ACUTE-PHASE PROTEINS IN IMMUNE RESPONSE

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Summary

Innate (natural/nonspecific) processes of defense are accomplished with participation of some barriers and cellular and molecular effectors which have extremely diversified functions. Among the effectors, molecules have an important place because of their direct actions and participation in almost all cellular and barrier-dependent mechanisms of innate immunity.

The pathways involved and their material components (i.e. cells, molecules) are common for defense response against all or almost all pathogens, thereby being nonspecific. Also, unlike specific mechanisms, the nonspecific ones are deprived of immunological memory. Innate immunity is common to all animals, vertebrates and nonvertebrates, and adaptive immunity is a characteristic only for vertebrates, starting with fishes.

Innate defense molecules are synthesized by numerous populations and types of cells, according to many criteria appertain to multiple categories (groups), and have diversified structures. In this review study are presented the most important acute-phase proteins and their role in various species defense natural mechanisms.

Key words: acute-phase proteins, immune response

Acute-phase proteins (APPs) are serum molecules which are synthesized by many cells categories, especially by hepatocytes. Usually, the structure of APPs and acute-phase responses are similar in all species, having universal character in animal kingdom.

Biochemical structure of APPs is distinct, many of them being glycoproteins. Glycosylation are higher in molecules synthesized during the acute-phase responses. Exceptions are C-reactive protein (CRP) and serum amyloid A (SAA), important APPs which are not glycosylate. SAA has a particular structure (apolipoprotein), being characterized by high polymorphism. SAA is synthesized by other cells (non-hepatic types).

Synthesis of APPs and changes of their composition are fast processes that intervene in the stage named “acute phase reaction” or “acute phase response”, present in initial period and during the evolution of pathologic phenomena (e.g. inflammations, trauma, immunopathies, and bacterial or neoplastic disease). Acute phase response is an animal reaction to homeostasis changes during the diseases. Recently, it was described some association between APPs synthesis and atherosclerosis. Initially it was believed that some dysfunctions in APPs synthesis are specific to acute disease, but the anomalies were observed also in chronic disease, especially in active ones. In spite of these
observations, the term of “acute-phase proteins” was conserved, referable to an ample group of molecules with non-homogeneous structural and functional features, with differences between species and diseases, and with one common characteristic – changes in intensity of their synthesis during various pathological phenomena, irrespectively of their evolution type.

Increases or decreases in APPs serum concentration are observed after 2-4-6 days of disease. Increases of concentration predominate, with wide range - one-fold to two-fold in some complement proteins, five-fold to ten-fold in coagulation factors and protease inhibitors, hundred-fold to thousand-fold in CRP and SAA. The maximal level could be seen after 8-10 days and it is maintained during all evolution of disease. Also, there are some proteins that decrease the concentration in serum, which are named “negative acute-phase proteins”.

The level of some molecules (CRP, SAA) increases and decreases rapidly, spontaneously or after treatment, having a 24-30 hours halftime. Concentrations of other APPs increase slowly and achieve normal values equally slowly.

Anomalies in APPs synthesis are not characteristics for all mentioned diseases. Thereby, in human was characterized severe disease with no significant changes in concentration of APPs (e.g. systemic erythematous lupus, dermatomyositis, ulcerative colitis, and leukemia).

Simple measurement of the serum APPs is not enough to evaluate their involvement in diseases. APPs concentration is a resultant of equilibrium between synthesis and secretion rate on one hand, and elimination rate on the other hand (i.e. ration between production and catabolism). Additional to measuring serum APPs, the crucial element for defining functions and effects of APPs is their availability. This can be increase because of increased synthesis, but intense synthesis can be dissembled by tisular consumption or by the massive and fast elimination, therefore the APPs serum concentrations have normal values. Diseases have a low influence on elimination and catabolism of CRP and SAA. Therefore, CRP and SAA levels are determined mostly or exclusive by the synthesis rate, contributing to their special value in diagnostic and prognosis.

