia that transports foreign material collected from material suspended in the inspired air back out of the system (Figure 5.3).

Myeloid System

The cellular (myeloid) system provides the next line of defense against any pathogens or foreign materials that succeed in penetrating the epithelial or mucosal barriers. This system consists of three cell types that all originate from the bone marrow: polymorphonuclear granulocytes (the most important of which is the heterophil), thrombocytes and mononuclear cells, which differentiate into macrophages.

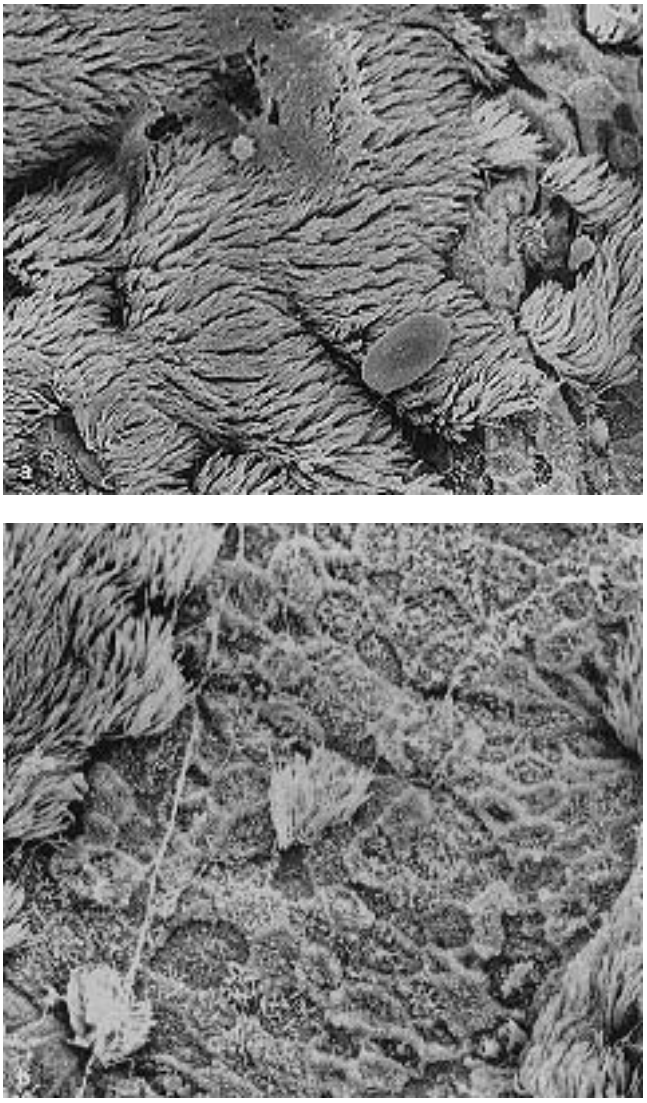

FIG 5.3 **a)** The normal tracheal mucosa is covered with a mucociliary blanket that serves to remove foreign material that enters the trachea and also serves as a barrier that prevents microbes from entering the circulation. **b)** When the mucociliary blanket is damaged, the patient becomes increasingly susceptible to infectious agents. In this case, the mucociliary blanket has been destroyed by indiscriminate exposure to disinfectant fumes (courtesy of Jean Sanders).

Leukocytes

Generally speaking, the nucleus of the avian heterophil is multi-lobulated when it leaves the bone marrow. Therefore, the left shift seen in mammals is difficult to ascertain. Heterophils are rather short-lived (a few hours or days), and their granules are packed with a variety of enzymes (peroxidases, proteases, hydrolases) and lactoferrin. Lactoferrin serves to bind free iron, which is a required growth factor for many bacteria. The main function of heterophils is to phagocytize and destroy "foreign" mate-

rial or damaged cells without having to process them as antigens for presentation to the immune system. The ability of heterophils to ingest some pathogens depends on their being coated with opsonins. Several blood proteins, including fibronectin, antibodies or C3b complexes derived from the complement cascade (in the case of the alternate pathway, this may occur independently of antibodies) can function as opsonins.[15] A depletion of fibronectins, induced by trauma or large numbers of bacteria can block the phagocytic cells and suppresses the nonspecific cellular defense system.

The second way in which heterophils eliminate bacteria is by producing OCl^-, hydroxyl radicals ($OH^\bullet$), and singlet oxygen. OCl^- causes oxidation of bacterial capsular proteins, and the latter two substances are highly unstable and react with lipids to form toxic, bactericidal hydroperoxides. These reactions are called the "respiratory burst."[15] Animals genetically deficient in superoxide dismutase or myeloperoxidase lack this defense mechanism and frequently suffer from recurrent bacterial infections. Heterophils have only limited energy resources that cannot be replenished. They are consistently reproduced and serve merely as the first line of internal defense. When a pathogen is able to resist these defense systems, the macrophage system, with prolonged diversified functions, assumes the responsibility for protecting the host.

