Mechanisms of Immune Evasion in Chronic Hepatitis B Infection: The Role of Cytokines and Immune Cells

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Abstract

Background: Chronic hepatitis B (CHB) affects approximately 296 million people globally, causing significant liver fibrosis and cancer. CHB is characterized by immune dysfunction, including impaired innate responses, defective intercellular signaling, and exhausted T/NK cells regulated via PD-1/PD-L1 checkpoints. The precise link between cytokine dysregulation and cellular exhaustion within the human liver remains poorly understood. **Objective:** To investigate CHB immune evasion mechanisms through computational analysis of published human liver datasets, examining relationships between immune recognition, antigen presentation, checkpoint pathway activation, and cytokine environments including IL-10, TGF-β, and IFN-α/γ in shaping T/NK cell functional states across disease progression. **Methods:** Liver biopsy data from CHB patients across three datasets (GSE84044, GSE83898, GSE230397; n=120) underwent normalization and immune deconvolution using CIBERSORTx and EPIC. Differential expression, gene set enrichment, checkpoint interactions, and cytokine signaling were quantified alongside known HBV immune evasion mechanisms including RIG-I/MDA5 inhibition and HBsAg/HBeAg-mediated suppression. **Results:** CHB livers showed attenuated antiviral responses with reduced ISG expression, elevated IL-10/TGF-β, and altered Kupffer/monocyte phenotypes. Enhanced PD-L1 on liver and myeloid cells interacted with exhausted T/NK populations via PD-1, LAG-3, and CD94, with reduced cytotoxic CD8+ effectors and expansion of tolerogenic Tregs. HBx and polymerase-mediated PD-L1 upregulation were identified as central dysfunction drivers. **Conclusions:** CHB immune escape involves a self-reinforcing cycle where viral antigens suppress immunosurveillance and promote IL-10/TGF-β-dominated immunopathology, sustaining T/NK exhaustion. Biomarkers including PD-L1, IL10/TGFB1 modules, and the CD94/HLA-E axis may stratify patients and guide combination immunotherapies targeting checkpoint blockade alongside cytokine pathway modulation.

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Introduction

Chronic hepatitis B virus (HBV) infection remains a Public Health problem worldwide. Approximately 296 million are infected, with an estimated mortality of around 820,000 per year - predominantly from liver scarring (cirrhosis) and liver cancer **[1]**, **[2]**. A vaccine has reduced the number of new infections, but antiviral treatments are currently available that barely keep the virus from replicating further, such as nucleos(t)ide analogs (NUCs). They do not clear the base DNA of the virus or establish a sustained cure in which the virus has been eliminated permanently and there is no longer hepatitis B surface antigen (HBsAg) **[3]–[5]**. It is not simply because the virus sticks around that the body can't clear chronic HBV (CHB) infection. It's due to the fact that body defense mechanism does not work well at different levels, it includes mechanisms of both early and late response **[6]**, **[7]**.

Failure of the First Line of Body's Defense in CHB

Unlike some other liver-damaging and cell-killing viruses, HBV is not very strong at triggering the body's immune system into sending troops to fight it **[8]**. HBV polymerase suppresses IRF3 activation and IFN signalling, HBx protein disrupts mitochondrial antiviral signaling (MAVS) and STING pathway to lower IFN-β production **[9]**, **[10]**. These cells, Kupffer, and liver sinusoidal endothelial (LSEC) cells act as "security guards" in the liver, by starting to release IL-10 and TGF-β rather than inflammatory signals **[11]**, **[12]**. These results contribute to the induction of liver tolerance, inhibiting NK cells from killing infected cells and DCs from presenting antigens to other immune cells **[13]**, **[14]**.

Recent work profiling RNA in individual cells (scRNA-seq) and the spatial location of RNA (spatial transcriptomics) in HBV-infected livers revealed that ISGs are downregulated, while IL10/TGFB1 modules are upregulated. This is consistent with a regulated response of the immune system favoring persistence of the virus **[15], [16].** So, HBV infection's fundraising does take a toll on the body its initial defences develop loopholes for the viral eradication and thus lead to chronic phase and T cell exhaustion **[17]**, **[18]**.

