Chemokine-Regulated Recruitment of Antigen-Specific T-Cell Subpopulations to the Liver in Acute and Chronic Hepatitis C Infection

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Background. In hepatitis C virus (HCV) infection, virus-specific CD8+ T cells are recruited to the liver for antiviral activity. Multiple chemokine ligands are induced by the infection, notably interferon-inducible chemokine, CXCL10. In HCV, intrahepatic T cells express chemokine receptors (CCRs), including CXCR3, CXCR6, CCR1, and CCR5, but CCR expression on antigen-specific effector and memory T cells has not been investigated.

Methods. Paired blood and liver samples were collected from subjects with chronic HCV for flow cytometric analysis of CCR expression on CD8+ T cells. Expression of these CCRs was then examined on HCV-specific CD8+ T-cell subpopulations in the blood from subjects with acute or chronic HCV.

Results. Relative to peripheral blood, the liver was enriched with CD8+ T cells expressing CCR2, CCR5, CXCR3, and CXCR6 either singly or in combinations. CXCR3 was preferentially expressed on HCV-specific CD8+ T cells in both acute and chronic phases of infection in blood. Both CXCR3 and CCR2 were overexpressed on HCV-specific CD8+CCR7+CD45RO+ (central memory) cells, whereas effector memory (CD8+CCR7−CD45RO+) cells expressed more CXCR6.

Conclusions. CXCR3-mediated signals support the accumulation of HCV-specific CD8+ memory T cells in the infected liver, and emphasize the importance of the CXCL10/CXCR3 trafficking pathway during acute and chronic HCV infection.

Keywords. chemokine receptor; CXCR3; CD8+ T cells; hepatitis C; liver.

CD8+ T cells play a pivotal role in viral clearance in primary hepatitis C virus (HCV) infection, and in liver injury in chronic HCV infection. T-cell recruitment to tissue sites of infection is controlled by multifaceted interactions between regional expression of chemokine ligands and T-cell expression of cognate receptors. In primary HCV infection, HCV-specific T-cell responses are higher in subjects who achieve spontaneous clearance than in those who develop chronic infection [1–3]. In addition, plasma levels of the chemokine ligands CXCL10 and CCL4 have been found to be elevated [4], with the former being associated with subsequent clearance [5]. A single chimpanzee study found that although levels of the chemokines CXCL10, CXCL11, CCL4, and CCL5 were increased in both blood and liver samples within 1 month of HCV infection, hepatic recruitment of HCV-specific CD8+ T cells expressing CXCR3 and CCR5 was delayed by 8–12 weeks [6].

In chronic HCV infection, multiple chemokine ligands are expressed, notably CCL3, CCL5, and CXCL10, with the latter being preferentially expressed by hepatocytes within the lobules, and the former in the portal tracts [7]. HCV-specific T cells are detectable at higher frequencies in the liver than in the blood, and the HCV-infected liver is enriched with CD3−CD45RO− (memory) T cells, including both CD4+ and CD8+ cells expressing CXCR3, CCR5, and CXCR6 [8–11], and accumulation of T cells with these phenotypes correlates with portal and lobular inflammation [12].

A growing body of evidence suggests that expression of specific CCRs can shape CD8+ cytotoxic T-cell differentiation, leading to subsets with distinct functions [13]. CXCR3+ antigen-specific CD8+ T cells have been shown to be preferentially recruited to the liver in murine Cytomegalovirus infection [14]. In murine influenza, CXCR3+ CD8+ T cells predominantly had a resting phenotype and ultimately became the memory population, whereas the CXCR3− subpopulation showed more activation and effector status, and rapidly reduced in frequency during the infection [15]. Paradoxically, in response to vaccinia virus challenge in the mouse, CXCR3 has also been implicated in promoting antigen-specific CD8+ T cells to an effector rather than memory fate by regulating T-cell trafficking to sites of inflammation and antigen presentation, such as the spleen [16].

This report describes analysis of the expression of CCRs on CD8+ T cells from the blood and liver of subjects with chronic...
HCV, and also on HCV-specific effector and memory subpopulations in the peripheral blood in acute and chronic HCV infection.

