

## Review Article

# Dysfunction of Immune Systems and Host Genetic Factors in Hepatitis C Virus Infection with Persistent Normal ALT

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Patients with chronic hepatitis C (CHC) virus infection who have persistently normal alanine aminotransferase levels (PNALT) have mild inflammation and fibrosis in comparison to those with elevated ALT levels. The cellular immune responses to HCV are mainly responsible for viral clearance and the disease pathogenesis during infection. However, since the innate and adaptive immune systems are suppressed by various kinds of mechanisms in CHC patients, the immunopathogenesis of CHC patients with PNALT is still unclear. In this review, we summarize the representative reports about the immune suppression in CHC to better understand the immunopathogenesis of PNALT. Then, we summarize and speculate on the immunological aspects of PNALT including innate and adaptive immune systems and genetic polymorphisms of HLA and cytokines.

## 1. Introduction

Hepatitis C virus (HCV) is noncytopathic virus that causes chronic hepatitis and hepatocellular carcinoma (HCC) [1]. Approximately 70–80% of those acutely infected will become persistently infected with HCV [1]. Around 30% of CHC patients exhibit PNALT and show milder disease activity and slower progression to hepatic cirrhosis [2–6]. However, it is reported that about 40% of these progress to the active stage of inflammation, but the incidence of HCC in the PNALT was lower than that in those with elevated ALT levels [7]. Cellular and humoral immune responses to HCV play an important role in the pathogenesis of active and nonactive chronic hepatitis [8].

Numerous studies have indicated that failure of the cellular immune response, including type 1 helper T cells (Th1) hypo-responsiveness, cytotoxic T lymphocyte (CTL) exhaustion, excessive function of CD4<sup>+</sup> CD25<sup>+</sup> FOXP3<sup>+</sup> regulatory T cells, and failure of lymphoid cells via direct binding and/or infection in B cells, T cells, NK cells, and DCs occurs in CHC patients [9–21]. Since the liver damage in CHC is mainly induced by Th1 and/or CTL related responses [22–24], these responses might be strongly suppressed in PNALT.

Given the involvement of immune responses, genetic factors including polymorphism of HLA and cytokine-related

genes could also contribute to the activity of inflammation in CHC patients [25–37]. Many groups, including us, have reported on the relationship between certain HLA and ALT levels in CHC patients [25, 27–29]. Moreover, some groups indicated the polymorphism of certain cytokines-related genes contributed to the level of inflammation [30–37]. In a genomewide association study (GWAS), IL28B polymorphism was shown to influence the outcome of Peg-IFN and RBV therapy [38]. However, although the relationship between IL28B polymorphism and PNALT is still unclear, a possible relationship between IL28B polymorphism and the activity of inflammation has been reported [39, 40].

In this review, we focused on the possible immunopathological aspects of PNALT and summarize various studies about the mechanism and genetic host factors involved in the immune suppression in CHC patients.

## 2. Mechanism of Immune Suppression in CHC Patients

### 2.1. Innate Immune System in Hepatocytes

2.1.1. *Immune Suppression in CH-C.* Innate immune systems are important for the initial step of viral infection [41, 42] (Figure 1). Toll-like receptors (TLRs) are a family of

