

REVIEW ARTICLE

CYTOKINES AND THE HEPATIC ACUTE PHASE RESPONSE

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SUMMARY

The acute phase response is an orchestrated response to tissue injury, infection or inflammation. A prominent feature of this response is the induction of acute phase proteins, which are involved in the restoration of homeostasis. Cytokines are important mediators of the acute phase response. Uncontrolled and prolonged action of cytokines is potentially harmful, therefore mechanisms exist which limit the activity of cytokines; these include soluble cytokine receptors and receptor antagonists. The cytokine signal is transmitted into the cell via membrane-bound receptors. Different intracellular signalling pathways are activated by different cytokine-receptor interactions. Eventually, cytokine-inducible transcription factors interact with their response elements in the promoter region of acute phase genes and transcription is induced. Systemic inflammation results in a systemic acute phase response. However, local inflammatory or injurious processes in the liver may also induce an acute phase response, for example after partial hepatectomy and during hepatic fibrosis. The acute phase proteins induced in these conditions probably act to limit proteolytic and/or fibrogenic activity and tissue damage. The possible function of the acute phase protein α 2-macroglobulin in hepatic fibrosis is discussed in some detail. © 1997 by John Wiley & Sons, Ltd.

KEY WORDS—acute phase proteins; acute phase response; cytokines; HGF; liver fibrosis; liver regeneration; TGF- β ; α 2-macroglobulin; extracellular matrix; growth factors; inflammation; interleukins; signal transduction

INTRODUCTION

Under normal circumstances, maintenance of homeostasis in mammals, including man, is guaranteed by a number of mechanisms. Deviations from this stable situation may occur which impose a serious threat to the health of the organism. The organism responds to these challenges, such as tissue injury and infection, by a coordinated sequence of systemic and metabolic changes, or by local changes such as inflammatory reactions, collectively known as the acute phase response.^{1–3} The purposes of these responses are to restore homeostasis and to remove the cause of its disturbance. Characteristic features of the systemic acute phase response include (i) fever, (ii) neutrophilia, (iii) changes in lipid metabolism, (iv) hypoferraemia and hypozincaemia, (v) increased gluconeogenesis, (vi) increased (muscle) protein catabolism and transfer of amino acids from muscle to liver, (vii) activation of the complement and coagulation pathways, (viii) hormonal changes, and (ix) induction of acute phase proteins.^{1–3}

ACUTE PHASE PROTEINS

One of the prominent features of the acute phase proteins is the appearance, or rapid increase in concen-

tration, of a number of plasma proteins, collectively known as acute phase proteins. The synthesis and plasma level of some proteins, notably albumin, are decreased, hence their designation as negative acute phase proteins. Acute phase proteins are synthesized almost exclusively in the liver and most are glycosylated. They serve important functions in restoring homeostasis after infection or inflammation.^{1–3} These include haemostatic functions (e.g., fibrinogen), microbicidal and phagocytic functions (e.g., complement components, C-reactive protein), antithrombotic properties (e.g., α 1-acid glycoprotein), and antiproteolytic actions which are important to contain protease activity at sites of inflammation (e.g., α 2-macroglobulin, α 1-antitrypsin, and α 1-antichymotrypsin).

Acute phase proteins can be divided into two groups: type I and type II acute phase proteins.^{2,3} Type I proteins include SAA (serum amyloid A), CRP (C-reactive protein; human), complement C3, haptoglobin (rat), and α 1-acid glycoprotein, and are induced by interleukin-1 (IL-1)-like cytokines, which comprise IL-1 α , IL-1 β , tumour necrosis factor (TNF)- α and TNF- β . Type II proteins include fibrinogen, haptoglobin (human), α 1-antichymotrypsin, α 1-antitrypsin, and α 2-macroglobulin (rat). Type II proteins are induced by IL-6-like cytokines which include IL-6 and its family members LIF (leukaemia inhibitory factor), IL-11, OSM (oncostatin M), CNTF (ciliary neurotrophic factor), and CT-1 (cardiotrophin-1).

In general, IL-6-like cytokines synergize with IL-1-like cytokines in the induction of type I acute phase proteins,

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whereas IL-1-like cytokines have no effect on, or even inhibit, the induction of type II acute phase proteins.²

CYTOKINE RECEPTORS

IL-1-like cytokines

Two types of IL-1 receptor (IL-1R) exist, the 80 kD IL-1R type I and the 60–68 kD IL-1R type II.^{4,5} Both receptors belong to the immunoglobulin superfamily, but represent distinct gene products. The type I receptor is a low-affinity receptor. After binding of IL-1 to the type I receptor, the complex interacts with the IL-1R-accessory protein to yield a high-affinity receptor.⁴ The type I receptor is responsible for the transmission of the IL-1 signal, including the induction of type I acute phase proteins.^{4,6} The type II receptor is considered to function as a decoy receptor for IL-1 and does not generate a signal.^{4,6} The type II IL-1 receptor is present on HepG2 hepatoma cells.^{5,6} It has been suggested that the type II IL-1R partly mediates the effects of IL-1 on the human hepatoma cell line HepG2,⁷ but the biological significance of this finding remains to be determined. After ligand binding, the type I IL-1R is rapidly phosphorylated at serine/threonine residues.⁸ The IL-1 ligand–receptor complex is internalized and translocated to the nucleus.⁴ The significance of these translocation events for the execution of the IL-1 signal is not clear, since signalling events are initiated before translocation to the nucleus.⁴