The majority of APPs quantitative changes are resultant of hepatocytes dysfunctions correlated with plasma proteins synthesis. There are mostly changes of APPs genes transcription induced by cytokines (interleukins, interferons, tumor necrosis factors). Thereby, IL-6 regulates fibrinogen, serum amyloid A and P and complement component C3 synthesis. Also, IL-1 and TNF-α regulate SAA synthesis. It was observed that synthesis of APPs induced by IL-6 is inhibited by IL-1 and synthesis of some APPs is decreased by IL-4 action. Also, nervous and endocrine system has some influence in APPs synthesis by effects of glucocorticoids and steroids hormones. Adrenocorticotropic hormone (ACTH) increases level of rabbit fibrinogen and fibrinonecutin, but not the level of calf same proteins and SAA. Neuropeptides influence acute phase responses (e.g. α-melanocites stimulating hormone inhibit fewer, inflammations and cytokines activity).
Some of APPs are fetal proteins and are not expressed in significant quantities in adults (e.g. human and rat fetal FAPA molecules - acute phase agent - and serum α1-glycoprotein in cattle and swine).

**APPs role in natural defense mechanisms**

According to Pepys et al. (25, 26), APPs activities belong to natural defense mechanisms networks, having synthesis and concentration adaptive regulated. The most important results of APPs activities are:

- practical effects - homeostasis rehabilitation and bacterial development inhibition; APPs generate responses with participation of tisular elements and vascular system: decreasing damages intensity, amplifying resistance, opsonization, decreasing inflammation, recovery of morphopathological changes and tisular protection (by anti-proteases); these responses are correlated with cytokines and other molecular mediators synthesis; also, these mediators activate surface cells receptors, resulting systemic responses;

- local or systemic responses against biotic and abiotic pathogens manifested by fewer and reactions of immune, hematopoietic and endocrine systems (related to stress);

- dysfunctions in proteins, lipids and carbohydrates metabolism.

All these data suggest that APPs (CRP, fibrinogen, SAA, complement components etc.) are important constituent of pathologic mechanisms, having favorable or harmful effects. It is accepted that APPs have influences that can be enframe mainly in protective mechanisms of the organism.

Systemic acute phase reaction are characterized especially by fewer, anorexia, negative natrium balance, decreased plasma lipoproteins concentration, increased ACTH and glucocorticoid hormones secretion, complement and coagulation mechanisms activation, decreased calcium, zinc, iron and A vitamin levels, and changes in some plasma proteins concentration (including APPs concentration). APPs positive RNAm synthesis is correlated with a significant decrease in synthesis of normal serum proteins (e.g. albumin and prealbumin which are negative APPs).

Principal actions of positive APPs are hemoglobin, free radicals and aggregate cells elimination, binding of some bacterial components, and amplification of immunoglobulins synthesis.

In the past few years were developed more specific and sensitive methods for measuring APPs (e.g. immunoenzymatic tests for CRP). Extensive application of these techniques, correlate with clinical and epidemiological observations, revealed the importance of apparent benign chronical disease in establishment of general morbidity indexes in human, especially in developed countries.

APPs detection and measuring techniques are applied also in domestic animals (13) for diagnosis in some diseases – neoplastic diseases, immunopathies, and glomerulonephritis – especially in dogs and horses. Measuring of some APPs
Acute-phase proteins classification

Assortment of APPs based especially on functional criteria, proposed by Pepys et al. (24), comprise:

a. Plasma proteins with increased concentration during the acute phase response (positive acute-phase proteins):
   - Protease inhibitors: α1-antitrypsin, α1-antichymotrypsin;
   - Coagulation proteins: fibrinogen, prothrombin, factor VIII, plasminogen;
   - Complement proteins: C1s, C2, B, C3, C4, C5, C6, C9, C1, and INH;
   - Transport proteins: haptoglobin, hemopexin;
   - Other proteins: CRP, SAA, α1-acid glycoprotein, Gc-globulin, ceruloplasmin.

