JGH Journal of Gastroenterology and Hepatology JGHF

doi:10.1111/jgh.12543

HEPATOLOGY

Regulation of T cell recruitment and inflammation in the human immunodeficiency virus/hepatitis C virus coinfected liver

Nam Nguyen,* Taline Hampartzoumian,* Barbara Cameron,* Clovis Palmer,* Sandra O'Toole,[†,‡] Jeffrey Post,[§] Amany Zekry*[,¶] and Andrew Lloyd*[,§]

*Inflammation and Infection Research Centre, School of Medical Sciences, ¶St George Hospital Clinical School, University of New South Wales, Sydney, †Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, ‡Department of Anatomical Pathology, Royal Prince Alfred Hospital, Camperdown, University of Sydney, Sydney, and §Department of Infectious Diseases, Prince of Wales Hospital, Randwick, New South Wales, Australia

Key words
chemokines, HIV/HCV coinfection, immunohistochemistry, liver, T cells.

Accepted for publication 13 January 2014.

Correspondence
Dr Barbara Cameron, Centre for Infection and Inflammation Research, School of Medical Sciences, University of NSW, Sydney, NSW 2052, Australia. Email: b.cameron@unsw.edu.au

Abstract

Background and Aim: Patients coinfected with both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) have accelerated liver disease compared with HCV mono-infected patients. In chronic HCV infection, it is known that chemokines play a key role in T cell recruitment and in determining the extent of hepatic injury.

Methods: In this study, we determined by quantitative real-time reverse transcriptase polymerase chain reaction and immunohistochemistry the intrahepatic phenotype of the cellular infiltrate and its associated chemokine profile and localization in a cohort of relatively immune competent coinfected HIV/HCV subjects.

Results: Increased lobular expression of CD8+ cytotoxic T cells was found in the coinfected liver in conjunction with increased expression of the T cell chemoattractant, chemokine (C-C motif) ligand (CCL)5, compared with the HCV mono-infected liver. Furthermore, the number of lobular-infiltrating CD8+ T cells was positively correlated with the expression of CCL5. Immunohistochemical staining of CCL5 showed it to primarily localize to the hepatocytes. Within the inflammatory infiltrate, proliferating (Ki-67+) and apoptotic terminal deoxynucleotidyl transferase 2′-deoxyuridine 5′-triphosphate nick end labeling + (TUNEL)+ cells were sparse.

Conclusions: Collectively, the data suggest that even in the setting of relatively immune competent coinfected subjects, a pro-inflammatory milieu exists ,which can potentially drive the increased T cell recruitment found in the HIV/HCV coinfected liver. This profile is likely to contribute to the accelerated progression of liver disease observed in HIV/HCV coinfection.

Introduction

Liver disease in subjects coinfected with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) is characterized by increased necro-inflammation and fibrogenesis, leading to increased mortalitybecause of liver failure and hepatocellular carcinoma.[1] Although some reports have suggested that the HCV viral load in HIV/HCV coinfected patients is increased, HCV itself is not considered to be cytopathic.[2] It is widely accepted that the dominant mechanism of HCV-induced liver injury is primarily immune-mediated, yet necro-inflammatory scores and fibrosis grades have been shown to be higher in HIV/HCV coinfected patients. Moreover, accelerated fibrosis is most evident in the setting of decreased peripheral CD4+ T cell counts. Thus, effective antiretroviral therapy leading to CD4 T cell recovery has been shown to result in slower progression of fibrosis. However, overall, the mechanisms of accelerated necro-inflammatory activity in the HIV/HCV coinfected liver remain unclear.[3]

Liver injury in chronic HCV is characterized by T cell infiltration into the portal and lobular compartments. These T cells are believed to be recruited from the circulation via chemokine-dependent pathways, in which the chemokine (C-C motif) ligand (CCR)5 and C-X-C motif chemokine receptor (CXCR)3 ligands are heavily implicated (reviewed in Heydtmann and Adams[4]). In the setting of HIV/HCV coinfection, differential localization of T cells within the liver has been observed. In this regard, profound depletion of periportal CD4+ T cells has been demonstrated histologically.[5] Additionally, other studies have shown by reverse transcriptase polymerase chain reaction (RT-PCR) that the HIV/HCV coinfected liver was enriched with CD8+ T cells, along with increased expression of chemokines such as chemokine (C-C motif) ligand (CCL)3, CCL5, and C-X-C motif chemokine ligand

(CXCL)10.[6] The data suggest that a different chemokine milieu operates in the liver of HIV/HCV coinfection to that seen in HCV alone. In parallel, a question arise whether the enrichment of T cells in the liver of coinfected subjects is due to a viral mediated imbalance in the proliferation/apoptosis pathways of T cells per se or whether it is primarily dependent on chemokine recruitment. Thus, further characterization of the hepatic inflammatory infiltrate and associated pro-inflammatory mediators is likely to help us gain insight into the molecular events underlying the progression of liver disease in the HIV/HCV group.

In this report, we have compared the phenotype of resident liver cells (lymphocytes and macrophages) in a relatively immune-competent cohort of HCV/HIV coinfected subjects compared with HCV mono-infected ones. In parallel, we determined by quantitative RT-PCR (qRT-PCR) and immunohistochemistry staining the associated expression of key chemokines mediating lymphocyte recruitment to the liver. We further utilized immunohistochemistry to localize relevant chemokines and enumerate CD3+, CD4+, and CD8+ T cells and CD68+ macrophages. In addition, proliferating and apoptotic leukocytes were examined by immunohistochemistry to estimate the relative contributions of recruitment, proliferation, and survival to the inflammatory infiltrate in the liver.

Methods

Subjects and samples. Liver biopsy samples were obtained from eight HIV/HCV coinfected and 16 HCV mono-infected patients prior to anti-HCV treatment (Table 1). Each coinfected case was matched with two HCV mono-infected control patients based on liver histopathology, as read by a pathologist masked to their disease status, including the grade (necro-inflammation) and stage (fibrosis) as determined by the Scheuer score.[7] All patients provided written informed consent. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review board.

qRT-PCR. Total RNA was extracted from snap frozen liver with Trizol (Invitrogen, Carlsbad, CA, USA). Messenger RNA expression of CCL3, CCL5, CXCL10, and interferon (IFN)-γ gene transcripts were amplified by qRT-PCR from all coinfected and 16/18 HCV mono-infected liver RNA samples. Five hundred nanograms of RNA was treated with Turbo DNase (Ambion, Austin, TX, USA) before complementary (c)DNA synthesis with SuperScript III kit (Invitrogen, Melbourne, Australia). qRT-PCR amplification of target gene transcripts were conducted in duplicate in 96-well plates with the Roche Light Cycler 480 (Roche Diagnostics, Basel, Switzerland). Primer sequences and PCR cycling conditions were performed as previously described.[8] Gene expression was analyzed using the ΔCt relative to the expression of the housekeeping gene β-actin.

