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Review

Immunological and molecular aspects of liver fibrosis in chronic hepatitis C virus infection

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Summary. Chronic C hepatitis represents a major health problem worldwide, mainly because progression of the tissue damage leads to the development of cirrhosis and hepatocellular carcinoma. In this review we discuss the molecular mechanisms underlying the development of liver fibrosis. In particular we consider some immunologic aspects that regulate the interaction between HCV and the host immune defense. Reflections are made about the roles played by the host capacity to respond to the viral infection during therapy and the consequences of the deposition of extracellular matrix (ECM) proteins leading to the development of fibrosis. The involvement of inflammatory cytokines in regulating the proteolytic remodeling of the liver and the ECM turn-over is essential for the activation of hepatic stellate cells (HSCs), that have an important role in the progression of liver fibrosis. Finally, we analyze one of the aspects involved in the activation of the HSCs, namely the proteolytic remodeling of the surrounding environment.

Key words: Immune response, Extra-cellular matrix proteins, Matrix metalloproteinases, Cytokines, Lymphocytes, Chronic hepatitis, Liver fibrosis

Introduction

Hepatitis C virus (HCV) represents a major health problem worldwide, owing to the fact that following the primary HCV infection, chronic hepatitis develops in about 85% of infected individuals (Alter et al., 1992). As a consequence, patients are at risk of progressive liver disease which favors the generation of long-term complications such as cirrhosis, end-stage liver disease and hepatocellular carcinoma (Bruix et al., 1989).

Despite advances in the knowledge of the epidemiological and virological characteristics of HCV, the mechanisms accounting for viral persistence and/or hepatocellular damage have not yet been fully clarified, above all due to the lack of a reliable in vitro cell culture models on which to investigate HCV infection. With reference to this point, either viral or host factors have been suggested to play a role in the clinical outcome of the disease. Several studies have indicated that older age, race, gender, alcohol intake and high body weight might promote the development and the progression of liver injury (Alberti and Benvegnu, 2003; Pawlotsky, 2004). Certainly, replication efficiency, nucleotide substitution rate and viral heterogeneity (quasispecies induction) negatively influence the disease pattern (Boyer and Marcellin, 2000; Freeman et al., 2001). On the other hand, the observation that the recovery from acute infection is associated with the effectiveness of the host immune response implies that the immune system is involved in the pathogenesis and outcome of the infection (Gruner et al., 2000; Lechner et al., 2000; Thimme et al., 2001; Rosen et al., 2002).

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Relationship between HCV and immune system

The immune defense against viral infection usually requires the activation of two different effector mechanisms. First of all, engagement of the innate immune response, which occurs after a few hours or days from the infection, and is mediated by a wide array of cellular and humoral inflammatory mediators, such as Natural Killer (NK) cells, Natural Killer T (NKT) lymphocytes, dendritic cells, macrophages and complement factors (Biron, 1999). Moreover, in the immediate antiviral defense pathways the production of type I interferon (IFN), i.e. IFN-α/ß plays a key role by exerting modulatory effects. In the early phase, in fact, IFNα/ß inhibits interleukin 12 (IL-12) and IL-12-dependent IFN-γ production, while it triggers blastogenesis and NK activity (Biron, 1999). These events give rise to the induction of genes that encode proteins related to the adaptive immune response, such as Major Histocompatibility Complex (MHC) class II antigens, immunoproteasome subunits and chemokines. As a consequence, the activation of the innate response is in turn responsible for the secretion of IFN-γ by NK cells and/or the upregulation of CXCL9 and CXCL10

chemokines production. The binding of the latter soluble factors to CCR5 and CXCR3 chemokine receptors on liver-infiltrating lymphocytes favors their recruitment into Disse's space and in the parenchymal tissue (Racanelli and Rehermann, 2003). Since very few preliminary data are available about the innate immune response (Bertoletti and Ferrari, 2003), herein, we outline the relationship between adaptive immunity and chronic HCV infection.

In this regard, the activation of T cell functions is a dynamic process which occurs by a series of two-step signals. Firstly, T lymphocytes must receive a signal through the interaction of T cell receptors (TCR) with peptides associated with MHC class I and class II molecules on antigen-presenting cells (APC). This gives rise to the upregulation of different structures located on the lymphocyte membrane, i.e. CD28, IL-2 receptor ß and γ subunits, and favors the expression of the CD40 ligand. Therefore, the cross-linking of these structures with their homologous ligands on APC, CD28 with CD80 and the CD40 ligand with CD40, respectively, generates costimulatory signals which fully activate T cells, even in the presence of suboptimal TCR-driven stimulation (Lenschow et al., 1996; Whitmire and Ahmed, 2000).

