Review

Recent Trends in the Immune Response against Hepatitis C Virus

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SUMMARY

Hepatitis C Virus (HCV) represents a viral pandemic infecting 170 million people worldwide, 80% of whom develop persistent infection and approximately 20% cirrhosis. HCV is present in numerous quasispecies in each individual patient, caused by its very high mutation rate. The development of quasispecies has, as a consequence, the development of escape mutants to humoral immunity. Cellular immunity, on the other hand, is believed to be the immune system's effector arm that is utilized the most in the fight against HCV. Recent data suggest that a vigorous, polyclonal and multispecific proliferative CD4+ T-cell response, and especially a Th1 shift in the cytokine profile of peripheral blood is associated with viral clearance. CD4+ Th1 immune responses are needed to prime and maintain the CD8+ cytotoxic T lymphocytes (CTL) response which is responsible for eliminating infected cells. Unfortunately, the response of cytotoxic T lymphocyte in persons with chronic hepatitis C infection seems to be insufficient to contain viremia but sufficient to cause collateral damage through the elaboration of inflammatory cytokines in the liver. A better understanding of the immunity in conjunction with the assessment of viral replication may facilitate further immunotherapeutic and vaccine strategies against HCV infection.

Key words: CD4, CD8, chronic hepatitis C, HCV (Hepatitis C Virus), immune response, immunity, cytokines

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INTRODUCTION

Hepatitis C virus (HCV) was identified in 1989 as a new viral agent responsible for post-transfusion non-A and non-B hepatitis. The virus infects an estimated 170 million people worldwide and thus represents a viral pandemic, one that is five times as widespread as infection with the human immunodeficiency virus type 1 (HIV-1). Furthermore, deaths from HCV infection are expected to more than triple over the next two decades, eventually becoming responsible for greater mortality than AIDS.[1]

Course of the infection

HCV transmission occurs primarily through exposure to infected blood and is usually detected incidentally since most patients with acute hepatitis C infection are either asymptomatic or express only a mild symptomatology.[2] HCV infection becomes chronic in about 80% of the individuals infected. Chronic infection is also characterized by a prolonged asymptomatic period.[3] It is usually only when complications of chronic liver disease occur that patients become symptomatic.[4] As many as 20% of patients with HCV infection develop cirrhosis in the first or second decade of the disease and 1 to 4% of patients may further develop hepatocellular carcinoma.[5, 6]

HCV infection is diagnosed primarily by the presence of anti-HCV antibodies in the serum. Anti-HCV can be detected by enzyme-linked immunoassays (ELISA) or recombinant immunoblot assays (RIBA) but no sooner than the 6th week of infection. On the other hand, HCV RNA can be used for earlier diagnosis since it is detectable in the serum by polymerase chain reaction (PCR) within one to two weeks after infection. Most patients have persistently or intermittently increased serum aminotransferases which rise approximately two to eight weeks after infection. The correlation, though, between severity of liver injury and the degree of raised serum aminotransferase activity is poor and the outcome

of therapeutic protocols is similar in patients with chronic hepatitis C and normal ALT levels and those with elevated ALT levels.[7,8] (Fig.1)

