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Vδ1 T Lymphocytes Expressing a Th1 Phenotype Are the Major γδ T Cell Subset Infiltrating the Liver of HCV-infected Persons

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Abstract

Background: Hepatitis C infection induces an acute and chronic liver inflammation that may lead to cirrhosis, liver failure, or hepatocarcinoma. Since the role of αβ T lymphocytes in hepatitis C virus (HCV) immunopathology has been analyzed extensively, we investigated the distribution and functional activation of γδ T cell subsets in chronically HCV-infected patients.
Materials and Methods: Blood samples and liver biopsies from 35 patients with compensated chronic HCV infection were compared in terms of T cell subset distribution, expression of activation markers, γδ T cell receptor (TCR) repertoire, and pattern of cytokine production. Moreover, we analyzed whether these immunological parameters were associated with other clinical observations (plasma viremia, ALT levels, Ishak index).
Results: Differing from peripheral blood distribution, a specific compartmentalization of Vδ1 T cells ($p < 0.001$) was observed in the liver of HCV patients. These cells represented a relevant fraction of intrahepatic T lymphocytes (1.8–8.7%) and expressed the memory/effector phenotype (CD62-L$^-$ CD45-RO$^+$CD95$^+$). This phenotype was consistent with selective homing upon antigen recognition. Mitogenic stimulation of Vδ1$^+$ T lymphocytes recruited in the liver revealed the T helper cell type 1 (Th1) pattern of cytokine secretion. Interestingly, the frequency of interferon-γ (IFN-γ)-producing Vδ1 T cells was associated with an higher degree of liver necroinflammation, measured by the Ishak index. Finally, the T-cell repertoire analysis revealed the absence of Vγ selection in the TCR repertoire of intrahepatic Vδ1 T cells.
Conclusions: γδ T cell distribution in the peripheral blood differs from the Vδ1 T cell subset because it is policlonally activated and recruited in the liver of chronic HCV-infected patients. During HCV-infection, this T cell subset may release Th1 cytokines and contribute to the necroinflammatory liver disease.

Introduction

Among hepatitis viruses, hepatitis C virus (HCV) is the main factor responsible for chronic liver disease often progressing to cirrhosis and/or hepatocellular carcinoma (1,2). Unfortunately, the majority of HCV-infected persons develop chronic hepatitis that is difficult to eradicate with the current therapeutic protocols (2). In contrast, less than 10% of patients with acute hepatitis B virus infection will become chronic (3). This distinct difference implies that different survival strategies of hepatitis viruses and/or different host immune responses may exist. Specifically, progression of chronic hepatitis C is characterized by a dramatic lymphocyte infiltrate in the liver (4,5). Several lines of evidence argue in favor of immune-mediated hepatic damage triggered by infiltrating lymphocytes, rather than a direct HCV-mediated cytopatic effect in chronic hepatitis C virus infection. Further studies are required to determine which of the infiltrating T cell subset play cytotoxic or inflammatory functions that could be helpful, useless or even harmful.

Although γδ T lymphocytes represent a minor population of human peripheral lymphocytes, recent studies have demonstrated a predominant localization of lymphocytes expressing the γδ T cell receptors (TCR) in the liver (6,7,8). Moreover, gd T cells

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mediate a potent antiviral response against different viruses (9). In peripheral blood, the majority of γδ T cells express the Vγ9Vδ2 rearrangement. This subset exerts potent cytotoxic activity controlled by specific natural killer (NK)-receptors and may recognize nonpeptidic antigens in a major histocompatibility complex (MHC)-unrestricted manner, without the need for antigen processing (10). The remaining fraction of γδ T lymphocytes express the Vδ1 chain associated with different Vγ-chains (11). Vδ1 T lymphocytes were shown to proliferate in response to stimulation with antigens from cytomegalovirus (CMV) or Epstein Barr virus (EBV)-infected cells (12,13,14). However, whether the stimulatory ligand is of cellular or viral origin is not defined clearly. Interestingly, the proliferative response is independent of the Vγ chain coexpressed with Vδ1 (13). In healthy donors, Vδ1 T cells are distributed mainly throughout the hu-man intestinal epithelium (70–90% of intestinal γδ T cells) (15) and may recognize self-antigens, such as (MIC)-A,B or CD1 molecules (16,17), which provide a link between antiviral immunity and autoreactivity.

In this study, the phenotype and functional characteristic of intrahepatic γδ T cells were analyzed. We observed a polyclonal localization of effector/memory $V\delta1^+$ T lymphocytes expressing a T helper cell type 1 (Th1) phenotype in the liver of HCV-infected persons with a necroinflammatory process. Thus, Vδ1 T cells may contribute to the liver inflammation and autoreactivity frequently occurring during hepatitis C.