MATERIALS AND METHODS

Subjects and Samples

There were 2 cohorts of study subjects.

Subjects with Chronic HCV for Paired Blood and Liver Analysis

An initial cohort of 13 subjects with chronic HCV infection was recruited from specialist clinics. Relevant demographic and clinical information was recorded at the time of presentation. Percutaneous liver biopsy was performed for histopathological assessment using the METAVIR scoring system [17]. Paired peripheral blood and liver biopsy tissue were collected in either acid citrate dextrose-containing vacuum tubes or sterile phosphate buffered saline (PBS) cryovials, respectively. Additionally, samples were collected from 5 subjects with nonalcoholic fatty liver disease (NAFLD) who served as comparators.

Subjects with Acute or Chronic HCV for HCV-Specific Circulating CD8+ T-Cell Analysis

A second cohort of 28 subjects with stored peripheral blood mononuclear cells (PBMCs) was selected from the Hepatitis C Incidence and Transmission Study in prisons prospective cohort [18]. This group included 13 subjects with acute HCV infection (<180 days postestimated date of infection) and 15 subjects with samples collected after establishment of chronic infection (>180 days post-infection). These subjects were also selected on the basis of prevalent class I HLA types (A1, A2, B7, B8, B57) to allow identification of HCV-specific CD8+ T cells using class I dextramers in flow cytometry (Table 1). PBMCs from healthy control donors were utilized as negative controls for these analyses.

All subjects provided written informed consent. Ethical approvals were obtained from the relevant Human Research Ethics Committees of Justice Health (reference number GEN31/03), the New South Wales Department of Corrective Services (reference number 05/0884), and University of New South Wales (reference numbers 05094, 08081, 13237, 09075, and 14170).

Isolation of Fresh PBMC and Liver Infiltrating Mononuclear Cells

PBMC were isolated from peripheral blood via density-based centrifugation, and resuspended in RPMI (Sigma-Aldrich, MO) supplemented with penicillin, streptomycin, l-glutamine, and fetal bovine serum at 1 × 10^6 cells/mL. Liver-infiltrating mononuclear cells (LIMC) were isolated from liver specimens by gentle mechanical disruption in supplemented RPMI until the tissue was entirely homogenized, as previously described [19]. Disrupted tissue and media were passed through a 0.5-µm filter and resuspended at a concentration of 1 × 10^6 cells/mL in supplemented RPMI. Isolated PBMC and LIMC were rested for an hour at 37°C with 5% CO₂ prior to antibody staining for flow cytometry.

HLA Typing

Molecular HLA typing was performed at the Institute of Immunology and Infectious Diseases in Perth, Australia, using second-generation sequencing of HLA-A/B/C genes.

Chemokine Receptor Expression on Paired Samples of PBMC and LIMC

Fresh PBMC and LIMC were stained with the following antibodies in 10-color flow cytometry: CCR1-biotinylated (75 µg/mL, R&D Systems); CCR2-biotinylated (75 µg/mL, R&D Systems); CCR5- allophycocyanin (APC)-Cy7 (Becton Dickinson Biosciences, NJ); CCR7-phycoerythrin (PE) (BD Biosciences); CXCR3-PerCP-Cy5.5 (BD Biosciences); CXCR6-APC (R&D Systems); CCR6-PE-Cy7 (R&D Systems); CCR7-APC (BD Biosciences); and CXCR4-PerCP-Cy5.5 (BD Biosciences).