The information on avian eosinophils is still rather poor. Responses by eosinophils are at least partly species-specific. In contrast to mammals, birds are not generally thought to respond to parasitic invasion with an increase of eosinophils. The fact that avian eosinophils are difficult to distinguish from heterophils may explain some of the literature reports suggesting parasite-induced eosinophilia. It appears that avian eosinophils participate in hypersensitivity reactions. The degree of involvement is thought to be dependent on the species, inciting antigen and age of the individual.[9]

Avian basophils are morphologically and functionally identical to tissue mast cells. Their granules contain vasoactive amines and proteins, prostaglandins and activators for the coagulating cascade, as well as anticoagulants such as heparin. These cells function to accelerate inflammation at the site of antigen deposition.

Thrombocytes

Unlike mammalian platelets, avian thrombocytes are capable of phagocytosis. It is currently undetermined if the phagocytic process is the same as that used by heterophils.

Macrophages

All macrophages are derived from the bone marrow and enter the peripheral blood as monocytes. The morphologies of the macrophages vary according to their location and functional state (Table 5.1).

TABLE 5.1 Macrophage Morphology by Location

Morphology	Location
Monocyte	Peripheral blood
Histiocyte	Various tissues
Kupffer's stellate cell	Liver sinus
Multinucleated giant cell	Granulomatous tissue
Langhans' giant cell	Tuberculous granuloma
Epithelioid cell	Macrophages with intraplasmatic inclusion
Microglial cells	Brain

Cells corresponding to the alveolar macrophages of mammals have not been demonstrated in the avian lung. However, as a compensatory mechanism the entire epithelial surface in the parabronchi, atria and part of the infundibulum is capable of taking up particulate matter and transporting it into phagosomes, which are subsequently processed by interstitial macrophages. This defense system may partially explain the relatively high resistance of the avian lung to infectious agents[6] (see Chapter 22).

Macrophages have a long life-span unless they are destroyed by the material they ingest. They are equipped with lysosomes containing various substances that can be set free according to their respective functions. These substances are involved in phagocytosis, promoting fever (much rarer in birds than in mammals), inducing inflammation, processing antigens to stimulate an immune response and tissue healing. Phagocytosis is the fundamental process of macrophages. These cells possess receptors for complement factor (C) 3 and antibodies. Foreign particles that are opsonized (covered) with either of these substances can then bind to specific receptors on macrophages and be ingested. Functional macrophages isolate ingested material within a specialized intracytoplasmic compartment called a phagosome. These cells secrete a protein called interleukin 1 (IL-1), which seems to function in mobilizing hetero-

phils, altering host metabolism and inducing inflammation. Macrophages are activated by τ-interferon released by T-lymphocytes. Activation causes macrophages to increase in size, mobility and metabolic activity. The phagosomes enlarge and produce increased amounts of hydrolytic enzymes. As a consequence, more IL-1 is secreted, causing intracellular microorganisms engulfed by phagosomes to be destroyed more easily. Some microorganisms are able to survive inside the macrophage.

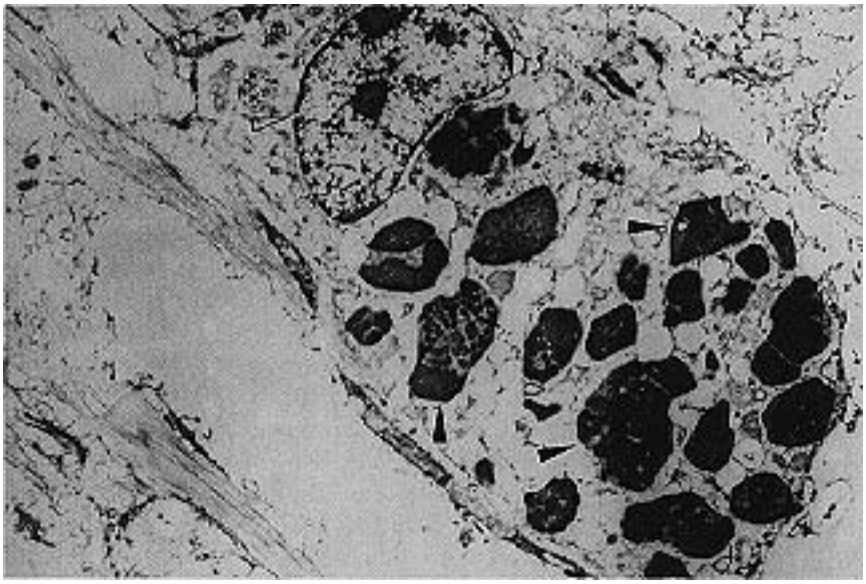

FIG 5.4 The macrophage engulfs and processes foreign material that enters a bird. The macrophage then activates the immune response by stimulating the propagation of B-cells and T-cells. If a pathogen persists in the macrophage, then a bird's immune system will not be stimulated to destroy the invading pathogen. Such is the case with PBFD virus. The PBFD virus present within the intracytoplasmic inclusion bodies (arrows) in this macrophage is able to persist rather than stimulate an immune response. Nucleus = open arrow.