Both humoral and cell-mediated immune responses are involved in the host protection against invading virus. However, the presence of circulating anti-HCV antibodies even during persistent infection clearly indicates a limited efficiency of humoral immunity in achieving viral clearance (Cerino et al., 1997). As far as the cell-mediated response is concerned, immunophenotyping analysis of liver specimens from chronic HCV infected patients provides strong evidence for the recruitment of T cells at liver sites. A significant infiltration of CD4 cells occurs, in fact, in portal and periportal areas, while lymphocytes displaying CD8 antigen are detected at lobular and periportal sites (Onji et al., 1992; Fiore et al., 1997). At the same time, positive staining for Histocompatibility Complex Antigen (HLA) class I and class II has been observed at the tissue level, with an increased expression of HLA I when compared to its HLA class II counterpart (Onji et al., 1992; Fiore et al., 1997). The finding of hepatocytes carrying CD80 and CD40 molecules (Mochizuki et al., 1997, Fiore et al., 1999) confirms the overall induction of accessory molecules, which are required for the development of an efficient immune response.

The evidence of a high rate of chronic infection, despite the tissue recruitment of T cells, implies that an infiltration of both HCV-specific and non-specific T lymphocytes likely occurs in infected liver. Nevertheless, these cells may trigger a cascade of events resulting in either hepatocellular damage or an imbalance of their reactivity, at least in terms of the immune mechanisms devoted to viral clearance. In this regard, a spectrum of alterations, in the form of an impairment of cytotoxic functions related to the overcoming of cytotoxic T lymphocytes (CTL) frequency, the stunning of HCV-specific CTL, the suppression of the CTL response by high levels of soluble HLA-I antigens and a reduced IFN-γ release due to a dysfunction of IL-12 production, has been demonstrated using different experimental systems (Antonaci and Schiraldi, 1998; Cerny and Chisari, 1999; Piazzolla et al., 2000; Eisen-Vandervelde et al., 2004).

The possibility of a CD4-driven dysregulation of the CD8 effector cell function should also be taken into consideration. A strong polyclonal and persistent CD4 responsiveness to HCV proteins has, in fact, been found in subjects who recover from acute infection, whereas individuals with chronic HCV hepatitis display only a weak responsiveness (Chang et al., 2001). In addition, recovered individuals display a reactivity towards non-structural proteins, whereas an occasional focused response has been determined in chronically infected patients (Diepolder et al., 1996; Cooper et al., 1999). In this framework, novel findings suggest a possible role for CD4+CD25+ T regulatory lymphocytes in the down-modulation of immunoresponsiveness during chronic HCV infection. These cells usually bear the IL-2 receptor α chain and exert a suppressive activity through the secretion of IL-10 and transforming growth factor ß (TGF-ß) (Dieckmann et al., 2002; Shevach, 2002). The high frequency of CD4+CD25+ lymphocytes in chronic HCV subjects in comparison to recovered individuals or healthy donors, and the demonstration that these cells specifically suppress IFN-γ production by HCV-specific CD8 lymphocytes, without affecting T cell reactivity to phytohemagglutinin or tetanus toxoid, points out a critical role for T regulatory cells in the CD8 imbalance (Sugimoto et al., 2003).

With regard to CD4+CD25+ cells, recent data indicate that T regulatory lymphocytes might hamper dendritic cell function (Misra et al., 2004), implying that an impairment of APC priming might possibly occur in chronic HCV infection. It should be emphasized that in normal liver, dendritic cells exhibit an immature phenotype with low expression of both HLA class I antigens and costimulatory molecules. On the contrary, when they take up an antigen they undergo a maturation process characterized by the expression of CD83, CD80, CD86, HLA class I and class II antigens at surface sites (Carbone and Heath, 2003). In liver samples from chronic HCV patients, dendritic cells displaying a mature phenotype are usually detected in portal infiltrates in close contact with CD8 lymphocytes and are drained by newly formed lymphatic capillaries into lymphatic vessels in portal areas (Galle et al., 2001). In the light of these findings, the question arises whether such cells exhibit an impairment of their functional capacity. Evidence has been provided that chronic HCV infection is associated with an inhibition of the allostimulatory capacity exerted by monocyte-derived dendritic cells (Bain et al., 2001). On the other hand, the occurrence of productive HCV infection in either myeloid or plasmacytoid-derived dendritic cells seems to indicate that dendritic cell dysfunction is likely related to a direct cell infection by HCV and not to a general imbalance due to viral persistence (Bain et al., 2001;

Goutagny et al., 2003).

