ACUTE PHASE PROTEINS OF VETERINARY IMPORTANCE – REVIEW

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ABSTRACT

A review of selected acute phase proteins (APPs) with veterinary importance is given. It appears that APPs represent as suitable analytes for evaluation of animal health. Acute phase proteins are usually glycoproteins. Exceptions are C-reactive protein (CRP) and serum amyloid A (SAA). Acute phase proteins can be used as diagnostic tool in many diseases like bovine respiratory syncytial virus, prostate cancer, bronchopneumonia, multiple myeloma, mastitis, Streptococcus suis infection, starvation, or lymphatic neoplasia and thus, APPs may provide an alternative means of monitoring animal health. The functions of the APPs are varied but generally relate to the defense of the animal to pathological damage and restoration of homeostasis. Indeed the acute phase response is an integral component of the innate immune response forming the first reaction of the host to pathogens and tissue damage. The innate response and the APP predate the acquired immune response during evolution.

Keywords: APPs, cancer, bronchopneumonia, multiple myeloma.

INTRODUCTION

Monitoring the levels of the acute phase proteins (APP) can provide a means to assess the innate immune system’s response to disease and in the ability of the APP to provide a “molecular thermometer.” As these proteins change their serum concentration by 25% in response to inflammation, infection, and trauma, many conditions can cause their elevation or decrease. Therefore, as quantitative markers for disease they can be used for prognosis and monitoring responses to therapy, for general health screening, as well as for diagnosis of
disease. The APP are highly sensitive for the presence of pathological lesions while having a low specificity for a particular disease\textsuperscript{[1]}. The APP are now recognized as having an important role to play in the diagnosis of disease in animals, but there are major differences between species in the pathophysiological change in their concentrations during an acute phase reaction. Furthermore, although initial interest focused on proteins that increase in concentration during this response (positive APP), a number of serum proteins decrease in concentration and can be considered to be negative APP\textsuperscript{[2]}. In any one species, positive APP have been found that have major, moderate, or minor responses. A major APP has a low concentration in the serum of healthy animals, often at 0.1 μg/dl (1 μg/liter) but with the concentration increasing over 100- or 1000-fold on stimulation, reaching a peak 24 to 48 hours after the insult and falling rapidly during recovery\textsuperscript{[3]}. A moderate APP is present in the blood of healthy animals, but on stimulation the concentration will increase 5- to 10-fold, reach a peak concentration 2 to 3 days after stimulation, and decrease more slowly than the major APP. A minor APP shows a gradual increase and only increases in concentration by 50% to 100% of the resting level. Production of APP is controlled by cytokines, with the proinflammatory cytokines interleukin-1, interleukin-6, and tumor necrosis factor–α released from the site of pathogenic or inflammatory damage stimulating the production of the APP\textsuperscript{[4]}. The mammary gland has been shown to be the source of significant amounts of the APP haptoglobin and mammary-associated serum amyloid A during infection of the gland in cattle. Though varied, the functions of many APP can be grouped together. A number of the APP (α 1-antitrypsin, α 2-macroglobulin) have antiprotease activity designed to inhibit proteases released by phagocytes and other cells of the immune system to minimize damage to normal tissues. Number of APP (haptoglobin, SAA, CRP) have scavenging activities and bind metabolites released from cellular degradation so they can reenter host metabolic processes rather than be utilized by pathogen. Other APP actions include antibacterial activity and the ability to influence the course of the immune response\textsuperscript{[5]}

**Selected APP’S of veterinary importance**

Haptoglobin (Hp)

C- reactive protein (CRP)

Serum amyloid A (SAA)
Haptoglobin (Hp)

a. Biochemistry

Haptoglobin is a glycoprotein composed of 2 α and 2 β subunits with the α subunit having a molecular weight of 16 to 23 kDa and the β subunit 35 to 40 kDa. The subunits combine in the form of a β – α – α – β chain. Human Hp has three subtypes known to be genetic polymorphisms (Hp 1-1, Hp 1-2, Hp 2-2). Canine Hp is thought to be similar to human Hp 1-1, whereas bovine Hp has closer similarities to Hp 2-2. Bovine and ruminant Hp in general have noticeable species differences from the Hp in carnivores and omnivores. In the ruminants, Hp tetramers form polymers with other Hp tetramers and a macromolecular complex with a molecular mass of 1000 to 2000 kDa is formed. The mechanism of polymer formation in ruminant serum is thought to depend on the presence of a gene duplication in the α -chain, which results in a free cysteine residue capable of forming disulfide bridges between Hp tetramers as occurs in the human Hp 2-2 phenotype. Another difference between Hp in ruminants and many other species is that this protein is not present in serum from healthy animals, only appearing during the acute phase response.