Macrophages are active factors in the inflammatory process. They are chemotactically attracted to the site of microbial invasion where they help to eliminate the intruder. In addition, they secrete factors such as complement factors C2, C3, C4 and C5, enzymes such as lysozyme, elastase and collagenase and plasminogen activators. Cyclic AMP, prostaglandins and leukotrienes are also released. Macrophages also activate fibroblasts and stimulate wound healing. The ability of a host to survive an infection is usually dependent on the functional capacity of the macrophages (Figure 5.4) An increase of monocytes in the peripheral blood can therefore indicate that the host successfully resisted microorganisms.

Not all foreign material is totally ingested or destroyed in macrophages. Some antigen molecules remain on the cell surface for long periods of time. The surface of this macrophage subpopulation expresses special antigens (class II histocompatibility antigens: cell membrane antigens on macrophages, B-cells and activated T-cells) that regulate the interaction between the antigen-presenting macrophage and the antigen-recognizing cells (lymphocytes). If an antigen evades the macrophages and reaches the antigen-sensitive cells, then the host either will manifest a poor immune response or will be tolerant of the antigen.

Macrophage-like cells (called dendritic cells) are characterized by long, filamentous cytoplasmic processes and are distributed throughout the spleen and lymph follicles in the parenchymal organs. The dendritic cells have poor phagocytic activity, but they have surface receptors for complement, antibodies and class II histocompatibility antigens. They can absorb antibody into their cytoplasmic filaments in such a way that antigens can be trapped. Antigens that are bound to dendritic cells are very powerful immunostimulants that may play a major role in anamnestic response to antigens.

Immune Modulators

Several immune modulators, including adjuvants and paramunity inducers, function principally at the macrophage level. Adjuvants function in various ways to enhance the immune response to antigens. Many of them, such as aluminum hydroxide or some oils, function only to inhibit the resorption of antigens, causing a prolonged local antigenicity. Adjuvants are usually insoluble and provoke local inflammatory reactions (granulomas). The tissue inflammation (severe inflammation is an undesirable adverse response) increases the number of antibody-producing cells in the affected tissues. These cells then contribute a significant portion of the total antibody produced. Some adjuvants cause increased phagocytosis activity; in particular, Freund's adjuvant can activate the alternative complement pathway, leading to undesirable results. Some adjuvants enhance the "trapping" of lymphocytes in the spleen, ie, lymphocytes are rendered incapable of escaping from the spleen, causing an accumulation of lymphocytes and stimulation of the immune response. The

lymphocytes are released from the spleen approximately seven days later.

Paramunity inducers, especially those consisting of inactivated components from various poxviridae, can stimulate phagocytosis by macrophages and granulocytes, natural killer (NK) cells and, depending on the strain of poxvirus used, the production of τ-interferon and the prostaglandins E and A. Complement and interleukins can also be activated. The activation of NK cells seems to be an important factor in the nonspecific defense mechanism, especially against virus infections and virus-induced tumor cells. NK cells destroy target cells by cytotoxicity.[5] They seem to play a distinct role in "genetic" resistance to virus-induced neoplasms. Very recent results show that the avian NK cells are independent of the thymus but have CD3 and CD8 antigens at their surfaces.

Antibiotics, especially some tetracycline preparations, inhibit the immune system to varying degrees. An indirect effect is a transient increase in the serum corticosterone level, which depresses macrophage activity. Direct effects include interference with protein synthesis, phagocytosis and antigen processing. Because of their potential side effects, antibiotics should be used only when absolutely necessary, particularly when treating secondary bacterial infections associated with viral diseases. Antibiotics should not be used prophylactically, but only when specifically indicated.

Specific Defense

Nonspecific defense mechanisms function to destroy foreign material the moment it touches or enters the host. Specific defenses have a prophylactic quality in defending the host against ubiquitous microorganisms and recurrent infections. The specific defense mechanism relies mainly on antigen-sensitive cells, B- and T-lymphocytes, to recognize each antigenic epitope (antigenic determinant) and to produce organism-specific antibodies (humoral immune system), or to provoke cell-mediated reactions (cellular immune system). Depending on whether the host has been exposed to the particular antigen before or if it is an initial encounter, specific defense responses to an antigen may be delayed for two to three or even five to ten days, respectively.

Antigens are the trigger for stimulating the specific defense response. For a substance to be immunogenic, it must be a structurally stable macromolecule and foreign to a host, and it must possess surface structures (epitopes) against which the immune response will be focused. The specific site on an antigen that reacts with antibodies is called an epitope and only comprises a few molecules (10 to 12 amino acids in proteins). Several epitopes may exist in each antigen molecule (approximately one epitope per 5,000 daltons). With such a defined antigenic site, the main portion of foreign macromolecule is nonantigenic, and the immune response of the host is dependent on recognizing defined epitopes as not "self." Individual hosts respond differently to the same epitopes. The type of reaction is mainly controlled by immune response genes, which code for regulatory proteins located on the surface of cells of the immune system. Epitopes may also stimulate a varying response depending on the manner in which the antigen is presented to the lymphocytes. Individual epitopes may induce antibody production, cell-mediated reactions, tolerance or immunosuppression. The immune response that follows natural infection is thus a mixture of responses (ie, polyclonal). Since epitopes are specifically defined and occupy rather small areas on an antigen, an antibody produced against one antigenic site may react with an epitope on another totally unrelated antigen. This cross-reaction between totally different antigens can create diagnostic problems in some serologic tests. It has been experimentally suggested that approximately ten million epitopes exist that can stimulate an immune response.

