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REVIEW ARTICLE

Single Nucleotide Polymorphisms of Cytotoxic T-lymphocyte Antigen 4 (CTLA-4) and Susceptibility to Chronic Viral Hepatitis B and C Infections

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Abstract

The cytotoxic T-lymphocyte antigen 4 (*CTLA-4)* gene is a negative regulator of T-lymphocyte activation and proliferation. Single nucleotide polymorphisms (SNPs) occurring on the *CTLA-4* gene can modify the ability to control the proliferation of T-lymphocytes, thereby impacting the clearance of hepatitis B (HBV) and hepatitis C (HCV) virus infections. The −319C/T and +49A/G SNPs of *CTLA-4* gene have been associated with autoimmune disorders and liver infections. Studies show that the +49G allele confers susceptibility to HBV and HCV infections in chronic disease (without cirrhosis), associates with the risk of chronic HCV infection in males, confers protective effect against the development of hepatocellular carcinoma, and favors viral elimination. Furthermore, the +49G allele alone or in haplotype with the −319C favors chronic infection with genotype 3 HCV, has an inverse association with HCV genotype 1, and decreases viral load in chronic hepatitis C associated with sustained virological response (SVR). These findings support an important role of the SNPs of *CTLA-4* gene in viral hepatitis; however, the mechanisms by which they influence immune response against viral infections are not fully understood. This review gives an overview of the current understanding of the association between *CTLA4* SNPs and HBV/HCV infections.

Keywords: cytotoxic T-lymphocyte antigen 4; CTLA-4; hepatitis C virus; hepatitis B virus; single nucleotide polymorphisms

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Introduction

Chronic viral hepatitis is most commonly caused by hepatitis B (HBV) or hepatitis C (HCV) viruses. Chronic infection is defined as a measurable viral load for more than 6 months (1). An estimated 700,000 people die each year due to complications of chronic HCV and HBV infections such as cirrhosis and hepatocellular carcinoma (HCC) (2). HCV infection affects approximately 180 million people worldwide

and 75% of infected patients with acute disease progress to chronic infection (3–5). Approximately 400 million people are chronic HBV carriers. It is estimated that 5–10% of individuals infected are unable to eliminate the virus; of these, 15–40% will develop cirrhosis or HCC (6–8). Both HBV and HCV have a similar natural history but with different propensities for chronic complications. This difference may be due to an imbalance between genetic factors, and viral, environmental, and host components (9). Interestingly, HBV and HCV do not directly cause hepatocellular damage. The interaction between the virus and the immune response of the host is responsible for liver damage and the clinical manifestations of disease (10, 11). This demonstrates the importance of the immune system in determining how the body will respond to these pathogens. This review aims to discuss the results of research evaluating how single nucleotide polymorphisms (SNPs) in *CTLA-4* gene may affect the course of HBV and HCV infections through their role in immune function.

CTLA-4 Immune Response against Viral Infections

Immunity against viral infections is determined through a variety of specific and nonspecific mechanisms. The predominant immune response is through T-lymphocyte activation. Although the activated T-lymphocytes play an important role in the control of HBV and HCV infections, they also contribute to liver damage. T-lymphocytes recognize infected cells and respond through cytokine release and coordinated lysis of the infected cells (12). When the immune system fails to wholly eradicate an infection, immunomodulatory pathways are activated to minimize the extent of immune-mediated tissue damage (13). Inappropriate induction of T-lymphocytes to recognize self-antigens at this stage can lead to autoimmune-driven damage.

The activation and function of T-lymphocytes in the immune response depends on two signals. In the first signal, the HCV and HBV antigens are processed and presented by major histocompatibility complex (MHC) to T-cell receptor (TCR). This signal in isolation does not cause activation of the T-lymphocytes and requires costimulatory signals (14). The well-characterized costimulatory signal is the interaction between CD28 receptor on the surface of T-lymphocytes, and CD80 and CD86 ligands on the antigen-presenting cell (APC). This interaction triggers a biochemical cascade of signals such as IL-2 production, induction of antiapoptotic protein Bcl-xL, and cell-cycle progression, which are necessary for the clonal expansion of T-cell populations (15, 16). CD28 is constitutively expressed on the surface of T-cells and provides a costimulatory signal rapidly following T-lymphocyte binding to the APC (17), promoting the immune response against HBV and HCV infections.