Materials and Methods

HCV Patients

Patients with compensated chronic HCV infection were involved in the study (mean age: 45 ± 13 years; 23 males and 12 females). All patients ($n = 35$) were from the National Institute for Infectious Diseases, L. Spallanzani, of Rome. They all had histological confirmation of chronic hepatitis and abnormal serum aminotransferase values (84 ± 68 UI/mm^3) for at least 6 months. Specifically, a total of 35 HCV-infected patients was analyzed (30 patients untreated, 3 patients treated with interferon α (IFNα), and 2 patients with IFNα plus ribavirin, in accordance with international guidelines). No significant differences between treated and untreated patients were observed regarding the parameters analyzed in this study. As control patients with different liver diseases, we analyzed four persons HCV-seronegative. This control group was composed of two hepatitis B virus (HBV)-infected persons, one HBV and human immuno deficiency virus (HIV)-infected person, and one patient without viral hepatitis and HIV seronegative. Blood samples and liver biopsies were always performed for diagnostic purposes and an aliquot of the samples (10–15 mm of length) was obtained upon informed consent of the patients. The local etichal committee approved the study.

Human Peripheral Blood Mononuclear Cells (PBMC) and Intrahepatic Lymphocytes (IHL) Isolation

Blood samples were diluted volume per volume (v/v) in RPMI medium (RPMI). PBMC were separated by density gradient centrifugation (Ficoll-Hypaque, Pharmacia Biotech, Uppsala, Sweden). The number of lymphocytes/mm3 was 2474 ± 530 cell/mm^3. Liver biopsy samples were washed three times with the complete medium [RPMI 1640 supplemented with 10% heat inactivated fetal calf serum (FCS), 10 U/ml penicillin/ streptomycin] to eliminate contaminating blood lymphocytes and then manually homogenized using a 70 μm filter (Becton Dickinson, Mountain View, CA). Mononuclear cells were separated by density gradient centrifugation with Ficoll-Hypaque, washed twice in the complete medium, and counted. The cell recovery was 243000 ± 95000 lymphocytes/biopsy sample.

Monoclonal Antibodies

Monoclonal antibodies coupled with fluorescein (FITC), phycoerythrin (PE), phycoerythrin-cyanin 5.1 (PE-Cy5), and allophycocyanin (APC) were combined for simultaneous staining. The anti-human antibodies (mAbs) used in this study were as follows: anti-CD3 mAb [immunoglobulin G1 (IgG1), clone UCHT-1, Immunotech, Marseille, France] was coupled with PE-Cy5; anti-CD4 (IgG1, clone SK3) and anti-CD95 (IgG1, clone DX2) mAbs were coupled with APC (Becton Dickinson, Mountain View, CA). The δTCS1 mAb (IgG1, clone 005768, Endogen, Woburn, MA), which recognized the Vδ1 region of the γδTCR, was coupled with FITC. The anti-HLA-DR (IgG2a, clone L243), the anti-CD45-RO (IgG2a, clone UCHL-1), and the anti-CD62L (IgG1, clone Dreg-56) mAbs coupled with PE, and the anti-CD45-RA (IgG1, clone L48) coupled with FITC were all purchased from Becton Dickinson. The CD81 (IgG2a, clone JS64, Immunotech, Marseille, France) was coupled with PE. The following anti-cytokines mAbs were all purchased from Becton Dickinson [anti-interleukin-4 (IL-4), IgG1, clone MP4-25D2; anti-IFNγ, IgG1, clone 4S.B3]. A PE-conjugated control mAb (IgG1 clone MOPC-21, Becton Dickinson) was used in all experiments. Anti-γ-chains mAbs were kindly provided by Dr. M. Bonneville (Nantes, France): anti-Vγ2/3/4 (23D12), Vγ3/5 (56.3), Vγ8 (R4.5.1), and Vγ9 (7A5). A goat anti-mouse, coupled with PE (Becton Dickinson) was used to detected the anti-Vγ-chain mAbs.

Flow Cytometry

Analysis of surface antigen (Ag) expression was performed as previously described (18). Briefly, 5×10^5 PBMC or 5×10^4 IHL were washed in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.1% sodium azide, and were incubated for 15 min at 4°C with the indicated FITC-, or PE-, or PE-Cy5-, or APC-conjugated mAbs.

Samples were fixed in 1% paraformaldehyde and immediately acquired by a FACScalibur flow cytometer (Becton Dickinson). A total of 20,000 events were acquired for each sample and analyzed with the CellQuest software (Becton Dickinson).

Single-cell Analysis of Cytokine Synthesis

Cytokine production was detected by flow cytometry analysis as previously described (19). Human IHL and PBMC were stimulated overnight with phorbol12-myristate13acetate (PMA); 50ng/ml) + ionomycin (1 μg/ml). Brefeldin A (10 μg/ml) was added 2 hr after from stimulation to block intracellular transport, allowing cytokine accumulation in the Golgi. Cells were washed twice in PBS, 1% BSA, and 0.1% sodium azide, and stained with mAbs specific for the membrane antigens described above (Vδ1-FITC, CD8-PerCP, CD4-APC) for 15 min a 4°C. To permeabilize the cell membrane, samples then were fixed in PBS/1% paraformaldehyde for 10 min at 4°C, incubated with anti-cytokine-specific mAb (IFN-γ-PE, IL-4-PE) diluted in 1% PBS, 1% BSA, and 0.5% saponin. The cells finally were washed twice in PBS, 1% BSA, and 0.1% saponin, and acquired on a FACScalibur (Becton Dickinson). Control for nonspecific staining always was monitored with isotype-matched mAbs and nonspecific staining always was subtracted from specific results.

