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Hepatitis B Virus—Specific and Global T-Cell Dysfunction in Chronic Hepatitis B

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BACKGROUND & AIMS: T cells play a critical role in viral infection. We examined whether T-cell effector and regulatory responses can define clinical stages of chronic hepatitis B (CHB). **METHODS:** We enrolled 200 adults with CHB who participated in the National Institutes of Health–supported Hepatitis B Research Network from 2011 through 2013 and 20 uninfected individuals (controls). Peripheral blood lymphocytes from these subjects were analyzed for T-cell responses (proliferation and production of interferon gamma and interleukin 10) to overlapping hepatitis B virus (HBV) peptides (preS, S, preC, core, and reverse transcriptase), influenza matrix peptides, and lipopolysaccharide. T-cell expression of regulatory markers FOXP3, programmed death-1, and cytotoxic T lymphocyte-associated antigen-4 was examined by flow cytometry. Immune measures were compared with clinical parameters, including physician-defined immune-active, immune-tolerant, or inactive CHB phenotypes, in a blinded fashion. **RESULTS:** Compared with controls, patients with CHB had weak T-cell proliferative, interferon gamma, and interleukin 10 responses to HBV, with increased frequency of circulating $FOXP3^{+}CD127^{-}$ regulatory T cells and $CD4^{+}$ T-cell expression of programmed death-1 and cytotoxic T lymphocyte-associated antigen-4. T-cell measures did not clearly distinguish between clinical CHB phenotypes, although the HBV core-specific T-cell response was weaker in hepatitis B e antigen $(HBeAg)^{+}$ than $HBeAg^{-}$ patients (percent responders: 3% vs 23%; $P = .00008$). Although in vitro blockade of programmed death-1 or cytotoxic T lymphocyte-associated antigen-4 increased T-cell responses to HBV, the effect was weaker in $HBeAg^{+}$ than $HBeAg^{-}$ patients. Furthermore, T-cell responses to influenza and lipopolysaccharide were weaker in CHB patients than controls. **CONCLUSIONS:** HBV persists with virus-specific and global T-cell dysfunction mediated by multiple regulatory mechanisms, including circulating HBeAg, but without distinct T-cell–based immune signatures for clinical phenotypes. These findings suggest additional T-cell–independent or regulatory mechanisms of CHB pathogenesis that warrant further investigation.

Keywords: HBRN; LPS; IFN; IL10.

Hepatitis B virus (HBV) is largely non-cytopathic, with host immune response playing a key role in liver injury and virus control.[1,2] As such, clinical stages of chronic hepatitis B (CHB) are generally classified as immune active, immune tolerant, or clinically inactive by serum alanine aminotransferase (ALT) activity and HBV DNA levels.[3] This nomenclature is based on the concept that these clinical measures reflect varying levels of host immune activation in response to HBV that mediate both liver injury and virus control.[4] For example, patients with immune-active CHB display elevated ALT activity and active hepatic necroinflammation. By contrast, immune-tolerant (IT) patients "tolerate" high levels of HBV viremia without ALT elevation and respond less well to interferon (IFN)-based immune modulatory therapy than immune-active patients.[5] These immunologically conceptualized but clinically defined CHB phenotypes have been a cornerstone for clinical management of patients with CHB.[6,7] However, the underlying mechanisms or immune correlates for clinical CHB phenotypes are not well defined.

Abbreviations used in this paper: **ALT, alanine aminotransferase; CHB, chronic hepatitis B; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; HBeAg, hepatitis B e antigen; HBRN, Hepatitis B Research Network; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IA^{+}, $HBeAg^{+}$ immune active; IA^{-}, $HBeAg^{-}$ immune active; IFN, interferon; IL, interleukin; IT, immune tolerant; LPR, lymphoproliferation; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; PD-1, programmed death-1; PHA, phytohemagglutinin; RT, reverse transcriptase; SFU, spot-forming unit; SI, stimulation index.**

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HBV is believed to be a "stealth" virus that is not readily sensed by the innate immune defense.[8] By contrast, a critical role for T cells was shown in animal models of HBV infection and/or replication.[2,9] Successful HBV clearance in patients is associated with robust and broad HBV-specific proliferative and IFN gamma$^+$ effector T-cell responses compared with weak, dysfunctional responses in CHB.[10] Multiple inhibitory pathways have been implicated for HBV-specific T-cell dysfunction in CHB, including extrinsic regulation through regulatory T cells, cytokines, and serum factors; intrinsic regulation through co-inhibitory molecules, such as programmed death-1 (PD-1) or cytotoxic T lymphocyte-associated antigen-4 (CTLA-4); and deletion of virus-specific T-cells.[11–20] Suppressive CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells were induced in patients with CHB in direct correlation with disease progression in some[21,22] but not all studies.[23,24] Furthermore, HBV-specific T-cell dysfunction in CHB was associated with increased T-cell expression of PD-1.[18–20] Importantly, antibody-mediated blockade of these inhibitory receptors restored HBV-specific effector T-cell function in vitro, raising hope for potential therapeutic application.[18–20]