Receptors for TNF- α are also in two forms, type I (55 kD) and type II (75 kD). Both receptor types belong to the immunoglobulin superfamily, but are not homologous to the IL-1 receptors.^{9–11} Both are present on hepatocytes, although the basal level of expression is low;^{12,13} their expression is increased during acute or chronic liver inflammation.¹³ Induction of acute phase proteins by TNF- α is probably mediated by the type I (55 kD) receptor, which is also involved in TNF- α -induced apoptosis.^{14,15} Trimeric TNF- α probably binds to dimeric receptors.⁹ Internalization of complexes of TNF- α and its receptor and subsequent translocation to a lysosomal compartment have been described.¹⁶

IL-6-like cytokines

The prototypical IL-6 receptor (IL-6R) is composed of an 80 kD α -subunit, IL-6Ra, which, after binding of IL-6, complexes with two signal-transducing gp130 β -subunits.¹⁷ This constitutes the active ligand–receptor complex. Dimerization of the β -subunits is essential for receptor activation.¹⁷ Receptors for the other members of this family have one gp130 β -subunit in common and one LIFR- β subunit (LIFR, CNTFR, and CT-1R) or another gp130 β -subunit (IL-11)^{17–26} (Table I). Two types of OSM receptor exist. Type I is identical to the LIF receptor, while type II is composed of a gp130 β -subunit and a putative OSM-specific β -subunit.^{18–22} α -Subunits are known only for IL-6, IL-11, and CNTF^{17,25} (Table I). These ligands first bind their α -subunits and subsequently induce homo- or heterodimerization of the signalling β -subunits. LIF, OSM,

Table I—IL-6-like cytokine receptors

Cytokine	α -Subunit	Signalling β -subunits
IL-6	IL-6Ra	2 \times gp130
LIF		1 \times gp130+1 \times LIFR β
OSM		1 \times gp130+1 \times LIFR β (type I) 1 \times gp130+1 \times OSMR β (type II)
CNTF	CNTFRa	1 \times gp130+1 \times LIFR β
IL-11	IL-11Ra	2 \times gp130
CT-1		1 \times gp130+1 \times LIFR β

and CT-1 bind directly to their signalling subunits and induce heterodimerization.^{17–26} Owing to the sharing of identical subunits, a particular cytokine can bind to different receptors and a particular receptor can bind different cytokines. This explains the pleiotropic and redundant actions of IL-6-like cytokines. Because not all cell types express the same pattern of receptor subunits, some degree of specificity of cytokine action is achieved.

SIGNAL TRANSDUCTION

Since a detailed discussion of cytokine signal transduction pathways is beyond the scope of this review, a simplified summary, highlighting the key events, will be presented.

IL-1-like cytokines

Activation of TNF/IL-1 receptors initiates the conversion of membrane sphingomyelin to ceramide via sphingomyelinase.^{4,27,28} Subsequently, ceramide-activated protein kinases connect to several signalling pathways which ultimately lead to activation and translocation of transcription factors AP-1 (activating protein-1; *c-jun/c-fos* heterodimer) and nuclear factor (NF)- κ B. NF- κ B is activated and translocated to the nucleus after phosphorylation and degradation of the inhibitory subunit I κ B. In addition, the IL-1 signal connects to the mitogen activated protein (MAP)-kinase pathway, ultimately activating transcription factor NF-IL-6 (syn. LAP, IL-6DBP, C/EBP β). Many type I acute phase protein genes contain NF- κ B, NF-IL-6, and AP-1 response elements in their promoter regions.^{4,27,28}

IL-6-like cytokines

Activation of the IL-6 receptor complex activates JAK tyrosine kinases.^{28–31} Subsequent tyrosine phosphorylation of STAT proteins (signal transducers and activators of transcription), including STAT3, also known as APRF (acute phase response factor), induces STAT protein homo- and heterodimerization and translocation to the nucleus, where they bind to their response elements.^{29,32–34} Type II acute phase protein genes contain a hexanucleotide motif (CTGGGA) which is an IL-6 response element.^{32–34} This motif is recognized by APRF and other IL-6-inducible transcription factors.^{29,34} In addition, the IL-6 signal transduction

pathway activates the MAP-kinase pathway, connecting the IL-6 and IL-1 signalling pathways and converging on NF-IL-6, another IL-6 response element.²⁸ In common with type I proteins, many type II acute phase proteins contain NF-IL-6 binding sites in their promoter regions.

