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Review

Cellular immune response in HCV infection

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Introduction

Hepatitis C virus (HCV) is a positive-strand RNA virus that is hepatotropic. It infects an estimated 2 million people in Japan, 4 million people in the United States, and 400 million people worldwide. It causes chronic hepatitis (CH), liver cirrhosis (LC), and, finally, hepatocellular carcinoma (HCC). The cumulative appearance rates of HCC in liver cirrhosis patients with HCV were reported to be 4%–8%/year, which about twice as high as those in liver cirrhosis patients with HBV.[1–3]

The HCV genome is approximately 9500 nucleotides long. The structural proteins encoded by HCV include the core protein, envelope protein 1 (E1), and envelope protein 2 (E2). E2 contains two regions, termed hypervariable regions (HVR1 and HVR2), that exhibit significant amino acid variation between viral genotypes and even within the same host (quasispecies). The HVR1 is expressed on the viral surface and its variability is helpful for allowing the virus to escape neutralizing antibodies. Six nonstructural proteins exist, which are called NS2, NS3, NS4A, NS4B, NS5A, and NA5B.

When the virus infects a cell, the coordinated efforts of various host immune responses are required for its eradication. Several terms for the immune responses are used in textbooks of immunology: (1) innate immunity and the acquired immunity; (2) antigen-specific and non-specific responses, (3) humoral immunity and cellular immunity, and (4) major histocompatibility complex (MHC)-restricted and unrestricted immune response.

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In acute hepatitis B, it has been shown that a strong cellular immune response eliminates infecting viruses, while neutralizing antibodies can prevent secondary infection. In hepatitis C, the responses of cytotoxic T lymphocytes (CTLs) has also been reported to suppress viral replication. However, in most cases of hepatitis C, the virus is not eradicated during the acute phase of infection, and thus tissue damage of varying degrees continues to occur, depending on the balance between the viral antigen load in infected cells, and the activity of virus-specific T cells.[4]

Much is still unknown about the immune responses to HCV. This review will concentrate on the immune responses to HCV, especially cellular responses, focusing on the determinants that establish chronic liver infection and on promising approaches in immunomodulation therapy.

Innate immunity and acquired immunity in viral infection

When viral infection occurs, the defense system of innate immunity nonspecifically combats pathogens. This system does not require prior exposure. Natural killer (NK) cells are activated and nonspecifically recognize cells which have undergone changes caused by infection and act to kill them. Interferons (IFNs) α/β are produced by the cells with viral infection, which leads to the suppression of viral replication.

If the infection cannot be controlled at this earlier stage, neutralizing antibodies and CTLs are subsequently induced and play an important role in eliminating the virus. These are the responses of acquired or adaptive immunity, which is specific to the virus. Neutralizing antibodies bind to viral particles in body fluids and eliminate them (humoral immunity). The viruses in the infected cells are eliminated by CTLs by the killing of the infected cells.

Cellular immune responses depend on direct interactions between T cells and the cells bearing the antigen that the T cells recognize. The function of CTLs is the most direct. They recognize viral antigens expressed on the surface of infected cells and attack the cells to eliminate the virus. They release a protein called perforin, which makes holes in the cellular membranes of the target cells, and another protein called granzyme, which may be delivered into the target cells by a mechanism analogous to receptor-dependent endocytosis and activates death substrates in the target cells. Granzyme and perforin cooperatively cause the death of virus-infected cells, and several models are proposed.[5–8]

Activated CTLs show increased expression of Fas ligand (FasL) and tumor necrosis factor (TNF)-α. If their target cells are sensitive to Fas ligand or TNF-α, activated CTLs damage these cells, regardless of whether or not they are infected with virus, by releasing apoptotic signals mediated through the Fas ligand-Fas antigen and TNF-α systems.[9,10] The cytotoxity of the Fas antigen-Fas ligand system and TNF-α systems is lower than that of the perforin system. The former Fas antigen-Fas ligand and TNF-α systems are also involved in attacking cells which are not virus-infected, but have acquired sensitivity as a result of cellular damage occurring through some mechanism at the site of inflammation, whereas perforin mainly damages cells infected by viruses. This molecular mechanism causing damage to virus-infected cells has been clarified by research using clonal CTLs specific for HCV.[11]

