Immune-Regulatory Events in the Clearance of HBsAg in Chronic Hepatitis B: Focuses on HLA-DP

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ABSTRACT

Successful clearance of hepatitis B virus (HBV) is a promising event in which host's immune system will attempt to get rid of the virus. The immunological events of HBsAg seroclearance have attracted great attention in both natural history investigations and therapeutic trials. Recent genome-wide association studies (GWAS) has confirmed polymorphisms in the human leukocyte antigen (HLA)-DP locus associated with spontaneous HBV clearance. In this review the impact of host immune response in declining HBsAg during the natural history of the infection has been discussed.

KEYWORDS
Chronic hepatitis B; Immune response; Polymorphism; Human leukocyte antigens DP (HLA-DP)

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INTRODUCTION

The combination of viral, environmental, and host genetic factors contribute to the heterogeneous outcome of chronic hepatitis B infection including spontaneous HBsAg seroclearance. This self-limiting outcome of infection has been detected in a very small proportion of patients with chronic HBV infection. Annual HBsAg seroclearance rate has reported between 0.1% and 2.3% depending on the endemicity of the area for HBV infection, mean age of participants at study entry, years of follow up and serum levels of HBV DNA and HBsAg.[1]

The effect of HBV genotype with its distinct geographical distribution on the aforementioned self-limited outcome of infection has been well documented in several studies.[2,3] However, resolution of HBV infection is mainly related to interplay between virus and the complex interactions between host innate and adaptive immune response. The subsequent studies have shown that the group of cytidine deaminases family enzymes have practical role in the host anti-HBV defense system.[4] The initiate antiviral immunity is strongly associated with the function of plasmacytoid dendritic cells (pDCs) and natural killer (NK) cells as the first line defense and non-specific immune response.[5] Moreover, a polyclonal strong response of T-helper 1 (Th1) and cytotoxic T-lymphocytes (CTL) implies in the clearance of HBV infection through adaptive immune response.[6] With some discrepancy, twin studies and segregation analysis strongly support the role of host

genetic factors in HBV infection outcome and the chance of HBsAg loss.[7] Association studies of candidate genes including HLA class I and II alleles and non-HLA genes have been revealed the effect of a number of genes in the persistence or clearance of HBV. Recently several genome-wide association studies (GWAS) demonstrated some genetic variants of HLA-DP in chronic hepatitis B populations. Meta-analysis of these results showed the strong influence of rs3077-A and rs9277535-A alleles of HLA-DP gene for an increased risk of spontaneous HBV clearance from persistent HBV infection.[7]

A greater understanding of the viral and host immunity factors and their influencing on HBsAg clearance will enable us to improve our therapeutic strategies in the treatment of chronic HBV infection. In this study we provide an in-depth review of the most important new data on immune-regulatory mechanisms which can play a role in the HBsAg seroclearance.

Functional Genomics of Hepatitis B virus

Human HBV belongs to the family of hepadnaviridae. These viruses have a strong preference for infecting hepatocytes as definite site of replication.[8] Small amounts of hepadenaviral DNA can be found in other organs such as the kidney, pancreas, and mononuclear cells, but infection at these sites does not correlate with extrahepatic disease.[9] In 1988 the entire nucleotide sequences of 18 HBV isolates were compared in Japan.[10] These isolates were found to cluster into four genotypes defined by more than 8% sequence divergence in the entire HBV genome. The genotypes were named A, B, C and D. In 1994, Norder et al. identified a further two genotypes, E and F.[11] In 2000, Stuyver et al. discovered genotype G from blood donors in France and the United States.[12] Then the eighth genotype (H) was identified by Arauz-Ruiz et al.[13] Hepadenaviridae are unique because they contain a partially double-stranded DNA genome that replicates via an RNA intermediate using its own encoded reverse transcriptase.[14] The genome is 3 kbp in length and contains four overlapping genes that encode for the nucleocapsid (precore and core), polymerase with reverse-transcriptase activity, envelope (pre S and S) and hepatitis X proteins. The core contains the viral genome and a polymerase that is essential for viral DNA replication. The envelope proteins which consist of two or three subspecies are essential for envelopment of nucleocapsids. The most abundant protein is the S protein which is known as HBsAg. The X open reading frame encodes the viral X protein. Although many roles have been postulated for the X protein, its exact role in HBV infection is unknown, however, it is essential for replication. An estimation of mutation rate was reported by okamoto (1987) to be between 1.4 to 3.2x10-5 substitutions per site per year. This mutation rate is larger than that for DNA viruses and more similar to certain RNA viruses.[2,14]