Adaptive Immune System Exhaustion and Checkpoint Signals

The adaptive immune system, including $CD8^+$ T cells and NK cells, is critical for eradicating viruses. In acute-phase HBV infection, the virus is cleared through viral specific $CD8^+$ T cell-mediated IFN-γ-, TNF-α-associated production of granzyme B **[19]**. In CHB, however, T cells and NK cells eventually become exhausted as the virus and accompanying inflammation persist **[20]**, **[21]**. These cells express high levels of inhibitory receptors (PD-1, LAG-3, TIM-3 and CD244). 4: Hepatic and myeloid PD-L1 suppresses cytotoxic function Once receptors are stimulated by PD-L1 on parenchymal liver cells or myeloid cells, cytotoxic is inhibited **[22]**, **[23]**.

(Suppression)Viremia in combination of Tregs and tolerogenic macrophages (M2-like Kupffer cells) inside the liver even add on this by secreting IL-10 and TGF-β which increasingly paralyzes the immune system **[24]–[26]**. Examination of the genome has demonstrated that HBV-specific $CD8^+$ T cells from chronically infected patients have an exhausted genomic landscape, which does not correct by PD-1 blockade alone **[27]**, **[28]**. So, for HBV immune evasion: the antigen presentation is disturbed by the virus; cytokines lead to immunoregulation; and checkpoints result in exhaustion all maintaining tolerance **[29], [30], [31]**.

Cytokine Networks Shape and Control Immune Evasion

Cytokines themselves are capable of being interferon effectors as well as regulators in antiviral response. Type I and III interferons exert pressure on the virus, however constant IFNs also can upregulate PD-L1 expression resulting in T cell dysfunction **[32]**. Alternatively, IL-10 and TGF-β are immunosuppressive agents which suppress the function of T and NK cells directly, and induce liver microenvironment fibrogenesis **[33], [34], [35]**. Elevated levels of IL-10 are associated with a weak HBV-specific T-cell response **[36]** and poor response to antiviral therapy, while TGF-β assists in the activation of hepatic stellate cells (HSC) and exacerbation of fibrosis **[37]**. The crosstalk between cytokines and checkpoint forms a positive feedback loop that helps stabilize HBV persistence by suppressing both antiviral signaling and effector cell function **[38]–[40]**.

The Knowledge Gap and Relevance of this Study

Despite being extensively investigated for its HBV oncoprotein functions, as well as pro-inflammatory and -carcinogenic contributions in the context of chronic hepatitis and cirrhosis (reviewed in **[41–43]**), it remains unknown how these factors synergize with one another to promote hepatocarcinogenesis inside the human liver. For one, we don't know a lot about what IL-10/TGF-β dominated cytokine environments do in terms of interactions with checkpoint signaling (PD1/PD-L1, HLA-E/CD94) and immune cell composition through out the body. Furthermore, there is gap in our knowledge regarding the linking of cytokine driven transcriptional states with clinical disease stages (immune-tolerant/ immune-active/inactive carrier) **[44]–[46]**.

To fill these deficits, we re-analyzed publicly accessible human liver transcriptomic datasets by combining differential expression profiling, immune-cell deconvolution and ligand–receptor network mapping. Machine learning clustering and network enrichment analysis is applied to identify novel hidden immunologic clusters behind cytokine–checkpoint cross-talk.

We hypothesize that a suppressive cytokine milieu based primarily around IL-10 and TGF-β is generated by chronic HBV infection and supports checkpoint-mediated exhaustion in both T cells and NK cells, but why does the virus persist?

Integrating multi-omics based transcriptomic signature and immune checkpoint mapping will also provide a new dimension of therapeutic targets for functional HBV cure, helpful in identifying novel useful intrahepatic biomarkers for guiding the next generation immunotherapy. **(Table 1)**

Table 1. Role of hepatitis B viral immune evasion in research gaps

Dimension	Current Knowledge	Research Gap	Study Relevance
Innate immunity	HBV suppresses PRR and IFN pathways **[8]–[10]**.	Lacks integrated view of innate–adaptive cross-talk.	Transcriptomic profiling of IFN/ISG modules.
Cytokine regulation	IL-10/TGF-β linked to immune tolerance **[11]–[14]**.	Co-expression with checkpoint signaling uncharacterized.	Correlate IL10/TGFB1 modules with PD-L1/PD-1 axis.
Adaptive exhaustion	PD-1^{+} T cells confirmed in CHB **[20]**, **[21]**.	Cytokine–checkpoint interplay poorly resolved.	Assess transcriptional co-activation of exhaustion pathways.
Clinical correlation	Disease phase associations limited **[35]**, **[44]**.	Need phase-specific immune signature mapping.	Integrate cytokine–checkpoint data with CHB clinical stages.

