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Acute Phase Proteins – Analysis, Clinical Applications and Potentials

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1. Introduction

Immune response is a universal and evolutionarily conserved mechanism of host defense against infection. It is the first line of defense found in all multicellular organisms and is adaptive only in vertebrates (Medzhitov, 1997).

The body responds to tissue injury through two different defense systems, the innate (non-specific or non-adaptive) and adaptive (specific) immune system. The adaptive immune response is highly specific and has a memory which helps the system to remember the invading agent but the innate immune system is an evolutionary rudiment whose only function is to contain the infection regardless of the type of invading pathogen until the "real" immune response can kick in. Adaptive immunity developed because of the inflexibility of the nonclonal receptors used by the innate immune response. It uses receptors and effectors that are ancient in their lineage and must provide protection against a wide variety of pathogens. Defects in innate immunity are very rare and almost always lethal.

Property	Innate immune system	Adaptive immune system
Receptors	Fixed in genome Rearrangement is not necessary	Encoded in gene segments Rearrangement necessary
Distribution	Non-clonal All cells of a class identical	Clonal All cells of a class distinct
Recognition	Conserved molecular patterns (LPS, LTA, mannans, glycans)	Details of molecular structure (Proteins, peptides, carbohydrates)
Self-Nonself discrimination	Perfect: Selected over evolutionary time	Imperfect: Selected in individual somatic cells
Action time	Immediate activation of effectors	Delayed activation of effectors
Response	Co-stimulatory molecules Cytokines (IL1β, IL-6) Chemokines (IL-8)	Clonal expansion or anergy IL-2 Effector cytokines (IL-4, INFγ)

Table 1. Innate and adaptive immunity

Some of the consequences of the innate immune response are sodium and water retention in response to hypovolaemia, aldosterone and ADH release, pyrexia (elevation of body temperature by 1 to 4° C) for 24-48 hours, increase energy expenditure, increase glucose and fat turnover, breakdown of adipose tissue as principal energy source, decrease in iron concentration, activation of monocytes and macrophages, catabolism of skeletal muscle to provide amino acid for gluconeogenesis and synthesis of acute-phase proteins. The properties of innate and adaptive immune systems are compared in Table 1.

Increased expression of MHC class I related genes is demonstrated in hepatocytes after LPS treatment proposing as indicative of a possible link between innate and adaptive immune response (Yoo and Desiderio, 2003).

In the CNS, cytokines induce a cascade of events which potentiate the cytokine-induced response, so favouring the appearance of the three hallmarks of the APR, namely fever, leucocytosis and changes in the concentration of serum acute phase proteins (APPs). In addition, the stimulation of the CNS results in activation of a variety of responses, mostly mediated by the hypothalamo– pituitary–adrenal and hypothalamo–pituitary–gonadal axes, inducing behavioural changes such as including lethargy, anorexia, adipsia and a disinterest in social and sexual activities (Paltrinieri 2007).

Some of the factors modifying the response to injury include: severity of injury, nature of injury/infection, genetic factors, nutritional status, and coexisting diseases (Paltrinieri 2007).

Inflammation can be classified into two categories: acute and chronic. Acute inflammation is a highly regulated defense mechanism of immune system possessing two well-balanced and biologically opposing arms termed apoptosis ('Yin') and wound healing ('Yang') processes. Unresolved or chronic inflammation (oxidative stress) is perhaps the loss of balance between 'Yin' and 'Yang' that would induce co-expression of exaggerated or 'mismatched' apoptotic and wound healing factors in the microenvironment of tissues. Unresolved inflammation could initiate the genesis of many age-associated chronic illnesses such as autoimmune and neurodegenerative diseases or tumors/cancers (Khatami, 2009). As it is in the Daoism theory Yin and Yang can change in to one another.

The term "acute phase" was introduced in 1941 to describe serum in which C-reactive protein was present (Petersen et al., 2004).

Acute phase response (APR), an evolutionary conserved and nonspecific response, refers to a series of complex physiological events occurring shortly after a tissue injury, inflammation and infections, infection, trauma, malignant neoplasms, burns, tissue infarction, immunologically mediated inflammatory states, crystal-induced inflammatory states (gout), strenuous exercise, childbirth, and marked psychological stress (Hirvonen, 2000).

The purpose of the APR is to prevent further injury of an organ, to limit the growth of the infective organism, to remove harmful molecules, and to activate the repair processes to return the organ to normal function (Hirvonen 2000). APR is mediated by cytokines and signaling molecules which are produced and secreted by hepatocytes, macrophages, fibroblasts, and epithelial cells (Burgess-Beusse and Darlington, 1998). They are able to release a broad spectrum of inflammatory mediators, such as cytokines, lipid mediators, vasoactive amines, products of the complement and coagulation cascades, proteases, reactive oxygen species, and nitric oxide (Hirvonen, 2000).

These inflammatory mediators set off both the local and systemic inflammatory processes. Activated macrophages release a broad spectrum of mediators of which cytokines appear to be uniquely important in initiating the next series of reactions (Koj, 1996).

Cytokines which act as the messengers between the local site of injury and hepatocytes, have different sources and functions and are present in mammals, birds, fish, and reptiles and starfish (Peterson et al., 2004).

At the reactive site, cytokines act on stromal cells, including fibroblast and endothelial cells, to cause a secondary wave of cytokines. This secondary wave augments the homeostatic signal and initiates the cellular and cytokine cascades involved in the complex process of the APR (Baumann and Gauldie, 1994).

The synthesis and release of plasma APP from liver is regulated by inflammatory mediators. These mediators fall into four major categories: interleukin-6-type cytokines, interleukin-1-type cytokines, glucocorticoids, and growth factors. Cytokines mainly stimulate the APP gene-expression, while glucocorticoids and growth factors function more as modulators of cytokine action (Baumann & Gauldie 1994). Interleukin-6 (IL-6) has been recognized as the principal regulator of most APP genes (Hirvonen 2000).

The cytokines act in a synergistic manner: TNF-α mobilizes peripheral amino acids by activating a proteolytic process in muscles, thus increasing the molecules available for the liver to synthesize new proteins. IL-1 is a key element in modulating hepatic protein synthesis since it has an inhibitory effect on the synthesis of negative APPs and, by contrast a stimulatory activity on the synthesis of positive APPs (Paltrinieri, 2007).

This latter effect depends also on a permissive action of glucocorticoids.
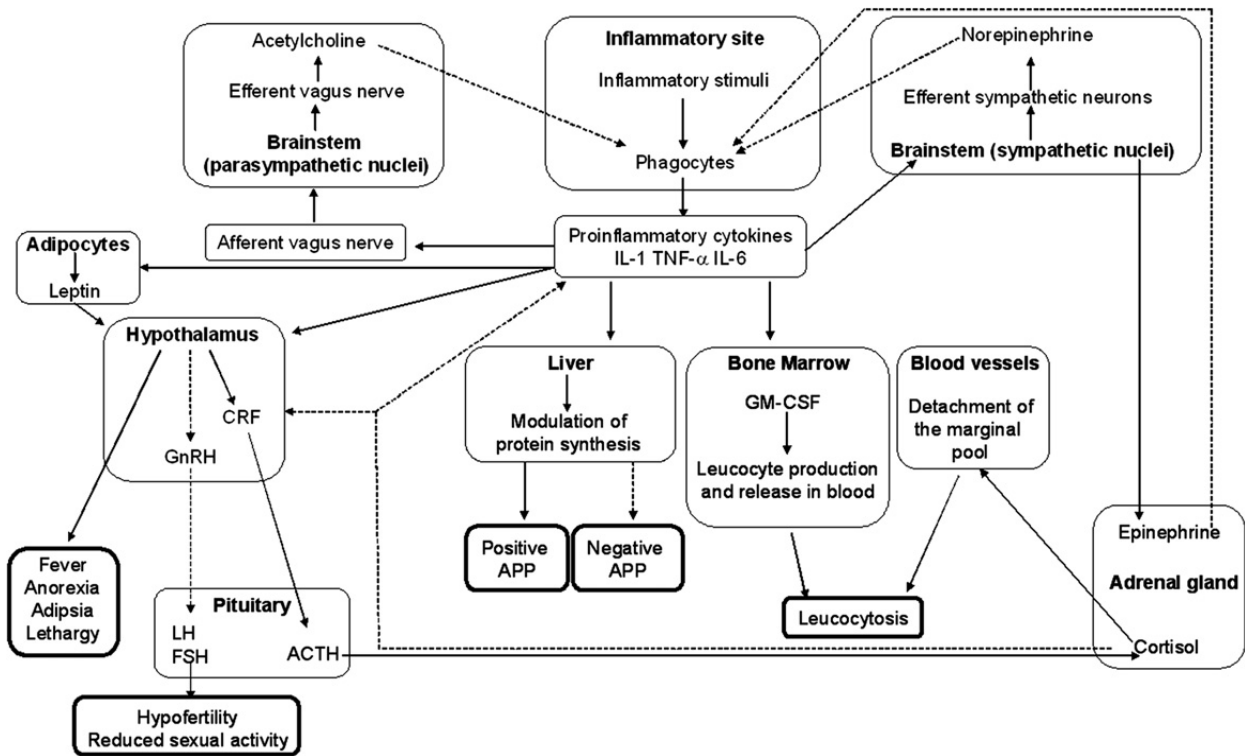

Fig. 1. Summary of the mechanisms responsible for the clinical signs and laboratory findings in the acute phase reaction (circled by the thick line). Solid lines indicate stimulatory effects; dashed lines indicate inhibitory effects. IL-1, interleukin-1; TNF-a, tumour necrosis factor a; IL-6, interleukin-6; CRF, corticotrophin releasing factor; GnRH, gonadotropin releasing hormone; LH, luteinising hormone; FSH, follicle stimulating hormone; ACTH, adrenocorticotropic hormone; APP, acute phase protein (Paltrinieri, 2007).