There are some classifications of APPs based on amplification of synthesis rate during acute phase responses. Thereby, additionally to previous data about APPs production rate, Gruys et al. (13) admitted the existence of three APP groups, differentiated based on synthesis intensification and other parameters:
   - Approximately half-fold increase: ceruloplasmin, complement component C3;
   - Increase from a two-fold to three-fold: haptoglobulins, fibrinogen, α-globulins with protease activity; these are detected in 8 hours after stimulation of generative cells;
   - Rapidly increase to a thousand-fold: CRP, SAA; these are detected 4 hours after the stimuli and attain a maximum level at least 24 hours.

b. Plasma proteins with decreased concentration during the acute phase response (negative acute-phase proteins):
   - Protease inhibitors: inter α1-antitrypsin;
   - Complement proteins: properdin;
   - Lipoproteins: lipoproteins with high density, lipoproteins with low density;
   - Other proteins: albumin, prealbumin, transferrin.

Felsburn et al. (9) categorized principals dogs APPs in only two categories, according to importance of their participation in innate immunological mechanisms:
   - Major APPs: CRP and SAA;
   - Other APPs: α1-acid glycoprotein, haptoglobin, β2-macroglobulin, ceruloplasmin, fibrinogen, hemopexin, α1-proteinase inhibitor, α1-antitrypsin, α1-antichymotrypsin, cystein protease inhibitor, fetuin and serum mucoid.

All of these APPs are considered systemic and tardily mediators, unlike other cytokines with APPs signification which appear early and have local action (IL-1, IL-6, and TNF-α). Additionally to these molecules, some of the cytokines and complement can be added because a part of their functions are APPs-like.

All mentioned classifications include plasma proteins with important physiological activities. Their changes, registered in various diseases, furnish...
significant clues about ethiopathogenesis and elements for establishing diagnosis and prognosis.

The main acute-phase proteins

C-reactive protein (CRP) is the most important APP. CRP is a α-globulin characteristic to vertebrates, including teleosteen fishes, that was discovered by Tillet and Francis in 1930 in serum of the patients with acute pneumonia produced by Streptococcus pneumoniae (23). CRP is the first protein described in relation with an acute pathological process with resultant of the entire group name. The term “C-reactive protein” derived from capacity to react with pneumococcal protein C (polyside somatic antigen) through phosphorylcholine. Binding of CRP to the bacteria, fungi and parasites membranes are dependent to Ca²⁺, being associated with the classical activation pathway of the complement, resulting C3b formation. After interaction with various ligands, CRP exerts other functions, similar to antibodies like agglutination but without specificity. CRP reacts also like opsonin. It amplified chemotaxis mechanisms and macrophages and neutrophils phagocytosis. CRP binds damaged cells nuclear chromatin, participating in degradation of their nuclear components (9). CRP also binds some endogenous compounds, like membrane phospholipids.

Similar to other APPs, CRP is synthesized by hepatocytes, synthesis amplified in acute infections, inflammations, neoplastic and autoimmune diseases, and diseases with immune complexes. In these pathological conditions, CRP serum concentration increases rapidly, being obvious in a few hours after infection.

Conclusively, CRP has nonspecific functions, independent to immune system, in early response and defense mechanisms against infectious diseases and other pathological conditions.

Coglutinin (CGT) is an ample plasma molecule which binds C3b of the complement in multiple combination sites; thereafter, CGT contributes in agglomeration (coagglutination) of C3B covered particles, followed by their phagocytosis and elimination.

Serum amyloid A (SAA) has various functions, including decrease of IL-1 and TNF-α induced fewer, inhibition of thrombocytes aggregation, and inhibition of oxidative reaction in neutrophils. Higher plasma concentration of SAA determined amyloidosis, disease characterized by deposition of this APP in various tissues, in fibrils that interfere with normal functions of the organs (e.g. myocardial contraction and glomerular filtration).