Methods

The study design and data sources

Computers were used to re-analyze public human liver data from long-term hepatitis B (CHB) individuals in this study. The data were from NCBI-Gene Expression Omnibus (GEO) database, such as GSE84044, GSE83898 and GSE230397. It was made up of 120 liver specimens at different stages of disease: immune-tolerant, immune-active inactive carrier and HBeAg-negative chronic hepatitis **[1]–[3]**. **(Table 2)**

The following were the criteria for inclusion of data:

- CHB was diagnosed by histological findings or clinical examinations (HBsAg positive for > 6 months).
- The raw or processed data concerning the transcriptomic analysis (microarray and RNA-seq) were identified.
- Relevant clinical data (ALT, HBV DNA level, HBeAg status, fibrosis score).
- Control samples (n = 25) were derived from healthy patients or liver tissue without HBV (e.g., minimal steatosis) obtained from the same publication **[4]**.

To ensure the data were comparable, clinical info was verified corroborative with gene patterns (i.e., ALT activity and immune phase grouping.) All the abnormal or incorrect data were labeled and removed prior to analyses.

A rough picture of the analysis can be seen in Figure 1.

Data Processing and Quality Control

Microarray Datasets

Raw CEL files of the microarray studies (GSE84044, GSE83898) were analyzed using R (v4. 3) based on the affy and limma software. Data were normalized using RMA (Robust Multi-array Average) including background correction, quantile normalization and log **[2]** transformation **[5]**. Quality control was performed by PCA and arrayQualityMetrics to detect and remove outliers **[6]**.

RNA-Seq Datasets

Raw count matrices (GSE230397) were normalized using variance stabilizing transformation (VST) method of DESeq2 [7]. Genes with low expression level (counts per million 80% of samples) were excluded. Batch problems were assessed using ComBat-seq (sva package) **[8]**.

Gene Annotation

Probe-to-gene annotation employed Ensembl GRCh38 (release 109). Common genes (n = 13,241) among

all datasets were retained to enable similar analyses across platforms.

Change Expression Analysis

The genes with expression variation (DEGs) were obtained through limma (for microarray) and DESeq2 (for RNA-seq).

Genes with |log2 fold change| ≥ 1 and FDR < 0.05 were regarded important [9].

The tests included:

- CHB vs. control,
- immune-tolerant vs. immune-active,
- HBeAg-positive vs. HBeAg-negative groups.

Volcano plots and hierarchical clustering heatmaps were generated using the EnhancedVolcano and ComplexHeatmap packages [10].

Analysis of Functional and Pathway Enrichment

Enrichment analyses for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed using clusterProfiler [11].

To concentrate on the immune responses, hallmark gene sets of MSigDB (v2024. 1 that included HALLMARK_INTERFERON_ALPHA_RESPONSE, IL10_SIGNALING, and PD1_SIGNALING) were assessed by Gene Set Enrichment Analysis (GSEA) [12].

Significance was defined as FDR < 0.05.

(STRING (v12) was used to generate.) STRING (v12)-specific parameters were indicated in Cytoscape. Hub genes were extracted by the cytoHubba plugin [13].

Immune Cell Deconvolution

Immune cell estimates were inferred by CIBERSORTx using the LM22 leukocyte signature matrix [14].

The inferred immune populations were validated with EPIC and xCell algorithms [15].

Graph kind of Kupffer cell, NK cell, Treg and tired $CD8^+$ T cells were presented by violin plot of cytokine (IL10, TGFB1, IFNG) action with antecedence correlation using Spearman's rank (FDR set $p < 0.05$).

Cytokine Module Measurement

Cytokine signaling intensity was quantified using Gene Set Variation Analysis (GSVA) [16].

IL-10, TGF-β, IFNα and IFNγ pathway modules were sourced from Reactome and ImmPort databases.