Finally, IL-6 facilitates the release of APPs in blood (Ceciliani et al., 2002). This complex pattern of induction is defined as 'type I response' but some APPs are induced mainly by IL-6, according to a 'type II response' (Petersen et al., 2004).

There are two categories of APP genes based on their cytokine responsiveness. Type 1 genes respond to IL-1, IL-6, tumor necrosis factor alpha (TNF-α), and glucocorticoids, while type 2 genes respond to IL-6 and glucocorticoids but not IL-1 or TNF-α (Burgess-Beusse B.L. and Darlington G.J., 1998). Some authors categorize the pro-inflammatory cytokines into two major groups with respect to acute phase protein induction, namely IL-1 type cytokines (including IL-1α, IL-1β, TNF-α, TNF-β, etc) and IL-6 type cytokines (including IL-6, oncostatin M, IL-11, leukemia inhibitory factor, oncostatin M, cardiotrophin-1, etc) acting through different receptors located on the membrane of the hepatocytes. IL-1 type cytokines elicit a primary autostimulatory signal stimulating the release of a secondary cytokine signal, IL-6 type cytokines, in various cell types (Mackiewicz, 1997) (Fig 1).

IL-6 type cytokines also seem to exert a negative feed-back on the production of IL-1 type cytokines (Petersen et al., 2004). IL-6 is released by many cells when they are injured. β –adrenergic agonists also can enhance IL-6 release from stimulated cells (Munford, 2000). These cytokines all elicit similar responses because of the association of their respective individual receptors with a common membrane-spanning signal-transduction molecule, gp 130 (Uhlar and Whitehead, 1999). Serum concentrations of cytokines increase during hours after stimulation (Petersen et al., 2004) and would be cleared from blood soon. Cytokines mainly stimulate the APP gene-expression, while glucocorticoids and growth factors function more as modulators of cytokine action (Baumann & Gauldie 1994).

Hepatocytes have a high density of cytokine receptors per cell, and the liver has the largest number of cells with receptors, making it a primary organ involved in the APR. In response to cytokines, changes in expression of various acute-phase protein (APP) genes occur, with an up regulation of positive APP genes (those genes whose expression increases during the APR) and a down regulation of negative APP genes (genes whose expression decreases during the APR). The protein products of these genes are secreted from hepatocytes and, in combination with the effects of the cytokines themselves, bring about the systemic and metabolic changes seen in response to inflammation. These changes include clinical, systemic inflammatory signs as fever, inappetite and depression, which are reflections of multiple endocrinological, haematological, immunological, metabolic and neurological changes in the diseased animal (Stadnyk and Gauldie, 1991).

2. Acute phase proteins (reactants)

One of the main phenomena during the APR is the production of acute phase proteins (APPs) (Baumann and Gauldie, 1994). APPs play a role in the defense response of the host. Monitoring the blood concentrations of APPs can provide information on the progression of the inflammatory reaction (Kent, 1992). The APR may result in changes in more than 200 proteins grouped as either positive APP or negative APP (Cray et al., 2009).

It has been proposed that the term 'acute phase protein' should be replaced with 'acute phase reactant'. The two terms are generally considered to be synonymous, but the latter would also include non-protein molecules such as total serum syalic acid, which increases during inflammation, or proteins involved in APRs but traditionally considered separately from APPs, such as the same APR-inducing cytokines or hormones ghrelin, leptin, and gonadotropins, the concentrations of which vary during APR (Paltrinieri, 2007). Here we use the more common nomenclature (acute phase proteins, since only the classical APPs are discussed).

Interleukin-6 (IL-6) has been recognized as the principal regulator of most APP genes. The APPs produced are termed type-2 APPs; in most species these include fibrinogen (Fb), haptoglobin (Hp) and at least one of the major antiproteases, like α1-proteinase inhibitor (α1-PI). The group of APP genes regulated by interleukin-1-type cytokines (IL-1a, IL-1b, TNFα) is clearly different from that regulated by IL-6-type cytokines. The APP produced are called type-1 APP, and they include alpha1-acid glycoprotein (α1-AG), serum amyloid-A (SAA), and C-reactive protein (CRP), depending on the species.

Binding of the inflammatory mediators to their respective receptors on hepatocytes and transduction of this signal induce changes in APP gene expression that are primarily regulated at a transcriptional level. Under certain conditions post-transcriptional mechanisms, translation, APP modelling and export, may also be involved in this process (Kushner 1993, Pannen & Robotham 1995). Some APPs are also produced extrahepatically, e.g. α1-PI, ceruloplasmin (Cp), complement components, and SAA (Raynes 1994).

By definition, APPs are proteins which their serum concentrations change by >25% in esponse to inflammation or infection (Eckersall and Bell, 2010). The APPs can be divided into minor, moderate and major APPs depending on their increase in concentration (Eckersall, 2000; Peterson, et al., 2004) (Table 2).

Major APP (10-100 fold increase)	Haptoglobin (Hp) Serum amyloi-A (SAA)
Moderate APP (2-10 fold increase)	α₁- acid glycoprotein (α₁-AG) α₁- proteinase inhibitor (α₁-PI)
Minor APP (1-5 fold increase)	Fibrinogen Ceruloplasmin α₂- macroglobulin (α₂-M) complement component 3 (C3) Bovine lipopolysaccharide binding protein (bLBP)

Table 2. Illustration of bovine plasma APP according to their responsivity during APR (Hirvonen, 2000).

A major APP has a low serum concentration (<1 μg/L) in healthy animals and rises significantly by 100-1000 fold on stimulation, peaking at 24-48 h and then declining rapidly during the recovery phase. Moderate APPs increase some 5-10-fold on activation, peak after 2-3 days, and decrease more slowly than major APPs. Minor APPs gradually increase by between 50% and 100% of its initial level. Negative APPs include albumin, transferrin and retinol binding protein which apart from albumin, their use in veterinary medicine is not common (Eckersall and Bell, 2010).

This way of classifying positive APPs partly overlap since in many cases the protein that most frequently increases in a given species also shows a higher magnitude of increases compared to other species. The important concept is that each animal species has its own 'major' APP(s) that must be considered the marker of choice for diagnostic purposes (Table 3).

The serum concentration of the rapid reacting APPs (such as SAA and CRP), which are primarily induced by IL-1 type cytokines, increases within four hours (Petersen et) and return to the normal levels rapidly. Type 2 APPs (fibrinogen and haptoglobin in most species) are characterized by a latter increase in serum concentration remaining up to two weeks (Petersen et al., 2004). The above mentioned classification is not complete: bovine Hp is stimulated by IL-6 and TNF, but not by IL-1 (Nakagawa-Tosa et al. 1995).

The group of positive APPs tends to increase continuously with the inclusion of newly discovered molecules, such as hepcidin, a protein involved in regulating iron metabolism during inflammation (Nemeth et al., 2003; Fry et al., 2004), or of molecules that are involved in processes different from inflammation but that are characterised by the typical 'APP behaviour' (e.g. showing an increase of >25% during inflammation). This is the case with antithrombin III, which may work as an APP in cats (Paltrinieri, 2007).

	Swine	Cattle	Dog	Cat	Man	Mouse	Rat	Rabbit
Albumin	NEG	NEG	NEG	NEG?	NEG	NEG	NEG	NEG
Haptoglobin	III	III	II	I/II	II	III	II	III
α_1-acid glycoprotein	0	II	II	II	II	II	III	II
Fibrinogen	I	I	II	?	II	II	II	II
C-reactive protein	II	0	III	0	III	I	II	II
Serum amyloid A	III	II	III	II	III	III	0	III
Serum amyloid P	?	0	?	?	0	I-III*	0	0
Major acute phase protein	III	II	?	?	?	?	II	?

*Normal level as well as acute phase concentration changes of SAP differ strongly between different mouse strains. ?: Nothing has been reported about the reaction of the protein indicated in the literature. NEG: Decrease in concentration (10-30%); 0: No change; I: 50% to 100% increase; II: Between 100% and 10 time increase; III: More than 10 times increase (Petersen et al., 2004).

Table 3. Acute phase proteins in different species.

The APP profiles vary among different animal species (Kushner 1982, Hayes 1994) and also within them. The profiles can be affected e.g. by age, sex, pregnancy, and polymorphism (Alsemgeest et al. 1993, Hayes 1994). Table 4 shows the physicochemical properties and reference intervals of different APP responders in some animal species.