Results

Recruitment of CD62-L$^-$ CD45-RO$^+$ Effector/Memory Cells In the Liver of HCV+ Patients

The surface expression of markers for naive/memory cells (CD45-RA/RO and CD62-L) and human leucocyte antigen-DR (HLA-DR) were analyzed by flow cytometry on PBMC and IHL. Activation of naive T cells through TCR was followed by loss of the CD45-RA isoform and transition to the CD45-RO phenotype (20,21,22). Figure 1 shows the cytometric panels from a representative HCV-infected patient. Naive lymphocytes were double-stained, expressing both CD45-RA$^+$ and CD62-L$^+$ (Fig. 1A and D). This subset represented 47% of the circulating lymphocytes (Fig. 1A). In the liver of the same HCV-infected patient, the percentage of naive lymphocytes was reduced markedly (8.1%, Fig. 1D), compared with the peripheral blood compartment. In contrast, memory and recently activated lymphocytes (CD45-RO$^+$ CD62-L$^-$) (23) represented 8.8% of the circulating cells (Fig. 1B). Interestingly, the fraction of memory/activated cells in the liver of the same HCV-infected donor increased to 36.8% (Fig. 1E). HLA-DR was expressed constitutively at high levels on the surface of B lymphocytes (24,25). As shown in Figure 1C, few HLA-DR brightly positive B cells were present in the liver of HCV-infected persons. However, HLA-DR represented an activation marker for T cells and was expressed moderately on T cells upon activation (24,25). Consistent with a functional recruitment of activated T cells, the increase of memory IHL was paralleled by an induction of HLA-DR on T lymphocytes (Fig. 1F). The bottom graph shows that the reported higher percentage of activated/ memory T cells in the liver of HCV$^+$ patients, compared with the circulating pool, which was statistically significant ($p < 0.01$). No differences were observed in the expression of activation markers on PBMC from healthy donors, HCV+ patients, and HCV− patients with liver diseases (data not shown). Interestingly, the frequency of activated cells was increased similarly in IHL from HCV+ or HCV− patients with liver diseases, compared with PBMC. Specifically, CD45RO$^+$CD62L$^-$ memory/activated IHL were 36.6 ± 8.6% in HCV+ patients and 46.0 ± 4.9% in HCV− persons with liver diseases. Moreover, CD3$^+$HLA-DR$^+$-activated IHL were 35.6 ± 14.9% in HCV+ patients and 40.0 ± 16.6% in HCV− persons with liver diseases. Altogether, these data confirmed that liver inflammation induced a broad increase of memory/ activated intrahepatic T lymphocytes (6).

Selective Recruitment of CD8 and Vδ1 T Cells In the Liver of HCV+ Patients

The T cell subsets infiltrating the liver of HCV-infected persons were further characterized by flow cytometry. In the liver of HCV-infected persons, the percentage of CD3$^+$ T lymphocytes was reduced slightly, compared with the peripheral blood (Table 1). Moreover, the CD4/CD8 ratio also was inverted, with a predominance of CD8 T lymphocytes in the liver of HCV patients. Interestingly, an increased fraction of T cells expressing the γδ TCR was observed in the liver of HCV patients (Table 1), suggesting that this T cell subset may play a relevant role at the site of infection (6). We then analyzed the Vδ1 and Vδ2 T cell subset distribution in IHL and PBMC by flow cytometry. Double-staining was performed with anti-CD3 and anti-Vδ1-TCR or anti-Vδ2-TCR-specific mAbs. Our observations confirmed that Vδ2 T lymphocytes were the main subset of γδ T cells in the peripheral blood and showed that the number of CD3$^+$ cells expressing the Vδ1 TCR was increased markedly in the liver of HCV+ patients (Table 1 and Fig. 2A). The frequency of Vδ1 T cells in PBMC was similar both in healthy donors, HCV-infected persons, and HCV-seronegative patients with other liver (1.0 ± 0.2%, 0.9 ± 0.6%, 1.5 ± 0.2%, respectively). Moreover, the percentage of Vδ1 T cells in the liver was significantly higher in HCV-infected persons vs. HCV-seronegative patients with other liver diseases (3.5 ± 2.1% vs. 1.5 ± 0.3% respectively, $p < 0.05$.), suggesting that liver infiltration by Vδ1 T lymphocytes specifically was induced by HCV-infection.