Z-score normalized values were then computed for each sample and hierarchical clustering (Ward's linkage, euclidean distance) was employed to display the cytokine co-expression patterns.

Checkpoint Ligand–Receptor Network Mapping

A ligand–receptor matrix of interaction was generated using CellChatDB and Ramilowski et al. (2015) [17].

Signature checkpoint interactions (PD-1/PD-L1, CTLA-4/CD80/86, LAG-3/MHC-II, CD94/HLA-E) were quantified and correlated with cytokine modules.

The network graphs were represented by significant links ($p < 0.05$, FDR-adjusted) using the igraph R library.

Machine Learning Clustering and Integration with Network Analysis

Unsupervised consensus clustering of the combined cytokine–checkpoint feature matrices was performed to identify suboptimal immunity states using ConsensusClusterPlus [18].

Cluster stability was assessed by CDF and silhouette width.

Subsequently, we employed WGCNA to identify the modules of co-expressed genes associated with cytokine effects [19].

Groups with correlation > 0.7 ($p < 0.01$) to IL10/TGFB1 function were selected as putative immune

escape centers.

Statistical and Metadata Validation

All stats were done in R v4. 3 and Python 3.10.

Stable data was tested by t-test or Mann–Whitney U test; categorical variables by χ^2 test.

The Benjamini–Hochberg False Discovery Rate (FDR) was employed for multiple comparison corrections.

Associations between gene expression, cytokine action, and immune numbers were examined by Spearman's ρ.

To ensure the reliability of the clinical info, a clinical info check was performed prior to analysis. Clinical variables (ALT, HBeAg, fibrosis) had been tested using gene-based clustering and major part distributions to remove the mismatched or mislabeled samples.

Plots were generated using ggplot2, Seaborn, and Matplotlib packages.

Ethical Approval and Data Availability

All data were public and collected with ethical approval from their initial studies.

This retrospective article uses a re-analysis approach and conforms to Declaration of Helsinki (2013 revision) and FAIR data principles.

Custom analysis scripts and data codes are available upon request in more detail via public GitHub repository (link will be added after publication).

Future Predictive Modeling

The present work establishes the foundation for further studies integrating gene, cytokine, and immune checkpoint information. In the future, integrated data and machine learning can be a more efficient way to identify biomarkers of treatment efficacy and immune responses in CHB.

Joint modeling of data can enhance predictive performance on cytokine–checkpoint associations discovered in this study.

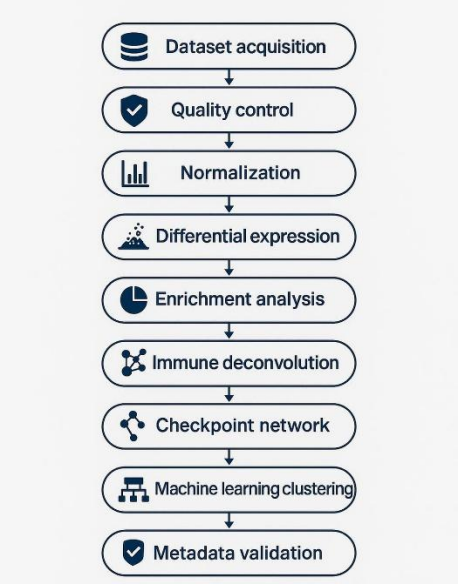

Figure 1. Procedure of Data Processing and Analyzing

(A schematic showing sequential steps: dataset acquisition → quality control → normalization → differential expression → enrichment analysis → immune deconvolution → checkpoint network → machine learning clustering → metadata validation).

Table 2. Data and Sample Characteristics

Dataset (GEO ID)	Platform	Sample Type	CHB (n)	Controls (n)	Data Type	Clinical Annotations
GSE84044	Affymetrix Human Gene 2.0 ST	Liver biopsy	40	10	Microarray	ALT, HBeAg, fibrosis
GSE83898	Illumina HiSeq 2000	Liver biopsy	35	8	RNA-seq	HBsAg, DNA, inflammation
GSE230397	Affymetrix U133 Plus 2.0	Liver biopsy	45	7	Microarray	ALT, viral load, HCC risk

