

EDITORIAL

**INFLAMMATORY MARKERS: SERUM AMYLOID A, FIBRINOGEN
AND C-REACTIVE PROTEIN – A REVISITED STUDY**

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The acute phase response is the part of the innate defence system of an animal against trauma, inflammation or infection. During this response, there is increased production and release of certain plasma proteins known as acute phase proteins, which include C-reactive protein (CRP), serum amyloid A (SAA) and fibrinogen (Fg). CRP consists of five identical subunits of 206 amino acids with a molecular weight of approximately 23 kDa. There is strong evidence from numerous studies that CRP is a predictor of inflammation. The acute-phase protein serum amyloid A (SAA) is a clinically useful marker of inflammation. SAA plays not only an important role in the development of AA amyloidosis (an important complication of rheumatoid arthritis) but also interacts with events closely involved in the metabolic syndrome as a high- and low-grade inflammatory modulator. Fibrinogen (Fg) is a high molecular weight plasma adhesion protein and is a biomarker of inflammation. It is synthesized and assembled in hepatocytes and fibroblasts and when secreted into the circulation, its plasma half-life ranges from 3 to 4 days. Several cytokines, are involved in the induction of acute phase protein synthesis, but the mutual importance of these cytokines seems to be cell-type specific and to vary in various experimental settings. Here we revisited the classic acute phase proteins SAA, C-Reactive protein and fibrinogen in their role in inflammation and their interrelationship with some cytokines.

Systemic and localized inflammation plays an important role in the development of acute and chronic inflammatory syndromes. The following inflammatory markers were studied: C-reactive, protein, fibrinogen, and serum amyloid A (SAA). Traumatic tissue injury from infection provokes a systemic inflammatory response termed acute-phase response, which is accompanied by hepatic

synthesis of certain plasma proteins. Increased levels of serum amyloid A (SAA), C-reactive protein (CRP), and fibrinogen have been observed during the acute-phase response. The normal physiological homeostasis of the organism is perturbed in response to inflammatory stimuli, leading to alterations in a wide variety of biochemical processes. These changes are collectively referred to as the 'acute-

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phase response'; they represent the organism's first line of defence and precede the more specific changes of the immune response. C-reactive protein (CRP) and serum amyloid A (SAA) are the two major human acute-phase proteins; their plasma concentrations may increase by 1000-fold or more following an inflammatory stimulus.

Fibrinogen (Fg) is a biomarker of inflammation (1-5) which, when elevated, indicates the presence of inflammation and identifies individuals with a high risk for cardiovascular disorders. Fg is synthesized and assembled in hepatocytes and fibroblasts, and when secreted into the circulation, its plasma half-life ranges from 3 to 4 days. Fibrinogen (Fg) is a high molecular weight plasma adhesion protein and a biomarker of inflammation (6). Many inflammatory disorders are accompanied by an increased blood content of Fg (7-9). Increased levels of Fg result in changes in blood physical properties such as increases in plasma viscosity, erythrocyte aggregation, platelet thrombogenesis, alterations in vascular reactivity and compromises in endothelial layer integrity. These alterations exacerbate the complications in peripheral blood circulation during systemic inflammatory diseases (10-11). In addition to affecting blood viscosity by altering plasma viscosity and erythrocyte aggregation, growing experimental evidence suggests that Fg alters vascular reactivity and impairs endothelial cell layer integrity by binding to its endothelial cell membrane receptors and activating signalling mechanisms.

The increase of fibrinogen results in the following symptoms: a) an increase of blood viscosity, b) an increase in vascular reactivity, and c) a decrease in endothelial integrity. All these symptoms lead to vascular dysfunction. Infections, trauma and inflammatory processes induce a host response with increases in a large group of structurally and functionally diverse plasma proteins. Parenteral administration of foreign proteins also induces an increase in plasma fibrinogen.

Serum amyloid A (SAA)

The acute-phase protein serum amyloid A (SAA) is a clinically useful marker of inflammation and is strongly associated with increased risk of cardiovascular events (12). Chronically elevated SAA concentrations may contribute to physiological

processes that lead to atherosclerosis, including endothelial dysfunction, an early and predictive event in the development of cardiovascular disease (13-15). Studies suggest that SAA can be a direct mediator in the development and progression of atherogenesis and atherothrombosis (16). SAA may affect key events underlying acute coronary syndromes, including cholesterol transport, contribute to endothelial dysfunction, promote thrombosis, and enhance leukocyte trafficking and activation (17-18). Novel therapeutic strategies to reduce SAA levels and/or oppose the actions of SAA may have beneficial effects on patients with inflammatory disease. SAA plays not only an important role in the development of AA amyloidosis (an important complication of rheumatoid arthritis), but also interacts with events closely involved in the metabolic syndrome as a high- and low-grade inflammatory modulator (19-20).

