PUNTO DE VISTA

Cytokines – their pathogenic and therapeutic role in chronic viral hepatitis

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ABSTRACT

Cytokines make up a network of molecules involved in the regulation of immune response and organ functional homeostasis. Cytokines coordinate both physiological and pathological processes occurring in the liver during viral infection, including infection control, inflammation, regeneration, and fibrosis. Hepatitis B and hepatitis C viruses interfere with the complex cytokine network brought about by the immune system and liver cells in order to prevent an effective immune response, capable of viral control. This situation leads to intrahepatic sequestration of nonspecific inflammatory infiltrates that release proinflammatory cytokines, which in turn favor chronic inflammation and fibrosis. The therapeutical administration of cytokines such as interferon alpha may result in viral clearance during persistent infection, and revert this process.

Key words: Cytokines. Chronic hepatitis. HBV. HCV. Immunopathogenesis.

INTRODUCTION

Cytokines are small soluble proteins secreted by immune system cells and other body cells, and are part of an intercellular communication system responsible for developmental regulation, tissue repair, and immune response in pluricellular organisms (1). These proteins play their role in an autocrine or paracrine manner by binding specific cell receptors that either induce or inhibit cytokine-regulated genes. Over 100 different cytokines have been reported, which are classified according to their primary role (Table I). These proteins are involved in all immune response aspects, and play a key role in immune response polarization and regulation. The combination of cytokines resulting from a specific antigenic stimulus determines the kind of immune response that will develop.

Table I. Cytokine classification

Group	Abbreviation	Example
Interleukins	IL	IL-1; IL-2...
Interferons	IFN	
Type I		IFN α; IFN β
Type II		IFN γ
Tumor necrosis factor	TNF	TNF α; TNF β
Colony-stimulating factors	CSF	M-CSF; G-CSF..
Growth factors	GF	EGF; NGF...
Chemokines	CCL, CXCL, CX3CL, XCL	CCL5; CXCL10…

Cytokine nomenclature reflects the function initially described when a molecule was first discovered, as well as its chronological order of discovery.

During viral infection various cytokines play a role both in viral clearance and tissue damage mechanisms. Viruses may interfere with the normal function of this complex cytokine network as an escape route to avoid destruction.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic, non-cytopathic viruses of the *hepadnavirus* and *flavivirus* families, respectively, that induce both acute and chronic necro-inflammatory liver disease (2,3). HBV escapes immune control in 10% of adult infections, whereas HCV successfully evades the immune system in 60-80% of cases. Changes in various cytokine activities have been reported for both viral infections, which might favor viral persistence.

THE ROLE OF CYTOKINES IN EFFECTIVE IMMUNE RESPONSE AGAINST HBV AND HCV

When infecting the liver parenchyma hepatotropic viruses such as HBV or HCV continuously release viral particles into the blood stream. The first line of defense viruses will encounter includes natural killer (NK) cells and natural killer T (NKT) cells, which abound in the liver (4). These cells are activated by type-I interferon (IFN) (α and β) released by infected liver cells. NK and NKT cells both can eliminate infected cells, but also constitute a relevant source of IFN-γ and tumor necrosis factor (TNF) alpha (5). These cytokines inhibit viral replication through non-cytolytic mechanisms, that is, can eliminate viruses without destroying liver cells. NK cells are activated by IL-12 released from dendritic cells (DCs), and thus become empowered to eliminate both infected cells and immature DCs with no Th1 cytokine profile, which would not result in appropriate stimulation of a specific response (6). According to the balance between cytokines released by innate immune system cells resident in or recruited by the liver (IL-4/IFNα/IL-12), NK cells may induce partial or total DC maturation (7).

DCs can process viral antigens and present them to specific immune system cells via class-I and class-II ma-jor histocompatibility complex (MHC) molecules. DCs capture viral particles through Toll-like receptors (TLRs). Upon activation these cells secrete several types of cytokines (IL-12, TNF-α, IFN-α, IL-10) that will regulate and polarize the response of adjacent cells (8). Two types of DC have been described; myeloid DCs mainly produce IL-12 or TNF-α, whereas plasmacytoid DCs release IFN-α (9). Mature DCs leave the liver after viral epitope collection and head for lymph nodes, where they will activate T cells in the specific immune system (10).

Cytokines released in the liver parenchyma induce chemokine release by liver cells, including interferon-inducible protein (IP-10/CXCL10), interferon-induced monokine (Mig/CXCL9), macrophage inflammatory protein (MIP/CCL3)-1α, and MIP 1-β/CCL4, which recruit inflammatory infiltration (11) including specific cells capable of infection control.

