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Cytokines in Autoimmune Liver Diseases

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Abstract: Constitutive production of cytokines is absent or minimal in the normal liver but, in response to various types of injury, hepatocyte and cholangiocyte damage causes the local recruitment of neutrophils and macrophages that produce cytokines and chemokines and the former mediate the inflammatory response that leads to the regeneration of the liver tissue and ultimately, to the deposition of extracellular matrix by activated stellate cells. Cytokine production can have both beneficial and harmful effects, depending on the amount and duration of cytokine release. Within the spectrum of chronic liver diseases, cytokines play a pivotal role in the loss of immune tolerance characterizing chronic autoimmune hepatitis and primary biliary cirrhosis. This study will provide a general overview on cytokines and focus on their role in initiating and driving the inflammatory infiltrate in autoimmune liver diseases. A better understanding of this process might allow therapeutic interventions to switch off the peculiar inflammatory response which occurs in these conditions before irreversible damage occurs.

Key words: Cytokines, autoimmuine, liver diseases, chemokines

INTRODUCTION

Cytokines are soluble peptides secreted by several cell types to mediate several immune and inflammatory reactions, thus regulating several biochemical processes in and around the cells that produce them. They can act on different cell types (pleiotropic effects) and have overlapping effects (redundancy); furthermore, their action may be local or systemic. In most tissues, including the liver, constitutive production of cytokines is absent or minimal. However, as physiologic and pathologic stimuli activate cells, the production of these molecules increases and they orchestrate the tissue response to the stimulus. Phenotype of the immune response is a function of the repertoire of cytokines produced in the early phases (Borish and Steinke, 2003).

Monocytes and tissue-resident macrophages are major cytokine sources. Macrophages are found in many tissues, but the largest number resides in the liver where they are called Kupffer's cells (Bioulac-Sage *et al*., 1996). Nearly 80% of all macrophages in the body are Kupffer cells (Sheth and Bankey, 2001). Together with other immune cells these generate an acute inflammatory reaction, that is the body's first line of defence. Another important source of cytokines are $CD4^+$ (helper) T lymphocytes. The interaction between monocytes/ macrophages and T lymphocytes activate T lymphocytes, determining their multiplication and production of cytokines. Two distinct subsets of $CD4^+$ helper T cells exist, Th1 and Th2, which can be distinguished by their cytokine patterns, with Th1 cells producing mainly Interleukin (IL)-2 and Interferon (IFN)γ (which activate $CD8^+$ cytotoxic T cells and macrophages) and Th2 cells producing IL-4, IL-5, IL-6, IL-10 and IL-13 (which activate B lymphocytes for antibody production) (Mosmann and Sad, 1996). Th1 cells and their relative cytokine products are thought to be involved in delayed type hypersensitivity reactions and organ-specific autoimmune disorders; in contrast, Th2 cells and their cytokine products are considered to participate in allergic reactions and systemic autoimmune disorders. The signature cytokines of Th1 and Th2 subsets inhibit each other's secretion and consequently influence in opposite yet complementary ways the lymphocyte proliferation, resulting in a dynamic balance of the subsets within inflamed tissues. Since, the original description of the Th1

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and Th2 sets of cytokines, it was recognized that cells other than CD4` lymphocytes can produce similar cytokine patterns, which has prompted a broader classification of the respective immune responses into type 1 and type 2, rather than strictly Th1 and Th2. Furthermore, a subset of cells producing both type 1 and type 2 cytokines and a subset characterized by IL-10 and TGFβ production have been identified and designated as Th0 and a Th3 (Hoyne and Lamb,1996; Seder *et al*., 1998), respectively. Though a clear-cut distinction between type 1 and type 2 immune responses is more difficult in human than in the mouse, altered Th1/Th2 balances have been demonstrated in various autoimmune diseases not only in representative animal models but also in human conditions (Liblau *et al*., 1995; Borchers *et al*., 1999). Finally, a regulatory role is also played by CD4`CD25` T lymphocytes, which mediate antigen-specific suppression of T lymphocyte responses by local secretion of IL-10 and Transforming Growth Factor β (TGFβ) (Roncarolo *et al*., 2003).

The cytokine network activated in response to pathologic conditions acts through the local recruitment of distinct combinations of effector cells (Fig. 1 and 2). A distinct cytokine subfamily with a crucial role in determining which leukocyte subsets are recruited from the circulation to injured tissue in different conditions is represented by chemokines (short for chemotactic cytokines), acting as chemoattractants that induce target cells migration along a gradient. The chemokine system includes about 50 members which can be divided into 4 families on the basis of their molecular structure. The largest family includes 28 members mainly active on mononuclear cells (i.e., lymphocytes and monocytes), all characterized by the presence of 2 cysteine residues adjacent to each other in the N-terminal portion of the molecule, thus indicated as CC chemokines. The second family includes 16 members where one intervening aminoacid separates the first 2 cysteine residues (Kunkel, 1999), thus indicated as the CXC family. This family can be further subdivided into two groups, based on whether or not a molecule carries an ERL (glutamic-leucine-arginine) motif that immediately precede the first cysteine residue. ERL` CXC chemokines are important in neutrophil chemotaxis and angiogenesis, while ERL-are angiostatic and act mainly on T lymphocytes (Rollins, 1997). Two minor families, called C and CX3C chemokines, include a limited number of members and are mainly involved in the recruitment of selected T lymphocyte subsets and NK cells. Classically, the chemokines have been named according to their expression patterns or functions, but due to the rapid discovery of new chemokines by Zlotnik and Yoshie (2000) proposed a