Table 1. Clinical and Laboratory Characteristics of Subjects Included in the Hepatitis C Virus (HCV)-Specific T-Cell Analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Genotype</th>
<th>Viral Load (IU/mL)</th>
<th>Phase of Infection</th>
<th>Epitope and HLA Restriction</th>
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<tr>
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<td>30</td>
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<td>1a</td>
<td>3218</td>
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<td>CINGVCWTV(A2) VLSDFKTLUA2</td>
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*All the subjects selected for this study progressed to become persistently infected with HCV.
CD3-AmCyan (BD Biosciences); CD4-fluorescein isothiocyanate (FITC) (BD Biosciences); and CD8-PE-Cy7 (BD Biosciences). Cells were first labeled with all antibodies, excluding CCR2, for 15 minutes, washed with PBS/0.5% bovine serum albumin, and then labeled with streptavidin-Texas Red (Invitrogen, CA) for 10 minutes, before washing again. The cells were then labeled with CCR2 antibody for 15 minutes, washed, labeled with streptavidin-Alexa Fluor 700 for 10 minutes, washed again, and then finally fixed in 1% paraformaldehyde. Corresponding isotype-matched antibodies were used as negative controls. Acquisition included all available LIMC, and 200,000 events gated on the lymphocyte population of PBMC, acquired on an LSR II cytometer with FACS Diva software (BD Biosciences).

Chemokine Receptor Expression on HCV-Specific CD8+ T Cells
A panel of 8 HCV-specific, PE-labeled dextramers were obtained from Immudex (Denmark): A01-restricted (ATDALMTGF_{NS3:1435\textsuperscript{a}} ATDALMTG_{NS3:1435\textsuperscript{b}}); A02-restricted (CINGVCWTV_{NS3:1073\textsuperscript{a}} KIVALGINAV_{NS1:1460\textsuperscript{b}} VLSDFKTLW_{NS1:1995\textsuperscript{b}}); B07-restricted (GPRLGVRAT_{GEndo\textsuperscript{a}}); B08-restricted (HPVTKYM_{NS3:1639\textsuperscript{b}}), and B57-restricted (KKSTRTMFG_{NS5B:2629\textsuperscript{a}}). These dextramers were used to screen samples from subjects with acute or chronic HCV infection, revealing 7 acutely infected individuals and 8 chronically infected individuals with dextramer-positive CD8+ T cells, which were selected for further phenotyping using an 11-color polychromatic flow panel.

Cryopreserved PBMC were thawed, resuspended in supplemented RPMI, then rested, before staining with: HLA-relevant Dextramer-PE; Fixable Blue viability dye; CD3-APC-Cy7; CD8-AF700; CD19-PE-Cy5; CD45RO-PE-Cy7; CCR5-PE-CF594; CCR2-AF647; CCR5-BV711; CXCR6-BV421; and CXCR3-FITC (all antibodies were purchased from BD Bioscience). Briefly, cells were labeled with the dextramer for 15 minutes at room temperature, washed, then stained with Fixable Blue for 25 minutes at 4°C. The cells were washed and labeled with all surface antibodies for 30 minutes at 4°C, before a second wash and labeling with streptavidin-PE-CF594 for 20 minutes at 4°C, followed by a final wash and fixation with 1% paraformaldehyde. A minimum of 200,000 events in the lymphocyte gate were acquired on a LSR Fortessa cytometer before analysis with FACS Diva software (BD Biosciences).

Data Analysis
Flow cytometry data were further analyzed using FlowJo software v7.6 (Tree Star Inc., OR). Dead cells were first gated out on the basis of low forward scatter and high side scatter, after which forward scatter area versus height was used to eliminate doublets from the lymphocyte gate. T cells were then selected by CD3+ versus CD19+ expression. For the blood and liver comparisons, CD4+ and CD8+ T cells were separately assessed for CCR expression. Single CCR expression on T-cell subsets was first identified, then all combinations of double receptor expression on individual CCR-positive cells. T-cell subsets with triple CCR expression were gated on double-receptor-positive cells for all possible triple combinations of CCRs. Overall, 26 unique combinations of single, double, and triple CCR-expressing T cells were described.

HCV-specific cell data were also analyzed using FlowJo software v10 (TreeStar Inc). The threshold for each CCR stain was identified by fluorescence minus 1 staining. The threshold for positive dextramer staining was determined using a HCV-specific T-cell line spiked into PBMC of a health control donor at cell concentrations between 0.001% and 10% of the final CD3+ T-cell population. Single, live CD3+ lymphocytes were selected as above, before CD8+dextramer+ T cells were gated. CD8+dextramer+ T cells were analyzed for single CR expression and then double expression combinations ensuring a minimum of 25 events were counted in the quadrants of interest [20].