Inflammation and tissue damage

The tissue damage represents the result of the interaction between HCV replication and host response. This balance is very different in each patient, so that patients are observed with viral replication with or without elevated levels of transaminases. Several different studies have attempted to investigate the molecular mechanisms underlying such differences, but so far it seems more likely that the individual ability to respond to the viral infection is crucial. Nevertheless, the main goal of antiviral therapy is to block or to slow down the inflammation and consequently the occurrence of liver fibrosis (Manns et al., 2001; Lee et al., 2002; Poynard et al., 2002). In this regard, the role of cytokine (CK)-mediated modulation of the immune response against HCV needs to be addressed. The characterization of Th1 and Th2 cells within the CD4 subset according to different CK release has offered a suitable approach for the evaluation of additional regulatory mechanisms involved in the antiviral defense (Mosmann and Sad, 1996). With reference to CK levels, several reports provide clearcut evidence for an increased serum concentration of IL-2, IL-4, IL-10 and IFN-γ in chronic hepatitis C (Cacciarelli et al., 1996; Fan et al., 1998). In these studies, Th2-type CK levels are higher than those of the Th1 counterpart, suggesting the involvement of Th2 lymphocytes in the persistence of the infection. However, analysis of the CK profile of T cells at the liver tissue level outlines a predominance of IFN-γ and IL-2 synthesis during the chronic phase of the disease (Bertoletti et al., 1997; Schweyer et al., 2000). At the same time, it is noteworthy that during acute hepatitis C infection, a prevalence of Tumor Necrosis Factor α (TNF-α), IFN-α and IL-2 secretion by liver-infiltrating cells occurs, giving rise to the activation of cellular genes responsible for antiproliferative and antiviral effects (Koziel, 1999). Such a pattern is usually associated with virus clearance and recovery from acute infection (Zeuzem, 1999). Both observations suggest that the high mutation rate of HCV may lead to persistent T cell activation with the subsequent production of Th1 CK. On the other hand, the prevalence of a Th1-driven inflammatory liver microenvironment triggers exaggerated cytotoxic and/or apoptotic functions which account for the damage of either HCV-infected or bystander hepatocytes (Antonaci and Schiraldi, 1998; Jacobson Brown and Neuman, 2001). On this basis, the production of Th2 CK may represent a host attempt to confine the Th1 response to the liver site in order to prevent systemic effects.

Proteolytic remodelling, tissue repair and liver fibrosis

The tissue repair that follows on from the HCV replication/ host immune response is at the end-stage responsible for the occurrence of fibrosis that underlies the development of cirrhosis. In fact, the legend of Prometheus and his never-ending liver regeneration cannot be applied to reality. The necrosis that occurs as a consequence of the inflammation has to be healed by the accumulation of extracellular matrix proteins (ECM), mainly secreted by the hepatic stellate cells (HSCs). This process, although apparently very simple, has actually proved very complicated and is not yet fully understood. The HSCs, that are well recognized to play a central role in liver fibrosis (Friedman et al., 1985), in a healthy liver state are basically "inactive", while when tissue repair is needed they become "active", proliferate and migrate toward the site of necrosis and deposit ECM proteins (Bissell and Choun, 1988). How HSCs convert their phenotype and become active is not yet known, but some evidence suggests that a family of proteolytic enzymes named matrix metalloproteases (MMPs) is likely involved (Giannelli and Antonaci, 2002). In this scenario, Kuppfer cells are also very important since they are a major source of MMPs production. Their production is stimulated by inflammatory cytokines, that feature an altered network in chronic hepatitis (Koziel, 1999; Haruyama et al., 2000; Freeman et al., 2001; Han et al., 2004). Furthermore, other cell types including the HSCs have been reported to produce and secrete MMPs (Benyon et al, 1999). These enzymes play a central role because of their proteolytic activity towards ECM components which are physiologically expressed in hepatic tissue, and whose degradation products have been shown to activate HSCs cells toward a myofibroblastic phenotype, triggering the secretion and deposition of new ECM proteins with a particularly rich content of Collagen type I and III, the main components of the fibrotic tissue in the cirrhotic liver (Theret et al., 1999; Yoshida et al., 1999; Neubauer et al., 2001; Yang et al., 2003; Han et al., 2004). This hypothesis is further supported by an in vivo experimental model where acute lethal hepatitis in mice was blocked by MMP inhibitors such as BB-94, and by the better survival of MMP-2 knock-out animals (Wielockx et al., 2001). In addition, MMP-2 seems to play a role in allowing Ito cell migration within Disse's spaces or toward the inflammation area in response to the degradation of the BM-like structure present in Disse's spaces (Yang et al., 2003). Loss of contact with a BM-like structure, either by remodeling of the surrounding microenvironment or disruption of the adhesive substrates, is also responsible for the activation of the Ito cells and consequent deposition of ECM proteins, migration, or MMP production (Friedman et al., 1989; Sohara et al., 2002; Issa et al., 2003). In addition, proliferation of Ito cells is important in the progression of liver fibrosis, and MMP-2 seems to work as a paracrine stimulator factor or by activating other growth factors such as transforming growth factor (TGF)-ß1, that directly stimulates cell proliferation (Yu and Stamenkovic, 2000; Giannelli et al., 2002a). Hepatocytes have also been implicated in the production of MMPs with consequent activation of HSCs (Haruyama et al., 2000), and therefore a better understanding of the fine balance between MMPs and

