THE SYSTEMIC REACTION DURING INFLAMMATION: THE ACUTE-PHASE PROTEINS

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Abstract: The acute-phase response consists in a large number of behavioural, physiologic, biochemical, and nutritional changes involving many organ systems distant from the site, or sites, of inflammation. One of the most investigated, but still not well understood, characteristic of the acute phase is the up-regulation, or down- regulation, of many plasma proteins, known as the acute-phase proteins. The changes in the concentrations of these positive acute-phase proteins and negative acute-phase proteins are due to changes in their liver production. Their increase may vary from 25 percent to 1000 fold, as in the case of C-reactive protein and serum amyloid A. This review summarises the recent advances that have been acquired on the acute-phase proteins, in particular their function in pathologies such as infections or inflammatory lesions.

Keywords: Acute-Phase, Serum Amyloid, Inflammation, Systemic Response

INTRODUCTION

The response to infection and injuries usually involves a large number of changes both local and distant from the site of inflammation. This physiological condition takes place at the very beginning of the inflammatory process and lasts for 1-2 days. After that, the host returns to normal functions. The systemic response can also be prolonged, if acute inflammation becomes too chronic [1]. These events lead to a wide-ranging systemic response that is also called acute-phase.

The purpose of acute-phase reaction is to counteract the underlying challenge in order to restore the homeostasis as soon as possible. This result is accomplished by isolating and destroying the infective organisms, or removing the harmful molecules, and activating the repair process. Acute-phase reaction includes a wide range of neuroendocrine, hematopoietic, metabolic and hepatic changes, summarized in Table 1.

One of the most interesting features of the acute-phase is the change in the concentrations of many plasma proteins, known as the acute-phase proteins. An acute-phase protein (APP) has been defined as one whose plasma concentration increases (positive acute-phase proteins) or decreases (negative acute-phaseproteins) by at least 25 percent during inflammatory disorders [2]. The very first APP to

be described, C-reactive protein (CRP), was discovered in 1930 [3] in the plasma of patients during the acute phase of pneumococcal infection. In some pathologies, CRP may increase more than 1000 times.

TABLE I **ACUTE-PHASE PHENOMENA**

Currently, approximately 40 proteins are considered APP [4]. The following review will focus on the knowledge that has been acquired on acute-phase reaction, in particular acute phase proteins in human and domestic animals.

The regulation of acute-phase changes by cytokines

The synthesis and release of plasma APP from the liver is regulated by inflammatory mediators. These mediators fall into four major categories: IL-6-type cytokines, IL-1-type cytokines, glucocorticoids, and growth factors. Cytokines mainly stimulate the APP gene-expression, while glucocorticoids and growth factors modulate cytokine activity. Binding of the inflammatory mediators to their respective receptors on hepatocytes and the transduction of this signal induce changes in APP gene expression that are primarily regulated at a transcriptional level [1].

Cytokines are a group of proteins acting as intracellular and intercellular signalling molecules. The role of cytokines during inflammation is both initiation and fine-tuning of the whole process: some cytokines initiate and amplify the response, others sustain or attenuate it, and some of them cause it to resolve.

During inflammation, inflammatory cells, mainly macrophages and neutrophils that assemble at the site of challenge, together with endothelial cells, secrete the so-called pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, in that order [5]. This order is important, since each cytokine fulfills a precise role in up-regulating or down-regulating the expression of the others.

Figure 1: The acute phase reaction. Green arrow indicates the promoting activity. Red arrow indicates the inhibitory activity.

Pro-inflammatory cytokines induce a number of local and systemic responses (Table 1 and Figure 1): a) the expression of selectins on local endothelial cells, that recruit inflammatory cells from the bloodstream; b) the activation of recruited cells in developing their optimal defensive activity, i.e. increased expression of receptors (Complement receptors), or cell metabolism, such as oxidative burst, and c) expression of other cytokines, such as chemokines, that recruit more and more defensive cells to the inflammation site.