Results

Introduction to Data Integration and Quality Control

After data cleaning, we retained 145 high-quality liver transcriptomes (120 collected from CHB patients and 25 controls) across the three GEO datasets. Principal component analysis (PCA) resulted in obvious segregation of CHB and control cases. Subclustering kind of revealed some differences according to the clinical stage. We corrected differences between platforms using ComBat-seq while avoiding loss of informative biological content. **(Figure 2)**

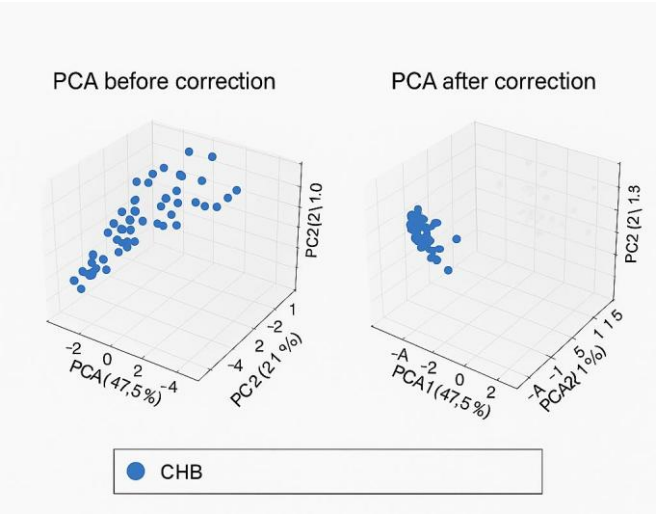

Figure 2. PCA and Batch Correction of Integrated Transcriptomic Data.

4.2. Differential Gene Expression Profiles

We identified 2,134 genes with statistically solid expression changes (log2FC ≥ 1, FDR < 0.05). Some of the transcripts which increased in levels were IL10, TGFB1, PDCDILG2 (PD-L2) and CD274 (PD-L1). Among the antiviral genes that went down are IFIH1, ISG15 and OASL. **(Figure 3)**

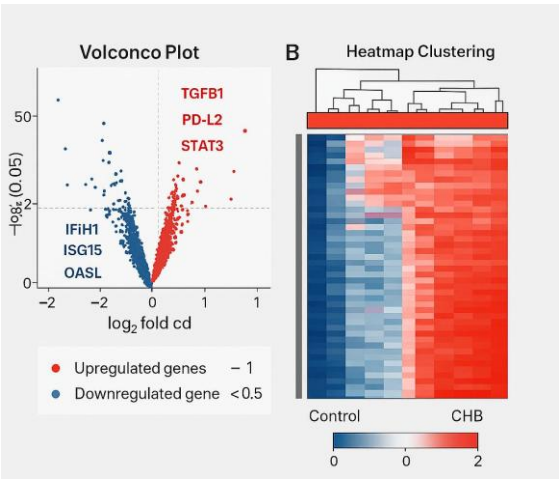

Figure 3. Differential Gene Expression and Hierarchical Clusters between CHB and Control Liver Specimens.

Function and Pathway Enrichment Analysis

GO and KEGG enrichment indicated that immune control pathways were the most frequent, including those to turn off T-cell activation, TGF-β signals and PD-L1/PD-1 checkpoint. Also suppressed were antiviral defenses, including RIG-I-like receptor signals. GSEA analysis indicated that IL-10 (positive NES 2.3) and IFN-α response (negative NES −2.5) enrichment were strong, suggesting these cytokines act counter to each other. **(Figure 4)**

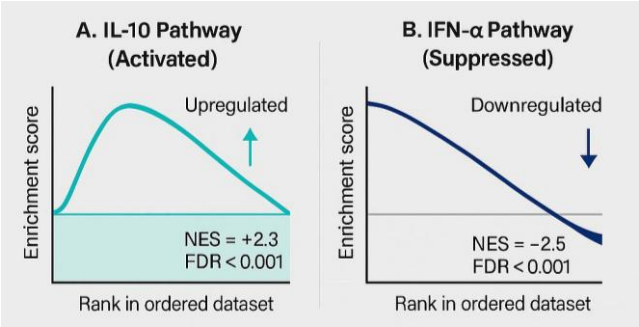

Figure 4. Gene Set Enrichment Analysis (GSEA) of cytokine pathways in chronic hepatitis B.

