

## Leading article – Hepatology series

# Hepatocyte and immune system: acute phase reaction as a contribution to early defence mechanisms

Cytokines, a variety of hormone-like molecules, include a group of soluble factors – the interferons, interleukins, and hematopoietic growth factors. They build a complex network of interactive signals and are important for the regulation of hematopoiesis and many immune reactions. The interaction of the hepatocyte with the immune system may occur at different levels (Fig 1). For example viral infections often induce interferon (IFN) production, but can also stimulate synthesis of class II molecules, tumour necrosis factor and interleukin (IL) 2.<sup>1,2</sup> IFN activates other cytokines directly by interacting with IFN sequence motifs in their promoters.<sup>3</sup> Little information exists on the role of cytokines in the initiation and perpetuation of liver diseases. The level and activity of different cytokines such as IL 6 can be abnormal and may contribute to the injury in immunologically mediated liver diseases.<sup>4</sup> Another mechanism is the 'acute phase response', in which a panel of cytokines regulates different liver specific genes. There is detailed knowledge on how the signals of these cytokines are transduced from the surface of the hepatocyte membrane to the promotor of the liver specific gene: the mechanisms will be reviewed in this article.

### Acute phase response and protein

The acute phase response has been known for centuries. The ancient Greeks gave an initial description as they observed an increased sedimentation rate of erythrocytes in the blood of severely ill patients.<sup>5</sup> Today, we can explain this phenomenon as an increase in the plasma concentration of fibrinogen and other acute phase proteins.<sup>6</sup>

Most of the acute phase proteins are synthesised in hepatocytes, non-hepatic tissue is only a minor source of the acute phase proteins. Depending on their regulation during inflammation, they are called either positive or negative acute phase proteins. *Positive* means the plasma concentration is *increased* and *negative* means it is *decreased* during inflammation. In humans the most prominent positive acute phase proteins are C reactive protein (CRP) and serum amyloid A. In man an increase of up to 100 fold is observed during inflammation, whereas in rats the most

prominent changes are seen with  $\alpha$ 2-macroglobulin and  $\alpha$ 1 acid glycoprotein.<sup>5,7</sup> The table shows the most important acute phase proteins in man and rat and their maximum regulations due to cytokine induction during the acute phase response.

The acute phase response is well preserved throughout phylogeny and is believed to play an important protective role in the host defence against tissue damage and infection. CRP, a clinically useful diagnostic marker for patients suffering from an infection, has been shown to opsonise bacteria, parasites, foreign particles, and immune complexes and to facilitate their clearance by the immune system. Additionally, CRP binds to chromatin and mediates solubilisation of chromatin.<sup>8,9</sup> These observations have led to the suggestion that CRP might have a role as a 'garbage man' for chromatin fragments released from damaged cells during inflammation. Fibrinogen is involved in blood clotting and wound healing. Others like  $\alpha$ 2 macroglobulin,  $\alpha$ 1 antitrypsin and  $\alpha$ 1 antichymotrypsin are proteinase inhibitors.<sup>10-12</sup>

Based on our present knowledge, the acute phase proteins have a physiological role in the early stage of infection. They are immediately induced after infection and form a non-specific first line of defence against a broad range of invaders. This first line of defence is important as it provides the body with the time to activate the more specific part of the immune response, for example, humoral and cellular antigen specific response.

### Cytokines involved in the acute phase response

The first idea that blood-borne mediators are responsible for inducing the acute phase response in the liver was proposed in 1974.<sup>13</sup> Clear evidence that CRP was induced by blood-borne factors at local sites of inflammation was published in 1978.<sup>14</sup> Since then the list of factors inducing

### Acute phase proteins

Increase in plasma concentration			
10-100 fold	2-10 fold	Up to 2 fold	Decrease in plasma concentration
<b>Human:</b>			
C reactive protein	$\alpha$ 1 proteinase inhibitor	Coeruloplasmin	Inter $\alpha$ antitrypsin
Serum amyloid A	$\alpha$ 1 acid glycoprotein $\alpha$ 1 antichymotrypsin Fibrinogen Haptoglobin	C <sub>3</sub> of complement $\alpha$ 2 antiplasmin C <sub>1</sub> inactivator	Transferrin $\alpha$ 1 lipoprotein Prealbumin Albumin
<b>Rat:</b>			
$\alpha$ 2 macroglobulin	Fibrinogen Haptoglobin	$\alpha$ 1 proteinase inhibitor	$\alpha$ 1 inhibitor Transferrin
$\alpha$ 2 acid glycoprotein	Cysteine proteinase inhibitor	Prekallikrein Haemopexin C reactive protein Coeruloplasmin	Prealbumin Albumin Cytochrome P450s