Both mature DCs and immature T cells, both of which express chemokine receptor CCR7, are recruited towards lymph nodes by secondary lymphoid-tissue cytokine (SLC/CCL21) (10). In the lymph node T cells expressing T-cell receptors (TCRs) appropriate for the recognition of epitopes presented by DCs in their MHC molecules are activated. The interaction between the TCR and MHC-viral epitope complex, together with appropriate co-stimu-
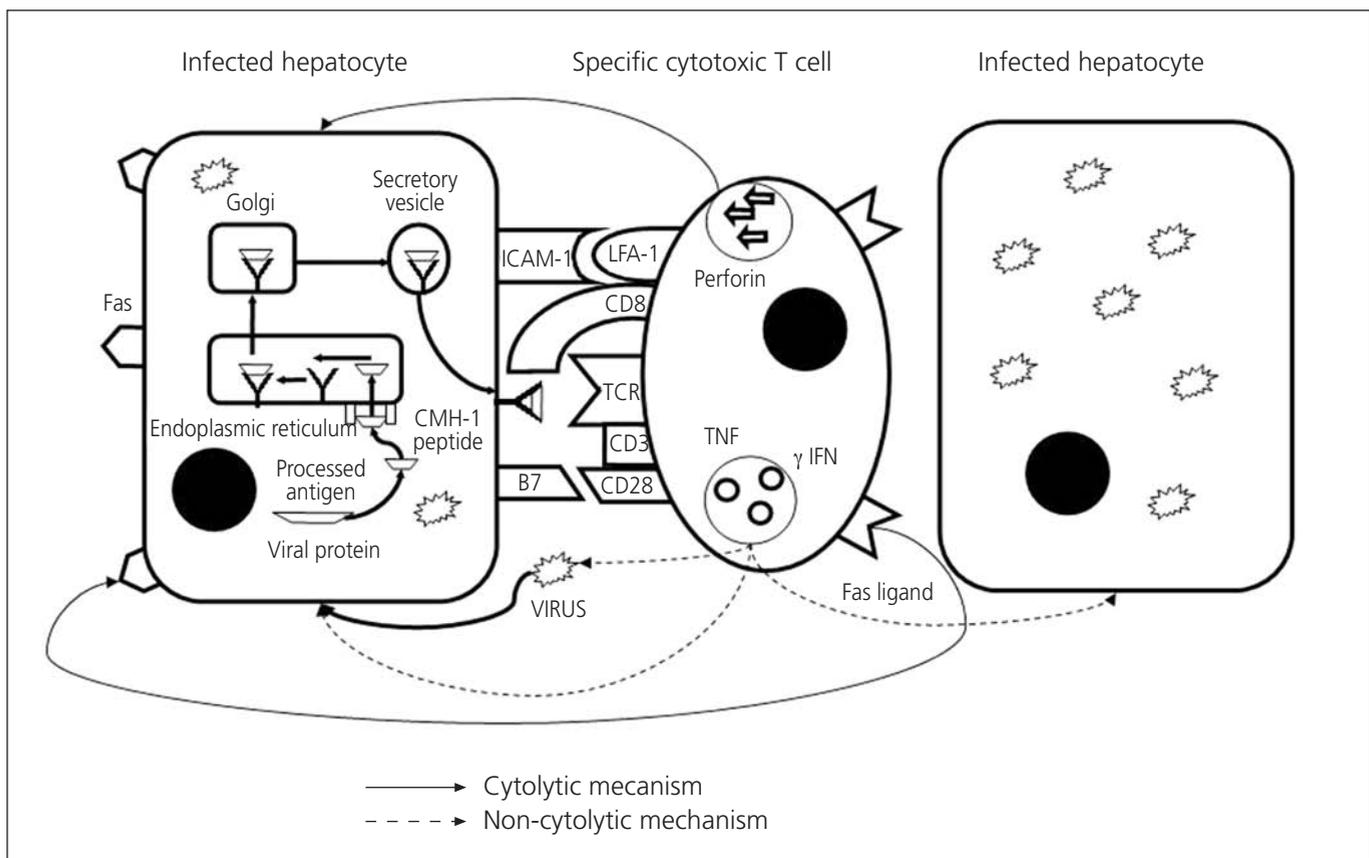

Fig. 1. *Cytolytic mechanisms for viral control based on perforin activity and Fas-FasL interaction.* ------- Non-cytolytic mechanisms for viral control through type-I cytokine production.