Although neutralizing antibodies and CTLs are directly involved in the eradication of viruses from body fluids, and the killing of virus-infected cells, respectively, the production of antibodies and the activation and proliferation of CTLs are controlled by helper T cells. Helper T cells are activated when they recognize viral antigens that are presented by antigen-presenting cells, such as dendritic cells, macrophages, and B cells. When activated, type 1 helper T cells (Th1 cells) produce interleukin (IL)-2 and IFN-γ to accelerate the activation and proliferation of CTLs and NK cells. Type 2 helper T cells (Th2 cells) produce IL-4, IL-5, IL-6, and IL-10, which promote B-cell differentiation into antibody-producing plasma cells, and the proliferation of such cells. Antigen-presenting cells produce IL-12 when stimulated by activated T cells, and IL-12 acts on Th1 cells, CTLs, and NK cells, leading to viral elimination and suppression of viral replication. IL-10 produced by Th2 cells acts on antigen-presenting cells to suppress the activation of Th1 cells by decreasing IL-12 production, leading to termination of the cellular immune response to the virus.

T cells are specialized to recognize foreign antigens as peptide fragments bound to cell-surface glycoproteins called MHC (Fig. 1). There are two types of MHC molecules. One is the MHC class I molecule, which is expressed in various types of cells and recognized by CTLs with CD8 molecules. The other is the MHC class II molecule, which is expressed in the antigen-presenting cells such as dendritc cells, macrophages, and B cells, and recognized by helper T cells with CD4 molecules. In humans, there are three main class I genes, called HLA-A, -B, and -C, and three pairs of MHC class II α and β-chain genes, called HLA-DR, -DP, and -DQ. The endogenous proteins, produced in the cytosol, are degraded to peptide fragments by the proteasome, and the fragments are actively transported from the cytosol into the endoplasmic reticulum (ER) by proteins called transporter associated with antigen processing (TAP). Subsequently, they are bound by MHC class I molecules, and the peptide: MHC complexes are transported through the Golgi apparatus to the cell surface. TAP transporter in the endoplasmic

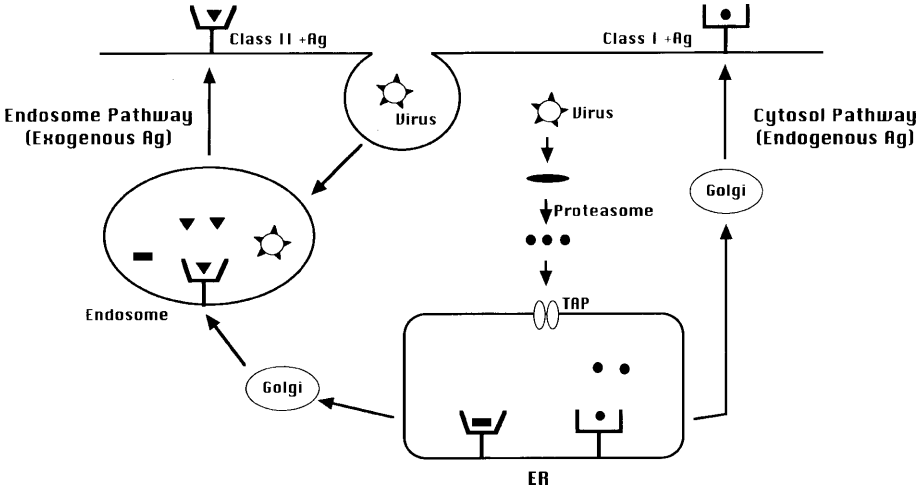

Fig. 1. Antigen-presenting pathways (cytosol pathway and endosome pathway). *Ag*, Antigen; *class I*, MHC class I molecule; *class II*, MHC class II molecule; *ER*, endoplasmic reticulum; *TAP*, transporter associated with antigen processing

reticulum is a heterodimer of TAP1 and TAP2 protein, whose genes lie in MHC class II gene. Exogenous antigens are taken up into intracellular vesicles of the professional antigen-presenting cells such as dendritic cells, macrophages, and B cells, degraded, and presented by MHC class II molecules to CD4+ T cells. But these rules described above are not necessarily strict. Therefore, endogenous antigens can be presented with class II molecules and exogenous antigens can be presented with class I molecules.