CTLA-4, another receptor structurally similar to CD28, also binds the CD80 and CD86 ligands with greater avidity than CD28. CTLA-4 can inhibit the activation of T-cells, in competition with CD28, and subsequently inhibit the costimulatory effect of CD28. The *CTLA-4* gene is encoded in human chromosome 2q33 and is made up of four exons and three introns (18). It is a member of the immunoglobulin superfamily and is a costimulatory molecule expressed in activated T-cells (19). The CTLA-4 is a negative regulator of T-cell activation and proliferation through mechanisms such as reducing interleukin 2 (IL-2) and IL-2 receptor (IL-2R) expression, promoting G1 cell-cycle arrest of T-lymphocytes, and inducing FAS-independent apoptosis of activated T-lymphocytes (20, 21). Previous studies have reported that mice deficient of *CTLA-4* develop lymphoproliferative disorders and die from multiorgan failure and cytokine storm, suggesting the indispensable immunoregulatory role of CTLA-4 (22, 23). CTLA-4 is not constitutively expressed in resting T-lymphocytes. Upon TCR stimulation, CTLA-4 expression is induced, peaking 48–72 h following stimulation (24). A greater stimulation of T-cells induces greater levels of membrane-localized CTLA-4 expression (19). The increase in the expression of CD28 occurs during the activation of the T-lymphocytes; this effect induces the expression of CTLA-4 and increases the stability of CTLA-4 mRNA (25, 26). After T-cell activation, CTLA-4 is endocytosed (19) in order to maintain a fast T-cell activation response. The interaction between T-lymphocytes, costimulatory molecules, and the effect of CTLA-4 SNPs is shown in Figure 1.

CTLA-4 SNPs in HBV/HCV Infection

SNPs in cytokine and receptor genes (27) may be playing an important role in the outcome of HBV and HCV infections. More than 10 million polymorphisms in the human genome have been identified, some with the potential to influence the development of disease (28). In the *CTLA-4* gene, SNPs have been reported at positions −1722, −1661, −319, +49, and CT60 (29). However, the most studied CTLA-4 SNPs in patients infected with HCV and HBV are −319 C/T (rs5742909) and +49 A/G (rs231775) (30–34). Studies in patients with HBV- and HCV-associated chronic liver disease (CLD) have reported differences in the allelic and genotypic frequencies of the −319 C/T and +49 A/G polymorphisms (Tables 1 and 2).

The −319 C/T SNP is named in many studies as −318 C/T; however, in the GenBank SNP database, it is described as −319 C/T (rs5742909) (35). We therefore consider the correct form as −319 C/T. The functional significance of −319 C/T *CTLA-4* SNP in the promoter region it is not well defined; however, studies suggest participation in the binding of cis -acting elements to the promoter (NF-1 and c/EBP beta), thus affecting the gene activity (36, 37). The presence of the −319T allele of −319 C/T SNP is associated with greater promoter activity than the −319C allele, contributing to a greater expression of the *CTLA-4* gene, representing a mechanism to inhibit the exaggerated cellular immune response (38) and contribute to hepatocellular damage in HBV and HCV infections (Figure 1A) (30, 39–42). The −319T allele is associated

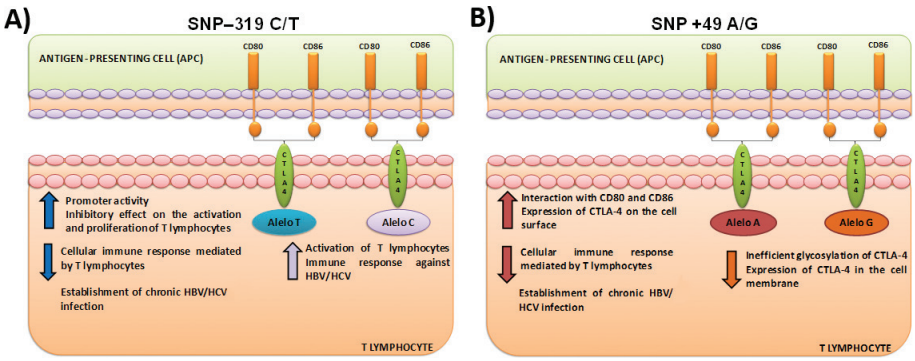

Figure 1. Function of −319 C/T and +49 A/G SNPs in the immune response against HBV/HCV infection. (A) −319 C/T SNP in the immune response against HBV/HCV Infection; (B) + 49A/G SNP in the immune response against HBV/HCV infection.

