Quantification and Functional Analysis of Plasmacytoid Dendritic Cells in Patients with Chronic Hepatitis C Virus Infection

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Background. Plasmacytoid dendritic cells (PDCs) are the major producers of interferon (IFN)–α within peripheral blood mononuclear cells (PBMCs).

Methods. We analyzed whether chronic hepatitis C virus (HCV) infection could be linked to a defective function or number of PDCs. We evaluated the capacity of PBMCs from 5 cohorts of subjects to produce IFN-α after viral stimulation. We concomitantly analyzed the frequency of PDCs and the levels of IFN-α transcripts within the PBMCs from the same cohorts.

Results. PBMCs from patients with chronic HCV infection receiving antiviral therapy displayed a reduced capacity to release IFN-α, compared with those from healthy individuals, those from long-term responders to therapy, and those from nontreated patients. This defect was significantly correlated with the percentage of PDCs. In addition, PDCs from patients with chronic HCV infection receiving therapy displayed a reduced intrinsic capacity to produce IFN-α, which could be linked to the level of IFN-α transcripts.

Conclusion. Our observations point to an effect of the therapy on either the survival or the localization of PDCs, rather than a direct detrimental effect due to the viral infection during chronic HCV infection.

Hepatitis C virus (HCV) belongs to the Flaviviridae family and is the main etiologic factor of non-A, non-B hepatitis. Its genome is a positive, single-stranded RNA encoding for a large polyprotein processed into structural and nonstructural proteins [1]. HCV is highly variable and exists in infected individuals as a quasi species, which usually consists of a predominant viral variant and a pool of highly related but genetically distinct variants [2].

The World Health Organization estimates that ~170 million individuals are infected with HCV worldwide [3]. Viral infection is characterized by a high rate (50%–70%) of chronicity that, in 20% of cases, eventually evolves to liver cirrhosis, with complications that may lead to hepatocellular carcinoma [4]. Current treatments that use interferon (IFN)–α or pegylated IFN-α, in combination with ribavirin, allow resolution in 55% of treated patients. Although immune correlates linked to spontaneous or therapeutic resolutions are not yet clearly defined, several data suggest that rapid, strong, and maintained type 1–mediated cellular immune responses are needed to control viral infection [5, 6]. The phenotype and function of HCV-specific CD8+ lymphocytes, ranging from the onset of acute infection to the resolution of infection or the establishment of chronicity, have recently been analyzed in longitudinal follow-up studies [7, 8]. These studies indicate that most of the tetramer-positive CD8+ T cells display a phenotype of memory T cells during the acute phase of infection but are blocked in their maturation and thus cannot exert their effector functions (e.g., lytic activity and production of IFN-γ). This impairment nonetheless appears to be only transient in patients who will eventually recover [7]. This feature strongly suggests that some defects in the priming of the antiviral
immune response occur very early during acute infection.

Dendritic cells (DCs) play a key role in the induction of innate and adaptive immune responses. In humans, DCs are widely distributed throughout the organism and play the role of a sentinel that rapidly traps invading pathogens and migrates to secondary lymphoid tissues, where priming of specific T cells occurs [9]. Two major subsets of DCs described in peripheral blood can be differentiated by their differential expression of the cell-surface markers CD11c and CD123: the CD11c+CD123- myeloid DCs (MDCs) and the CD11c-CD123+ plasmacytoid DCs (PDCs) [10]. During viral infection, MDCs play an important role in the initiation of adaptive antiviral immune response by acquiring and processing antigens for presentation to specific T cells. In addition, PDCs participate in antiviral responses by producing large amounts of IFN-α/β when exposed to enveloped viruses, including herpes simplex virus (HSV), HIV, and influenza virus [11–13]. In turn, IFN-α initiates a cascade of biological effects, including a direct viral inhibition, the activation of other cells from the innate immune system (such as macrophages or NK cells), the induction of the Th1 polarization, and the survival of activated antigen-specific T cells [14]. Furthermore, a recent study suggests that PDCs also play a role in the adaptive immune response against viruses, since they have the capacity to expand anti–influenza virus cytotoxic T lymphocytes and Th1 CD4+ T cells as efficiently as CD11c+ MDCs [15].

