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The character, quality, and progression of inflammatory reactions in non-mammalian vertebrates (fish, amphibians, reptiles, and birds) differ from the mammalian paradigm more markedly than any other aspect of general pathology. Although the tendency for granuloma formation in response to a wide variety of insults has been described (Montali, 1988), the mechanisms underlying these and other inflammatory reactions are less well understood than in mammals. However, there is an increasing body of literature investigating non-mammalian inflammatory responses particularly in domestic poultry, fish (primarily salmonids), and amphibians. The fundamental mechanisms of inflammation in non-mammalian vertebrates presented in this review are intended as an overview for the pathologist that may find themselves working with these species in either a diagnostic or research setting.

Innate Immunity
Prior to the origin of "jawed" vertebrates, which occurs in the fish (the Class Agnatha is "jawless" and includes lampreys and hagfish), all animals relied exclusively on innate immunity to defend against infections. As a result, innate immune responses are well developed in many of the "lower" vertebrates.

Cellular Components
The cellular components of the innate immune response include granulocytes, natural killer cells, and macrophages. The function of natural killer cells and macrophages appears to be similar in the non-mammalian vertebrates; however, there are important differences in the types of granulocytes within the different classes of animals. Fish (Classes Agnatha, Chondrichthyes, Osteichthyes) have neutrophils but most species also possess eosinophilic granular cells. Eosinophilic granular cells appear to function similarly to mammalian mucosal mast cells (Ellis, 2001) and are present within the skin, gills, and mucosa of the gastrointestinal tract. Fish neutrophils contain large amounts of myeloperoxidase while macrophages produce nitric oxide and reactive oxygen species (Lamas and Ellis, 1994; Campos-Perez et al, 2000). The major granulocytic cell of both reptiles and birds (Classes Reptilia and Aves) is the heterophil. Heterophils contain two, possibly three, types of granules (reviewed in Harmon, 1998). "Specific" granules are elliptical and contain cationic proteins, lysozyme and acid phosphatase. Spherical granules contain the acid hydrolases. A third, smaller, round vacuole has been identified by electron microscopy. Functionally, heterophils lack myeloperoxidase, catalase as well as alkaline phosphatase and therefore depend primarily on nonoxidative mechanisms of killing. When exposed to a phagocytic stimulus, heterophils undergo a respiratory burst but do not produce hydrogen peroxide or superoxide anions (Penniall and Spitznagel, 1975). Reactive oxygen species can, however, be produced by avian and reptilian macrophages (reviewed by Dietert and Golomboski, 1998). B-defensins (gallinacins), which are absent from mammalian neutrophils, are present in heterophils (Harwig et al, 1994). The B-defensins have a broad spectrum of activity against infectious agents binding to negatively charged sites on microbial cell walls and inserting ion channels into membranes (Kagan et al, 1990). Reptiles also have circulating monocytes that are often called azurophils due to their staining characteristics. Azurophils appear to be functionally similar to mammalian monocytes.

Very little has been described regarding amphibian (Class Amphibia) hematology. Amphibians have heterophils as well as very small numbers of cells that have staining characteristics of neutrophils. Little is known about the characteristics of these "neutrophils". Azurophilic monocytes are also present.
Melanomacrophages -
Aggregates of melanin containing macrophages normally occur within the liver, spleen, and kidney of fish and the liver of reptiles and amphibians (reviewed by Agius and Roberts, 2003). With systemic inflammation, these aggregates can proliferate and even develop in other sites, such as the atrium of fish. In addition to melanin granules, cells may also contain hemosiderin and lipofuscin. In higher teleosts, amphibians, and reptiles, melanomacrophage aggregates may have a reticulin capsule. Melanomacrophages are phagocytic. The specific role of melanin granules has not been elucidated, but these granules are thought to be important in the production of free-radicals and bacterial killing. In fish, melanomacrophage centers have been shown to trap and retain antigens and immune complexes thus functioning as a primitive analogue to the lymphoid germinal center (Press et al, 1996). The atrial and renal endothelium of fish are also important components of the innate immune response. The endothelium in each of these sites is phagocytic. As previously mentioned, in conditions of a systemic inflammatory response, melanomacrophage aggregates can develop in the atrial endothelium.