Finally, we analyzed whether the increased percentage of Vδ1 T cells in the liver of HCV-infected patients was associated with other clinical parameters. The percentage of peripheral Vδ1 T cells correlated

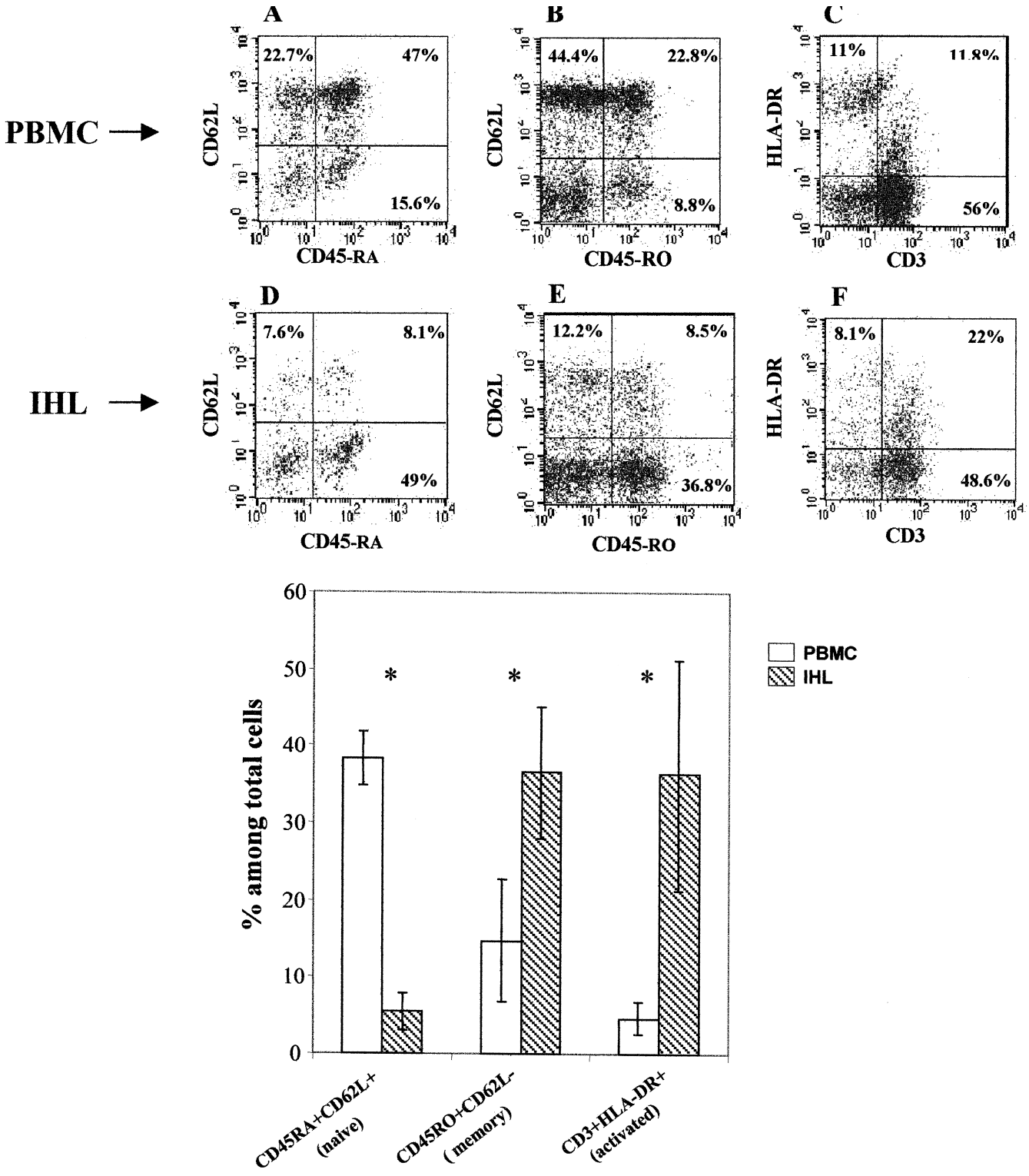

Fig. 1. Phenotypic characterization of naive, memory and activated lymphocytes in the peripheral blood and liver of hepatitis C virus (HCV)-infected patients. (A–F) The percentage of naive ($CD45RA^+CD62L^+$), memory ($CD45RO^+CD62L^-$) and activated ($HLA\text{-}DR^+$) cells was analyzed by flow cytometry. (A,B,C) The percentage of these cell subsets in peripheral blood mononuclear cells (PBMC) and (D,E,F) intrahepatic lymphocytes (IHL) compartments from a representative patient is shown. The percentage of naive, memory, and activated cells in blood (white bars) and liver (hatched bars) on 10 HCV+ patients are shown in the bottom graph. Data represent the arithmetic mean percentage ± standard deviation (SD). Significance refers to Student's *t*-test. *p*-values are indicated as follows: * = $p < 0.001$.