	EF	MW (kDa)	g/dL	Group	Major	Minor
Haptoglobin (Hp)	α_2	100–400	1–2.6	II	$B_{(III)}, S_{(III)}, M_{(III)}$	$H_{(II)}, C_{(II)}, F_{(I/II)}, R_{(II)}$
Complement fraction C3	$\alpha_1-\beta$	185	0.8–1.4	I	None	$All_{(I/II)}$
Complement fraction C4	α_1	206	0.2–0.4	I	None	$All_{(I/II)}$
Ceruloplasmin (Cp)	α_2	51	0.2–0.6	I	None	H,C,F,B
Fibrinogen	$\beta-\gamma$	341	2.0–4.5	II	None	$H_{(II)}, E_{(II)}, B_{(II)}, S_{(II)}, M_{(II)}, R_{(II)}$
α_1-Acid glycoprotein (AGP)	α_1	41	0.5–1.4	II	$F_{(II)}, R_{(II)}$	$H_{(II)}, C_{(II)}, B_{(II)}, M_{(II)}$
C reactive protein (CRP)	α_2	106	<0.01	II	$H_{(III)}, C_{(III)}, E_{(II)}, S_{(II)}, M_{(II)}, R_{(II)}$	$F_{(II)}, B_{(I)}$
Serum amyloid A (SAA)	α_2	14	0.01	III	$All_{(III)}$	None
Serum amyloid P (SAP)	–	–	–	III	$R_{(I/II)}$	None
α_2-Macroglobulin (2MG)	α_2	–	–	III	None	$B_{(II)}, R_{(III)}$
Pig major acute phase protein (Pig-MAP)	α_2	115	–	III	$S_{(II)}$	$B_{(II)}, R_{(II)}$

EF, electrophoretic migration; MW, molecular weight; g/dL, physiological concentration in serum; Group: proteins are listed according to the usual increase during the acute phase reaction (APR): I = increase up to 100%, II = increase up to 10·, III = increase >10· during APR. Major or Minor = species in which each APP is considered major or minor based on the frequency of increase during APR and on the magnitude of elevation (see I, II and III above); B = bovine; C = canine; E = equine; F = feline; H = human; M = mouse; S = swine; R = rat (Paltrinieri, 2008).

*Modified from Gruys and Toussaint (2001) and Petersen et al. (2004).

Table 4. Summary of physico-chemical characteristics of the most important positive acute phase proteins in different animal species.

3. Selected acute phase proteins of clinical importance

3.1 Haptoglobin

The name haptoglobin (Hp) is derived from its ability to form a stable complex (haptein = to bind) with hemoglobin. Hp, an α_2-globulin with a molecular weight of approximately 125 kDa, is one of the APPs in the blood of humans and animals and its concentration varies according to the health status (Whicher and Westacott 1992; Kushner & Mackiewicz, 1987). Hp was first described as a protein with the ability to increase the stability of the peroxidase activity of hemoglobin to low pH. Hp consists of light (α) and heavy (β) chains ($\alpha\beta$)$_2$, linked by disulfide bonds (Petersen et al., 2004). In humans, 16 different subtypes have been observed which have three different phenotypes, Hp 1-1, Hp 2-1 and Hp 2-2, with molecular weights of 100, 220 and 400 kDa, respectively (Putman, 1975). Porcine Hp has an electrophoretic mobility similar to that of human Hp phenotype 1-1; bovine Hp exists as a tetramer in association with albumin with a molecular weight above 1000 kDa in cattle serum which is most similar to human Hp 2-2 (Eckersall & Conner, 1990). Equine Hp consists of a pair of polypetides with molecular weights of 108 and 105 kDa. Dogs have only 1 phenotyoe of Hp, which closely resembles human Hp 1-1 with molecular weight of 81 KD but the 2 $\alpha\beta$ chains are joined by a noncovalent interaction rather than by a disulfide bridge (the noncovalent linkage also exists in feline HP) (Petersen et al., 2004).

The main function of Hp is to prevent the loss of iron via urine by the formation of a very stable complex with free hemoglobin. Hp together with hemopexin and transferrin helps to reduce the detrimental effects of free iron and to restrict the availability of free iron and to invading pathogens (Petersen et al., 2004). Free hemoglobin released from erythrocytes has oxidative and toxic properties (Putman, 1975). The Hp-hemoglobin complex is recognized via a specific cell surface receptor located on macrophages and when bound the complex will rapidly be removed from circulation. Hp also inhibits bacteria dependent on hem iron for growth (Eaton et al., 1982). In cattle, Hp has been proposed to be involved in the regulation of lipid metabolism and as a immunomodulator. Stimulation of angiogenesis, role in lipid metabolism/development of fatty liver in cattle and inhibition of neutrophils respiratory burst activity have been proposed for Hp (Petersen et al., 2004).

Hiss et al., (2004) showed that Hp in milk not only originates from serum Hp, which is produced by hepatocytes, but also from mammary glands in which Hp mRNA is expressed. Hp levels increase dramatically during infection, inflammation or trauma and measurement of its concentration in serum provides valuable diagnostic information to clinicians in both human and veterinary medicine.

3.2 Serum amyloid A

The SAA family was originally considered to comprise only a single circulating precursor of the amyloid A protein from which its name is derived. SAA constitutes a protein family related to the A proteins of secondary amyloidosis (Betts et al., 1991). The family is known to contain a number of differentially expressed apolipoproteins which can be divided into two main classes based on their responsiveness to inflammatory stimuli: acute phase serum amyloid A (A-SAA) and constitutive SAAs (C-SAAs). The A-SAAs are highly conserved across evolutionarily distinct vertebrate species and it is generally accepted that they have a crucial protective role during inflammation. C-SAAs have been described in two species, human and mouse and are minimally induced during APR. Both SAAs associate with HDL. Multiple SAA genes and proteins have been described for several mammalian species (including human, mouse, hamster, rabbit, dog, mink, fox, cow, sheep, goat and horse) and

non-mammalian vertebrates (including marsupials and fish which provide evidence that they are likely to have important biological functions (Uhlar and Whitehead, 1999).

The four human and five mouse SAA family members were originally named in numerical progression according to the order in which they were identified. According to the Nomenclature Committee of the International Society of Amyloidosis, extrahepatically-produced SAA should be referred to as SAA3 (Sipe, 1999). The human SAA1 and SAA2 and the mouse Saa1 and Saa2 genes have 90 percent nucleotide identity (Betts et al., 1991) and all encode A-SAAs.   SAA3 show approximately 70 percent identity with SAA1 and SAA2 (Kluve-Beckerman et al., 1986) but no mRNA or protein product specified by human SAA3 has been identified. So human SAA3 protein, if it exists, cannot be a molecule with functions analogous to those of the SAA3 molecules in other species. The human SAA4 and the mouse Saa5 gene are the only constitutively expressed genes, which produce C-SAA (Uhlar and Whitehead, 1999). Intestinal epithelial cells, convoluted tubules of the kidney, bone marrow stromal cells, jejunal mucosa stomach, muscle, spleen, brain, heart, lung, ovary, testis, uterus and mammary glands are among the extrahepatic sites of A-SAA synthesis (Uhlar and Whitehead, 1999; McDonald et al., 2001). Different names have been used for SAA occurring in milk. Some authors refer to mammary-associated serum amyloid A as M-SAA 3 (McDonald et al., 2001, Larson et al., 2005) while others use MAA (milk amyloid A) (O'Mahony et al., 2006; Safi et al., 2009). Many studies only use SAA regardless if it is hepatically or locally produced (Gronlund et al., 2003).

The induction profiles of the SAA mRNAs in the liver and other tissues, vary considerably with different inflammatory agents. In humans, extra hepatic synthesis of both A-SAA and C-SAA mRNAs are expressed in monocytic/macrophages cell lines, endothelial and smooth muscle cells and also in adipocytes. These findings suggest a possible role for SAAs as an immunological defense molecule at local sites against inflammatory stimuli during the time taken to mount a systemic response by increased hepatic synthesis (Uhlar and Whitehead, 1999).

Like Hp, the biological function of SAA is not fully understood but it is known that SAA is involved in lipid transport /metabolism (Malle et al., 1993) and also in alteration of cholesterol metabolism under inflammatory conditions (Pannen & Robotham, 1995). SAA binds Gram-negative bacteria (Hari-Dass et al., 2005), possibly to facilitate the uptake by macrophages and neutrophils (Larson et al., 2005). Inhibitory effect on fever, on the oxidative burst of neutrophilic granulocytes and on in vitro immune response, chemotaxic effect on monocytes, PMN and T cells, and induction of calcium mobilization by monocytes and inhibition of platelet activation are among the functions attributed to SAA. Different conditions and diseases lead to increased SAA concentration in human, pig, cattle, horse, dog, cat, mouse, rabbit and chicken (Peterson et al. 2004, Murata et al., 2004), which will be addressed in the following sections.

SAA is synthesized intraarticularly during inflammatory conditions, and this intraarticular SAA may lead to induction of metalloproteinase activity. The intraarticularly produced SAA isoform had a highly alkaline pI value, which is in accordance with pI values reported for extrahepatically synthesized murine and bovine SAA3 (Jacobsen et al., 2005).

In chickens, SAA is likely to be a reliable APP for diagnosing inflammatory lesions (Chamanza et al., 1999).

3.3 C-reactive protein (CRP) and serum amyloid-P component (SAP)

CRP and SAP are pentraxins, belong to the highly conserved pentraxin family of plasma proteins with a pentameric organization of subunits (Steel and Whitehead, 1994).