In addition to macromolecules, small molecules (called haptens) that are linked to a carrier may also provoke an immune response. Haptens of particular interest to the clinician are small, metabolized molecules of drugs, which may bind to serum (or other) proteins. These molecules are recognized as foreign and often induce hypersensitivity responses. Classical examples of hapten-induced reactions in mammals are reactions to penicillins and cephalosporins. Hypersensitivity responses appear to be less common in birds but have been linked to some antimicrobial sulfonamides. Responses to hapten-carrier molecules indicate that production of antibodies to epitopes of the haptens is possibly independent of the carrier molecule itself. Nevertheless, cell-mediated response may be initiated against the hapten-carrier as such, and is therefore called "carrier-specific." The latter

point is of practical importance, since binding of haptens to carriers is a common occurrence *in vivo*.

Humoral System

Immunoglobulins

The primary function of the humoral immune system is the production of antibodies directed mainly against extracellular phases of antigens. Antibodies are immunoglobulins, with the major part of the molecule containing ligands for membrane receptors, complement activation and isotype-specific (antigenically unique) structures. Immunoglobins can be differentiated into isotypes (IgM, IgG, IgA and, in mammals, also IgD and IgE). No subclasses of any of the isotypes has currently been demonstrated in birds. In ducks and some geese, the predominant immunoglobulin is called IgN, a 5.7 S protein molecule that does not fix complement (which occurs in some fishes, turtles, marsupials and rabbits). Although the other avian isotypes are not the same as those in mammals, they do share the same functions and are termed similarly.[1] However, there are some biochemical differences between mammalian and avian immunoglobulin. Avian immunoglobulins aggregate in a 8% NaCl solution. This is in contrast to mammalian immunoglobulin, which will aggregate in a 0.8% NaCl solution. Although chicken complement is fixed by immune complexes, it is not affected by complement from the guinea pig. This means that the routine complement fixation test cannot be carried out in many avian species.

- ***IgG:*** (synonym IgY because of its structural and weight difference from mammalian IgG) is the most common antibody in the serum, and due to its small size (7 S), it can penetrate into tissue spaces and across body surfaces. IgG can opsonize, agglutinate and precipitate antigen.

- ***IgM:*** is the major isotype produced following the initial contact with an antigen. Because of its size (19 S), IgM is normally confined to the peripheral bloodstream and is more active than IgG in opsonization, agglutination, virus neutralization and complement activation.

- ***IgA:*** exists in both monomeric and polymeric forms and when coupled with a secretory component, is excreted onto the mucosal surfaces of the respiratory, genitourinary and digestive tracts. In the chicken, IgA also occurs in the bloodstream and in pigeons, this immunoglobulin is found in high concentrations in the crop milk.[7] IgA does not activate the complement cascade, nor can it act as an opsonin. It can agglutinate particulate antigens and neutralize viruses. Its major task is to prevent antigens from adhering to the mucosal surfaces of the body.

FIG 5.5 Neonatal birds depend on IgG absorbed with the yolk to protect them from environmental pathogens until they become immunocompetent. In some cases, improper hatching will prevent the egg-yolk from being absorbed and it must be surgically removed. Neonates that do not properly absorb the yolk sac are considered to be immunologically naive and are more susceptible to infectious disease.

Antibody Production

Although antibody production is the main feature of the humoral defense system, the concentrations of IgM and IgG in the serum are generally not indicators of immunity. As a rule, birds with high antibody titers against a certain infection are better protected than those with a low titer. However, there are many exceptions to this generality, particularly with respect to antibodies directed against bacteria. In many instances, an effective response requires the interaction of antibodies and components of the cell-mediated immune system. The fate of the antibody-antigen complex is either phagocytosis (binding to macrophages on the Fc fragment of the antibody) or lysis with the aid of the complement cascade. In the case of phagocytosis, the immunoglobulins can be recycled by virtue of the noncovalent complex bonds.

The humoral immune system requires time to respond to a pathogen and the respective lymphocytes responsible for humoral immunity do not migrate

into the secondary lymphatic organs (where they mature) until around the time of hatching. The newborn chick is, therefore, ill prepared to respond to all environmental antigens. For compensation, the newly hatched chicks receive maternally derived antibodies (IgG) transmitted via the yolk (Figure 5.5). The type and quantity of antibodies that the chick receives depend on the immunologic status of the hen. Vaccination (plus a booster) of the breeder hens with the appropriate antigens is carried out four to six weeks prior to the beginning of egg production in order to ensure significant levels of IgG in the yolk. The antibodies are absorbed from the yolk by the third day of life, and their purpose is to help protect the chick before it achieves immunological maturity around 20 to 25 days of age. The half-life of the maternally transmitted antibodies is four to six days. Neonates from hens vaccinated against PBFD virus were found to have HI antibody titers that decreased 20 to 45 days post-hatching, suggesting maternal antibodies may have a longer half-life in psittacine chicks.[16]

Maternal antibodies may present an obstacle to early vaccination programs by neutralizing the vaccinal antigen and, at the same time, depleting the chick's natural protection. Early vaccination successfully inhibits the production of immunoglobulins.[15] It is also known that IgM and IgA secreted by the oviduct diffuse from the albumen into the amniotic fluid where they are swallowed by the embryo, thus coating the surface of the intestine with a protective covering of these immunoglobulins.