The Clinical Course of Hepatitis B Virus Infection

The initial step in the life cycle of hepadnaviruses is attachment of the viral particle to the hepatocytes. The PreS1 domain in viral envelope contains the major cell attachment epitope for interaction with the cellular receptors.[15] The replication of HBV is exclusive in DNA viruses of animal nonetheless in comparison with cauliflower mosaic virus in which they use RNA intermediate and a reverse transcription step.[16] The genome of HBV allow to encode about 50% proteins by varying the frame code of its genome. The significant feature of all hepadenaviruses is the secondary structure of their pregenomic RNA in the precore region, the stem-loop structure, which highly conserved among the hepadenaviruses family. At 5' end, this structure forms the encapsidation signal for packaging of pregenomic RNA into the immature core particles during replication.[17] Mutations influencing base pairing in the stem regions result in reducing the amount of replication or non-feasible virus. It has been believed that different range of HBeAg to anti HBe seroconversion between genotypes, are related to precore translational mutation, G 1896 A mutation, that stop the expression of HBeAg) or formation of a double mutation at nucleotides 1762 and 1764 in the upstream core promoter.[2,18] As predicted by the molecular studies of encapsulation signal in the

pre-core region, pre-core stop codon mutation was found to be more frequent within genotype D.[2]

The clinical course of HBV infection can be variable depending on the patient's age at infection and the host immune responses. Approximately 0-10% of infected adults (with adult –onset infection) become chronic carriers of HBV, whereas the prenatal transmission leads to up to 90% of chronic carriers that is a primary cause of end-stage liver disease.[3,19] In infected patients with acute liver disease hepatocytes regeneration is an effective mechanism for eliminating the virus from hepatocytes.[20] In chronic HBV infection, however, the clearance of the virus is rare because telomere shortening and senescence of hepatocytes limits the number of cell divisions and regeneration.[21] Telomeres are specialized nucleoprotein structures at the end of eukaryotic chromosomes. Continuous shortening of telomeres during each cell division limits the life span of primary human cells in vitro. However, an intriguing possibility is that the replacement of infected hepatocytes by proliferation of uninfected population of progenitor cells might help eliminate the virus.[22] Meanwhile the reduced expression of viral proteins and nucleic acids in hepatocytes, which is induced by certain cytokines form CTL and major histocomplatibility complex type-I (MHC-I) response that kill many infected cells before virus production, increases the kinetics of clearance.[23]

Viral Predictors of HBsAg Seroclearance

Spontaneous HBsAg seroclearance, defined as HBsAg level measuring less than 0.05 IU/mL by available commercial assays, is a favorable outcome in the natural history of chronic HBV infection if there is no preexisted advanced fibrosis/cirrhosis at the time of seroconversion.[24] It is also an important endpoint following HB antiviral therapies like interferon-based therapy. Therefore, it is not surprising that HBsAg seroclearance has attracted considerable attention in natural history studies as well as therapeutic trials of chronic HBV infection.[25] HBsAg serum level is a reflection of not only infectious spherical HBV virion (Dane particle) but also of defective HBsAg particles (spheres and filaments). The association between serum HBsAg levels and HBsAg clearance has been well documented. It has also been shown that significant decrease in HBV viral load is an important predictor of HBsAg seroclearance in chronically infected patients.[1] In some chronic hepatitis patients with an appropriate immune response and serum HBV-DNA levels below the cut-off 2000 IU/mL, serum HBsAg may become negative together with the normalization of alanine aminotransferase (ALT).[26] HBV-DNA levels are being reduced as low as 100 IU/mL in this group of patients.[1,24,25]