lating molecules and an adequate cytokine environment, results in specific T-cell activation. Certain specific CD8 T cells, cytotoxic T lymphocytes (CTLs), become cytolytic, secrete type-I cytokines (Fig. 1), and express chemokine receptors that will let them travel to the in-fected liver for infection control (12-14) (Fig. 2). Specific CD4+ T cells will regulate the adaptive response by secreting Th1 cytokines (IL-2, IFN-γ, TNF-α) to facilitate a cytotoxic response, and Th2 cytokines (IL-4, IL-10, IL-13) to regulate humoral response (15).
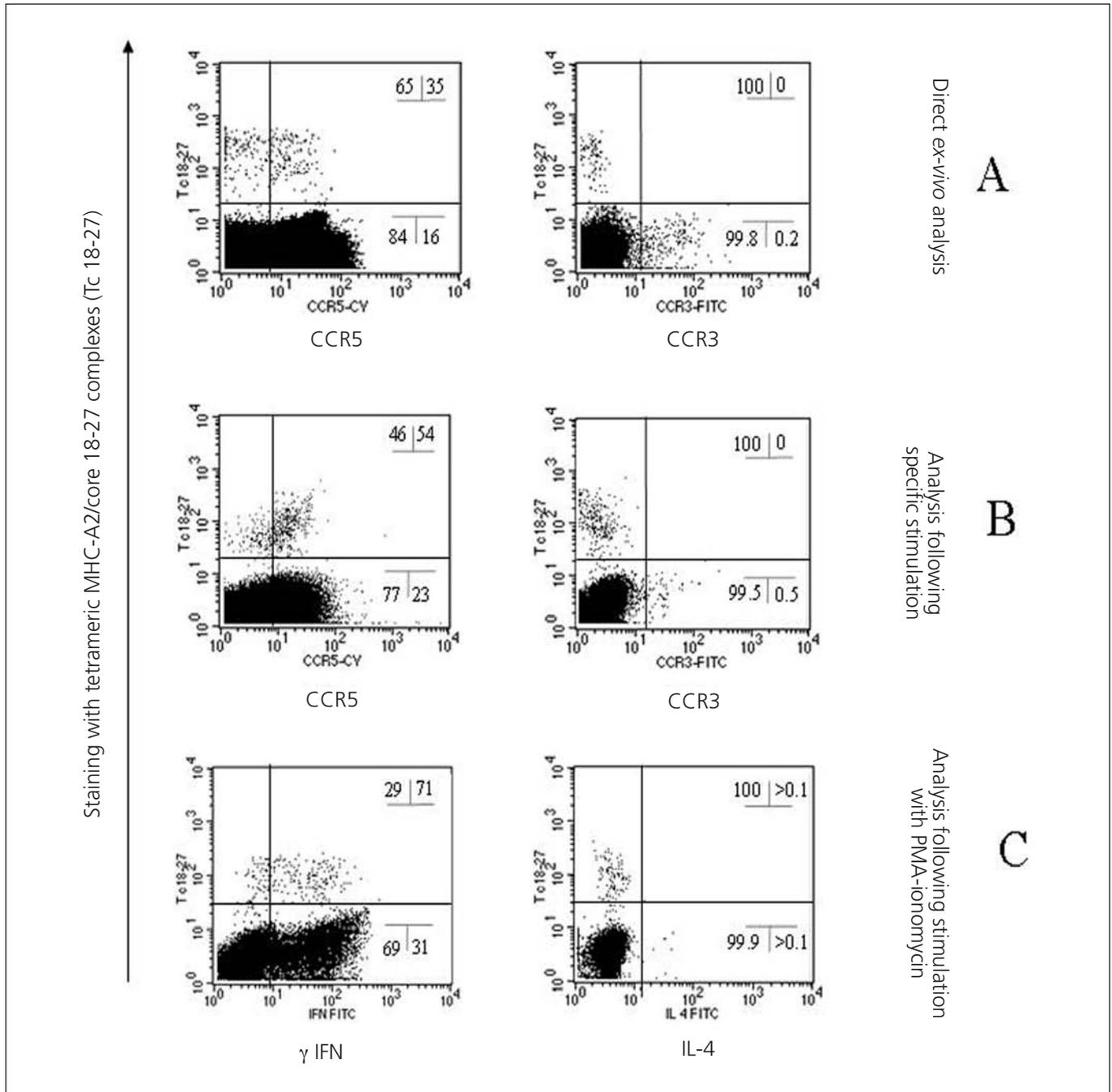

Fig. 2. *Cytokines and chemokyne receptors in the specific cytotoxic cell response associated with viral control during infection with HBV.* Flow cyto-metry data diagrams showing the staining of peripheral blood CD8+ T cells with tetrameric MHC-A2/core 18-27 complexes (Tc 18-27) and monoclo-nal anti-CCR5, anti-CCR3, anti-IFNγ, and anti-IL-4 antibodies. Note how HBV-specific CD8+ T cells controlling the infection may overexpress Tc1 res-ponse-associated receptors, and release type-1 cytokines following antigen contact. A. An *ex-vivo* analysis of CCR5 and CCR3 expression by HBV-specific CD8+ T cells (Tc 18-27). B. An analysis of CCR5 and CCR3 expression by HBV-specific CD8+ T cells (Tc 18-27) following *in-vitro* stimula-tion with HBV core peptide 18-27. C. An analysis of IFNγ and IL-4 production by HBV-specific CD8+ T cells (Tc 18-27) following nonspecific stimulation with PMA and ionomycin.