Hepatitis C Virus

Viral replication is extremely robust, and it is estimated that more than 10 trillion virion particles are produced per day, even in the chronic phase of infection.[9] HCV encodes a single polyprotein of 3011 amino acids, which is then processed into 10 mature structural and regulatory proteins. Structural components include the core and the two envelope proteins (E1, E2) while regulatory proteins are the 6 non-structural proteins (NS2, NS3, NS4A/B, NS5A/B). Two regions of the envelope E2 protein, designated hypervariable regions 1 and 2, have an extremely high rate of mutation, believed to be the result of selective pressure by virus-specific antibodies.[10] The core protein is thought to interact with RNA to form the virion nucleocapsid,[11] while the non-structural regions are likely to have a role in viral replication and encode for proteases (NS2, NS3), for a helicase (NS3) and an RNA-dependent RNA polymerase (NS5).[12,13] (Fig.2)
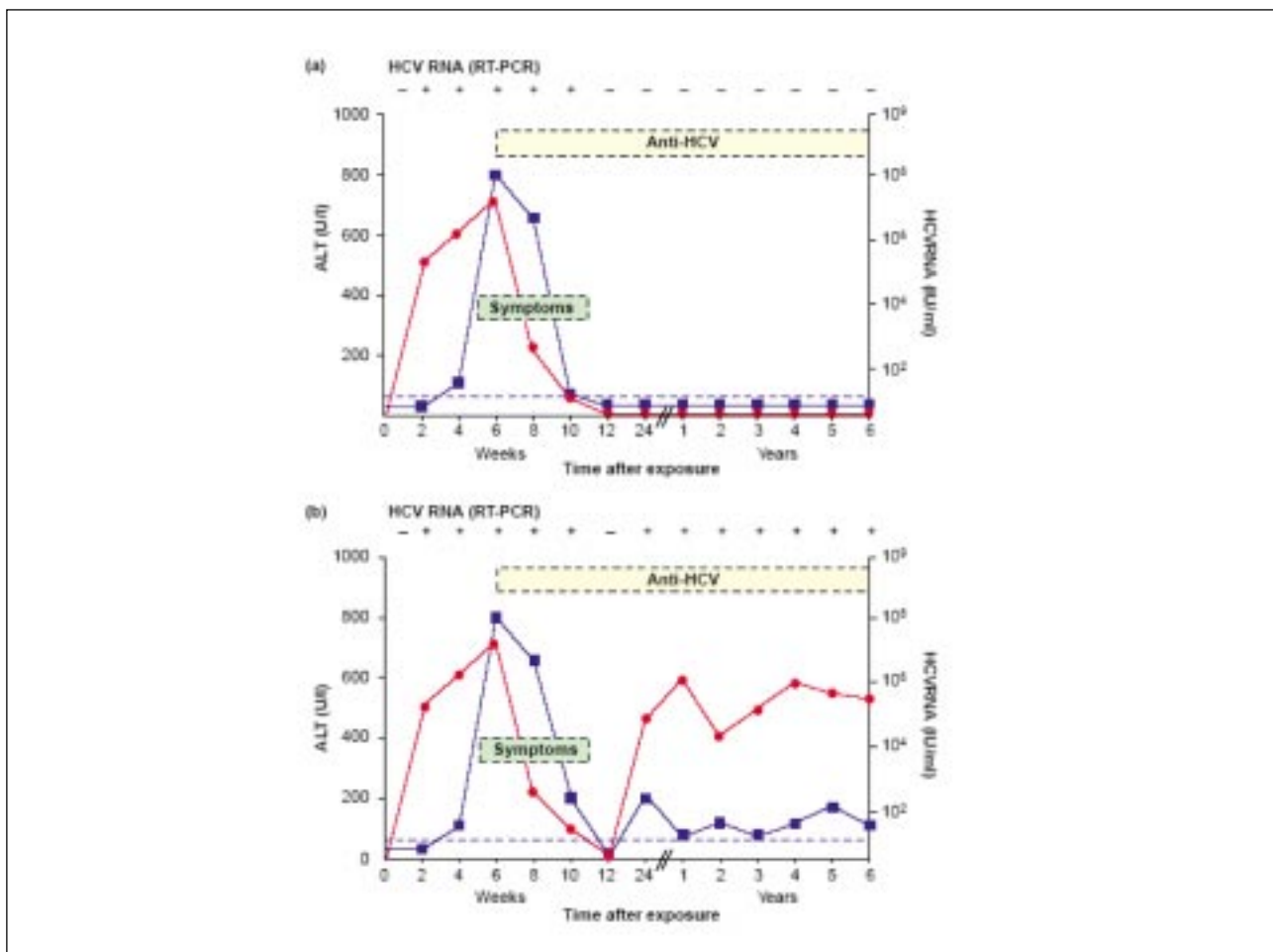