α₁-acid glycoprotein (α₁-AGP) suppresses lymphocytes blastogenesis response and antibodies synthesis.

β₂-macroglobulin (β₂-M) is a proteases inhibitor, neutralizing neutrophils and macrophages lysosomal hydrolases, similar to other anti-proteases.

Ceruloplasmin (CPL) eliminates neutrophils superoxides.
Fibrinogen (FNG) is implicated in coagulation and wounds healing. Higher concentration of FNG determined erythrocytes agglomeration, fast deposition, and increase of their sedimentation velocity.

Haptoglobin (HGB) inhibits bacterial development by binding iron, depriving bacteria of this element. In some species, authors describe other acute-phase proteins.

**Responses by acute-phase proteins in various species**

There are only few data about this subject. It was described defense natural mechanisms characterized by changes in APPs concentration partially similar in animals, and also some differences between species APPs molecules and their functions. Therewith, some authors described pathological condition in which intervened APPs like innate immunity factors (13).

**Cattle** – APPs synthesis is initiated and regulated by IL-1, IL-6 and TNF (11). According to Godson et al. (11), CRP does not have increases of serum concentration during the acute phase responses; therefore CRP is not an APP. CGT is the main APPs in ruminant.

The main plasma proteins whose concentrations increase during acute phase responses and their functions are:

- Haptoglobin – this APP binds free hemoglobin and composes polymers in association with albumin; it is an paraclinical marker for inflammatory processes severity; increases of haptoglobin concentration could be seen in hepatic lipodosis, defective nutrition, mastitis, experimental inflammation and in Dexamethasone treatment; presence of haptoglobin in serum is useful paraclinical sign in acute inflammations, acute infections, traumatic reticulitis (20);
- Fibrinogen - the fibrin precursor in coagulation process;
- Serum amyloid A - the fibrillar amyloid proteins precursor; it is associated with serum high density lipoproteins;
- Ceruloplasmin - binds copper ions, participates in oxygen radicals elimination;
- α₁-antitrypsin, α₁-antichemotrypsin and β₂-macroglobulin - all with proteases inhibitor functions;
- α₁-acid glycoprotein - with proteins transport function.

Acute phase response is attended by other blood changes (hypoalbuminemia, leucocytosis and decrease of zinc and iron concentrations), as well as by fewer.

McNair et al. (20) were used competitive immunoassay and an indirect test for hemoglobin fixation in purpose to measure bovine haptoglobin in different groups: normal and experimental infected with *Haemophilus somnus*, having various pathologic condition. Infected animals had higher HGB serum concentration, especially at immunoassay, correlated with lung lesions severity.

Hirvonen et al. (15) studied the response by some APPs (haptoglobin and α₁-acid glycoprotein) in dairy cow with *postpartum* acute metritis. In healthy
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animals, HGB serum concentration is less than 0.05 g/l, being higher (more than 0.7 g/l) in the individuals with severe clinical signs.

Using immunoassay, Funke et al. (10) observed fast increases of HGB serum concentration in animals with tissue damages associated with infection and inflammations. They consider that APPs measuring ensure information about organ diseases and microbial infections evolution, inclusive about treatment efficiency.

Responses through synthesis of some APPs (FNG and HGB) were studied by Hirvonen et al. (15) in dairy cows with abdominal diseases (traumatic reticuloenteritis, abomasal displacement, and other gastrointestinal diseases) surgically treated. It was established that measuring of serum FNG is helpful in assessment of peritonitis and other post surgery complications.

SAA serum concentration is increased in cows fed with endotoxin, during the pneumonia and also in association with parturition (1, 11, 16).

Sheep. There was described multiple APPs whose concentration are increased during endotoxin shock and various pulmonary diseases: HGB, CPL, and FNB. Synthesis of these APPs and their actions appertain to systemic and tardy period of acute phase response (14).