Pentraxins are able to clear nuclear material released from necrotic tissue; they are also involved in opsonization, activation of classical pathway of complement (C1q), chemoattraction, and enhancement of phagocytosis. CRP binds released bacterial or host DNA (Hirvonen, 2000).

Canine AGP has been described as a very unusual protein of 43 KD with a very low pI of 2.8-3.8 and a very high carbohydrate content of 45% (Ceron et al., 2005).

CRP and SAP are found in all mammals and presumptive homologues have been found in a number of nonmammalian vertebrates and invertebrates (Steel and Whitehead, 1994). CRP behaves like a broad-specificity antibody. It has been called an "ante" antibody, since it has been found in invertebrates that do not make either immunoglobulins or complement. It also is one of the key pattern recognition molecules that enable prompt host recognition of invading pathogens. In humans, CRP and SAP share only 51% amino acid identity and 59% nucleotide sequence identity (Steel and Whitehead, 1994).

CRP is the first APP to be described as an APP in human. Crystallization of the CRP isolated from human serous fluids was first described in 1947. The crystalline protein was obtained from two pathological specimens, one a pleural fluid from a patient with streptococcal pneumonia and the other an abdominal fluid from a cirrhotic patient suffering from an intercurrent infection. CRP has been described in ruminants, dog, pig, rat, rabbit and to a lesser degree, horse (Petersen et al., 2004) and was originally named for its ability to bind the C-polysaccharide of *Pneumococcus pneumonia*. CRP has been shown to act as an opsonin for bacteria, parasites and immune complexes, can activate the classical pathway of complement, can modulate the behavior of several cell types, including neutrophils, monocytes, natural killer cells and platelets and can bind to chromatin, histones, and small nuclear ribonucleoprotein particles (snRNPs) (Steel and Whitehead, 1994). CRP increases L-selectin shedding from neutrophils and prevents neutrophils-endothelial cell adhesion which leads to neutrophils release from marginated pool. CRP also stimulates monocytes to release an anti-inflammatory molecule, IL-1 receptor antagonist (IL-1Ra). Although CRP does not bind to normal cell membranes, it can bind avidly to cells that are undergoing apoptosis or necrosis, through its ability to recognize the lysophosphatidylcholine (lysoPC) that appears on the surfaces of dying cells (Munford, 2000).

SAP is a non-fibrillar plasma glycoprotein which is found in amyloid deposits due to its specific calcium dependent binding to motifs present on all types of amyloid fibrils. Amyloid deposits are present in a heterogeneous group of disorders, the amyloid A amyloidoses. The predominant amyloid A protein type found in amyloidotic tissues corresponds to the N-terminal two thirds of A-SAA (the first 76 residues of mature human A-SAA) (Uhlar and Whitehead, 1999). The SAP is also found to prevent fibrillar breakdown by enzymes and seems to be one of the factors that maintains the stability of the amyloid deposits. It interacts with inflammatory and complement factors and might be associated with the production of inflammation in specific cases. SAP concentration does not change in cattle, human, rat and rabbit during inflammation but normal level as well as acute phase concentration changes of SAP differ strongly between different mouse strains (Petersen et al., 2004). The exact role of SAP is uncertain.

3.4 Alpha-1-acid glycoprotein (AGP)

AGP is a sialo-glycoprotein synthesized and secreted mainly by hepatocytes and is the main protein component in seromucoid, the fraction of plasma that is most resistant to acid precipitation (Ceron et al., 2005).

Human AGP is characterized by low molecular weight (41–43 kDa), high solubility, very low pI (2.8–3.8) and high percentage of carbohydrates (45%). Its glycosylation pattern is very variable (12–20 glycoforms) depending on the physiological or pathological conditions, such as pregnancy, inflammation or cancer (Paltrinieri, 2008).

AGP is considered a moderate APP in most species and is more likely to be associated with chronic conditions rather than acute inflammation.

AGP is mostly synthesized during inflammation in rat, rabbit, mouse, man, cat, dog, horse and cattle but mammary-associated AGP has also been identified in bovine colostrum and milk (Ceciliani et al., 2005).

AGP is a immunocalin; a group of proteins that shows significant immunomodulatory effects (Logdberg and Wester, 2000). AGP regulates the inflammatory response of leucocytes (e.g., inhibits platelet aggregation, proliferation of lymphocytes and activation of neutrophils (Hochepied et al., 2003). Immunomodulating effects of AGP are primarily downgrading the local inflammatory response to reduce tissue damage caused by inflammatory cells (Hochepied et al., 2003). Suppression of cattle leucocytes was also found to correlate with AGP concentrations during mastitis (Sato et al., 1995). One of the many functions of AGP is to protect cells from apoptosis, and an anti-apoptotic effect on cattle monocytes has recently been reported (Ceciliani et al., 2007). There is some evidence that AGP might contribute to the net charge on microvessel walls and could decrease albumin leakage from circulation during the acute phase. In cattle, AGP is known to suppress lymphocyte blastogenesis and thus possess immunosuppressive properties (Sato et al. 1995). Systemic AGP has two major physiological functions: drug binding and immunomodulation. Like serum albumin, AGP binds with and carries endogenous and exogenous substances such as heparin, histamine, serotonin and steroids. This ability might help to keep total drug binding levels unaffected in APR in which serum albumin decreases in concentration. AGP may help to enhance the clearance of lipopolysaccharide (LPS) by binding with it and neutralizing its toxicity (Murata et al., 2004).

3.5 Fibrinogen (Fb)

Fibrinogen is a soluble β-globulin present in the plasma of all vertebrates. It is composed of 3 nonidentical polypeptide chains linked by disulfide bridges and a glycoprotein that contains 3-5% carbohydrates. Fb specifically binds to CD11/CD18 integrins on the cell surface of migrated phagocytes, thereby triggering a cascade of intracellular signals that lead to enhancement of degranulation, phagocytosis, antibody-dependent cellular cytotoxicity and delay of apoptosis (Murata et al., 2003). Fb was one of the earliest recognized APPs. It is a coagulation protein produced by liver, serving as a matrix for wound healing (Raynes 1994). Fibrinogen is a minor APP in most species including humans and cattle and horse. Although determination of plasma Fb concentration has long been used for detecting inflammatory diseases, its relatively slow reaction during inflammatory insult hampers its clinical utility. Nevertheless, Fb measurements are easy and inexpensive to perform, and this fact has likely secured its continued wide use in veterinary medicine (Crisman et al., 2008).

3.6 Negative acute phase proteins: albumin and transferrin

Albumin is the most abundant protein in blood constituting the major band observed in serum protein electrophoretograms.

Transferrin is a plasma glycoprotein that is responsible for the transport of iron in the circulation and has a single polypeptide chain of about 700 amino acids. It binds iron as a ferric

ion in 2 binding sites at a neutral pH (Ceron et al., 2005). Nutritional status affects production of negative APPs but the APR has a stronger effect than the nutritional plane on concentration of transferrin, albumin, and other negative APPs (Ceron et al., 2005). In mammals transferrin is down-regulated during inflammation (Kushner and Rzewnicki, 1999).

Ovotransferrin in birds appears to have special characteristics differing from those of mammals. It has been shown that following injection of croton oil to induce inflammation a 65-kDa glycoprotein with an N-terminal sequence matching to that of chicken ovotransferrin, a major egg white protein is induced. In laying hens, ovotransferrin is synthesized under the control of estrogen (Palmiter et al., 1981) and is a major constituent of egg white. Its major physiological function, like other members of the transferrin family, is presumed to be iron transport, and its antimicrobial activities are probably related to its ability to sequester iron, an essential element for bacterial growth. However, unlike in mammals, in birds it appears to be a positive APP (Xie et al., 2002).

Other APPs like proteinase inhibitors, α_1-antitrypsin, α_1-antichymotrypsin, α_2-macroglobulin, mannan-binding lectin (MBL), ceruloplasmin, etc have been dexcribed as APPs in different species but have a less diagnostic importance.

4. Methods of analysis

Although inflammatory status can be measured indirectly by measuring serum concentrations of albumin, globulins and copper (kaneko et al., 2008), but more specific and sensitive methods to determine the status are based upon direct measurement of individual APPs. The development of assays to quantify APPs has been pioneered by a number of laboratories throughout the world, in which calibration of the assays has been achieved by isolation of the protein and determinations of its concentration prior to its use as a standard (Skinner, 2001). The European Commission Directorate General Research Concreted Action Group was established in 2000 to harmonize the calibration of APP assays and to disseminate strategic and applied research results on the use of these assays. For those laboratories that are unable to set up specific and sensitive methods for APP measurement, electrophoresis on agarose or cellulose acetate gels can be used to identify changes in APP concentrations during inflammation or infection. (Carapeto et al., 2006).

4.1 Haptoglobin

Assays for serum Hp concentration can be divided into 2 groups: a) spectrophotometric assays and b) immunoassays. Although some ELISA assays have been established for Hp measurement, the convenience of methods based on the binding of Hp to hemoglobin and the potential for the development of automation, has lead to biochemical Hp assay becoming a routine biochemical test in many veterinary diagnostic laboratories. Spectrophotometric assays have been based on ability of Hp to bind hemoglobin, forming Hp-Hb complexes that either alter the absorbance of Hb in proportion to the concentration of Hp or preserve peroxidase activity at an acidic pH (Ceron et al., 2005). An automated spectrophotometric multispecies assay based on the peroxidase activity of Hp-Hb complexes has been described and validated by Eckersall et al., (1999), in which interference by serum albumin is eliminated. The methodology has been developed into a commercial biochemical assay kit for routine analysis, giving satisfactory results.