In addition, several genes, such as complement components C2, Factor B, and C4, TNF-α, and TNF-β (lymphotoxin), which map within MHC and have important functions in immunity, have been termed MHC class III genes.

Immune response in HCV infection (Fig. 2)

HCV-Specific T-cell responses

CD4+ T-cell (helper T-cell) responses. HCV-specific T-cell responses with CD4 (helper T cells) are analyzed by cell proliferation assay to measure the antigen-specific proliferative responses of peripheral blood mononuclear cells (PBMCs) or liver-infiltrating lymphocytes (LILs) to HCV antigens, or cytokine production by T cells in response to HCV antigen stimulation is analyzed using enzyme immunoassay (EIA), immunofluorescent intracellular cytokine analysis, enzyme-linked immunospot (ELISpot), or reverse transcription-polymerase chain reaction (RT-PCR).

Patients with self-limited HCV infection have been reported to show more vigorous peripheral blood proliferative CD4+ T-cell responses to HCV-derived proteins. In contrast, patients who develop a chronic HCV infection show weaker HCV-specific helper T-cell responses.[12–15] CD4+ T cell response is directed against several HCV antigens, such as core, E2, NS3, NS4, and NS5.[16] Among these, it has been reported that the helper T-cell response to NS3 was frequently strong and sustained in a self-limited course of acute HCV infection.[14] Amino acids 1248 to 1261 in the NS3 region were identified as the immunodominant epitope of CD4+ T cells which can be presented by several HLA-DR molecules.[17] Amino acids 21 to 40 in the core region; 1253 to 1272 in the NS3 region; and 1767 to 1786, 1907 to 1926, and 1909 to 1929 in the NS4 region have also been revealed to be helper T-cell epitopes which can be presented by broad HLA-DR molecules.[4] Several reports have shown that the Th1 response is predominant and more vigorous in self-limited hepatitis and the Th2 response is more vigorous in the chronic hepatitis patient.[4,18,19] When a patient who initially had displayed a strong HCV-specific CD4+ T-cell response and had lowered HCV RNA below the detectable level lost the specific T-cell response, HCV recurrence occurred promptly.[12] The results of these studies indicate that the HCV-specific CD4+Th1 T-cell response that eliminates the virus during the acute phase has to be maintained permanently to achieve long-term control of the virus. Therefore, the induction and maintenance of the HCV-specific CD4+ T cell response could