Besides their prominent role in the initiation of antiviral immunity, DCs also are involved in the pathogenesis of viral infections responsible for either chronic disease or immunosuppression, including HIV, measles virus, and HCV [16–21]. Several lines of evidence now stress the importance of the PDC subset in the pathogenesis of HIV infection. PDC counts, as well as the type I IFN response, seem to be key factors in controlling HIV replication in cohorts of long-term survivors [22, 23]. Furthermore, defects in PDC function, in concert with absolute CD4 cell counts, have been associated with the occurrence of opportunistic infections [22]. These data indicate that type I IFN responses can be crucial in the control of viral and nonviral infections.

In the present study, we analyzed the contribution of innate immunity and, more particularly, of type I IFN responses in the course of HCV natural infection. To address this issue, we evaluated the capacity of PBMCs from patients with chronic HCV infection, with different disease outcomes, to produce IFN-α/β in response to viral stimulation. We further tried to correlate this capacity to the proportion of PDCs present within PBMCs and that of IFN-α transcripts present within the PDC subset.

### SUBJECTS, MATERIALS, AND METHODS

#### Study population.

Five groups of subjects were included. Patients with chronic HCV infection were divided into 3 groups according to their treatment and response to therapy at the time blood was obtained: patients who had never been treated (nontreated [NT]; n = 12), patients receiving antiviral therapy who responded to treatment (responders [R]; n = 13), and patients receiving therapy who did not respond to it (nonresponders [NR]; n = 10). The clinical characteristics of these patients are summarized in table 1. Concomitantly to these patients, 2 cohorts of HCV-negative individuals were studied: patients who had cleared viremia for at least 6 months after the end of therapy (long-term responders [LTR]; n = 12) and seronegative, healthy individuals (S; n = 12). The subjects from the 2 latter cohorts had a median age of 51 years (range, 38–

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**Table 1. Characteristics of hepatitis C virus (HCV)-infected patients, by treatment subgroup.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NT (n = 12)</th>
<th>NR (n = 10)</th>
<th>R (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV genotype/subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>ND</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>HCV RNA level, median (range), copies/mL</td>
<td>6.44 × 10^6 (2.143 × 10^7–2.02 × 10^7)</td>
<td>8.61 × 10^5 (2.143 × 10^3–9.935 × 10^8)</td>
<td>2.143 × 10^6</td>
</tr>
<tr>
<td>ALT level, median (range), UI/mL</td>
<td>56.5 (13–93)</td>
<td>58.5 (37–166)</td>
<td>57 (9–85.5)</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribavirin</td>
<td>NA</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>IFN + ribavirin</td>
<td>NA</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>PEG-IFN + ribavirin</td>
<td>NA</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients, except where noted. ALT, alanine aminotransferase; IFN, interferon; NA, not applicable; ND, not determined; NR, nonresponder; NT, nontreated; PEG, pegylated; R, responder.
77 years) and 33 years (range, 18–51 years), respectively, and showed no evidence of hepatic disease.

For the majority of the patients, blood samples of 20–40 mL were obtained. For some patients undergoing therapeutic phlebotomy for iron overload, 400–500 mL of whole blood was obtained. Informed consent was obtained from all these subjects before being included in the present study.

**HCV load.** HCV load was quantified from serum samples by use of real-time polymerase chain reaction (PCR), as described elsewhere [24]. The sensitivity of this assay is 4 copies/capillary, which is equivalent to 2143 copies/mL of serum. For the convenience of the analysis, virus loads beneath this threshold value were considered to be 2143 copies/mL.

**Quantification of PDCs.** PBMCs were isolated by use of density-gradient separation using Ficoll Hypaque (Pharmacia) and were resuspended in complete RPMI medium (RPMI 1640 medium [Invitrogen] supplemented with 2 mmol/L L-glutamine [Invitrogen], 50 IU/mL penicillin [Invitrogen], 50 μg/mL streptomycin [Invitrogen], and 10% fetal calf serum [FCS; Hyclone]).