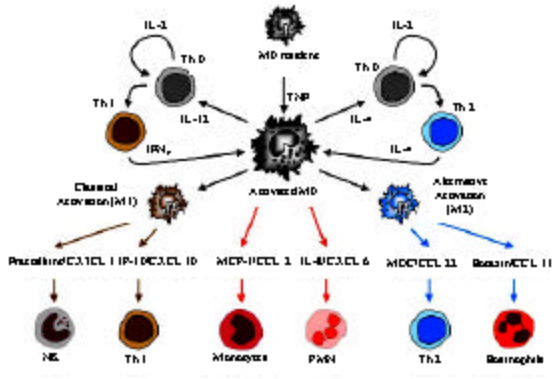

Fig. 1: Cytokine-chemokine circuitry acting in polarized immune responses

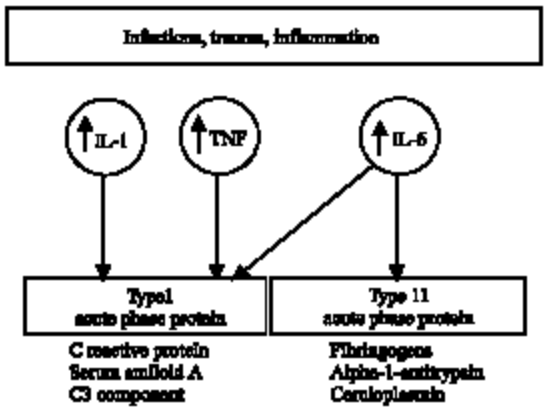

Fig. 2: Schematic representation of the liver acute phase

new classification system for chemokines that is based on the subfamily followed by a number provided by the position of the corresponding coding gene in the cluster. Thus, chemokines now are identified by a name providing information on the respective structural subfamily, corresponding also to the type of receptor they engage, followed by a number provided by and referring to the respective coding gene. Chemokines biological effects are mediated by a subfamily of G protein-coupled transmembrane domain receptors. Though each chemokine receptor usually binds more than one ligand, thus, having redundant activity, nonetheless they respect ligand family boundaries; therefore, chemokine receptors are classified as CC chemokine Receptors (CCR; 10 at present), CXC chemokine Receptors (CXCR; 6 at present), C chemokine Receptors (XCR; 1 at present) and CX3C receptors (CX3CR; 1 at present) (Bone-Larson *et al*., 2000). Some chemokines are expressed at high levels in specific tissues (tonic chemokines) and are involved in homeostatic functions such as thymocyte maturation/ selection and lymphocyte recirculation.

Table 1: Characteristics of cytokines involved in liver diseases

Cytokine	Main source	Effects	Implicated in	Ref.
IL-1	Macrophages Ag presenting cells	Pro-inflammatory Fever acute phase response	Alcoholic disease Liver regeneration Ischemia-reperfusion	(Bode *et al*., 2005; Neuman *et al*., 2005) (Fausto *et al*., 2006; Shirasugi *et al*., 1997)
IL-6	Ag presenting cells Th2 cells	Pro inflammatory Fever activates T lymphocytes Differentiates B lymphocytes Acute phase response	Alcoholic disease Liver regeneration	(Bode *et al*., 2005; Neuman *et al*., 2005) (Fausto *et al*., 2006)
TNFα	Macrophages NK cells	Similar to IL-1	Alcoholic disease PSC Liver regeneration Ischemia-reperfusion	(Bode *et al*., 2005; Neuman *et al*., 2005) (Tjandra *et al*., 2000; Bo *et al*., 2001), (Spengler *et al*., 1992; Sprengers *et al*., 2005) (Fausto *et al*., 2006; Shirasugi *et al*., 1997)
IL-12	Activated hepatocytes	Stimulates NK cells and T lymphocytes Stimulate IFNγ production	Ischemia reperfusion Viral hepatitis AIH	(Lentsch *et al*., 1999; Bertoletti and Ferrari, 2003; Tanaka *et al*., 1996; Trinchieri *et al*., 2003) (Fausto *et al*., 2006)
TGFβ	Macrophages Th3 cells	Anti-inflammatory Inhibits B,T and NK cells Stimulates fibrogenesis	Liver regeneration Liver fibrosis	(Reddy *et al*., 2001; Friedman *et al*., 1999)
IL-10	B and Th2 cellss Macrophages	Anti-inflammatory Inhibits IFN production Stimulates B lymphocytes	Control of inflammation	(Mosmann and Sad, 1996)
IFNα	Macrophages	Inhibits viral replication Stimulates NK cells	Viral hepatitis	(Bertoletti *et al*., 2003; Foster, 1997)
IFNγ	Th1 cells NK cells	Modulates IL-1 and TNFα Increases MHC expression Inhibits viral replication	Viral hepatitis PBC	(Bertoletti *et al*., 2003; Foster, 1997) (Harada *et al*., 1997; Martinez *et al*., 1995; Shindo *et al*., 1996)