TABLE 2 HUMAN ACUTE-PHASE PROTEINS

The systemic response develops in two waves: [6], at the local reaction site these cytokines activate cells such as fibroblasts and endothelial cells to initiate the secondary release of cytokines that also induce endocrine effects [1]. This secondary wave and the consequent appearance of these cytokines in the circulation are responsible for the start of a wide range of systemic inflammatory effects described in Table 1. [7]

Cytokines operate in a very complex signalling network that is still not completely understood. For example IL-1β induces the production of IL-6, but IL-6 inhibits the expression of the other pro-inflammatory cytokine, TNF-α, thus introducing a controlling step in the amplification of inflammation.

Circulating IL-6 is believed to play the most important role in the induction of acute phase reactions [8]. The synthesis of IL-6 is induced by other cytokines, including IL-1β and TNF-α, but also directly by bacterial endotoxins. Figure 2 shows the regulation of IL-6 secretion [9].

Figure 2: Regulation of IL-6 synthesis. TNF-α is produced first, IL-1β second, and IL-6 last. Each of these inflammatory cytokines stimulates its own production. TNF-α and IL-1β stimulate each other, and both stimulate IL-6. IL-6 inhibits the secretion of both TNF-α and IL-1β. IL-6 stimulates also its own production. Glucocoticoids, the end products of the hypothalamic-pituitary-adrenal axis, inhibit the production of all three pro-inflammatory cytokines. A line with a solid circle at the end indicates an inhibitory effect; a line with an arrowhead at the end indicates a stimulatory effect.

Pro-inflammatory cytokines act by binding to their respective receptors on the cell surface. Then transmit the signal into the cell. The expression of the different APP is accomplished either by activation of resident pools of inactive factors in the cytoplasm and/or by increased factor biosynthesis.

Figure 3: Scheme of signalling pathways that mediate the expression of APP. TNF-α and IL-1β binding to their receptor results in the activation of IkB kinase, which in turn phosphorylates IkB, the inhibitor associated to NF-kB in cytoplasm. IkB-P is then degraded by the ubiquitin-proteasome pathway, thus allowing the now free NF-kB to translocate to the nucleus, where it promotes transcription of acute-phase genes. IL-6 binds to IL-6 receptor. The activation of the receptor induces the phosphorylation of NF-IL6. Phosphorylated NF-IL6P traslocates to the nucleus, where it promotes transcription of acute-phase genes. The activation of IL-1β receptor may induce the phosphorylation of NF-IL6.

Figure 3 shows the signaling pathways that mediate the overexpression of most APP. The signal transmitted by IL-6 binding to their receptors results in a phosphorylation of transcription factor NF-IL-6, which translocates in the nucleus and mediates the transcription of APP genes. Also NF-IL-6b gene, a de novo synthesized nuclear factor that enters the nucleus, contributes to the amplification of APP genes expression. TNF-α binds to TNF-α receptor, which activates a different nuclear factor, the NF-kB. NF-kB is one of the main factors involved in the induction of gene

transcription during AP-response. NF-kB resides in the cytoplasm, associated with its inhibitor IkB. The signal transduction eventually leads to an activation of IkB kinases that phosphorylate IkB. This phosphorylation targets IkB for degradation via the ubiquitin-proteasome pathway. The NF-kB can therefore be released in the cytoplasm, and move into the nucleus, where it promotes the transcription of AP-genes. IL-1β binds to its receptor IL-R, which can activate both NF-IL6 and NF-kB pathways. [10]

The liver is not the only organ able to produce APP: many of them are also produced extrahepatically, e.g. ceruloplasmin (Cp), complement components, and serum amyloid A protein (SAA) [11].

Classification of APP

Several classifications of acute phase proteins have been proposed. All the up-regulated proteins have been called “positive APP”, in order to differentiate them from the so-called “negative APP”, that are down-regulated. The most physiologically expressed protein, albumin, and several other proteins usually present in blood belong to the latter group.