Table 1. Allelic and genotypic frequencies of −319 C/T SNP in HBV/HCV infected patients and control subjects

−319 C/T Population, n = total	Genotype			C allelen n (%)	T allele n (%)	
	C/C n (%)	C/T n (%)	T/T n (%)			
Mexican						
Control, n = 215	193 (89.8)	21 (9.7)	1 (0.5)	407 (94.6)	23 (5.4)	Enciso-Vargas et al. (30)
HCV, n = 205	183 (89.3)	22 (10.7)	0 (0)	388 (95.0)	22 (5.0)	
Brazilian						
Control, n = 183	152 (83.1)	31 (16.9)	0 (0)	335 (91.5)	31 (8.5)	Danilovic et al. (39)
HCV, n = 112	92 (82.1)	19 (17.0)	1 (0.9)	203 (90.6)	21 (9.4)	
Iranian						
Control, n = 65	55 (48.61)	9 (13.84)	1 (1.53)	119 (91.54)	11 (8.46)	Sepahi et al. (42)
HCV, n = 65	55 (48.61)	10 (15.38)	0 (0)	120 (92.30)	10 (7.70)	
Iranian						
Control, n = 150	134 (89.3)	10 (6.7)	6 (4.0)	278 (92.6)	22 (7.4)	Mohammad Alizadeh et al. (59)
HBV, n = 51	41 (80.4)	10 (19.6)	0 (0.0)	92 (90.2)	10 (9.8)	

Values are presented in frequencies (%) and genotypes and alleles in number (n). Total alleles were determined considering that each homozygote would contribute two copies of the allele toward the total fraction, while each heterozygote would only contribute one copy toward the total fraction.

with the likelihood of HBV persistence and susceptibility to progression of chronic liver damage, which is consistent with its emerging role in the T-regulatory cells in the pathogenesis of disease (31, 43). Conversely, some studies report that the −319 C/T SNP was underrepresented in patients with chronic HBV infection compared with healthy controls, and that it was not associated with autoimmune liver disease (33). In addition, these SNPs have been associated with the risk of developing B-cell non-Hodgkin lymphoma in patients with HCV and various autoimmune disorders (44–48). Few studies have documented the *CTLA-4* SNPs in HCV infection, as most evaluate HBV infection and autoimmune diseases, in particular the +49 A/G SNP.

A transition of the +49A allele by the +49G allele in exon 1 causes an amino acid change from threonine to alanine in the CTLA-4 protein. This change is associated with a reduction in CTLA-4 mRNA, increased T-cell activation, and instances of autoimmune disorders (49, 50). The presence of the +49G allele in the +49 A/G SNP favors inefficient glycosylation in the sequence of the leader peptide of CTLA-4, altering the process in the endoplasmic reticulum resulting in the reduced expression of the molecule on the cell surface (Figure 1B) (51).

Table 2. Allelic and genotypic frequencies of +49A/G SNP in HCV/HBV infected patients and control subjects

+49 A/G Population, n = total	Genotype			A allele n (%)	G allele n (%)	
	A/A n (%)	A/G n (%)	G/G n (%)			
Mexican						
Control, n = 215	93 (43.3)	89 (41.4)	33 (15.3)	275 (64.0)	155 (36.0)	Enciso-Vargas et al. (30)
HCV, n = 205	67 (32.7)	104 (50.7)	34 (16.6)	238 (58.0)	172 (42.0)	
Brazilian						
Control, n = 183	81 (44.3)	67 (36.6)	35 (19.1)	229 (62.5)	137 (37.5)	Danilovic et al. (39)
HCV, n = 112	59 (52.7)	34 (30.3)	19 (17.0)	152 (68.0)	72 (32.0)	
Iranian						
Control, n = 65	39 (60.0)	17 (26.15)	9 (13.85)	95 (73.07)	35 (26.93)	Sepahi et al. (42)
HCV, n = 65	51 (78.46)	0 (0.0)	14 (21.54)	102 (78.46)	28 (21.54)	
Iranian						
Control, n = 150	57 (38.0)	52 (34.7)	41 (27.3)	166 (55.3)	134 (44.7)	Mohammad Alizadeh et al. (59)
HBV, n = 51	26 (51.0)	16 (31.4)	9 (17.6)	68 (66.6)	34 (33.4)	
Chinese						
Control, n = 407	45 (11.0)	179 (44.0)	183 (45.0)	269 (33.0)	545 (67.0)	Gu et al. (34)
HBV, n = 203	24 (12.0)	85 (42.0)	94 (46.0)	133 (26.0)	273 (74.0)	
Chinese						
Control, n = 145	16 (11.0)	68 (47.0)	61 (42.0)	100 (34.5)	190 (65.5)	Zhang et al. (57)
HBV, n = 172	33 (19.0)	89 (52.0)	50 (29.0)	155 (45.0)	189 (55.0)	
Chinese						
Control, n = 361	33 (9.14)	177 (49.03)	151 (41.83)	243 (33.65)	479 (66.35)	Chen et al. (60)
HBV, n = 304	47 (15.46)	125 (41.12)	132 (43.42)	219 (36.0)	389 (64.0)	
Tunisian						
Control, n = 358	40 (11.2)	142 (39.7)	176 (49.2)	222 (31.0)	494 (69.0)	Ksiaa et al. (58)
HCV hemodialyzed, n = 500	77 (15.4)	198 (39.6)	225 (45.0)	352 (35.2)	648 (64.8)	