Table 2: Characteristics of chemokines involved in liver diseases

Chemokine	Family	Receptor	Target	Implicated in	Ref.
IL-8/CXCL8 (CINC)	CXC (ELR+)	CXCR1/ CXCR2	Neutrophils	Alcoholic disease GVDH disease Baterial hepatitis Ischemia-reperfusion	(Huang *et al*., 1996) (Huang *et al*., 1993) (Zhang *et al*., 1995) (Colletti *et al*., 1998)
ENA-78/CXCL5 (MIP-2)	CXC (ELR+)	CXCR2	Neutrophils	Bacterila hepatitis Ischemia-reperfusion	(Shrotri *et al*., 1999) (Colletti *et al*., 1998)
GRO/CXCL1 (KC)	CXC (ELR+)	CXCR2	Neutrophils	Ischemia-reperfusion Bacterial hepatitis	(Lentsch *et al*., 1998) (Tilg *et al*., 1992)
IP-10/CXCL10	CXC (ELR-)	CXCR3	NK cells Th1 cells	Alcoholic disease Viral hepatitis PBC; AIH	(Nanji *et al*., 1999) (Kakimi *et al*., 2001) (Chuang *et al*., 2005; Nicoletti *et al*., 2007)
MIG/CXCL9	CXC (ELR-)	CXCR3	NK cells Th1 cells	Viral hepatitis Liver cancer Graft rejection PBC	(Shields *et al*., 1996) (Yoong *et al*., 1999; Goddard *et al*., 2001) (Chuang *et al*., 2005)
SDF-1α_/ CXCL12	CXC (ELR-)	CXCR4	Multiple	Graft rejection Liver cancer	(Goddard *et al*., 2001) (Shibuta *et al*., 1997)
MCP-1/CCL2	CC	CCR2	Monocytes	Immature DC Ischemia-reperfusion Alcoholic disease Liver fibrosis bacterial hepatitis	(Afford *et al*., 1998) (Narumi *et al*., 1997) (Marra *et al*., 1998) (Salkowski *et al*., 1998)
MIP-1α_/CCL3	CC	CCR1/ CCR5	Monocytes Immature DC Th1 cells	GVDH disease Bacterial hepatitis Viral hepatitis Alcoholic disease	(Murai *et al*., 1999) (Adams *et al*., 1996) (Salkowski *et al*., 1998; Salazar-Mather *et al*., 1998; Fisher *et al*., 1999)
RANTES/CCL5	CC	CCR1/ CCR5	Monocytes Immature DC Th1 cells	Autoimmune diseases Viral hepatitis Graft rejection	(Hirano *et al*., 2001) (Kusano *et al*., 2000) (Muruve *et al*., 1999; Nagral *et al*., 1998)
Eotaxin/CCL11	CC	CCR3	Eosinophils	Fulminant hepatic failure (acetaminophen toxicity)	(Pham *et al*., 2001)
TARC/CCL17 and MDC/CCL22	CC	CCR4	Th2 cells	Fulminant hepatic failure (post-infection model)	(Yoneyama *et al*., 1998; Chvatchko *et al*., 2000 Matsukawa *et al*., 2001)
LARC/CCL20	CC	CCR6	Immature DC Tm cells	Viral hepatitis	(Shimizu *et al*., 2001)
Fractalkine/ CX3CL1	CX3C	CX3CR1	Th1 cells	Fulminant hepatic failure (acetaminophen toxicity) PBC	(Simpson *et al*., 2003) (Chuang *et al*., 2005)

However, most chemokines are not expressed in homeostatic conditions and are rapidly induced in pathologic conditions (fasic or inflammatory chemokines). In this case, tissue damage induces a specific cytokine milieu which in turns defines the composition of the inflammatory response acting on the combination of chemokines present in the microenviroment (Fig. 1 and 2). Master cytokines, which activate polarized responses differentially, regulate chemokine production. For instance, the type 2 cytokines, IL-4 and IL-13, induce production of chemokines which interact with receptors that are preferentially expressed on polarized type 2 T cells, including MDC/CCL22 and TARC/CCL17 (agonists for CCR4), eotaxin/CCL11 (agonist for CCR3) and I-309/CCL1 (agonist for CCR8). Conversely, interferon (IFN)-γ inhibits production of MDC/CCL22 in different cell types and induces expression of CXCR3 agonists. that are active on receptors expressed on type 1 T cells. Hence, these chemokines supporting selective recruitment of polarized T cells and specific type I and II effector cells expressing distinct panels of chemokine receptors are involved in the amplification of polarized responses (Bonecchi *et al*., 1998) (Table 1 and 2).

CYTOKINES IN THE HEALTHY LIVER

A number of inflammatory chemokines have been associated with liver diseases (Locati *et al*., 2005) (Table 2 for selected references) and in most cases their role is clearly linked to selective recruitment of leukocyte subsets, thus playing a direct (mostly negative) role in the pathogenesis. Chemokine receptor inhibitors are in advanced development and might be available for treatment in the next years. However, it is worth mentioning that though chemokine patognomonic biological activity is leukocyte recruitment, some members of this large family also have other non-chemotactic biological activities, some of which of possible relevance in liver diseases (Kunkel, 1996). For example, CXC chemokines regulate angiogenesis (being ELR^+ CXC chemokines pro-angiogenetic and ELR-CXC chemokines anti-angiogenetic), CC chemokines have been associated with fibrosis and some chemokines have been demonstrated to control apoptosis and cell survival in specific cases. Thus, caution must be exerted inferring a negative role for chemokine expression in the pathogenesis of liver diseases. This is consistent with some experimental data in gene-targeted animal models showing that some chemokines may play a positive role acting as hepatocyte protectors or sustaining parenchyma regeneration (Bone-Larson *et al*., 2000; Shirasugi *et al*., 1997).

The liver cells under normal conditions produce only minimal levels of cytokines and as a consequence only a small quantity of cytokines are detected by immunohistochemistry on liver sections. The weak staining of chemokine is confined to the vascular endothelium and to inflammatory cells around blood vessels. This observation suggests that low-level chemokine secretion occurs in normal liver and could be important for the regulation of leukocyte recruitment during physiological immune surveillance. An exception is represented by the homeostatic CC chemokine Liver and Activation-Related Chemokine (LARC/CCL20), which acts on CCR6 regulating the homeostatic recirculation in the liver of memory T cells (Schutyser *et al*., 2003).

CYTOKINES IN LIVER DISEASES

In response to various liver injury (i.e. viral agents, alcohol consumption, hepatotoxins, autoimmunity, ischemia) hepatocytes damage causes the recruitment of neutrophils and macrophages that produce cytokines and chemokines and the latter mediate the inflammatory response that leads to the regeneration of the liver tissue and ultimately to the deposition of extracellular matrix by the activation of HSC. Under normal conditions, the levels of these proteins promoting inflammation decrease once the infection is under control. However, if the inflammation continues for a long time, persistent production of cytokines may lead to fibrosis and liver cirrhosis. Thus, cytokines production can have both beneficial and harmful effects, depending on the amount and duration of cytokine release. The main liver cells that produce cytokines are the resident macrophages, i.e., Kupffer's cells, that constitute the largest reservoir of tissue macrophages in the body. Particulary important cytokines for the liver are TNFα, IL-1, IL-6, IFNs, TGFβ and chemokines (Canbay, 2004).