Nephelometric immunoassays, in which the rate of precipitation of the Ab-Ag complex is measured, have been validated for Hp measurement in dogs (Ceron et al., 2005) and pigs (Lipperheide et al., 1998). Other methods for estimation of serum Hp include capillary zone

electrophoresis, turbidimetry (Ekersall, 2000), biosensor assay (Åkerstedt et al., 2006), single radial immunodiffusion (SRID) (Hanzawa et al., 2002), latex agglutination test (In Murata et al., 2003) and capillary zone electrophoresis (Pirlot et al., 1999). Most of these assays depend on the cross reactivity of antiserum to human Hp with the analogus protein in animal serum and must be properly validated before use.

Canine serum samples must be diluted in many cases when Hp assays developed for other species are used, as the concentrations of Hp in health and disease are significantly higher in dogs than in other species (Ceron et al., 2005).

Other methods of liquid phase analysis with the option of recycling of the sample (Philips, 2001), such as those possible with Surface Plasmon Resonance (Biacore system), have been tested for Hp in bovine milk (Åkerstedt et al., 2005).

Bovine serum Hp has been traditionally analyzed indirectly by measurement of Hb bound to Hp (Makimura & Suzuki 1982). Morimatsu et al. (1992) introduced a single radial immunodiffusion assay for bovine serum Hp. Monoclonal antibodies against bovine Hp have been characterized and used for analysing bovine serum Hp by several immunotechniques (Hirvonen, 2000).

4.2 Serum amyloid A

Previously, SAA measurements were primarily the domain of research laboratories. Nowadays there are several methodologies for SAA measurement in different species. Monoclonal antiserum against human SAA has been used in a sandwich ELISA that can be used successfully for other species as well. An ELISA for equine SAA using chemiluminescent substrate has been established. Other methodologies for measurement of equine SAA include slide-reversed passive latex agglutination, latex agglutination immunoturbidimetric assay. A commercially developed immunoturbidimetric assay for human SAA has been evaluated for use in horses with good precision (Crisman et al., 2008). A commercially available ELISA for SAA measurement in veterinary species using monoclonal antiserum against human SAA has been proven to be useful for canine and feline SAA quantification (Ceron et al., 2005). Multispecies SAA kit has been developed by Tridelta Development Ltd which is suitable for most species except murine.

Bovine SAA can be analyzed immunologically, and enzyme-linked immunosorbent assays (ELISA) for the determination of bovine SAA have been developed (Boosman et al. 1989; Horadagoda et al. 1993).

Other methods include electroimmunoassay, single radial immunodiffusion (SRID) and a sandwich enzyme immunoassay (Hultén et al., 1999).

4.3 C –reactive protein

Measurement of serum CRP is generally performed by immunoassays using species-specific CRP antibodies with several formats such as immunoturbidimetric assay, ELISA, slide/capillary reverse passive latex agglutination test, and time-resolved fluorometry (TRFIA) (Ceron et al., 2005). There is a commercially available automated turbidometric immunoassay for human serum CRP which has been validated for measuring canine CRP; however, in other investigations very weak cross-reactivity of canine CRP with antihuman CRP antibodies has been found (Ceron et al., 2005). Different nephelometry systems are compared during application for human CRP (Maggiore et al., 2005). Turbidimetry is developed for CRP in the dog (Kjelgaard-Hansen et al., 2003).

A protein chip has been developed for measurement of haptoglobin and SAA in human patients (Tolson et al., 2004).

4.4 Alpha-1-acid glycoprotein (AGP)

Although AGP can be estimated by precipitation of the majority of serum proteins by perchloric acid and quantification of the remaining soluble proteins, AGP is measured routinely by single radial immunodifffusion (SRID) on agarose gel impregnated with anti-species AGP rabbit serum (Tamura, 1989). The kits are species-specific and are available for humans, dogs and cats but have the disadvantage of time consuming process (24 to 48 hours to be complete). Immunoturbidimetric assays for canine and feline AGP have been developed that are rapid and adaptable to biochemical analyzers (Ceron, et al., 2005). Nephelometric and turbidometric immunoassay methods have been also described for AGP measurement (Komine et al., 1994).

Preliminary experiments with a monoclonal anti porcine CRP and pig acute phase sera using protein microarray methodology on slides (Timmerman et al., 2004), offered the possibility to measure more than 1000 pig blood sample spots on a single slide.

In chickens, AGP is an APP of clinical significance. High AGP levels have been observed in chickens with various bacterial or viral pathogens (Murata et al., 2004).

4.5 Ceruloplasmin

Many quantitative methods based on different principles have been used for Cp measurement in plasma or serum. Assays based on oxidation of different compounds such as *p*-phenylenediamine (PPD) or its N-dimethyl derivative and *o*-dianisidine dihydrochloride have been used most often in veterinary medicine. Manual and automated methods based on PPD-oxidase activity have been reported for measuring Cp (Ceron et al., 2005). Ceruloplasmin can also be estimated biochemically by measuring its endogenous oxidase activity.

One of the main problems with Cp assays is the lack of commercially available reference materials to standardize Cp measurements (Ceron et al., 2005).

4.6 Fibrinogen

Fibrinogen (Fb) is a large protein of 340,000 Da, which constitutes about 5% of the total proteins of plasma. It is most simply and rapidly estimated by the heat precipitation-refractometer method proposed by Kaneko and Smith in 1967. The heat precipitation method is now extensively used as a routine screening method but more accurate methods include modifications of the Ratnoff-Menzie assay, measurement of clot weight, and immunoprecipitation method (Crisman, et al., 2008).

4.7 Methods of analysis: alternatives

Indirectly, acute phase protein formation may be measured in biopsies by methods to assess upregulation of protein synthesis (quantitative PCR) (Gruys et al., 2005).

A possible future challenge for veterinarians would be the development of high throughput techniques, such as protein microarray methodology, which would allow simultaneous measurements of thousands of samples per batch, as has already proposed for some species (Gruys et al., 2005). In addition, techniques able to detect qualitative or structural changes to the APPs, such as two-dimensional gel electrophoresis, high performance liquid chromatography (HPLC), Western blotting and lectin staining, are available (Paltrinieri, 2008).

Although these techniques are currently used mainly for research purposes due to high cost and to the need for specialized equipment and trained personnel but they may have crucial importance in diagnostics in the future if done rapidly, and at low costs, making it possible to run many samples at the same time.

5. Clinical applications of major APPs

APP concentrations are elevated in many diseases with different pathogeneses. The fact cause APPs to have poor specificity in detecting the cause for a particular disease but some studies have been performed to increase the specificity of APPs, using group analysis of APPs (acute phase index) (Gruys et al., 2005). On the other hand APPs have very high sensitivity in detecting different conditions that cause subclinical infection or inflammation. So APPs can provide 1) an alternative means for monitoring animal health, as well as for human patients 2) an objective information about the presence and extent of ongoing lesions in individual animals, 3) an information about the prevalence of a disease/diseases indicated by the high serum concentration of selected APP(s), 4) a prognostic tool, with the magnitude and duration of the APR reflecting the severity of infection (Petersen et al., 2004), 5) a practical means to identify the effectiveness of antibiotic therapy (Wittum et al., 1995), 6) a useful tool for separation of suspect from non-suspect animals during ante mortem inspection at slaughterhouses, to improve food safety for public health (Toussaint et al., 1995).

A further likely use of the APPs as diagnostic markers in production animals is that they might have a role to play in monitoring health for optimal growth, by detection of small changes in APPs concentrations, using acute phase index (Toussaint et al., 1995).

The interpretation of APP results from haemolytic, icteric and lipaemic samples should be interpreted with caution, due to the possible influence of these interfering substances on analytical results (Ceron et al., 2005).

5.1 Acute phase proteins in human medicine

Shortly after it was discovered that a protein in human serum binds to the pneumococcal "C" polysaccharide, Arvey and others found that the serum concentrations of this "C-reactive" protein (CRP) can rise dramatically during illness. Although ESR, an indirect measure of APR, had been introduced as by Westergren in 1921, but its wide reference interval, moderate specificity and sensitivity, low to moderate reproducibility, being time-consuming to be complete and the fact that its results may be affected by gender, age, temperature, drugs, level of plasma proteins and RBC factors (hematocrit, morphology, size,...) (Collares and Vidigal, 2004) made it unsuitable to be a marker of choice for monitoring inflammation or infection; however it is a simple and inexpensive laboratory test.

While plasma levels of CRP in most healthy subjects is usually 1mg/L with normal being defined as <10 mg/L (0.06-8 mg/L) its concentration is exponentially increases (100-500 mg/L), doubling every 8-9 hours and remains elevated during APR. So serial CRP measurements can be used as a diagnostic tool for infection, monitoring of treatment or early detection of relapse in humans.

Today, CRP remains an APP of primary interest in humans, where it is a major marker of infection, autoimmune disease, trauma, malignancy, and necrosis including myocardial infarction. Furthermore, CRP has been proposed as a marker for wellness assessments, which is a common role proposed in many studies of human and animal APP (Cray et al., 2009).