b. Function and Pathophysiology

The primary function of Hp is to bind free hemoglobin in the blood. The affinity of Hp for hemoglobin is one of the highest among transport proteins, and by removing from the circulation any free hemoglobin, which has inherent peroxidase activity, Hp prevents it causing oxidative damage to tissues. The Hp-hemoglobin binding also reduces the availability of the heme residue and its iron from bacterial use, and therefore Hp has an indirect antibacterial activity. The Hp-hemoglobin complex is recognized by CD163, a surface receptor on macrophages, which leads to its rapid removal from the circulation. In cattle, it has been shown to be an effective marker for the presence, severity, and recovery in cattle with mastitis, enteritis, peritonitis, pneumonia, endocarditis, and endometritis, and for monitoring processes such as tail docking and surgical castration. Elevations have also been reported in cows with fatty liver syndrome, at parturition, during starvation, and following the stress of road transport. Increases of APP during noninfectious disease that involve lipid metabolism may be explained by the release of cytokines from adipose tissue or adipose tissue macrophages that have been implicated in human obesity-related diseases. In pigs, raised Hp was found to be associated with clinical signs of lameness, respiratory disease, diarrhea, tail bite, and ear necrosis, and at slaughter it was found to be related to the presence of lesions and chronic abnormalities. Experimental or natural infection with Actinobacillus
pleuropneumoniae, Mycoplasma hyorhinis, Toxoplasma gondii, Bordetella bronchiseptica, Pasteurella multocida, and porcine reproductive and respiratory syndrome virus leads to increased Hp concentration in serum\textsuperscript{10}.

C-Reactive Protein

a. Biochemistry

C-reactive protein (CRP) was the first acute phase protein to be recognized. It is named from its ability to bind to C-polysaccharide of Gram negative bacteria, but it has since been shown that CRP has a high affinity for phosphorylcholine and related membrane lipids as well as for DNA\textsuperscript{11}. The protein is a pentraxin, being composed of five subunits (20 kDa) combining in the same plane to form a pentametric structure which can be seen as a distinct structure using an electron microscope. Each subunit of CRP contains a binding site for ligand. Human CRP is nonglycosylated and the subunits migrate as a single band on SDS-PAGE, whereas canine CRP migrates as a double band and two of the five subunits are glycosylated.

b. Function and Pathophysiology

Following bacterial infection, CRP binds to pathogen and activates the classical complement pathway leading to the opsonization of the bacteria. Binding of CRP to pathogen also interacts with specific receptors on phagocytes, induces anti-inflammatory cytokine production, and modulates neutrophil function (Du Clos., 2004). There is considerable species variation in the pathophysiology of CRP\textsuperscript{12}. In a number of species such as dog and pig, CRP is a major APP, and its serum concentration can increase rapidly from 0.5 mg/dl (0.5 mg/liter) to more than 10 mg/dl (100 mg/liter). In other species such as cow and cat, CRP has been reported to be a constitutive serum protein with only a minor increase during disease. A number of infectious diseases lead to an increase in CRP in the dog including babesiosis, leishmaniasis, leptospirosis, parvoviruses, trypanosomiasis, and infection with Bordetella bronchiseptica, Ehrlichia canis, and Escherichia coli sepsis\textsuperscript{13}. Relatively moderately raised levels of CRP have been found in inflammatory bowel disease and in hematological and neoplastic diseases of the dog. Elevated levels of canine CRP have been observed in serum from midgestation of pregnant bitches with its appearance coinciding with the implantation of the embryo in the endometrium. It has been postulated that sufficient damage is caused to the endometrium by this process to stimulate the acute phase response in the maternal circulation. In the pig, CRP concentration increases following aseptic inflammation\textsuperscript{14} and with experimental infection with Actinobacillus pleuropneumoniae.
where plasma levels also correlated with clinical findings and were reduced following antibiotic treatment\(^\text{[15]}\).