The production of TNFα is one of the earliest events in several types of liver injury (Tilg and Diehl, 2001). It can initiate hepatocyte apoptosis and triggering the production of other cytokines and chemokines, that together recruit inflammatory cells, kill hepatocytes and initiate a healing response that include fibrogenesis (Locksley, 2001) (Fig. 1 and 2). Apoptosis is a form of cell death characterized by organized nuclear and finally cellular fragmentation. It is regulated by a great number of pathways. The interaction between TNFα and its cellular receptor is one of these ways; moreover the engulfment of apoptotic bodies by Kupffer's cells induces the expression of death ligands that continue the apoptotic stimulation (Canbay *et al*., 2003; Rust and Gores, 2000). TNFα perpetuates inflammation through the activation of

Nuclear Factor kappa B (NF-kB), a transcriptional factor that regulates the expression of several cytokine and chemokine genes (Reddy *et al*., 1994). Further, TGFβ is the most potent cytokine for enhancing hepatic fibrinogenesis by stimulating the activation of HSC (Reddy *et al*., 1996) and is generated when apoptotic bodies are encountered (Friedman, 1999). Under normal condition HSC are resident perisinusoidal mesenchymal cells that mainly serve to store fat and vitamin A in the liver. When activated they assume the features of fibrogenic, contractile myofibroblasts and produce collagen, the major component of fibrotic tissue. In addition, activated HSC mediate the inflammatory response by the production of several number of cytokines and chemokines (Eng *et al*., 2000; Pinzani and Marra, 2001). Finally, IL-1 and IL-6 are also involved in the hepatic acute phase response (Moshage, 1997) and in liver regeneration (Fausto *et al*., 2006).

Recent studies have suggested an important role, specially in inflammatory bowel diseases, of the IL-17. This cytokine may be implicate in liver immune-mediate damage too.

AUTOIMMUNE LIVER DISEASES

It has been suggested, that a skewed immune response towards a type 1 or type 2 pattern plays a role in the pathogenesis of several human autoimmune diseases such as multiple sclerosis, type 1 diabetes and rheumatoid arthritis (Borchers *et al*., 1999; Selmaj *et al*., 1991; Foulis *et al*., 1991; Matthews *et al*., 1993). Primary biliary cirrhosis, primary sclerosing cholangitis and autoimmune hepatitis are the main chronic autoimmune liver diseases in adult. Diverse cytokines have been shown to be overexpressed in the liver and serum of patients with such diseases. Despite the progress in the area of lymphocyte homing, the mechanisms involved in the enrichment of T cells observed in inflammatory liver diseases are still poorly understood. Available data implicate both selective recruitment and selective retention in this process. It is also possible that, at different stages, the migration of T lymphocytes into the liver is controlled by different pathways, as indicated by evidence in cellular and cytokines studies.

Primary biliary cirrhosis: In chronic cholestatic disorders such as Primary Biliary Cirrhosis (PBC), T lymphocytes and cytokines mediate persistent bile duct damage. Bile duct epithelial cells reaction actively contribute to the promotion and regulation of biliary type liver fibrogenesis. They synthesize and release a number of paracrine mediators such as TGF-beta, connective tissue growth factor, platelet-derived growth factor-BB and endothelin-1 that target different liver cell types, including HSC and portal fibroblasts. Through these interactions, bile duct epithelial cells and peribiliary myofibroblasts cause periportal fibrosis. There is still some controversy concerning the cytokine pattern characteristic of PBC. *In situ* hybridization revealed that PBC liver presents a significantly higher prevalence of IFNγ and IL-4 mRNA positive cells when compared to controls (Harada *et al*., 1996). Yet, there were considerably fewer cells with detectable levels of IL-4 mRNA than cells expressing IFNγ mRNA in PBC liver and the intensity of staining for IFNγ expression was highly correlated with the degree of portal inflammation. Moreover, IFNγ mRNA-positive cells were detected primarily in the lymphoid aggregates surrounding damaged bile ducts and in areas of piecemeal necrosis. Analysis of RNA extracted from PBC liver have also indicated an upregulation of IFNγ mRNA expression (Martinez *et al*., 1995; Shindo *et al*., 1996; Nagano *et al*., 1999).

Fractalkine/CX3CL1 production is demonstrated in biliary epitelial cells and upregulated by several cytokines including IFNγ and TNFα. In PBC the expression of Fractalkine/CX3CL1 is upregulated in injured bile ducts and T lymphocytes expressing CX3CR1 are found in portal tracts and within the biliary hepitelial layer of injured bile ducts. In addition, the expression of IP-10/CXCL10 and MIG/CXCL9 is increased in the portal tracts of PBC livers; these chemokines are involved in the selective recruitment of Th1 cells via CXCR3 (Chuang *et al*., 2005).

In contrast, mitogen-stimulated T lymphocytes infiltrating the liver of PBC produce significantly higher levels of IL-4 and IL-10 compared to control T cells, but little IFNγ. However, we note that several reports propose an upregulation of specific type 2 cytokines, such as IL-5, IL-6 and IL-10 in PBC (Martinez *et al*., 1995; Nagano *et al*., 1999), although this was not an entirely consistent finding (Shindo *et al*., 1996).

Overall, these results suggest that type 1 cytokines might constitute the dominant pattern in PBC. However, the mechanisms for such dysregulation of the Th1/Th2 cytokines ratio in biliary hepitelial cells is still unclear (Fig. 3).