Lymphocyte Activity

The cellular basis of humoral immunity is the B-lymphocyte, which is the antigen-sensitive cell. Precursor cells colonize and develop in the cloacal bursa during embryonic life. In chickens, the bursal microenvironment is established from the fourth day of incubation onward as a special outgrowth of the cloacal epithelium. The bursa is thought to attract lymphoid precursor cells through the secretion of bursin (and maybe other mediators from the bursal epithelial cells). The first antigen that is localized on B-lymphocytes is the histocompatibility complex class II antigen, followed by cells with surface IgM. Unlike in mammals, no pre-B-cells with intracytoplasmic μ-chains have been described in chickens.[12] Between the 14th and 16th days of incubation, the first IgG- and IgA-carrying B-cells can be demonstrated within the bursa (Figure 5.6).

Around hatching time, the mature B-lymphocytes migrate in large numbers from the bursa into the secondary lymphatic organs (spleen, cecal tonsils, Peyer's patches, Meckel's diverticulum, lymph follicles in the various organs, paraocular and paranasal lymphatic tissue) where they start to function. Here the Harderian gland is particularly important, and parts of the cloacal bursa, which act as a secondary lymphatic organ. There are some indications for the existence of extrabursal sites of B-cell differentiation (suggested to be gut-associated lymph tissue, Harderian gland, and bone marrow). Around hatching, the Harderian gland has already accumulated actively secreting plasma cells within the interstitial space prior to antigen exposure. By this point in

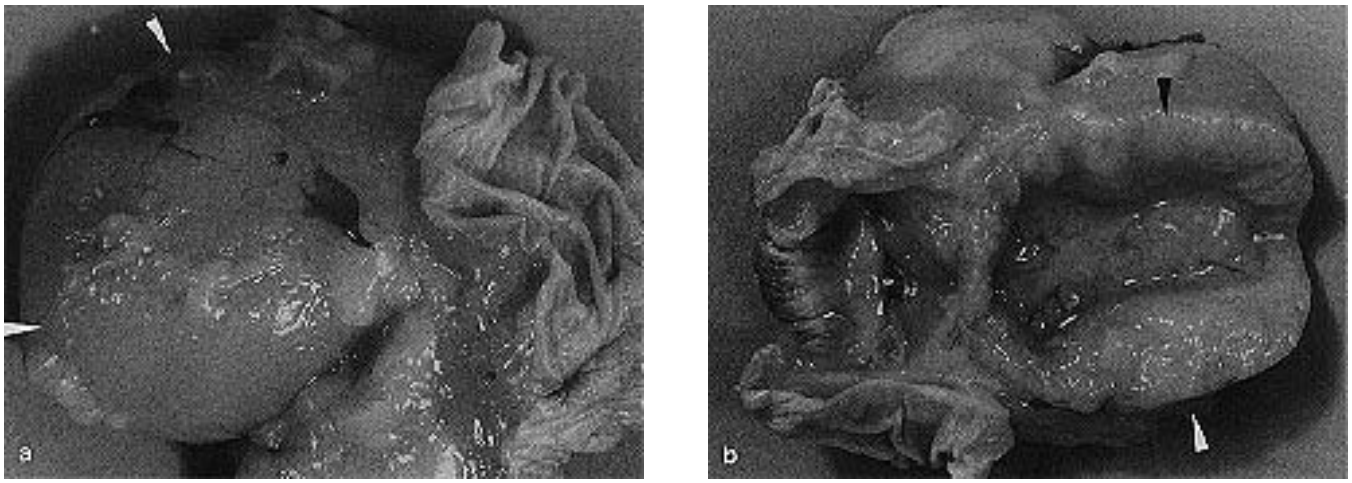

FIG 5.6 **a)** The cloacal bursa is the site of B-lymphocyte differentiation and growth (arrows). The bursa is large in neonates (as in this five-week-old Umbrella Cockatoo) and decreases in size as the bird matures. **b)** The hollow bursa is located in the dorsal wall of the cloaca to which it connects (arrows). The bursa functions as an immunologic organ by taking up particulate matter from the cloaca and stimulating an immune response to the organisms that pass through the cloaca.

