Acute phase response

Inflammation is the major and complex reaction of the body against infection upon tissue injury. It consists of using and activation of leukocytes and plasma proteins at the site of infection to eliminate the infectious agent

Acute phase response is the sum of the systemic and metabolic changes occurred by release of acute phase proteins in response to inflammatory stimulus.

Acute phase proteins are plasma proteins which increase in concentration following infection, inflammation, or Trauma, Moreover they 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).

acute phase response (APR) is a complex reaction, involving local and systemic effects. One of these effects corresponds to changes in the concentration of some plasma proteins, basically synthesized in the liver, which are called acute phase proteins (APP). The APR is induced by protein hormones called cytokines acting as messengers between the local site of injury and the hepatocytes responsible for synthesizing the APPs

The classes of acute phase proteins include ….

Haptoglobin
C-reactive protein
Serum amyloid
Ceruloplasmin
Ferritin
Fibrinogen
α1 acid glycoprotein
Alpha-2-Macroglobulin
Prothrombin
ESR

Major Functions of acute phase response

1-Opsonisation and trapping of microorganisms and their products
2-Inactivating complement system
3-In binding cellular remnants
4-In neutralizing enzymes
5-Scavenging free hemoglobin and radicals
6- Modulating the host’s immune response

Haptoglobin

is an α2-glycoprotein acute phase reactant that binds to free hemoglobin and forms a stoichio metrically stable complex. The name haptoglobin comes from "hapto,"or to bind, and globin. Polonovski and Jayle2 discovered haptoglobin in 1938, and later Smithies3 determined its genetic variations.

Several functional properties of haptoglobin have been described. The major biologic function of haptoglobin is to bind hemoglobin in an equimolar ratio with very high affinity to prevent hemoglobin-mediated renal parenchymal injury and loss of iron following intravascular hemolysis.4 In addition,haptoglobin can inhibit prostaglandin synthesis and is believed to have anti-inflammatory and antioxidant properties in the body. Haptoglobin is present in the serum of all mammals, but polymorphism is found only in humans. Three major haptoglobin phenotypes have been identified by gel electrophoresis: Hp 1-1, Hp 2-1, and Hp 2-2.3,6 These phenotypes have different biologic activities, as described in the following text.

Physiology and Functions of Haptoglobin

The liver is the principal organ responsible for synthesis of haptoglobin. In addition to the liver, the *Hp* gene is expressed in other tissues, such as lung, skin, spleen, kidney,and adipose tissue in mice. Haptoglobin synthesis is increased by growth hormone, insulin, bacterial endotoxin,prostaglandin, and cytokines such as interleukin-6.5, Plasma haptoglobin levels change during life. Haptoglobin levels in healthy infants are lower than in healthy adults. In healthy adults, the haptoglobin concentration in plasma is between 38 and 208 mg/dL (0.38 and 2.08 g/L).16 People with Hp 1-1 have the highest plasma concentrations, those with Hp 2-2 the lowest plasma concentrations, and those with Hp 2-1 have concentrations in the middle.1,16 The half-life of haptoglobin is 3.5 days, and the half-life of the haptoglobin-hemoglobin complex is approximately 10 minutes.5 The haptoglobin-hemoglobin complex is removed by binding to the CD163 receptors on the surface of macrophages and monocytes.17,18 Macrophage expression of CD163 is increased by glucocorticoids, interleukin-6, and interleukin-10.19-21 In addition to being detected in plasma,haptoglobin can be detected in urine, synovial fluid, ascetic fluid, cerebrospinal fluid, and pleural fluid.1

Antioxidant Activity

As mentioned, haptoglobin binds to free hemoglobin,with extremely high affinity (*Kd* ~1 × 10–15 mol/L), probably the highest in nature.22 Hemoglobin is the richest source of iron in the body.23 The major hazard posed by iron and iron containing compounds is formation of reactive

oxygen species. Free hemoglobin also enhances the peroxidation of purified arachidonic acid and other polyunsaturated fatty acids within neuronal cell membranes. Iron released from heme proteins can catalyze oxidative injury to neuronal cell membranes and might have a role in post traumatic central nervous system (CNS) damage. Also, oxidation of low density lipoprotein is catalyzed by heme and leads to vascular endothelial cell damage and atherosclerosis. Haptoglobin, by binding hemoglobin and removing it from the circulation, prevents iron-stimulated formation of oxygen radicals and has an important role as an antioxidant.28 The antioxidant capacity of Hp 2-2 is lower in circulation than that of Hp 1-1 because Hp 2-2 binds hemoglobin with lower binding affinity than does Hp 1-1.29

Prevention of Renal Damage

Free hemoglobin can cause oxidative damage in renal tissues following intravascular hemolysis. In the presence of haptoglobin, free hemoglobin binds to haptoglobin, forming a haptoglobin-hemoglobin complex, which is too large to pass through the glomeruli of the kidney and will be removed via the reticuloendothelial system. Thus, haptoglobin prevents hemoglobin-induced injury to the renal parenchyma.1

Antibacterial Activity

of hemorrhagic injury and infection can have a fatal outcome because the presence of blood in injured tissue can provide the required iron to the invading microorganisms.