Fig. 1. (a) Course of acute, resolving hepatitis C virus (HCV). HCV RNA (red circles) is detectable in the serum by PCR within one to two weeks after infection. Serum alanine aminotransferase (ALT) levels (blue squares), indicative of liver cell injury, rise approximately two to eight weeks after exposure. Clinical manifestations include malaise, weakness, jaundice and pain in the upper-right quadrant of the abdomen and appear three to twelve weeks after exposure in about a third of patients. As ALT levels decline, symptoms usually subside and HCV-specific antibodies become detectable by enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA). (b) Course of acute, chronically evolving hepatitis C. During the evolution of acute to chronic infection, HCV RNA and ALT levels fluctuate considerably. Chronic hepatitis C is diagnosed if HCV RNA persists in the serum for at least six months. Abbreviation: RT-PCR, reverse transcription-PCR. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Course and outcome of hepatitis C; Hoofnagle JH; Copyright © [2002, Wiley Publishers].[99]
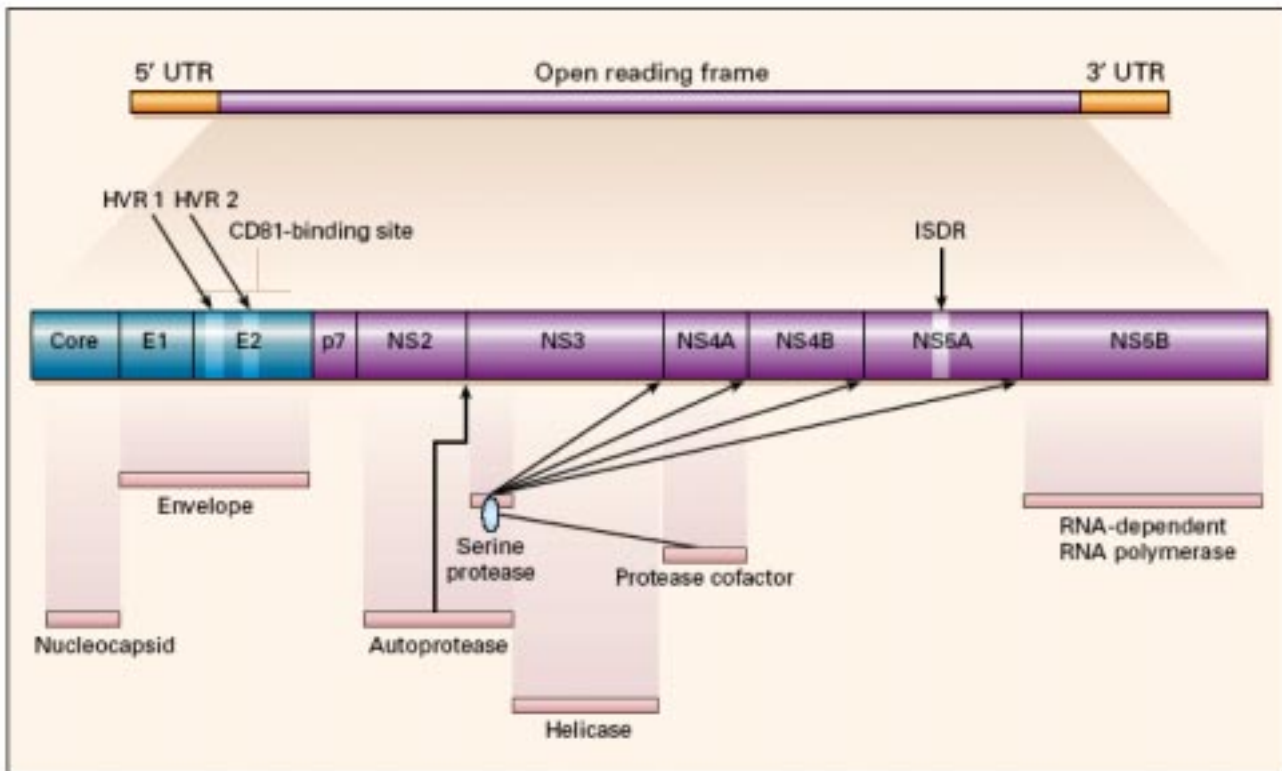
Fig. 2. HCV, a single-stranded RNA virus of 9.5 kb, consists of a single open reading frame and two untranslated regions (UTRs). It encodes a polyprotein of approximately 3000 amino acids, which is cleaved into single proteins by a host signal peptidase in the structural region and the HCV-encoded proteases in the non-structural (NS) region. Reprinted with permission from: Lauer G.M. & Walker B.D. (2001) Hepatitis C virus infection. N Engl J Med, 345, 41; Copyright © 2001 Massachusetts Medical Society.[3]