Statistical Analysis
Statistical analysis was performed using GraphPad Prism software (v7 source, La Jolla) using the nonparametric Mann-Whitney test with a value of P < .05 being significant. Paired data were analyzed with nonparametric paired Wilcoxon rank sum tests.

RESULTS
Subjects and Samples
Paired blood and liver samples were collected from 13 subjects with chronic HCV including 11 males, with a mean age of 43.8 years (range 36–63). These subjects had a mean log10 HCV viral load copies/mL of 6.32 (range 5.28–6.9), and included 12 infected with genotype 1, and 1 infected with 3a. The liver biopsies featured mild to moderate inflammation and fibrosis. The median METAVIR scores were: portal necrosis 1.0 (interquartile ranges [IQR] 1.0–2.0); lobular necrosis 1.0 (IQR 0.0–1.0); inflammatory activity 1.0 (IQR 1.0–2.0); and fibrosis 2.5 (IQR 1.25–3.0).

Preferential Expression of CCR2, CCR5, and CXCR6 on Liver CD8+ T Cells in Chronic HCV
The expression of 6 CCRs were examined on CD8+ and CD4+ T-cell subsets in the blood and liver of patients with chronic HCV infection. All 6 CCRs were detected on both T-cell subsets in the blood and liver. The liver showed a significant enrichment of CCR2+, CCR5+, and CXCR6+ on CD8+ T cells (P = .005, P = .0002, and P = .009, respectively) (Figure 1A), whereas CCR2-, CCR3-, CXCR3-, and CXCR6+ were enriched on CD4+ T cells (Supplementary Figure 1A). Additionally, in comparison to CD4+ T cells, CXCR3 expression was higher on peripheral blood CD8+ T cells (P = 0.0006), and CCR5 was higher on both peripheral blood (P = 0.0035) and liver-infiltrating CD8+ T cells (P = .0003) (data not shown). In the NAFLD samples, only CD8+ T cells were enriched in the liver compared to the blood (data not shown), and when compared with HCV-infected livers, CD8+ T cells coexpressing CCR5+
Similar comparisons were made for double CCR expression patterns in the blood and liver of HCV-infected patients. Combined expression of CCR1+CCR2+, CCR1+CCR5+, CCR1+CXCR3+, CCR1+CXCR6+, CCR2+CCR5+, CCR2+CXCR3+, CCR2+CXCR6+, CCR5+CXCR3+, and CCR5+CXCR6+ were enriched in intrahepatic CD8+ T cells (Figure 1B). The most frequently expressed double CCR combinations on intrahepatic CD8+ T cells were CCR5+CXCR3+ (55.5%, SD 19.6), CCR2+CCR5+ (45.6%, SD 16.9), and CCR2+CXCR3+ (41.2%, SD 18.3). Compared with CD4+ T cells, combined expression of CCR5+CXCR3+ was significantly higher on peripheral blood CD8+ T cells (P \(<\) .0018), and CCR2+CCR5+ on both peripheral blood (P \(<\) .02) and liver-infiltrating CD8+ T cells (P \(<\) .0018) (data not shown).

For triple CCR expression in the blood and liver of HCV-infected patients, the 2 most prevalent triple CCR combinations on CD8+ T-cell subsets in the liver were CCR2+CCR5+CXCR3+ (29.6%, SD 18.0) and CCR5+CXCR3+CXCR6+ (16.0%, SD 18.7). The CD8+ T-cell expression of CCR1+CCR2+CCR5+, CCR1+CCR2+CXCR3+, CCR1+CCR2+CXCR6+, CCR1+CCR5+ CXCR3+, CCR2+CCR5+CXCR3+, CCR2+CCR5+CXCR6+, and CCR5+CXCR3+CXCR6+ was enriched on intrahepatic CD8+ T cells compared to peripheral blood (Figure 1C). In comparison to CD4+ T cells, the peripheral blood CD8+ T-cell population had significantly lower frequencies of CCR1+CCR5+CXCR3+ (P \(<\) .027) and CCR2+CCR5+CXCR3+ (P \(<\) .01), whereas no statistical differences were found between intrahepatic T-cell subsets.