Table 1. T cell subsets infiltrating the liver of HCV-infected persons

	PBMC % (± SD)	IHL % (± SD)
CD3	79.2 (±7.7)	**72.2** (±11)*
CD4	50.9 (±12)	**29.7** (±11)*
CD8	30.5 (±10)	**42** (±10)§
CD4/CD8 ratio	1.7	**0.7**
αβ TCR	77.6 (+/3.6)	73.6 (+/3.5)
Vδ1 TCR	0.9 (±0.6)	**3.8** (±2.5)*
Vδ2 TCR	2.3 (+/−0.9)	2,6 (+/−1.5)
Vδ2/Vδ1 ratio	2.5	**0.7**

The percentage of different lymphocyte subsets in the blood (PBMC) and liver (IHL) of 19 HCV+ persons was evaluated by flow cytometry. Results are expressed as arithmetic mean percentage ± SD. Significance refers to Student's *t*-test. *p*-values are indicated as follows: * $p < 0.01$, § $p < 0.03$ PBMC, peripheral blood mononuclear cells; IHL, TCR, T cell receptor; SD, standard deviation.

with a higher distribution of intrahepatic Vδ1 T lymphocytes (Fig. 2B), suggesting that Vδ1 T cells entering the liver may originate from blood circulation under the influence of chemo-attractant factors (26). Altogether, these observations suggested that Vδ1 T cells may contribute to liver damage through the secretion of proinflammatory cytokines. No correlation between plasma viremia or alanine aminotransferase (ALT) levels was observed (data not shown).

Cytokine Production by Intrahepatic Vd1$^+$ T Cells and Liver Injury In HCV-infected Patients

The pattern of cytokine production displayed by peripheral and intrahepatic Vδ1 T cells was determined at single-cell level by intracellular staining, monitoring the production of IFN-γ and IL-4. Th1 cells promoted inflammatory cellular responses and were biased toward IFN-γ secretion; whereas, Th2 cells were prone to IL-4 production and favored humoral immunity. The percentage of Vδ1$^+$IFN-γ$^+$-producing T cells was calculated among the percentage of total Vδ1$^+$ T cells. As shown in Fig. 3A, a relevant fraction of Vδ1 T lymphocytes released IFN-γ upon stimulation, independent of their origin (liver tissue or peripheral blood). No difference in term of cytokine production by Vδ1 T lymphocytes was observed comparing PBMC from healthy donors with HCV-infected persons (Fig. 3A). However, an increased number of IFN-γ-producing-Vδ1 T lymphocytes was observed in IHL from HCV-infected patients with high liver inflammation (Grading, Gr > 4), compared with peripheral cells or IHL from patients with moderate liver diseases (1 < Gr < 2). These observations confirmed that IHL displayed a higher frequency of Th1 effectors (27,28). In contrast, only a minor fraction of PBMC and IHL produced IL-4 upon stimulation (Fig. 3B). Thus, the inflammed liver of HCV patients was enriched with Vδ1 T lymphocytes displaying a Th1 phenotype.

Characterization of Vδ1 T Cell Phenotype and Vγ-repertoire

To investigate whether the intrahepatic Vδ1 T cells were expressing particular differentiation and

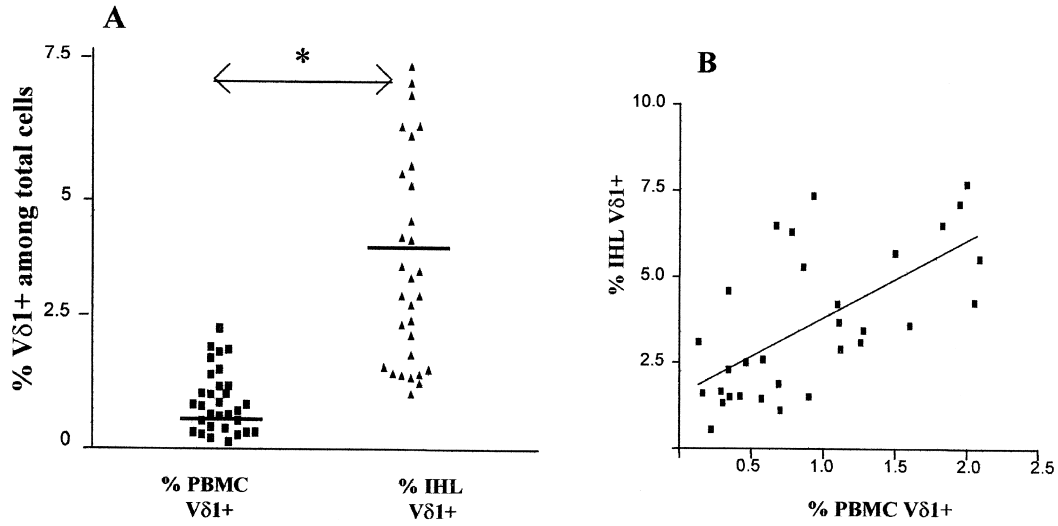

Fig. 2. Vδ1$^+$ T cells compartmentalisation in the liver of hepatitis C virus (HCV)-infected patients. The percentage of Vδ1$^+$ T lymphocytes in peripheral blood mononuclear cells (PBMC) and intrahepatic lymphocytes (IHL) from 31 HCV-infected patients was evaluated by flow cytometry. (A) The percentage of Vδ1$^+$ T cells subset in the blood and liver was analyzed. Each dot represents one HCV-infected patient. Significance refers to Student's *t*-test. *p*-values are indicated as follows: * = $p < 0.001$. (B) The presence of intrahepatic Vδ1 T lymphocytes was correlated with the percentage of peripheral Vδ1 T cells ($r^2 = 0.4221$, $p < 0.0001$). Each dot represents one HCV-infected patient.