Six distinct but related genotypes and over 100 subtypes have been identified throughout the world on the basis of molecular relatedness. In Western Europe and the United States genotypes 1a and 1b are the most common, followed by genotypes 2 and 3. Knowledge of the genotype is important because it has predictive value in terms of the response to antiviral therapy, with better responses associated with genotypes 2 and 3 than with genotype 1.[14] Furthermore, several distinct but closely related HCV sequences, referred to as quasi-species, coexist within each infected individual. HCV genotypes vary by as much as 30% in nucleotide sequence while HCV quasispecies vary by less than 5%.[15]

Liver as an immune organ

Liver tissue is organized into hexagonal hepatic lobules separated by portal tracts. Blood supply from the hepatic portal vein and the hepatic artery mix in the hepatic sinusoids, where the blood percolates from the portal tracts to the central veins, passing between plates of hepatocytes through spaces that are lined by liver sinu-soidal endothelial cells (LSECs). The latter comprise an unusual type of vascular endothelial cells which act as antigen presenting cells and provide a biofilter between the sinusoidal blood and plasma within a sub-endothelial space, known as the space of Disse.[16,17] This organization maximizes the exchange of molecules between the sinusoidal space and hepatocytes, allowing the liver to carry out its functions of digestion, detoxification and synthesis of plasma proteins. Blood plasma, lymphocytes and dendritic cell precursors pass from the sinusoids into the space of Disse. From this space, lymph is collected, and it flows through lymphatic vessels that run in the portal tracts to the draining lymph nodes.[18] The liver also contains a large population of resident macrophages, known as Kupffer cells. Kupffer cells attach to the LSEC layer and are activated by endotoxin-type stimuli, including bacterial lipopolysaccharides (LPS) and superantigens with release of acute phase proteins and cytokines capable in turn of activating resident Natural Killer (NK) and Natural Killer T (NKT) cells.[19,20] NK and NKT cells participate in innate immune responses to intracellular

pathogens by antibody dependent cytotoxicity (ADCC) and the production of cytokines.[21]

Innate immune responses

Control of an infection requires a rapid and specific immune response. The innate immune system acts rapidly and provides the first line of defense against a pathogenic threat. Conversely, adaptive immune responses require days to weeks to develop, but provide the specificity component. In the best case scenario, i.e., leading to elimination of the infectious pathogen, these discrete subsets function as part of a coordinated and complementary system, and their importance for host defense is seen in secondary responses in which speed and specificity are united in the form of immunologic memory.[22]

NK and NK T cells are considered to be of particular interest in the innate immune responses against HCV infection because the human liver contains significantly greater proportions of these cells than other organs. Both NK and NK T cells are found to decrease in parallel with the histological progression of HCV.[23, 24] Dendritic cells are also detected in significantly lower numbers in patients with chronic HCV infection.[25] However the importance of these findings is still unclear. More data about the role of the innate immune responses in HCV infection are just beginning to emerge.