ACUTE PHASE RESPONSE AND IL-6 FAMILY MEMBERS

IL-6 is the prototypical member of the IL-6-like cytokine family. IL-6 induces all type II acute phase proteins. In addition, all members of the IL-6-like cytokine family are able to induce (a subset of the) type II acute phase proteins. These cytokines were originally identified and characterized by their actions on non-hepatic cells.

Leukaemia inhibitory factor [syn. hepatocyte-stimulating factor-III (HSF-III), differentiation factor, etc.] maintains the pluripotentiality of embryonic stem cells, promotes the survival of neurons, induces a switch in neurons from an adrenergic phenotype to a cholinergic phenotype, promotes the proliferation of megakaryocyte progenitors and myoblasts, and promotes monocytic differentiation of leukaemia cell lines.^{35,36} LIF induces type II acute phase proteins.³⁷⁻³⁹ In several inflammatory conditions and during an acute phase response induced by endotoxin administration or septic shock, levels of LIF are increased in plasma and inflammatory body fluids.^{40,41} Interestingly, LIF pretreatment protects against lethal septic shock in mice induced by challenge with *Escherichia coli* bacteria.^{41,42} This protective effect was accompanied by a reduced peak of serum TNF- α and a decreased number of viable bacteria after challenge.^{41,42} The protective effect of LIF was only present when given before challenge and was enhanced by simultaneous administration of IL-1 or TNF- α . This phenomenon resembles endotoxin tolerance, a transient state characterized by unresponsiveness to stimulation by endotoxin, IL-1, or TNF- α . Endotoxin tolerance is induced by pretreatment with low doses of endotoxin, TNF- α , or IL-1 and is mediated in part by increased expression of IL-10 and TGF- β .⁴³ Together, these results suggest that systemic LIF production plays an important role in the host response against infection. However, the continuous presence of high levels of LIF induces severe cachexia in mice, thus resembling the action of TNF- α or inducing TNF- α .^{42,44}

Oncostatin M is expressed by leukaemic and lymphocytic cell lines, activated lymphocytes, and normal adherent macrophages, and has variable effects on the proliferation and differentiation of normal and tumour cells. OSM inhibits the proliferation of aortic endothelial cells, melanoma cells, and other carcinoma cell lines. OSM also increases LDL-receptor expression in HepG2 cells and stimulates plasminogen activator activity in endothelial cells. Induction of type II acute phase proteins has been reported.^{17,45}

Interleukin-11 was originally identified as a plasmacytoma growth factor and is produced by bone marrow stromal cells. Its activities include the promotion of

megakaryopoiesis and erythropoiesis, the enhancement of antibody production of mature B cells, and the induction of acute phase proteins.^{17,23-25,46}

Ciliary neurotrophic factor is normally expressed in non-neuronal cells of the central and peripheral nervous tissue. It promotes the survival of ciliary and motor neurons and induces the differentiation of oligodendrocytes into astrocytes.^{17,47,48} Owing to the restricted expression of CNTF and its receptor, its relevance in the induction of acute phase proteins is not clear.^{47,48} As suggested by Schooltink *et al.*, CNTF may either induce acute phase proteins in extrahepatic cells, such as choroid plexus cells in the nervous system, or act locally in the liver when peripheral nerves are damaged.⁴⁸

Cardiotrophin-1 was originally identified as a factor which induces hypertrophy of cardiac myocytes. It has several actions in common with LIF, OSM, and CNTF. CT-1 promotes the survival of neurons, maintains the pluripotentiality of embryonic stem cells, and inhibits the proliferation of leukaemia cell lines.^{26,49-51} The actions of CT-1 are mediated by the LIF receptor. Expression of CT-1 mRNA has been reported in whole liver tissue, but not in hepatoma cell lines or isolated hepatocytes, indicating that CT-1 expression in the liver may be restricted to non-parenchymal cells.⁴⁹⁻⁵¹

Although IL-6-like cytokines are able to induce type II acute phase proteins, their true relevance for the acute phase response is not clear. It is unknown whether the liver is exposed to all these cytokines *in vivo*. It is possible that some of these factors (e.g., CNTF) act very locally at extrahepatic sites and will never attain systemic plasma levels sufficient to induce acute phase proteins *in vivo*, like IL-6 and LIF. Alternatively, these cytokines may be released locally in the liver under very specific conditions (e.g., CNTF and CT-1).

In this respect, it is interesting to note that mice deficient in IL-6 show a defective local inflammatory response elicited by turpentine injection, including a severely diminished induction of acute phase proteins.^{52,53} However, in a model of systemic inflammation (endotoxin administration), induction of acute phase proteins in IL-6-deficient mice is not impaired. Interestingly, induction of TNF- α after endotoxin administration in IL-6-deficient mice is increased several-fold compared with normal mice, suggesting a possible mechanism of compensation or release from a suppressive force in TNF- α regulation.⁵³ In addition, weight loss and hypoglycaemia occur during the systemic acute phase response in wild-type and IL-6-deficient mice. In contrast, during the local acute phase response, the weight loss and hypoglycaemia occur in wild-type mice but not in IL-6-deficient mice.⁵³ These results indicate that IL-6 is the predominant mediator of the local acute phase response. By contrast, in the systemic acute phase response, at least with regard to weight loss, hypoglycaemia, and induction of acute phase proteins, IL-6 is not essential. This may indicate that other cytokines take over the function of IL-6 during the systemic acute phase response. These cytokines need to be identified, but LIF is a likely candidate. Interestingly, the pattern of cytokine expression in monocytes, macrophages, and Kupffer cells during a systemic acute phase response

induced by endotoxin is different from a local acute phase response induced by turpentine injection.⁵⁴ The significance of differential expression patterns of cytokines remains to be elucidated.

