Human Type 2 Myeloid Dendritic Cells Produce Interferon-λ and Amplify Interferon-α in Response to Hepatitis C Virus Infection

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BACKGROUND & AIMS: The type III interferons (IFN-λs: interleukin [IL]-28a, IL-28b, and IL-29) have important roles in hepatitis C virus (HCV) infection, but little is understood about what cells produce these cytokines or how production is activated. We investigated whether human immune cells recognize HCV-infected cells and respond by producing IFN-λ. **METHODS:** We cultured healthy human peripheral blood mononuclear cells (PBMCs) with different populations of immune cells and Japanese fulminant hepatitis-1 (JFH-1) HCV-infected Huh7.5 (cell culture–derived HCV particles [HCVcc]/Huh7.5) cells. **RESULTS:** Human PBMCs recognized HCVcc/Huh7.5 cells and responded by producing IFN-α, IFN-γ, and IFN-λ. A rare subset of myeloid dendritic cells (mDCs), which are blood DC antigen (BDCA)+ (also called *mDC2 cells*), were the major source of IL-28 and IL-29 production in response to HCVcc/Huh7.5 cells. Plasmacytoid DCs produced IFN-α, whereas natural killer and natural killer T cells were the main source of IFN-γ production in co-culture experiments. Of the endosomal Toll-like receptors (TLRs)3, 7, 8, and 9, only TLR3 or double-stranded HCV RNA induced production of IL-28 and IL-29 by mDC2s; endosomal maturation was required. Production of IFN-α and IFN-λ were linked—IFN-λ increased production of IFN-α by plasmacytoid DCs and IFN-α significantly increased production of IFN-λ. **CONCLUSIONS: mDC2s are a major source of IFN-λ production by PBMCs in response to HCVcc/Huh7.5 cells. mDC2s are activated through the TLR3 pathway, indicating that human DCs efficiently can initiate an immune response against HCV infection. IFN-λ therefore has an important role in HCV infection.**

Keywords: IL-28B SNP; NK Cells; NKT Cells; Viral Immune Regulation.

Hepatitis C virus (HCV) infection affects 120 million people worldwide, typically resulting in chronic infection. Early and high induction of interferon (IFN)-stimulated genes (ISGs) in HCV infection is a marker of decreased response to therapy,[1] however, the coordinated role of IFNs during HCV infection and the signaling pathways leading to their production are only partially understood. The IFNs are divided into type I (IFN-α and β), type II (IFN-γ), and type III subtypes (IFN-λs: interleukin [IL]-28a, IL-28b, and IL-29) and have immunomodulatory and antiviral activities through their respective receptors that induce ISGs.[2] All IFNs contribute to the coordination of antiviral immunity in the control of HCV. IFN-α has anti-HCV activity and is widely used to treat chronic HCV infection.[3] IFN-γ is produced by natural killer (NK) cells or HCV-specific T cells and orchestrates anti-HCV immune responses.[4] IFN-λs may play a unique role in clearing HCV infection because several single-nucleotide polymorphisms (SNPs) near the IL-28b region predict the outcome of natural HCV infection or IFN-α therapy.[5–8] Despite using different receptors, IFN-λ shares a common downstream Janus kinase-Signal transducer and activator of transcription (JAK-STAT) pathway with type I IFNs and induces very similar antiviral immune responses.[2] IFN-λ is currently in clinical trials for treatment of HCV infection.[9]

Although recent reports have suggested that human hepatocytes produce type I and III IFNs in response to HCV infection,[10,11] it also is well recognized that HCV disrupts IFN synthesis though NS3-4–mediated cleavage of 2 crucial adaptor proteins, mitochondrial antiviral-signaling protein and Toll/IL-1 resistance-domain-containing adapter-inducing interferon-β, in infected hepatocytes.[12] Thus, it is crucial for host immune cells to recognize ongoing HCV infection and initiate an immune response. Human dendritic cells (DCs) bridge the innate and adaptive immune systems and play an irreplaceable role in immune defense against viral infections. According to their distinct phenotype and functional characteristics, human peripheral DCs can be divided into 3 subsets: myeloid CD1c+ DC (mDC1), myeloid CD141+ DC or myeloid blood DC antigen (BDCA)3+ DC (mDC2), and plasmacytoid DC (pDC).[13] The mDC1 (conventional DC)

Abbreviations used in this paper: BDCA, blood DC antigen; CpG, cytosine-phosphate-guanosine; CXCL, CXC-chemokine ligand; DC, dendritic cell; dsRNA, double-strand RNA; HCV, hepatitis C virus; HCVcc, cell culture–derived hepatitis C virus particles; IFN, interferon; IL, interleukin; IP, interferon-inducible protein; ISG, interferon-stimulated gene; JFH-1, Japanese fulminant hepatitis-1; mDC, myeloid dendritic cell; mRNA, messenger RNA; MX1, myxovirus resistance 1; NK, natural killer; NKT, natural killer T; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; PHH, primary human hepatocytes; PRR, pattern recognition receptor; RANTES, regulated on activation, normal T cell expressed and secreted; RLR, RIG-I like receptor; SNP, single-nucleotide polymorphism; ssRNA, single-strand RNA; TLR, Toll-like receptor; TRAIL, TNF-related apoptosis-inducing ligand.