Values are presented in frequencies (%) and genotypes and alleles in number (n). Total alleles were determined considering that each homozygote would contribute two copies of the allele toward the total fraction, while each heterozygote would only contribute one copy toward the total fraction.

Therefore, the +49G allele seems to favor the activation and proliferation of T-lymphocyte, while the +49A allele is associated with the decrease of T-lymphocyte activation in HBV and HCV infections.

In relation to the previously mentioned observations, it has been reported that +49 A/G polymorphism plays an important role in the expression of the *CTLA-4* gene. Individuals with the +49 A/A genotype have increased expression of CTLA-4 on the cell surface compared with the genotypes +49 A/G and +49 G/G (52). In addition, the presence of the +49A allele in T-cells increases the interaction with the B7.1 ligand and increases the inhibitory function of CTLA-4. Therefore, the +49G allele may favor the activation and proliferation of T-lymphocytes at a high rate. This implies that

the +49G allele can favor viral elimination. In contrast, T-cells carrying the +49 A/A genotype have significantly lower rates of activation compared to T-cells with the +49 G/G genotype (53). These findings illustrate that molecular changes in *CTLA-4* can modify the ability to control the proliferation of T-lymphocytes, promoting the persistence or elimination of HBV/HCV infection.

In kind, the +49G allele confers protection for the development of CLD in HBV/HCV infections (11, 12), suggesting inhibition of CTLA-4 could facilitate control of these pathogens. We previously found that the +49G allele is associated with HCV chronic infection but not with the presence of liver cirrhosis in the Mexican population (30). A report revealed spontaneous HCV clearance to be higher in patients carrying the +49G allele (54). The above is supported by the natural history of the infection, where the majority of patients with acute infection have progressive chronic liver damage, and approximately 20% of patients develop liver cirrhosis (10, 55), highlighting the importance of the cellular immune response in elimination and/or persistence of HCV infection.

Danilovic et al. reported that alleles −319C and +49G interfere with the ability to block the immune response of CTLA-4, favoring chronic infection with genotype 3 HCV in a Brazilian population (39). These findings are consistent with our results, where subjects with the −319C/+49G haplotype are associated with genotype 3 infection of HCV (30). Moreover +49G allele has been associated with the risk of chronic HCV infection in males (30). Males, compared to females, are three to five times more likely to develop HCC. This risk can be attributed to the production of androgens in men, while the production of estrogens provides protection against the development of HCC in women (34). These sex differences can be attributed to factors encoded in the sex chromosomes, giving rise to differential immune activation (56). Male sex is considered an important factor in the progression and risk of HCV infection. Another study report *CTLA-4* polymorphisms have been associated with risk to HCV infection in an Iranian population (42). Homozygotes with +49 G/G show a persistent infection with HBV (57). Furthermore, it has been reported that the +49G allele has a protective effect against the development of HCC, and the subjects with the +49A allele have greater susceptibility to the development of HCC in HBV infection (34). The +49G allele was also detected more frequently in patients who recovered from HBV infection (32). However, another study shows no association between the polymorphisms studied and spontaneous clearance or persistence of HCV infection. The authors demonstrated that *CTLA-4* SNPs (+49GG/CT60 haplotype) could influence susceptibility to HCV infection in the Tunisian hemodialyzed population (Tables 1 and 2) (58). A meta-analysis showed that the +49G allele can positively influence the elimination of the virus, while the presence of the +49A allele may increase the risk of infection with HBV (61). Accordingly, multiple studies have reported that the +49 A/G SNP is associated with HBV and HCV infection.

