Intrahepatic Expression of Interleukin-1β and Tumor Necrosis Factor–α in Chronic Hepatitis C

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The intrahepatic expression of interleukin (IL)–1β and tumor necrosis factor (TNF)–α was studied in liver specimens from patients with chronic hepatitis C (n = 29) and primary biliary cirrhosis (PBC; n = 12) and from normal controls (n = 19). IL-1β and TNF-α immunoreactivity was predominantly localized in sinusoidal cells, with IL-1β immunoreactivity being weaker in chronic hepatitis C samples than in PBC or control samples, whereas no difference in staining intensity could be observed for TNF-α. On semiquantitation by reverse transcription/competitive polymerase chain reaction, IL-1β mRNA levels were significantly lower in chronic hepatitis C than in PBC or control samples (chronic hepatitis C, 0.87 ± 0.77; PBC, 7.96 ± 3.32; control, 3.78 ± 2.56 amole IL-1β mRNA/fmole β-actin mRNA; P < .001). In contrast, no significant differences in TNF-α mRNA levels were observed between the groups. The data suggest insufficient IL-1β production by sinusoidal cells in chronic hepatitis C, which might facilitate viral persistence.

Hepatitis C virus (HCV) is the major cause of non-A, non-B hepatitis and leads to chronic liver disease with persistent infection in the majority of patients [1]. Viral persistence may be facilitated by selective defects in the host’s immune response [2]. Thus, it could be shown that patients with viral replication display low HCV antigen–specific T cell responses, whereas strong antigen-specific responses were associated with a more benign course of disease [3]. Data from other chronic viral infections suggest that impaired function of antigen-presenting cells, in particular a low monokine production, may be an important pathogenetic factor for viral persistence [4]. In support of this hypothesis, resolution of chronic viral hepatitis during interferon treatment is associated with increased production of interleukin (IL)–1β and tumor necrosis factor (TNF)–α [5], further emphasising the importance of these cytokines in the control of viral hepatitis.

In patients with chronic hepatitis C, a reduction of both spontaneous and stimulated release of monokines has been observed in peripheral blood mononuclear cells [6–8]. In addition, both a reduced monokine production and an impaired monocyte antigen presentation have been reported for HCV-seropositive, hemophilic boys [9]. However, there is also evidence for a compartmentalization of specific immune cells to the liver in chronic hepatitis C [10], and thus the studies on peripheral cells may not necessarily reflect the events at the site of inflammation. We have therefore studied the intrahepatic production of IL-1β and TNF-α in liver specimens from patients with chronic hepatitis C by use of immunohistochemistry to determine the cellular source and reverse transcription/competitive polymerase chain reaction (PCR) to semiquantitate steady state mRNA levels of these cytokines.

Materials and Methods

Tissue specimens. Liver-tissue specimens from patients with chronic hepatitis C (n = 29) were obtained during routine diagnostic liver biopsies with a 1.4-mm Menghini needle after informed consent was obtained from the patients. Tissue from patients with primary biliary cirrhosis (PBC; n = 12) was obtained from explant livers at the time of transplantation. Normal liver specimens (n = 19) were from unaffected areas of liver resections for secondary hepatic malignancy (10/19) and from cadaveric donors at the time of hepatectomy for liver transplantation (9/19). Tissue specimens were snap frozen in liquid nitrogen either as native tissue for RNA extraction or embedded in Tissue Tek OCT compound (Miles Laboratories, Naperville, IL) for immunohistochemistry.
**Patient characteristics.** Patients with chronic hepatitis C were consistently positive for anti-HCV antibodies (Matrix-ELISA; Abbott Laboratories, Wiesbaden, Germany) and for HCV-RNA. Risk factors were hemophilia (n = 11), intravenous drug use (n = 7), blood transfusion (n = 2), or unknown (n = 9). The mean virus load was 10.7 × 10^6 copies/mL (Amplicor HCV Monitor; Roche Molecular Systems, Branchburg, NJ). HCV genotypes were 1a (n = 9), 1b (n = 11), 2a (n = 2), 3a (n = 1), and mixed (n = 3; genotypes 1a/1b; 1a/2a; 2a/3a) as determined by the Inno-Lipa HCV II line probe assay (Innogeneics, Zwijndrecht, Belgium); in 3 cases further virological data were unavailable. All HCV patients were negative for markers of hepatitis B virus or human immunodeficiency virus coinfection and showed liver histologic evidence of chronic hepatitis. The diagnosis of advanced PBC had been made according to internationally accepted criteria, including antimitochondrial antibodies and liver histologic findings; in the normal control group, there was no clinical, laboratory, serologic, or histologic evidence of chronic liver disease, including markers of viral hepatitis.