Humoral Immunity

The humoral immune response to HCV is polyclonal and directed against virtually every viral antigen. Antibodies directed against the envelope proteins of the virus, and especially the E2 protein, are considered the prime candidates for virus-neutralizing antibodies. In several other viral infections as exemplified in hepatitis B, envelope antibodies induced either after a self-limited infection or vaccination, confer long-lasting immunity. However, their presence in more than 90% of the HCV chronically infected patients and the observation that chimpanzees can be repeatedly infected by the identical HCV strains argue against the possibility of producing an efficient anti-E2 virus neutralizing antibody in vivo in hepatitis C infection.[26,27] Due to mutations occurring in the E2 protein region, it contains the two most variable sequences in the HCV genome, named hypervariable regions 1 and 2 (HVR1 and HVR2), which are responsible for the loss of antibody recognition of the E2 epitopes in HCV infection.[21,28-32]

It is, hence, doubtful whether the humoral immune response contributes to viral elimination in chronic HCV infection. This is in accordance with the observation that after acute HCV infection, a spontaneous viral clearance has been described in patients with hypogammaglobulinaemia.[33-35] Additionally, antibody titers to all studied HCV antigens including E1 and E2 tend to decline during successful interferon therapy.[36] It has been reported that even after a second infection of an HCV-recovered chimpanzee, a rapid control of the virus is achieved before a boost in serum anti-envelope antibodies, providing evidence that cellular immune responses alone are sufficient for the protection from HCV persistence.[37]

Cellular Immunity

In recent years evidence has accumulated that HCV – similar to HBV- is a non-cytopathic virus and that, therefore, both successful viral clearance and chronic liver injury are mediated by a more or less potent antiviral immune response.[38] Indeed, patients with acute symptomatic disease clear HCV more frequently than asymptomatic patients, suggesting the presence of a more vigorous immune response.[39, 40] The deterioration of the disease in human immunodeficiency virus co-infection, also provides proof of immune mediated mechanisms playing a crucial role in viral elimination of HCV infection.[41] Cellular immunity, in particular, is believed to be the immune system's effector arm utilized the most in the fight against HCV.

CD4+ T helper cells: guiding the course of the immune response

CD4+ T cells are thought to be centrally involved in the resolution of HCV infection. Very strong evidence supporting this comes from the fact that perturbations in the MHC class II allele frequencies of the Antigen Presenting Cells have been observed in patients who have overcome HCV infection.[42, 43] CD4+ T cells are MHC class II restricted, suggesting a better activation of them in those cases. Studies of T-cell responses in patients with acute HCV infection have shown a close association of a vigorous, polyclonal and multispecific proliferative CD4+T-cell response, directed mainly against the nonstructural proteins of the virus, with viral clearance and resolution of the disease.[39,44-46] On the other hand, loss of the HCV-specific CD4+ T-cell response results in the recurrence of viremia.[47] Likewise, viral clearance after a course of IFN-α treatment alone[48-51], and combination IFN-α and ribavirin treatment[52] appears to relate to the induction of a vigorous T-helper cell response. On the contrary, in chronically infected individuals, there seems to be a state of functional impairment or anergy of HCV-specific CD4+T cells.[53]

Activated CD4+T cells can be divided into two subsets based on the cytokine secretion profile. The T helper 1 (Th1) subset of CD4+T cells produces interleukin (IL)-2 and interferon (IFN)-γ and participates in cell-mediated immune responses supporting CD8+T cells generation, while the T helper 2 (Th2) subset of CD4+T cells produces IL-4 and IL-10 and mediates humoral immune responses through the production of antibodies by B cells.[54,55](Fig.3) It is known that intracellular immunity is critical for the defense against viral infections and that this function lies within the Th1-type immune response. The Th1/Th2 cytokine profile of a patient infected with HCV is thus important for determining the chronicity of the infection.[56,57] This cytokine profile needs to be shifted towards Th1 cytokines to eliminate HCV.[47,52, 58-60] This notion that is easily explained by the fact that CD4+ Th1 immune responses are needed to prime and maintain the CD8+ cytotoxic T lymphocyte (CTL) response which is responsible for eliminating infected cells.[61-63] It has been shown that in patients who exhibit a sustained response during treatment of HCV infection, IL-4 and