Successful Immune Responses Inhibit HBV Replication

Patients with chronic hepatitis B are allocated into different medical phases: the immune-tolerance phase, the immune-clearance phase, and the inactive carrier state.[26] These phases reveal apparent differences of penetrate of immune cells in liver. The composition of liver-derived lymphocytes in fine needle aspiration liver biopsies of patients with chronic HBV infection showed that the proportion of NK cells in immune-tolerant compare to patients in immune-clearance and asymptomatic carriers.[26,27] HBV-specific CD4+ and CD8+ T-cell play an important role in self-limited process of the disease and viral clearance in acute hepatitis however, in individuals with chronic HBV infection the response of these kind of cells are significantly diminished.[5,6,28] In acute viral hepatitis, the produced antiviral cytokines contribute to the viral clearance in noncytopathic mechanisms by eliminating the replicative form of virus in infected cells. It has been shown that the residues 18–27 as core epitopes for CD8+ T-cells that are almost undetectable in HBeAg-positive patients have important role for the clearance of HBeAg. Smaller number of specific CD8+ T-cells are associated with reduced ability of Interferon gamma (IFN-γ) production that are important to inhibit viral replication.[6,28] Likewise, in some adefovir dipivoxil (ADV) treated chronic hepatitis B patients, HBV-specific T cell reactivity temporally increases that this trend is strongly associated with HBeAg loss. The functional effect

of HBV-specific CD8+ cells for inhibition of virus replication can be independent of its effect for liver damage.[20,29] Thus the exhaustion of intrahepatic naive lymphocyte reservoir is important for the development of a weak antiviral immune response and the inability to control viral replication in chronic hepatitis B patients. When CD8+ T-cells responses are unable to control virus replication, they may contribute to liver pathology not only directly but also by the recruitment of nonvirus-specific T cells.[29]

Dendritic cells (DCs) are the most effective antigen presenting cells and play a pivotal role to induce antiviral responses. The exogenous antigens are phagocytized, and then loaded on both HLA-class II and HLA class I by dendritic cells. Though this mechanism in vaccination is able to present exogenous HBsAg for efficient immunity in healthy people and in a few patients with chronic hepatitis B, however HBsAg vaccine could not induce immunity and consequently eradication of HBV in the majority of these patients probably by impaired function of intradermal dendritic cells.[30] Patients with chronic HBV infection show an impaired stimulatory capacity for HBV-inoculated monocytoid drived dendritic cell (MoDC) and therefore impaired production of Interleukin-2, tumor necrosis factor alpha (TNF-α) and IFN-γ.[31] However, inhibition of viral replication is directly mediated by a specialized DC subpopulation, the pDCs, that produces high levels of type I IFNs and determine outcomes of HBV infections. Conversely type I IFNs, regardless its source, negatively controls pDC numbers in vivo via the intrinsic apoptosis pathway that may explain the loss of pDCs in chronic HBV infections.[32] It has been reported that HBV interferes with the functional interaction between NK cells and pDCs in chronic hepatitis B and therefore the production of IFN-α by NK cells is reduced.[5] Most likely, the activation of complex networks of innate and adaptive immune-related cells like NK, NK-T, monocytes, dendritic cells, T cells and B cells can be enabled by the function of innate immune-recognition receptors, such as Toll-like receptors (TLRs) for intracellular signaling transduction that concomitantly increase IFN-γ and cytotoxicity of NK.[33,34]

NK cells are essential innate immune cells as they are able to produce NK cell-derived cytokines for stimulating Th1 cell development and subsequent cytotoxic CD8+ T-cell response to inhibit viral replication.[33] Modifications in hepatocyte CD1D lipid antigen that induce by HBV, lead to activation of natural killer T (NKT) cells and contribute to protective immunity.[35] Killer immunoglobulin-like receptor (KIR) genes may be protective genes that facilitated the clearance of HBV. In that case the activation of NK and T cells can be regulated by KIR gene upon interaction with HLA class I molecules.[36] These data indicated that well-organized immune cells in T-cell response.are responsible in both viral clearance and liver disease in the course of chronic HBV infection.

During recent years the established role of cytidine deaminases in protein diversity and immunity has directed scientists to hypothesize that these proteins facilitate the clearance of both pathogenic and non-pathogenic foreign DNA.[37] Since a number of these enzymes are induced by IFNs, it has been suggested that the family of APOBEC3 have important role in antiviral effect of the cytokines. APOBEC3G (A3G), the well-studied member of APOBEC3 family, is being part of intrinsic immunity to DNA viruses such as HBV. However, these enzymes do not necessary in anti-HBV action of type I and II IFNs in vital cells.[4,38]

HLA Polymorphism and HBV Spontaneous Clearance

The highly polymorphic HLA class I and II molecules that influence the capacity of HLA molecules to trigger immune responses, affects the outcome of infection with any given pathogen.[39] Multiple viral peptides are presented by HLA class I molecules to activate epitope-specific CTL populations which are varied in size and clonal composition.[8,36] The immunodominant epitopes have central effect for immunopathology related to T cells. These epitopes stimulate the largest number of antiviral CTL and have protective role against the infecting virus.