**Immunohistochemistry.** Immunostaining was performed on 4 randomly chosen tissue samples from each group. Briefly, cryosections of 4–7 μm were stained by an indirect immunoperoxidase technique after blocking of endogenous peroxidase activity with 0.03% H2O2/NaNO2 (Peroxidase Blocking reagent; Dako, Carpinteria, CA). The sections were incubated with the primary antibodies for IL-1β (mouse monoclonal ILB1-H67 and rabbit polyclonal P420B; Endogen, Woburn, MA) or for TNF-α (mouse monoclonal ICC TNF 10B and rabbit polyclonal ICT TNF 5a; IC Chemikalien, Ismaning, Germany), and tissue sections were counterstained with Meyer's hematoxylin (Sigma). Irrelevant mouse monoclonal antibodies (IgG1 and IgG2; Becton Dickinson, Heidelberg, Germany) were used as isotype controls.

**RNA extraction, reverse transcription, and competitive PCR.** Semiquantitation of intrahepatic mRNA species was performed as described elsewhere [11]. Briefly, after extraction of total cellular RNA from tissue specimens (Trizol; Life Technologies, Eggenstein, Germany), random primed reverse transcription was done with 1 μg total RNA with 200 U reverse transcriptase (Superscript Plus RT; Life Technologies). A cDNA equivalent to 50 ng of total RNA was then amplified for 27 (β-actin) or 30 (IL-1β, TNF-α) cycles, respectively, along with 312 (β-actin) or 1.22 amole (IL-1β, TNF-α) of the multispecific competitor fragment pHCQ2 (a generous gift from Dr. C. Platzer, University of Jena, Germany). PCR products were size fractionated on agarose gels and visualized under ultraviolet light after ethidium bromide staining. Control experiments were conducted to rule out contamination with genomic DNA or amplification products.

**Calculation of steady state mRNA levels and statistics.** Band intensities of competitor and sample amplicon were quantified directly from agarose gels by use of a video camera imaging and densitometry software system (Intas, Göttingen, Germany). For each group, levels of IL-1β and TNF-α mRNA are given in attomoles per femtomole of β-actin mRNA as mean ± SD. The normalization relative to the housekeeping gene β-actin allowed a correction for variations in the efficiency of RNA extraction and reverse transcription. Statistics were calculated by use of the SPSS for Windows software (SPSS, Chicago). After checking for normal distribution, the mean mRNA levels from each group were compared by Student’s t test. Correlations were calculated as Pearson’s correlation coefficients.

**Results**

**Localization of intrahepatic IL-1β and TNF-α immunoreactivity.** Immunohistochemistry showed that IL-1β immunoreactivity was predominantly localized to sinusoidal cells. In PBC, heterogeneously distributed granulomas were an additional source of IL-1β (data not shown). Compared with the samples from normal controls and patients with PBC, IL-1β immunoreactivity on sinusoidal cells was only faint in all 4 specimens from patients with chronic hepatitis C (figure 1A–1C). Likewise, TNF-α immunoreactivity was present mainly on sinusoidal cells, although no difference in the overall TNF-α immunoreactivity could be detected between the groups (data not shown).