Primary sclerosing cholangitis: Patients affected by Primary Sclerosing Cholangitis (PSC) have a redominantly Th1 response (Tjandra *et al*., 2000; Bo *et al*., 2001) with high levels of TNFα (Spengler *et al*., 1992) and IL-1 in all stages of disease. Colangiocytes secrete CXCL10 and CCL2 chemokines in response to these pro-inflammatory cytokines. These chemokines recruit effector lymphocytes

	IFNγ	TNFα	IL-4	CX3CL1	IP-10	IL-12	IL2/IL10
PBC	+++		++	++	+		
CSP	--	+++					--
AIH					++	++	

Fig. 3: Schematic repersentation of the cytokines involvoed in a autoimmune liver diseases

and monocytes, thereby promoting inflammation. Periportal mononuclear cell infiltration with more than 80% of T lymphocytes is found in most liver biopsy of PSC patients. Liver-derived T cells from PSC patients have greater intracytoplasmic TNFα levels compared to those derived from patients with primary biliary chirrosis, autoimmune hepatitis and healthy subjects and have significantly lower levels of IL-2, IL-10 and IFNγ (Bo *et al*., 2001). T cells are unlikely to cause direct biliary cells injury in PSC since the assessment of the functional capacity of liver-infiltrating T cells from PSC patients demonstrated a decreased cytolytic activity; the NK killing capacity was also abolished. The reduced T-cells reactivity in liver infiltrating cells obtained from patients with PSC is due to high local production o TNFα. More likely, the biliary cells injury in PSC is mediated by TNFα which may act synergistically with IFNγ to induce biliary epithelial cells to produce nitric oxide, that contributes to ductal cholestasis thorough the inhibition of cAMP-dependent HCO3-secretion (Spirli *et al*., 2003). Moreover, expression of receptor TNF-R1 on biliary cells supports the possibility of induction of apoptosis by high levels of TNFα in PSC. In addition the absence of IL-2 in concert with chronic TNFα and IL-1 production may cause further injury to the biliary cells (BO *et al*., 2001). The treatment with anti TNF antibodies restores a normal level of IL-1 and of IL-2, IFNγ and IL-10 in this scenario. The impaired cytolytic activity of both NK and T cells was also partially restored (Fig. 3).

Autoimmune hepatitis: Very limited data on intrahepatic cytokine expression are available in Autoimmune Hepatitis (AIH). Patients with AIH display an increased expression of HLA class II antigens in their hepatocytes (Vergani *et al*., 2002) and a preponderant $CD4^+$ T lymphocyte infiltration of the portal spaces. These findings might indicate the involvement of T helper cells in the pathogenesis of this disease. In response to the antigenic peptide/HLA class II complex, naive $CD4^+$ T cells differentiate into either IFNγ-secreting Th1 or IL-4/IL-10-producing Th2 lymphocytes. The IL-12 produced mainly by macrophages and dendritic cells is required not only for their differentiation into Th1 cells but also to sustain the presence of memory/effector Th1 cells capable of mediating a biologic outcome. It was shown in a murine model of autoimmunity that IL-12 plays a pivotal role in the Th1-dependent liver injury (Tanaka *et al*., 1996; Nicoletti *et al*., 2000). IL-12 is part of a family of cytokines that shares important functions in the regulation of both innate and adaptive immunity (Nicoletti *et al*., 2007).

In addition an increase of liver IP-10 and the presence of CXCR3+ lymphocytes have been observed in murine model of AIH (Bode and Bode, 2005) (Fig. 3).

CONCLUSION

Liver cytokines and chemokines represent the components of a complex scenario in liver physiology and pathology. As indicated by the large amount of data available, interaction networks appear to be more important to the final outcome of immune unbalance compared to single mediator alterations. As a result, cytokine and chemokine response to several types of chronic and acute injury ensues in an attempt to counteract the damage but often result in pathological effects, as in the case of TGFβ and fibrosis. Importantly, cytokines are being studied as potential targets for novel treatments in several liver conditions. We note, however, that results obtained thus far with monoclonal antibodies (such as infliximab targeting TNFα) are disappointing; yet we warrant a vigorous effort in the near future to unravel new aspects of cytokine defects in liver diseases to ultimately develop new and effective treatments.

The table reports chemokines (old/new nomenclature) associated to liver diseases, with the indication of main target leukocytes and receptors involved. References supporting a pathogenetic role of a specific ligand/receptor in liver diseases, mostly inferred by animal models using blocking antibodies or gene-targeted animals, are provided. The name of rodent chemokines which differ from the human counterpart are provided in parenthesis.

REFERENCES

Adams, D.H. S. Hubscher, J. Fear, J. Johnston, S. Shaw and S. Afford, 1996. Hepatic expression of macrophage inflammatory protein-1 alpha and macrophage inflammatory protein-1 beta after liver transplantation. Transplantation, 61: 817-825.

Afford. S.C., N.C. Fisher, D.A. Neil, J. Fear, P. Brun, S.G. Hubscher and D.H. Adams, 1997. Distinct patterns of chemokine expression are associated with leukocyte recruitment in alcoholic hepatitis and alcoholic cirrhosis. J. Pathol., 186: 82-89.

Borish, L.C. and J.W. Steinke, 2003. Cytokines and chemokines. J. Allergy Clin. Immunol., 111: 460-475.

Bertoletti, A. and C. Ferrari, 2003. Kinetics of the immune response during HBV and HCV infection. Hepatology, 38: 4-13.

Bioulac-Sage, P., J. Kuiper and T.J. Van Berkel, 1996. Balabaud C. Lymphocyte and macrophage populations in the liver. Hepatogastroenterology, 43: 4-14.

Bo, X., U. Broome, M. Remberger, S. Sumitran-Holgersson, 2001. Tumour necrosis factor alpha impairs function of liver derived T lymphocytes and natural killer cells in patients with primary sclerosing cholangitis. Gut., 49: 131-341.

Bonecchi, R., G. Bianchi, P.P. Bordignon, D. D'Ambrosio, R. Lang, A. Borsatti, S. Sozzani, P. Allavena, P.A. Gray, A. Mantovani and F. Sinigaglia, 1998. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J. Exp. Med., 187: 129-1234.

Borchers, M.T., S. Wesselkamper, S.E. Wert, S.D. Shapiro and G.D. Leikauf, 1999. Monocyte inflammation augments acrolein-induced Muc5ac expression in mouse lung. Am. J. Physiol., 277: 489-497.

Bone-Larson, C.L., K.J. Simpson, L.M. Colletti, N.W. Lukacs, S.C. Chen, S. Lira, S.L. Kunkel and C.M. Hogaboam, 2000. The role of chemokines in the immunopathology of the liver. Immunol. Rev., 177: 8-20.