Table 2 shows a list of APP that considers the defensive systems that are activated during systemic inflammation. A different classification of APP production depends on the rate of their expression. The apolipoproteins serum amyloid SAA1 and SAA2 (commonly called SAA) are considered “major acute-phase proteins”. Their concentration can increase as much as 1000-fold [12]. Other major APPs are CRP and α1 acid-glycoprotein (AGP).

Complement components are modestly induced during acute-phase [13,14]. Both coagulation and fibrinolytic proteins and transport proteins, together with the proteases inhibitors proteins, are also slighty up-regulated (up to three fold).

Post-translational regulation of APP production

Post- translation mechanisms, APP modelling and export, may also be involved in this process [6], for example in AGP expression. An alteration of carbohydrate moiety, with increased sialylation, has been described for AGP, as previously mentioned. An increase in fucose content has also been described in the glycosylation pattern of haptoglobin (Hp) in the dog [15]. This post-translation mechanism is very complex and still not well understood, but it is likely very important during inflammatory response. Increases in glycoforms expressing di-antennary glycans, and an increase in the degree of 3-fucosylation are apparent from the very first moment of AP. More interesting, pro-

inflammatory cytokines (IL-1β, IL-6, TNF-α) and glucocorticoids seem to be involved in these modifications [16].

Proteins whose plasma concentrations decrease: Albumin, Transferrin, Transthyretin, *a*2-HS glycoprotein, Alpha-fetoprotein, Thyroxine-binding globulin, Insulin-like growth factor I, Factor XII.

The function of Acute Phase proteins in the defense of the organism.

Probably all the acute-phase proteins have the potential to influence one or more stages of inflammation. For some of them the beneficial effects are evident.

The classic Complement system proteins, most of which are APP, have an important pro-inflammatory role in immunity. The mannose-binding lectin, that activates the alternative pathway of the complement, is also overexpressed during an acute phase. The function of complement pathways during inflammation are well known, and include chemotaxis, plasma protein exudation at inflammatory sites, and opsonization of infectious agents and damaged cells. Inflammation is a very complex and finely orchestrated process involving many cell types and molecules. A number of the participating molecules are multifunctional and contribute to both the initiation and the control of inflammation at different points during its evolution [17]. In the coagulation and the fibrinolytic system, the overall improvement of defense and restoration of tissue integrity is evident. The requirement for wound healing can be satisfied by the up-regulation of several acute-phase proteins: fibrinogen, for example, causes activation of the endothelium, including an increase in cell adhesion, spreading and proliferation. One of the most striking characteristics of the overexpression during acute phase is that the whole coagulatory and wound-healing system, i.e. fibrinogen, plasminogen, tissue plasminogen activator (TPA), urokinase and plasminogen activator inhibitor-I (PAI-1), is overexpressed, in order to avoid the unbalancing of the repair process. Hp, whose main function is to bind hemoglobin and thus prevent loss of iron, is also considered an angiogenic factor [18].

Inflammation is a very violent process: the defense of homeostasis from internal and external enemies is often accomplished at the cost of wide “collateral damages” , such as large destruction of tissues. This is the reason why several APP may also have anti-inflammatory activity. Hp, hemopexin and ceruloplasmin exhibit an anti-oxidant function focused against reactive oxygen intermediate (ROI) species.

Several proteases are activated during AP, like phagocytic lysosomal proteases, and the clotting cascade proteases. If not controlled, their adverse effect can override their beneficial one. Moreover, bacterial proteases, such as collagenases, are also active in septic AP. Therefore, the overexpression of protease inhibitors is functional to control both the physiological repair mechanisms and bacterial aggressiveness.