Serum amyloid A (SAA) protein is a major acute phase reactant in humans and many other species. Infections and traumatic inflammation are characterized by a rapid increase of SAA; its concentration in the plasma may augment many-fold (21-22). We have previously shown that the induction of synthesis of the two major human acute phase proteins, serum amyloid A (SAA) and C-reactive protein (CRP), are produced *in vitro* by hepatocytes stimulated by certain cytokines (23-24). Neither of these cytokines alone caused significant induction of either SAA or CRP. Serum amyloid A is an acute phase protein complexed to high density lipid protein (HDL) as an apoprotein (25-27). The molecular weight is 11.4-12.5 kDa in different species, and the protein has from 104 to 112 amino acids, with or without an insertion of eight amino acids at position 72. The protein is very well conserved throughout evolution, indicating an important biological function (28-30). The N-terminal part of the molecule is hydrophobic and probably responsible for the lipid binding properties. The most conserved part is from position 38 to 52, and this part is therefore believed to be responsible for the until now unknown biological function. The protein is coded on chromosome 11p in man. Acute phase SAA is first of all produced in hepatocytes after induction by cytokines, but extrahepatic expression of both acute phase and constitutive SAA

proteins has been demonstrated (31-35).

C-reactive protein (CRP)

C-reactive protein was first identified by Tillet and Francis more than 80 years ago. CRP consists of five identical subunits of 206 amino acids with a molecular weight of approximately 23 kDa. There is strong evidence from numerous studies that CRP is a predictor of inflammation (36-38).

Measurement of serum C-reactive protein (CRP) level is in widespread clinical use as a sensitive marker of inflammation. CRP has a role in the clearance of bacteria and of dying and altered cells, and might also have more complex immunomodulatory functions (39-41). Considerable evidence suggests CRP is an independent predictor of future cardiovascular events, but direct involvement in atherosclerosis remains controversial.

The plasma concentration of C-reactive protein (CRP), the first acute-phase protein to be identified, increases dramatically following tissue injury or inflammation. Although the physiological role of CRP is still not fully known, it has been suggested that concentrations might increase as part of the acute-phase response for facilitating non-specific immune functions, defence against bacterial pathogens, clearance of apoptotic and necrotic cells to prevent immunization against autoantigens and acceleration of the repair process (42-44). In agreement with the evidence that inflammation plays a pivotal role in the pathogenesis of atherosclerosis, CRP concentrations have been associated with cardiovascular disease, and measurement of CRP has therefore been proposed as a valuable aid to predict and stratify the risk of myocardial infarction and stroke. Native and monomeric CRP exert several prothrombotic activities, including activation of blood coagulation, impairment of the endogenous fibrinolytic capacity, and stimulation or enhancement of platelet adhesiveness and responsiveness (45-46). Inflammatory markers are ideal markers which enable early detection of patients with inflammatory syndrome, whereas inflammation markers are helpful in diagnosing and assessing the severity of inflammation.

Cytokines and acute-phase proteins

Several cytokines, first of all IL-1, IL-6 and TNF, are involved in the induction of SAA synthesis, but

the mutual importance of these cytokines seems to be cell-type specific and to vary in various experimental settings (47). The role of corticosteroids in SAA induction is somewhat confusing. In most *in vitro* studies corticosteroids show an enhancing or synergistic effect with cytokines on SAA production in cultured cells. However, in clinical studies and *in vivo* studies on animals an inhibitory effect of corticosteroids is evident, probably due to the overall anti-inflammatory effect of the drug (48). To date, no drug has been found that selectively inhibits SAA production by hepatocytes. Effective anti-inflammatory or antibacterial treatment is the only tool for reducing SAA concentration in serum and reducing the risk of developing secondary amyloidosis (49). The function of SAA is still unclear. Interesting theories, based on current knowledge of the lipid binding properties of the protein and the relation to macrophages, in the transportation of cholesterol from damaged tissues has been advanced (50). The potential that SAA is a modifying protein in inflammation influencing the function of neutrophils and platelets is interesting and more directly related to the inflammatory process itself. One possible mediator of the acute-phase response is interleukin-1, a pro-inflammatory monokine released in response to traumatic tissue injury or infection. There is evidence that partially purified macrophage supernatants containing interleukin-1 activity stimulate hepatocyte secretion of SAA, CRP, and fibrinogen (51).

Interleukin-6 (IL-6) is a monocyte-derived mediator and has regulatory effects on acute phase protein genes which result in the induction of fibrinogen synthesis in primary hepatocytes, while the addition of interleukin-1 (IL-1) exerts a negative modulating influence on the IL-6-stimulated fibrinogen (52). It is clear that IL-1 inhibits IL-6-stimulated fibrinogen transcription and translation. We studied the effects of IL-1ra on the down-regulation of IL-6 stimulated fibrinogen by IL-1, using an Fg ELISA method (53).