It is widely accepted that adaptive immune response plays a key role in the control of infection with hepatotropic, non-cytopathic viruses. Infection control correlates to a multispecific polyclonal response capable of secreting type-I cytokines and of expressing Th1/Tc1 response-associated chemokines (16).

However, both HBV and HCV often manage to escape immune response. To this end they interfere with various immune mechanisms including cytokine activity modulation.

THE ROLE OF CYTOKINES IN PERSISTENT INFECTION WITH HBV AND HCV

Cell tropism and entry into the host cell

Liver parenchyma is the place for HBV and HCV replication. Receptors for viral entry have not been fully identified, but potential candidates include some cytokine receptors. During infection with HBV IL-6 receptor has been seen to interact with polypeptides included in hepatitis B surface antigen (17), and this may thus be an entry route into the liver cell.

Innate response (Fig. 3)

Production of type-I interferon (IFN): IFN α/β

A primary cell defense mechanism during initial infection is the synthesis of anti-viral cytokines such as type-I interferon (IFN α/β) (18). On binding its receptor this cytokine activates a number of intracellular mechanims that can prevent viral replication and spread to other liver cells. *In vitro*, HCV can block type-I IFN induction, which is however not the case *in vivo*. HCV is a good inducer of IFN α/β expression, possibly because this virus replicates via dsRNA intermediaries (4,19,20). These intermediaries rapidly activate the dsRNA-sensitive cell apparatus, and thus stimulate IFN α/β expression induction (21). In HCV chimpanzee models a high expression of type-I IFN-induced genes has been seen early during infection. However, HCV seems to be unresponsive to IFN α/β effects, and effectively replicates in the liver despite such gene induction. This possibly results from the fact that non-structural proteins NS3 and NS5A, and structural protein E2 may both potentially block the expression and transcription of IFN α/β-induced genes. This has been demonstrated *in vitro*. HCV NS5A protein has also
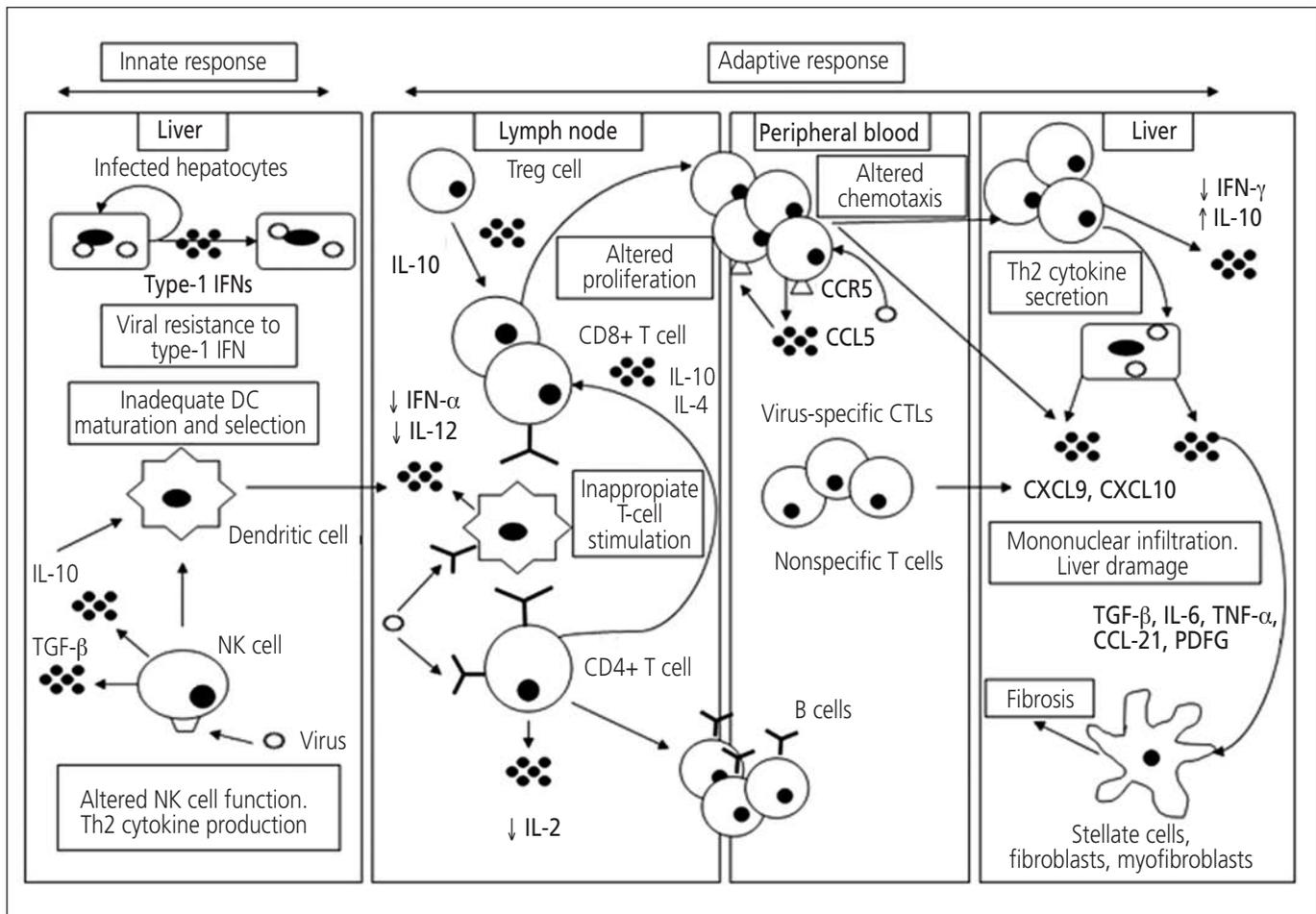