Haptoglobin is a significant paraclinical parameter in sheep dystocia prognosis. Measuring of HGB, like auxiliary diagnosis element, is useful in infections (28). Changes in HGB, CPL, and FNB concentration are correlated with decreases in production during diseases accompanied by inflammatory phenomena (27).

Goat. It was described significant increases of serum HGB in goat with dystocia unlike those with normal births and in the nonpregnant. Instead it was not observed changes in this APP concentration associated with helminthiasis (4).

Swine. There are some studies about response through multiple APPs in swine: α1-AGP, soluble acid glycoprotein (AGPs), CLP, HGB, and CRP. Authors induced sterile inflammations and these were associated with increases in APPs concentrations, especially in CRP, AGPs, and HGB. CRP and HGB is the most useful marker for inflammatory damages diagnosis in swine.

Bürger et al. (5) observed a close immunological relation between human and pig CRP. Using SDS-PAGE it was determined that pig CRP has 23.4 kDa. Authors observed an increase in CRP concentration after ACTH or corticosteroids administration, the level of this protein achieving normal value after treatment interruption. They consider that CRP is a recommended parameter in assessment of non-infectious and non-inflammatory stress.

HGB level is increase in animal with atrophic rhinitis (Eurell et al, 1990 – authors that purified pig HGB by affinity chromatography) and with Actinobacillus pleuropneumoniae infection.

Similar observations were published by Asai et al (3) for natural and experimental infection with porcine reproductive and respiratory syndrome virus. Level of serum HGB increased seven-fold to 21-fold, but α1-AGP concentration
was preserved. Intense synthesis of HGB was correlated with intense IL-6 production, level of TNF-α being unchanged.

**Horses.** Published data about this species revealed that APPs have significant changes in synthesis during some pathological condition. These changes can be observed in plasma FNG, with intense synthesis, and in CPL, with a decrease level in inflammations. The serum levels of some APPs increases in laminitis. In severe colic can be observed high concentrations of serum lipids, lipoproteins and TNF, all associated with high mortality (17).

*Coté et al.* (7) described the role of β_{2}-macroglobulin in joint inflammation processes, emphasizing correlation between this APP and TGF-1β (transforming cells grow factor). β_{2}-M had high concentration in septic and degenerative arthritis. In horses with colic induced by inflammatory conditions or intestinal strangulation, *Topper and Prasse* (29) observed significant responses through intense synthesis of some common APPs (FNG and CRP) and coagulation factors, and also synthesis of other molecule - α_{2}-antiplasmin, plasminogen, and C1-esterase inhibitor - which are considered APPs by the mentioned authors.

It is considered that HGB, CRP, ad SAA measuring is useful in diagnosis of horses inflammatory diseases.

**Dogs.** Normal values of CRP serum concentration in 1-2 years old Beagle were established by *Otabe et al.* (22) between 0.8 and 16.4 mg/ml (4.5-5.06 mg/ml average). There are no diurnal oscillations, but significant individual differences are present. Instead, CRP concentration was modified in some diseases. Relatively recent, dog’s CRP was isolated and described in comparison with human homologue. Immunoassay was used in measuring of CRP serum level. Measure of CRP, together with α1-AGP and HGB, was used in trypanosomiasis and ehrlichiosis monitoring. CRP concentration increases after the middle of gestation (being an auxiliary parameter in gestation diagnosis), and after parturition.

Using isoelectric focusing and immunotransfer techniques, *Andersson et al.* (2) observed one single type of HGB in dogs. This APP has a characteristic micro-heterogeneous structure in healthy animals. It was observed HGB structural changes in dogs with hepatic diseases, especially chronic hepatitis, and in various types of anemia. Some fractions have different values for carbohydrates content.

Surgical traumas are associated with significant changes in HGB, CPL and serum mucoid, but not in CRP.

Characterization of dog’s serum amyloid A structure revealed that this APP has 80% similarity with human and mouse SAA.

**Cat.** In feline infectious peritonitis it was observed that activity of serum and ascitic fluid IL-6 is high, being associated with intense synthesis of some APPs (12).