Some metal chelating proteins, such as ceruloplasmin, that binds copper, and hemopexin, that binds heme, also accomplishes direct defense against pathogens. As usual, their role is multifunctional. Both reduce the availability of copper and iron for bacterial growth, meanwhile avoiding the loss from the organisms. Other proteins are directly involved in the innate immunity against pathogens. LPS-BP, for example, interacts with bacterial lipopolisaccharides and transfers them to CD-14, a receptor on the surface of macrophages and B-cells. Following the presentation of LPS by LPS-BP, a lipopolisaccharides recognition complex is formed on the membrane via the recruitment of a second receptor, Toll Like Receptor 4 (TLR4). These events drive the signaling pathway of TLR, that induce the activation of several inflammatory and immune-response genes, including pro-inflammatory cytokines. [19].

For other proteins, such as CRP, SAA and AGP, the biological advantage is less evident and still not precisely defined. These three proteins belong to the “major APP” group.

Pathophysiologic roles of CRP during the inflammatory process are complex and apparently inconsistent. C-reactive protein is a component of the innate immune system. It binds phosphocholine and therefore can recognize some foreign bacteria as pathogens, but also the phospholipid constituents of damaged cell [20].

CRP can activate the complement system when bound to one of its ligands and can also bind to phagocytic cells thus initiating the elimination of targeted cells. Finally, CRP induces the expression of inflammatory cytokines and tissue factor in monocytes [21,22]. Interestingly, the net effect of an overexpression of CRP may result in an anti-inflammatory effect [23], probably due to the down-regulation of the surface expression of L-Selectin [24]. At high concentration CRP also inhibits the generation of superoxide in neutrophils, and stimulates the synthesis of IL-1R antagonist by monocytes.

The function of SAA is not known. No individual lacking the capacity to up-regulate SAA during AP has been described, thus confirming that the protein carries out an essential function. Following AP, SAA associates with HDL_3, replacing Apo-A1 as the predominant apoprotein on this class of HDL [25].

SAA associated HDL_3 seems to facilitate the uptake and removal of cholesterol from monocytes/macrophages at the inflammatory site [26]. Lindhort *et al* [27] demonstrated that mouse SAA levels during inflammation peak at the same time as plasma cholesterol levels. Moreover, cholesterol directly injected into an experimental abscess is redirected to the plasma: therefore it has been suggested that SAA reversibly controls cholesterol transport to optimize its clearance from dead cells at inflamed sites.

Several other SAA activities have been described: it increases the cleaving of triacylglicerols into glycerol and fatty acids on HDL_3, by enhancing the activity of secretory phospholipase [28]. SAA directly acts on cholesterol molecule, decreasing its esterification [29] and increasing its uptake by hepatocytes [16].

Free SAA, but not HDL_3 associated, mediates chemotaxis of monocytes, granulocytes and T-cells [30,31]. Also, SAA exhibits also some inhibitory effects on inflammation [32, 33], including down-regulation of fever, pgE_2 synthesis, platelet activation and oxidative burst.

The very high expression rate of SAA raises a completely different pathological problem in the organism: the continuous high expression of SAA is the prerequisite for the development of secondary amyloidosis, caused by the conformational change of SAA in an insoluble proteolytic peptide, AA, that deposits as insoluble plaque in major organs [34]. Moreover, since SAA is also synthesized by cells involved in inflammation (monocytes and endothelial cells), and is closely associated with platelets and cholesterol, the possibility that SAA is involved in the pathogenesis of atherosclerosis cannot be ruled out, [35].

α-1-acid glycoprotein is usually considered one of the major APPs, even if the concentration rises only 2-5 fold [36.]. AGP belongs to the lipocalin family, a group of proteins sharing a similar three-dimensional structure capable of binding and carrying hydrophobic molecules.

AGP is a very unusual protein indeed. Its pI is very low (2.8-3.8) and the carbohydrate content is very high (45%) [37]. AGP is considered a natural anti-inflammatory and immunomodulatory agent due to its anti-neutrophil and anti-complement activity [38]. AGP inhibits neutrophil activation [39] and increases the secretion of IL-1R antagonist by macrophages. [40].