Certain HLA class I allotypes also regulate the response of natural killer cells.[39]

Numerous studies have been investigated to identify the distribution of HLA alleles in spontaneous recovered subjects from HBV infection. The importance of HBe/HBcAg-specific T helper to recognize the viral epitopes on MHC class II molecules for an efficient immune response in HBV infection was first reported by Lohr et al.[40] Then it was shown that the MHC class II allele DRB1*1302 was associated with the clearance of HBV among both children and adults in the Gambia.[41] This result was also established in European and Caucasians population.[42] Positive associations between multiple alleles DRB1*1302, DRB1*1502, DQB1*0302, DQB1*0609, and related-haplotypes with viral clearance have been reported in Korean studies which demonstrated that DR13 was associated with self-elimination of HBV.[43] Attempting to investigate a possible link between HLA polymorphism with the outcome of HBV (sub) genotypes infection, Li X et al showed that HLA-B*15, DRB1*11 and 14 were associated with spontaneous recovery of HBV subgenotype C2 infection.[44] Recent Meta-analysis to assess the relationship between HLA-DR alleles and spontaneous recovery from prior HBV infection showed that HLA-DR*04 and DR*13 alleles were significantly associated with HBV clearance.[45]

Impact of HLA-DP Gene Variants on Risk of Spontaneous HBV Clearance

Genome-wide association studies (GWAS) have greatly contributed to the identification of common genetic variants related to common diseases. Candidate gene association studies have implicated the relationship between clearance or persistence of HBV and the host genetic polymorphisms, including HLA classes I and II alleles and non-HLA genes.[7] Current investigations highlighted the importance of single-nucleotide polymorphisms (SNPs) in HLA-DP (rs3077 and rs9277535) and HLA-DQ (rs2856718 and rs7453920) with risk of chronic infection.[7,46] These SNPs were then genotyped in different group of patients as well as in persons with HBV natural clearance from China to search the associations of the variants of HLA-DP/DQ and the probability of both HBV clearance and HCC development. Further analyses revealed that the variant rs2856718 significantly diminished host HCC risk, whereas three SNPs rs9277535, rs7453920 and rs2856718 of HLA-DP gene more found in cases that clear HBsAg. More association analysis in the Japanese and Korean population identified the protective effects of alleles rs3077 and rs9277542 respectively in genes HLA-DPA1 in HLA-DPB1 to infection of hepatitis B and clearance of HBV.[47]

To find more evidence on the relevance of these HLA-DP polymorphism in HBV infection outcome, two meta-analyses were conducted to evidence for the relevance of these HLA-DP polymorphisms in HBV infection outcome. The results showed that subjects bearing at least one A allele of HLA-DPB1 rs9277535 and -DPA1 rs3077 variants had an increased susceptibility to spontaneous HBV clearance compared with those with G alleles. Also, it was specified that the strength and effect size of rs3077T is more powered to efficient viral clearance, instead for decreased susceptibility to infection.[7] Further work was completed to sequence the coding exons and the prime untranslated regions (3'-UTRs) of HLA-DPB1 and DPA1 in originally persons of European-American and African-American.[48] The 3'-UTRs in proximate to translation termination codon of messenger RNA (mRNA) is regulatory regions in post-transcriptional and gene expression process. and stability of the mRNA.[49] The candidate variant rs9277535 correspond to 550 A/G in the 3'-UTRs of the HLA-DPB1 gene that accompanied with chronic hepatitis B and outcomes to HBV infection predominantly in Asian carrier had a minimal effect on HBV recovery in the European- and African-American subjects. However, the novel variant rs9277534 in the 496A/G of HLA-DPB1 3'-UTRs region in both European and African-American populations were identified significant associated with recovery of HBV infection. Compare to 550A/G variants, the protective HLA-DPB1 allele (DPB1*04:01) could be distinguishes

from the susceptible allele (DPB1*01:01) by 496A variant that showed the strong effect of 496A on HBV recovery. Interestingly, it was shown that samples with 496AX genotypes had considerably lower expression of HLADPB1 than samples with the 496GG genotype, may signify the important role of this locus in outcome to HBV infection.[48] These results elucidate the effect of higher expression of HLA-DP in Th2 response for enormous antibody production but fewer efficacy of CTL activity and consequently the persistence of HBV.