SOLUBLE CYTOKINE RECEPTORS AND RECEPTOR ANTAGONISTS

Another layer of complexity in the regulation of the acute phase response is added by the presence of cytokine receptor antagonists and soluble cytokine receptors. IL-1 receptor antagonist (IL-1Ra) competes with IL-1 for binding to the IL-1 receptors, in particular IL-1R type I.^{4,55-57} Binding of IL-1Ra to IL-1R type I does not elicit a signal. IL-1Ra pretreatment attenuates the inflammatory response induced by IL-1 and the symptoms of septic shock and endotoxin administration, including the rise in serum IL-6 and induction of acute phase proteins.^{55,56,58-61} Induction of type I, but not type II, acute phase proteins by IL-1 was completely blocked by IL-1Ra in hepatoma cells lines.^{60,61} However, in cultured human hepatocytes IL-1Ra does not block the IL-1 induction of the type I acute phase proteins CRP and SAA, suggesting that these proteins do not behave as true type I proteins in human hepatocytes.⁶¹ In contrast, IL-1Ra opposes the inhibitory effect of IL-1 on IL-6-induced type II acute phase proteins such as fibrinogen.⁶² IL-1Ra is expressed in the liver and in hepatoma cells, suggesting a role in regulating IL-1 activity in the liver.⁶³ In summary, IL-1Ra attenuates the acute phase response both *in vivo* and *in vitro*, although species differences may exist.

Soluble cytokine receptors have been described for IL-1, TNF- α , IL-6, CNTF, and possibly IL-11. Soluble (s) IL-1 receptors have been detected in plasma, urine, and inflammatory fluids, and identified as the extracellular domains of the type I and type II IL-1 receptors.^{4,64-66} sIL-1R type II levels are about ten-fold higher than sIL-1R type I levels. Levels of sIL-1 receptors (type II >> type I) are increased during sepsis or inflammatory disorders.⁶⁴⁻⁶⁶ Soluble IL-1 receptor type II is shed into the circulation and is able to bind IL-1, yielding an inactive ligand-receptor complex. Shedding is induced by TNF- α and endotoxin.⁶⁵ Interestingly, sIL-1R type I, but not sIL-1R type II, is able to abolish the inhibitory action of IL-1Ra.⁶⁷ This is a unique example of two inhibitors of IL-1 activity cancelling each other's effect. Two soluble TNF- α receptors have been identified which correspond to the extracellular domains of the 55 kD and 75 kD TNF- α receptors.⁹⁻¹¹ They act as TNF- α antagonists and have been found in the serum and urine of normal individuals and patients with various inflammatory disorders and cancer. Interestingly, a soluble TNF- α :Fc fusion protein, able to bind and inactivate circulating TNF- α , protected experimental animals against septic shock, but was ineffective in patients with septic shock and even increased mortality in these patients when given at a high dose.^{68,69}

In contrast to soluble IL-1 and TNF receptors which act as antagonists, sIL-6Ra and sCNTFR α act as agonists. The soluble IL-6- and CNTF-receptors (sIL-6Ra

and sCNTFR α) bind their respective ligands, attach to the signal transducing receptor β -subunits, and induce cellular responses.¹⁷ Indeed, sIL-6Ra in combination with IL-6 has been shown to induce the synthesis of acute phase proteins *in vitro*.⁷⁰ This effect is partly dependent on the expression of membrane-bound IL-6Ra on the target cell. The agonistic effect of sIL-6Ra is maximal on cells with low membranous IL-6Ra expression (e.g., hepatoma cells) and minimal on cells with high membranous IL-6Ra expression (e.g., primary hepatocytes).⁷⁰ Interestingly, addition of sIL-6Ra alone also induced a response, indicating the presence of trace amounts of IL-6 (either endogenously produced or present in culture medium) or the ability of sIL-6Ra to activate directly the gp130 signal transducer.⁷⁰ sIL-6Ra is present in the circulation and inflammatory body fluids and a major fraction of circulating IL-6 is complexed to its soluble receptor.⁷¹ During the acute phase response, mRNA levels for IL-6Ra are increased.⁷² This increase could be mediated by IL-6 and glucocorticoids, which are increased during the acute phase response and induce IL-6Ra-mRNA *in vivo*. *In vitro*, glucocorticoids and OSM, but not IL-6, are potent inducers of IL-6R mRNA.⁷³ IL-1 inhibited the glucocorticoid-induced IL-6Ra mRNA but enhanced the expression of sIL-6Ra mRNA.⁷³ The mRNA level of the signal-transducing gp130 subunit in HepG2 cells is enhanced by IL-6 and glucocorticoids.⁷⁴ sIL-6Ra is produced from the intact IL-6Ra either by shedding of the membrane-bound form or by alternative splicing yielding only the extracellular domain.⁷⁰ Since sIL-6Ra acts as an agonist, the results indicate that during the acute phase response increased levels of both IL-6 and its receptor are present, thus potentially enhancing the effects of IL-6 during the acute phase response. Clearly, the presence of cytokine receptor antagonists and soluble receptors constitutes an important mechanism to regulate cytokine activity.