One of the most interesting characteristics of AGP is that its immunomodulatory activity has been shown to depend on its glycosylation. Inflammation induces not only an overexpression of AGP, but also a modification of its carbohydrate moiety, the sialyl-Lewis X being the most represented.

The sialyl-Lewis X form of AGP induced during inflammation reduces both complement and neutrophil mediate injuries [41], while the non-sialyl Lewis X form does not.

Sialyl Lewis X is the ligand for the cell adhesion molecules E- and P-selectin involved in the rolling of leukocytes to endothelial cells and platelets. Since it has been demonstrated that AGP expressed during inflammation actually bind an E-selectin-IgG chimeric molecule, it has been hypothesized that the inflammation-induced increase in sialyl-Lewis X glycans on AGP can be considered a mechanism for feedback inhibition of leukocytes extravasation [42].

AGP exhibits also a potent platelet aggregation inhibitory activity [43]. Finally, inhibition of oxidative metabolism has also been reported [39]. For all these activities, it may be suggested that the main function of AGP consists in reducing the cellular damage that usually happens as a consequence of the inflammation process.

Why negative APP?

There are no clear reasons to explain the down-regulation of some proteins. It is possible to speculate on the need to divert available amino acids to the production of other acute-phase proteins during systemic response. The amino acids necessary for APP synthesis derive in part from reduced synthesis of other proteins that are not considered important in that very defensive moment, since the decreased production of negative acute phase plasma proteins is not important for host defense, and in part from muscle protein degradation via the ubiquitin-pathway. For what concerns the other down-regulated proteins, has been demonstrated that transthyretin exhibits inhibitory activity of IL-1β production by monocytes and endothelial cells [44] and therefore a decrease in its plasma concentration may be considered as a proinflammatory mechanism.

Other systemic effects of AP phenomena.

Apart from the hepatic APP overexpression, the other two main AP effects are fever and leukocytosis. Several cytokines may induce fever, but the final step is driven by IL-6 produced in the brain stem [45]. Esogen pyrogens, such as LPS, activate macrophages and other leukocytes to release endogen pyrogens (the pro-inflammatory) which stimulate the anterior hypothalamus to produce prostaglandins which lead to sympathetic nerve stimulation, vasoconstriction of skin vessels and increase in body temperature, fever. Fever is beneficial up to a certain point. It can inhibit the growth of some microorganisms and increase the rate of enzyme reactions, thus speeding up the body's metabolism. An increase in the rate of metabolism can increase the rate of phagocytosis, immune responses, and tissue repair. Too high a body temperature, however, may cause serious damage. Leukocytosis can be defined as an increased concentration of white blood

cells [46]. The phenomenon of leukocytosis is essential in the primary host defense against infections. The leukocytosis during AP is essentially induced by IL-1β and TNF-α, which mobilize hematopoietic cells egress into the peripheral circulation. IL-1β up-regulates also GM-CSF gene, indispensable for the growth and development of granulocyte and macrophage progenitor cells. Moreover, it stimulates myeloblasts and monoblasts and triggers irreversible differentiation of these cells.

Down-regulation and control of APR and pathological consequence of its failure.

Optimal APR should begin after several minutes, the first APP being detected within one-two hours, and last for 1-2 days. Afterwards, the host should return to normal function. The down-regulation of APR involves many inflammatory mediators, such as glucocorticoids, cytokines including IL-4 and IL-10, and receptor antagonists for certain pro-inflammatory cytokines, such as IL-1Ra and IL-6Ra [7, 47]. Glucocorticoids (cortisol) play a major role in modulating the APR. Cortisol enhances IL-6-mediated APP production. It also reduces the release of pro-inflammatory cytokines, decreases capillary permeability and leucocyte recruitment, stabilises lysosomal membranes, and suppresses cells of the immune system. If acute inflammation becomes too chronic the acute-phase reaction can also be prolonged [1].