One million PBMCs in 50 μL of PBS (Invitrogen) supplemented with 10% FCS were incubated with monoclonal antibodies for 30 min in the dark at 4°C. To estimate the percentage of PDCs within PBMCs, we used a combination of antibodies purchased from BD Biosciences: phycoerythrin (PE)–conjugated anti-CD123 (9F5) with peridinin-chlorophyll protein (PerCP), anti-HLA-DR (L243), and a cocktail of 6 fluorochrome isothiocyanate (FITC)–conjugated antibodies (lineage [lin] cocktail) comprising anti-CD3 (SK7), anti-CD14 (MfP9), anti-CD16 (3G8), anti-CD19 (SJ25C1), anti-CD20 (L27), and anti-CD56 (NCAM 16-2). Cells were washed twice in PBS supplemented with 10% FCS (Hyclone) and were fixed in 100 μL of PBS supplemented with 2% paraformaldehyde (Sigma Aldrich). Flow cytometric analysis was performed by use of a FACScalibur and CellQuest Pro software (both from BD Biosciences). Approximately 50,000 events corresponding to mononuclear cells by forward and side scatter characteristics were acquired (gate R1). The percentage of CD123+ PDCs (gate R3) was determined within the HLA-DR^{high}lin^{low} mononuclear cell population (gate R2).

**Production of IFN-α.** The capacity of total PBMCs to produce IFN-α was assessed by stimulating or not stimulating 2 × 10^5 cells, with 0.1 pfu/cell of HSV-1 (nonreplicative HSV-1Δ3 virus was a gift from A. Epstein, UMR 5534, Lyon, France) [25, 26], in 96-well U-bottom plates. Culture supernatants were harvested after 20 h of incubation at 37°C and were stored at −80°C until analysis. Cellular pellets for quantification of IFN mRNA were snap-frozen in liquid nitrogen after 8 h of stimulation with HSV-1 and were further stored at −80°C until analysis. IFN-α protein was assessed by use of an ELISA (PBL Biomedical Laboratories) that allows the detection of a majority of IFN-α subtypes. The sensitivity of this assay is 12.5 pg/mL.

**Relative quantification of IFN-α transcripts.** Total RNA was extracted from pellets of PBMCs after 8 h of stimulation with HSV-1 by use of the RNeasy extraction kit (QIAGen). Eighty nanograms of total RNA was reverse transcribed by use of Omniscript reverse transcriptase (QIAGen) and was amplified by use of real-time PCR performed on a Light Cycler instrument (Roche Diagnostics) by use of IFN-α and blood DC antigen (BDCA)–2–specific primers (Search LC). The IFN-α primers are described to be universal for most known IFN-α isoforms. In brief, the thermal profile was 95°C for 10 s, 68°C for 10 s, 58°C for 0.5 s, and 72°C for 16 s. Levels of IFN-α mRNA were gauged relative to the BDCA-2 mRNA [27] by use of RelQuant software (Roche Diagnostics) and are reported as arbitrary units (AU).

**Statistical analysis.** Because of sample sizes, nonparametric statistics were used. Comparisons between all groups were achieved by use of a Kruskal-Wallis test (a nonparametric analysis of variance), and, for a Kruskal-Wallis P < .05, pairwise comparisons were conducted by use of Fisher’s least significant difference test (a nonparametric analysis based on rank comparison). Spearman’s ρ was used to describe correlations, and P values were used to judge the strength of Spearman’s ρ. Data analyses were performed with SAS software (SAS Institute).

**RESULTS**

**Altered Capacity of PBMCs from Patients with HCV Receiving Therapy to Produce IFN-α**

Within the population of PBMCs, PDCs have been reported to be the main producer of IFN-α in response to stimulation with enveloped viruses [13, 28]. To evaluate whether PDCs from HCV-infected patients have retained the capacity to produce IFN-α, we compared the levels of IFN-α secreted by PBMCs from 59 subjects obtained from the following cohorts of individuals: NT patients (n = 12), R patients (n = 13), NR patients (n = 10), LTR patients (n = 12), and S subjects (n = 12).