Table 1 Clinical features of the subjects in the study

	HIV/HCV coinfected†	HCV mono-infected†
Number of subjects‡	8	16
Gender (M/F)	8/0	11/5
Age	40.5 (32–53)	38.4 (22–47)
HCV load	3.7×10^5–6.9×10^7	7×10^5–6.9×10^7
HCV genotype	1 and 4	1a, 3b, and 3a
HIV load	0–341	
CD4+ T cells/mm^3	544 (240–870)	
Total inflammatory score (0–8)	3.4 (1–7)	3.8 (1–5)
Fibrosis score (0–4)	1.8 (0–4)	1.8 (0–4)
Serum AST (U/L)	102.5 (33–202)	93.5 (6–308)
Serum ALT (U/L)	207.5 (36–497)	135.8 (28–462)

†Mean and standard deviation values are shown where applicable.
‡Subjects were matched according to their histopathology scores.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus; HIV, hepatitis C virus.

Immunohistochemistry. Immunohistochemical studies to quantitate the expression of chemokines, and the number of CD3, CD4, CD8, CD68, and Ki-67 (a marker of cell proliferation) positive cells were performed in all coinfected and 15/16 HCV mono-infected samples. Antigen retrieval was performed by microwave heating in either 10 mmol/L citrate buffer pH 6.0 or 1 mM Tris-EDTA buffer pH 9.0 for 20 min. Endogenous peroxidase and biotin was blocked with 3% H_2O_2 and with a biotin blocking kit (DAKO, Glostrup, Denmark) followed by a serum block. Primary antihuman antibodies for CCL3 (14 μg/mL, R&D Systems, Minneapolis, MN, USA), CCL5 (10 mg/L, R&D Systems), and CXCL10 (7.5 mg/L, R&D Systems) were incubated for 2 h at room temperature, while the CD3 (1:50, DAKO), CD4 (10 μg/mL, R&D Systems), CD8 (clone C8/144B, 1:50, DAKO), CD68 (clone EBM11, 1:50, DAKO), and Ki-67 (clone MIB-1, 8 mg/L, DAKO) antibodies were incubated for 1 h. Negative control sections were incubated with matched species immunoglobulin G isotype antibodies that were substituted for the primary antibodies. Bound complexes were visualized with 3,3′-diaminobenzidine (DAB) before counterstaining with hematoxylin and cover-slipping.

Terminal deoxynucleotidyl transferase 2'-deoxyuridine 5'-triphosphate nick end labeling (TUNEL) assay. Apoptotic cells were detected in tissue sections with the In Situ Cell Death Detection kit (Roche Diagnostics) that labels the terminal end of nucleic acids in fragmented DNA with 2′-deoxyuridine 5′-triphosphates conjugated with fluorescein isothiocyanate. DNase-treated sections were used as positive controls, while negative controls were incubated with buffer without terminal deoxynucleotidyl transferase. The sections were then cover-slipped with a 4′,6-diamidino-2-phenylindole-containing mounting medium (DAKO) and visualized with fluorescent microscopy.

Morphometric analysis. Intrahepatic CD3+, CD4+, and CD8+ T cells and CD68+ cells were enumerated in portal and lobular regions provided that each section stained for the individual leukocyte marker had more than five portal and three lobular high power (400×) fields. Expression levels of chemokine proteins was assessed and scored by an independent histopathologist (S.O.) masked to the HIV status of the samples. The score was based on the semiquantitative enumeration of positively stained hepatocytes, mononuclear cells, and other cells (biliary epithelia and endothelia) as well as an index of the intensity of the staining.

Statistical analysis. The Mann–Whitney *U* test was used for statistical comparisons between patient groups. Correlations were calculated using the Spearman's test (Prism Version 4.0; GraphPad, San Diego, CA, USA).

Results

Clinical characteristics of study subjects. The two subject groups were comparable in age, HCV viral load, histological inflammatory, and fibrosis scores (Table 1). Slightly higher, but not statistically significant, serum transaminase levels were found in HIV/HCV coinfected patients but did not correlate with T cell infiltration or chemokine levels. The HIV/HCV coinfected patients were relatively immune-competent with a median CD4+ T cell count of 544 cells/μL, and all had low or undetectable HIV viral loads (attributable to the combination antiretroviral [cART] therapy that all were receiving).

Liver infiltration of CD3+, CD4+, CD8+ T cells, and CD68+ macrophages. All leukocyte subpopulation markers, including for the T cell subsets (CD3, CD4, and CD8) and macrophages (CD68), showed immunoreactivity in the both the portal and lobular compartments in both HIV/HCV coinfected and HCV mono-infected liver samples (Fig. 1). The portal tracts were infiltrated by both CD4+ and CD8+ T cell subsets and macrophages at variable density. In contrast, the lobules were primarily infiltrated by macrophages, with CD8+ T cells as the predominant T cell subset.

The results of the morphometric analysis were consistent with the visual observations (Fig. 2). When compared with HCV mono-infected patients, lobular CD8+ T cell counts were significantly higher in the coinfected group (median count 34 cells/mm^2 *vs* 9 cells/mm^2, $P = 0.0082$). A somewhat higher number of CD4+ and CD68+ cells were also found in the lobules in the HIV/HCV coinfected liver samples, but this was not statistically significant (median count 13 and 338 cells/mm^2 *vs* 3 and 216 cells/mm^2, $P = 0.2345$ and $P = 3152$), respectively. CD8+, CD4+ T cells, and macrophage numbers were not significantly different in the portal tracts.

Intrahepatic expression of CXCL10, CCL3, and CCL5 mRNA. Semiquantitative analysis of intrahepatic mRNA expression of the T cell chemoattractants, CXCL10, CCL3, and CCL5 was performed by qRT-PCR in all HCV-infected patients. When compared with mono-infected subjects, coinfected patients had significantly higher intrahepatic expression of CCL5 mRNA ($P = 0.032$), but not CCL3 or CXCL10 mRNA (Fig. 3).