IL-10 levels where found to be decreased[52, 64] while a poor response to treatment correlated with higher IL-10 levels.[65] Viral antigens preferentially induce type 2 cytokines in patients with chronic HCV disease,[66] while a type 1 cytokine profile predominates in those patients with self limited HCV infection.[67] Specifically, among viral antigens, a type 1 T cell response to NS3 has been associated with clearing the virus and with a better clinical prognosis, while type 2 responses have been described *in vitro* responses to core antigen in chronically infected patients.[40, 60] Naturally occurring single point mutations in an immunodominant epitope of HCV NS3 antigen have been shown to be able to cause a transition from the Th1 to the Th2 phenotype.[68]

The differentiation of Th1 and Th2 cells from naove T-cells is promoted by IL-12 and IL-4, respectively.[69] It has been suggested that nonstructural protein 4 (NS4) of HCV inhibits IL-12 production and induces IL-10 production by monocytes, therefore inhibiting Th1 differentiation.[70] A key role in this process, although not fully understood, is believed to be played by T regulatory cells
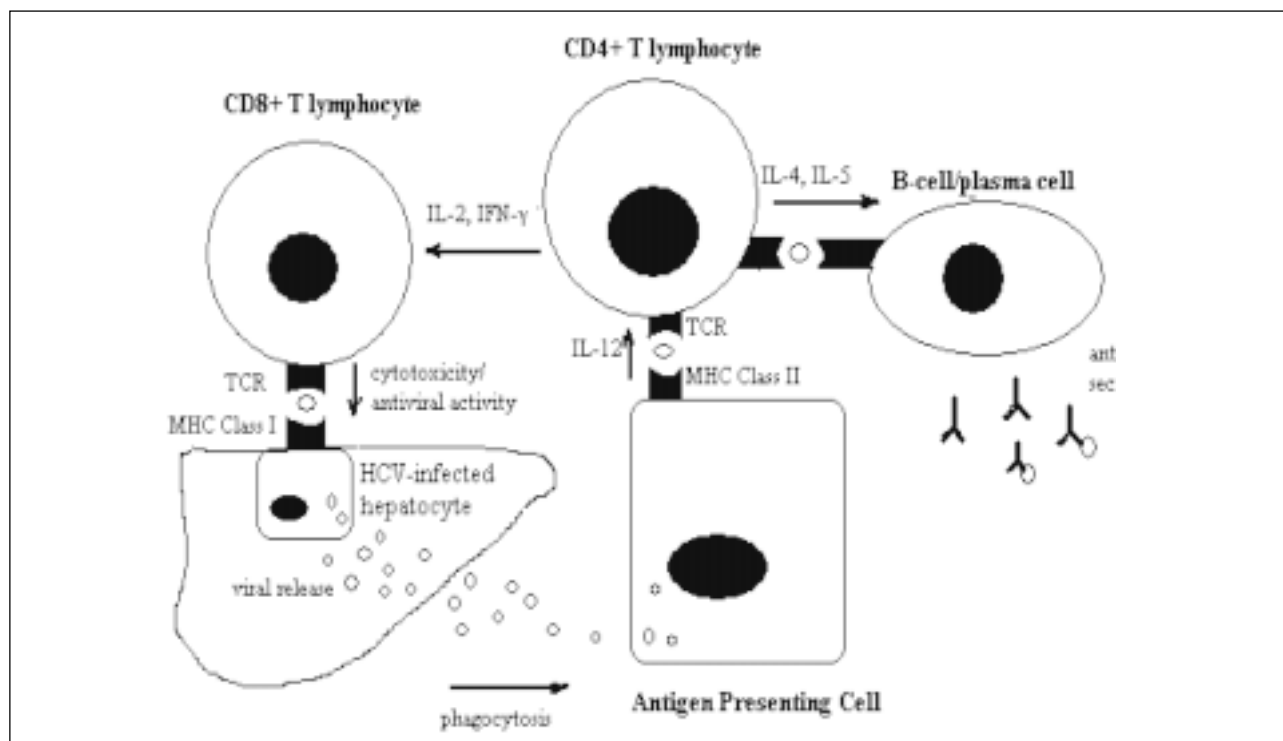

Fig. 3. The interaction of different arms of the HCV-specific immune response: Specific CD8+ T lymphocytes recognize viral peptides bound to major histocompatibility complex (MHC) class I molecules on the surface of infected hepatocytes leading to the elimination of infected cells or inhibition of viral replication. Specific CD4+ T lymphocytes recognize viral peptides bound to MHC class II molecules present on the surface of professional antigen presenting cells (e.g. monocytes, macrophages, B cells, dendritic cells). By the secretion of appropriate lymphokines CD4+ T lymphocytes can support cytotoxic effector mechanisms (IL-2, IFN-γ) as well as the production of virus-specific antibody (IL-4, IL-5).