CRP levels serve as an early marker of the magnitude of inflammation in events as dissimilar as appendicitis and myocardial infarction. The level of circulating CRP correlates with endovascular disease and may serve to identify otherwise asymptomatic patients at sufficient cardiovascular risk to warrant aggressive therapy. Determining whether CRP has a direct pathologic role in the vascular wall itself may have the most clinical relevance (Zimmerman et al., 2003). Measurements of plasma or serum C-reactive protein can help

differentiate inflammatory from noninflammatory conditions and are useful in managing the patient's disease, since the concentration often reflects the response to and need for therapeutic intervention. In some diseases, such as rheumatoid arthritis, serial measurements of C-reactive protein are of prognostic value (Gabay and Kushner, 1999). Lau et al., (2006) reported that levels of CRP were associated with HIV disease progression independent of CD4 lymphocyte counts and HIV RNA levels. Increased serum CRP level has been described to be associated with unstable angina or myocardial infarction, stroke, infection with *C. pneumoniae*, cytomegalovirus, hepatitis A, Herpes simplex, rubella virus, *Helicobacter pylori*, and *E.coli* lipopolysaccharide (Munford 2000), post-operative infection (Mustard et al., 1987), and heterotopic ossification (Sell and Schleh, 1999).

Major CRP response	
Infections	Bacterial
	Systemic/Severe fungal, mycobacterial, viral
Allergic complications of infection	Rheumatic fever
	Erythema nodosum
	Rheumatoid arthritis
	Juvenile chronic arthritis
	Ankylosing spondylitis
	Systemic vasculitis
	Acute sinusitis
	Acute otitis media
	Polymyalgia rheumatica
	Systemic vasculitis
	Behcet's syndrome
	Reiter's disease
	Psoriatic arthritis
	Crohn's disease
	Familial Mediterranean fever
Necrosis	Myocardial infarction
	Tumor embolization
	Acute pancreatitis
Trauma	Surgery, Burns, Fractions
Malignancy	Lymphoma, Carcinoma, Sarcoma
Modest or absent CRP response	Systemic lupus erythematosus
	Scleroderma
	Dermatomyositis
	Ulcerative colitis
	Leukemia
	Graft-versus-host disease

Modified from Dixon (1983) and Pepys & Hirschfield (2003).

Table 5. CRP response in human diseases

Larsson et al., (1992) performed a prospective study focused on CRP levels in 193 patients undergoing 4 types of uncomplicated elective orthopedic procedures and concluded that a

normalized CRP response that follows a typical biphasic response indicate an uneventful recovery. Meyer et al. (1995), proposed using CRP as a simple, reliable, and inexpensive screening test in detection of early infections after lumbar microdiscectomy. CRP levels are increased in patients with rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis and psoriatic arthritis, giant cell arthritis and rheumatic polymyalgia (Rosa Neto and Carvalho, 2009). CRP can be used as a tool to monitor the effect of antibiotic therapy (Peltola et al., 1984).

There is a wealth of literature supporting the use of CRP in the diagnosis and monitoring of treatment of infection in post-operative patients but it should be noted that single CRP reading has very limited value, and that a trend should be followed to have the full usefulness of CRP measurement. The exquisite responsiveness of CRP to acute phase stimuli, along with its ease of measurement, have led to CRP levels being used to monitor accurately the severity of inflammation and the efficacy of disease management during an infection. Covering all related studies is out of the scope of this chapter and readers are referred to review articles for more detailed information (Steel and Whitehead, 1994). Table 5 summarizes CRP response in different human diseases.

In humans, SAA is catabolized in the liver and has a half-life of 1 day but the capacity of the liver to degrade SAA decreases in acute or chronic inflammation (Ceron et al., 2005). SAA is a major APP in humans and has been reported to be as part of the host innate immune defense mechanisms against HCV infection. SAA was also shown to be an opsonin for gram-negative bacteria (Cai et al., 2007). The highest concentrations of SAA are generally found in connection with bacterial infections, but SAA has also proven useful as a marker in many other clinically important conditions in human medicine, including viral infections, rheumatic disorders, and neoplastic disease (Hulten et al., 1999). SAA has been found valuable in monitoring viral infections such as influenza and rhinovirus in human medicine (Petersen et al., 2004). However, due to difficulties in standardization and a need of more complicated and time-consuming assays compared with CRP, SAA has not yet gained full acceptance as an inflammatory marker in clinical practice (Hulten et al., 1999).

SAA and SAP have been implicated in a number of clinical conditions like secondary or reactive amyloidosis that is the occasional consequence of a variety of chronic and recurrent inflammatory diseases, for example leprosy, tuberculosis, systemic lupus erythematosis and rheumatoid arthritis (Steel et al.,). Pepys et al., (2004) have developed a drug that is competitive inhibitor of SAP binding to amyloid fibrils. The mechanism may provide a new therapeutic approach to both systemic amyloidosis and associated diseases, including Alzheimer's diseases and type 2 diabetes. The association of SAA with HDL3 suggests another area in which chronically high SAA concentrations may promote clinical disease. The sustained decrease in total HDL during the acute phase, could be a major risk factor for the development of atherosclerosis in patients with chronic recurrent inflammation (Steel and Whitehead, 1994).

There is an extensive body of reports concerning SAA responses in naturally occurring infectious and noninfectious inflammatory conditions, especially in humans, that has been reviewed by Malle and De Beer (1996).

Some investigators have advocated caution when using SAA in clinical cases in human medicine, stating that the highly sensitive and unspecific nature of the SAA response could lead to misjudgment of the severity of clinical conditions (Whicher et al., 1985). Zhao et al., (2009) in a meta-analysis and systematic review found that SAA levels are positively associated with BMI levels and that weight loss led to decreased SAA levels. Many studies

had been conducted on the relationship between SAA and deposition of reactive (AA) amyloid in patients with chronic arthritis, tuberculosis or Familial Mediterranean Fever (Gruys et al., 2005).

One of the most interesting features of AGP is that, not only is its gene expression up-regulated during an acute phase reaction, but the activity of the protein can be further fine-tuned by qualitatively controlling its post-translational processing by modifying its glycan microheterogeneity and, perhaps, also its phosphorylation status (Ceciliani and Pocacqua, 2007).

An elevated plasma haptoglobin level is seen following inflammation, trauma, and burns and with tumors. The plasma level increases 4 to 6 days after the beginning of inflammation and returns to normal 2 weeks after elimination of the causative agent. The plasma haptoglobin level in the initial phase of acute myocardial infarction is high, but later, owing to hemolysis, the plasma level decreases temporarily. The plasma haptoglobin level is decreased in hemolysis, malnutrition, ineffective erythropoiesis, hepatocellular disorders, late pregnancy, and newborn infants. In addition, the plasma haptoglobin level is lower in people with positive skin tests for pollens, high levels of IgE and specific IgE for pollens and house dust mites, rhinitis, and allergic asthma (Sadrzedeh and Bozorgmehr, 2004). The possible association of allelic polymorphism of haptoglobin with various pathologic conditions such as coronary artery disease, hematologic disorders, infectious diseases and other disorders has been studied in detail by Sadrzadeh and Bozorgmehr (2004).

5.2 Acute phase proteins in canine medicine

CRP is a major APP in dogs and its serum concentration can increase rapidly from <1mg/L to >100 mg/L in a number of conditions including surgical trauma, rheumatoid arthritis, polyarthritis, intestinal obstruction, inflammatory bowel disease, lymphoma, acute pancreatitis, pyometra, pneumonia, bacterial enteritis, turpentine oil injection, *E.coli* endotoxemia, babesiosis, leishmaniosis, leptospirosis, parvovirus infection, trypanosomiasis, *Bordetella bronchiseptica* and *B.canis* and *Ehrlichia canis* infection, bacterial and hemorrhagic enteritis and tumors (Ceron et al., 2005; Jergens et al., 2003 and Tecles et al., 2005, Yamamoto, et al., 1993). CRP levels increase from mid-gestation in pregnant bitches, coinciding with embryonic implantation (Eckersall et al., 1993; Vannucchi et al., 2002). Burton et al., (1991) showed a significant, but weak, correlation between CRP concentration and band neutrophils count and concluded that CRP could be more indicative of the extent of the inflammatory lesion. There is no circadian rhythm in CRP concentration in dogs (Otabe et al., 1998).

In a review of more than 900 cases of inflammation in dogs with various diseases, CRP concentrations were significantly correlated with disease, whereas only slight or no correlation was found with total WBC and band neutrophil counts (Ohno et al., 2008).

Hp is a moderate APP in dogs and is particularly sensitive to the effects of corticosteroids. This fact should be considered when Hp is used for monitoring inflammation as steroid treatment or hyperadrenocorticism interfere with test result. In canine hyperadrenocorticism a moderate increase occurs in serum Hp levels, probably due to endogenous glucocorticoid-mediated stimulation of Hp production (Ceron et al., 2005). Serum Hp levels have been shown to increase in dogs with leishmaniosis, trypanosomiasis, diabetes mellitus and diabetic ketoacidosis, and Cushing's syndrome (Ceron et al., 2005).

The reference intervals proposed for serum canine Hp are 0-3 and 0.3-1.8 g/L (Ceron et al., 2005).