However, once bound to haptoglobin, hemoglobin and iron are no longer available to bacteria that require iron, such as *Escherichia coli*. Indeed, Eaton et al30 showed that a fatal consequence of intraperitoneally injected *E coli* and hemoglobin in rats can be prevented by the administration of haptoglobin.30 In the lungs, haptoglobin is synthesized locally and is a major source of antimicrobial activity in the mucous layer and alveolar fluid and also has an important role in Iron is one of the essential elements for bacterial growth. The combination protecting against infection.32

Inhibition of Nitric Oxide

Nitric oxide (NO) is a potent vasodilator molecule that is produced by vascular endothelial cells and has an important role in the control of platelet aggregation and the regulation of cardiac contractility
.33 Also, a large amount of NO is produced by activated macrophages during immunologic reactions.34 NO not only is a potent vasodilator but also has an important role in the control of platelet aggregation
and the regulation of cardiac contractility.34 Haptoglobin,by binding to hemoglobin and limiting hemoglobin interaction with NO, inhibits

endothelium relaxation. This effect is not seen with unbound haptoglobin.35

Inhibition of Prostaglandins

Prostaglandins are produced from arachidonic acid by the action of lipoxygenase and cyclooxygenase enzymes.36 Prostaglandins have important roles in modulating platelet aggregation (both proaggregation and antiaggregation). In addition, some of the prostaglandins such as the leukotrienes have a proinflammatory role in the body.37 Again, haptoglobin,by binding to hemoglobin and limiting its access to the prostaglandin pathway enzymes such as prostaglandin synthase, has an important anti-inflammatory function in thebody.38,39

Conditions Associated With an Elevated Plasma Haptoglobin Level

An elevated plasma haptoglobin level is seen following inflammation, trauma, and burns and with tumors.1,58 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.59 The plasma haptoglobin level in the initial phase of acute myocardial infarction is high, but later, owing to hemolysis, the plasma level decreases temporarily.60

Conditions Associated With Decreased Haptoglobin Level

The plasma haptoglobin level is decreased in hemolysis,malnutrition, ineffective erythropoiesis, hepatocellular disorders,late pregnancy, and newborn infants.59,61 In addition,the plasma haptoglobin level is lower in people with positive skin tests for pollens, high levels of IgE and specific IgE forpollens and house dust mites, rhinitis, and allergic asthma.62 While working on the mechanism of hemoglobininduced CNS damage, Panter et al63 found a highly significant association between low or absent plasma haptoglobin levels and the presence of seizure in familial idiopathic epilepsy. This is a seizure disorder occurring without evident precedent cause and affecting 2 or more members of the same family. In several of the kindreds being studied, the affected individuals and some of their first-degree relatives had very low or absent levels of serum haptoglobin.63 Panter et al63 hypothesized that the very low levels of serum haptoglobin in affected individuals might impair the normal process of clearance of free hemoglobin from the CNS. Indeed, the clearance of radiolabeled hemoglobin, previously injected into the brains of hypohaptoglobinemic mice, was substantially less than that from the brains of animals with normal haptoglobin levels.63 The defect in hemoglobin clearance in the latterexperiment was corrected by injection of hemoglobin-haptoglobinpurified complex.63 In protein-losing nephropathies,the plasma level of only Hp 1-1 is decreased because the Hp 1-1 molecule is smaller and filters through the kidney, while Hp 2-2 and Hp 2-1 remain in the plasma.59,64

Cytokines….