their physiological tissue inhibitors (TIMPs) seems to be needed. In particular, another hypothesis suggests that MMP-2 could degrade Collagen type I, since it exhibits an interstitial collagenase-like function, as observed in rabbits where specific MMP-2 inhibitors reduced Collagen type I degradation (Kerkvliet et al., 1999). Thus, it has been proposed that MMP-2 could have an anti-fibrotic activity, although more data are needed to confirm this possibility. Similarly, it has been reported that MT1-MMP could also have a proteolytic activity toward Collagen type I, having a cleavage site in the a-chain identical to that reported for MMP-1 (Ohuchi et al., 1997).

Based on these data, TIMP-1 and TIMP-2 are likely involved in the progression of liver fibrosis in view of their correlation with the inhibition of ECM degradation. Increased levels of TIMP-2 in serum and hepatic tissue (mRNA) were found to correlate with progression of the disease and inflammatory status (Benyon et al., 1996; Ebata et al., 1997; Lichtinghagen et al., 2001). In particular, patients with liver cirrhosis had higher levels of TIMP-1 and TIMP-2 when compared to patients with chronic liver disease, and the latter had higher levels when compared to normal subjects (Benyon et al., 1996; Murawaki et al., 1999; Ninomiya et al., 2001; Giannelli et al., 2002b). An additional role for TIMP-1 in the course of chronic liver damage has been proposed, as it has recently been reported that TIMP-1 protects activated Ito cells from apoptosis both “in vitro” and “in vivo” by inhibiting MMPs (Murphy et al., 2002; Iredale et al., 2003). Furthermore, growth factors such as TGF-b1, which are known to increase in the course of liver fibrosis, play a central role in the pathogenesis of liver fibrosis through the exertion of several mechanisms including the regulation of TIMP-1 and TIMP-2 (Knittel et al., 1996; Moshage, 1997).

Clinical implications

There is no doubt that the main goal of antiviral therapies, including alpha-Interferon, is to reduce viral replication and the consequent inflammation, in order to reduce fibrosis and deterioration of the liver function (Poynard et al., 2002; Sanceau et al., 2002). While up to now the evaluation of fibrosis has been reserved to histological analysis of liver biopsy samples, there is an urgent need for other serum markers capable of quantifying and staging the development of fibrosis.

For this reason, the use of some proteolytic fragments of ECM proteins expressed in the liver has been proposed as a prognostic indicator of the status of fibrosis (Guechot et al., 1994; Murawaki et al., 1995). However, the utility of these fragments would be bolstered by an understanding of the underlying mechanisms and of the proteolytic enzymes that are responsible for their production.

Conclusions

In the course of chronic hepatitis C the liver damage represents the end result of the targeted immune response toward the HCV-infected hepatocytes. The deposition of ECM proteins occurs as a consequence of the cellular damage. The liver tissue remodeling is characterized by an altered ECM turnover that may lead to liver fibrosis, and its progression is responsible for the development of cirrhosis and eventually of hepatocellular carcinoma. Alterations in the surrounding microenvironment due to increased ECM protein production and/or increased proteolytic activity modulated by growth factors or cytokines in response to the inflammation could be responsible for the progression of the liver damage. More studies are needed to gain a better understanding of the mechanisms regulating fibrosis and the modifications of the microenvironment responsible for excessive or faulty tissue remodeling leading to liver failure.

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