Production of IFN-α by PBMCs after stimulation with HSV-1 was measured in the culture supernatants by use of ELISA and is reported in figure 1. These data first indicate that PBMCs from LTR patients produce equivalent amounts of IFN-α as do PBMCs from S subjects. On the contrary, among chronically infected patients, we could distinguish 2 subgroups of IFN-α producers. PBMCs from patients with chronic infection receiving therapy at the time blood was obtained produced significantly lower quantities of IFN-α than did those from HCV-negative individuals, including both S subjects (P < .0001) and LTR patients (P < .005). This defect in the release of IFN-α was equally observed whether the patients were in the R or NR group. In contrast, PBMCs from NT patients produced levels of IFN-α significantly higher than those from treated patients (P < .0001) and equivalent to those from the HCV-negative individuals.
Production of IFN-α by PDCs in HCV Infection

Figure 1. Decreased production of interferon (IFN-α) in patients with chronic hepatitis C virus (HCV) infection receiving therapy. Fifty-nine subjects were divided into 5 cohorts: patients with chronic HCV infection who were nonresponders (NR; n = 10), responders (R; n = 13), nontreated patients (NT; n = 12), and long-term responders (LTR; n = 12) and healthy seronegative subjects (S; n = 12). Peripheral blood mononuclear cells (PBMCs) obtained from the 59 subjects were evaluated for the level of IFN-α secreted after stimulation with herpes simplex virus–1. Results are shown as picogram per milliliter of IFN-α produced for 10^6 PBMCs. Each circle represents the value for 1 subject. Statistical analysis was performed by use of Fisher’s least significant difference based on rank comparison. Only significant P values are shown. Data and exact P values are also summarized in table 2.

Decreased Percentage of Blood PDCs in HCV-Infected Patients Receiving Therapy

To determine the origin of the reduced quantities of IFN-α produced by PBMCs from patients with chronic HCV infection receiving therapy, we first quantified the percentage of PDCs in total PBMCs for the different cohorts. By use of 3-color flow cytometry, the percentage of blood PDCs was estimated in total PBMCs from the same 59 subjects. The R1 gate containing PBMCs, excluding debris and polymuclear cells (figure 2A), was combined with an R2 gate, excluding cells strongly positive for lin-specific molecules (e.g., T cells, B lymphocytes, monocytes, and NK cells; figure 2B). PDCs could be distinguished among the HLA-DR^-lin^- population—comprising MDCs, PDCs, and some monocytes/macrophages—by their specific expression of the interleukin-3 receptor α chain or CD123 cell-surface marker (gate R3; figure 2C). Results of the overall PDCs quantification performed on the PBMCs issued from the different cohorts of individuals are indicated in figure 2D. More-precise data and statistical analysis are given in table 2.

First, figure 2D shows that, within any given cohort of individuals, the proportion of PDCs in PBMCs is quite variable (0.02%–0.8% of PBMCs; table 2). Second, our data also demonstrate variability between the different cohorts. HCV-negative individuals, comprising S subjects and LTR patients, have equivalent percentages of PDCs within PBMCs (median for LTR patients, 0.26%; median for S subjects, 0.29%). In addition, the proportion of PDCs within PBMCs from these individuals is not statistically different from that from the NT patients (median, 0.41%). In contrast, R and NR patients had a significant decrease in the percentage of PDCs in PBMCs, compared with healthy individuals (median for R patients, 0.22%; median for NR patients, 0.22%; P<.05) and NT patients (P<.005). No statistical difference was observed when the R or NR patients were compared with LTR patients (P>.05; data not shown). When these data are taken together, PBMCs from HCV-infected patients receiving therapy concomitantly display a lower capacity to produce IFN-α and a lower percentage of circulating PDCs.