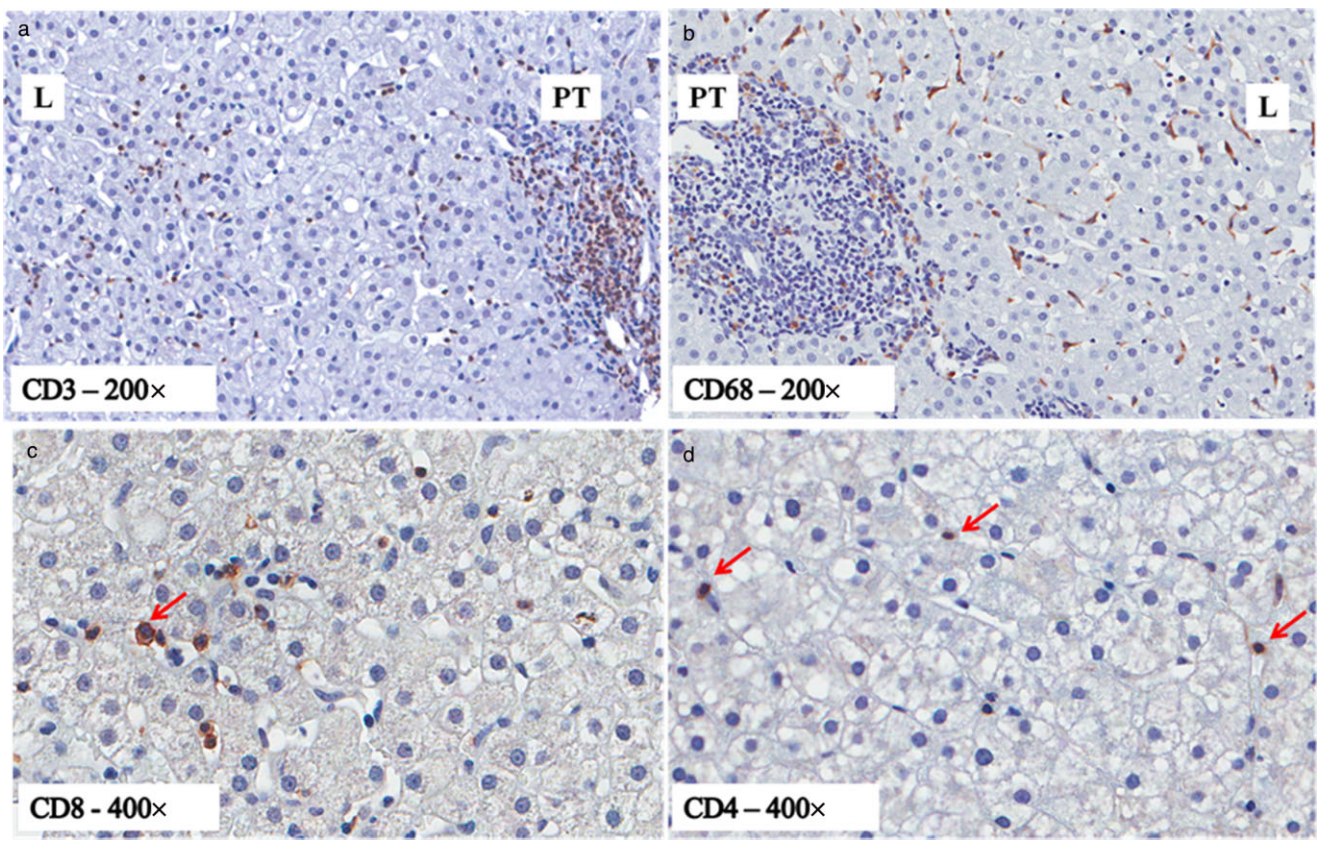

Figure 1 Immunohistochemical staining of CD3 (a), CD68 (b), CD8 (c), and CD4 (d) of hepatitis C virus (HCV)-infected patients. Infiltration of CD3+ T cells and CD68+ macrophages, identified by the brown 3,3′-diaminobenzidine chromogen (a,b, 200×), were found within the portal tracts (PTs) and lobules (L) in the liver of both human immunodeficiency virus/HCV coinfected and HCV mono-infected subjects. Similarly, cytotoxic CD8+ and helper CD4+ T cells (red arrows) were found in the portal tracts and lobules (c,d, 400×) of both coinfected and mono-infected patients.

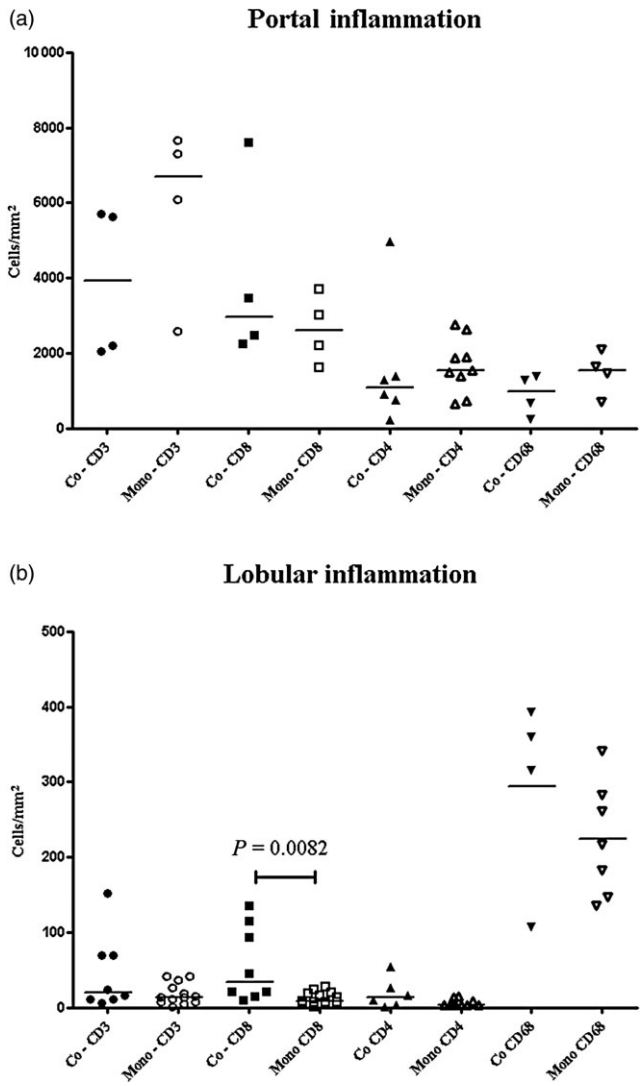

Figure 2 Morphometry results for CD3, CD8, CD4, and CD68 immunohistochemistal staining within the portal tracts (a) and lobules (b) of human immunodeficiency virus/hepatitis C virus (HCV) coinfected (closed shapes) and HCV mono-infected patients (open shapes). All leukocytes identified by positive immunoreactivity in the liver were counted and the area measured. The portal tracts were densely infiltrated with CD3+ T cells, including both CD4+ and CD8+ subsets, at a higher frequency than CD68+ macrophages (a). No differences were found in the number of the leukocyte subsets of any subset in portal tracts of coinfected and mono-infected patients. In contrast, CD8+ T cells were the predominant T cell subset within the lobules, and macrophages were the predominant cell type. In addition, a significantly higher number of CD8+ T cells were found in the coinfected liver samples compared with the mono-infected liver ($P = 0.082$).