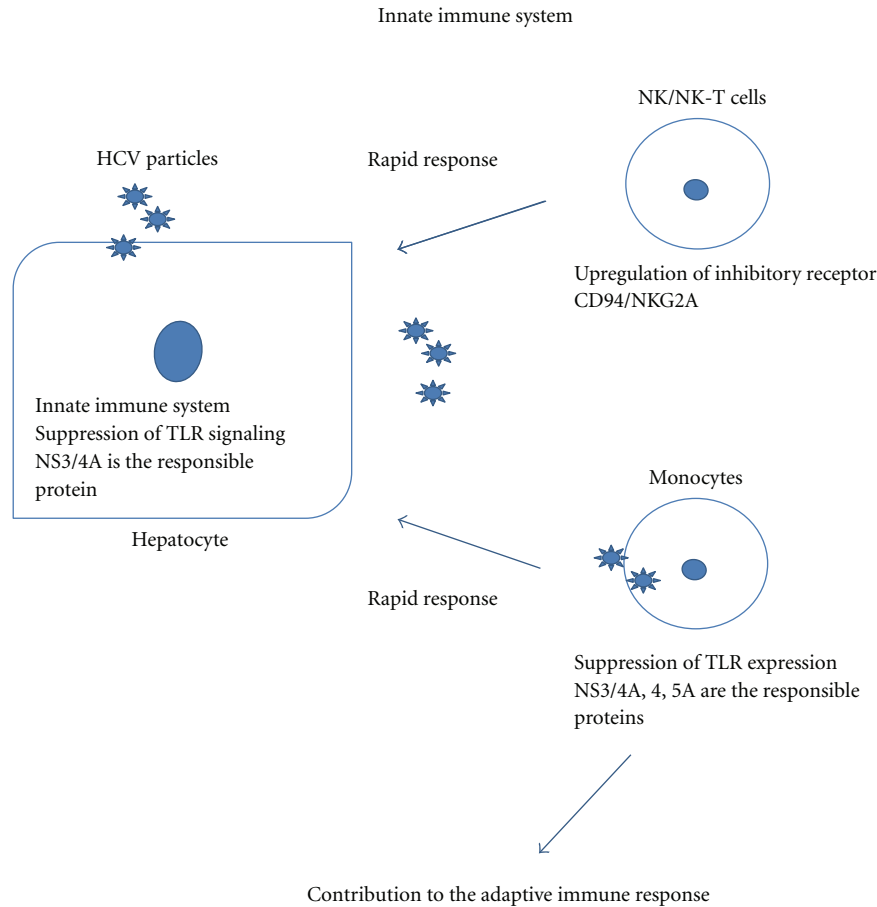


FIGURE 1: The schema of innate immune suppression in CHC is shown. The representative suppressive mechanisms are shown in this figure.

innate immune-recognition receptors that recognize molecular patterns associated with microbial pathogens including single and double-strand RNA. HCV interferes with the innate immune response and the induction of IFN-beta via the HCV NS3/4A protease activity, which inhibits the phosphorylation of IRF-3, a key transcriptional regulator of the IFN response [43, 44]. HCV NS3 protein interacts directly with TBK1, and this binding results in the inhibition of the association between TBK1 and IRF-3, which leads to the inhibition of IRF-3 activation [45].

**2.1.2. Immune Suppression in PNALT.** However, since little is known about the direct relationship between the suppression of TLR signaling and PNALT, the level of TLR signaling suppression might contribute to the immunopathogenesis of PNALT.

## 2.2. Monocyte, NK, and NK-T Cells

**2.2.1. Immune Suppression in CH-C.** In addition to the intracellular immune reaction of hepatocytes, monocytes, NK, and NK-T cells are responsible for the rapid reaction in HCV infection (Figure 1). Many groups have described that NK and NK-T cells were suppressed in CHC patients [46–53]. However, whether NK and NK-T cells would be suppressed

in CHC patients is still controversial [48]. The various backgrounds of CHC patients have resulted in controversial findings [49]. The function of NK cells is regulated by a balance of inhibitory and activating signals, which are mediated by the differential expression of receptors. Takehara and Hayashi recently reported that the expression of the inhibitory receptor CD94/NKG2A is upregulated on NK cells in CHC patients [53]. Another group reported that exposure to HCVcc modulates the pattern of cytokines produced by NK cells, leading to reduced antiviral activity [47].

**2.2.2. Immune Suppression in PNALT.** Previously, it was reported that HCV-core and NS3 protein triggered the inflammatory pathway via TLR2, which might affect viral recognition and activation of the immune system [54]. It was also reported that peripheral blood monocyte expression of TLR2 but not of TLR4 correlated significantly with the serum ALT levels [55]. Concerning the mechanisms of monocyte-suppression, it was clearly demonstrated that macrophage cell lines expressing NS3, NS3/4A, NS4B, or NS5A inhibited the activation of the TLR2, TLR4, TLR7, and TLR9 signaling pathways. Among the HCV individual proteins, NS5A bound to MyD88, a major adaptor molecule in TLR, inhibited the recruitment of interleukin-1 receptor-associated kinase 1 to MyD88 and impaired the cytokine production in response to