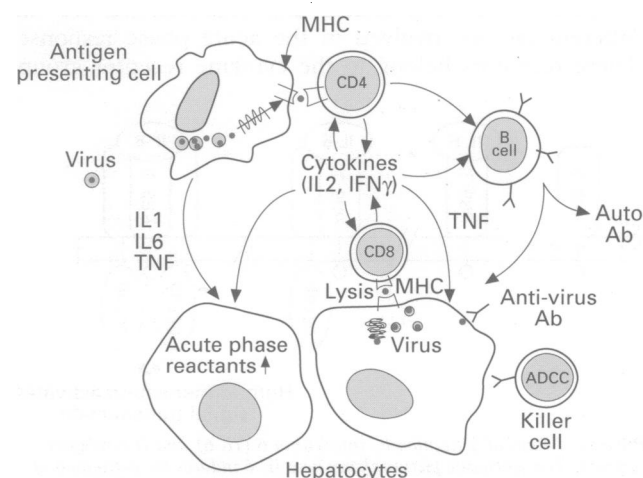


Figure 1: Potential interactions of cytokines released by the immune system and the hepatocytes. MHC=major histocompatibility complex; IL=interleukin; TNF=tumour necrosis factor; ADCC=antibody-dependent cell-mediated cytotoxicity; IFN $\alpha$ =interferon  $\alpha$ .

the acute phase response in the liver has increased rapidly and these have become well defined. At present the main mediators of the acute phase response in the liver are believed to be: IL 6 and IL 6-like mediators, IL 1, TNF, and glucocorticoids (Fig 1). Many different cell types may produce these cytokines, but the most important are activated macrophages, monocytes, fibroblasts, and endothelial cells.<sup>15-19</sup>

The term 'IL 6-like mediators' describes a group of cytokines, which change the expression of acute phase genes in a manner similar to IL 6. Some of these cytokines have been recently cloned and their role in the acute phase response has been described. IL 6-like mediators include IL 11, leukemia inhibitory factor, oncostatin M, and ciliary neurotropic factor.<sup>20-22</sup> This class of cytokines may have emerged from a common ancestral gene through gene duplication, since they show a significant similarity in the amino acid sequence, predicted secondary structure, and shared exon organisation.<sup>23</sup>

Two different forms of IL 1 exist –  $\alpha$  and  $\beta$ . They have a homology of 26% on the peptide level, but no difference in function. IL 1 is an important regulator of the acute phase response not only because of its direct effect on several acute phase genes, but also because it modulates the synthesis of IL 6. TNF has activities similar to IL 1,<sup>24</sup> but the changes seen with TNF are less potent than those observed after IL 1 administration in vivo and in vitro.<sup>25,26</sup> As with IL 1, two different forms,  $\alpha$  and  $\beta$  (lymphotoxin), exist.<sup>27</sup> They have a homology at 28% on the peptide level but no difference in function.

According to their differences in induction by cytokines, two classes of positive acute phase proteins are recognised. Class 1 genes are mainly regulated by IL 1 or by combinations of IL 1 plus IL 6 or IL 1, IL 6, and glucocorticoids. Examples of this class are CRP, serum amyloid A, complement C3, haemopexin, haptoglobin, and  $\alpha_1$  acid glycoprotein. Class 2 genes are regulated by IL 6 and glucocorticoids. Representatives of this class are  $\alpha_2$  macroglobulin,  $\alpha_1$  antichymotrypsin, fibrinogen,  $\alpha_1$  antitrypsin, thiostatin, and fibrinogen.<sup>28-30</sup> As IL 6 is involved in all the effects of liver acute phase genes, it is the main regulator of the acute phase response.<sup>5,31</sup>

### Receptors mediating the acute phase response

The cytokines inducing a change in the expression of an acute phase gene must activate a cascade, which transduces the signal from the outer cell membrane to the nucleus of the hepatocyte (Fig 2). Specific cytokine receptors have been cloned and characterised for the different factors involved in the acute phase response. These receptors belong to the cytokine receptor group.

