Altered Functions of Plasmacytoid Dendritic Cells and Reduced Cytolytic Activity of Natural Killer Cells in Patients With Chronic HBV Infection

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BACKGROUND & AIMS: Hepatitis B virus (HBV) modulates the immune system to escape clearance. Plasmacytoid dendritic cells (pDCs) initiate antiviral immunity and might determine outcomes of HBV infections. Functional defects in pDCs and natural killer (NK) cells have been reported in patients with chronic HBV infection. However, the mechanisms of these immune dysfunctions and the interactions between pDCs and NK cells have not been determined. We investigated features of pDCs from patients with chronic HBV infection and their interactions with NK cells. **METHODS:** We used flow cytometry and cytokine assays to analyze pDCs from patients with chronic HBV infection (118 aviremic and 67 viremic) and compared them with pDCs from uninfected individuals (controls). We performed coculture assays to analyze the ability of pDCs to activate heterologous NK cells. **RESULTS:** Circulating and hepatic pDCs from patients with chronic HBV infection had higher levels of activation than pDCs from controls and defective responses to stimulation with Toll-like receptor 9 ligand (TLR9-L), regardless of the patient's viral load. TLR9-L–activated pDCs from viremic patients with HBV did not induce cytolytic activity of NK cells. This altered function of pDCs was associated with reduced expression of OX40L and could be reproduced by incubating control pDCs with plasma from viremic patients with HBV. A high level of interferon-induced protein 10 (IP-10 or CXCL10) and hepatitis B surface and e antigens might induce these defective pDC functions. **CONCLUSIONS: HBV escapes antiviral immunity by altering pDC functions, to disrupt interactions between pDC and NK cells. This could reduce immune control of HBV and lead to chronic infection.**

Keywords: Immune Regulation; HBeAg; HBsAg; Virology.

BASIC AND TRANSLATIONAL LIVER

Approximately 350 million people worldwide are infected with hepatitis B virus (HBV), and control and eradication of HBV are considered major public health challenges. The pathophysiology of HBV infection is closely related to host immunity.[1–3] Patients who ultimately clear the virus elicit vigorous and efficient antiviral immunity involving multi-specific T-cell responses, a neutralizing humoral response, and efficient natural killer (NK) cells. In contrast, patients who go on to develop chronic infection only mount weak and dysfunctional immune responses. Immune control of chronic HBV infection appears to be essential for viral clearance and therapeutic success. The pathogenic mechanisms used by HBV to modulate the immune system remain largely unknown. To move closer to successful treatment of this disease, the mechanisms underlying impaired viral control and immunopathogenesis of chronic HBV infection must first be characterized.[4]

In the early stages of viral infection, the immune responses elicited are believed to be critical determinants of subsequent disease outcome. Dendritic cells (DCs) play an essential role in initiating such responses. There are 2 major DC subsets: myeloid or conventional DCs, the most potent antigen-presenting cells, and plasmacytoid dendritic cells (pDCs),[5,6] which cooperate to activate both innate and adaptive immune responses and shape antiviral immunity. pDCs have the unique ability to recognize viruses through the sensing of viral nucleic acids, single-stranded RNA, and unmethylated DNA motifs via Toll-like receptors (TLRs) TLR7 and TLR9.[6–8] Following triggering of their TLR, pDCs produce large amounts of type I interferon (IFN) and proinflammatory cytokines, which can inhibit viral infection and modulate innate and adaptive antiviral immunity.[9] In addition to direct detection of viruses, pDCs can capture and cross-present viral antigens from infected cells.[10–12] This allows them to induce virus-specific adaptive immune responses in vitro[13] and trigger cytotoxic T lymphocytes in vivo in response to viral infection.[14]

In many chronic viral infections, these crucial mediators eliciting cytotoxic effectors appeared to be defective.[15] In chronic HBV infection, the role of pDCs remains enigmatic and poorly explored[16] with conflicting results on circulating blood pDC numbers, which are variously shown to be unaffected[17] or decreased.[18] Nevertheless, functional impairments of pDCs in patients with chronic

Abbreviations used in this paper: CBA, cytometric bead array; DC, dendritic cell; IFN, interferon; IL, interleukin; NK, natural killer; NKR, natural killer receptor; pDC, plasmacytoid dendritic cell; PBMC, peripheral blood mononuclear cell; rh, recombinant human; RM-ANOVA, row-matching analysis of variance; TLR, Toll-like receptor; TLR9-L, Toll-like receptor 9 ligand.