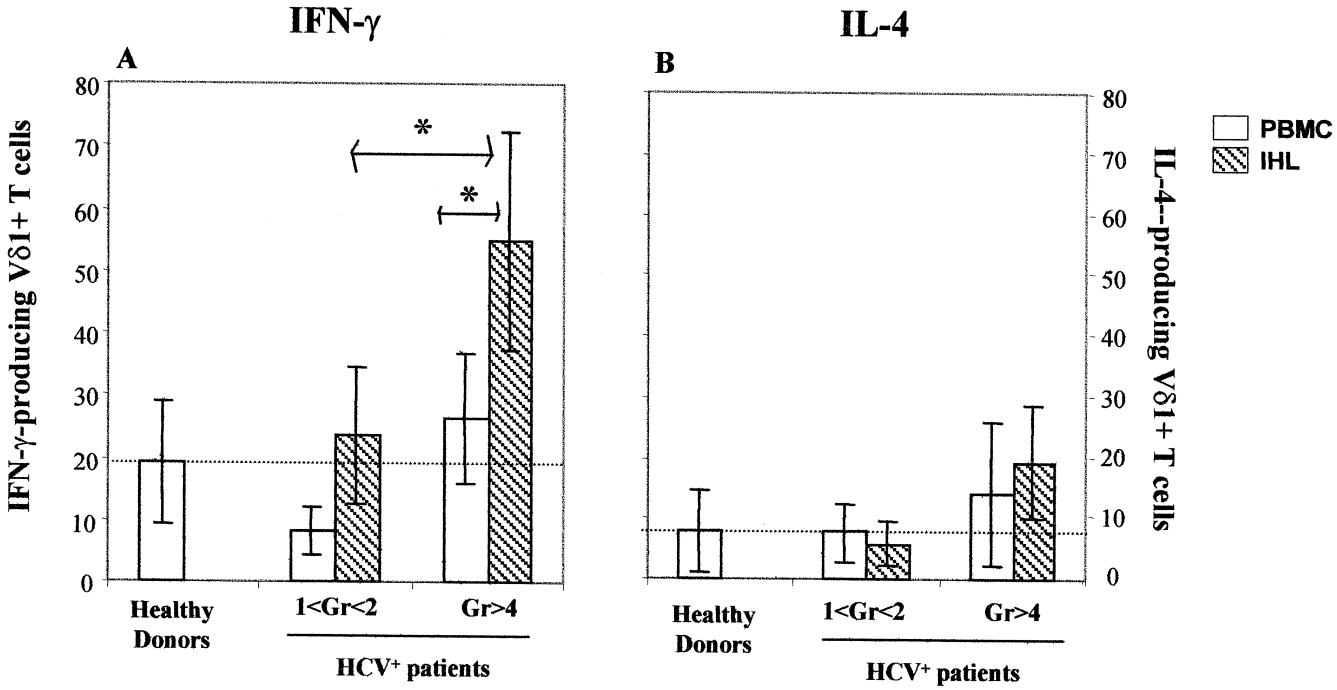

Fig. 3. Cytokine production by peripheral and intrahepatic Vδ1 T lymphocytes. Production of interferon-γ (IFN-γ) and interleukin-4 (IL-4) was determined at the single-cell level by intracellular staining with specific anti-bodies (mAbs) after stimulation with (PMA) and ionomycin. 10^6 peripheral blood mononuclear cells (PBMC) and 2×10^5 intrahepatic lymphocytes (IHL) were stimulated for 6 hr in the presence of brefeldin. (A) The percentage of IFN-γ-producing-Vδ1 T lymphocytes was evaluated in peripheral blood from healthy donors. Moreover, the frequency of Vδ1 T lymphocytes was monitored in PBMC and IHL of hepatitis C virus-positive (HCV+) persons presenting (A) low inflammation ($1 < Gr < 2$, $n = 4$) or (B) high inflammation of the liver ($Gr > 4$, $n = 5$). (B) The same analysis was performed to determine the percentage of IL-4-producing Vδ1 T lymphocytes. Results are expressed as arithmetic mean percentage ± standard deviation (SD) of positive cells among peripheral (white bars) and liver infiltrating (hatched bars) $V\delta1^+$ T cells. Significance refers to Student's *t*-test. *p*-values are indicated as follows: * = $p < 0.04$. Gr: Grading.