Bissell, D.M., D. Roulot and J. George, 2001. Transforming growth factor beta and the liver. Hepatology, 34: 859-867.

Bode, C. and J.C. Bode, 2005. Activation of the innate immune system and alcoholic liver disease: Effects of ethanol per se or enhanced intestinal translocation of bacterial toxins induced by ethanol? Alcohol. Clin. Exp. Res., 29: 166-171.

Canbay, A., S. Friedman and G.J. Gores, 2004. Apoptosis: The nexus of liver injury and fibrosis. Hepatology, 39: 273-278.

Colletti, L.M., M. Green, M.D. Burdick, S.L. Kunkel and R.M. Strieter, 1998. Proliferative effects of CXC chemokines in rat hepatocytes *in vitro* and *in vivo*. Shock, 10: 248-257.

Canbay, A., A.E. Feldstein, H. Higuchi, N. Werneburg, A. Grambihler, S.F. Bronk and G.J. Gores, 2003. Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine expression. Hepatology, 38: 1188-1198.

Chuang, Y.H., Z.X. Lian, C.M. Cheng, R.Y. Lan, G.X. Yang, Y. Moritoki, B.L. Chiang, A.A. Ansari, K. Tsuneyama, R.L. Coppel, M.E. Gershwin, 2005. Increased levels of chemokine receptor CXCR3 and chemokines IP-10 and MIG in patients with primary biliary cirrhosis and their first degree relatives. J. Autoimmun., 25: 126-132.

Chvatchko, Y., A.J. Hoogewerf, A. Meyer, S. Alouani, P. Juillard, R. Buser, F. Conquet, A.E. Proudfoot, T.N. Wells and C.A. Power, 2000. A key role for CC chemokine receptor 4 in lipopolysaccharide-induced endotoxic shock. J. Exp. Med., 191: 1755-1764.

Eng, F.J., S.L. Friedman and I. Fibrogenesis, 2000. New insights into hepatic stellate cell activation: The simple becomes complex. Am. J. Physiol. Gastrointest. Liver Physiol., 279: 7-11.

Friedman, S.L., 1999. Cytokines and fibrogenesis. Semin. Liver Dis., 19: 129-140.

Foulis, A.K., M. McGill, M.A. Farquharson, 1991. Insulitis in type 1 (insulin-dependent) diabetes mellitus in man-macrophages, lymphocytes and interferon-gamma containing cells. J. Pathol., 165: 97-103.

Fausto, N., J.S. Campbell, K.J. Riehle, 2006. Liver regeneration. Hepatology, 43: 45-53.

Foster, G.R., 1997. Interferons in host defense. Semin. Liver Dis., 17: 287-295.

Fisher, N.C., D.A. Neil, A. Williams and D.H. Adams, 1999. Serum concentrations and peripheral secretion of the beta chemokines monocyte chemoattractant protein 1 and macrophage inflammatory protein 1alpha in alcoholic liver disease. Gut., 45: 416-420.

Goddard, S., A. Williams, C. Morland, S. Qin, R. Gladue, S.G. Hubscher and D.H. Adams, 2001. Differential expression of chemokines and chemokine receptors shapes the inflammatory response in rejecting human liver transplants. Transplantation, 72: 1957-1967.

Hoyne, G.F. and J.R. Lamb, 1996. Peptide-mediated regulation of the allergic immune response. Immunol. Cell. Biol., 74: 180-186.

Hirano, F., A. Kobayashi, Y. Hirano, Y. Nomura, E. Fukawa and I. Makino, 2001. Bile acids regulate RANTES gene expression through its cognate NF-kappaB binding sites. Biochem. Biophys. Res. Commun., 288: 1095-1101.

Huang, Y.S., C.Y. Chan, J.C. Wu, C.H. Pai, Y. Chao and S.D. Lee, 1996. Serum levels of interleukin-8 in alcoholic liver disease: Relationship with disease stage, biochemical parameters and survival. J. Hepatol., 24: 377-384.

Harada, K., J. Van de Water, P.S. Leung, R.L. Coppel, A. Ansari, Y. Nakanuma and M.E. Gershwin, 1997. In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: Predominance of the Th1 subset. Hepatology, 25: 791-796.

Kusano, F., Y. Tanaka, F. Marumo and C. Sato, 2000. Expression of C-C chemokines is associated with portal and periportal inflammation in the liver of patients with chronic hepatitis C. Lab. Invest., 80: 415-422.

Kakimi, K., T.E. Lane, F.V. Chisari and L.G. Guidotti, 2001. Cutting edge: Inhibition of hepatitis B virus replication by activated NK T cells does not require inflammatory cell recruitment to the liver. J. Immunol., 167: 670-675.

Kunkel, S.L., 1999. Through the looking glass: The diverse *in vivo* activities of chemokines. J. Clin. Invest., 104: 1333-1334.

Koziel, M.J., 1999. Cytokines in viral hepatitis. Semin Liver Dis., 19: 157-169.

Lentsch, A.B., H. Yoshidome, A. Kato, R.L. Warner, W.G. Cheadle, P.A. Ward and M.J. Edwards, 1999. Requirement for interleukin-12 in the pathogenesis of warm hepatic ischemia/reperfusion injury in mice. Hepatology, 30: 1448-1453.

Lapierre, P., K. Beland and F. Alvarez, 2007. Pathogenesis of autoimmune hepatitis: From break of tolerance to immune-mediated hepatocyte apoptosis. Trans. Res., 149: 107-113.

Lentsch, A.B., H. Yoshidome, W.G. Cheadle, F.N. Miller, M.J. Edwards, 1998. Chemokine involvement in hepatic ischemia/reperfusion injury in mice: Roles for macrophage inflammatory protein-2 and KC. Hepatology, 27: 1172-1177.

Locksley, R.M., N. Killeen and M.J. Lenardo, 2001. The TNF and TNF receptor superfamilies: Integrating mammalian biology. Cell., 104: 487-501.