Fig. 3. A schematic of changes induced by persistent infection on the cytokine network involved in persistent infection with hepatotropic viruses.

been seen to induce proinflammatory chemokyne IL-8 expression, which is associated with IFN α inhibition both *in vitro* and *in vivo* (22). Membrane-sited Toll-like receptors (TLRs) allow cells to detect viral particles. HCV NS3/4A protease blocks TLR-3 signaling, and also blocks a dsRNA binding protein (RIG-1) that activates interferon regulatory factor 3 (IRF-3), a central mediator of IFN-β induction during a response to viral infection (23,24).

In contrast HBV induces no type-I interferon expression during initial infection (25). In HBV infection in chimpanzees, viruses remain hidden from the immune system during the first few weeks, and hence induce no effective innate response. Such relative invisibility results from various viral replication strategy components including transcriptional template retention in the nucleus, sequestration of the replicated genome in the cytoplasm, and similarity between mRNA molecules and normal hepatocyte transcripts (26,27). From all this hepatocytes will not release IFN α/β early during initial infection, hence allowing HBV replication and spread.

These data show two distinct mechanisms used by HCV and HBV to escape IFN α/β anti-viral effects in early infection, and thus propagate before the emergence of any specific response.

Blocked type-II interferon production in natural killer (NK) cells and natural killer T (NKT) cells