In this study, we hypothesized that clinical phases of CHB represent the balance between immune effector and regulatory factors that impact HBV-specific T cells. We looked for virus-specific effector T-cell function (proliferation, IFN gamma) relative to regulatory parameters, such as virus-specific IL10 response, FoxP3$^+$ regulatory T-cell frequency, and T-cell expression of PD-1 and CTLA-4 in peripheral blood of CHB participants enrolled in the National Institutes of Health–funded Hepatitis B Research Network (HBRN) Immunology Study. We also examined whether circulating hepatitis B e antigen (HBeAg) impacted antigen-specific T-cell tolerance and responses to immune inhibitory blockade in vitro.

Methods

Patient Recruitment

Between January 2011 and December 2013, two hundred of 1763 participants with CHB enrolled in the National Institutes of Health–funded HBRN Adult Cohort Study were recruited into the ancillary HBRN Immunology Cohort Study (Immunology Cohort Study) from 8 participating clinical centers in Toronto, Dallas, San Francisco, Richmond, Seattle, Minnesota, Boston, and Chapel Hill (see Supplementary Material for individual institutions).

The Adult Cohort Study protocol has been described elsewhere.[25] In brief, the Adult Cohort Study enrolled hepatitis B surface antigen (HBsAg)–positive patients that were 18 years or older without evidence for hepatic decompensation, hepatocellular carcinoma, liver transplant, or human immunodeficiency virus infection, and were not receiving antiviral therapy. The inclusion criteria for the Immunology Cohort Study were enrollment in the Adult Cohort Study; informed consent for the Immunology Cohort Study; absence of active conditions that preclude large-volume research blood draws; and absence of active autoimmune disease, medications, or comorbid illnesses that can impact immune response. Twenty HBV-uninfected healthy control subjects, including 17 prior HBV vaccinees (vaccinated 0–33 years from enrollment) were recruited from 2 HBRN clinical centers (Toronto, Dallas) and the HBRN Immunology Center in Philadelphia, with no known liver disease, conditions that preclude large-volume research blood draws, active autoimmune disease, or immunosuppression. All subjects were screened for HBsAg, antibody to hepatitis B core antigen, antibody to HBsAg, antibody to hepatitis C virus, antibody to human immunodeficiency virus, and liver enzymes. Serum HBV DNA was quantified by real-time polymerase chain reaction assays at each clinical center.

Participation in the Immunology Cohort Study involved 50-mL blood draws at weeks 12 and 24 (or week 48 in case of missed blood draw) in the first year of enrollment into the Adult Cohort Study. Participants with ALT flares (ALT 10 times the upper limit of normal) underwent 50-mL blood draws within 1–2 weeks of flare, at 4 weeks from the ALT flare and after flare resolution. Blood samples were collected in EDTA-coated tubes, shipped overnight in ambient air to the Immunology Center, and processed within 24 hours.

Chronic Hepatitis B Phenotype Groups

Baseline CHB phenotype was assigned by investigators from each clinical center based on available history and baseline laboratory results at the time of Immunology Cohort Study enrollment, using phenotype characteristics described in Supplementary Table 1. The 200 HBRN participants included 21 (11%) IT, 60 (30%) HBeAg$^+$ immune active (IA$^+$), 67 (34%) HBeAg$^-$ immune active (IA$^-$), 48 (24%) inactive carriers, and 4 (2%) subjects with "indeterminate" phenotype (Supplementary Table 2). Cross-sectional immune comparisons were made with the first available immune assay results. All assays were performed with the investigators at the HBRN Immunology Center blinded to the knowledge of CHB phenotype. Clinical and laboratory results were available only to the statistician at the Data Coordinating Center.

Viral Peptides and Controls

Four sets of 155 genotype-specific overlapping 15-mer peptides (genotypes A–D) were synthesized by Mimotopes (Victoria, Australia). Peptide sequences were determined by aligning 2 or more published HBV S, Core, or polymerase sequences from each HBV genotype (see Supplementary Material and Supplementary Table 3 for published HBV sequences used and strategy for peptide pools). T-cell response to influenza virus was measured using 41 overlapping 15-mers spanning the matrix M1 protein (residues 1–252) based on A/PR/8/34 (H1N1) virus.[26] Additional controls included lipopolysaccharide (LPS) and phytohemagglutinin (PHA) (Sigma Aldrich, St Louis, MO).

Antibodies

Fluorescent and blocking antibodies are listed in the Supplementary Material.

Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Histopaque (Sigma Chemical Co, St Louis, MO)[27,28] and used directly or cryopreserved.