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represents the largest mDC population in the blood that produces inflammatory cytokines and chemokines.[14] The mDC2, a minor subset, is the human homologue of the mouse CD8+ DC subset,[15,16] which produces IL-12 and cross–presents antigen for CD8 class 1 restricted cytotoxic T lymphocyte responses under Toll-like receptor (TLR)3 ligation.[17] pDCs, natural type 1 IFN-producing cells, produce high levels of IFN-α and have a central role in antiviral immune responses.[18]

Because HCV cannot effectively infect immune cells,[19] it is speculated that immune cells must recognize HCV virions and/or HCV-infected cells. Previous studies have found that cell culture–derived HCV particles (HCVcc) cannot or only weakly induce mDC or pDC activation.[20,21] A recent report showed that pDCs can be triggered to produce type I IFNs by Huh7.5 cells containing replicating HCV RNA.[22] We hypothesized that human immune cells can recognize HCV-infected hepatoma cells and produce IFNs or inflammatory cytokines in response. We found that all 3 types of IFNs were produced in co-cultures of human peripheral blood mononuclear cells (PBMCs) and HCV-infected hepatoma cells without significant inflammatory cytokine induction. We determined that induction of the different IFNs by HCV-infected hepatoma cells was cell-specific and involved different pattern recognition receptors (PRRs). We identified mDC2s as the main producer of IFN-λ and confirmed IFN-α production by pDCs and IFN-γ production by NK/natural killer T (NKT) cells. These findings broaden our view of anti-HCV immunity and reveal unique roles for different human dendritic cells in initiating immune responses in HCV infection.

Materials and Methods

Cells, Replicons, and Viruses

Japanese fulminant hepatitis-1 (JFH-1) genomic RNA was derived from pUC-vJFH-1 plasmid using in vitro transcription. JFH-1 virions were produced by JFH-1-RNA–transfected Huh7.5 cells. Detailed information and protocols are described in the Supplementary Materials and Methods section.

Preparation of Human PBMCs, DC Subsets, and Co-culture Experiments

Human PBMCs were isolated from peripheral blood from normal human volunteers and chronic HCV-infected patients after informed consent was obtained according to procedures approved by the Committee for Protection of Human Subjects in Research at the University of Massachusetts Medical School. Detailed protocols for DC isolation and co-culture experiments are described in the Supplementary Materials and Methods section.

Reagents, Bioplex, Enzyme-Linked Immunosorbent Assay, RNA Quantification, Flow Cytometry, HCV Double-Stranded RNA Generation, IL-28B SNP Genotyping, and Other Methods and Statistical Analysis

See the Supplementary Materials and Methods section for more detail.

Results

HCV-Infected Cells Induce Type I, II, and III IFNs in Human PBMCs

We hypothesized that HCV-infected hepatoma cells represent danger signals and stimulate interferons and inflammatory cytokines in human PBMCs. By using co-cultures between normal human PBMCs and HCVcc/Huh7.5 or control Huh7.5 cells we found that all 3 types of IFNs (IFN-α, IFN-γ, and IFN-λs [IL-28 and IL-29]) were produced in the presence of HCVcc/Huh7.5 but not in noninfected or apoptotic Huh7.5 cells (Figure 1*A*). There was no IFN production by PBMCs, Huh7.5 cells, or HCVcc/Huh7.5 cells alone (data not shown). IFN-α, IFN-γ, IL-28, and IL-29 induction was highest in the presence of HCVcc/Huh7.5 cells, with the highest percentage of HCV infection (87% HCV infected) and the extent of IFN induction directly correlated with the percentage of HCV-infected Huh7.5 cells (Figure 1*B*). We found a significant increase in the levels of interferon-inducible gene products, interferon-inducible protein (IP)-10 (CXC-chemokine ligand [CXCL] 10), and TNF-related apoptosis-inducing ligand (TRAIL) but no induction in inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, regulated on activation, normal T cell expressed and secreted [RANTES], and tumor necrosis factor [TNF]-α) in co-cultures with HCVcc/Huh7.5 cells (Supplementary Figure 1). We also confirmed the induction of *CXCL10* and no induction of *IL-1B*, *IL-6*, *IL-8*, *IL-10*, and TNF-α at messenger RNA (mRNA) levels (Supplementary Figure 2). Addition of concentrated JFH-1 virions to PBMCs resulted in no IFN-α, -γ, or -λ induction (data not shown), suggesting that HCV-infected hepatoma cells and not the isolated virus induced all 3 types of IFNs without inducing inflammatory cytokines.

IL-28, IL-29, and Myxovirus Resistance 1 Gene Are Induced Rapidly From Co-cultures Between Human PBMCs and HCV-Infected Cells

Time-course experiments revealed that *IL-28*, *IL-29*, and ISG (myxovirus resistance 1 [*MX1*]) genes were induced rapidly in co-cultures between human PBMCs and HCVcc/Huh7.5 cells, but not with noninfected Huh7.5 cells (Figure 1*C*). None of the type I or II IFNs (including *IFNA1*, *IFNB*, and *IFNG*) genes were induced at early time point (4 hours), indicating that the early type III IFN response against HCV infection was independent of type I/II IFN production. We found increased protein levels of IL-28 and IL-29 at 8 hours after co-culture, although IFN-α and IFN-γ levels remained low (Figure 1*D*). Furthermore, IP-10, another IFN-inducible gene, was detected at 4 hours, whereas IFN-α was detectable only at 8 hours after co-culture (Figure 1*D*), indicating the existence of IP-10 inducers that were different from pDCs and IFN-α.

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