APR is not uniformly beneficial. A failure to control acute-phase process has severe pathological consequences. Secondary amyloidosis due to elevated SAA concentration in patients with chronic inflammatory conditions has been previously mentioned. Inflammation-associated cytokines have been implicated in the pathogenesis of anemia in chronic diseases; examples of their involvement include the decreased responsiveness of erythrocyte precursors to erythropoietin, decreased production of erythropoietin, and impaired mobilization of iron from macrophages [48].

Septic shock is the consequence of an uncontrolled activation of host response against bacteria. Very aggressive stimulation, or an uncontrolled AP systemic response, inevitably results in an over-activation of pro-inflammatory cytokines that directly affect organ function, but also acts indirectly through secondary mediators.These secondary mediators include nitric oxide, thromboxanes, leukotrienes, platelet-activating factor, prostaglandins, and complement. Both these primary and secondary mediators cause the activation of the coagulation cascade, the complement cascade and the production of prostaglandins and leukotrienes. Endothelial cell damage occurs, which affects profusion of the organs and can lead to multiple organ system failure. Activation of the coagulation cascade can then lead to disseminate intravacular coagulopathy (DIC) and adult respiratory distress syndrome (ARDS). Leukocytosis may also be hazardous to the host, since it may be associated with

tissue destruction. In septic shock for example, uncontrolled leukocytosis and intravascular leukocyte activation lead to irreversible damage to endothelium and to organs and tissues [49].

Finally, the persistence of some systemic acute phase phenomena, such as fever, anorexia, gluconeogenesis and muscle protein catabolism because of long-term stimulation, such as in cancer, uncontrolled sepsis or immunological disorders, eventually lead to metabolic disorders that result in cachexia.

APP in clinical pathology and pharmacology

APP affects protein binding to several drugs, and therefore their distribution in blood and their therapeutic availability for organs and tissues. The most important protein in this respect is probably AGP, which binds basic and neutral drugs, like tamoxifen [50], trimethoprim and erythromycin [51, 52, 53]. The pharmacokinetic implications are important, since variations in AGP levels can consistently alter the free plasma level of the drug, without affecting its total plasma concentration. Moreover, many enzymes that metabolise drugs are down-regulated during the acute phase [54]. Interestingly, AGP also binds molecules that modulate its expression, such as retinoic acid [55] and cortisol [56].

APPs are of very valuable diagnostic significance. CRP is currently the most important analyte in humans, and provides diagnostic information on the presence of inflammatory lesions. In some diseases, like rheumatoid arthritis, the CRP values also possess prognostic meaning [57]. SAA concentration is also a marker of inflammation, being more sensitive than CRP for inflammatory diseases [58], but assays for this protein are not currently available for wide scale use.