**CXCR3 is Upregulated on Circulating Virus-Specific CD8+ T cells in Both Acute and Chronic HCV Infection**

CCR expression was examined on HCV-specific CD8+ T cells, in comparison to dextramer-negative CD8+ T cells, from peripheral blood samples of acute and chronically infected subjects (Table 1). Representative flow cytometry data showing the gating strategy employed for CCR analyses of HCV-specific and dextramer-negative CD8+ T cells are illustrated in Figure 2A. A typical staining pattern for both the dextramer and the CCRs from a subject with acute HCV infection is also shown in Figure 2A.

CXCR3 expression was significantly higher on HCV-specific CD8+ T cells from patients with both acute and chronic HCV infection, compared to the dextramer-negative CD8+ T cells of the same subjects (P \(<\) .024 and P \(<\) .038; Figure 2B and 2D). By contrast, CCR7 expression was significantly higher on dextramer-negative CD8+ T cells compared to HCV-specific CD8+ T cells in acute HCV infection (P \(<\) .006; Figure 2B). There was a trend towards higher expression of CXCR6 on HCV-specific CD8+ T cells in patients with acute infection (P \(<\) .25). The CXCR3+CXCR6+ double-positive CCR population was significantly increased within HCV-specific CD8+ T cells compared to dextramer-negative CD8+ T cells, during both acute and chronic phases of infection (P \(<\) .006 and P \(<\) .05, respectively; Figure 2C and 2E).
HCV Infection is Associated With an Expansion of the Effector and Central Memory CD8+ T-Cell Pools

The size and composition of the memory and effector CD8+ T-cell pools was also evaluated in HCV-specific and dextramer-negative CD8+ T-cell populations in PBMC samples from the acute and chronically infected subjects. In chronic infection, HCV-specific CD8+ T cells were identified at frequencies ranging between 0.01% and 0.03% of CD3+ T cells, whereas in the acutely infected subjects the frequencies ranged between 0.01% and 0.97% of CD3+ T cells.

As shown in Figure 3A, 4 subpopulations of CD8+ T cells were defined by CCR7 and CD45RO expression: naive (CCR7+CD45RO−), central memory (CCR7−CD45RO−), effector memory (CCR7−CD45RO+), and terminally differentiated effector memory (CCR7+CD45RO−). There was a higher proportion of the effector memory cells amongst HCV-specific CD8+ T cells compared to dextramer-negative cells in both acute and chronic phases of infection ($P = .004$ and $P = .002$, respectively). There were also higher proportions of cells with the central memory phenotype amongst HCV-specific CD8+ T cells compared to dextramer-negative CD8+ T cells in both acute and chronic infection samples ($P = .002$ and $P = .001$, respectively).

Conversely, dextramer-negative CD8+ T cells had significantly higher frequencies of the naive phenotype compared to HCV-specific CD8+ T cells in both acute and chronic infection samples (Figure 3). There was a slight increase in the size of the

Figure 2. Comparison of chemokine receptor expression patterns between hepatitis C virus (HCV)-specific and dextramer (Dex)-negative CD8+ T cells in acute and chronic HCV infection. A, Gating strategy for analysis of chemokine receptor expression on HCV-specific and dextramer-negative CD8+ T cells. B and D, Single chemokine receptor expression in samples collected from subjects with acute HCV (n = 7) (B) and chronically infected subjects (n = 8) (D) assessed on HCV-specific and dextramer-negative CD8+ T cells. C and E, Analysis of the coexpression of different chemokine receptors on HCV-specific CD8+ T cells in acute (C) and chronic (E) subjects, respectively. Differences in expression of chemokine receptors between HCV-specific CD8+ T cells and dextramer-negative CD8+ T cells were assessed using Mann-Whitney $t$ tests.
putatively naive population within HCV-specific CD8\(^+\) T cells in chronic infection in comparison to acute phase samples, potentially reflecting a phenotypic reversion from the memory (CD45RO) phenotype in chronic viral infection [21].