Locati, M., R. Bonecchi and M.M. Corsi, 2005. Chemokines and their receptors: Roles in specific clinical conditions and measurement in the clinical laboratory. Am. J. Clin. Pathol., 123: 82-95.

Liblau, R.S., S.M. Singer and H.O. McDevitt, 1995. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. Immunol. Today, 16: 34-38.

Marra, F., R. DeFranco, C. Grappone, S. Milani, S. Pastacaldi, M. Pinzani, R.G. Romanelli, G. Laffi and P. Gentilini, 1998. Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: Correlation with monocyte infiltration. Am. J. Pathol., 152: 423-430.

Murai, M., H. Yoneyama, A. Harada, Z. Yi, C. Vestergaard, B. Guo, K. Suzuki, H. Asakura and K. Matsushima, 1999. Active participation of CCR5 (+) CD8 (+) T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. J. Clin. Invest., 104: 49-57.

Matsukawa, A., M.H. Kaplan, C.M. Hogaboam, N.W. Lukacs and S.L. Kunkel, 2001. Pivotal role of signal transducer and activator of transcription (Stat) 4 and Stat 6 in the innate immune response during sepsis. J. Exp. Med., 193: 679-688.

Muruve, D.A., M.J. Barnes, I.E. Stillman and T.A. Libermann, 1999. Adenoviral gene therapy leads to rapid induction of multiple chemokines and acute neutrophil-dependent hepatic injury *in vivo*. Hum. Gene. Ther., 10: 965-976.

Narumi, S., Y. Tominaga, M. Tamaru, S. Shimai, H. Okumura, K. Nishioji, Y. Itoh and T. Okanoue, 1997. Expression of IFN-inducible protein-10 in chronic hepatitis. J. Immunol., 158: 5536-5544.

Nagral, A., Z. Ben-Ari, A.P. Dhillon, A.K. Burroughs, 1998. Eosinophils in acute cellular rejection in liver allografts. Liver Trans. Surg., 4: 355-362.

Mercer-Jones, M.A., M.S. Shrotri, J.C. Peyton, D.G. Remick and W.G. Cheadle, 1999. Neutrophil sequestration in liver and lung is differentially regulated by C-X-C chemokines during experimental peritonitis. Inflammation, 23: 305-319.

Mosmann, T.R. and S. Sad, 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol. Today, 17: 138-146.

Moshage, H., 1997. Cytokines and the hepatic acute phase response. J. Pathol., 181: 257-266.

Matthews, N., P. Emery, D. Pilling, A. Akbar and M. Salmon, 1993. Subpopulations of primed T helper cells in rheumatoid arthritis. Arthritis Rheum., 36: 603-607.

Martinez, O.M., J.C. Villanueva, M.E. Gershwin and S.M. Krams, 1995. Cytokine patterns and cytotoxic mediators in primary biliary cirrhosis. Hepatology, 21: 113-119.

Neuman, M.G., 2003. Cytokines-central factors in alcoholic liver disease. Alcohol. Res. Health, 27: 307-316.

Nanji, A.A., K. Jokelainen, A. Rahemtulla, L. Miao, F. Fogt, H. Matsumoto, S.R. Tahan and G.L. Su, 1999. Activation of nuclear factor kappa B and cytokine imbalance in experimental alcoholic liver disease in the rat. Hepatology, 30: 934-943.

Nicoletti, F., R. Di Marco, P. Zaccone, A. Salvaggio, G. Magro, K. Bendtzen and P. Meroni, 2000. Murine concanavalin A-induced hepatitis is prevented by Interleukin 12 (IL-12) antibody and exacerbated by exogenous IL-12 through an interferon-gamma-dependent mechanism. Hepatology, 32: 728-733.

Nagano, T., K. Yamamoto, S. Matsumoto, R. Okamoto, M. Tagashira, N. Ibuki, S. Matsumura, K. Yabushita, N. Okano and T. Tsuji, 1999. Cytokine profile in the liver of primary biliary cirrhosis. J. Clin. Immunol., 19: 422-427.

Pham, B.N., J. Bemuau, F. Durand, A. Sauvanet, C. Degott, L. Prin and A. Janin, 2001. Eotaxin expression and eosinophil infiltrate in the liver of patients with drug-induced liver disease. J. Hepatol., 34: 537-547.

Pinzani, M., F. Marra, 2001. Cytokine receptors and signaling in hepatic stellate cells. Semin. Liver Dis., 21: 397-416.

Rust, C. and G.J. Gores, 2000. Apoptosis and liver disease. Am. J. Med., 108: 567-574.

Reddy, S.A., M.M. Chaturvedi, B.G. Darnay, H. Chan, M. Higuchi and B.B. Aggarwal, 1994. Reconstitution of nuclear factor kappa B activation induced by tumor necrosis factor requires membrane-associated components. Comparison with pathway activated by ceramide. J. Biol. Chem., 269: 25369-25372.

Roncarolo, M.G., S. Gregori and M. Levings, 2003. Type 1 T regulatory cells and their relationship with CD4+CD25+ T regulatory cells. Novartis Found Symp., 252: 115-131, 203-210.

Rollins, B.J., 1997. Chemokines. Blood, 90: 909-928.

Sheth, K. and P. Bankey, 2001. The liver as an immune organ. Curr. Opin. Crit. Care, 7: 99-104.

Shirasugi, N., G. Wakabayashi, M. Shimazu, A. Oshima, M. Shito, S. Kawachi, T. Karahashi, Y. Kumamoto, M. Yoshida and M. Kitajima, 1997. Up-regulation of oxygen-derived free radicals by interleukin-1 in hepatic ischemia/reperfusion injury. Transplantation, 64: 1398-1403.

Seder, R.A., T. Marth, M.C. Sieve, W. Strober, J.J. Letterio, A.B. Roberts and B. Kelsall, 1998. Factors involved in the differentiation of TGF-beta-producing cells from naive CD4+ T cells: IL-4 and IFN-gamma have opposing effects, while TGF-beta positively regulates its own production. J. Immunol., 160: 5719-5728.