(Treg cells) which secrete IL-10 and/or transforming growth factor-β (TGF-β) and are capable of suppressing pathogen-specific immune responses and thus facilitating the development of persistent or chronic infections.[71]

An interesting schema has been proposed by Masaki *et al* for the Th1/Th2 cytokine imbalance in chronic hepatitis C.[72] Intrahepatic mRNA expressions of IFN-γ and IL-2 in chronic hepatitis C patients are up-regulated, strongly suggesting that in the HCV chronically infected liver, cytokine profiles are shifted to Th1 predominance.[57] On the other hand, *in vitro* cytokine responses of peripheral blood mononuclear cells to recombinant HCV antigens were confined to IL-4 and IL-10,[60, 67] suggesting that cytokine profiles are shifted to Th2 predominance in peripheral blood. Briefly, in the HCV chronically infected liver, cytokine profiles are shifted to Th1 predominance, while in peripheral blood they are shifted to Th2 predominance, thus allowing HCV infection to proliferate.[72] It is considered that peripheral blood may provide a repository of the virus that continually reinfects the liver throughout the course of the infection.[68] (Fig. 4)

CD8+ cytotoxic T cells: protective and hepatotoxic role

Both CD4+ and CD8+ T cells contribute to virus control.[73, 74] Virus specific CTL have been shown to be the main effector cells for the destruction of virally infected cells. The antiviral effect is mediated either by lysis of the infected cells or by cytokine-mediated suppression of viral mechanisms.[75-77] (Fig.3) The ability to mount a strong antiviral CTL response early in the disease is important for viral clearance.[78, 79] In experimentally infected chimpanzees, a vigorous, multispecific, intrahepatic CD8+T-cell response during the early phase of the infection was associated with subsequent HCV clearance while a more narrowly focused and delayed response was associated with persistent infection.[46, 80, 81] These HCV-specific CD8+ T cell responses are preserved in the majority of persons with resolved HCV infection (in the absence of detectable virus) in relatively high levels.[82] In chronically infected individuals, the cause of the delayed immune response is thought to be the lack of widespread tissue destruction early in the course of infection and the subsequent delay in effective antigen transfer to professional antigen-presenting cells.[37] In these patients, HCV-specific CD8+T-cells are present at higher frequency, however displaying an impaired effector function which might be related to the deficiencies in the CD4+ T cell response.[83]