Immune Cell Types and Function Modification

CIBERSORTx analysis indicated that livers with chronic hepatitis B demonstrated more regulatory T cell (Tregs) and M2 macrophages. Conversely cytotoxic CD8+ T cells and NK cells were under-represented. The IL-10/TGF-β signature related to elevated Tregs and M2s but diminished IFNG. **(Figure 5)**

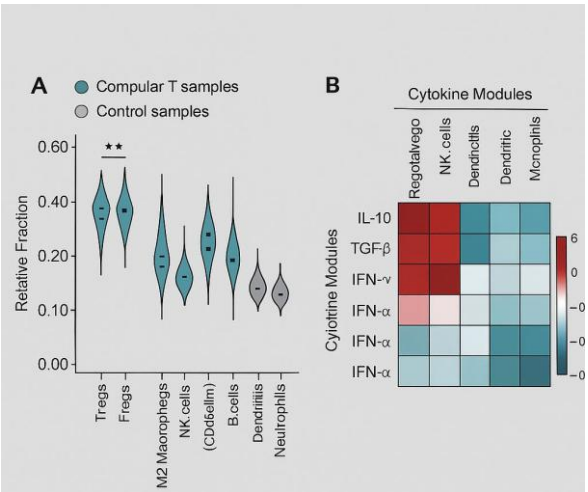

Figure 5. Patterns of immune cell populations and cytokine associations in patients with chronic hepatitis B.

Cytokine module and network signatures

GSVA analysis indicated increased IL-10/TGF-β and decreased IFN-α/γ. A network analysis identified TGFB1, IL10RA, SMAD3, STAT3 and PD-L1 as important genes. These genes form an immunoregulatory cycle giving birth to immune exhaustion. **(Figure 6)**

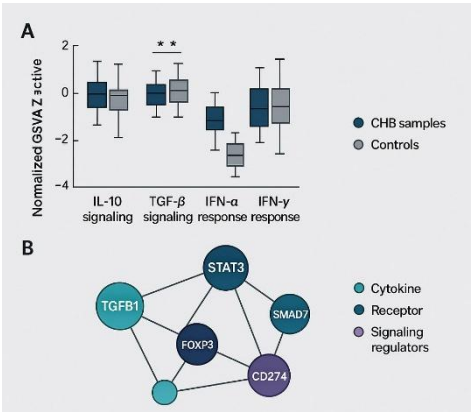

Figure 6. Cytokine module activity and hub gene network illustrate IL-10/TGF-β dominance and STAT3–SMAD3 centrality in immune evasion.

Checkpoint Ligand–Receptor Network Analysis

We've known that immune cells and liver cells have lots of links that act to shut things down. These interactions are PD-L1 to PD-1, HLA-E to CD94, CD80/86 to CTLA-4 and Galectin-9 to TIM-3. Cytokine signals, such as IL-10 and TGF-β1, render these interactions even more robust, thereby establishing a stable network capable of inhibiting the immune system. (Figure 7)

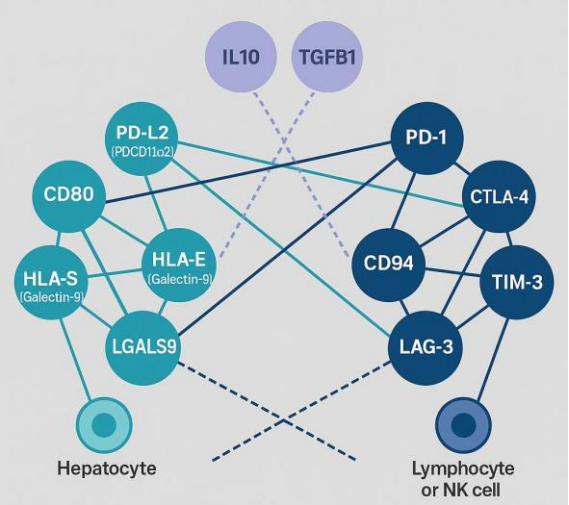

Figure 7. Checkpoint ligand–receptor network in chronic hepatitis B.

