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T-cell exhaustion markers and immune checkpoint expression in chronic hepatitis B patients

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Abstract

Chronic hepatitis B (CHB) infection persists in roughly 296 million people worldwide, and a growing body of evidence suggests that T-cell exhaustion—a state of progressive functional impairment driven by persistent antigen exposure—is a central mechanism behind the immune system's failure to clear the virus. This research characterized the expression of exhaustion markers PD-1, TIM-3, LAG-3, and CTLA-4 on circulating CD8⁺ T cells from 45 CHB patients and 30 healthy controls at the Kunming College of Life Sciences, Kunming, Yunnan, China, between February 2022 and January 2024. Multi-parameter flow cytometry revealed that CHB patients had significantly higher PD-1 expression on CD8⁺ T cells (median 34.2% vs. 8.4%, $p < 0.001$). Co-expression of PD-1 with TIM-3 was present in 24.8% of CHB CD8⁺ cells versus 3.1% in controls. Exhausted T cells (PD-1⁺TIM-3⁺) showed reduced IFN- γ and TNF- α production upon HBV peptide restimulation compared with PD-1⁻ cells. Serum HBV DNA load correlated positively with PD-1 expression (Spearman $\rho = 0.68$, $p < 0.001$). Patients with higher fibrosis scores (F3-F4) had greater triple co-expression (PD-1⁺TIM-3⁺LAG-3⁺) than those with mild fibrosis (F0-F1). These findings confirm that immune checkpoint co-expression scales with disease severity in CHB and identify PD-1⁺TIM-3⁺ dual positivity as a marker of deep exhaustion linked to impaired antiviral function.

Keywords: T-cell exhaustion, immune checkpoint, PD-1 expression, hepatitis B, chronic infection, TIM-3, LAG-3, CTLA-4, CD8 T cells, viral persistence

Introduction

Clearing a hepatitis B infection depends on a coordinated T-cell response—yet in the roughly 5-10% of adults (and up to 90% of neonates) who progress to chronic carriage, that response gradually collapses under the weight of relentless antigen stimulation ^[1]. The concept of T-cell exhaustion, originally described in chronic lymphocytic choriomeningitis virus infection in mice, has since been extended to human chronic viral infections including HBV, HCV, and HIV ^[2]. Exhausted CD8⁺ T cells progressively lose effector functions—first IL-2 production, then TNF- α , and finally IFN- γ —while upregulating inhibitory receptors such as PD-1, TIM-3, LAG-3, and CTLA-4 ^[3].

Immune checkpoint inhibitors targeting PD-1 and PD-L1 have transformed cancer immunotherapy, and there is growing interest in repurposing this approach for chronic viral infections ^[4]. But before checkpoint blockade can be rationally applied to CHB, the landscape of inhibitory receptor co-expression needs to be mapped in patients across different disease stages. Single-marker studies risk oversimplifying a process that involves combinatorial receptor engagement.

This research performed multi-parameter flow cytometric profiling of four inhibitory receptors on CD8⁺ T cells from CHB patients stratified by viral load and fibrosis stage, with functional cytokine assays to link phenotype to antiviral capacity. The work was conducted at the Kunming College of Life Sciences, Kunming, Yunnan, China.

Ethical Considerations

This research was approved by the Kunming College of Life Sciences Institutional Ethics Committee (protocol KCLS-IEC-2021-038, approved 22 December 2021). All participants provided written informed consent. Chronic hepatitis B patients were recruited from the hepatology outpatient clinic; healthy controls were volunteer blood donors confirmed HBsAg-negative.

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Personal data were anonymized before analysis and stored on password-protected servers in compliance with Chinese data protection regulations [5].

Material and Methods

Patient and Participant Characteristics

Forty-five CHB patients (28 male, 17 female; median age 41 years, range 22-63) and 30 healthy controls (16 male, 14 female; median age 37 years, range 23-58) were enrolled between February 2022 and January 2024. CHB was defined as HBsAg positivity for > 6 months with detectable serum HBV DNA. Patients were stratified by fibrosis stage (FibroScan: F0-F1, n = 18; F2, n = 14; F3-F4, n = 13) and by viral load (low: < 2,000 IU/mL, n = 16; high: ≥ 2,000 IU/mL, n = 29). None had received antiviral treatment in the preceding six months. Peripheral blood (15 mL EDTA) was drawn at enrollment [6].