ACUTE PHASE RESPONSE AND GROWTH FACTORS

Apart from the IL-1-like and IL-6-like cytokines, several other factors, usually associated with the regulation of proliferation, are able to modulate the synthesis of acute phase proteins, including hepatocyte growth factor (HGF) and transforming growth factor- β (TGF- β). HGF is a mitogen for normal hepatocytes.⁷⁵ Expression of HGF mRNA and HGF plasma levels are increased after liver injury and partial hepatectomy.⁷⁵ The effects of HGF on acute phase protein synthesis are very diverse. In rat hepatocytes cultured at high cell density (no proliferation), but not at low cell density (proliferation), HGF stimulated albumin synthesis.⁷⁶ In rat hepatoma cells, HGF stimulated both basal and IL-6-induced type II acute phase proteins, in particular α 2-macroglobulin, and reduced type I acute phase proteins.⁷⁷ In human hepatocytes, contrary to the effects of IL-6, HGF increased albumin synthesis and decreased α 1-antichymotrypsin and haptoglobin synthesis.⁷⁸ These effects of HGF could be partially reversed by IL-6. Like IL-6, HGF induced the synthesis of fibrinogen and

$\alpha 1$ -antitrypsin. However, in contrast to IL-6, HGF had no effect on CRP.⁷⁸ Surprisingly, HGF induced $\alpha 2$ -macroglobulin synthesis in human hepatocytes; this is not an acute phase protein in man.⁷⁸ These results indicate that the effect of HGF on both basal and cytokine-induced acute phase protein synthesis is variable and may depend on the species and proliferative state of the target cell.

The same is true for TGF- β . This growth factor is mitogenic to normal hepatocytes and plays an important role in local inflammatory reactions (fibrosis) and wound healing.⁷⁵ TGF- β has diverse and sometimes inconsistent effects on acute phase protein synthesis. In general, TGF- β decreases albumin synthesis and inhibits both basal and IL-6-induced fibrinogen synthesis.^{79–83} TGF- β increases basal and IL-6-induced synthesis of $\alpha 1$ -protease inhibitor and $\alpha 1$ -antichymotrypsin.^{81–83} The effects on $\alpha 1$ -acid glycoprotein and haptoglobin are conflicting.^{81–83} Again, it is likely that the modulatory effect of TGF- β on acute phase protein synthesis is dependent on the cell type, the species, and the proliferative state of the hepatocytes. Finally, several hormones are able to modulate the response of acute phase proteins to cytokines, notably glucocorticoids, which augment the response to cytokines and insulin, which attenuates the cytokine-induced rise in acute phase proteins.^{84–87}

In summary, the acute phase response is regulated in a highly complex fashion. This complexity accounts for the diversity of changes occurring during the acute phase response and is necessary for controlling the action of cytokines in order to avoid deleterious effects (Fig. 1).

ACUTE PHASE RESPONSE DURING LIVER REGENERATION

An acute phase response occurs during liver regeneration after partial hepatectomy. The pattern of acute phase proteins after partial hepatectomy resembles the pattern observed during a systemic acute phase response.^{88,89} The function of acute phase proteins during the regenerative phase is probably related to host defence against infection, which is compromised during the regenerative phase as a result of diminished clearance and phagocytic capacity of Kupffer cells. Alternatively, they may act to control proteolytic activity during the regeneration period. The situation of increased acute phase protein synthesis concomitant with regeneration is a particularly challenging one for the liver, since the liver has to express differentiated functions (acute phase protein synthesis) and to regenerate at the same time.^{90,91} In general, expression of differentiated functions and proliferation are mutually exclusive. The regenerating liver may circumvent this dilemma, since not all hepatocytes in the remnant liver divide at the same time. Indeed, at any time during regeneration, a substantial proportion of the hepatocytes are not proliferating and express differentiated functions.⁹⁰ Non-dividing hepatocytes may account for the plasma protein synthesis during regeneration and these hepatocytes presumably have enough capacity to compensate

for the proliferating hepatocytes and loss of liver mass. Indeed, after partial hepatectomy, mRNA and plasma levels of most acute phase proteins are comparable to the levels observed during a systemic acute phase response.^{88,89} After partial hepatectomy, an increase in the plasma level of IL-6 is observed, whereas the plasma levels of TNF- α do not change.⁹² Kupffer cell synthesis of both IL-1 and IL-6 is increased after partial hepatectomy and increased hepatic expression of TNF- α has been documented.^{92–94}

A possible trigger for cytokine induction after hepatectomy is the increased exposure to reactive oxygen species, resulting in activation of transcription factor NF- κ B and induction of TNF- α .^{90,91,95,96} This is considered to be an immediate early response to liver injury.^{95,96} TNF- α is able to induce IL-1 and IL-6 expression. In addition, cytokine expression could be induced or sustained by increased exposure to gut-derived endotoxin, due to increased supply or decreased clearance of endotoxin by the remnant liver. The precise sequence of events in the period immediately following partial hepatectomy remains to be established.