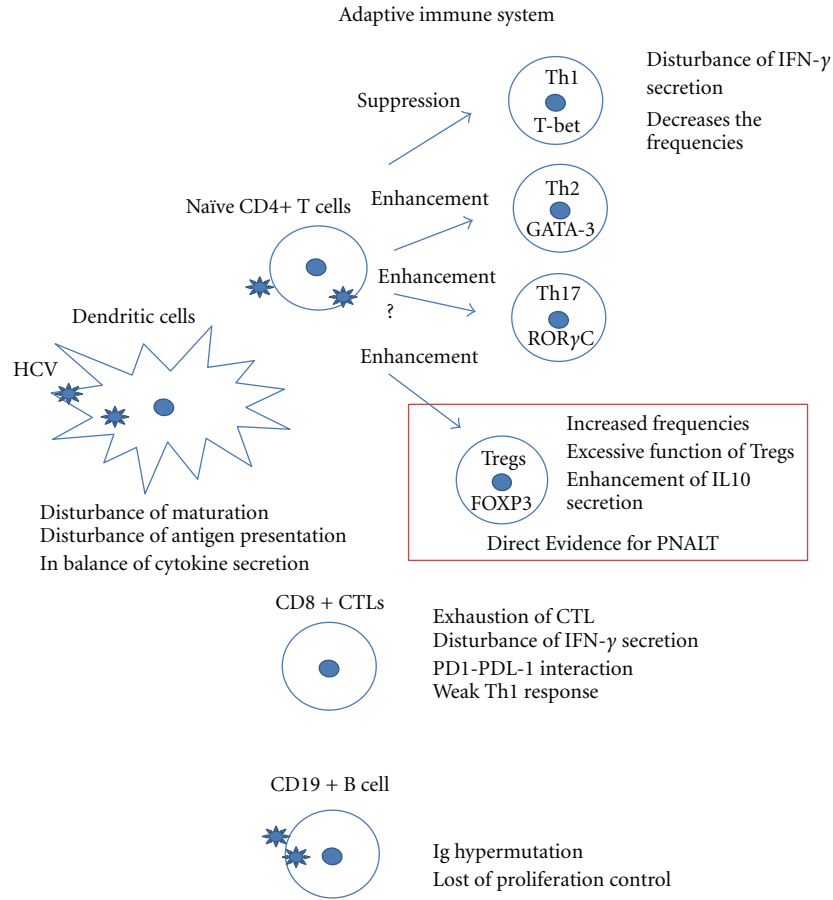


FIGURE 2: The schema of adaptive immune suppression in CHC is shown. The representative suppressive mechanisms are shown in this figure.

TLR ligands [56]. These results indicated that the activation of monocytes was probably suppressed in low ALT and PNALT CHC patients.

2.3. Adaptive Immune Systems: T Cells, B Cells, and Dendritic Cells

2.3.1. Immune-Suppression in CH-C. After the innate immune period, the adaptive immune system including CD4+ T cells, CD8+ cytotoxic T cells, B cells, and dendritic cells should be involved in a more effective immune response to HCV.

The HCV antigen-driven proliferation of CD4+ T cells is weak in patients who develop persistent HCV infection [57, 58]. It has been demonstrated that depletion of CD4+ T cells results in a weak CD8+ T cell response, which partly controls viremia, followed by viral persistence in chimpanzee infection studies [59]. In addition, an appropriate Th1 response is essential to eradicate HCV [60]. It has been reported that an increased Th2 cytokine response may reduce the inflammatory and biochemical activity [61]. Moreover, various studies have indicated that failure of the adaptive immune response, including Th1 hypo-responsiveness, CD8+ CTL exhaustion, excessive function of CD4+ CD25+

FOXP3+ regulatory T cells, and failure of lymphoid cell via direct binding and/or infection in B cells, T cells, and DCs occurs in CHC patients [9–21]. Previously, we reported the direct suppressive effect of HCV on T cell- and B cell-immunity in CHC by using a lymphotropic HCV strain [14–17]. However, since the contribution of lymphotropic HCV to the pathogenesis of PNALT is still not clear, the biological significance of lymphotropic HCV needs to be analyzed in future studies. Studies about the relationship between the infectivity of lymphotropic HCV and PNALT are ongoing in our laboratory. Tregs constitutively express CD25 (the IL2 receptor alpha-chain) in the physiological state [62]. In human, this Tregs population, defined as CD4+ CD25+ FOXP3+ cells, constitutes 5% to 10% of peripheral CD4+ T cells and has a broad repertoire that recognizes various types of self and nonself antigen. Antigens induced by HCV might induce Tregs to escape from immunological pressure as reported in persistent infection of EB virus, hepatitis B virus, and HIV [63–67] (Figure 2).