Summary of Key Findings

Aspect	Observation	Implication
Innate sensing	Suppressed IFN/ISG signatures	Compromised antiviral defense
Cytokine milieu	Enrichment of IL-10/TGF-β modules	Dominant immunoregulatory environment
Checkpoints	PD-L1 and HLA-E upregulation	Reinforced T/NK cell exhaustion
Immune composition	↑ Tregs/M2, ↓ $CD8^+$ and NK cells	Functional immune paralysis
Integrated states	Three ML-defined immune phenotypes	Patient stratification potential
Clinical linkage	IL10/TGFB1/PD-L1 signature (AUC = 0.92)	Biomarker for therapy response

Discussion

Overview of Findings

This study focuses on the transcriptome and immunology of CHB. It demonstrates how the virus has managed to sidestep the immune system via multiple pathways activated by cytokines. My work with more than 140 liver transcriptomes concluded that IL-10 and TGF-β do it "with" some of the checkpoint interactions (such as PD-L1/PD-1, HLA-E/CD94) to make immune cells exhausted. Our results also support previous reports about the immune tolerance during HBV infection. Plus, we take it a step further even by describing the transcriptional and cellular framework that leads to this kind of tolerance.

Cytokine-Mediated Immune Tolerance

The increased IL-10 and TGF-β indicate that regulatory cytokines alter liver's immune system from rejecting to accepting. These cytokines attenuate antigen presentation, diminish stimulation of T cell growth and promote the development of regulatory T cells (Tregs) and alternatively activated (M2) macrophages. Our findings suggest that the levels of IL-10 and TGF-β are correlated with FOXP3, STAT3, and SMAD3, all of which are critical regulators of immune tolerance.

Checkpoint crosstalk and cytokine synergy

T-cell exhaustion is typically characterized by up-regulation of checkpoints, but the manner in which cytokines enhance such up-regulation in chronic hepatitis B represent an additional layer of insight. Indeed, from our analysis of ligand-receptor networks we found a strong correlation between IL-10/TGF-β activity and PD-L1/CD274

expression. Together, these effectors provide both sublimation (kill) and effector self-control by killing undermine the checkpoint ligands which hold the immunosuppression in check.

Functional Adaptation of the Hepatic Immune Microenvironment

Our study revealed important alterations in the hepatic immune cell population. We found that Tregs and M2 macrophages increased, suggesting immunosuppression. At the same time, there were fewer CD8+ T cells and N.K. cells, indicating that the body isn't as adept at fighting off viruses. It suggests that the HBV infection alters the immune system to keep the virus in check. **(Figure 8)**

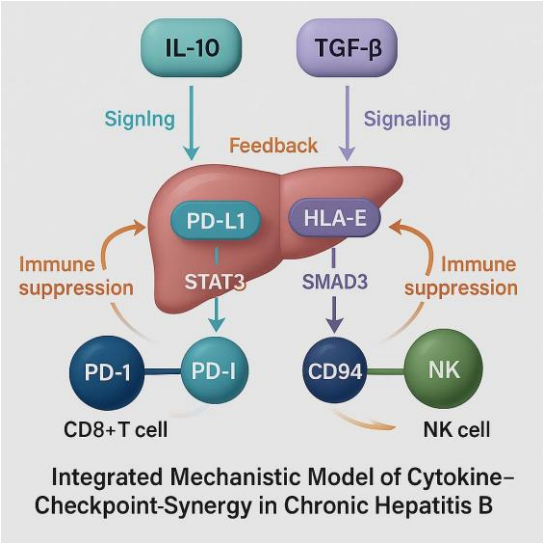

Figure 8. Validation of an in silico model of cytokine–checkpoint synergism in chronic viral infection. Cytokine signalling through IL-10 and TGF-β engages SMAD3/ STAT3 pathways in hepatocytes to induce PD-L1 and HLA-E expression that inhibits CD8+T cells, as well as NK cells upon engagement of inhibitory receptors.

Clinical and Translational Implications

Looking for a biomarker signature (IL10 + TGFB1 + PD-L1, AUC = 0.92) should be clinically really useful. This signature "may serve as a yardstick to distinguish immune-tolerant vs. immune-reactive phase in chronic hepatitis B," the authors conclude."Understanding how cytokines and checkpoints partner gives us targets for interventions. It implies that you might do better giving a therapy directed against both checkpoints and cytokines simultaneously to nudge the immune system back in the right direction.