Intrahepatic IFN-γ mRNA in HIV/HCV coinfection. The intrahepatic expression of the type I cytokine IFN-γ mRNA was not significantly different ($P = 0.1246$) in the HIV/HCV coinfected patients compared with the HCV mono-infected patients (data not shown).

Localization of intrahepatic chemokine expression in HIV/HCV coinfection and HCV mono-infection. Intrahepatic expression of CXCL10, CCL5, and CCL3 was detected by immunohistochemistry (Fig. 4). CXCL10 was abundant, featuring the most extensive and highest intensity staining in hepatocytes of the three chemokines studied (Fig. 5a). Occasional CXCL10 expression in mononuclear cells and biliary epithelia was also found. CCL5 staining was also localized mostly to hepatocytes, followed by mononuclear cells and biliary epithelia at variable frequencies and intensities (Fig. 5c,d). CCL3 expression was also found predominantly in hepatocytes and occasional infiltrating mononuclear cells, but at a much lower intensity in hepatocytes when compared with staining for CXCL10 and CCL5 (Fig. 5b). All isotype control-stained sections and normal liver sections showed no DAB staining, indicating that the chemokine staining was specific.

Semiquantitative analysis of intrahepatic CXCL10, CCL5, and CCL3 protein expression on immunostained sections revealed no statistical significance in the level of expression of these chemokines between the coinfected and the mono-infected group.

Correlations and associations between all T cell subsets and CD68+ cells with chemokine IFN-γ expression. We examined the association between the extent of T cell and macrophage infiltration (numbers) of the liver in relation to chemokine and IFN-γ expression. The number of CD3+ T cells infiltrating the lobules was correlated with the mRNA expression of CCL5 ($P = 0.04$, $r > 0.55$, Fig. 5a) and CXCL10 ($P = 0.043$, $r > 0.55$, Fig. 5c). Furthermore, CCL5 mRNA was correlated with lobular CD8+ T cell numbers ($P = 0.047$, $r > 0.55$, Fig. 5b) and also showed a positive trend with the number of portal CD8+ T cells ($P = 0.06$, data not shown). In addition, CD4+ T cell numbers were correlated with IFN-γ mRNA expression ($P = 0.008$, $r > 0.55$, Fig. 5d).

T cells apoptosis and proliferation in HIV/HCV coinfection compared with HCV mono-infection. We also wanted to determine whether the enrichment of T cells within the liver of coinfected subjects could be due to an imbalance in the apoptosis/proliferation process. Assessment of TUNEL-stained and Ki-67 expression showed a very low frequency of mononuclear cells that were either apoptotic or proliferating in both subject groups (Fig. 6). There was no difference in the extent of intrahepatic T cell or hepatocyte apoptosis or proliferation between groups.

Discussion

In this study, we have examined the intrahepatic localization and expression of T cell subsets and chemokines in a relatively immune competent group of HIV/HCV coinfected patients compared with those with HCV mono-infection. We have observed increased lobular CD8+ T cell infiltration and expression of CCL5 mRNA in the HIV/HCV coinfected liver. In addition, CCL5 and CXCL10 were widely expressed by hepatocytes in the HIV/HCV coinfected livers. Furthermore, CCL5 mRNA expression was strongly correlated with lobular CD8+ T cell infiltration, while