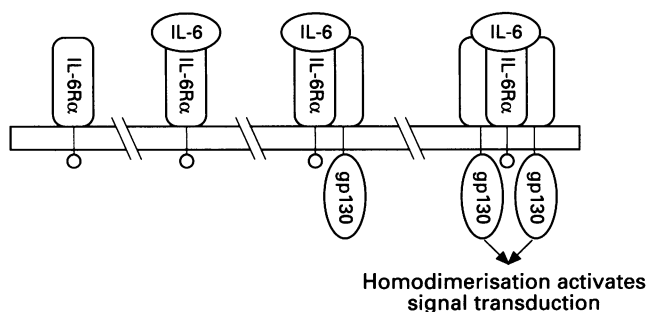


Figure 2: Model of activation by interleukin 6 (IL 6) and IL6 receptor complex. The schematic picture shows how IL 6 induces the activation of intracellular signal cascades. After the binding of IL 6 to the IL 6 receptor a gp 130 molecule binds to the complex. Addition of a second gp 130 molecule leads to homodimerisation of the intracellular parts of the two bound gp 130 molecules which in turn activates intracellular signal transduction.

In contrast to the family of growth factor receptors, this group of cytokine receptors contains no tyrosine kinase domain in the cytoplasmic region. Instead, the cytokine receptors need a second receptor associated molecule for high affinity ligand binding and transmission of cytoplasmic signals. One of the first receptors cloned was the IL 6 receptor.<sup>32</sup> This consists of one immunoglobulin like domain followed by an additional 200 amino acids in the extracellular domain. These 200 amino acids are essential for binding of IL 6 to the receptor. The intracellular domain is short and no function for signal transduction is known. Signal transduction is performed by the receptor associated molecule gp 130. The molecule is not involved in direct ligand binding, but functions as the only signal transducing element into the cell.<sup>33</sup> Additionally, the gp 130 molecule is important in stabilising the binding of IL 6 to the IL 6 receptor. The transfection of the IL 6 receptor together with gp 130 in a IL 6 receptor negative cell line results in the formation of high affinity binding sites for IL 6, whereas the transfection of the IL 6 receptor alone creates only a low affinity receptor for IL 6. Additionally, incubation of the transfected cells with a gp 130 antibody reduces the amount of high affinity IL 6 receptors.<sup>33</sup> Signal transduction is activated by homodimerisation with a second gp 130 molecule added to the complex.<sup>34</sup> Homodimerisation leads to the phosphorylation of associated tyrosine kinases and the activation of intracellular signal cascades.<sup>35</sup> The mechanism is illustrated in Figure 2.

Interestingly, the signal transducing molecule gp 130 is used by several other cytokines which mediate IL 6 like activity in the acute phase response of the liver. It has been shown that leukaemia inhibitory factor, oncostatin M, ciliary neurotropic factor, and IL 11 use gp 130 as a receptor associated molecule for signal transduction. However, the specific receptor for ligand binding is different for each cytokine, and different from the IL 6 receptor.<sup>36</sup>

Knowledge of the TNF receptor led to the definition of a new, small receptor family. The family is defined by an arrangement of three or four conserved, cysteine rich sequence segments of approximately 40 amino acids in the extracellular domain of the molecule. Two different TNF receptors are known; p55 TNFR and p75 TNFR.<sup>37-39</sup> Both receptors are normally expressed in various tissues and have a similar high affinity for TNF  $\alpha$  and  $\beta$ .<sup>40</sup> Different functions for the two receptors have been proposed,<sup>41-43</sup> but a definitive function has not yet been reached. A region of 44 amino acids in the cytoplasmic domain seems to be essential for receptor signalling.<sup>43</sup> No identifiable signalling function, such as kinases, has been described.