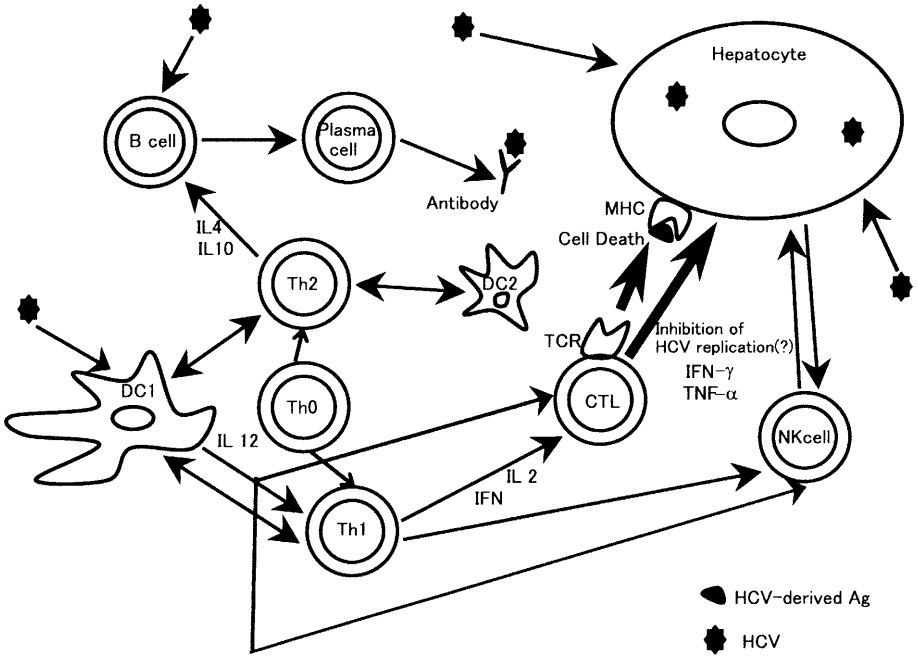

Fig. 2. Immune responses in hepatitis C virus (*HCV*) infection. *Ag*, antigen; *DC*, dendritic cell; *IFN*, interferon; *IL*, interleukin; *MHC*, major histocompatibility complex; *NK*, natural killer; *TCR*, T-cell receptor; *Th*, helper T cell; *TNF*, tumor necrosis factor; *CTL*, cytotoxic T-lymphocyte

present a promising therapeutic approach in HCV infection.

It has been reported that the cellular immune responses induced in IFN-treated patients with chronic hepatitis C may determine the therapy outcome. Löhr et al.[20] have reported that IFN-α treatment enhanced NS3-, helicase-, and NS4 antigen-specific helper T-cell responses in patients with viral clearance, whereas viral persistence was associated with increased T-cell reactivities against core and NS5 antigens. Other reports showed that the frequency of core-specific helper T-cell precursors was significantly higher and the helper T-cell response was stronger in patients with sustained biochemical and virological response after IFN therapy than in untreated patients or patients who remained viremic after completion of the treatment. Also, the number of core peptides recognized by helper T cells was higher in sustained responders than in non-responders or transient responders.[16,21] According to another report, the T-cell response to HCV core protein detected throughout the follow-up after IFN therapy was significantly more vigorous in genotype 2c- than in genotype 1b-infected patients.[22] The results suggest that the vigor of the helper T-cell response enhanced by IFN influences the effectiveness of IFN therapy in patients with different genotypes of HCV. Moreover, the main difference in HCV-specific (core, NS3, NS4 and NS5) CD4+ T-cell reactivity between patients treated with IFN and those treated with IFN plus ribavirin has been reported to be the different level of IL-10 production, but lymphocyte proliferation has been shown to be similar to that in patients receiving IFN monotherapy. The results suggest that the efficacy of therapy for HCV eradication may be related to its ability to enhance HCV-specific IFN-γ production (Th1) and suppress HCV-specific IL-10 production (Th2).[23] In regard to IL-10 gene polymorphism and the efficacy of IFN therapy, a highly significant relationship was found between inheritance of the IL-10 promoter alleles which show high IL-10 production and response to IFN therapy.[24] The results suggest that heterogeneity in the promoter region of the IL-10 gene has a role in determining the initial response of chronic hepatitis C to interferon therapy.

A recent report has shown that HCV-specific MHC class II restricted- CD4+ T cell responses are detectable in patients with minimal histological recurrence after liver transplantation. In contrast, PBMCs from patients with severe HCV recurrence, despite being able to proliferate in response to non-HCV antigens, fail to respond to the HCV antigens.[25] These results suggest that the inability to generate virus-specific helper T-cell responses plays a contributory role in the pathogenesis of HCV-related graft injury after liver transplantation.

Th1 responses accelerate HCV-specific CTL responses, as previously reported,[26] and, thus, they may indirectly affect viral eradication via the function of CTLs. However, they can also affect viral eradication directly by producing antiviral cytokines. In HBV infections, it has been reported that non-cytolytic mechanisms work for the eradication of HBV.[27–29] Thus, it may be possible that cytokines can suppress HCV replication by non-cytolytic mechanisms.

CD8+ T-cell (CTL) responses. Several epitopes of HCV for MHC class I-restricted CD8+ T cells (CTLs) in peripheral blood lymphocytes (PBL) and LIL have been determined.[26,30–44] The limitation of research on CD8+ T cells is due to the difficulty in quantification of CTL responses. Because the number of cells of interest is limited, expansion of the cells by lectin, IL-2, and other agents is needed to analyze CTL. A novel method has been developed using tetramer with MHC and epitope peptide. Using this method, Greenberg's group (He et al.[45]) reported that HCV NS3-specific CD8+ T cells existed at a frequency ranging from 0.01% to 1.2% in peripheral CD8+ cells, and the frequency of these specific CD8+ T cells in the liver was 1%–2%, at least 30-fold higher than in the peripheral blood. This approach is expected to be a useful method for the quantitative analysis of HCV-specific CD8+ T cells which exist both in the liver and in the peripheral blood.