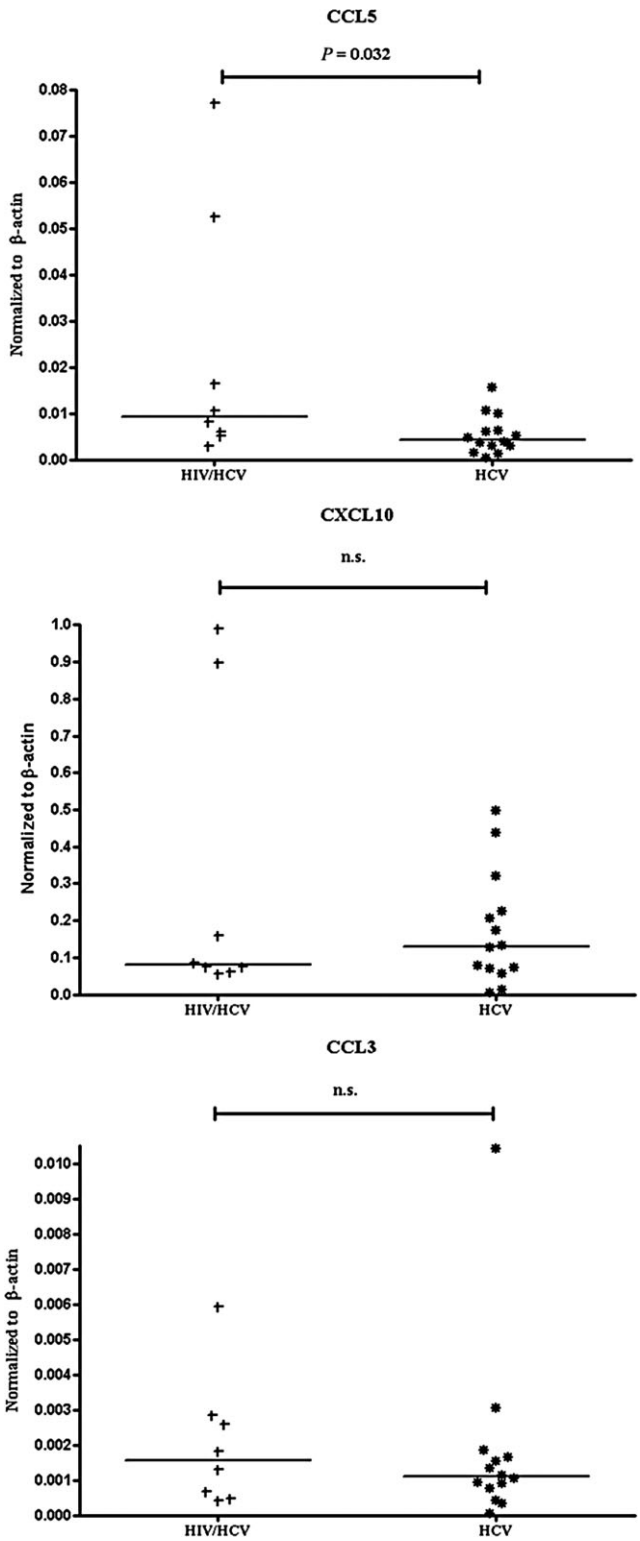

Figure 3 Intrahepatic chemokine messenger RNA (mRNA) expression in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection compared with HCV mono-infection. Chemokine mRNA expression was quantified in HCV mono-infected and HIV/HCV coinfected patients and normalized to the expression of the housekeeping gene, β-actin. The chemokine with the highest expression level was C-X-C motif chemokine ligand (CXCL)10, followed by chemokine (C-C motif) ligand (CCL)5 and CCL3. There was a significant increase in CCL5 mRNA ($P = 0.032$) expression in HIV/HCV coinfected samples compared with HCV mono-infected liver samples. No statistically significant differences were found between groups for either CXCL10 or CCL3 mRNA expression.

lobular CD4+ T cell numbers were positively correlated with IFN-γ. Finally, the processes of apoptosis and cell proliferation were not enhanced in the coinfected liver samples. These data indicate that liver injury in the setting of coinfection with HIV and HCV is characterized by increased CD8 T cell infiltration and is likely to be driven by chemokine expression.

The major limitation of this study is the small number of subjects; thus, a type 1 error is possible, and also the two subjects with very high CD8+ T cell infiltration skew the correlations. However, the enrichment of CD8+ T cells in the lobules of HIV/HCV coinfected patients is noteworthy as the patient groups studied here were closely matched by necroinflammatory grade. Previously, intrahepatic mRNA expression of the T cell markers, CD3ε, TCRα, CD8α, and CD8β was reported to be increased in HIV/HCV coinfection in comparison with HCV mono-infection and was more pronounced in coinfected patients who were not receiving cART.[6] Similarly, an increase in CD3 gene expression was more pronounced in HIV/HCV coinfected patients who were naïve to antiretroviral therapy, compared with treated coinfected patients and HCV mono-infected patients.[9] Furthermore, an increased ratio of intrahepatic CD8+ : CD3+ T cells have also previously been found in HIV/HCV coinfected patients.[10] Collectively, these data clearly show that HIV/HCV coinfection is associated with an increase in CD8+ T cell infiltration into the liver. Although CD8+ T cells did not directly correlate with histological markers of liver injury, it is known that cytotoxic CD8+ T cells suppress HCV replication through a combination of direct cytolysis via the perforin/granzyme pathway, or activation of Fas ligand, and the production of Th1 cytokines.[3,11–14] In chronic HCV, repeated bouts of inflammation driven by viral persistence lead to ongoing hepatocellular injury and the emergence of fibrosis via the activation of profibrogenic hepatic stellate cells.[3]

Chemokines are key mediators of leukocyte trafficking and are heavily implicated in the immunopathogenesis of chronic HCV infection.[15,16] The chemokines, CXCL10, CCL5, and CCL3 were selected for examination because they are well-documented chemoattractants for activated T cells and have previously been shown to be associated with T cell inflammation in the HCV-infected liver.[17–21] Furthermore, CCL3 and CCL5 are secreted by CD4+ T cells and macrophages after infection with HIV *in vitro*.[22] No differences in intrahepatic CXCL10 and CCL3 mRNA expression were found, yet CCL5 expression was significantly higher in the liver of HIV/HCV coinfected patients. Furthermore, there was a correlation between CCL5 mRNA expression and the number of lobular infiltrating CD8+ T cells. This result is intriguing as it suggests that HIV coinfection in HCV-infected individuals is associated with further induction of CCL5, which in turn is likely to drive increased recruitment of cytotoxic CD8+ T cells into the coinfected liver.