There are two different interleukin receptors. A larger 80 kDa form<sup>44</sup> and a smaller recently described 60 kDa form.<sup>45</sup> IL  $\alpha$  and  $\beta$  bind to the receptors with the same affinity.<sup>46</sup> The 80 kDa receptor belongs to the immunoglobulin like form of receptors. The 80 kDa protein consists of 557 amino acids. The 319 N-terminal amino acids belong to the extracellular part of the protein and are organised into three immunoglobulin like domains.<sup>44</sup> The intracellular 217 amino acids have no tyrosine kinase function or other described enzymatic function.

### Intracellular events which are involved in signal transduction

The intracellular pathways leading to the induction of acute phase genes are poorly understood. Recent research is trying to define mechanisms which connect

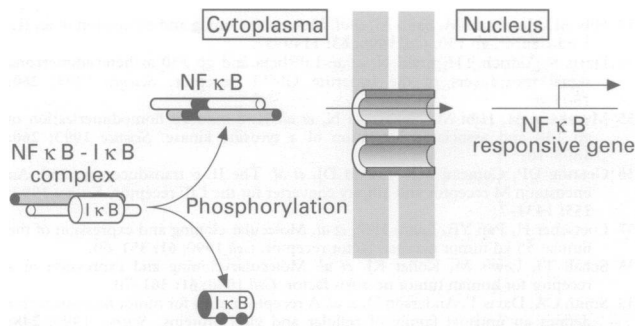


Figure 3: Activation and translocation of NF  $\kappa$ B. Extracellular signals like tumour necrosis factor  $\alpha$  or interleukin 1 lead to the activation of NF  $\kappa$ B in the cytoplasmic NF  $\kappa$ B-I  $\kappa$ B complex. I  $\kappa$ B is phosphorylated and can no longer bind and inactivate NF  $\kappa$ B. NF  $\kappa$ B translocates to the nucleus and activates NF  $\kappa$ B-responsive genes.

the activation of a specific receptor to the change in transcription of a certain gene. The activation of the different cytokine receptors involved in the acute phase response has shown a panel of signal cascades which may have importance for the acute phase response.

The TNF receptor leads to activation of phospholipase A 2 and diacylglycerol. Downstream of diacylglycerol is protein kinase C (PKC), a serine/threonine kinase and different proteases. The activation of these pathways leads over yet undefined signal cascades to the activation of the transcription factors NF  $\kappa$ B and c-jun (Fig 3). NF  $\kappa$ B especially has been shown to play an important role in the regulation of acute phase genes. The NF  $\kappa$ B complex seems to be a heterodimer that contains two proteins of 50 and 65 kDa which bind to the decameric  $\kappa$ B motif in the promoter of different genes.<sup>47, 48</sup> NF  $\kappa$ B pre-exists in the cytoplasm of most cells as an inactive form by the association with a family of inhibitor proteins called I  $\kappa$ B.<sup>49</sup> Stimulation by TNF or IL 1 and also agents like LPS or phorbol myristate acetate results in the dislocation of the I  $\kappa$ B-NF  $\kappa$ B complex by phosphorylation of I  $\kappa$ B. Subsequently, NF  $\kappa$ B translocates from the cytoplasm to the nucleus, binds to the promoter of a cognate DNA binding site, and activates the transcription of the target gene (Fig 3) (for review see<sup>50-51</sup>).

Examples of acute phase genes where NF  $\kappa$ B plays an important role in induction are serum amyloid A<sup>52</sup> and the angiotensinogen gene.<sup>53</sup> This same mechanism has also been shown for the regulation of the interleukin 6 gene itself<sup>54, 55</sup> and therefore NF  $\kappa$ B is one of the tools by which IL 1 and TNF stimulate the acute phase genes as well as the IL 6 gene.

NF-IL 6/LAP (IL 6-DBP, C/EBP  $\beta$ , CRP 2) is another important transcription factor which has been recently cloned and is involved in the regulation of different acute phase genes and the IL 6 gene itself.<sup>56-60</sup> NF-IL 6 is a member of the bZIP family of transcriptional activators and is highly enriched in liver nuclei. NF-IL 6 binds to a 14 bp nucleotide in the human IL 6 promoter, which is located between position -180 and -122. Other NF-IL 6 recognition sequences are present in liver specific genes such as the haptoglobin, CRP,  $\alpha_1$  acid glycoprotein or the albumin gene. The 14 bp recognition sequence has been found to be important for the class 1 acute phase genes. During the acute phase response, NF-IL 6 is regulated by LPS, IL 1, TNF, and IL 6 on the transcriptional level. The mRNA of NF-IL 6 starts to increase in the liver 15 minutes after IL 6 stimulation.<sup>61</sup> Besides direct stimulation on the protein level, NF-IL 6 is regulated on the post-translational level by protein phosphorylation. The gp 130 molecule which is associated with the IL 6 receptor has no tyrosine kinase activity itself, however there is evidence that after ligand binding, gp 130 activates a tyrosine kinase

