Defective T Helper Response of Hepatocyte-Stimulated CD4 T Cells Impairs Antiviral CD8 Response and Viral Clearance

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Background & Aims: **In hepatitis, hepatocytes gain the ability to express major histocompatibility complex (MHC) class II molecules and to present antigen to CD4 T cells. Here, we investigated whether MHC class II-expressing hepatocytes influence in vitro the differentiation of CD4 T cells and in vivo the T-cell response to and control of viral infection.** ***Methods:*** **Class II transactivator-transgenic hepatocytes that constitutively express MHC class II molecules were used to stimulate CD4 T cells in vitro, and the effector response type of the stimulated CD4 T cells was determined. The in vivo relevance of the obtained findings was confirmed by infecting nontransgenic or class II transactivator-transgenic mice with lymphocytic choriomeningitis virus.** ***Results:*** **MHC II-expressing hepatocytes induced T helper cell (Th) 2 differentiation of uncommitted CD4 T cells and abrogated the ability of previously differentiated Th1 to secrete interferon-γ, even in the presence of proinflammatory microbial signals. The suppression of Th1 responses by hepatocytes was associated with poor expression levels of Th1-promoting Delta-like Notch ligands. In vivo, MHC II expression by hepatocytes impaired the interferon-γ production by lymphocytic choriomeningitis virus-specific CD4 and CD8 T cells and prolonged viral persistence.** ***Conclusions:*** **By instructing infiltrating CD4 T cells to differentiate into a less inflammatory phenotype, MHC II-expressing hepatocytes seem to impair antiviral CD8 T-cell responses and viral clearance. Thus, hepatocytes may contribute to the chronicity of hepatitis virus infection.**

Hepatocytes are normally separated from portal blood-borne CD4 T cells by the sinusoidal endothelial cell layer, and, under healthy conditions, hepatocytes do not express class II molecules of the major histocompatibility complex (MHC II). However, during clinical hepatitis in patients or in mice, hepatocytes have contact to inflammatory CD4 T cells and often acquire MHC II expression.[1–3] Therefore, MHC II-expressing hepatocytes may interact with inflammatory CD4 T cells and influence their functional phenotype. Indeed, hepatocytes feature costimulatory molecules[4] and have the ability to process and present protein antigen to CD4 T cells.[5] Being the predominant liver cell population, hepatocytes may thus influence the type of hepatic immunity in clinical hepatitis.

The type of an immune response is shaped by differentiation of naive or uncommitted (T helper cell [Th] 0) CD4 T cells into various effector lineages; the best characterized lineages are interferon (IFN)-γ-producing Th1 cells, which promote cellular immunity, inflammation, and viral clearance, and interleukin (IL) 4-producing Th2 cells, which favor humoral immunity.[6] Lineage commitment of CD4 T cells is instructed by the context in which CD4 T cells are stimulated by antigen-presenting cells with peptide antigen complexed to MHC II molecules. The major factor that drives Th1 differentiation is IL-12[7]; Th2 differentiation is mainly driven by IL-4.[8] Besides these cytokines, the Notch ligand families Delta or Jagged, which are differentially expressed on antigen-presenting cells, have been shown to favor Th1 or Th2 differentiation, respectively.[9]

The type of hepatic immunity has been linked to the course of liver diseases; acute hepatitis virus infection and viral clearance,[10–12] as well as autoimmune liver disease,[13] seem to be associated with a preponderance of hepatic Th1 immunity. In contrast, chronic hepatitis virus infection seems to be associated with poor hepatic Th1 responses or even predominance of Th2 immunity.[14] Thus, the contextual signals delivered by hepatic antigen-presenting cells may contribute to the pathogenesis of hepatic diseases.

To explore the possibility that antigen-presenting hepatocytes may instruct CD4 T cells to differentiate into a certain immune effector type, we previously generated transgenic mice, which express the class II transactivator molecule (CIITA) under control of the C-reactive protein

Abbreviations used in this paper: APC, antigen-presenting cell; CIITA, class II transactivator molecule; CFSE, 5-,6-carboxyfluorescein diacetate, succinimidyl ester; IL, interleukin; IFN, interferon; LCMV, lymphocytic choriomeningitis virus; MHC, major histocompatibility complex; Th, T helper cell.

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promoter.[5] Hepatocytes from these mice constitutively express MHC II molecules and can process and present protein antigen to CD4 T cells.[5] Here, we report that antigen-presenting hepatocytes, in the absence of exogenous cytokines, induced Th2 differentiation of uncommitted CD4 T cells in vitro. Previously differentiated Th1 cells, when restimulated by hepatocytes, lost their ability to secrete IFN-γ. Thus, hepatocytes may instruct infiltrating CD4 T cells to obtain a less inflammatory phenotype. Indeed, lymphocytic choriomeningitis virus (LCMV) infection of CIITA-transgenic mice resulted in impaired IFN-γ production of LCMV-specific CD4 and CD8 T cells and delayed viral elimination. These findings indicate that hepatocytes may promote chronicity of hepatitis virus infection by modulating the functional phenotype of infiltrating CD4 T cells.