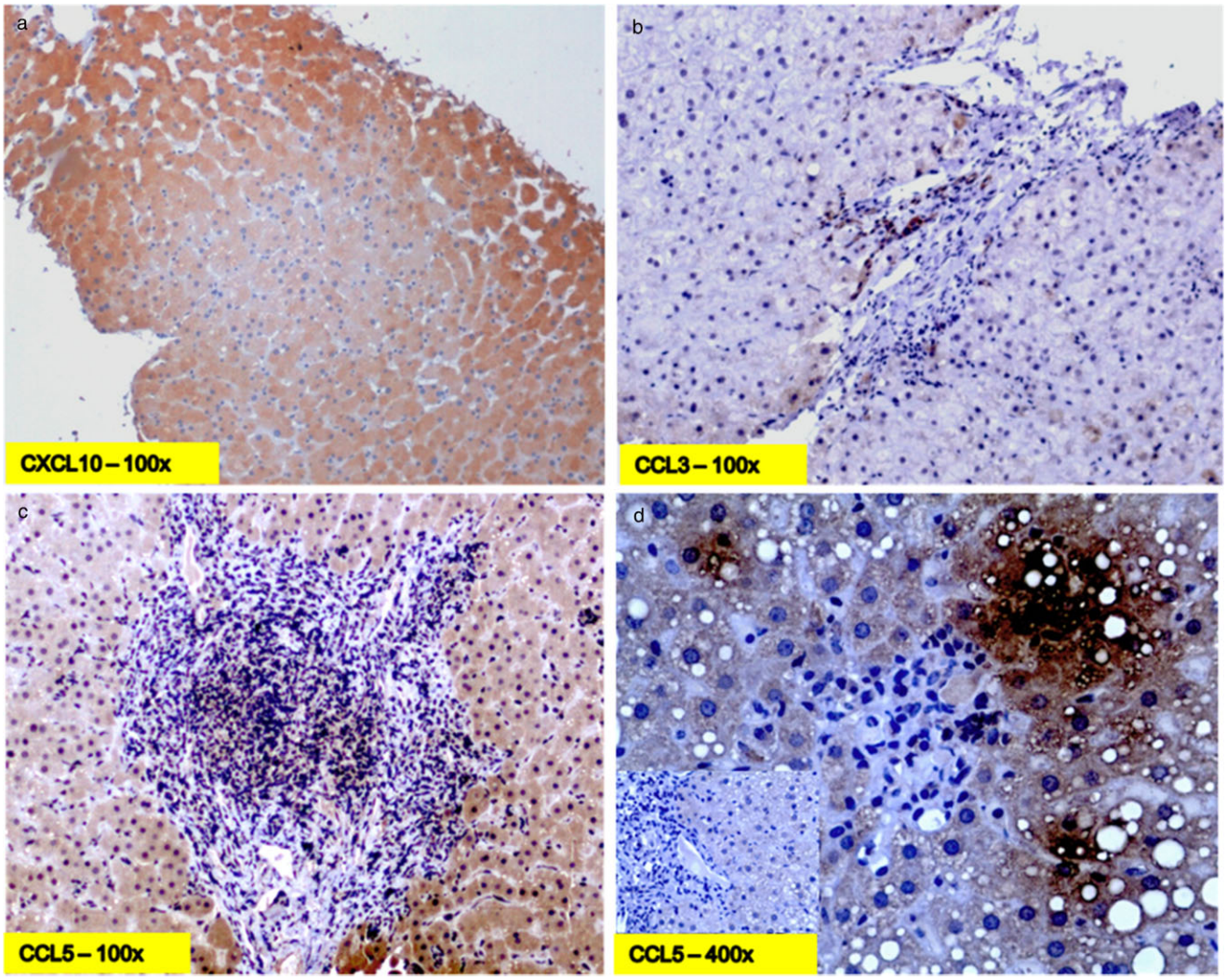

Figure 4 Immunohistochemical staining for C-X-C motif chemokine ligand (CXCL)10, chemokine (C-C motif) ligand (CCL)3, and CCL5 in HCV-infected patients. Intrahepatic chemokine protein expression was detected by immunohistochemistry for quantitative analysis. Positively stained cells for each chemokine were identified with the 3,3′-diaminobenzidine chromogen chromogen (brown staining). CXCL10 (a, 100×) was the most abundantly expressed chemokine with hepatocytes being the primary source, although some mononuclear cells and biliary epithelia also showed positive staining. CCL3 (b, 100×) was the least expressed chemokine in hepatocytes but was more prominently in mononuclear cells. CCL5 (c,d, 100× and 400×) was expressed in hepatocytes at varied intensity in addition to occasional mononuclear cells. Matched isotype control antibodies were included in each experiment and showed no immunoreactivity (see inserts in each panel), indicating the chemokine staining was specific.

Further analysis of liver infiltrating mononuclear cells indicated that they had a low frequency of markers of apoptosis (TUNEL-positive) or proliferation (Ki-67+), consistent with previous reports.[5,23] These collective results suggest that recruitment of effector cells from the circulation is the major determinant of T cell accumulation in the infected liver, as opposed to *in situ* proliferation. Once inside the liver parenchyma, these cells may well have enhanced survival as they appear to have low rates of apoptosis.

This is the first study to examine the expression intrahepatic chemokines in HIV/HCV coinfection by immunohistochemistry. Similarly to previous reports in the HCV mono-infected liver,[8,17,20] the primary sources of the chemokines studied in HIV/HCV coinfected liver were hepatocytes, followed by mononuclear cells, and in some patients, biliary epithelia also showed expression.

Another interesting result in this study was that the levels of intrahepatic IFN-γ mRNA were positively correlated with lobular CD4+ T cell numbers, which suggests that this lymphokine maybe derived from those cells. The secretion of the type I cytokine IFN-γ by effector CD4+ T helper cells is critical to the effective immune response against HCV.[3,12] Part of this response includes the induction of a number of interferon stimulated genes, including CXCL10 to recruit effector T cells into the liver.[24] In addition, *in vitro* studies have shown that co-stimulation of hepatocytes with HCV proteins in combination with IFN-γ induces the secretion of CXCL10 and CCL5.[25] The previous reports of intrahepatic