Chemical Mediators -
Some classes of chemical mediators of inflammation are similar among the vertebrates. In particular, the acute phase proteins appear to be conserved. Many of these, including C-reactive protein and transferrin, are strongly upregulated in fish and have been used diagnostically as they increase more rapidly than other hematologic parameters (reviewed by Bayne and Gerwick, 2001). C-reactive protein can also be induced in response to certain chemicals (Winkelhake et al, 1983). Both classical and alternative pathways of the complement cascade are present in the non-mammalian vertebrates, although the alternative pathway may be more important than the classical pathway in fish (Zarkadis et al, 2001). There are conflicting reports as to the role of histamine in fish, as some species do not appear to react to injections of histamine. Instead, degranulation of fish thrombocytes and eosinophilic granular cells releases 5-hydroxytryptamine (serotonin). Fish do not appear to possess histamine, metachromatic staining mast cells or basophils. Fish also lack IgE (Ellis, 1986). Cytokines and chemokines are being described with increasing frequency in domestic poultry and fish, with much less information available about reptiles and amphibians. In domestic poultry these include homologs of TNFα, IFNα, IFNβ, IFNγ, TGFβ, CC and CXC chemokines, and numerous interleukins including IL-1, IL-2, IL-6, IL-8, IL-15, IL-16, and IL-18 (reviewed by Staeheli et al, 2001). In fish TNFα, IL-1, and CC and CXC chemokines, including IL-8, have been described (reviewed by Secombes et al, 2001).

As in mammals, nitric oxide (NO) is an important inflammatory mediator, however there are interesting differences in its induction (reviewed by Dietert and Golemboski, 1998). For example, in chickens IFNγ but not Type I INF induces synthesis of NO but in turkeys Type I INF not IFNγ induces NO synthesis. In birds, the production of nitric oxide is sensitive to dietary levels of arginine because they are not able to synthesize arginine due to an incomplete uric acid cycle (Sung and Dietert, 1994).

Adaptive Immunity -
There are marked functional and anatomical differences in the adaptive immune response not just between the non-mammalian vertebrates and mammals but within phylogenetic classes. Anatomically, none of the species within these classes have organized lymph nodes but all have a thymus, spleen, and a reticuloendothelial system. Not all fish have bones, and many of those that do (Osteichthyes) lack a medullary cavity. Elasmobranchs have a specific hematopoietic organ located along the caudal dorsal abdomen near the gonads. In many of the bony fishes, the renal interstitium is a primary site of hematopoiesis with many species possessing a discrete anterior (head) kidney that is entirely devoted to hematopoiesis. The evolution of the adaptive immune system is evident even among the three major classes of fishes. The agnathans have no evidence of immunoglobulins, T cell receptors (TCR), or major histocompatability complex (MHC) molecules (Fujii et al, 1992), although they do have lymphocyte-like cells with surface homology to those of mammals. The elasmobranchs are the most "primitive" organisms to have TCR and MHC and to produce diverse immunoglobulins (IgM) (Bartl et al, 2003). Although their MHC is polymorphic, graft rejection is slow. Additionally, there is no evidence of an anamnestic response within the elasmobranchs (reviewed in Flajnik, 1996). The bony fishes do appear to have memory and within some species of this class there is evidence of maternal transfer (Bly et al, 1986). An important characteristic of the amphibian adaptive immune system is variability in MHC expression during different stages of development. Adult amphibians are similar to mammals with MHC class I expression in most tissues and MHC II expression primarily on hematopoietic cells. Tadpoles, in contrast, express MHC II in multiple organs and MHC I only at low levels in the gills, hematopoietic tissue and gastrointestinal tract (Du Pasquier and Flajnik, 1990). Additionally, terminal-deoxynucleotidyl transferase is not expressed until metamorphosis (Lee and Hsu, 1994), therefore the immunoglobulin repertoire of tadpoles is very restricted. Two classes of immunoglobulins are produced in amphibians: IgM and IgY, a functional equivalent to IgG.