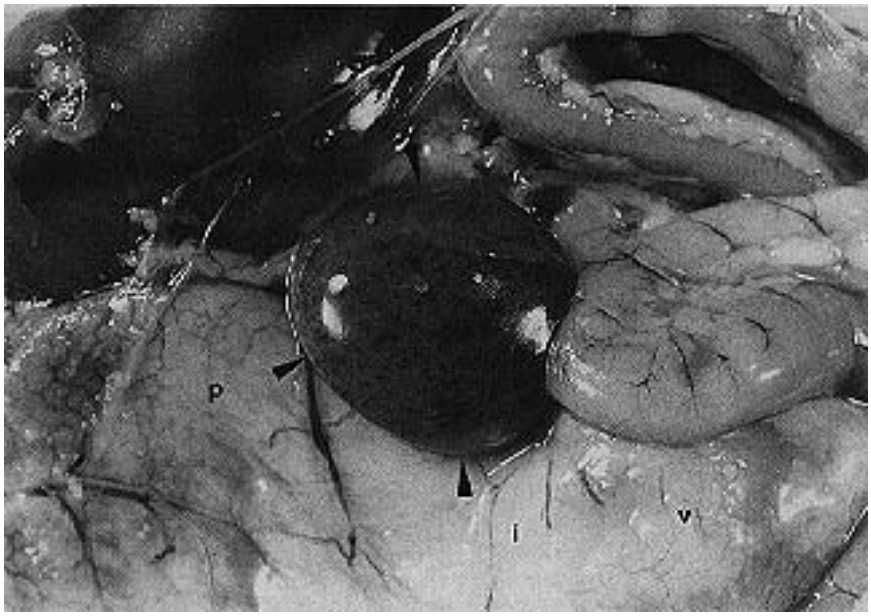

FIG 5.7 Splenomegaly (arrows) is a common finding in birds. The proventriculus (p), isthmus (i) and ventriculus (v) are also noted.

development, neither hormonal treatment nor surgical bursectomy can suppress the infiltration of these plasma cells.

It has also been suggested that mediator-secreting cells migrate from the bursa into the germinal centers of the spleen and the cecal tonsils (probably also into other secondary lymphatic tissues that are capable of forming germinal centers) to monitor the microenvironment (Figure 5.7). It is also assumed that ellipsoid and ellipsoid-associated cells are derived from the mediator-secreting cells. Further studies, particularly of the spleen, have shown that the penicilliform capillaries possess stomata that allow particulate antigen (probably soluble antigen as well) to enter the ellipsoid cells, which are surrounded by ellipsoid-associated cells and dendritic cells. The latter bind the antigen to their surfaces and migrate to the appropriate T- or B-cell-dependent regions of the periarteriolar sheath. In birds, the cloacal bursa is colonized by developing B-lymphocytes until four to six weeks of age. Subsequently, those lymphocyte clones spread to populate the peripheral lymphatic organs. These lymphocyte cells are capable of restoring humoral immunity in the long term, but only as long as the various kinds of "reticular" cells representing the ellipsoid and ellipsoid-associated cells are intact.

The binding of antigen to the membrane of a B-cell stimulates proliferation of the cellular clones and ends in the differentiation of two functional cell populations: plasma cells and memory cells. The proliferation of B-cells is rigorously controlled and occurs only if certain additional factors are present: 1) The antigen has to be presented fixed to the surface of certain cells, mainly macrophages (which secrete IL-1 and possess class II histocompatibility antigens); 2) T-helper cells must also respond to the same antigen and secrete soluble mediators.

The T-helper cells bind to the same macrophage (but not necessarily to the same epitope) as the B-cell. However, the T-cell binds not only with the antigen, but in combination with a class II histocompatibility antigen. The T-cell then secretes two proteins: the B-cell growth factor (IL-4) and IL-2. IL-2 binds to the IL-2 receptors (produced under the influence of IL-1) on the B-cell, where it stimulates DNA synthesis and division. The B-cell differentiation factor (IL-5) secreted by T-helper cells regulates the switch of isotypes. This cascade results in two cell subpopulations: plasma cells and structurally unchanged memory cells, although the latter have switched their isotypes, mainly to IgG.

Plasma cells differentiate from B-cells to form a series of intermediates until they attain their typical morphology (eccentric wheel-like nucleus and copious cytoplasm). The specificity of the immunoglobulin is the same as in the B-parent cell. Plasma cells can produce up to 2,000 Ig molecules per second, and these antibodies are normally secreted by reverse pinocytosis. Plasma cells survive for only three to six days due to gradual catabolism of the immunoglobulins.

In order to maintain high serum immunoglobulin levels, it is necessary to expose a bird to a second dose of antigen to achieve a so-called booster effect. The memory cells, which survive for many months or even years (perhaps not strictly as individuals, but as clones), are stimulated by the proper antigen, inducing the production of more antigen-sensitive cells. The resulting immunoglobulin production is both faster and more vigorous than the initial response. The booster effect, the duration of antigenic exposure, the half-life of the serum antibodies and the expected age of an individual or flock dictate the