Shibuta, K., N.A. Begum, M. Mori, K. Shimoda, T. Akiyoshi and G.F. Barnard, 1997. Reduced expression of the CXC chemokine hIRH/SDF-1alpha mRNA in hepatoma and digestive tract cancer. Int. J. Cancer, 73: 656-62.

Salkowski, C.A., G. Detore, A. Franks, M.C. Falk and S.N. Vogel, 1998. Pulmonary and hepatic gene expression following cecal ligation and puncture: Monophosphoryl lipid A prophylaxis attenuates sepsis-induced cytokine and chemokine expression and neutrophil infiltration. Infect. Immun., 66: 3569-3578.

Salazar-Mather, T.P., J.S. Orange and C.A. Biron, 1998. Early Murine Cytomegalovirus (MCMV) infection induces liver Natural Killer (NK) cell inflammation and protection through Macrophage Inflammatory Protein 1alpha (MIP-1alpha)-dependent pathways. J. Exp. Med., 187: 1-14.

Schutyser, E., S. Struyf and J. Van Damme, 2003. The CC chemokine CCL20 and its receptor CCR6. Cytokine Growth Factor Rev., 14: 409-426.

Selmaj, K., C.S. Raine, M. Farooq, W.T. Norton and C.F. Brosnan, 1991. Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. J. Immunol., 147: 1522-1529.

Shindo, M., G.E. Mullin, L. Braun-Elwert, N.V. Bergasa, E.A. Jones and S.P. James, 1996. Cytokine mRNA expression in the liver of patients with Primary Biliary Cirrhosis (PBC) and Chronic Hepatitis B (CHB). Clin. Exp. Immunol., 105: 254-259.

Spengler, U., A Moller, M.C. Jung, G. Messer, R. Zachoval, R.M. Hoffmann, J. Eisenburg, G. Paumgartner, G. Riethmuller and E.H. Weiss *et al*., 1992. T lymphocytes from patients with primary biliary cirrhosis produce reduced amounts of lymphotoxin, tumor necrosis factor and interferon-gamma upon mitogen stimulation. J. Hepatol., 15: 129-135.

Shields, P.L., C.M. Morland, M. Salmon, S. Qin, S.G. Hubscher and D.H. Adams, 1999. Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. J. Immunol., 163: 6236-6243.

Spirli, C., L. Fabris, E. Duner, R. Fiorotto, G. Ballardini, T. Roskams, N.F. Larusso, A. Sonzogni, L. Okolicsanyi and M. Strazzabosco, 2003. Cytokine-stimulated nitric oxide production inhibits adenylyl cyclase and cAMP-dependent secretion in cholangiocytes. Gastroenterology, 124: 737-753.

Sprengers, D. and H.L. Janssen, 2005. Immunomodulatory therapy for chronic hepatitis B virus infection. Fundam. Clin. Pharmacol., 19: 17-26.

Shimizu, Y., H. Murata, Y. Kashii, K. Hirano, H. Kunitani, K. Higuchi and A. Watanabe, 2001. CC-chemokine receptor 6 and its ligand macrophage inflammatory protein 3alpha might be involved in the amplification of local necroinflammatory response in the liver. Hepatology, 34: 311-319.

Simpson, K.J., N.C. Henderson, C.L. Bone-Larson, N.W. Lukacs, C.M. Hogaboam and S.L. Kunkel, 2003. Chemokines in the pathogenesis of liver disease: So many players with poorly defined roles. Clin. Sci. (Lond), 104: 47-63.

Tjandra, K., K.A. Sharkey and M.G. Swain, 2000. Progressive development of a Th1-type hepatic cytokine profile in rats with experimental cholangitis. Hepatology, 31: 280-290.

Tanaka, Y., A. Takahashi, K. Watanabe, K. Takayama, T. Yahata, S. Habu, T. Nishimura, 1996. A pivotal role of IL-12 in Th1-dependent mouse liver injury. Int. Immunol., 8: 569-576.

Trinchieri, G., S. Pflanz and R.A. Kastelein, 2003. The IL-12 family of heterodimeric cytokines: New players in the regulation of T cell responses. Immunity, 19: 641-644.

Tilg, H. and A.M. Diehl, 2000. Cytokines in alcoholic and nonalcoholic steatohepatitis. N. Eng. J. Med., 343: 1467-1476.

Tilg, H., M. Ceska, W. Vogel, M. Herold, R. Margreiter and C. Huber, 1992. Interleukin-8 serum concentrations after liver transplantation. Transplantation, 53: 800-803.

Uguccioni, M., R. Meliconi, S. Nesci, G. Lucarelli, M. Ceska, G. Gasbarrini and A. Facchini, 1993. Elevated interleukin-8 serum concentrations in beta-thalassemia and graft-versus-host disease. Blood, 81: 2252-2256.

Vergani, D., K. Choudhuri, D.P. Bogdanos, G. Mieli-Vergani Pathogenesis of autoimmune hepatitis. Clin. Liver Dis., 6: 727-737.

Yoneyama, H., A. Harada, T. Imai, M. Baba, O. Yoshie, Y. Zhang, H. Higashi, M. Murai, H. Asakura, K. Matsushima, 1998. Pivotal role of TARC, a CC chemokine, in bacteria-induced fulminant hepatic failure in mice. J. Clin. Invest., 102: 1933-1941.

Yoong, K.F., S.C. Afford, R. Jones, P. Aujla, S. Qin, K. Price, S.G. Hubscher and D.H. Adams, 1999. Expression and function of CXC and CC chemokines in human malignant liver tumors: A role for human monokine induced by gamma-interferon in lymphocyte recruitment to hepatocellular carcinoma. Hepatology, 30: 100-111.

Zlotnik, A. and O. Yoshie, 2000. Chemokines: A new classification system and their role in immunity. Immunity, 12: 121-127.

Zhang, P., M. Xie, J. Zagorski and J.A. Spitzer, 1995. Attenuation of hepatic neutrophil sequestration by anti-CINC antibody in endotoxic rats. Shock, 4: 262-268.