activation markers, we used three-color labeling with specific mAbs. In the liver of HCV patients, the majority of Vδ1 T cells lose their naive phenotype typical of peripheral Vδ1 T lymphocytes ($CD62\text{-}L^+$ $CD45\text{-}RO^-$) and clearly were stained as effector/memory cells ($CD62\text{-}L^-$ $CD45\text{-}RO^+$) (Fig. 4A). Accordingly, liver Vδ1 T lymphocytes expressed more CD95 antigen (Fig. 4A), a member of the tumor necrosis factor/nerve growth factor-receptor (TNF/NGF-R) family that is related to programmed cell death (29,30). Moreover, recent studies indicated that CD95 expression on T lymphocytes reflected previous or ongoing antigen-specific activation (31). Both peripheral and liver Vδ1 T cells expressed the T cell activation marker HLA-DR (Fig. 4A), suggesting that this subset already was activated in the peripheral blood of HCV infected persons. Furthermore, the expression of Fas antigen on Vδ1 T lymphocytes was significantly higher in PBMC from HCV+ patients, compared with healthy donors (51.0 ± 15.8% vs. 9.2 ± 7.6, respectively; $p < 0.01$). No differences were observed concerning the expression of other activation/differentiation markers (CD62L, CD45RO, HLA-DR, CD4, CD8; data not shown). Finally, we confirmed that the majority of Vδ1 T lymphocytes lacked the expression of both CD4 and CD8 coreceptors (Fig. 4A), indicating that antigen recognition by γδ T cells was largely MHC-independent.

The TCR repertoire of Vδ1 T cells in the liver of HCV patients then was analyzed to determine whether the increased expression of this subset in the liver was determined clonally. TCR Vδ1 and Vγ usage was assessed at the protein level by flow cytometry on peripheral and liver γδ T cells, using mAbs that specifically recognized each TCR V-γ region. Both in periphery and in the liver, the Vγ-gene segment usage was heterogeneous, with all variable regions represented and a slight predominance of Vγ3.5-Vδ1, Vγ8-Vδ1, and Vγ9-Vδ1 in the liver (Fig. 4B). Altogether, these data indicated that the γδ T cell recruitment in the liver of HCV patients was polyclonal, since the Vγ-usage largely was variable.

Discussion

Chronic hepatitis C usually occurs in more than 70% of HCV-infected persons and frequently is associated with complications, such as cirrhosis and hepatocarcinoma (2). However, the mechanisms in-

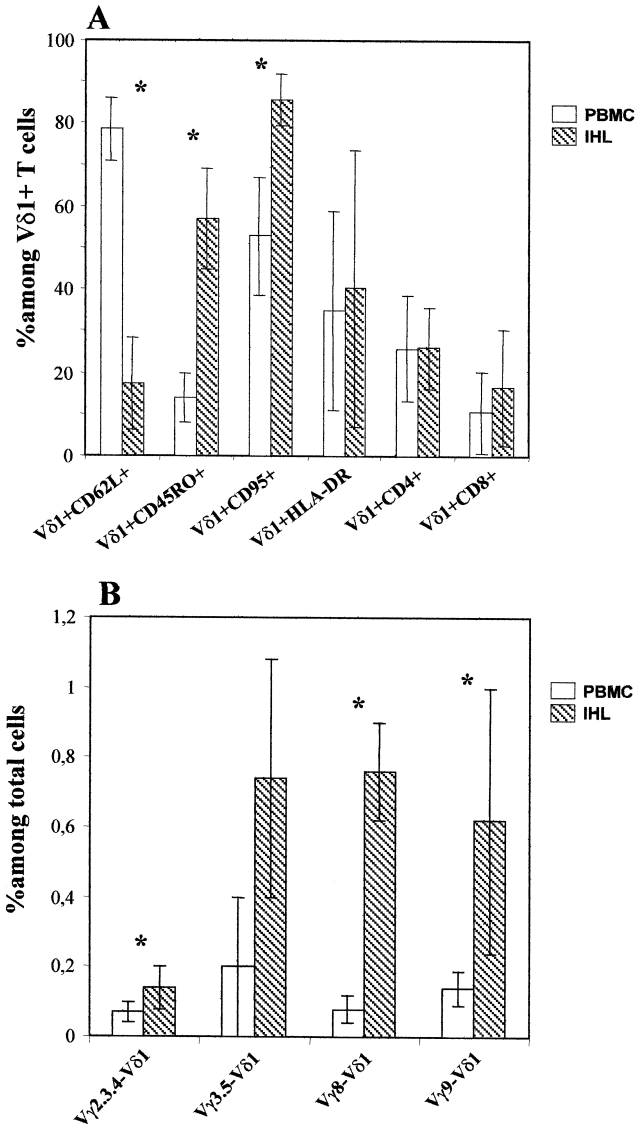

Fig. 4. Characterization of Vδ1 T cell phenotype and Vγ-rearrangements. (A) The expression of several differentiation markers on Vδ1 T cells was evaluated by flow cytometry after three-color labeling with anti-Vδ1, anti-CD62L, anti-CD45-RO, anti-HLA-DR, anti-CD95, anti-CD4, and anti-CD8 antibodies (mAbs). Results are expressed as arithmetic mean percentage ± standard deviation (SD) of positive cells among peripheral (white bars) and liver infiltrating (hatched bars) $V\delta1^+$ T cell subset from 10 hepatitis C virus (HCV)-infected patients. (B) The T cell receptor (TCR) repertoire of peripheral (white bars) and intrahepatic (hatched bars) Vδ1+ T cells was analyzed by flow cytometry using specific mAbs directed against Vγ regions (Vγ2-3-4, Vγ3-5, Vγ8, Vγ9) on six HCV-infected patients. Significance refers to Student's *t*-test. *p*-values are indicated as follows: * = $p < 0.05$. PBMC, peripheral blood mononuclear cells; intrahepatic lymphocytes (IHL).