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HBV infection have been reported.[19] Most studies indicate that HBV has no direct effect on pDCs. However, the virus is suggested to actively alter the function of pDCs by abrogating their ability to up-regulate the expression of costimulatory molecules and produce cytokines on stimulation with TLR9 ligand (TLR9-L).[20] These effects could be due to hepatitis B surface antigen (HBsAg) binding to the C-type lectin receptor BDCA2 on pDCs,[21] induced by hepatitis B e antigen (HBeAg)[20] or related to impaired TLR9 expression.[22]

NK cells also play a pivotal role in antiviral immunity through killing infected cells and activating other immune pathways.[23,24] In the context of HBV infection, NK cells contribute to controlling the virus in the early phases of infection and inducing subsequent adaptive immune responses through their cytolytic potential and production of cytokines.[25,26] Defects in activation and antiviral functions of NK cells have been described in patients with chronic HBV.[27,28] Both could be reversed by reducing the viral load[29] or blocking immunosuppressive cytokines.[28] However, NK cells have also been shown to contribute to liver damage through TRAIL-mediated apoptosis of hepatocytes.[4,30] The basis for this controversial functional dichotomy of NK cells remains poorly understood.[27,31]

Interestingly, DCs play an essential role in shaping NK cell–mediated immunity. Both interleukin (IL)-12 produced by myeloid DCs and type I IFN secreted by pDCs modulate NK activation, IFN-γ production, and cytolytic activity.[32–34] TLR9-engaged pDCs have been shown to specifically enhance NK cell activation and effector functions.[35] pDCs are also able to regulate NK cell activity through cell-cell contacts involving interactions between GITR/GITRL[36] and OX40/OX40L.[37] NK cell activity can also be regulated through ICOS/ICOSL[38] and 4-1BB/4-1BBL.[39] Activated NK cells reciprocate to induce pDC maturation and enhance IFN-α production.[32,33] How chronic HBV infection affects relations between pDCs and NK cells has not been explored.

Thus, pDCs, crucial mediators of effective NK-based and CD8 T cell–based immune responses, may orient the outcome of the immune response on HBV infection, resulting either in resolution or chronicity. Functional defects in both the pDC and NK cell compartments have been reported with HBV infection. However, pDC-NK cross talk and the mechanisms involved in pDC and NK cell dysfunctions have yet to be addressed. In this study, we investigated the pathophysiological role of pDCs from patients with chronic HBV infection for the first time. We also studied the functional consequences of these on subsequent pDC-NK cross talk. Our results indicate that pDCs from viremic patients with chronic HBV are poor activators of NK cytolytic function through an OX40L/IFN-α–dependent pathway. This dysfunction may be related to high plasma IP-10 levels, together with HBeAg, HBsAg, and HBV viral load. A better understanding of the mechanisms of immune dysfunction in chronic HBV infection will allow the development of new immunotherapeutic strategies for improved viral control and clearance.

Patients and Methods

Patient Samples

This study was approved by Grenoble University Hospital's (CHU Grenoble) Comite de Protection des Personnes and the French Blood Service's Institutional Review Board. All participants signed informed consent forms. Blood samples were obtained from patients with chronic HBV (185 in total, of which 118 were aviremic and 67 were viremic) and from healthy donors (280 in total). Exclusion criteria included infection with human immunodeficiency virus, coinfection with hepatitis C virus or hepatitis D virus, other liver diseases, and current treatment with IFN-α or immunosuppressive agents. Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll-Hypaque density gradient centrifugation (Eurobio, Courtaboeuf, France). Plasma samples were collected and stored frozen. Serum HBsAg was quantified using the Abbott Architect HBsAg QT assay (Abbott Diagnostics, Rungis, France). pDCs and NK cells were purified from PBMCs using the EasySep Human pDC (purity >96%) and NK (purity >97%) Enrichment Kits (StemCell Technologies, Grenoble, France), respectively. Excess liver tissues not needed for diagnosis were obtained from 6 patients with chronic HBV and 8 noninfected controls. Biopsy specimens were reduced to cell suspensions using a potter. Main patient features are shown in Supplementary Table 1.

Cell Lines and Reagents

All cultures were performed in RPMI 1640/GlutaMAX (Invitrogen, Saint Aubin, France) supplemented with 1% nonessential amino acids, 100 μg/mL gentamycin, 10% fetal calf serum (Invitrogen), and 1 mmol/L sodium pyruvate (Sigma, Saint-Quentin Fallavier, France). The K562 cell line was purchased from American Type Culture Collection (LGC Standards, Molsheim, France).

Phenotypic Analysis

PBMCs were stained with fluorochrome-labeled anti-human HLA-DR, Lin, OX40L, 4-1BBL, ICOS-L (BD, Pont de Claix, France), BDCA2 (Miltenyi Biotec, Paris, France), CD123, CD40, CD86, CD62L (Beckman, Villepinte, France), and GITR-L (R&D, Abingdon, United Kingdom) antibodies. Stained cells were analyzed by flow cytometry using a FACSCalibur and CellQuest software (BD). Cells derived from liver biopsy specimens were stained with anti-human HLA-DR, BDCA2, CD40, and CD86 antibodies before flow cytometry analysis using FACSCanto and FACSDiva software (BD). pDCs were defined as Lin-HLA-DR$^+$ CD123$^+$ cells or as HLA-DR$^+$ BDCA2$^+$ cells. NK cells were determined after staining with anti-human CD16, CD56, and CD3 antibodies (Beckman).

Cytokine Quantification

PBMCs were cultured for 24 hours with CpG_A ODN-2336 (10 μg/mL; Coley Pharma, Berlin, Germany) or 640 UHA/mL UV-formol–inactivated influenza virus strain A/H3N2/Wisconsin/67/05 (Sanofi Pasteur, Lyon, France). Production of IFN-α, IP-10, IL-6, and tumor necrosis factor α was measured in culture supernatants by a Cytometric Bead Array assay (CBA; BD). Plasma IP-10 concentrations were determined by CBA (BD).