NK cells and NKT cells play a central role in innate immune response against several viral infections, as is the case with murine cytomegalovirus infection in mice (28). These cells exert their anti-viral action through direct, non-MHC-restricted cytotoxic mechanisms and IFN-γ production (28). In addition, they play an edition role on dendritic cells, allowing maturation for DCs favoring the development of Th1/Tc1 responses (6). However, they do not seem to play a significant role in infection with HBV or HCV. In chimpazee models with acute HCV or HBV infection no expression of IFN-γ-inducible genes is seen when HBV or HCV is spreading across the liver parenchyma (20,29,30). Therefore, data suggest that these viruses can block NK-cell and NKT-cell functions thus preventing anti-viral cytokines such as IFN-γ from being produced. A potential mechanism for this blockade in HCV infection is via an interaction between HCV E2 protein and NK-cell CD81 molecule (31,32). This interaction would block the activation of NK cells, which subsequently would release no IFN-γ, would develop no cytolytic action, and would not contribute to appropriate dendritic cell maturation.
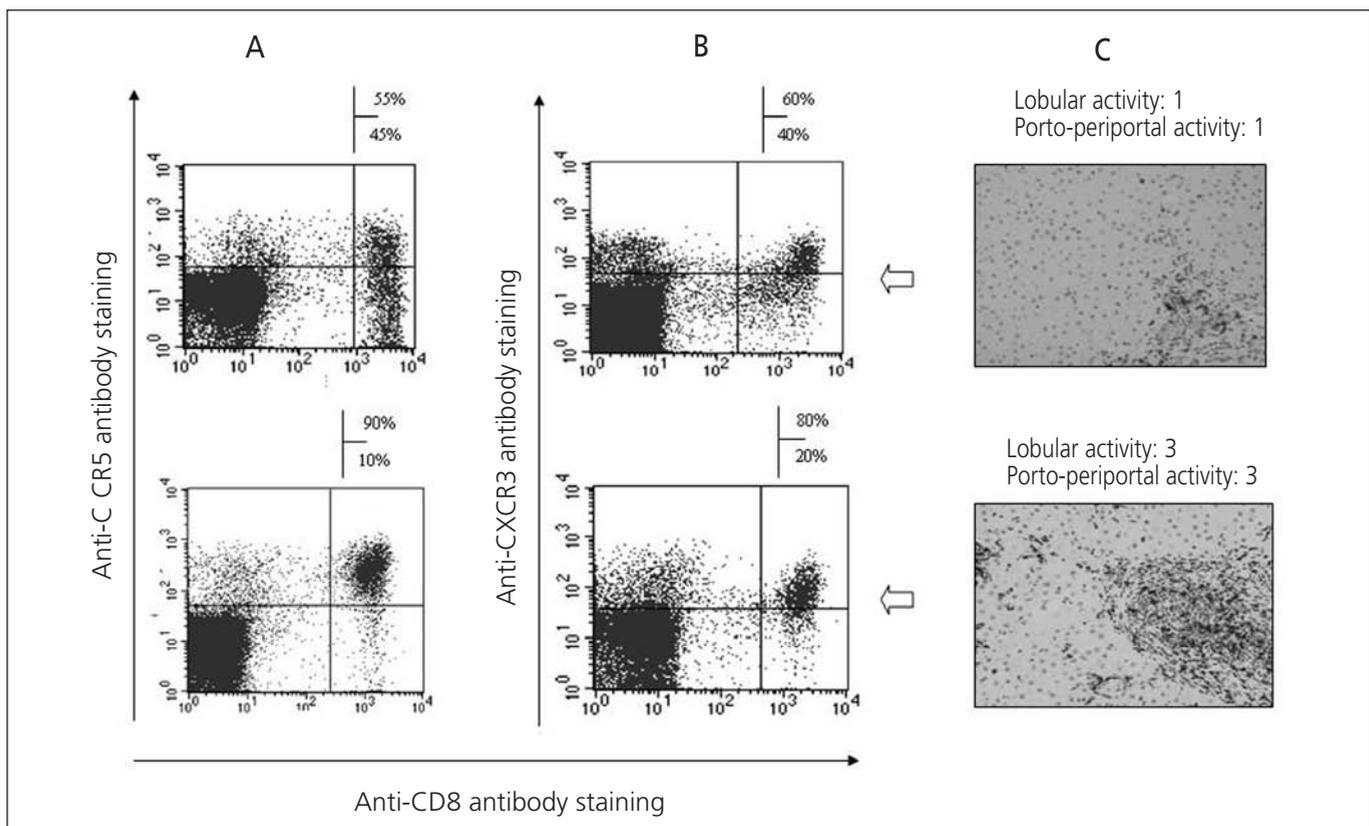

Fig. 4. *Positive correlation between chemokine receptor expression associated with Th1/Tc1 response in intrahepatic CD8+ cells and extent of liver inflammation in chronic hepatitis C.* A. An analysis of CCR5 expression by intrahepatic CD8+ T cells using flow cytometry. B. An analysis of CXCR3 expression by intrahepatic CD8+ T cells using flow cytometry. C. Immunohistochemical staining of intrahepatic CD8+ cells using the immunoperoxidase technique (X400). Scheuer's histological activity index.

Changes in cytokines produced by dendritic cells

Cytokine production by DCs is important for T-cell activation and innate immunity. During chronic infection with HCV a decrease in IFNα production by plasmacytoid DCs has been reported after stimulation with TLR-9 ligands (33). A decrease in IL-12 production by myeloid DCs has also been found during chronic infection with HCV in the presence of stimuli such as CD40L or poly-(I:C) (34). This decreased production of type-I IFN and IL-12 may explain the Th1-to-Th2 response shift seen in chronic hepatitis C (15). *In vitro* studies have shown that HCV structural proteins can interact with TLR2 in monocytes/macrophages, and induce IL-10 production, which eventually inhibits IL-12 production in myeloid cells and IFN-α production in plasmacytoid DCs (35). This decreased production of type-I cytokines contributes to inadequate NK-cell and NKT-cell activation. Furthermore, this dendritic cell-related cytokine profile cannot polarize T-cell responses towards a Th1/Tc1 phenotype (36).

Adaptive response (Fig. 3)

Virus-specific CTLs and helper CD4+ T cells play a key effector and regulatory role in immune responses against HCV and HBV. Specific CD4+ T cells play a key role in adaptive response in that they provide help in activating cytotoxic and humoral responses. They can secrete Th1 cytokines including IFN-γ, which favors neutrophil and macrophage recruitment, and leads to inflammatory response. They also may release Th2 cytokines such as IL-4 and IL-10, which limit Th1 cytokine-mediated response and favor the development of humoral response (37). A multispecific, strong, sustained, CD4+-T-cell-specific Th1 response may be seen in infections with hepatotropic viruses evolving to resolution (38,39,44). However, when infection becomes chronic a weak CD4-T-specific response with few specificities and scarce type-I cytokine production is observed (40,41).