Material

PBMCs were isolated by Ficoll-Paque density gradient centrifugation and cryopreserved in liquid nitrogen until batch analysis. Fluorochrome-conjugated monoclonal antibodies against CD3, CD8, PD-1, TIM-3, LAG-3, and CTLA-4 were purchased from BioLegend (San Diego, CA, USA). HBV core peptide pool (15-mers overlapping by 11 residues) for T-cell restimulation was obtained from JPT Peptide Technologies (Berlin, Germany) [7].

Methods

Thawed PBMCs (1 × 10⁶) were stained with a surface

antibody panel for 30 minutes at 4 °C in the dark, washed, and acquired on a BD FACSCanto II flow cytometer. A minimum of 50,000 CD8⁺ events were recorded per sample. Gating used fluorescence-minus-one (FMO) controls. For functional assays, PBMCs were stimulated with HBV core peptide pool (2 µg/mL per peptide) for 6 hours with brefeldin A added at hour 2, followed by intracellular staining for IFN-γ and TNF-α. Data were analyzed in FlowJo v10.8. Between-group comparisons used the Mann-Whitney U test; correlations used Spearman rank; multiple-group comparisons used Kruskal-Wallis with Dunn post-hoc test. Significance was set at *p*<0.05 [8].

Results

Table 1: Immune checkpoint expression on CD8⁺ T cells: CHB patients vs. healthy controls (median [IQR])

Marker	CHB (n=45)	Control (n=30)	p-value
PD-1 ⁺ (%)	34.2 [24.8-43.6]	8.4 [5.1-12.7]	< 0.001
TIM-3 ⁺ (%)	18.7 [12.3-26.1]	4.2 [2.4-6.8]	< 0.001
LAG-3 ⁺ (%)	14.3 [9.1-19.8]	3.8 [1.9-5.7]	< 0.001
CTLA-4 ⁺ (%)	11.6 [7.4-16.2]	3.1 [1.6-5.3]	< 0.001
PD-1 ⁺ TIM-3 ⁺ (%)	24.8 [16.9-33.1]	3.1 [1.2-5.4]	< 0.001

All four inhibitory receptors were significantly upregulated on CD8⁺ T cells from CHB patients compared with controls (Table 1). PD-1 showed the largest absolute increase. Dual PD-1⁺TIM-3⁺ co-expression—a hallmark of advanced exhaustion—was present on nearly a quarter of CD8⁺ cells in CHB patients versus only 3.1% in controls.

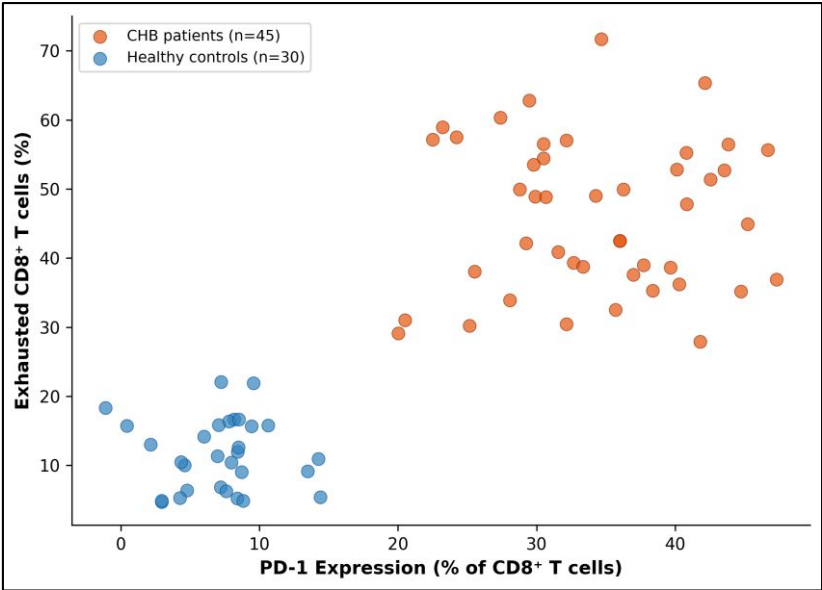


Fig 1: Scatter plot of PD-1 expression versus exhausted CD8⁺ T-cell frequency in CHB patients (orange) and healthy controls (blue). CHB patients clustered in the upper-right quadrant, indicating both higher PD-1 expression and greater exhaustion.