Correlation Between Secretion of IFN-α and Blood PDC Count

To confirm the potential link between the capacity of PBMCs to produce IFN-α and the level of PDCs within PBMCs, we performed a correlation analysis. The nonparametric Spearman’s test was used; Spearman’s ρ describes the correlation, whereas the P value quantifies the probability to obtain this ρ value by chance.

For the 59 subjects, a significant correlation was found between the percentage of PDCs in total PBMCs and the in vitro production of IFN-α by PBMCs in response to stimulation with HSV-1 (Spearman’s ρ = 0.338; P = .01; figure 3). Indeed, NT patients with a high percentage of PDCs also had the highest levels of production of IFN-α in response to stimulation with HSV. In addition, when considering only HCV-infected patients (NT, NR, and R patients), the strength of the correlation was increased (Spearman’s ρ = 0.499; P = .0022; data not shown). As predicted from the Fisher’s comparison test, the correlation...
Figure 2. Decreased frequency of blood plasmacytoid dendritic cells (PDCs) in patients with chronic hepatitis C virus (HCV) infection receiving treatment, quantified by 3-color flow cytometry. Peripheral blood mononuclear cells (PBMCs; 10⁶) were stained with anti-CD123-phycoerythrin (PE), lineage (lin) (CD3, CD14, CD16, CD19, CD20, and CD56)-fluorescein isothiocyanate, and HLA-DR-peridinin-chlorophyll protein (PerCP), and PDCs were identified as lin⁻/HLA-DR⁺/CD123⁺ cells. PBMCs were selected in the R1 gate, excluding debris and polynuclear cells (A). Lin⁻/HLA-DR⁺ cells were selected in the R2 gate (B). In this combined R1 and R2 gate (R3), the percentage of CD123⁺/HLA-DR⁺ events was determined by use of the quadrant-stat function (CellQuest Pro software; BD Biosciences) (C). Percentages of PDCs within PBMCs from 59 subjects were quantified by use of this 3-color flow cytometry assay (D). Statistical analysis was performed by use of Fisher’s least significant difference based on rank comparison. Only significant P values are shown. Data and exact P values are also summarized in table 2. FSC, forward scatter; LTR, long-term responders; NR, nonresponders; NT, nontreated patients; R, responders, S, seronegative subjects; SSC, side scatter.

analysis confirms that low levels of IFN-α released by PBMCs from patients with chronic HCV infection receiving therapy could be linked to a decrease in the proportion of PDCs in the blood of these patients.

Transcriptional Regulation of Production of IFN-α

We analyzed whether a transcriptional and/or a translational regulation of the expression of IFN-α genes may also play a role in the lower production of IFN-α observed in the case of HCV-infected patients receiving therapy.

Intrinsic defect in the capacity of PDCs to produce IFN-α in HCV-infected patients receiving therapy. By use of data from the PDC quantification and data from the IFN-α dosage (figures 1 and 2D), we compared the level of IFN-α produced per PDC between the 5 cohorts. As previously observed with PBMCs, the mean quantity of IFN-α produced per PDC was not significantly different between LTR and NT patients and S subjects (figure 4A; median for LTR patients, 0.289; median for NT patients, 0.286; median for S subjects, 0.481). However, the levels of IFN-α produced by PDCs were significantly lower for the cohort of HCV-infected, treated patients (median for R patients, 0.042; median for NR patients, 0.044), compared with those for NT patients (P < .05) and HCV-negative individuals, including both LTR patients (P < .005) and S subjects (P < .0005). Overall, these data show that PDCs from patients with chronic HCV infection receiving therapy have a lower intrinsic capacity to produce IFN-α than do PDCs from other cohorts.