Interestingly, the cytokines released after hepatectomy influence the regenerative response. Pretreatment of rats with anti-TNF- α inhibits the regenerative process after partial hepatectomy and attenuates the rise in hepatic *c-jun* expression and plasma IL-6.^{92,97} In addition, regeneration after hepatectomy in germ-free (and therefore endotoxin-free) mice is delayed, indicating that endotoxin-induced cytokines, in particular TNF- α , promote liver regeneration.⁹⁸ In accordance with this, TNF- α stimulates hepatocyte proliferation *in vitro*.⁹⁷ Kupffer cell depletion prior to partial hepatectomy accelerates liver regeneration.⁹⁴ This may be due to increased hepatic expression of TNF- α or HGF in Kupffer cell-depleted regenerating liver.^{94,99} Alternatively, increased regeneration may result from the absence of Kupffer cell-derived IL-1 (and possible IL-6), which is incriminated in the termination of liver regeneration.^{100,101} *In vitro*, IL-1 and to a lesser extent IL-6 inhibit hepatocyte proliferation.⁷⁵

CYTOKINES, ACUTE PHASE RESPONSE, AND LOCAL HEPATIC INFLAMMATION/FIBROGENESIS

Hepatic fibrosis is a local inflammatory process in the liver, characterized by increased deposition of extracellular matrix components, most notably collagens.^{75,102,103} Fibrogenesis is an extremely complex process in which locally produced cytokines and growth factors act on several cell types. A key event in the development of hepatic fibrosis is the activation of hepatic stellate cells (syn. Ito cells, fat-storing cells)^{75,102,103} and increased expression of TGF- β . Matrix synthesis in hepatic stellate cells is increased by TGF- β . TGF- β is expressed by many cell types, including activated stellate cells and Kupffer cells.^{102,103} Since TGF- β increases its own expression and secretion in activated stellate cells, an autocrine amplifying loop is created, which promotes the synthesis of matrix