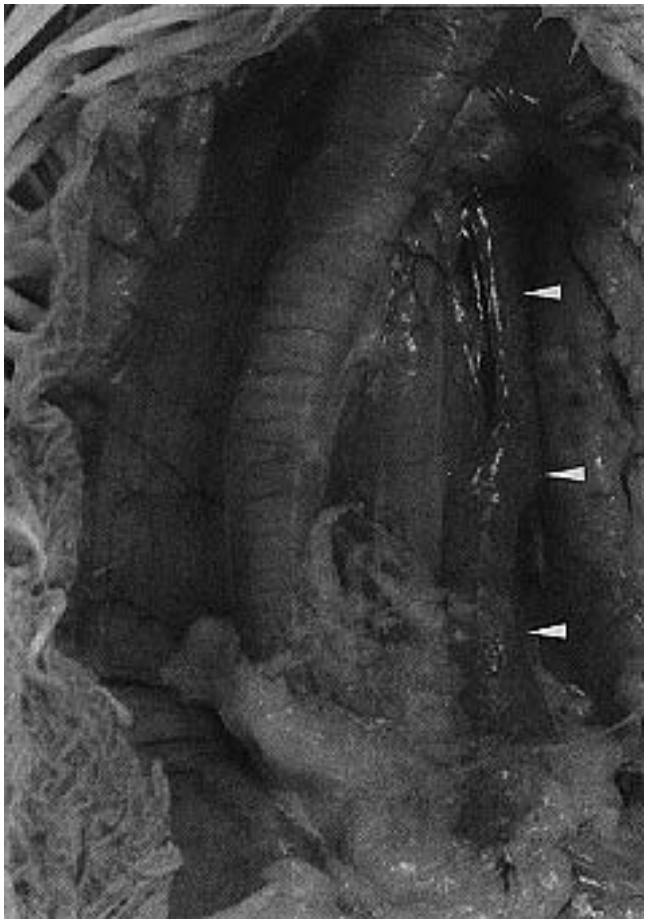

FIG 5.8 The thymus is the site of differentiation and development of T-lymphocytes. In neonates, the thymus is present bilaterally with seven lobes each at the lateral sides of the neck (arrows).

intervals of vaccination and revaccination that are necessary to achieve a desired level of individual and flock immunity.

Function of the Cell-mediated System

The cell-mediated system is essential for protection against viruses, virus-infected cells, intracellular bacteria, foreign tissue grafts, parasites, fungi and some tumor cells. Thymus-derived lymphocytes (T-cells) also mediate the inflammatory response known as delayed hypersensitivity. T-cells form the basis of the cell-mediated system, but in contrast to the uniform B-cells, T-cells form many subgroups including:

- effector cells, which produce lymphokines provoking cytotoxicity
- helper cells, which produce lymphokines
- suppressor cells, which inhibit the responses of other B- or T-cells.

During development, avian T-lymphocytes differentiate and mature in the microenvironment of the thymus (Figure 5.8). At least part of the microenvironment may be provided by nurse cells. The thymus also functions as a gland in secreting several mediators that participate in the T-lymphocyte maturation process.

T-cell subpopulations are characterized by the expression of surface molecules, designated CD1 to CD11, and by the T-cell antigen receptor TRC.[2]

CD4 and CD8 molecules are associative recognition structures for the major histocompatibility complex (MHC) class II and class I molecules, respectively. Antigens have to be presented in association with these MHC molecules for them to be recognized by T-cells. All T-cells express the CD3 molecule, which is noncovalently associated with the T-cell receptor and which represents a signal-transducing structure.

As in a B-cell response, three mutually interacting cells are necessary for a cell-mediated immune reaction to occur: the antigen-presenting macrophage, the effector cell (carrying the CD8 antigen) and the CD4 helper cell. The epitope must be closely linked to the class I histocompatibility antigen in order for the effector cell to be able to recognize the antigen. Once an antigen is recognized, the helper cell secretes IL-2, causing T-cell proliferation and leading to the production of both effector and memory cells. The effector cells, somewhat larger than the parent cell and with an activated metabolism, are capable of performing various functions. They can secrete several lymphokines, or they can cause direct toxic reactions on contact with foreign or modified cells.

In addition to the cells listed above, vascular endothelial cells, keratinocytes, and cutaneous Langerhans cells have also been found to be capable of presenting antigen. They can take up antigen and synthesize IL-1 under the influence of interferon, and express the class II histocompatibility antigen. Langerhans cells play an especially important role in the development of skin allergies, delayed hypersensitivity reactions, and allergic contact dermatitis.

Lymphokines produced by effector cells can be divided into several groups:

- regulatory factors, such as IL-2, IL-3, τ-interferon, IL-4, IL-5.

- inflammatory mediators that increase vascular permeability.
- macrophage activity modulators, such as migration inhibitory factor, leukocyte inhibitory factor, and macrophage fusion factor for inducing giant cell formation.
- cytotoxic factors, such as lymphotoxins, tumor necrosis factor, perforins,
- fibroblast stimulation factor.

Immune Tolerance

Tolerance is defined as a host's failure to respond to produce reactions against a specific antigen. The most important example of tolerance is the absence of antibodies against normal body components. Tolerance to self-antigens is established during embryonal life. The mechanism is not quite clear, but it may depend on the immature condition of the antigen-sensitive cells at the time of their first encounter with antigens. The same matured cell type is later fully responsive to antigens that it encounters for the first time. An embryo may develop tolerance to viruses or some bacteria that are egg-transmitted.

Tolerance in adults can be facilitated by administering either high doses of antigens (particularly polysaccharides) or very low doses of antigen, by giving antigen in the absence of antigen-presenting cells or by applying antigen that is free of aggregations.