Transcriptional regulation of IFN-α gene expression in HCV-infected patients receiving therapy. To discriminate between a transcriptional and/or a posttranscriptional regula-
Table 2. Summary of analysis comparing hepatitis C virus (HCV)–infected patient subgroups, long-term responders, and control subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCV-infected subgroups</th>
<th></th>
<th>HCV-uninfected subgroups</th>
<th></th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT (n = 12)</td>
<td>NR (n = 10)</td>
<td>R (n = 13)</td>
<td>LTR (n = 12)</td>
<td>S (n = 12)</td>
</tr>
<tr>
<td>IFN-α protein, pg/mL</td>
<td>1313 (232–2323)</td>
<td>87 (16–931)</td>
<td>78 (7–617)</td>
<td>1502 (79–3565)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>$P &lt; .0001$ (NT), $P &lt; .0001$ (S), $P = .0005$ (LTR)</td>
<td>$P &lt; .0001$ (NT), $P = .0006$ (S), $P &lt; .0001$ (S), $P = .0006$ (S), $P &lt; .0001$ (S), $P = .0006$ (S)</td>
<td>$&lt;.0001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDCs in total PBMCs, %</td>
<td>0.41 (0.11–0.8)</td>
<td>0.22 (0.10–0.58)</td>
<td>0.22 (0.02–0.34)</td>
<td>0.26 (0.13–0.62)</td>
<td>0.29 (0.11–0.65)</td>
</tr>
<tr>
<td>Median (range)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>$P = .0047$ (NT), $P = .0370$ (S)</td>
<td>$P = .0013$ (NT), $P = .0147$ (S)</td>
<td>$P = .0013$ (NT), $P = .0147$ (S)</td>
<td>$P = .0013$ (NT), $P = .0147$ (S)</td>
<td>$&lt;.0097$</td>
</tr>
<tr>
<td>IFN-α mRNA, AU</td>
<td>1.76 (0.22–3.15)</td>
<td>0.03* (0.01–0.96)</td>
<td>ND</td>
<td>2.57 (0.85–4.12)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>$P = .013$ (NT), $P = .005$ (S)</td>
<td>$P = .013$ (NT), $P = .005$ (S)</td>
<td>$P = .013$ (NT), $P = .005$ (S)</td>
<td>$P = .013$ (NT), $P = .005$ (S)</td>
<td>$&lt;.025$</td>
</tr>
</tbody>
</table>

**NOTE.** AU, arbitrary unit (see Subjects, Materials, and Methods for definition); IFN, interferon; LTR, long-term responder; ND, Not done; NR, nonresponder; NT, nontreated; PBMCs, peripheral blood mononuclear cells; PDCs, plasmacytoid dendritic cells; R, responder; S, seronegative.

* Overall $P$ value from Kruskal-Wallis test. If Kruskal-Wallis $P$ values were inferior to .05, pairwise comparisons were conducted by use of Fisher’s least significant difference.

* $P$ value from Fisher’s least significant difference were given only for significant differences.

* IFN-α mRNA quantification on treated patients, including R (n = 3) and NR (n = 2) patients.
tion of the expression of IFN-α, we evaluated the levels of IFN-α transcripts, after 8 h of stimulation with HSV-1, in PBMCs obtained from 15 patients—5 in the NT group, 5 in the R and NR groups, and 5 in the S group—for whom frozen PBMCs remained. Total mRNA was extracted from HSV-stimulated PBMC pellets, to determine levels of IFN-α transcripts in the PDC. Because PBMCs contain several cell subtypes, we selected a reference gene specifically expressed in PDCs to normalize the amount of IFN-α transcripts measured per PDCs within total PBMCs. This gene is the recently identified BDCA-2 gene, which encodes a new C-type lectin that is probably involved in antigen uptake and inhibition of type I IFN pathway [27].

To assess the validity of the BDCA-2 gene as a reference gene in the present study, different experiments were performed. We first purified BDCA-4+ PDCs from the blood of 6 healthy individuals and confirmed, by use of real-time PCR, that BDCA-2 was expressed at the same level in these different samples (data not shown). The expression of BDCA-2 transcripts was compared between PBMCs derived from HCV-infected patients (n = 10) and healthy control subjects (n = 10), before and after stimulation with HSV-1. Our data show that the expression of BDCA-2 transcripts is stable among the cohorts and is not modulated by either HCV infection or stimulation with HSV-1 (data not shown). Overall, these results confirm that this gene can be used to normalize data from IFN-α mRNA quantification.