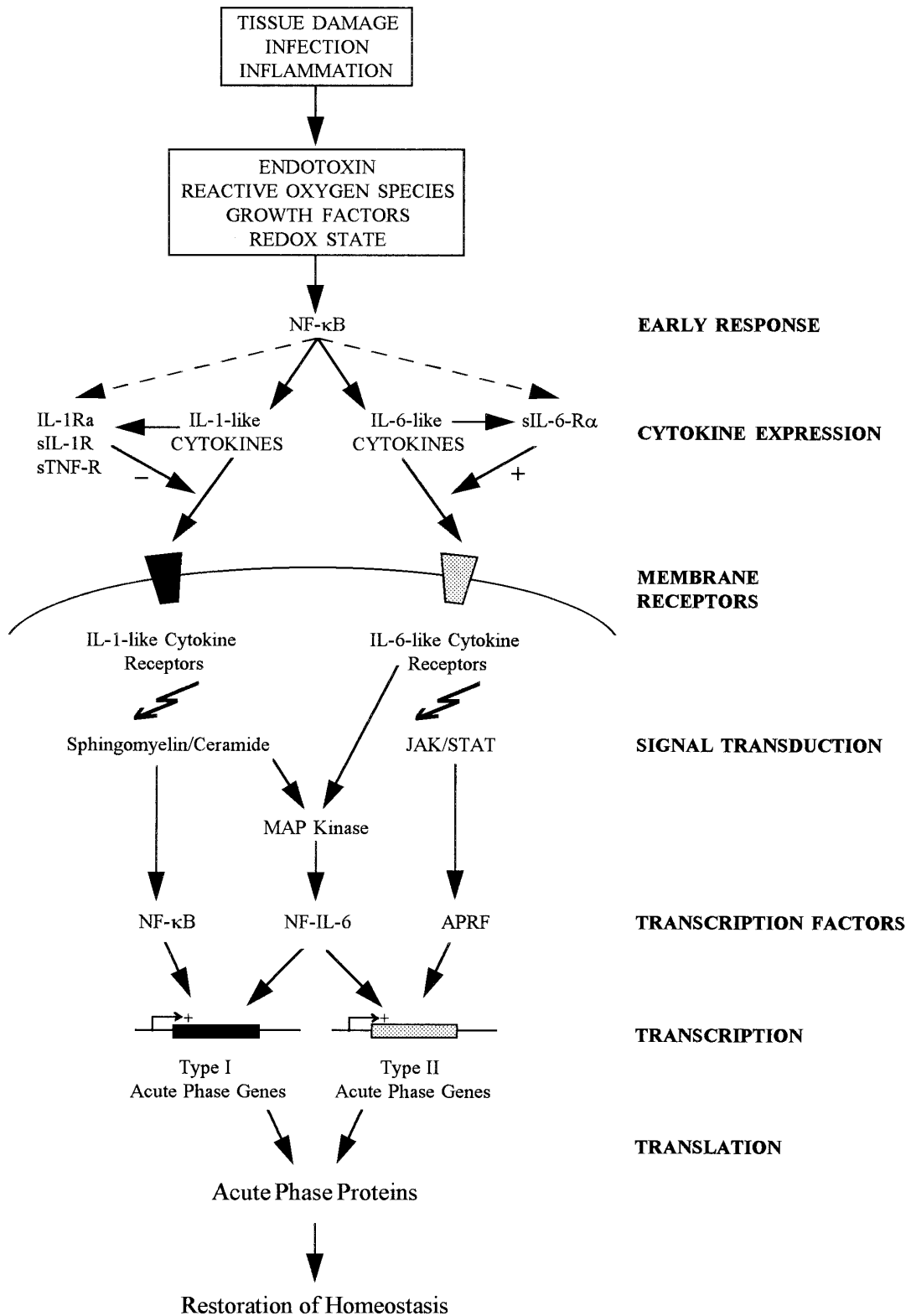


Fig. 1—Flow-chart depicting the main events leading to induction of the acute phase response. The initial trigger leads to rapid activation of transcription factor NF-κB, resulting in increased expression of cytokines. Binding to cytokine receptors initiates signalling events, leading to activation of several transcription factors. These factors bind to their response elements in the promoter regions of acute phase genes. Transcription of acute phase genes is induced and acute phase proteins are secreted. In the extracellular milieu, acute phase proteins function to restore homeostasis

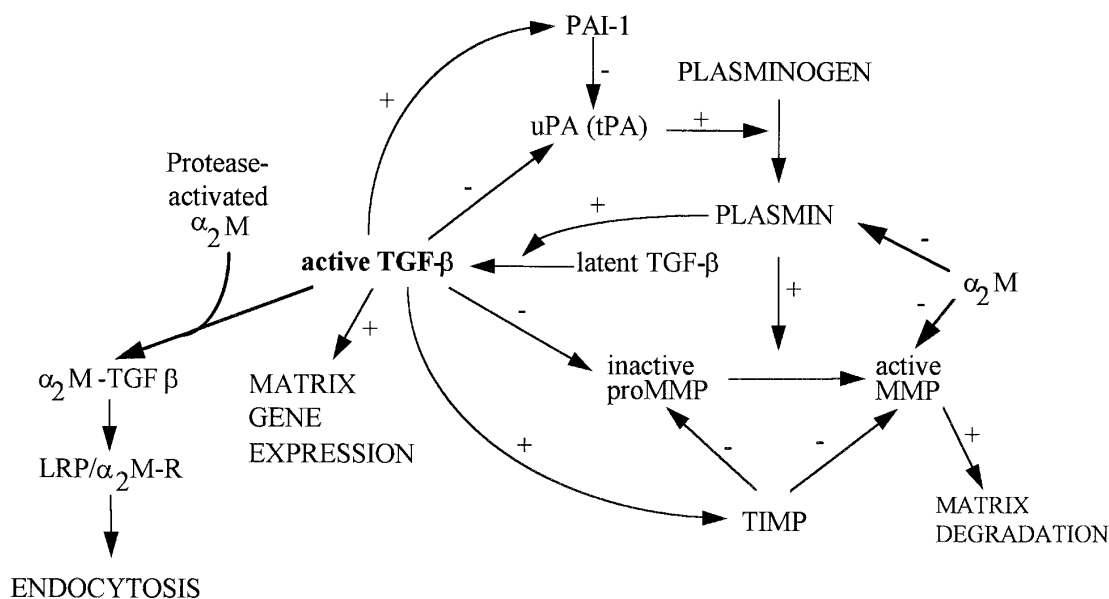


Fig. 2—Simplified scheme depicting the key events resulting in excessive matrix deposition during liver fibrosis. The profibrogenic actions of TGF- β include increased matrix synthesis, decreased matrix metalloproteinase (MMP) synthesis, and increased TIMP (tissue inhibitor of metalloproteinase) synthesis. Plasmin activates latent TGF- β and latent proMMP. The antifibrogenic actions of α_2 -macroglobulin (α_2 -M) include inhibition of plasmin-mediated TGF- β activation and scavenging of TGF- β . Profibrogenic actions of α_2 -M could be inhibition of MMP (activation). Feed-back loops: (1) TGF- β stimulates PAI-1 (plasminogen activator inhibitor type 1) synthesis and inhibits uPA expression, resulting in decreased plasmin activity; (2) TGF- β increases α_2 -M synthesis. + denotes stimulation or increase; - denotes inhibition or decrease. tPA=tissue type plasminogen activator

components. TGF- β also induces the expression of platelet-derived growth factor (PDGF) receptors on activated stellate cells, rendering these cells responsive to the mitogenic effect of PDGF.¹⁰³ Increased expression of PDGF and its receptor has been reported in fibrotic liver.¹⁰⁴ In contrast, expression of HGF by activated hepatic stellate cells is decreased.⁷⁵ HGF is a powerful mitogen for hepatocytes. Together, these changes result in a progressive proliferation of matrix-producing stellate cells, whereas the regenerative potential of hepatocytes is diminished.