CD8+ CTLs can clear viruses using apoptosis-related cytolytic mechanisms, and non-cytolytic mechanisms mediated by type-I cytokines (IFN-γ, TNF-α) (5) (Fig. 1). In chronic infection with HBV or HCV specific CTLs are few and engage few specific targets; they also display anergic characteristics with reduced type-I cytokine secretion (42-44). Changes in CTLs to allow secretion of these cytokines are multifactorial, but a number of cytokines doubtless play a role. The interaction of the HCV core protein with the globular domain of C1q receptor in T cells has been seen to reduce IL-2 production in T cells (45). This change decreases cell spread and maturation, and prevents these cells from reaching their cytotoxic and IFN-γ secreting potential (43,46). On the other hand, intense T-cell receptor stimulation during persistent infection results in an overexpression of PD-1, a negative co-stimulatory molecule, which favors the development of

cell anergy with failed type-I cytokine secretion (47-49) (Fig. 5). The presence of inadequate activation mediated by antigen-presenting cells in the setting of Th2/Tc2 cytokine expression is also a factor (36). Another potential mechanism of blocked type-I cytokine production results from regulatory T cell (Treg) activity. These cells can release IL-10 and TGF-β, and inhibit proliferation and cytokine synthesis in T cells, either directly or through other cytokines, in both hepatitis B and C (50,51). The presence of CD8+, CCR7- regulatory T cells has been demonstrated in the liver of patients with chronic hepatitis C; these cells can inhibit HCV-specific CD8+ T cells via IL-10 production (52).

Cytokines produced by T cells play a role in the regulation of humoral responses with both neutralizing and non-neutralizing antibodies (53). Nevertheless, these responses cannot control chronic viral hepatitis, even though they play a role in the pathogenesis of extrahepatic manifestations (15).

CYTOKINES AND LIVER DAMAGE (Fig. 3)

When specific immune response fails to control viral replication nonspecific inflammatory infiltrates are recruited into the liver that are responsible for liver damage (54). The infected liver secretes IFN-γ-induced chemokines such as CXCL9 and CXCL10, which results in the migration of nonspecific mononuclear cells into the liver (11,55). These cells are unable to control infection but result in sustained low-grade liver damage. A positive correlation has been reported between the expression of both these chemokines and their receptors, and histological damage (56-58) (Fig. 4). Inhibiting these chemokines limits nonspecific cell migration, and hence reduces inflammation with no impact on the actions of anti-viral specific CTLs (59). Hepatotropic viruses block the expression of chemokine receptors associated with Tc1/Th1 response in order to hinder the migration of specific and nonspecific responses to the liver, thus favoring viral persistence (60). By binding CD81, HCV E2 protein has been seen to induce RANTES/CCL5 expression in T cells. Overexpressed RANTES/CCL5 binds its CCR5 receptor, which results in receptor internalization. This reduced expression of chemokine receptors associated with Tc1 response in T cells may impair these cells' chemotaxis into the liver (61).