Quantitative PCR was performed on a Light Cycler instrument to quantify BDCA-2 and IFN-α mRNA for 15 of 59 subjects, including S subjects (n = 5), NT patients (n = 5), and R and NR patients (n = 5). IFN-α mRNA levels were gauged relative to the BDCA-2 mRNA by use of RelQuant software and are reported as AU.

Statistical analysis of our results reported in figure 4B and summarized in table 2 indicated that IFN-α transcripts are down-regulated in HCV-infected patients receiving therapy (median, 0.03 AU), compared with both NT patients (median, 1.76 AU; P = .013) and S subjects (median, 2.57 AU; P = .005). For these 15 subjects, the levels of transcripts are correlated with the IFN-α protein secretion (Spearman’s ρ = 0.70; P = .005). No correlation could be established with genotypes, viremia, or hepatic inflammation of the HCV-infected patients (data not shown).

Overall, the statistical and correlation analyses show that patients with chronic HCV infection receiving therapy display a decreased capacity to produce IFN-α after viral stimulation. This function is linked to both a lower proportion of PDCs present in the blood of these patients and an altered capacity of these cells to produce IFN-α.

DISCUSSION

The role of the immune system and, more particularly, of DCs, in therapeutic or spontaneous resolutions of HCV infections, is still poorly understood. In patients with chronic HCV infection, defective functions of monocyte-derived DCs that normalize after successful therapeutic viral clearance have been
reported elsewhere [20, 21, 29]. It has been suggested that the presence of virus [22] or its components, such as core and nonstructural protein 3 [30], could be responsible for the observed monocyte-derived DC dysfunctions. Although monocyte-derived DCs have been the model of choice for the study of the interaction between HCV and DCs so far, the involvement of circulating DCs in HCV pathogenesis has never been addressed. We have recently shown that circulating DCs from patients with chronic HCV infection contain replicating HCV [31]. Functional defects of these peripheral blood DCs and, more particularly, of the PDC subset, during HCV natural infection, could dramatically impair innate and, consequently, adaptive anti-HCV immune response, leading to viral persistence. In the present study, we have analyzed the capacity of PDCs derived from different cohorts of HCV-seropositive patients to produce IFN-α when exposed to viral stimulation.

We have shown that IFN-α levels produced by PBMCs from HCV-infected patients receiving therapy are significantly lower than those produced by PBMCs from S subjects and LTR patients. However, this defect was observed in both R and NR patients but not in NT patients. These observations suggest a detrimental effect of the treatment on the capacity of PDCs to secrete IFN-α after viral stimulation. Most of the patients receiving therapy in the present study were receiving a treatment combining IFN-α (pegylated or not) and ribavirin. Little is known about the primary mechanism of action of ribavirin in the treatment of viral infections such as that with HCV. Recent microarray results on respiratory syncitial virus (RSV) infection show that ribavirin essentially potentiates virus-induced signaling of the IFN-stimulated response element to enhance the expression of antiviral IFN-stimulated response genes [32]. On the other hand, that same study also reports that ribavirin down-regulates expression of IFN-β in RSV-infected epithelial cells [32]. Although quite unique so far, these preliminary data emphasize a potential relationship between ribavirin and the type I IFN signaling pathway. It is interesting to note that, in

Figure 4. Transcriptional regulation of the intrinsic defect in the capacity of plasmacytoid dendritic cells (PDCs) to secrete interferon (IFN)-α. With our results of IFN-α secretion (figure 1) and from PDC quantification (figure 2D), we calculated the level of IFN-α secreted per PDC (picogram of IFN-α/cell) for the 59 subjects of our study (A). The level of IFN-α mRNA gauged to the blood dendritic cell antigen (BDCA)–2 mRNA was measured for 15 of 59 subjects, including treated (nonresponders [NR] plus responders [R]; n = 5) and nontreated (NT; n = 5) patients with hepatitis C virus (HCV) infection, as well as seronegative healthy subjects (S; n = 5) (B), and are reported as arbitrary units (AU). Each circle represents the value for 1 individual. Statistical analysis was performed by use of Fisher’s least significant difference based on rank comparison. Only significant P values are shown. Data and exact P values are also summarized in table 2.
the present study, 3 of the HCV-infected patients receiving therapy were receiving monotherapy with ribavirin at the time blood samples were obtained. One could thus question whether ribavirin by itself could be responsible for the decrease in production of IFN-α that we observed in treated patients. Administration of recombinant human IFN-α for various indications has been reported to decrease the capacity of blood lymphoid cells to produce IFN-α [33–35].