The CTL activities for HCV amino acid residues 88–96 in HLA B44-positive patients have been shown to be significantly higher in patients with a low titer of serum HCV RNA than in those with a high viral load.[44] Another report has also shown that CTL responses were stronger in patients whose HCV RNA was below the detection threshold by branched-chain DNA assay than in those whose HCV was detectable; however no linear correlation between viral load and CTL response was detected.[46] In a chimpanzee HCV infection model, the CTL activity of LIL in the animals with self-limited HCV infection was shown to be stronger than that in the animals with chronic HCV infection.[47] It was reported that, among the IFN-treated patients with the HLA A2 molecule, responders showed significantly higher HCV-specific CTL precursor frequencies during viral clearance than non-responders.[48] This suggests that the CTL response to HCV has an influence on the efficacy of IFN therapy. Also, patients with intrahepatic HCV-specific CTL activity have been reported to have not only lower levels of viremia but more active liver disease, as reflected by a higher histologic activity index and serum aminotransferase levels compared with those without this CTL activity.[49] Interferon-α has been shown to up-regulate perforin in the T cells of healthy volunteers and in chronic HCV patients, which finding supports the elimination of virally infected cells via the

perforin pathway.[50] However, whether the control of HCV viral load by CTL depends on a cytolytic or a noncytolytic mechanism is unknown.

TAP2*0103 has been reported to be closely associated with low serum alanine aminotransferase (ALT) activity in HCV-infected patients, which suggests a relationship between TAP gene polymorphisms and disease progression.[51] TAP gene polymorphisms and the function of TAP may have an influence on the HCV-specific CTL response which may be related to the establishment of chronic infection.

The HCV-specific CTL precursor frequency in the peripheral blood is quite low, suggesting that their frequency is quantitatively inadequate to destroy all of the infected hepatocytes, thereby facilitating HCV persistence.[52] The potential for inducing immune response such as CTL reaction of HCV-derived protein may be weak compared with that of HBV-derived protein, as also suggested by other reports.[41,53]

HCV variants with altered peptide ligands in hypervariable region 1 capable of antagonizing CTL activity have emerged in the course of acute phase of HCV infection in patients in whom chronicity developed.[54] By the follow-up analysis of chronic HCV patients over a period of up to 46 months, it was revealed that, in contrast to the relatively high frequency of escape variants initially observed, the subsequent emergence rate of CTL escape variants was very low. In that report, the one escape variant that was detected proved to be a CTL antagonist. These observations suggest that CTL selection of epitope variants may have occurred in these patients before their entrance into the study, and that this may have played a role in HCV persistence.[43,55]

In experiments with a murine model, it has been shown that HCV core protein can suppress the HCV-specific CTL responses and depress the production of IFN-γ and IL-2.[56] This suggests that the HCV core protein plays an important role in the establishment and maintenance of HCV infection by suppressing host immune responses, in particular, the generation of virus-specific CTL.

Impaired function of HCV-dendritic cell (DC)

In the liver, which is the major site for HCV infection and proliferation, a considerable number of NKT cells exist. NKT cells produce IL-4, which induces the maturation of dendritic cells, and IFN-γ, which can induce Th1 predominance.[57] In addition, the existence of dendritic cells producing a high level of IL-12 was expected. However, it has been reported that the potential of dendritic cells generated from HCV-infected patients to stimulate allogeneic CD4 T cells was lower than that of dendritic cells from healthy donors. One of the reasons for this may be the low expression of CD86 and/or IL-12 in dendritic cells from HCV-infected patients.[58] It has also been reported that the stimulatory capacity of murine dendritic cells transfected with HCV genes using adenovirus vector in the allogeneic mixed leukocyte reaction was significantly lower than that of dendritic cells infected with control vectors. The murine dendritic cells transfected with HCV were also reported to produce significantly lower levels of IL-12 than dendritic cells infected with control vectors.[59] Interferon-α produced by type 2 dendritic cells in the early phase of viral infection is known to be involved in the suppression of viral proliferation. HCV envelope protein E2 has been reported to inhibit the kinase activity of double-stranded RNA-activated protein kinase (PKR) induced by IFN, and this may be one of the mechanisms by which HCV circumvents the antiviral effect of IFN and allows the establishment of chronic infection.[60] The HCV antigen-specific functions of dendritic cells in HCV infection are yet to be clarified.