SAA, a major APP in dogs, increases in the serum samples of dogs with leishmaniosis, parvovirus and *Bordetella bronchiseptica* infection. Serum and CSF concentrations of SAA are increased in dogs with steroid-responsive meningitis-arteritis (SRMA). In response to prednisolone therapy serum CRP and SAA concentrations fall within reference intervals; while in patients that relapse with SRMA, the concentrations increase. The use of APPs as biomarkers of remission and relapse of SRMA has proved less expensive and invasive than previous monitoring methods (Eckersall and Bell, 2010). Reference intervals for SAA concentrations in canine serum have been reported as nondetectable to 2.19 mg/L in one study and 1.15 ± 2.53 mg/L in another one (Ceron et al., 2005).

AGP, a moderate APP in dogs, in a health screen was shown to be useful for identifying dogs with subclinical disease that, after 2 weeks, had clinical signs or even died of diseases such as parvovirus infection (Ceron et al., 2005). AGP has been reported to increase in diseases such as babesiosis, lymphoma, carcinoma, sarcoma, parvovirus and *Ehrlichia canis* infection (Ceron et al., 2005). Clinically healthy Yorkshire Terriers and Dachshunds have lower levels of AGP compared with Poodle, Cocker Spaniel, Labrador Retriever, or German Shepherd. This finding could explain the wide range of AGP values (40-1070 mg/L) in healthy dogs (Ceron et al., 2005). Different authors have been proposed different reference intervals for serum AGP concentrations in dogs 322 ± 202 µg/ml, 509 ± 117 µg/ml, 302 ± 74 µg/ml, <380 µg/ml, 480 ± 149 µg/ml (Ceron et al., 2005). Phenobarbital, in a therapeutic dosage regime, induces a significant increase in canine AGP concentration, which should be considered during the interpretation of AGP results. Dogs with hepatitis or with turpentine treatment have raised serum AGP concentrations (Murata et al., 2004).

5.3 Acute phase proteins in feline medicine

Contrary to reports in other species, data regarding APP levels in cats are scarce and mostly focused on general aspects of feline APP biology, such as the demonstration of the lack of age-related changes in APPs concentrations in feline serum, basic and comparative information about APP gene and protein structures or methodological aspects of measurement of feline APPs (Paltrinieri, 2008). Most feline APP studies have been focused on AGP, SAA and Hp, the three major APPs in cats. AGP and Hp are considered as moderate APPs in cat by some authors. The peak time and magnitude of an APP response vary depending on the type of stimulus. Kajikawa et al., (1999) studied the changes in concentrations of SAA, AGP, Hp and CRP in feline sera following injection of lipopolysachharide or turpentine oil and showed a 4.2-, 5.7- and 2.9-fold increase in SAA, AGP and HP concentration, while CRP concentration didn't show any significant change. The time of detecting a significant increase in concentrations of SAA, AGP, and Hp was 8, 24 and 24 hours, respectively. Sasaki et al., (2003) found increases of >10-fold in AGP concentrations in cats with FIP and concluded that SAA and AGP could be considered as major APPs in the cat. Quantification of AGP has been demonstrated to be a reliable aid in the diagnosis of FIP, although not pathognomonic for the disease because high levels are also found in cats with feline immunodeficiency virus (Duthie, et al., 1997). Increases in other APPs (Hp and SAA) have been detected in FIP-infected cats. Increases in Hp concentration have been described in cats with abscesses and upper respiratory tract infections; while Hp concentration decreased after hemolysis caused by hemobartonellosis (Ceron et al., 2005). Concentrations of SAA, AGP, and Hp increased significantly 1 day after surgery in cats (Ceron et al., 2005).

Some feline pathological or pathophysiological conditions in which increases in APPs have been reported are as follows:

Anemia of inflammatory diseases (localised purulent infections), diabetes, experimental inflammation, feline coronavirus (FCoV) infection (non-symptomatic), feline calicivirus infection, chlamydiosis, feline leukaemia virus, feline infectious peritonitis, feline immunodeficiency virus (FIV), hospitalization, infectious diseases (miscellaneous), injury, lymphoma , amyloidosis in Somali, Oriental and Abyssinian cats, renal failure, splenectomy, surgery, and tumors (Paltrinieri, 2008).

The results of the studies on feline APPs indicate that Hp, SAA and, especially, AGP should be included in laboratory panels to diagnose inflammation in cats.

5.4 Acute phase proteins in ruminant medicine

Hp is a major APP in ruminants. Its serum level is negligible in healthy animals, but increases over 100-fold on immune stimulation (Conner et al., 1989).

Bovine Hp was first documented by Bremner (1964) who reported that plasma samples from healthy calves contained very little Hp, and that local inflammation induced by injection of turpentine elevated Hp concentrations greatly. In healthy cattle the serum concentration is below 20 mg/L but it can increase in concentration to over 2 g/L within a couple of days of infection (Eckersall and Bell, 2010). Many studies have indicated the significance of Hp as an clinically effective marker for evaluating the presence, severity and recovery of cattle with trauma, inflammation, experimental and natural inflammatory diseases, foot and mouth disease, fatty liver (hepatic lipidosis), mastitis, pneumonia, enteritis, peritonitis, endocarditis, abscesses, endometritis, clinical respiratory tract disease, bacterial contamination of uterus and delayed uterine involution, infections with *Mannheimia haemolytica, Pastuerella multocida,* bovine viral diarrhea (BVD) virus, bovine herpes virus 1 and bovine respiratory syncytial virus (Murata et al., 2004, Petersen et al., 2004). Hp is also a useful marker for monitoring processes such as tail docking and surgical castration. Elevations have also been reported in cows at parturition, during starvation and following the stress of road transport (Eckersall, 2006). Alsemgeest et al. (1994) found a significant difference (P<0.001) in Hp levels between healthy animals and animals with inflammatory diseases. Godson et al., (1996) and Young et al. (1996) found Hp to be a valuable diagnostic aid in bovine respiratory disease (BRD), and Wittum et al. (1996) suggested Hp to indicate response of respiratory tract disease to antimicrobial therapy (In: Hirvonen, 2000). Hp is used to monitor the treatment efficacy of antibiotics in cows with toxic puerperal mastitis. Hp is also used to determine the effect of anti-inflammatory drugs following the castration of bull calves, the effects of treatment in transport-stressed feedlot cattle, and the changes in the blood profile of neonatal calves (Murata et al., 2004). Hp can be used to investigate the relationship between uterine involution and the presence of intrauterine bacteria in ewes; however, Hp levels remained unchanged following castration or tail-docking in lambs (Murata et al., 2004).

Scott et al. (1992) reported serum Hp to have prognostic value in ovine dystocia cases, where serum Hp concentration of above 1.0 g/L indicated a reduced survival rate (Hirvonen, 2000).

In cattle SAA has been identified as a marker of inflammation being elevated more in acute rather than chronic conditions. It was raised also by experimental infection with *Mannheima haemolytica*, with bovine respiratory syncytial virus and in experimental and natural cases of mastitis ((Eckersall, 2006). SAA is a valuable APP in diagnosing cattle with inflammation

(Murata et al., 2004). Elevated SAA levels are also found in conditions unrelated to inflammation, in cows at parturition or in cattle subjected to physical stress (Murata et al., 2004).

When screening dairy cow herds, SAA was found to reflect systemic inflammatory disease. A good agreement (i.e. high specificity) between ruling out inflammation by a clinical examination performed by field veterinarians and SAA negative serum samples was observed (Höfner, 1994).

5.4.1 Bovine APPs: detection of mastitis and subclinical mastitis

The most important and common disease among dairy cows is mastitis and is still a big challenge for the dairy industry all over the world. There are two forms of mastitis: a) clinical, which is often easy to detect and b) subclinical mastitis, which shows no visible changes in the udder or in the milk and should be detected through different diagnostic tests including somatic cell count (SCC), California mastitis test, electroconductivity, measurement of lactose, LDH, alkaline phosphatase, plasminogen, NAGase, pH, lactate, alpha-1-antitrypsin, etc in milk.The most common way to detect subclinical mastitis is by measuring the SCC. Since SCC is influenced by other factors than mastitis, including lactation number, stress, stage of lactation, etc., there is a need for new biomarkers for detection of subclinical mastitis (Akerstedt, 2008). A lot of research has been done to find the biomarker with high sensitivity and specificity for diagnosis of subclinical mastitis. Recently there has been an increased interest in the potential of APP in milk (Eckersall et al., 2001; Grönlund at al., 2003; Petersen et al., 2004; Pyörala, 2003; Jacobsen et al., 2005; Eckersall et al., 2006; O'Mahony et al., 2006; Hiss et al., 2007; Kováč et al., 2007, Safi et al., 2009). Hp serum concentrations increased in experimentally induced mastitis and in field infections of different etiology (Petersen et al., 2004). Serum amyloid A has also been found to be a marker of experimentally induced and naturally occurring mastitis (Petersen et al., 2004, Eckersall and Bell, 2010). It has been shown that SAA is also produced by the mammary gland epithelial cells and a mammary-associated isoform of SAA (SAA3) has been identified in milk (Akerstedt, 2008).