Materials and Methods

Mice

CIITA-transgenic mice were generated as described.[5] DO11.10 BALB/c TCR transgenic mice[15] have CD4+ T cells that express a T-cell receptor, which recognizes the p323–339 chicken ovalbumin peptide complexed with the MHC class II molecule I-A^d (detected by the clonotypic monoclonal antibody KJ1.26). To allow for MHC-matched antigen-specific stimulation of T cells, antigen-presenting hepatocytes were derived from the F1 generation of CIITA-transgenic FVB mice crossed to BALB/c mice, which express H-2^d molecules. Mice were bred and kept in the animal facilities at the Johannes Gutenberg-University Mainz or at the University Medical Centre Hamburg-Eppendorf and used at the age of 8 to 12 weeks; the LCMV experiments were performed at the animal facilities of the Swiss Federal Institute of Technology in Zurich. The experiments were approved by the institutional animal experimentation committee.

Isolation and Purification of Hepatocytes

Hepatocytes were isolated from mouse livers after perfusion with 0.05% NB8 Collagenase (Serva, Heidelberg, Germany) via the portal vein. The livers were then mechanically dissected and centrifuged (30*g*). To remove residual accessory cells from the hepatocyte preparation, the cells were then incubated with a mixture of monoclonal antibodies to murine CD8,[16] F4/80,[17] CD13 (from Dr Veelken, Institute for Immunology, Johannes Gutenberg University, Mainz, Germany), B220,[18] Mac-1,[19] and mouse anti-rat IgG[20] and rabbit complement for 20 minutes at 37°C. Hepatocytes were then resuspended in Dulbecco's modified Eagle medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS), 1% glutamine, and 1% penicillin/streptomycin and cultured in 96-well Primaria flat-bottom plates (Becton Dickinson, Heidelberg, Germany), 5×10^3 cells per well, or in collagen-R (Serva)-coated 24-well culture plates (Greiner, Solingen, Germany), 5×10^4 cells per well. Hepatocytes were cultured overnight, and experiments were performed on the next day.

Isolation of Dendritic Cells From Spleen or Liver

Isolation of dendritic cells (DC) from spleen was performed applying a modification of a previously described protocol.[21] Spleens were cut into small fragments and digested with collagenase A (0.5 mg/mL; Roche, Mannheim, Germany) and DNAse I (40 μg/mL; Sigma, Taufkirchen, Germany) in RPMI 1640 medium supplemented with 5% FCS for 30 minutes at 37°C. Spleen cells were then suspended and washed in phosphate-buffered saline (PBS), supplemented with 5% FCS and 5 mmol/L EDTA. Alternatively, liver nonparenchymal cells were prepared from the supernatant of the hepatocyte preparation (see above) by centrifugation over a 17% Optiprep (Sigma) gradient at 400*g*. DC were then isolated from spleen cells or liver nonparenchymal cells by positive selection of CD11c+ cells using magnetic cell separation (Miltenyi, Bergisch-Gladbach, Germany) according to the manufacturer's instructions.

Preparation and Stimulation of T Cells

CD4 T cells were purified from the spleens of DO11.10 BALB/c mice by positive selection of cells labelled with CD4 antibody attached to FITC-labelled magnetic multisort-microbeads using the MACS system (Miltenyi). Residual non-CD4 T cells were depleted with magnetic Dynabeads (CD8, B220, and Mac1; Dynal, Hamburg, Germany). The purity of the isolated primary CD4 T cells was above 98%.

Purified ovalbumin-specific DO11.10 CD4 T cells (1×10^6/mL) were activated in the presence of CIITA-transgenic hepatocytes and the specific p323–339 ovalbumin peptide (1 μg/mL). As indicated, lipopolysaccharide (LPS) (100 ng/mL; Sigma) or CpG-ODN (500 pmol/mL) was added. Seven days after primary stimulation, cells were restimulated either on freshly isolated hepatocytes or by plate-bound antibody to CD3 (145-C11, 5 μg/mL). Cells of the purified protein derivative (PPD)-specific LNC2 CD4 T cell line,[22] 5×10^5 per well, were stimulated with 20 μg/mL PPD (kindly provided by Behringwerke, Marburg, Germany) presented by hepatocytes or splenic DC.

Nonparenchymal liver cells from perfused livers of LCMV-infected mice were prepared as described,[23] and the included hepatic lymphocytes were then stimulated for 6 hours with purified WE (10^7 plaque-forming units [pfu]/mL) or with the np118–126 peptide (RPQASGVYM; 1 μg/mL), which is an immunodominant H-2^d-restricted CD8 T-cell epitope derived from the LCMV nucleoprotein.