Humoral immunity

Unfortunately, an HCV neutralizing antibody, which could protect against HCV infection, has not yet been detected.[61] Moreover, the decrease of antibody levels during successful IFN therapy argues for a decisive role of antibodies in viral clearance.[62] In an animal model, chimpanzees can be repeatedly infected when they are exposed to the same infectious inoculum of HCV after recovery from a previous infection.[63,64]

Liver damage in viral hepatitis

An experimental study in HBV transgenic mice[65] suggests that liver tissue may be damaged in hepatitis virus infection in vivo through several processes. CTLs which have recognized virus-infected cells are activated and proliferate under stimulation by cytokines such as IL-2 and IFN-γ, which are produced and secreted by activated helper T cells. As described above, perforin is considered to have an important role in this type of hepatocellular injury. The production of Fas ligand and TNF-α is increased when CTLs are activated, while liver cells become sensitive to Fas ligand and TNF-α when there is increased expression of Fas and TNF receptors in association with the development of hepatitis, although there is little sensitivity in the normal state. Consequently, in addition to the perforin system, the Fas ligand/Fas and TNF-α/TNF receptor systems are also considered to be related to the development of tissue injury in viral hepatitis.

In fact, investigation of chronic HCV patients has shown that the expression of Fas antigen is increased at the site of intense inflammation, and it has been

reported that perforin, Fas ligand, and TNF-α are all required for the development of severe liver damage in an experimental hepatitis model.

IFN-γ, which is secreted by activated CTLs and helper T cells, activates macrophages. Activated macrophges then secrete TNF-α, thus activating themselves further, and subsequently mobilize inflammatory cells such as neutrophils through the activation of endothelial cells and fibroblasts. The mobilization of more potent inflammatory cells such as activated macrophages results in aggressive hepatitis. Such a mechanism of liver damage is thought to work mainly in acute infection. The mechanism may be the same irrespective of the virus involved. However, the severity of hepatitis may vary depending on the replication potential and antigenicity of the hepatitis virus, as well as the intensity of the host's immune response.

Fas ligand transcripts have been reported to be detected in liver infiltrating mononuclear cells and PBLs obtained from patients with chronic hepatitis C.[66] However, it has also been reported that few intrahepatic cytotoxic T lymphocytes expressed perforin and granzyme in patients with chronic HCV infection, while a large number of Kupffer cells in these patients were positive for both proteins.[67] It is possible that perforin in the CTLs in the liver of chronic HCV hepatitis patients was exhausted because of the persistent stimulation of HCV-derived antigens.

The severity of liver damage is determined by the vigor of the CTL response and the helper T-cell response, which are mostly MHC-restricted. It has been shown that the HLA haplotype may influence the eradication of HCV,[68–75] the severity and progression of chronic liver damage,[76–80] and the response to IFN therapy.[81–84]

Immunomodulation therapy

A DNA-based T-cell-mediated vaccine could be of prophylactic and therapeutic value for HCV infection. However, an effective vaccine against HCV has not been developed so far.[85]

Several epitopes derived from HCV can be presented with different MHC class II molecules, as described above.[4] This depends on the fact that the cleft of the MHC class II molecule is open at both ends. This feature will be beneficial for the development of a T-cell-mediated HCV vaccine. Also, considering that the conserved regions among different genotypes are immunogenic, a vaccine that induces an HCV-specific CD4 T-cell response may be one of the promising approaches for the development of treatment for HCV patients.

In a murine model, it has been reported that HCV recombinant plasmid injection with boost by recombinant HCV core protein induced both humoral and cellular immune responses to HCV.[86] In HBV vaccination, CpG-oligodeoxynucleotides (ODNs) were reported to overcome reduced responsiveness to hepatitis B vaccine in orangutans.[87] These approaches may represent one of the ways to overcome the obstacles in the development of an HCV vaccine.

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