Amyloidosis encompasses a spectrum of diseases in which there is disordered folding of certain proteins that lead to them being deposited as insoluble fibrils in the extracellular space (54). The result of this process is impaired tissue structure and function. Amyloidosis may be acquired or hereditary and local or systemic, and is defined according to the identity

of the fibril precursor protein (55). Amyloidosis is a clinical disorder caused by extracellular deposition of proteins that are normally soluble as insoluble, abnormal fibrils that impair organ function

More than 20 unrelated proteins can form amyloid fibrils *in vivo* (56). Local amyloid deposition occurs in the brain in Alzheimer's disease and in the pancreas in maturity-onset diabetes, but a direct role in the pathogenesis of these diseases remains unproven. Systemic amyloidosis, with amyloid deposits in the viscera, blood vessel walls and connective tissues, is usually fatal and is the cause of about one death per thousand in developed countries. Recent elucidation of fundamental aspects of the pathogenesis of amyloidosis, and developments in diagnosis and monitoring of this disorder have greatly improved outcome for patients (57).

Osteoarthritis is a worldwide heterogeneous group of conditions that leads to joint symptoms associated with defective integrity of articular cartilage (58), in addition to related changes in the underlying bone at the joint margins. The prevalence of the disease after the age of 65 is about 60% in men and 70% in women. The aetiology of osteoarthritis is multifactorial, with the end result being mechanical joint failure and varying degrees of loss of joint function. The pathophysiological events associated with osteoarthritis are beginning to be understood. Essential inflammatory cytokines, such as IL-1 β and TNF- α , are involved in initiating a vicious cycle of catabolic and degradative events in cartilage, mediated by metalloproteinases, which degrade cartilage extracellular matrix (59). The role of inflammation in the pathophysiology and progression of early osteoarthritis is supported further by the observation that C-reactive protein levels are raised in women with early knee osteoarthritis, and higher levels predict those whose disease will progress (60).

Increased levels of SAA, CRP, and fibrinogen have been observed during the acute-phase response (61). One possible mediator of the acute-phase response is interleukin-1, a pro-inflammatory monokine released in response to traumatic tissue injury or infection. There is evidence that partially purified macrophage supernatants containing interleukin-1 activity stimulate hepatocyte secretion of SAA, CRP, and fibrinogen. It is clear that recombinant interleukin-1, when injected intraperitoneally into mice, induced

specific production of SAA mRNA and hence one phase of the acute-phase response. However, maximal induction of the acute-phase proteins C-reactive protein (CRP) and serum amyloid A (SAA) in the human hepatoma cell line Hep3B requires the combination of interleukin (IL)-6 and IL-1. In contrast, IL-1 inhibits fibrinogen induction by IL-6.

The synovium from osteoarthritis joints stains for IL-1 β and TNF- α . Nitric oxide, which exerts pro-inflammatory effects, is released during inflammation. Cartilage from patients with rheumatoid arthritis and osteoarthritis spontaneously produces nitric oxide *in vitro*. In experimental osteoarthritis, nitric oxide induces chondrocyte apoptosis, thus contributing to cartilage degradation. Hence, unregulated nitric oxide production in humans plays a part in the pathophysiology of the disease. These recent observations suggest that therapy can now be targeted at specific sites of pathophysiological pathways involved in the pathogenesis of osteoarthritis (60, 62-63). The novel strategies under consideration for the treatment of osteoarthritis can be divided into five main areas. These are COX-2 inhibitors, nitric oxide synthesis inhibitors and anti-oxidants, chondrocyte and bone growth promoters, metalloproteinase and cytokine inhibitors and gene therapy.

Interleukin-6 (IL-6) is the major cytokine so far characterized that is capable of regulating synthesis of almost all acute-phase proteins (64). IL-6 exhibits pleiotropic biological functions and is produced by a variety of cell types, including monocytes/macrophages, fibroblasts, endothelial cells and epithelial cells. IL-6 alone was capable of inducing synthesis of these two acute-phase proteins as well as fibrinogen and other cytokines, including interleukin-1 (IL-1), tumour necrosis factor α , interferon γ , transforming growth factor β , leukaemia inhibitory factor, interleukin-11 and oncostatin M, and also modulating synthesis of acute-phase proteins (65-66). These cytokines can act independently or, as is often the case, interact with each other and other extracellular signals, such as hormones, resulting in the production of specific patterns of acute-phase proteins.

However, beyond the utility of measuring markers of inflammation: SAA, reactive C protein and fibrinogen, to assess patients with subclinical inflammatory diseases who are at higher risk of

more severe events, further studies are needed to evaluate the therapeutic implications in this category of patients.

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