Association of −319 A/G and +49 A/G SNPs with SVR

The determination of the seven main genotypes of HCV is used as a strong and independent predictor of the sustained virological response (SVR) to standard therapy (pegylated interferon and ribavirin). Patients with genotypes 2 and 3 respond better to treatment than those infected with HCV genotype 1 (62, 63). The *CTLA-4* SNPs have been associated with SVR to standard treatment in chronic HCV infection, whereas the +49G allele or the −319C/+49G haplotype decreases viral load in white patients with HCV genotype 1 infection (40). Concordantly, the *CTLA-4* SNPs were associated with treatment response in multivariable analysis, where the presence of favorable +49GG genotype conferred greater rate of response (64). Likewise, female sex can be an important factor in response to treatment for chronic HCV infection (65). However, in our study, we did not find an association with SVR (30), probably due to the low number of patients undergoing treatment. Thus, the discrepancy in the association studies of *CTLA-4* SNPs in HBV/HCV may be due to the difference in ethnic background of populations studied, as well as biased study designs, influenced by a failure to control for important factors such as duration of infection, disease severity, co-infections, and specific treatment for HCV and HBV infections.

Function of *CTLA-4* SNPs on HBV/HCV Infection

Although HBV- and HCV-associated hepatic diseases are not determined solely by genetic factors of the host, the *CTLA-4* SNPs offer the basis for the knowledge of the immunomolecular mechanisms involved in viral elimination or the development of CLD (Figure 1). The major mechanisms by which these SNPs can have a functional effect on the response against viral infections can be summarized as follows:

- the −319C and +49G alleles, either individually or as a haplotype, interfere with the ability to block the immune response by CTLA-4 (39)
- the −319T/+49A haplotype increases expression of *CTLA-4* (52)
- the −319C, +49G alleles, or the −319C/+49G haplotype downregulates CTLA-4 (38, 49) and therefore can be potentiating the T-cell response in individuals with HBV and HCV infection
- the CTLA-4 ligands CD80 and CD86 may play an important role in the development of Th1/Th2 cells (66) and, therefore, CTLA-*4* SNPs could shift the Th1/Th2 balance
- these SNPs are in linkage disequilibrium that, by themselves or as haplotypes, may alter CTLA-4 gene expression and function (40).

Immunotherapy for Viral Infections

The expression of CTLA-4 and other inhibitory receptors has been found in virally infected cells of HBV, HCV, and human immunodeficiency virus (HIV) patients, and is associated with dysfunction of cellular immune response by T-cells, and an increased viral load. Blockade of the CTLA-4 pathway *in vitro* restores the antiviral capacity of exhausted T-cells in chronically infected patients (13). The drugs which block CTLA-4 are currently in use for cancer patients (67) but not for HBV- or HCV-infected patients due to the liver damage that can generate the CTLA-4 inhibition (2). It has been reported that melanoma patients with HBV or HCV, when treated with ipilimumab (human α-CTLA-4), experienced lower viral titers. Patients who did not develop progressive melanoma during treatment with ipilimumab responded to immunotherapy and experienced dramatic reductions in viral load (68). Another study reports that immunotherapy with tremelimumab (human α-CTLA-4 antibody) of HCC patients with HCV reduces viral load more than 10 times (in 10 of 17 patients). It should be noted that the decrease in viral loads also correlates with the appearance of HCV mutants (69). It is possible that immunotherapy could be the next therapeutic tool to treat chronic HBV or HCV infections. However, more research is needed to evaluate the side effects and determine the safety profile of CTLA-4 blockade in patients infected with viral hepatitis.

Conclusion

In conclusion, most studies find −319 C/T SNP in the form of +49G/−319C haplotype. This is directly associated with SVR and chronic infection in genotype 3 HCV patients but not in HCV genotype 1 patients. These patients have the lowest SVR. This suggests that identifying individuals with the +49G/−319C haplotype for treatment may improve prognostication. Taken together, studies of CTLA-4 SNPs in viral hepatitis propose that the +49G allele is associated with chronic infection (HBV/HCV) but is protective against the development of HCC. Therefore, although the +49G allele is the most prevalent in chronic infection, it does not mean that this allele presents a poor prognosis. The +49G allele is also associated with viral elimination and SVR; therefore, it can be proposed as a marker of better prognosis in HBV and HCV infections. In addition, the knowledge of the role of *CTLA-4* SNPs in viral hepatitis may assist in the development of specific immunotherapy for these infections. Nonetheless, more studies are needed to elucidate the implication of these SNPs in the pathogenesis and progression, and susceptibility to HBV and HCV infections.

Conflict of interest

The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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