and a ras-encoded protein further downstream of the intracellular signal transduction pathway.<sup>33, 62</sup> Recent data of Kakajima *et al*<sup>63</sup> show that the ras-MAP pathway leads to the phosphorylation of NF-IL 6 at threonine 235 and subsequently to its transactivation. Additional pathways have as a target NF-IL 6. Wegner *et al*<sup>64</sup> demonstrated the activation of a Ca<sup>2+</sup>-dependent pathway and the phosphorylation of Ser 276. It was recently shown that the activation of PKC and the transactivation of LAP/NF IL 6 is linked to the phosphorylation of serine 105 in the molecule.<sup>65</sup> As TNF leads to the activation of PKC, the phosphorylation of serine 105 could be a target of the TNF pathway. With CREB<sup>66</sup> and C-jun,<sup>67</sup> NF-IL 6 is the third transcriptional regulator for which phosphorylation determines its transcriptional regulation, further proving the generality of this regulatory mechanism.

More recently the cloning of an additional member called NF-IL 6  $\beta$  (C/EBP- $\delta$ ) was reported. The protein has been shown to heterodimerize and thereby cooperate with NF-IL 6 in the transactivation of acute phase genes.<sup>58, 68</sup>

The cloning of transcription factors regulating mainly the promoters of class 2 acute phase genes will soon be finished. A hexanucleotide motif found in the promoters of several genes was shown to be required for responsiveness to IL 6, and has been called IL 6 responsive element (IL6-RE) or acute phase response element (APRE). The  $\alpha_2$  macroglobulin promoter for example is composed of two APREs which functionally cooperate to confer the full IL 6 response of the gene. The transcription factor is rapidly activated by IL 6 at the post-translational level and a role of phosphorylation has also been suggested for this factor. This transcriptional activator has been shown to have a molecular weight of approximately 100-110 kDa.<sup>30, 69</sup>

## Outlook

The hepatocyte is the main liver cell, accounting for 65% of all liver cells: minority cells include Kupffer cells, endothelial cells, ITO (fat storing) cells, and bile duct epithelia. The hepatocyte's involvement with the immune system is multifactorial. On one hand, the hepatocyte may become a target of liver infiltrating T lymphocytes as the major effector limb of the immune system in viral hepatitis and autoimmune liver diseases. In viral hepatitis, T cells are believed to attack those hepatocytes presenting viral peptides through HLA-class I molecules on their surface. In autoimmune hepatitis, HLA-class II molecules are believed to present surface autoantigens to the immune system. On the other hand, the hepatocyte is the site of synthesis of important components of the immune system such as complement factors. The hepatocyte is also the dominant cell of synthesis of the acute phase proteins that mediate the body's first line of defence against external insults such as infectious organisms. Thus studying the molecular basis of the acute phase response also means studying hepatocellular and thus organ specific gene expression and regulation. Therefore, an understanding of the activation of acute phase protein synthesis not only improves our understanding of the body's early defence mechanisms against infection, but also gives new insights into mechanisms regulating other organ specific genes such as those involved in metabolism, liver regeneration, and viral replication. Finally, the analysis and identification of factors mediating organ specific gene transcription may show new diagnostic or prognostic markers for liver diseases in particular acute liver failure. The understanding of these molecular mechanisms might enable

us to develop new therapeutic strategies for liver diseases. Hopefully, when the dust clears physicians will emerge with new therapeutic agents in their hands.

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#### Postscript

Meanwhile the APRF (acute phase response factor) has been cloned by two groups independently.<sup>70,71</sup> It belongs to the STATs proteins (signal transducers and activators of transcription). After phosphorylation by Jak-1 kinase APRF translocates from the cytoplasm into the nucleus and activates genes with an APREs in the promoter.

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