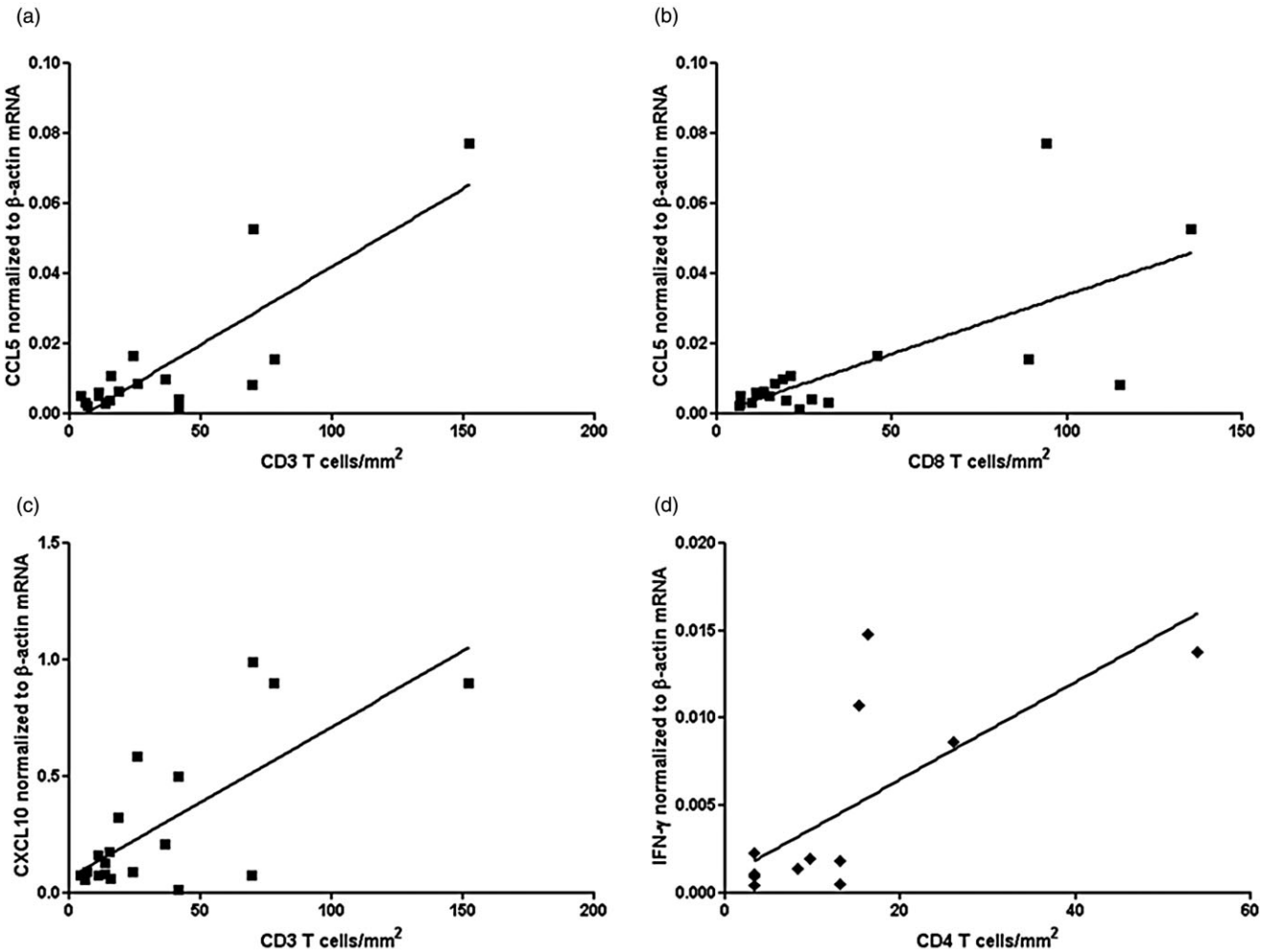

Figure 5 Correlations between chemokine and interferon (IFN)-γ messenger RNA (mRNA) expression with lobular infiltrating T cell subsets in both human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected and HCV mono-infected patients. Expression levels of both CCL5 mRNA were correlated with the number of CD3+ and CD8+ T cells infiltrating the lobules (a,b, $P = 0.04$ and 0.047). In addition, CD3+ T cell numbers were correlated with CXCL10 (c, $P = 0.043$) and CD4+ T cell numbers strongly correlated with IFN-γ expression (d, $P = 0.008$).

cytokine expression, in particular IFN-γ, in the HIV/HCV coinfected liver compared with the HCV mono-infected liver are conflicting with both enhanced and reduced expression reported. These discrepant results are likely to be attributable to the immune status of the HIV/HCV coinfected patients included in the reports. Immunocompromised patients (CD4+ T cell counts <400 cells/mm^3) have been found to have low IFN-γ expression compared with HCV mono-infected patients[26,27] consistent with the progressive CD4 T cell depletion of HIV disease. In addition, IFN-γ mRNA expression has been shown to increase with increasing peripheral CD4+ T cells counts.[9,28] Paradoxically, it has been reported immunocompromised patients have a greater risk of advanced fibrosis, although this may related to a longer duration of HIV infection and exposure to factors potentially associated with liver injury, including antiretroviral therapy (ART) and opportunistic infections.[29] In the present study, the coinfected subjects were relatively immune competent with a median CD4+ T cell count of 544 cells/mm^3. In combination, these data suggest CD4+ T cells are likely to be a major source of IFN-γ and are likely to play an important role in exaggerating the pro-inflammatory environment within the liver, particularly when immune restoration has been achieved via cART.

In summary, we have shown an enhanced pattern of inflammation in the HIV/HCV coinfected liver as evidenced by enrichment of CD8+ T cells and increased IFN-γ and chemokine expression. These data provide further insights into the immunopathogenesis of liver injury in HIV/HCV coinfected patients. Further studies are warranted to examine the chemokine receptor utilization on the infiltrating leukocytes and to extend the spectrum of HIV/HCV coinfected patients to include those with advanced immunodeficiency.

Acknowledgments

All patients enrolled in the study provided written informed consent. The study conformed to the ethical guidelines of the 1975

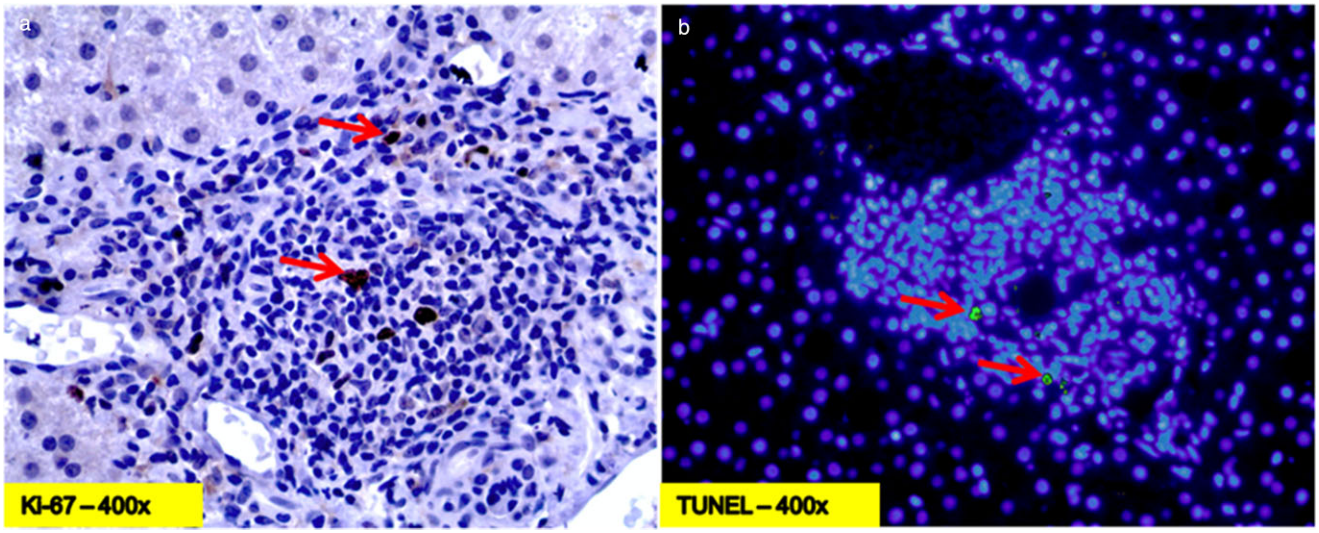

Figure 6 Localization of proliferating (Ki-67+) and apoptotic (terminal deoxynucleotidyl transferase 2′-deoxyuridine 5′-triphosphate nick end labeling [TUNEL]+) lymphocytes. Proliferating (a) and apoptotic (b) lymphocytes were detected at very low frequencies, as indicated by the arrows in both HCV mono-infected and HIV/HCV coinfected subjects.

