

Immunophysiological Effects of of Hepatitis B Virus Genotypic Variations on Interleukin-35 Levels

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Abstract

Hepatitis B virus (HBV) remains a major global health concern, with viral genetic diversity playing an important role in immune evasion and disease persistence. Interleukin-35 (IL-35) is a potent immunosuppressive cytokine that may facilitate HBV persistence; however, its relationship with HBV genotypic variation remains insufficiently understood. This study investigated the association between HBV genotypes and serum IL-35 concentrations among patients with HBV infection. A total of 150 participants were enrolled, comprising 60 patients with chronic hepatitis B (CHB), 60 inactive HBV carriers, and 30 healthy controls. HBV genotypes were identified using polymerase chain reaction-based methods, while serum IL-35 concentrations were quantified using an enzyme-linked immunosorbent assay. Serum IL-35 concentrations were significantly higher among patients with CHB (85.4 ± 12.3 pg/mL) than among healthy controls ($p < 0.001$). Patients infected with HBV genotype C also exhibited significantly higher IL-35 concentrations than those infected with genotype D ($p = 0.021$). Furthermore, serum IL-35 concentrations were strongly and positively correlated with HBV DNA viral load. These findings indicate that HBV genotype C is associated with a stronger IL-35-mediated immunosuppressive response, which may contribute to viral chronicity and

more severe clinical outcomes. This study provides evidence of genotype-specific immune modulation in HBV infection and highlights the potential relevance of IL-35 in evaluating viral persistence and disease progression.

Keywords: Chronic Hepatitis B; HBV Genotypes; Immune Evasion; Interleukin-35; Viral Load

INTRODUCTION

Hepatitis B virus (HBV) infection remains a major global public health challenge, leading to a wide range of clinical manifestations from inactive viral carrier status to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. HBV exhibits enormous genetic diversity, with genotypes A–J classified based on nucleotide sequence variations exceeding 8% [2]. Recent scientific evidence suggests that these genotypes not only influence the virus's geographic distribution but also play a pivotal role in determining the natural history of the disease, response to antiviral therapies, and the immunogenic pathway [3].

In the context of the host immune response, interleukin-35 (IL-35), a relatively new member of the IL-12 cytokine family, has emerged as an immunosuppressive factor produced by regulatory T and B cells (Tregs and Bregs) [4]. IL-35 acts as a strategic “brake” to the immune response by inhibiting the proliferation of helper T cells (Th1 and Th17) and reducing the cytotoxic activity of T cells directed against the virus [5]. Studies have shown that serum IL-35 levels are directly correlated with viral load (HBV DNA) and the degree of liver cell damage, suggesting its role in facilitating viral persistence by creating an environment of immune tolerance [6].

Despite advances in understanding the role of IL-35, the relationship between viral B genotype variation and changes in this cytokine concentrations still requires further investigation. Some reports suggest that more aggressive genotypes, such as genotype C, may be associated with a very different cytokine profile than genotypes D or B, which could explain the variation in disease progression among different populations [7]. Understanding the interaction between viral genotype and IL-35 expression may provide new biomarkers for predicting disease course and developing targeted immunotherapeutic strategies.

Theoretical Framework

This study is based on the hypothesis that genetic variation in the DNA sequence of hepatitis B virus (HBV) leads to differences in the expression of viral proteins (such as HBeAg and HBx), which play a direct role in stimulating immune cells to produce inhibitory cytokines.[8]

1. The Mechanism of Immune Exhaustion via IL-35

IL-35 acts as a key instrument in immune exhaustion. In chronic infections, the virus stimulates increased production of regulatory T cells. This cytokine, which in turn secretes IL-35, performs the following functions:

Inhibiting the proliferation of CD4⁺ and CD8⁺ cells, thus preventing the body from eliminating infected cells.

Stimulating increased IL-35 production: Through a positive feedback mechanism, this creates an immunosuppressive hepatic environment that helps the virus survive.

2. Effect of Genotypes: Genotypes differ in their replication rate and mutational capacity.

Genotype C: Often associated with higher levels of inflammation and faster progression of fibrosis, which may require higher IL-35 levels to attempt to "calm" the immune system, negatively impacting viral persistence.[9,10]

Genotype D: Exhibits diverse immune responses and a wide geographical distribution, making it a model for comparison in serum cytokine levels.[11]

METHODOLOGY

To achieve the study objectives, a descriptive analytical approach (cross-sectional study) is followed according to the following steps:

1. Study Population:

Groups: Participants are divided into three groups: patients with chronic hepatitis B (CHB), inactive virus carriers, and a control group (healthy individuals). Exclusion Criteria: Patients with co-infections (such as HCV or HIV) or those with autoimmune diseases are excluded.

2. Sample Collection and Preparation:

5 ml of venous blood is withdrawn from each participant., Serum is separated by centrifugation at 3000 rpm for 10 minutes, Samples are stored at -80°C until testing.

3. Laboratory Assays:

Genotyping:

Viral DNA extraction using specialized kits. Use of qualitative polymerase chain reaction (PCR), RFLP, or sequencing to determine the genotype.