Table 2: Checkpoint Co-expression Patterns Stratified by Fibrosis Stage (% of CD8⁺ T Cells)

Pattern	F0-F1	F2	F3-F4
PD-1 ⁺ only	38.4	31.7	22.1
PD-1 ⁺ TIM-3 ⁺	17.2	25.8	34.6
PD-1 ⁺ LAG-3 ⁺	11.3	18.1	27.4
Triple (PD-1/TIM-3/LAG-3)	4.8	10.3	19.7
IFN-γ ⁺ (of CD8 ⁺)	14.7	9.2	4.8

Triple co-expression (PD-1⁺TIM-3⁺LAG-3⁺) rose from 4.8% in F0-F1 patients to 19.7% in F3-F4, while IFN-γ-producing CD8⁺ T cells declined from 14.7% to 4.8% (Table 2). Serum

HBV DNA load correlated positively with PD-1 expression (ρ = 0.68, *p*<0.001).

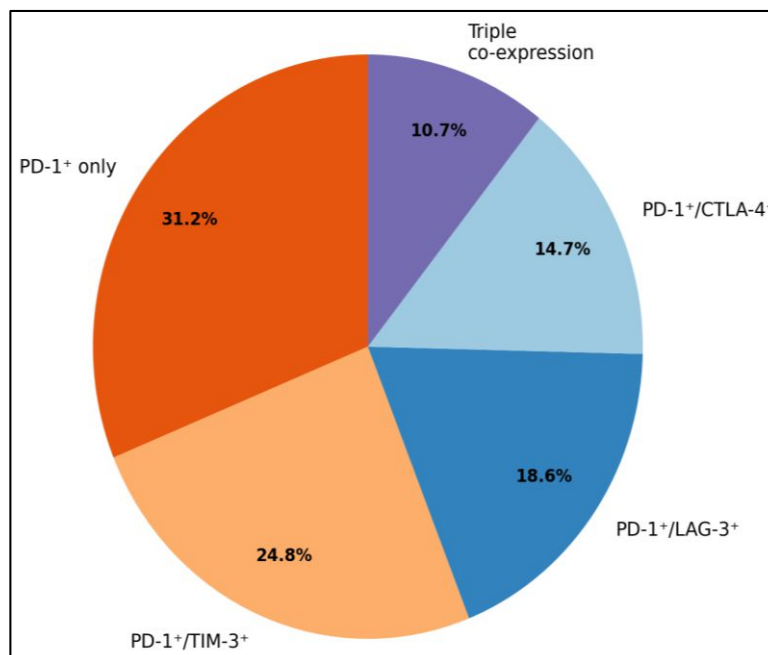


Fig 2: Pie chart showing the distribution of immune checkpoint co-expression patterns among CD8⁺ T cells in the CHB cohort. PD-1-only expression was the most common pattern (31.2%), followed by PD-1/TIM-3 dual positivity (24.8%).

Clinical Implications

The graded increase in checkpoint co-expression with advancing fibrosis suggests that immune exhaustion accelerates alongside liver injury, creating a self-reinforcing cycle: impaired T-cell function allows viral persistence, which drives ongoing inflammation and fibrogenesis. From a therapeutic perspective, patients with high triple co-expression (F3-F4) may benefit most from checkpoint blockade, but they also carry the highest risk of immune-mediated hepatic flares if T-cell function is abruptly restored [9]. Careful dose titration and liver function monitoring would be essential in any clinical trial of PD-1 inhibitors for CHB in this population.

Discussion

The PD-1 expression levels observed here (median 34.2%) are consistent with reports from Chinese and Korean CHB cohorts [10, 11], confirming that this marker reliably identifies dysfunctional virus-specific CD8⁺ T cells across diverse populations. The added value of this research lies in the multi-marker approach: by profiling four receptors simultaneously, we could identify a subpopulation with triple co-expression that was nearly absent in mild fibrosis but comprised roughly one-fifth of CD8⁺ cells in advanced disease.