2.3.2. Immune Suppression in PNALT. More recently, Itose et al. reported that the frequencies of naturally occurring-Treg in CHC patients were significantly higher than those in healthy individuals. The FOXP3 and CTLA4 transcripts

were higher in PNALT than those in CHC patients [21]. Another group also described that their suppressor ability is stronger in patients with PNALT than that in those with active CHC hepatitis [68]. These two studies could be direct evidence about the relationship between PNALT and the function of Tregs. Moreover, a unique subset of lymphocytes might contribute to the immune suppression in PNALT [69] (Figure 2).

### 3. HLA and Cytokine-Related Gene Polymorphism and ALT Level

**3.1. HLA Polymorphism.** CD8<sup>+</sup> CTLs are able to recognize viral antigens synthesized within infected cells in the form of short peptides associated with HLA class I molecules [70]. On the other hand, CD4<sup>+</sup> Th cells are able to recognize antigens associated with HLA class II molecules [70, 71]. Individuals that are heterozygous at HLA class I loci are able to present a greater variety of antigenic peptides to CTL resulting in a broader immune response [72]. HLA class I heterozygosity was found at a higher rate in patients with slow progression to AIDS in HIV-1 infection [73]. However, HLA class I heterozygosity did not affect the inflammatory levels of CHC patients in our previous study [27]. The frequency of HLA-A2 tended to be higher in patients with PNALT than in those with elevated ALT level [27]. A specific HLA class II allele has been reported to influence the disease severity [25] or viral clearance in chronic hepatitis C [26]. Yoshizawa et al. reported that the frequencies of DRB1\*12 (\*1201 and \*1202), DQB1\*0301, and DRB3\*03 alleles were higher in patients with asymptomatic HCV carriers than those in liver cirrhosis patients [28]. Large-scale studies might be able to verify the ethnic, gender, age, and other genetic factors and determine the influence of the HLA allele on PNALT.

**3.2. Cytokine-Related Gene Polymorphism.** Numerous studies indicated that various kinds of cytokine-related gene polymorphism were involved in the immunopathogenesis of CHC. IL10 is a suppressive cytokine that could contribute to the persistence of HCV infection and low inflammation level in CHC. Mangia et al. reported that the IL-10 ATA haplotype was more frequent in patients with spontaneous HCV RNA clearance (36.0%) than that in patients with persistent infection (23%) [74]. On the other hand, another group reported that no effect of IL-1beta and IL-10 gene polymorphism on the degree of hepatocellular injury was apparent based on the ALT levels [37]. However, a gender effect is clearly observed in women carrying the GG high IL-10 producer genotype. The higher levels of IL-10 present in such individuals are associated with a higher risk of inefficient clearance of the HCV and the development of a chronic HCV infection together with a lower risk of progression to cirrhosis in female patients [75]. These reports indicated that IL-10-related gene polymorphism might affect the level of inflammation in certain conditions. Another important gene is CCR5Delta32. CCR5Delta32, a 32-base pair deletion of the CC chemokine receptor (CCR) 5 gene, is associated with slower human immunodeficiency virus

disease progression in heterozygotes and protection against infection in homozygotes. Goulding et al. reported that heterozygosity for CCR5delta32 was significantly associated with spontaneous hepatitis C viral clearance and with significantly lower hepatic inflammatory scores [33]. In addition to these gene polymorphisms, polymorphism of TGF-beta, TNF-alfa, IL2, IFN-g, and OAS-1 genes might contribute to the level of inflammation [30–32, 35, 74]. More recently, GWAS revealed promising results. A genomewide association of IL28B with the response to pegylated interferon and ribavirin was reported [38]. Then, another group reported that different cytokine profiles induced by the IL28 polymorphism resulted in different Interferon stimulated genes and IL28 expression during chronic HCV infection [76]. They reported that the expression of IL28, MxA, PKR, OAS1, and ISG15 in hepatic cells was significantly lower in patients with the response-favorable (rs8099917) T/T genotype compared to those with T/G or G/G genotypes [76]. A future study might be able to determine the relationship between IL28 polymorphism and PNALT.

### 4. Concluding Remarks

In CHC patients, there are many kinds of immune-suppressive mechanisms. However, although the immunopathogenesis of PNALT has not been clarified yet, the complexities of immune reactions likely contribute to the difficulties of determining the detailed mechanisms of PNALT. Recently, the technologies of GWAS, immunoassay with increased numbers of multicolor flow cytometry analysis, and chimera mice with human hepatocytes and lymphocytes have been developed. These technologies, together with previous data, might be able to clarify the immunopathogenesis of PNALT in CHC.

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