Cytokines are polypeptide produced in response to microbes and other antigens that mediate and regulate immune and inflammatory reactions. Actions of cytokines are often pleiotropic and redundant (Dinarello CA, 1997). From the site of infection local tissue cells release some proinflammatory cytokines TNF, IL-1 and chemokines, which mediate many of the effect or functions of innate immunity and also the principle mediators of acute inflammatory response. TNF is secreted by activated mononuclear phagocytes, natural killer {NK} cells and local mast cells while IL-1 is secreted also by neutrophils, endothelial cells and epithelial cells (Wolf M,et.al. 1996). Lipopolysaccharides potently stimulate the production of TNF and IL-1. Both the proinflammatory cytokines induce the expression of adhesion molecules (ICAM-1, VCAM-1) for leukocytes. They also stimulate macrophages to secrete chemokines that enhances affinity to leukocyte integrins for their ligands (Moldawer LL, 1997).TNF and IL-1 when secreted in large amounts, exerts endocrine effects, by causing fever, chachexia and increased synthesis of APP. Chemokines act as a chemoattractants for leukocutes and are produced by endothelial cells, epithelial cells, fibroblasts, leukocytes and other cytokines (Rollins BJ, 1997). Chemokines act cooperatively in the process of leukocute migration towards chemical gradient. They are classified into two major families; CC and CXC family. Each family having distinct receptors on leukocytes. IL-6 works both in innate and adaptive immunity. It is synthesized in response to microbes and other cytokines such as IL-1 and TNF. It stimulates the synthesis of APP by hepatocytes, production of white blood cells and thus contributes to the efforts of inflammation (Bernardini G, et.al. 1998).

Erythrocyte Sedimentation Rate (ESR)

Although not used for establishing a clinical diagnosis in the clinic, it is a commonly used and cost efficient test, which can assist in following-up with the progress and response to treatment of a disease. It indirectly reflects the increased concentrations of the acute phase proteins. The major determinant of sedimentation rate, is the rouleaux formation of erythrocytes, in which cells are lined up in a singleaxis [6]. The aggregation of erythrocytes is determined by the electrostatic forces. The erythrocytes are normally negatively charged and repel each other.On the other hand, many of the plasma proteins are positively charged, and neutralize the charge on the erythrocyte membrane, therefore lessening the repellant force, contributing to aggregation [6]. The proteins, which contribute most to erythrocyte sedimentation are fibrinogen, albumin, alpha and beta globulin. Among these proteins, fibrinogen with an asymmetric molecular structure has the highest contribution [6, 7]. A

slight increase in the fibrinogen levels can bring about a great increase in ESR.An increase in the monoclonal immunoglobulin levels,as in multiple myeloma, may also increase ESR,independent of an acute phase reaction. Thus, ESR may not always reflect an acute phase reaction correctly (Table 2). Polycythemia vera, secondary polycythemias,sickle cell disease, hereditary spherocytosis,acanthocytosis, microcytosis, cachexia, and hipofibrinogenemia due to disseminated intravascular coagulation and massive hepatic necrosis may cause a decrease in ESR. Anemia and macrocytosis increase ESR (Table 3).

C-Reactive Protein (CRP)

CRP is the prototype of human acute phase proteins and the most frequently studied one [11]. It has been named as C-reactive protein because it adheres to the "capsule" antigen of pneumococcus [12]. Plasma CRP production occurs via the stimulation of IL-6 in the liver. It assists in the recognition of damaged host cells and foreign pathogens, and their removal. When CRP binds to its ligand, it activates the complement system via the classical pathway and increases phagocytosis [11]. While it is present in minute quantities in the plasma, after an acute inflammatory stimulation, it rises within a few hours. It peaks within 2-3 days. Its half-life is 19 hours [12]. The increase in CRP levels is proportional to the inflammatory stimulus. With a greater stimulus, a higher and longer lasting level of CRP will be measured. After the inflammatory stimulus is removed, the CRP levels will quickly decrease [4, 11, 12]. The causes for increased CRP levels are summarized in Table 4.In healthy individuals In healthy individuals the CRP level is generally below 0.2 mg/dl. Due to micro-traumas that occur during the day, this level can increase up to 1 mg/dl. After a single stimulus it can increase up to 5 mg/dl within 6 hours, and can reach a peak value within 48 hours. While a value between 1-10 mg/dl is considered as mild, and any value above 10 mg/dl is considered a very high increase [3, 4, 11-13].

CRP is not specific for a certain disease. It shows inflammation and its degree. Although not always,,it mostly demonstrates inflammation and the degree of tissue damage, and the acute phase reaction more than any other parameter with more accuracy.Acute phase CRP reaction does not show diurnal variation, and is not affected by diet [11-13, 14]. CRP is mainly useful for following the response to treatment, hence the decrease in inflammation in organic disorders accompanied by mainly inflammation.CRP reflects an inflammatory process and its degree. Therefore, it is very important in the follow up of rheumatologic disorders. In RA, high CRP values are almost always seen, and its titer correlates with the disease activity index [15]. A high CRP value at the initial presentation implies that a progressive erosive course is possible,

and hence,guides the treatment selection [15]. In RA, CRP levels do not decrease with the use of non-steroidal anti-inflammatory drugs (NSAIDs), and onlydecreases with drugs that provide remission [3]. In only 2-30% of the SLE patients, an increased CRP is observed. Because of this, CRP level is not helpful in following up disease activity in SLE. It has been shown that an elevated CRP level in a SLE patient usually is seen if there is accompanying synovitis and serositis. If there is a CRP increase from the very beginning, then perhaps the CRP level may be used to evaluate the disease activity. If a patient, whose CRP level is known to be negative beforehand, afterwards becomes CRP positive, especially if the patient is admitted to a hospital, it should bring to mind a possible bacterial infection.The reason why CRP is not elevated in SLE is