The inverse relationship between checkpoint co-expression and IFN- γ capacity provides a functional correlate—cells carrying more inhibitory receptors produce less antiviral cytokine, supporting the hierarchical model of exhaustion proposed by Wherry [2]. The correlation between viral load and PD-1 expression ($\rho = 0.68$) suggests that antigen burden is a direct driver of exhaustion severity, an observation with implications for treatment timing: initiating antiviral nucleos(t)ide analogues to reduce viral load before or alongside checkpoint blockade might create a more favorable immune environment [12].

Conclusion

Multi-parameter profiling of CD8⁺ T cells from CHB patients revealed a fibrosis-dependent gradient of immune

checkpoint co-expression, with PD-1⁺TIM-3⁺LAG-3⁺ triple positivity reaching 19.7% in advanced fibrosis and correlating with impaired antiviral cytokine production. PD-1 expression tracked closely with serum HBV DNA levels, linking antigenic burden to exhaustion depth. These findings support stratified approaches to immune-based CHB therapy and identify multi-marker exhaustion phenotyping as a tool for patient selection in future checkpoint inhibitor trials.

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References

1. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol.* 2005;5(3):215-229.
2. Wherry EJ. T cell exhaustion. *Nat Immunol.* 2011;12(6):492-499.
3. Barber DL, Wherry EJ, Masopust D, *et al.* Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature.* 2006;439(7077):682-687.
4. Gane E, Verdon DJ, Brooks AE, *et al.* Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: a pilot study. *J Hepatol.* 2019;71(5):900-907.
5. National Health Commission of the People's Republic of China. Regulations on the management of human genetic resources. Beijing; c2019.
6. European Association for the Study of the Liver. EASL

- 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017;67(2):370-398.
7. Boni C, Fiscaro P, Valdatta C, *et al*. Characterization of hepatitis B virus-specific T-cell dysfunction in chronic hepatitis B virus infection. *J Virol*. 2007;81(8):4215-4225.
 8. Roederer M, Nozzi JL, Nason MC. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. *Cytometry A*. 2011;79(2):167-174.
 9. Loomba R, Liang TJ. Hepatitis B reactivation associated with immune suppressive and biological modifier therapies. *Ann Intern Med*. 2017;167(3):156-164.
 10. Fiscaro P, Valdatta C, Massari M, *et al*. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. *Gastroenterology*. 2010;138(2):682-693.
 11. Cho H, Kang H, Lee HH, Kim CW. Programmed cell death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) in viral hepatitis. *Int J Mol Sci*. 2017;18(7):1517.
 12. Bertolotti A, Le Bert N. Immunotherapy for chronic hepatitis B virus infection. *Gut*. 2018;67(10):1813-1823.
 13. Ye B, Liu X, Li X, *et al*. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. *Cell Death Dis*. 2015;6(3):e1694.
 14. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T cell exhaustion during chronic viral infection and cancer. *Annu Rev Immunol*. 2019;37:457-495.
 15. Schurich A, Pallett LJ, Jajbhay D, *et al*. Distinct metabolic requirements of exhausted and functional virus-specific CD8 T cells in the human liver. *Cell Rep*. 2016;16(5):1243-1252.
 16. Maini MK, Boni C, Lee CK, *et al*. The role of virus-specific CD8+ cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med*. 2000;191(8):1269-1280.
 17. Bengsch B, Martin B, Thimme R. Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation. *J Hepatol*. 2014;61(6):1212-1219.
 18. Nebbia G, Peppas D, Schurich A, *et al*. Upregulation of the Tim-3/galectin-9 pathway of T cell exhaustion in chronic hepatitis B virus infection. *PLoS One*. 2012;7(10):e47648.
 19. Raziorrouh B, Schraut W, Gerlach T, *et al*. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. *Hepatology*. 2010;52(6):1934-1947.
 20. Protzer U, Maini MK, Knolle PA. Living in the liver: hepatic infections. *Nat Rev Immunol*. 2012;12(3):201-213.
 21. Zong L, Peng H, Sun C, *et al*. Breakdown of adaptive immunotolerance induces hepatocellular carcinoma in HBsAg-tg mice. *Nat Commun*. 2019;10:221.