The recruitment of persistent mononuclear infiltrates leads to the development of chronic inflammation, which results in sustained liver damage. Finally, chronic inflammation induces regenerating mechanisms in the liver parenchyma. Several factors influence this process, including cytokines such as IL-6, TNF-α, TGF-β, HGF, and EGF. These and other factors activate transcription factors such as NF-κβ, STAT3, AP-1, and C/EBP, which initiate the gene expression cascade leading to hepatocyte proliferation (62).
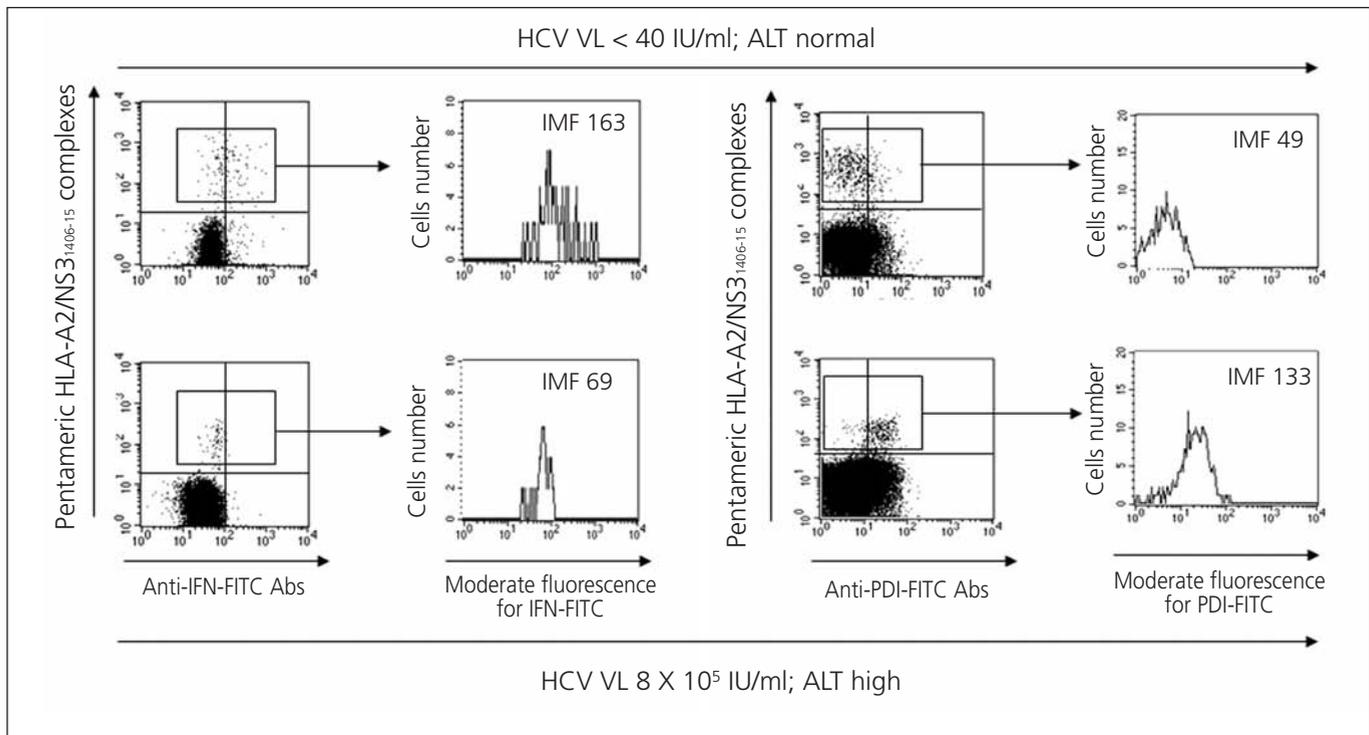
Fig. 5. *Negative correlation between co-stimulatory PD-1 expression and IFN-γ production in HCV-specific cytotoxic cells.* A flow cytometric analysis of CD8+ T cells specific against epitope NS31406-15 in peripheral blood. PD-1 expression and IFN-γ production following specific stimulation are shown for CD8+ cells stained with tetrameric MHC-A2/NS3 1406-15 complexes. In the patient with a high viral load specific cytotocix T cells strongly express PD-1 and produce no IFN-γ; the patient who is controlling infection has cells that can produce IFN-γ with a negative PD-1 phenotype.

Persistent inflammation also activates hepatic stellate cells, myofibroblasts, and fibroblasts, which initiate collagen, laminin, fibronectin, and proteoglycan production and deposition, which favors the development of liver fibrosis. The activation of these cells is regulated by pro-inflammatory cytokines such as TGF-β, IL-6, TNF-α, CCL-21, and PDFG, among other stimuli (63). A dysregulation of liver regeneration processes ultimately occurs, which results in liver cirrhosis.

THERAPEUTIC ROLE OF CYTOKINES IN CHRONIC VIRAL HEPATITIS

INFα is the only cytokine currently used in the treatment of chronic viral hepatitis. In chronic hepatitis C pegylated INFα combined with ribavirin leads to sustained viral clearance in 50% of patients (64). In monotherapy it leads to anti-HBe seroconversion in 25% of patients with e+ chronic hepatitis B (65). INFα has direct anti-viral and immunomodulating actions that favor Th1/Tc1 response restoration (66-68). On the other hand ribavirin, a wide-spectrum antiviral agent used in combination therapy for hepatitis C, has immunomodulating effects that induce type-I cytokine production (69). Sustained viral load reduction with antiviral agents has also been seen to facilitate specific T response recovery with type-I cytokine production in both hepatitis B and C (70).

An exogenous administration of Th1-inducing cytokines such as IL-12 (71) or anti-inflammatory cytokines such as IL-10 has also been attempted to reduce intrahepatic inflammation severity (72). However, such therapies remain experimental, and their effectiveness is unclear.

From a theoretical standpoint Tc1-associated chemokine receptors may represent an interesting therapeutic target in the development of drugs for patients with chronic hepatitis unresponsive to antiviral agents, their aim being a reduction of liver inflammation and progression to fibrosis by blocking inflammatory cell migration into the liver (55,59).

CONCLUSIONS

Cytokines are inter-cell mediators involved in viral control and liver damage as induced by infection with HBV or HCV. The complex cytokine network operating during initial infection allows a coordinated, effective development of both innate and adaptive immune responses. However, both HBV and HCV interfere with cytokines at various levels, and escape immune response by

inducing a Th2/Tc2 cytokine profile. Inability to control infection leads to the recruitment of inflammatory infiltrates into the liver parenchyma by pro-inflammatory chemokines, which results in sustained liver damage, and eventually in liver cirrhosis.

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