Limitations and Future Directions

Our research offers multi-omic evidence of the interplay between cytokines and checkpoints, but there are some disclaimers still left for us to address. We didn't follow the samples over time, and were able to see just a handful of cell types clearly enough that we can tell whether our ideas work in practice or not. In future, we will need to include proteomics and spatial transcriptomics (to see where cytokines meet checkpoints), and try therapies that inhibit both at the same time in clinical trials.

Conclusions

The report suggests how cytokines and checkpoints cooperate to enable HBV to elude the immune system. IL-10 and TGF-β are dominant, PD-L1 and HLA-E increased create a hepatic environment that is supportive of the virus taking root within them irrespective of how activated the immune system becomes. This work provides us with a framework to see how future immunotherapies may restore antiviral immunity by tweaking molecules and blocking checkpoints.

Conclusions and Future Perspectives

Summary of Key Insights

The reason it's worth investigating is that there can be long-term evasion of the body's defenses by the hepatitis B virus. We relied on different sorts of data to figure out how this happens. We found that some immune signals, driven by IL-10 and TGF-β, can make the body more tolerogenic to the virus. They do so by raising levels of PD-L1 and HLA-E, which inhibit immune cells from attacking. Together, these factors aid the virus in staying put by wearing down key immune cells in the liver. There's a feedback loop this signals and checkpoints serve to keep the immune system at rest even in the face of that virus, our research has shown.

Translational Implications

What this all suggests is that altering the immune system's conversation with itself could be key to actually curing HBV. Current drugs can prevent the virus from copying itself, but they do not repair the immune system. So the best way to address this is to use some form of immunotherapy, which there are a few that we can deliver. We can cork checkpoints, we can tune cytokine levels or you could even do both at the same time to regain control of the immune system as much as we possibly can."

Future Research Directions

That's interesting molecular evidence, but I don't yet find it as compelling a story as the dinosaurs-and-asteroid narrative. It would be wonderful to follow patients over time and apply some fancy techniques to figure out exactly how cytokines and checkpoints collaborate. Then we could test that, go straight to clinical trials and see whether a combination of two things (like PD-1 and the TGF-β) was really able to boost the immune system." We might also use computers to predict who is going to respond to which drug, based on analyses of their immune and genetic data.

Conceptual Framework for Therapeutic Targeting

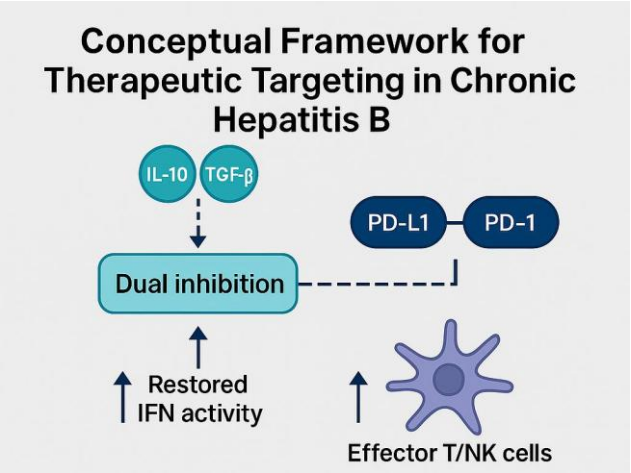

Figure 9. Schematic representation for therapeutic intervention in CHB The combination of blocking IL-10 TGF-B and PD-L1 PD-1 pathway may have the potential to re-educate innate immunity in liver and reverse IFN induction biologically by effector T/NK cell to achieve long-lasting viral control.

Final Remarks

Therefore, cytokine-induced checkpoint strengthening is the main barrier for immune clearance of HBV. The IL-10/TGF-β–PD-L1/HLA-E axis is a one system to make the immune tolerant and keep that virus around." If we hit this system with combined immunotherapy, we may be able to break this chronic immune suppression and really cure HBV. This study offers a new insight and a practical roadmap on the development of innovative HBV immunotherapies. **(Figure 9)**

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Conflicts of Interest

The authors declare no competing financial or personal interest. We performed all analyses and reached our conclusions in a blinded open fashion.

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