BIOMARKERS OF INFLAMMATION IN EXOTIC PETS

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Abstract

The acute phase response (APR) is a key part of the innate immune system and acute phase proteins (APPs) represent the core of the early response to stimuli such as trauma, infection, stress, neoplasia, and autoimmune disease. These biomarkers have a different timeline and magnitude of expression vs traditional means of examining inflammation (e.g., total white blood count and albumin/globulin (A/G) ratio). Extensive studies conducted in companion and large animals have demonstrated many clinical applications for inflammatory biomarkers including diagnosis, prognosis, detection of subclinical disease and chronic inflammation, and monitoring stress. This article provides information regarding the APR and the uses of APP quantitation, as well as the growing body of information on APPSs in exotic animals.

Key words: acute phase protein; acute phase response; biomarker; haptoglobin; inflammation; serum amyloid A

The general knowledge on acute phase proteins (APPs) and their application in veterinary medicine has greatly increased since the mid-1990s. APP have been well documented in studies on laboratory, companion, and large animals.[1-6] Clinical applications for APPs have been widely demonstrated for prognostication as well as for the detection of subclinical disease and chronic inflammation, diagnosis, and stress.[3] Importantly, APP assays have repeatedly demonstrated the ability to enhance the diagnostic sensitivity for inflammatory processes compared with more traditional measures found in routine hematologic and clinical chemistry testing.[3,7] In more recent years, assay validation has been undertaken for most major APPs in the common companion animal species.[7] Although APPs are just being identified and studied for their clinical application in exotic and wildlife species, many reagents have been found to be cross-reactive and validated using automated and highly reproducible methods. The goals of this article are to provide a review of the acute phase response (APR) and basic information concerning APPs and their clinical applications. An emphasis has also been placed on the expanding literature of APP expression in companion exotic species.

APR

The innate immune system includes physical barriers, phagocytes, complement, and toll-like receptors that aid in preventing infection and initiating the inflammatory process. The APR is a complex systemic inflammatory reaction that begins with a local response to a stimulus (i.e., the initiating steps of the early defense system).[2,3] Proinflammatory cytokines and other factors including interleukin 1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF-α), which are produced by the first-responding macrophages and monocytes, stimulate chemotaxis for further leukocyte accumulation and result in protease release and vessel dilation. In addition, the cytokines signal the hepatocytes to initiate and/or upregulate the expression of a number of APPs.

The other primary effects of the APR are focused on the nervous and endocrine systems and the bone marrow. In animal models of inflammation, behavioral, physiologic, biochemical, and nutritional changes are observed.[8] A large part of the APR is the modulation of the hypothalamic-pituitary-adrenal axis. Corticotrophin-releasing hormone is produced in the hypothalamus as a

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response to IL-6, which in turns results in the release of adrenocorticotropic hormone from the pituitary gland and glucocorticoids from the adrenal gland. Other responses include fever, lipid alterations, anorexia, and muscle catabolism. An immediate leukocytosis may be apparent as part of a stress response, but a true leukocytosis does not occur until after cytokine stimulation of the bone marrow, which happens several days later. Several lines of feedback continue to upregulate the response as needed. As the initiating stimulus (tissue damage) is addressed, impressive downregulation of the APR occurs through cortisol, cytokines, and APPs. The goals of successful APR are to promote healing and restore homeostasis.

APPs

The APR is the core of the innate immune response and has been observed across all animal species. APPs are highly conserved, and counterparts to mammalian APPs have been documented in invertebrates and fish.[9-11] APR may affect the production of over 200 APPs.[12] APPs are classified as either positive or negative and are further subcategorized as major, moderate, and minor. The expression of positive APPs increases with the APR.[1-3] Major APPs can increase by 10 to 1000-fold but are present only in negligible levels in normal animals.[3,7] The increase in expression is rapid, with peak levels often reached within 24 hours of stimulation. The half-life of these proteins is often quite short; consequently, the levels decrease rapidly after the cessation of inflammation. Examples of major APPs in domestic animals are serum amyloid A (SAA) in horses and C-reactive protein (CRP) in dogs.[1,3]

Mild and moderate APPs occur in significant concentrations in normal animals. Moderate APPs may increase by 2 to 10-fold and mild APPs increase by less than 2-fold in response to inflammation. In further contrast to major APP, mild and moderate APP increases are delayed with peak expression observed 4 to 6 days after stimulation and these proteins have longer half-lives. Haptoglobin (HP) is a common mild to moderate APP found in all species and is a preferred marker for chronic inflammation.[3]

Albumin exemplifies a negative APP, which decreases in concentration based on ongoing APR. This change may be related to the increased use of amino acids for the production of positive APPs, resulting in a selective decreased synthesis and/or loss due to renal or gastrointestinal changes.[12] In mammals, transferrin is a negative APP, although it appears to be a positive APP in avian species.[13]

Many different types of APPs have been characterized, and their biological functions are numerous and vary by species (Table 1). CRP acts as an opsonin to activate the complement system and phagocytosis and was the first-described APP in the early 1900s in humans and nonhuman primates infected with *Streptococcus pneumoniae*.[14] SAA has chemotactic activity and induces extracellular matrix–degrading enzymes to aid in tissue repair.[3,15] HP binds hemoglobin; this complex is effectively cleared by macrophages and thus serves to inhibit hemoglobin's oxidative activity.[16] Other APPs bind to lipopolysaccharides, inhibit trypsin, aid in the coagulation cascade, scavenge free radicals, and inhibit proteases.[3]

In veterinary medicine, the APR causes changes in protein fractions reflected in protein electrophoresis.[12] The protein fractions represent the multitude of APPs that change levels of expression during an ongoing APR. Newer, automated methodologies (e.g., enzyme-linked immunosorbent assay, immunoturbidity, and colorimetry) can specifically quantitate APP represented in these protein fractions.

APPLICATIONS OF APPs

The origin of the APR stimulation is tissue damage. As summarized in Table 2, the list of varied stimuli emphasizes the importance of the APR and reflects the potential clinical applications of APP testing in veterinary medicine. Many basic studies have been conducted using powerful inflammatory stimuli

TABLE 1. Summary of acute phase proteins (APP) by species. Notable APPs are those that are major APPs, are commonly used in APP assessments, and/or have otherwise been indicated in the literature as APPs with clinical applications

Species	Notable APP	Other Described APP
Bird	SAA and HP	AGP and TN
Cat	SAA	AGP and HP
Dog	CRP	AGP, CP, HP, and SAA
Fish	SAA	A2M, HP, and TN
Horse	SAA and FIB	HP
Mouse	SAA, SAP, and HP	CRP and FIB
Rabbit	CRP	AGP, FIB, HP, and SAA
Rat	AGP and A2M	CRP, FIB, and HP
Turtle	SAA	

A2M, α-2 macroglobulin; AGP, α1-acid glycoprotein; CP, ceruloplasmin; FIB, fibrinogen; SAP, serum amyloid P; TN, transferrin.