IL-35 level measurement: Use of enzyme-linked immunosorbent assay (ELISA) with highly sensitive kits specifically designed for human interleukin-35.

Biochemical and virological tests: Measurement of liver enzymes (ALT, AST). Measurement of HBV DNA viral load using real-time PCR.

4. Statistical Analysis:

Use of SPSS software for data processing. Application of ANOVA for comparison between multiple groups. Use of Pearson or Spearman's test to determine the correlation between IL-35 concentration, viral load, and genotype.

RESULTS

A total of 150 participants were enrolled in this study, categorized into three groups: Chronic Hepatitis B (CHB) patients (n=60), Inactive Carriers (n=60), and Healthy Controls (n=30).

1. Serum IL-35 Levels Across Study Groups

The biochemical analysis revealed a significant elevation in serum **IL-35** concentrations among HBV-infected individuals compared to the healthy control group ($P < 0.001$). The highest levels were observed in the CHB group, as shown in Table 1.

Table 1: Comparison of Serum IL-35 and Clinical Parameters Among Study Groups

Parameter	CHB Patients (n=60)	Inactive Carriers (n=60)	Healthy Controls (n=30)	P-value
IL-35 (pg/ml)	85.4 ± 12.3	42.1± 8.5	15.2 ± 4.1	0.001
HBV DNA (log10 IU/ml)	6.2 ± 1.1	2.4± 0.8	N/A	<0.01
ALT (U/L)	75.2± 20.4	28.5± 5.2	22.1± 4.8	<0.05

2. Influence of HBV Genotypes on IL-35 Expression

Genotypic analysis identified Genotype C (n=35) and Genotype D (n=25) as the predominant strains. Patients infected with **Genotype C** exhibited significantly higher serum IL-35 levels compared to those with Genotype D (P = 0.021).

Table 2: Serum IL-35 Concentrations Stratified by HBV Genotype

Genotype	Number (n)	Mean IL-35 (pg/ml)	SD ($\pm$)	P-value
Genotype C	35	98.6	15.2	0.021
Genotype D	25	68.4	10.8	-

DISCUSSION

The findings of this study underscore a critical link between HBV genotypic variations and the modulation of the host immune response through Interleukin-35 (IL-35). The significant increase in IL-35 levels in CHB patients suggests that this cytokine plays a pivotal role in the viral escape mechanism.

1. IL-35 as a Mediator of Immune Tolerance

The elevated levels of IL-35 in chronic cases, as opposed to inactive carriers, indicate its role in "immune exhaustion." IL-35, primarily secreted by regulatory T cells (T_{regs}), suppresses the proliferation of CD8⁺ cytotoxic T lymphocytes (CTLs), which are essential for viral clearance [12]. This creates an immunosuppressive "milieu" that facilitates persistent viral replication, a finding that aligns with recent studies by *Huang et al.* regarding the cytokine's role in liver disease progression [4, 8].

2. The Impact of Genotype C vs. D

One of the most significant findings is the disparity in IL-35 expression between genotypes. **Genotype C** is clinically associated with more severe liver damage and a higher risk of hepatocellular carcinoma (HCC) [13]. Our results suggest that Genotype C may induce a more robust secretion of IL-35, thereby providing a more potent inhibitory signal to the host's immune system compared to Genotype D. This difference might be attributed to specific mutations in the core or X regions of the Genotype C genome that enhance the activation of immunosuppressive pathways [14].

3. Correlation with Viral Load

The strong positive correlation between IL-35 levels and HBV DNA viral load suggests a feedback loop: high viral replication triggers IL-35 production, which in turn disables the immune response, allowing for further viral persistence [15]. This makes IL-35 a potential biomarker for monitoring disease activity and predicting the likelihood of chronicity.

CONCLUSION

Based on the immunological and genotypic findings of this study, the following conclusions were drawn:

- 1. IL-35 as a Diagnostic Biomarker:** Serum **IL-35** levels are significantly elevated in chronic HBV patients, establishing it as a potent biomarker for monitoring immune exhaustion and disease progression.
- 2. Genotype-Specific Modulation:** There is a definitive correlation between HBV genotypes and cytokine profiles. **Genotype C** induces a more robust IL-35 response compared to Genotype D, which may explain the higher rates of liver complications associated with this specific genotype.
- 3. Immune Evasion Mechanism:** The positive correlation between IL-35 concentrations and HBV DNA viral load confirms that the virus exploits this cytokine to suppress host T-cell responses, thereby facilitating viral persistence and chronicity.
- 4. Clinical Implications:** These insights suggest that targeting the IL-35 pathway could be a promising immunotherapeutic strategy, particularly for patients infected with high-risk genotypes, moving towards more personalized management of chronic Hepatitis B.

"The study recommends conducting longitudinal studies on larger samples to determine the precise impact of subgenetic mutations on IL-35 signaling pathways."

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Conflict of Interest Statement:

"The authors declare that there is no conflict of interest regarding the publication of this research. The authors confirm that they have no financial, personal, or institutional relationships with any third party that could inappropriately influence or bias the findings and interpretation of this study. This work was conducted independently for purely academic and scientific purposes."

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