volved with HCV persistence in the infected host and the factors mediating liver injury are poorly understood. It is likely that both viral and host factors are involved in the pathogenesis of chronic hepatitis C. In contrast to the normal γδ T cell distribution in the peripheral blood, we observed an intrahepatic recruitment of T cells expressing the Vδ1 TCR rearrangement and a memory/activated phenotype. This T cell subset reveals an increased frequency of IFN-γ precursors, compared with peripheral Vδ1 T cells from the same donors. Moreover, the percentage of intrahepatic Vδ1 T cells releasing IFN-γ is higher in the liver of HCV-infected patients going through the necroinflammatory process, suggesting that this T cell subset may contribute to the immunopathology of HCV infection. The stimulatory ligand may to be of cellular (rather than viral) origin, and the proliferative response could be independent of the Vγ-chain co-expressed with Vδ1. The ligand recognized by Vδ1 T lymphocytes is probably a stress antigen of cellular origin (16). Possible ligands are MHC class I-related proteins MICA and MICB that could function as self antigens and are recognized broadly by intestinal epithelial Vδ1 T cells without Vγ chain restriction. Their expression observed on intestinal epithelial cells and hepatocyte-derived cell lines, may be stress-induced, rather than constitutive (16). The stress-induced expression of MICA and the closely related MICB, and their recognition by polyclonal Vδ1 T cells through TCR or the NKG2-D natural killer receptor may serve as an immune surveillance mechanism for the detection of damaged, infected, or transformed intestinal epithelial cells. Our observations support the lack of antigenic selection for the Vγ-chain of the γδ TCR that could be consequent to MICA/TCR and/or MICA/NKG2-D interactions, promoting a polyclonal superantigen-like activation. Moreover, tissue Vδ1 T lymphocytes recently were shown to recognize nonpolymorphic CD1c molecule (17). Specifically, Vδ1 T cells were found to proliferate in response to $CD1^+$-presenting cells, lyse $CD1c^+$ targets, and release Th1 cytokines. Recognition of CD1c is TCR-mediated, as recognition was transferred by transfection of the Vδ1 TCR. The γδ T cell lines and clones show highly specific, direct reactivity to CD1c proteins that is not dependent on the presence of an exogenous foreign antigen. Interestingly, CD1 molecules particularly are expressed in the liver where the hepatocytes express very low levels of classical MHC molecules (32).

A significant increase of systemic Vδ1 T cells without clonal selection previously was observed in HIV infection (33). It is interesting to note that systemic HIV infection induces a Vδ1 mobilization from tissue to the peripheral blood; whereas, the Vδ1 T cell localization is preferentially hepatic during HCV infection. Moreover, the preactivated Vδ1 T cells observed in the peripheral blood of HIV patients always conserve a naive phenotype (33). In contrast, the expression of markers specific for memory cells is a common characteristic of Vδ1 and all intrahepatic T cells in HCV+ patients, confirming that CD62-L selectin is lost by lymphocytes coming from

bloodstream circulation to the hepatic tissue. These effector cells tend to home in the region of infection by recognizing the altered vascular endothelium and chemoactractant molecules that are generated during the inflammatory response (26). Parallely, these intrahepatic T cells acquire the CD45-RO antigen, suggesting antigen-mediated stimulation. Whether the compartmentalization of memory Vδ1 T lymphocytes in the liver of HCV-infected persons may be consequent to antigen exposure or to chemoattractant factors remain to be seen.

A critical event in the inflammatory process is the recruitment and migration of a discrete subpopulation of T cells to the site of infection. IHL can play two opposite role on HCV-infection: in the acute phase, they could be critical for the resolution of the disease (very rare); whereas, in the chronic phase, the IHL could be dangerous, damaging, infected and uninfected hepatocytes. Accordingly, we observed an intrahepatic recruitment of Vδ1 T lymphocytes in patients with chronic hepatitis C infection, displaying an increased frequency of IFN-γ-releasing Vδ1 T cells that parallel liver inflammation. IFN-γ is able to exert a potent antiviral function. However, it is already known that this cytokine may exert potent cytotoxic effect on hepatocytes. Thus, the overall role of Vδ1 T cells homing in the liver of chronic HCV-infected patients produces pathogenic, rather than protective, results. The elucidation of the MHC-unrestricted mechanisms involved in the activation and homing of Vδ1 T cells during chronic hepatitis C infection may help with understanding the immunopathology of this disease.

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