APP in domestic animals

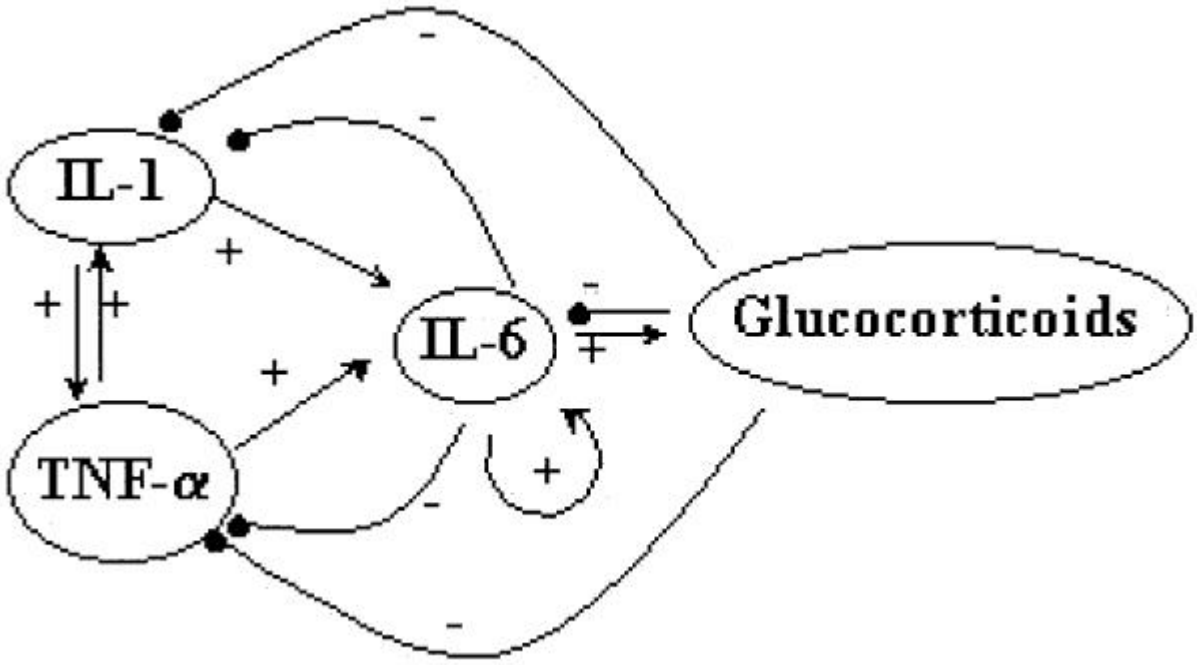
APR is characterized by a uniform nature. Nevertheless, expression levels can differ widely from species to species, and some proteins that are considered acute phase proteins for one species, may not be the same in another. Plasma APP families have their representatives in different species. For example CRP is practically negligible in healthy humans but is a major APP in man, dog and pig, whereas it is present in healthy ruminants and its serum concentration is only slightly modified during inflammation [59]. Hp is a major APP in ruminants, but in dogs is a constitutive serum protein, and its increase is very modest during disease. Human Hp is a constitutively secreted plasma protein that exhibits only a moderate increase during APR.

CONCLUSIONS

APPs play a very important defensive role. Their expression represents one of the most important effector mechanisms of innate immunity. This non-specific host mechanism is very effective. It can be readily mobilised (few minutes) and provide the tools necessary for rapid control of aggression from the pathologic agent, containing the damage until lymphocytes and other defensive mechanisms can begin to deal with it (4-6 days). However the role of APP is not only that of support to adaptive immunity. It is definitely more complex. The wide range of defensive and repair function fulfilled by APP not only reduces pathologic damage, but also restores the homeostasis. The complex network of agonist, antagonist and feedback loops ensure that this response is optimised for counteracting the menace, and assure the strictest control to avoid detrimental effects.

Several questions relating to the control of transcription factors, in particular the precise unfolding of the cytokine network remain unanswered. The knowledge of these very complex APP expression regulatory pathway is essential in order to develop safe and effective anti-inflammatory therapies against pathologies, such as amyloidosis, or pathological conditions, i.e. cachexia, that are often a consequence of the failure of defensive mechanism regulation.

Neuroendocrine changes	Fever, Poor appetite and Somnolence. Increased secretion of ACTH, Cortisol and Catecholamines.
Metabolic changes	Increased protein catabolism Hepatic production of APP Increased hepatic lipogenesis Increased adipose tissue lipolysis Decrease in bone mass Increased gluconeogenesis
Hematopoietic changes	Anemia (in chronic disease) Leukocytosis Thrombocytosis
Changes in non-protein plasma constituents	Hypozincemia, hypoferremia, and hypercupremia Increased plasma retinol and glutathione concentrations


IL-1
IL-6
TNF-α
Glucocorticoids
+
+
+
+
+
+
-
-
-
-
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