Proteolytic events play an important role in the initiation and perpetuation of fibrosis. TGF- β is secreted as an inactive latent precursor. Activation of latent TGF- β into active TGF- β is protease-mediated, probably by plasmin provided by endothelial cells.^{102,105} Plasmin activity is regulated by plasminogen activators, in particular urokinase type plasminogen activator (uPA), which convert inactive plasminogen into active plasmin (Fig. 2). The plasminogen activators are under the control of plasminogen activator inhibitor type 1 (PAI-1). An inhibitory feed-back loop is established, since active TGF- β induces PAI-1 and reduces plasminogen activator synthesis, thus limiting its own activation^{106,107} (Fig. 2).

TGF- β is a powerful profibrogenic factor (Fig. 2). TGF- β directly stimulates matrix gene transcription and synthesis,^{102,108} while it inhibits interstitial matrix degradation via inhibition of matrix metalloproteinase (MMP) synthesis and increased synthesis of TIMPs (tissue inhibitor of metalloproteinases)¹⁰⁸ MMPs are synthesized as inactive precursors (proMMPs) which are activated by plasmin.¹⁰⁸ TIMPs inhibit both the active

MMPs and the conversion of latent proMMPs into active MMPs (Fig. 2).

Cytokines modulate both the proliferative responses and the matrix-producing processes. Some of those known to induce acute phase proteins are locally released during the development of liver fibrosis. However, it is not clear whether the complete spectrum of acute phase proteins is induced and what role these proteins have in the fibrotic process. Several acute phase proteins, in particular protease inhibitors, have the potential to influence the fibrotic process. In this respect, the acute phase protein and protease inhibitor α_2 -macroglobulin has received considerable attention. α_2 -Macroglobulin is able to interfere in the fibrotic process in several ways (Fig. 2):

(1) α_2 -Macroglobulin inhibits plasmin, thus limiting the activation of TGF- β and the plasmin-catalysed conversion of latent proMMPs into active MMPs.¹⁰⁹

(2) Protease-activated α_2 -macroglobulin binds TGF- β , yielding an α_2 -macroglobulin-TGF- β complex which can be endocytosed and degraded via the low-density lipoprotein-receptor related protein (LRP)/ α_2 -macroglobulin receptor on hepatocytes, stellate cells, and other inflammatory cells.¹¹⁰⁻¹¹² Interestingly, TGF- β induces α_2 -macroglobulin synthesis in hepatic stellate cells, establishing an inhibitory feed-back loop.¹¹⁰

(3) α_2 -Macroglobulin is known to bind and modulate the activity of several cytokines and growth factors, including IL-1, IL-6, TNF- α , TGF- β , and PDGF.^{111,112} In general, native α_2 -macroglobulin (not activated by protease) functions as a cytokine and growth factor

carrier or reservoir. PDGF bound to native $\alpha 2$ -macroglobulin is not recognized by its receptor, but is protected from degradation. In contrast, protease-activated $\alpha 2$ -macroglobulin functions as a cytokine and growth factor scavenger. Protease-activated $\alpha 2$ -macroglobulin, complexed to cytokines or growth factors, is recognized and endocytosed by the LRP/ $\alpha 2$ -macroglobulin receptor. Scavenging of active PDGF via this pathway could constitute a mechanism to control the proliferation of matrix-producing cells. Whether the 'reservoir' or 'scavenger' function prevails will depend on the balance between native and activated $\alpha 2$ -macroglobulin.^{111,112}

$\alpha 2$ -Macroglobulin can be synthesized by both hepatocytes and hepatic stellate cells. In hepatocytes, $\alpha 2$ -macroglobulin is regulated as an IL-6-inducible type II acute phase protein. In hepatic stellate cells, $\alpha 2$ -macroglobulin is induced by TGF- β . Both IL-6 and TGF- β are secreted by activated hepatic stellate cells.^{75,113} Together, these results suggest that $\alpha 2$ -macroglobulin is induced during hepatic fibrosis. Indeed, the induction of $\alpha 2$ -macroglobulin in stellate cells has recently been demonstrated in a model of hepatic fibrosis.¹¹⁴ Although it has been suggested that $\alpha 2$ -macroglobulin has antifibrotic properties *in vitro*,¹¹⁰ it remains to be determined whether modulation of $\alpha 2$ -macroglobulin activity could be of therapeutic value.

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