**Semiquantitation of intrahepatic IL-1β and TNF-α mRNA levels.** Semiquantitative analysis of intrahepatic mRNA levels showed significantly lower IL-1β intrahepatic steady state mRNA levels in samples from chronic hepatitis C than in samples from normal controls. In contrast, intrahepatic IL-1β mRNA levels were significantly higher in the disease control group than in normal controls (chronic hepatitis C, 0.87 ± 0.77; disease controls, 7.96 ± 3.32; normal controls, 3.78 ± 2.56 amole IL-1β mRNA/fmole β-actin mRNA; P < .001; figure 2). In contrast, there were no statistically significant differences in the steady state levels of TNF-α mRNA between the groups (chronic hepatitis C, 2.03 ± 1.12; PBC, 2.17 ± 1.09; controls, 2.37 ± 1.68 amole TNF-α mRNA/fmole β-actin mRNA; figure 2). Within the control group there were no significant differences in the cytokine mRNA levels from donor liver versus liver resection. In particular, mean intrahepatic IL-1β mRNA levels from the HCV group were still significantly (P < .001) lower when compared with the mean IL-1β mRNA levels from the subgroups of either donor liver (4.43 ± 2.90 amole IL-1β mRNA/fmole β-actin mRNA) or liver resection tissue (3.19 ± 2.19 amole IL-1β mRNA/fmole β-actin mRNA). Although a weak correlation could be calculated for intrahepatic IL-1β mRNA levels and virus load (r = .55; P = .04) and IL-1β and TNF-α mRNA levels (r = .24; P = .06), there were no significant correlations between intrahepatic monokine mRNA levels and patient characteristics (age, risk factor), aminotransferase levels, or HCV genotype.

**Discussion**

In the present study, we have identified sinusoidal cells as the major source of hepatic IL-1β immunoreactivity and have demonstrated lower IL-1β immunoreactivity on sinusoidal cells...
Figure 1. Immunostaining for interleukin (IL)–1β in liver specimens from patients with chronic hepatitis C (A), primary biliary cirrhosis (PBC) (B), and normal controls (C). In all samples immunoreactivity is predominantly localized to sinusoidal cells. However, as compared with samples from patients with PBC (B) and normal controls (C), only faint IL-1β immunoreactivity was observed in all 4 specimens from patients with chronic hepatitis C (A). Magnification, ×250.
in liver tissue from patients with chronic hepatitis C than in samples from patients with PBC or normal controls. In support of this, intrahepatic IL-1β steady state mRNA levels were significantly lower in samples from both control groups. In contrast, no such differences were found with respect to intrahepatic TNF-α immunoreactivity or mRNA levels.

The finding of low intrahepatic IL-1β production in chronic hepatitis C most likely reflects a reduced IL-1β production rather than inadvertent IL-1β induction in our normal controls. To exclude the latter possibility, liver tissue from different sources (donor livers and liver resections) had been included as a normal control group, and no significant difference in the mean IL-1β mRNA levels was observed between the normal specimens from different origins. The significance of our observation is further emphasized by the fact that intrahepatic IL-1β steady state mRNA levels and immunoreactivity in chronic hepatitis C were lower in the presence of an inflammatory infiltrate, whereas higher IL-1β levels were seen in the normal control group where inflammatory changes were absent. Thus, our data suggest that, in addition to previously published data from peripheral blood [6-8], there is also an impaired IL-1β production by sinusoidal cells (e.g., Kupffer cells, stellate cells) in chronic hepatitis C.

The data presented here are in contrast to previous studies reporting an increased TNF-α production [12] or increased intrahepatic TNF-α mRNA levels in chronic hepatitis C [13]. These differences may have resulted from several factors. Thus, a different composition of the study population must be considered, in particular because our study included a relatively large (11/29) number of hemophiliacs. In addition, the methods of in situ hybridization [12] and reverse transcription/noncompetitive PCR [13], which were used in other studies, must be regarded as less precise for the determination of mRNA levels than the reverse transcription/competitive PCR method employed here.

The reasons for the impaired IL-1β production in chronic hepatitis C both from peripheral blood and from sinusoidal cells are unclear. A viral infection of monocytes/macrophages with consecutive downregulation of IL-1 production at the transcriptional or translational level has been proposed as a possible mechanism [6]. In line with this hypothesis, the HCV core protein has been shown to repress various cellular promoters, including those of cytokine genes [1]. However, such an effect is less likely to account for the apparently reduced intrahepatic IL-1β production in chronic hepatitis C, because there is only scant evidence for an infection of sinusoidal cells by HCV [14]. Therefore, other more indirect mechanisms might be operative.
Because of its pleiotrophic effects, impaired IL-1β production will be of strategic importance to the outcome of infectious diseases [15]. Given the importance of IL-1 as a costimulus for the propagation of T cell responses [15], a reduced IL-1β production might at least in part contribute to the defective immune functions reported in chronic hepatitis C [2].

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References