Declaration of Helsinki and was approved by the institutional review board. The authors do not have any associations with any commercial body that may potentially present a conflict of interest. This work was supported by a Project grant from National Health and Medical Research Council (NHMRC) Australia (No. 400920). NN is supported by a postgraduate research scholarship from the School of Medical Sciences, University of New South Wales. ARL is supported by a Fellowship from NHMRC (No 510246).

References

1 Petrovic LM. HIV/HCV co-infection: histopathologic findings, natural history, fibrosis, and impact of antiretroviral treatment: a review article. *Liver Int.* 2007; **27**: 598–606.
2 Bani-Sadr F, Goderel I, Morand P *et al*. High hepatitis C virus viral load in HIV/hepatitis C virus-co-infected patients: a different influence of protease inhibitor and non-protease inhibitor-based HAART? *AIDS* 2007; **21**: 1645–8.
3 Freeman AJ, Marinos G, Ffrench RA, Lloyd AR. Immunopathogenesis of hepatitis C virus infection. *Immunol. Cell Biol.* 2001; **79**: 515–36.
4 Heydtmann M, Adams DH. Chemokines in the immunopathogenesis of hepatitis C infection. *Hepatology* 2009; **49**: 676–88.
5 Canchis PW, Yee HT, Fiel MI *et al*. Intrahepatic CD4+ cell depletion in hepatitis C virus/HIV-coinfected patients. *J. Acquir. Immune Defic. Syndr.* 2004; **37**: 1125–31.
6 Kuntzen T, Tural C, Li B *et al*. Intrahepatic mRNA expression in hepatitis C virus and HIV/hepatitis C virus co-infection: infiltrating cells, cytokines, and influence of HAART. *AIDS* 2008; **22**: 203–10.
7 Scheuer PJ, Ashrafzadeh P, Sherlock S, Brown D, Dusheiko GM. The pathology of hepatitis C. *Hepatology* 1992; **15**: 567–71.
8 Palmer C, Corpuz T, Guirguis M *et al*. The effect of obesity on intrahepatic cytokine and chemokine expression in chronic hepatitis C infection. *Gut* 2010; **59**: 397–404.
9 Sitia G, De Bona A, Bagaglio S *et al*. Naive HIV/HCV-coinfected patients have higher intrahepatic pro-inflammatory cytokines than coinfected patients treated with antiretroviral therapy. *Antivir. Ther.* 2006; **11**: 385–9.
10 Rullier A, Trimoulet P, Neau D *et al*. Fibrosis is worse in HIV-HCV patients with low-level immunodepression referred for HCV treatment than in HCV-matched patients. *Hum. Pathol.* 2004; **35**: 1088–94.
11 Einav S, Koziel MJ. Immunopathogenesis of hepatitis C virus in the immunocompromised host. *Transpl. Infect. Dis.* 2002; **4**: 85–92.
12 Elliot LN, Lloyd AR, Ziegler JB, Ffrench RA. Protective immunity against hepatitis C virus infection. *Immunol. Cell Biol.* 2006; **84**: 239–49.
13 Heydtmann M, Shields PL, McCaughan G, Adams DH. Cytokines and chemokines in the immune response to hepatitis C infection. *Curr Opin Infect Dis* 2001; **14**: 279–87.
14 Leroy V, Vigan I, Mosnier JF *et al*. Phenotypic and functional characterization of intrahepatic T lymphocytes during chronic hepatitis C. *Hepatology* 2003; **38**: 829–41.
15 Wald O, Weiss ID, Galun E, Peled A. Chemokines in hepatitis C virus infection: pathogenesis, prognosis and therapeutics. *Cytokine* 2007; **39**: 50–62.
16 Zeremski M, Petrovic LM, Talal AH. The role of chemokines as inflammatory mediators in chronic hepatitis C virus infection. *J Viral Hepat* 2007; **14**: 675–87.
17 Harvey CE, Post JJ, Palladinetti P *et al*. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. *J. Leukoc. Biol.* 2003; **74**: 360–9.
18 Kusano F, Tanaka Y, Marumo F, Sato C. Expression of C-C chemokines is associated with portal and periportal inflammation in the liver of patients with chronic hepatitis C. *Lab Invest* 2000; **80**: 415–22.
19 Nischalke HD, Natterman J, Fischer HP, Sauerbruch T, Spengler U, Dumoulin FL. Semiquantitative analysis of intrahepatic CC-chemokine mRNAs in chronic hepatitis C. *Mediators Inflamm.* 2004; **13**: 357–9.

20 Shields PL, Morland CM, Salmon M, Qin S, Hubscher SG, Adams DH. Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. *J. Immunol.* 1999; **163**: 6236–43.
21 Zeremski M, Petrovic LM, Chiriboga L *et al*. Intrahepatic levels of CXCR3-associated chemokines correlate with liver inflammation and fibrosis in chronic hepatitis C. *Hepatology* 2008; **48**: 1440–50.
22 Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 1995; **270**: 1811–15.
23 Kasprzak A, Adamek A, Biczysko W *et al*. Intracellular expression of the proliferative marker Ki-67 and viral proteins (NS3, NS5A and C) in chronic, long lasting hepatitis C virus (HCV) infection. *Folia Histochem Cytobiol* 2007; **45**: 357–66.
24 Helbig KJ, Lau DT, Semendric L, Harley HA, Beard MR. Analysis of ISG expression in chronic hepatitis C identifies viperin as a potential antiviral effector. *Hepatology* 2005; **42**: 702–10.
25 Apolinario A, Majano PL, Lorente R, Nunez O, Clemente G, Garcia-Monzon C. Gene expression profile of T-cell-specific chemokines in human hepatocyte-derived cells: evidence for a synergistic inducer effect of cytokines and hepatitis C virus proteins. *J. Viral Hepat.* 2005; **12**: 27–37.
26 Abbate I, Cappiello G, Rosati S *et al*. Intra-hepatic messenger RNA levels for interferons and related genes in hepatitis C virus/HIV co-infected patients. *AIDS* 2004; **18**: 691–2.
27 Blackard JT, Komurian-Pradel F, Perret M *et al*. Intrahepatic cytokine expression is downregulated during HCV/HIV co-infection. *J. Med. Virol.* 2006; **78**: 202–7.
28 Gonzalez SA, Zhang C, Fiel MI *et al*. Hepatic inflammatory cytokine mRNA expression in hepatitis C virus-human immunodeficiency virus co-infection. *J. Viral Hepat.* 2008; **15**: 331–8.
29 Benhamou Y, Bochet M, Di Martino V *et al*. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. *Hepatology* 1999; **30**: 1054–8.