FIBRINOGEN

One of the acute phase proteins that originate from the liver is fibrinogen. Fibrinogen plays two importantroles in the body. Firstly, it is an important component of the common pathway of coagulation.Secondly, it takes part in the acute phase response after tissue inflammation and damage [3].High fibrinogen levels are also seen in heart or circulatory system disorders. Additionally, in stomach,breast and renal system malignancies, and inflammatory diseases such as rheumatoid arthritis, high fibrinogen levels may be observed. Fibrinogenis also used as a disease activity marker in Familial Mediterranean fever [19-21]. Additionally, exogenous use of estrogen and oral contraceptives is also associated with high levels [22].Low levels of fibrinogen may be seen in liver diseases, prostate and lung cancers, bone lesions,malnutrition and some bleeding disorders.Afibrinogenemia, hypofibrinogenemia and dysfibrinogenemia congenital diseases which are characterized with the lack or low levels of fibrinogen.In obstetric complications and traumas, low levels may be seen, too. The plasma fibrinogen levels may also decrease in response to massive amountsof transfusion due to blood provided from the banks.Drugs such as steroids, androgens, phenobarbital,urokinase, streptokinase and valproic acid may also cause low levels of fibrinogen.Plasma fibrinogen level is normally around 200-400 mg/dl. It is not a test that initially reflects the acute phase response. Its delayed increase, long half life, prolongedhigh levels after inflammation has passed, lack of stability in frozen plasma and preserved plasma, are some of the disadvantages of this test [3].

FERRITIN

Normally it reflects the iron stores in the body. Its normal values are 27-329 ng/ml for men and 9-125 ng/ml for women. However, in the presence

of inflammation,it may increase as an acute phase response.It increases in cases of liver damage and malignancies

serum amyloid A
is known to be one of the major acute phase proteins, its role in the acute phase response has not been elucidated. Serum amyloid A is a member of the apolipoprotein family.During inflammation, it binds to high-density lipoproteins and alters the cholesterol metabolism.There are some studies which show that serum amyloid A may be effecting the adhesion of phagocytic cells and lymphocytes, and is responsible of their chemotaxis, causes the oxidation of high-density lipoproteins, hence playing a role in the development of inflammation in atherosclerotic coronary arteries [5]. Another serum amyloid protein is serum amyloid P. It has a pentameric structure. It comprises about 14% of the amyloid deposits. It has a role in the inflammation process [22].

"Positive" acute-phase proteins:

Protein	Immune system function
C-reactive protein	Opsonin on microbes[3] (not an acute-phase reactant in mice)
Serum amyloid P component	Opsonin
Serum amyloid A	• Recruitment of immune cells to inflammatory sites • Induction of enzymes that degrade extracellular matrix
Complement factors	Opsonization, lysis and clumping of target cells. Chemotaxis
Mannan-binding lectin	Mannan-binding lectin pathway of complement activation
Fibrinogen, prothrombin, factor VIII, von Willebrand factor	Coagulation factors, trapping invading microbes in blood clots. Some cause chemotaxis
Plasminogen	Degradation of blood clots
Alpha 2-macroglobulin	• Inhibitor of coagulation by inhibiting thrombin.[4] • Inhibitor of fibrinolysis by inhibiting plasmin
Ferritin	Binding iron, inhibiting microbe iron uptake [5]
Hepcidin[6]	Stimulates the internalization of ferroportin, preventing release of iron bound by ferritin within intestinal enterocytes and macrophages
Ceruloplasmin	Oxidizes iron, facilitating for ferritin, inhibiting microbe iron uptake
Haptoglobin	Binds hemoglobin, inhibiting microbe iron uptake
Orosomucoid	Steroid carrier

(Alpha-1-acid glycoprotein, AGP)	
Alpha 1-antitrypsin	Serpin, downregulates inflammation
Alpha 1-antichymotrypsin	Serpin, downregulates inflammation

The most important ones of these acute phase reactants are erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen and ferritin

The major determinant of sedimentation rate, is the rouleaux formation of erythrocytes, in which cells are lined up in a sing axis

Functions of acute phase response

Positive APPs are regarded as having major functions in opsonisation and trapping of microorganisms and their products, in activating complement system, in binding cellular remnants, in neutralizing enzymes, scavenging free hemoglobin and radicals, and in modulating the host's immune response