Observations reported in the present study would suggest that IFN-α plus ribavirin therapy could have a direct incidence on the pool of circulating PDCs. Our data show that the percentage of PDCs within PBMCs is also decreased in patients with chronic HCV infection receiving therapy, compared with the other groups, and is significantly correlated with the levels of IFN-α released after viral stimulation. Similar defects in both the levels of IFN-α produced and the number of circulating PDCs have been reported in HIV infection [22, 36–41]. In the majority of the reports, a negative correlation was found between PDC counts and plasma HIV-1 RNA levels [22, 36–39], which contrasts with our data on HCV infection. The effect of highly active antiretroviral therapy on the level and function of PDCs is still controversial [23, 40, 41]. The decrease in circulating PDCs in HCV-infected patients receiving therapy could involve either apoptosis or relocalization of these cells. Although IFN-α is described as a potent survival factor for PDCs in vitro [42], its effect on PDCs, in the context of viral infection, has never been studied. IFN-α/β can greatly sensitize different cell types to apoptosis, more particularly in response to double-stranded RNA or certain virus infections, such as influenza virus [43]. Whether Fas-mediated apoptosis could be potentiated by IFN-α, as observed in the case of influenza, remains to be determined. Besides its proapoptotic effect, recombiant IFN-α, when injected into mice, induces a “chemokine-to-cytokine-to-chemokine cascade,” which is critical in antiviral defense, first driving the accumulation of macrophage inflammatory protein (MIP)-1α-producing cells in the liver [44, 45]. Macrophages are the major producers of MIP-1α within the liver, but PDCs can also produce MIP-1α after stimulation with CpG, CD40 ligand, or virus [46]. Thus, migration of PDCs from the blood to organs, such as the liver, after administration of IFN-α could explain the decrease in the proportion of PDCs in the blood of HCV-infected patients receiving therapy. Indeed, IFN-α-positive cells are observed in the liver-infiltrating mononuclear cells from patients with chronic HCV infection receiving IFN-α/α2 therapy [47].

Finally, when combining the IFN-α ELISA results with the quantification of PDCs within PBMCs, we could calculate the level of IFN-α produced per PDC and found that this level was significantly lower in the cohort of HCV-infected patients receiving therapy. Quantitative PCR evaluation of normalized IFN-α transcripts, although preliminary since they were performed on a small fraction of the patients, revealed that the expression of the IFN-α genes is down-regulated at the transcriptional level in HCV-infected patients receiving therapy. Furthermore, the levels of IFN-α transcripts were correlated to the levels of IFN-α protein secreted after stimulation with HSV-1. Together, our results suggest that antiviral therapy for HCV inhibits both the pool of circulating PDCs and the intrinsic expression of IFN-α genes in these cells.

Overall, we have reported a decrease in the proportion of PDCs, together with a reduced intrinsic capacity of these cells to produce IFN-α, in patients with chronic HCV infection receiving therapy. This defect appears to be most likely due to a detrimental effect of combination therapy with IFN-α and ribavirin, because it was only found in patients receiving therapy and because there was no correlation between viremia and IFN-α production (data not shown). Although a loss of PDCs and of their IFN-producing function seems to be directly correlated with HIV immunopathogenesis and, more particularly, with susceptibility to opportunistic infections, our data do not allow us to conclude that HCV has a direct role in determining PDC count and IFN-α–producing function of PDCs. However, we cannot, at this point, rule out the possibility that HCV may exert an effect either early after the onset of infection (i.e., during the acute phase of infection) or on other functions of circulating DCs.

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References


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