A characteristic of the reptilian inflammatory responses is their temperature dependence, including the synthesis of immunoglobulins. In some species, there are also seasonal differences in splenic lymphocytes with lymphocytolysis and
impaired immune function in the winter (Saad and el Ridi, 1988). Although they lack organized lymph nodes, there are lymphoid aggregates throughout multiple organ systems. Two classes of immunoglobulins: IgM and IgY are produced by reptiles.

The adaptive immune system in birds includes a unique organ, the bursa of Fabricius. The bursa, which is important for B cell development and education, involutes with age and cannot repopulate. Therefore, diseases that affect the bursa early in life have profound effects on the functional ability of the adaptive immune system. At least 3 immunoglobulin isotypes are present including IgM, IgG, and IgA. Differential Ig responses are noted not only between species but also between breeds (Koonen et al, 2002).

**The Morphology of the Inflammatory Response**

As previously mentioned, formation of granulomas is a characteristic feature of the inflammatory response to infectious and noxious agents in all of the non-mammalian vertebrates (Montali, 1988). There are two basic types of granulomas: heterophilic and histiocytic. Heterophilic granulomas develop when heterophils accumulate at a site of injury, degranulate and elicit a macrophage response. Histiocytic granulomas develop from aggregates of foamy macrophages and are the more characteristic response to mycobacteria and parasitic agents. Despite the presence of eosinophils in some reptiles and all avian species, eosinophilic inflammation is rare even in cases of widespread parasitemia.

**The Morphology of Wound Healing**

The character and pattern of wound healing responses in fish, amphibians and reptiles differs significantly from the mammalian paradigm. Because the cutaneous integrity of fish and amphibians is so important to their survival, there is a rapid epidermal response with epithelial cell migration covering the wound surface (Radice, 1980). In amphibians this response is temperature dependent while in fish the response is temperature independent (Dias and Sinhornini, 1991). As a result, wounds in fish and amphibians do not produce a "scab". In snakes, cutaneous wound healing in response to experimental surgical incisional and excisional wounds has been described (Smith and Barker, 1988). The rate of wound healing in these snakes was found to be temperature dependent, similar to amphibians (Smith et al, 1988). In contrast to birds and mammals in which macrophages predominate as healing progresses, heterophils are the predominant cell type throughout the healing process. Fibroplasia and neovascularization progress laterally from the adjacent dermis rather than from the underlying subcutis. Similar to fish and amphibians, horizontal migration of the epithelium precedes reparative fibroplasia and does not require the formation of granulation tissue. In fact, formation of true granulation tissue is variable in reptiles. Scales often do not form in areas of healed wounds and often the skin overlying healed wounds is more darkly pigmented. In both birds and reptiles, small lymphoid aggregates within the dermis surrounding wounds are common, particularly if these wounds are contaminated. Mixed cell populations, including small lymphocytes and plasma cells, suggests that these aggregates may be performing the role of local "lymph nodes".

**Concluding Comments**

Non-mammalian vertebrates of all classes are no longer primarily exhibited in zoological and aquarium collections. Increasing numbers of these species are found in private collections and as pets. As a result, there will be an increasing demand for pathologists with an understanding of non-mammalian pathology. Additionally, many of these species are being utilized in laboratory settings. The susceptibility of fish and amphibians, in particular, to environmental disturbances has resulted in their use in toxicologic research. Although the morphologic characteristics of non-mammalian vertebrate inflammatory response have been described, there are still many questions concerning the mechanisms underlying these responses. Given the concern that environmental perturbations may impact immune function, a greater understanding of these fundamental mechanisms is critical.

**References**


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