The clinical accuracies of different tests such as CMT, SCC, SAA, serum Hp and Hp and SAA in milk for detection of subclinical mastitis have been studied and SAA in milk was shown to be the most accurate test for the diagnosis of subclinical mastitis with area under curve (AUC) of 0.998 (Safi et al., 2009). Most of the studies have suggested SAA and Hp as potential biomarkers for milk quality; so there is considerable potential for the use of a biological marker, such as SAA and Hp, which is present in milk and can be measured routinely, rapidly and reliably, for the objective and early diagnosis of mastitis. Such a marker could be particularly important for the continued development of robotic milking systems in which the manual examination of milk and cows is not practicable; it might also provide a more accurate and earlier diagnosis of intramammary infection, reducing the time to treatment, and thus possibly reducing the adverse effects of mastitis in both economic and welfare terms (Eckersall et al., 2001).

5.5 Acute phase proteins in equine medicine

Equine SAA is an acute phase apolipoprotein that increases over 100 fold after tissue injury, infection, or inflammation. Hepatocytes are the main source of SAA synthesis, but

extrahepatic production of several isoforms of SAA (specifically SAA3, has been demonstrated in the mammary gland and synovial fluid of horses (Jacobsen et al., 2006, Mcdonald et al., 2001).

SAA has been shown to be a sensitive marker of inflammation and its increased concentrations have been reported in foals with various bacterial infections, septicemia, localized infections and arthritis (Crisman et al., 2008). It is generally agreed that SAA determinations proved superior when compared with classical markers of inflammation (Fb and leukocyte counts) in distinguishing infectious from noninfectious causes of systemic inflammatory response syndrome (SIRS) (Crisman et al., 2008). Increased levels of SAA has been shown in surgical trauma, experimental aseptic arthritis, equine herpes virus serotype 1 and *Streptococcus equi*, equine influenza virus serotype A2, infectious arthritis, tenovaginitis, experimental infection with equine herpes virus, various infections in foals and experimentally induced arthritis (Petersen et al., 2004), *Rhodococcus equi* pneumonia and colic. Jacobsen and Andersen in their excellent paper (2007) reviewed different conditions that result in increased equine SAA.

The reference range of SAA in clinically healthy adult horses reported as <7 mg/L in one study (Hulten et al., 1999) and <0.5 to 20 mg/L in other ones (Crisman et al., 2008). Due to the short half-life of SAA, sequential SAA measurements could be potentially useful in patient management and prognostication.

Hp, a moderate APP in horses, increases during infection, stress, trauma, allergy, surgery, noninfectious arthritis, rhabdomyolysis, traumatic incidents, colic, enteritis, grass sickness, castration, inhalation of equine influenza virus, experimental local aseptic inflammation and carbohydrate-induced laminitis (Petersen et al., 2004) while a substantial decline in serum concentration of free Hp is seen during intra- or extravascular hemolysis. The reference interval of serum Hp is 2-10 g/L (Crisman et al., 2008).

Little work has been done on AGP in horses. Increased AGP levels has been reported in colts 2 to 3 days after castration, in adult horses after jejunostomy and in ponies fed with high carbohydrate diets result in laminitis (Crisman et al., 2008).

CRP has been reported to increase in horses with pneumonia, enteritis, and arthritis, carbohydrate induced laminitis and also following intramuscular injection of turpentine (Petersen et al., 2004).

Fibrinogen has a wide reference interval in healthy horses (2-4 g/L) and is a slow reacting APP, therefore Fb is considered a fairly insensitive APP.

A limited and late response to stimuli with peak values 2-4 times normal concentration 4-6 days after stimuli and a slowly declining concentration during recovery are the drawbacks of Fb in horses (Campbell et al., 1981).

Measurement of major APPs in horses can provide an objective determinant of the heath, estimate the severity of underlying conditions and allow monitoring of the chosen therapy.

5.6 APP in the meat industry

One potential indication for the use of APP is to improve the quality of the meat inspection process. According to Saini & Webert (1991), incorporation of APP tests to *ante mortem* and *post mortem* inspection process allows screening of all animals to identify those with disease activity, confirm presence of disease in suspect animals at *ante mortem* inspection, and confirm the presence of a systemic illness at *post mortem* inspection. For these purposes, the non-specific nature of the APR is a major advantage (Hirvonen 2000). With their low

specificity for a particular disease, the APP would not be used as a front line test for specific infection. However, as a screening tool for monitoring the general health and welfare of animals at slaughter the APP could be valuable and have potential advantages in being able to be adapted to assays of the required accuracy, precision and robustness (Eckersall, 2006). Public health is another concern affecting the introduction of APP tests to the meat industry. Control of pathogens that are able to create food-borne epidemics, like *Salmonella*, *Listeria*, *E. coli*, *Toxoplasma*, and *Campylobacter* are of specific interest. Furthermore, traditional meat inspection methods are not effective in detecting some other diseases, like tuberculosis or cysticercosis. On-line APP tests would improve the sensitivity of traditional meat inspection protocols and prevent the contamination of meat processing plants (Saini et al., 1991).

Six-fold increases were found in Hp concentration comparing dairy cows with infectious, metabolic or traumatic disease at slaughter to those with only minor lesions. Another study showed a 40-fold increase in Hp and a 7-fold increase in SAA concentration between healthy beef cattle and dairy cattle culled with acute pathological lesions (Tourlomousis, et al., 2004). In calves to be slaughtered, haptoglobin concentration in serum indicates gross lesions at post mortem inspection (Young et al., 1996). In another study on emergency slaughtered dairy cows, a higher Hp concentration was observed (Hirvonen et al., 1997).

To detect cows with severe pathologic conditions, Tourlomousis et al. (2004) proposed 107 mg/L and 0.18 g/L, as the cut off points for SAA and Hp, respectively. Safi et al., (unpublished data) studied serum SAA and Hp in healthy cows (n=30) and cows with different pathological conditions (n=50) and proposed 60 mg/L and 0.96 g/L, as the cut off points for SAA and Hp, respectively. Such results demonstrate a significant potential of APPs to help ante-mortem differentiation of "suspect" and "non-suspect" animals, which could enable use of a simplified postmortem inspection for the latter group.

5.7 Acute phase index

Because APPs vary in their response to inflammation and tissue damage, group analysis of APPs (serum APP profile) may be more meaningful than measuring a single protein (Eckersall, 1995). Use of APP profiles involving at least 1 major (CRP or SAA), 1 moderate (Hp, AGP, or Cp), and 1 negative APP are likely to become more widely used instead of the determination of individual APPs. Such an approach may improve the sensitivity of individual assays, as has been shown for canine leishmaniosis (Ceron et al., 2005).

Calculation of an index from values of rapid- and slow-reacting positive and negative APPs has been repeatedly mentioned (Gruys and Toussaint, 2001; Gruys, 2002; Tousaint et al., 1995, 2002, 2004; Niewold et al., 2003), because it appeared to increase statistical sensitivity and specificity for detecting non-healthy subjects (Gruys et al., 2005). Use of an acute phase index, by combining the results of both positive and negative APP has been suggested for differentiation of different pathogens that cause subclinical mastitis (Safi et al., unpublished data).

6. Conclusion

Early detection of systemic inflammation is essential to devise and implement an effective treatment plan. Early diagnosis of subclinical diseases that subsequently impair health and performance is of great importance in both humans and animals. So the search for early

markers of inflammation has been expanded in human and veterinary medicine over the past several decades.

For production animals, monitoring APPs on a herd basis by including it in schemes for metabolic and nutritional profiling would be logical, not only for the identification of individual animals with disease, but also as a means to categorize the level of any sub-clinical disease present. Conditions identified by APP investigation have been related to the hygiene level on farms and so have a direct bearing on animal welfare as well as health (Eckersall, 2004).

APP may also serve in the follow-up of medical treatment, where sequential APP determinations would provide accurate information of the course of the disease. Early detection of subclinical diseases and, patient management and the monitoring of treatment would be more successful using APP analysis.

The harmonization of assay calibration between laboratories around the world in order to produce comparable results has been started (Skinner et al., 2000) and has caused a major practical advance for the future prospects in the application of APP assays.

Understanding better the basic pathophysiologic mechanisms by which APR is induced would help us to develop novel drugs that specifically block the proinflammatory effects of APPs.

New discoveries on technological possibilities for rapid chemical multianalyses have a key role to widespread use of APPs in human and veterinary medicine as well as in animal production. The current advances which have taken place in proteomics could also identify low abundance APPs, not recognized up to now as the APPs currently measured have plasma concentrations in the mg/L to g/L range. If proteomic methods are used to examine serum protein in the ng/L or pg/L range then a host of new APPs may well be revealed (Eckersall, 2004). This could be a new era in APP research.

At the present time there is a wealth of literature supporting the potential of APPs in the regular health monitoring of humans and animals.

David Eckersall is right. "The time is right for acute phase protein assays" (Eckersall, 2004).

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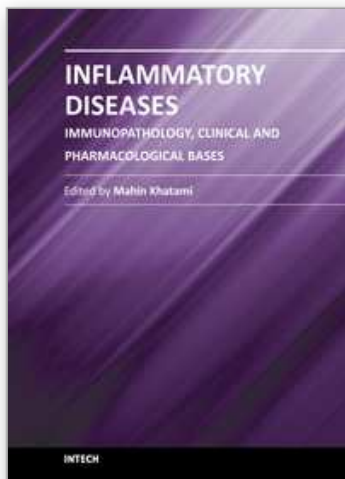
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