However this finding was in contrast with previous result from O'Brien et al. that the rs3077AA genotype (strongly related to mRNA expression of HLA-DPA1) and rs9277535AA genotype (strongly associated with HLA-DPB1 expression) were conversely associated with risk of chronic HBV infection.[50] Further work will be required to determine the association of these alleles with varied expression of HLA-DP molecules and how they are affecting the immune response for spontaneously clearance of HBV.

T-cell Epitopes in HBV Capsid Bind to HLA-DP

MHC class II molecules are cell surface glycoproteins on antigen presenting cells that present specific peptides, derived from extracellular proteins, to T-helper cells. HLA-DP is an HLA class II heterodimeric molecule belongs to HLA-DPA1 and -DPB1 genes with chromosomal locus 6p21.31, that encodes for alpha and beta chains of HLA-DP. The alpha chain is encoded by 5 exons with 33-35 kDa MW, while the gene of beta chain contains 6 exons that encode beta chain protein with 26-28 kDa MW. There are 6 exons in the gene of beta chain that encodes the leader peptide, the extracellular domains, the transmembrane domain and the cytoplasmic tail. The polymorphisms in both alpha and beta chains result in different T helper cell response.[51] Several studies discuss the effect of peptides that binds to HLA-DP molecules on the stability and expression of HLA-DPA1 and HLA-DPB1 alleles.[7,45,48] In this regard, interaction of HLA-DP with HBV peptides could be a critical step in immune regulation of HBV infection and clearance. Therefore, evaluation of T-cell epitopes for binding to HLA-DP molecules is important to design new strategies for controlling immune response. Previous studies have revealed that genotypes A and B of HBV have higher frequency of HBsAg seroclearance than genotypes D and C respectively.[52,53] Hence, the different rate of HBsAg seroclearance among different HBV genotypes could be explained by derivative epitopes of protein sequences of HBV genotypes. By the way, computational prediction of antigenic peptide that bind to human MHC class II protein HLA-DP have become supportive by epitope discovery methods in NetMHCII 2.2 server.[54] Using this server, similar epitopes of core proteins of HBV identified for genotypes A/B and also for genotypes C/D. As indicated in table 1, one epitope was found in all genotypes and the other one was found only in genotype C/D. Therefor it has been suggested that in adaptive immune response, epitopes of viral peptides specially in core protein of genotype C/D are presented by HLA class II molecules to activate epitope-specific T-helper population vigorous antibody production along with poor CTL activity. Genotype D is very common in the Mediterranean region and is also the dominant HBV genotype in Iran. More investigation needs to clarify the association between HLA-DP and HBsAg seroclearance between genotypes of HBV.

Concluding Remarks

Mechanisms of spontaneous HBV clearance are most determined by the interaction between the HBV and both the innate and adaptive immune response. The role of T lymphocytes response to HBV epitopes presented by the HLA is important for immunity against HBV infection. The impact of specific polymorphisms in human HLA gene loci that alter peptide epitope binding has been reported by several studies. However, GWAS data from new investigations repetitively showed the association HLA-DP genetic variations in different population with clearance of chronic HBV infection. Moreover, the assessment of HLA-DP binding to T-cell

Table 1: Predicted epitopes in capsid protein among HBV genotypes A, B, C and D for HLA-DPA10103-DPB10401 allele.

Capsid protein	Epitope core	Affinity (nM) •	%Rank ••
sp|P0C695|CAPSD_HBVA6	FLPSDFFPS	0.6676	36.5
sp|P17391|CAPSD_HBVB1	FLPSDFFPS	0.6676	36.5
sp|P0C6H4|CAPSD_HBVC2	FLPSDFFPS	0.6680	36.3
	FRQLLWFHI	0.7225	20.1
sp|P0C6I3|CAPSD_HBVD7	FLPHDFFPS	0.6667	36.8
	FRQLLWFHI	0.7240	19.8

• Affinity as IC50 value in nM (only for Artificial Neural network predictions)
•• Binding Level

epitopes would be helpful to design of new strategies in therapy of chronic hepatitis B. According to these finding, future research should clarify how varied expression of HLA-DP molecules and viral peptides that bind to this molecule among different HBV genotypes will affect the HBsAg seroclearance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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