The development of tolerance also implies a rigorous control mechanism to maintain balance between the various components of the defense system in order to avoid depressed immune reactivity (ie, increased susceptibility to infection and spontaneous tumors) or excessive immune reactivity (which results in autoantibodies, amyloidosis, lymphoid tumors and allergies). Immune response regulation is a complex mechanism.[8,13] Principally it can be divided into:

- genetic control by immune response genes located at the major histocompatibility complex B (which means that reactivity to antigens can be subject to genetic selection by a breeder),
- activity of T-suppressor cells bearing the CD8 antigen,
- control of B- or T-cell metabolism by insulin and/or prostaglandins and by adjusting the ratio of cyclic adenosine monophosphate and guanosine monophosphate,
- regulation by the amount and structure of the antigen,
- regulation by antibody or antigen-antibody complexes via a feedback mechanism.

Disturbance of the Defense System

Both the nonspecific and the specific defense systems can be impaired at almost any site. Depending on whether a stimulatory or suppressing portion of the system is damaged, impairment can result in either deficiency or exaggeration of the system. In addition to the aforementioned immunosuppression caused by antibiotics, certain mycotoxins and many tumors, particularly the virus-induced tumors, can decrease the efficiency of the defense system.[15] A variety of viruses (Newcastle disease virus, several herpesviruses, adenovirus and reovirus) are known to inhibit the immune system. This inhibition is often at the level of the T-cells, resulting in a bird that is prone to secondary infections. Other viruses, such as PBFD virus and polyomavirus that frequently cause degeneration or necrosis in lymphatic organs are almost certainly also immunosuppressive.

Genetic defects of the immune system in birds are infrequently reported, possibly because of insufficient information concerning the immune system of birds. In chickens, hypo- or dysgammaglobulinemia have been described; in obese strain chickens, the first step in the cascade of events appears to be a dysfunction of immune regulation.

Autoimmunity

Autoimmune antibodies are directed against self-antigens. At a very low level they can be considered as normal, but in higher concentrations they may cause disease. Autoimmune diseases have rarely been reported in birds. This may be a result of our inability to recognize autoimmune disease rather than a resistance to autoimmune problems. The obese strain chickens produce antibodies against thyroid cells, thus causing hypofunction and thyroiditis.

Hypersensitivity

An excessive immunologic reaction can cause a type of inflammation called hypersensitivity. Four differ-

ent types of hypersensitivity reactions have been described in mammals. Type I, the mechanism of which is not fully clear, occurs rarely in birds, because birds do not have IgE, which is essential for the reaction in mammals. Nevertheless, birds have large numbers of mast cells in their lymphatic tissues, particularly in the thymus. The latter does not always become completely involuted in avian species.

Type I = immediate hypersensitivity. IgE isotypes can attach to mast cells (= basophils) by their Fc fragment. If antigen is fixed to such a cell-bound antibody, the basophil releases vasoactive substances (including histamine), which causes a local inflammation within minutes.

Type II = cytotoxic hypersensitivity. The destruction of cells can be carried out either by antibodies activating complement or by cytotoxic cells. Heterophils, macrophages and some lymphocytes have receptors for Fc immunoglobulin fragments and may, therefore, lyse target cells that are coated with immune complexes. Both forms of lysis release many biologically active products from the doomed cells, causing inflammation as is also seen in graft rejection.

Type III = immune complex hypersensitivity. Immune complexes are able to activate complement, even in tissues. The C5a component, which leads to vasoactive anaphylatoxins, is also a potent heterophil attractor. As these heterophils try to digest the immune complexes, they release proteolytic enzymes, thus causing tissue damage. This triggers a vicious circle: heterophil-activated plasmin activates the complement system, which causes aggregation of thrombocytes and the release of more vasoactive factors, while mast cell degranulation may be caused by anaphylatoxin. The end result is inflammation and tissue destruction. The Arthus phenomenon and immunogenic glomerulonephritis are common examples.

Type IV = delayed hypersensitivity. This reaction is caused by cell-mediated immune responses occurring at least 24 hours after antigen contact with sensitized T-cells. The local inflammatory response is caused by vasoactive lymphokines and substances released by mast cells. This type of reaction is caused by various bacterial antigens and virus-infected cells.

Immune Complex Reactions

Chronic lesions caused by immune complexes can occur in the form of amyloidosis. In ducks, geese and swans, reactive amyloidosis is quite frequently associated with chronic suppurative disease processes. Although amyloids differ in their composition, the amyloid proteins share the common feature of having polypeptide chains arranged in ß-pleated sheets. This uniquely stable molecular configuration renders the fibers virtually insoluble and almost completely resistant to proteolysis. As a consequence, amyloid is deposited in tissues and cannot be eliminated, resulting in a loss of parenchymal cells and tissue destruction.

Avian defense mechanisms are rather complex, and many of the intricacies of the systems have not been defined for poultry, much less for pet or aviary birds. Understanding the avian immune system is further complicated because of the involvement of hormonal and nutritional factors. Since antibodies and several of the mediators are proteins, deficiencies in essential amino acids may cause immunosuppression. Some of the vitamins, in particular vitamins A and C, influence both the epithelial nonspecific defense and the interaction between the humoral and nonspecific systems.[12] Of the trace elements, zinc is essential for one of the mediators in the thymus. Therefore, a well-balanced diet free of immunosuppressive mycotoxins is essential for birds that are to be capable of adapting satisfactorily to their environment with its multitude of infectious agents.

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