As has been recently shown for other viruses, for a noncytopathic virus like HCV to persist, it must either not induce an effective antiviral immune response or it must overwhelm or evade it.[84] Since HCV mutation rate is at least 10-fold higher than that in HBV, escape mutants in the form of quasispecies may play an important role in the primary establishment of HCV persistence, greater than that in chronic hepatitis B. The occurrence of a strong, functionally monoclonal CTL response that is focused on a single viral epitope is the most important condition for a mutant virus to be selected by the CTL-mediated immune response. This scenario would favour the outgrowth of variant viruses not expressing the epitope because they would be invisible to the immune system.[85-87] Many researchers, though, support that viral persistence favours the selection of escape mutants and not the reverse.[55, 88, 89] The fact that other flaviviruses have
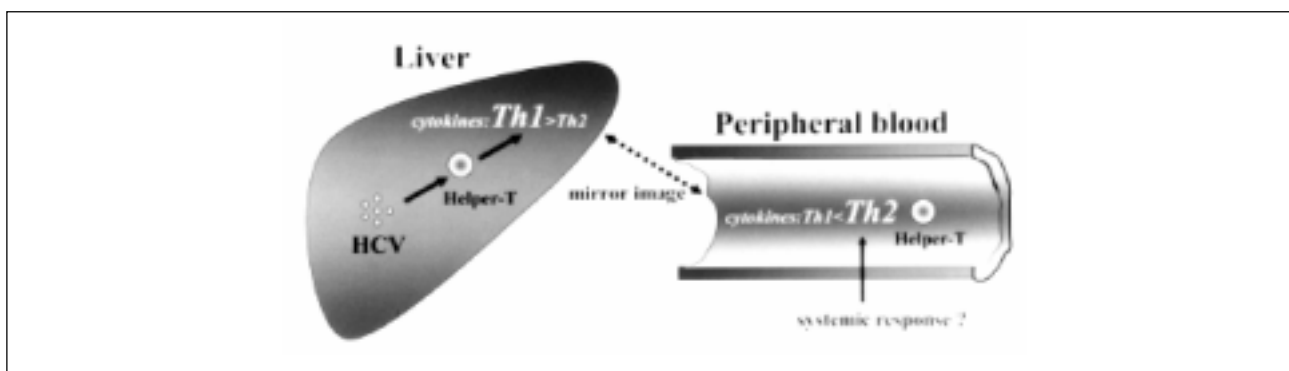

Fig. 4. Proposed schema for Th-1/Th-2 cytokine imbalance in chronic hepatitis C. There seems to be a mirror image between the two compartments in patients with chronic hepatitis C. (Reproduced from Masaki N., Fukushima S. & Hayashi S. (2002) Lower th-1/th-2 ratio before interferon therapy may favor long-term virological responses in patients with chronic hepatitis C. Reprinted with permission from: Masaki N, Fukushima S, Hayashi S. Lower th-1/th-2 ratio before interferon therapy may favor long-term virological responses in patients with chronic hepatitis C. Dig Dis Sci. 2002;47(10):2163-9.(72)

the same error-prone RNA dependant RNA-polymerase, which is responsible for the quasispecies nature of HCV, and yet rarely establish persistent infection, supports the above notion.[90]

The response of cytotoxic T lymphocytes in persons with chronic hepatitis C infection seems to be insufficient to contain viremia and genetic evolution of the virus, but sufficient to cause collateral damage through the elaboration of inflammatory cytokines in the liver.[91,92] It has been well documented that hepatotoxicity in chronic hepatitis C is causally related to enhanced immune recognition of viral antigens. A greater hepatic parenchymal concentration of activated CD4+ T-cells[93,94] and an up-regulation of intrahepatic Th1 cytokines[57] corresponds with more severe hepatitis, a fact showing that Th1 cells mediate tissue destruction through activation of CD8+ T and NK cells.[92] Immunosuppression of patients is generally associated with transient normalization of serum transaminase levels and a surge in viremia, while removal of immunosuppression can lead to an acute exacerbation of hepatitis.[95] In a trial by Nelson et al. administration of recombinant IL-10 to HCV chronically infected patients improved hepatic inflammation and fibrosis sub-scores (through the activation of Th2 responses), but unfortunately it also led to increased viral load.[96]

Prospects for the future

Worldwide, the best hope for a solution to the HCV infection epidemic is the development of an effective vaccine. It is clear that the quasispecies nature of HCV and the multiple prevalent genotypes pose a major challenge for the development of such a vaccine.[97] It is generally believed that an effective vaccine would have to induce a vigorous, multispecific immune response in order to eradicate HCV before the selection of escape mutants.

Therapies aimed at enhancing the strength of HCV-specific Th1 cell responses, particularly against NS epitopes, may facilitate the resolution of HCV infection. An impressive reduction of HCV-RNA plasma levels was established by the administration to infected patients of a recently discovered NS3 protease inhibitor, offering hope that in the future new drugs might be able to cure chronic hepatitis C infection.[98] A better understanding of the immunity in conjunction with the assessment of viral replication may facilitate further immunotherapeutic and vaccine strategies against HCV infection.

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