**Serum Amyloid A**

**a. Biochemistry**

Serum amyloid A (SAA) is a small hydrophobic protein (9 to 14 kDa) that is found in serum associated with highdensity lipoprotein (HDL). In humans, four isoforms have been identified that are separate gene products\(^\text{[16]}\). Of these, SAA1 and SAA2 respond to an acute phase reaction with increasing production from the liver. In contrast, SAA4 is a constitutive protein that is produced normally at a low level and is not affected by the acute phase response\(^\text{[17,18]}\). The SAA3 is expressed in nonhepatic tissues during the acute phase response with increases found in lung, adipose tissue, ovarian granulosa and in the mammary gland\(^\text{[19,20]}\). This isoform has also been detected in bovine colostrums\(^\text{[21]}\). Serum amyloid A is the precursor of amyloid A and is therefore implicated in the pathogenesis of amyloidosis\(^\text{[22]}\).

**b. Function and Pathophysiology**

A number of functions have been ascribed to SAA including reverse transport of cholesterol from tissue to hepatocytes, inhibition of phagocyte oxidative burst, platelet activation, and a number of *in vitro* immune responses\(^\text{[23]}\). A direct antibacterial action of SAA was described in which SAA was found to bind to Gramnegative bacteria leading to opsonization of the target bacteria\(^\text{[24]}\). It has been demonstrated that the M-SAA3 isoform found in colostrum stimulates the production of mucin from intestinal cells assisting the initiation of secretions from the neonatal intestine and helping to prevent bacterial colonization\(^\text{[25]}\). It is only relatively recently that immunoassays became available for measuring the concentration of SAA, but it is already apparent that this analyte will be of great value in monitoring the acute phase response, especially in species in which CRP is not a major APP. Therefore, in ruminants, horses, and cats, SAA assay may become a routine analysis included in the assessments of infection and inflammation. In cattle, SAA has been identified as a marker of inflammation being elevated more in acute rather than chronic conditions\(^\text{[26]}\). It was raised also by experimental infection with *Mannheimia haemolytica*, with bovine respiratory syncytial virus, and in experimental and natural cases of mastitis. The mammary isoform of SAA (M-SAA3), which is expressed and secreted in milk from mammary glands of dairy cows with mastitis\(^\text{[27]}\) is also found in milk from ewes with this condition. In the horse, SAA is a major APP with a large dynamic range between the resting level in the healthy animal...
and the concentrations obtained in serum from horses with infection or inflammation. Increased SAA concentrations have been observed in horses following surgery, with aseptic inflammation or arthritis, septicemia, enteritis, pneumonia, and diarrhea[^28]. Measurement of the SAA concentration was found to be of value in diagnosis of horses with colic, especially where inflammation was the primary component of the pathogenesis. Experimental infections with equine herpesvirus and influenza virus have also resulted in an increase in the SAA concentration. Determination of the SAA concentration may be similarly useful in cats as it was shown to be the most rapidly responding APP in a variety of inflammatory and infectious conditions, and the cat is another species where CRP does not show a major response. In the dog, the circulating concentration of SAA does increase during an acute phase response and has been observed in experimental parvovirus infection and in leishmaniasis. However, with CRP becoming the primary canine APP, it is likely that SAA will be used in a secondary role in monitoring the acute phase response in this species. The relationship of serum concentration of SAA with familial amyloidosis as encountered in Siamese and Abyssinian cats and Chinese shar-pei dogs remains to be fully elucidated[^29,30].

**APPLICATIONS**

Quantitative marker for prognosis, Monitoring response to therapy, General health screening, Disease diagnosis.

**CONCLUSION**

Non-specific markers of inflammation, A tool for studying pathogenesis, To test the efficacy of vaccines and pharmaceuticals, Possible use as a marker of pig selection, Detection of subclinical mastitis

**REFERENCES**