**Rat.** APPs are synthesized by hepatocytes, similar to other species. Decrease of serum transferrin concentration was described in inflammations. Typical acute phase responses are induced by systemic administration of LPS or local administration complete Freud’s adjuvant or turpentine. The same effects can
be obtained by cytokines inoculation, especially associated with glucocorticoids (19, 30).

In rat was described the majority of known APPs, classified in two types of acute phase reaction:
- Type 1 – APPs induced by IL-6 or IL-1/TNF-α: CRP, α1-AGP, HGB, hemopexin, serum amyloid A and P, and complement proteins;
- Type 2 – APPs induced by IL-6 (and also by IL-6-like cytokines - oncostatin M, leukemia inhibitory factor, and IL-11): CPL, FNG, β2-M (with ten-fold to hundred-fold increases, characteristic for this species), α1-antichemotrypsin etc.

Birds. SAA and transferrin concentrations were measured by polyacrylamide gel electrophoresis, immunotransfer and densitometry techniques in broilers infected by *Staphylococcus aureus* or inoculated with turpentines, in comparison with control groups. SAA was detected only in experimental group individuals, and transferrin concentration doubled only in birds inoculated with turpentines. This APP is considered an important variable in diagnosis (6).

Nielsen et al. (21) described a lectin which reacts with mannan (MBL), considering that is a minor acute phase protein in chickens. The concentration of serum MBL increased about twofold in 3-7 days after infection with infectious bronchitis virus, and in 3-5 days after infectious laryngotracheitis virus infection.

Fish. The main APP in fishes is CRP, being described in many species, including rainbow trout (*Onchorhynchus mykiss*) and Japanese eel (*Anguilla japonica*). The CRP effects are bacterial agglutination, complement activation, inhibition of some pathogen bacteria development, macrophages activation, emphasize of bacterial phagocytosis by peritoneal exudate cells, and nonspecific cytotoxic action targeted on myeloma cells.

Kodama et al. (18) described similar functions of CRP. They observed CRP opsonin effect in rainbow trout, this APP increasing phagocytic and chemokinetic activities of head kidney cells.

CRP of seal (*Phoca vitulina*), purified by Funke et al. (10) by affinity chromatography, has 25 kDa molecular weight and a amino acid sequence similar to other species, including mammals.

References


An acute phase response (APR) was experimentally induced in Atlantic cod (Gadus morhua L.) by intramuscular injection of turpentine oil. The change in the expression of immune related genes was monitored in the anterior kidney and the spleen over a period of 7 days. The genes examined were two. Studied at our institute and their participation in immune response, ontogeny or reaction to inherent or environmental changes examined [20e23]. Recent emphasis has been on examining the early APR of cod to infection and turpentine injection with particular attention to the possible role played by the classical acute phase proteins, the pentraxins. The results so far indicated that neither CRP-PI nor CRP-PII act as typical APPs in cod serum [17,24]. Phase I-II clinical trials of the vaccine have started that consist of two stages: Stage 1 is an open study of the safety, reactogenicity, and immunological activity of the vaccine with the involvement of 14 volunteers aged 18-30 years; Stage 2 is a single blind, comparative, randomized placebo-controlled study with the involvement of 86 volunteers. The study involved volunteers aged 18-60 years; the vaccine was injected intramuscularly twice, spaced 21 days apart between injections. However, three cases of acute respiratory viral infections were reported during the study, with two of them being laboratory-confirmed as COVID-19. These were the only severe adverse events registered. A N protein-specific CD4+ T-cell responses were also associated with humoral immune... Acute-phase proteins (APPs) are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction (also called acute-phase response). For acute-phase reaction is characteristic fever, acceleration of peripherals leukocytes, circulating neutrophils and their precursors.[1] The terms acute-phase protein and acute-phase reactant (APR) are often used synonymously, although some APRs are (strictly speaking) polypeptides rather than proteins. Inflammatory cells and red blood cells.