**Preferential Expression of CXCR3 and CCR2 on Circulating HCV-Specific Central Memory CD8\(^+\) T Cells**

HCV-specific effector and memory CD8\(^+\) T cells were identified by firstly gating on the positive and negative CCR7 and CD45RO populations, before evaluation of the expression of CCR2, CXCR3, CXCR6, and CCR5 (Figure 4A). The frequencies of CCR2 and CXCR3 expression on central memory HCV-specific CD8\(^+\) T cells were significantly higher than on effector memory subsets \((P = .0093, P = .0019)\). The mean proportions of CCR2\(^+\) and CXCR3\(^+\) cells within the central memory subset were 13% (SD = 14) and 62% (SD = 16), and for effector memory were 1% (SD = 1) and 30% (SD = 18), respectively (Figure 4B). In contrast, CXCR6 was preferentially expressed on effector memory CD8\(^+\) T cells compared to central memory cells (Figure 4B). There were no significant differences in the expression levels of CCR5 on central and effector memory subpopulations.

**DISCUSSION**

The chemokine system plays a fundamental role in governing the optimal CD8\(^+\) T-cell response to viral invasion of an organ by controlling recruitment, localization, and thence the interaction of antiviral T cells with targets [22]. These CCR-dependent mechanisms of trafficking remain incompletely defined in
relation to selective recruitment of antigen-specific CD8^+ T cells, including to the livers during HCV infection. This study has revealed the CXCL10-CXCR3 pathway to be key for both HCV-specific effector and memory CD8^+ T-cell trafficking to the infected liver, with cooperative roles for CCR2 and CXCR6 for these subpopulations, respectively.

Previous studies characterizing chemokine ligand and CCR expression patterns on LIMC and PBMC in chronic HCV infection have identified multiple molecular participants in the regulation of lymphocyte trafficking into the HCV-infected liver. The most substantive previous study examined simultaneous expression of 4 CCRs: CCR5, CCR7, CXCR3, and CXCR6 [11]. In the data presented here with simultaneous analysis of CCR1, CCR2, CCR5, CCR7, CXCR3, and CXCR6, there was enrichment in LIMC of CXCR3^+ , CCR2^+ , and CCR5^+ CD8^+ T cells. The combined findings are generally consistent in demonstrating that the HCV-infected liver is infiltrated with CCR5^+ , CXCR3^+ , and CXCR6^+ CD8^+ T cells [11, 23, 24]. However, the findings here of a relatively high frequency of both circulating and intrahepatic CCR2^+ cells, and the significantly higher frequency of CCR2^+ cells in the liver compared to the blood has not been reported previously. CCR2, along with CCR1 and CCR5, is a receptor for the promiscuous ligands, CCL3, CCL4, and CCL5, in addition to its unique ligand CCL2. All 4 are known to be upregulated in the HCV-infected liver [25]. The novel finding of higher frequencies of CCR5^+CXCR3^+ , CCR2^+CCR5^+ , and CCR2^+CXCR3^+ expressing CD8^+ T cells in the HCV infected liver is intriguing, as it suggests that initial CD8^+ T-cell recruitment via CXCL10-CXCR3 may be followed by fine positioning, or retention, in the liver mediated via CCR2 and/or CCR5. Furthermore, CCR2^+CCR5^+ CD8^+ T cells were the second highest double-receptor expressing population, suggesting that

Figure 4. Comparison of chemokine receptor expression patterns on hepatitis C virus (HCV)-specific CD8^+ T cells in effector and central memory subsets during acute HCV infection. Differentiation phenotype as well as CCR2, CCR5, CXCR3, and CXCR6 expression on HCV-specific CD8^+ T cells was measured. A, Peripheral blood mononuclear cells from acute infection subjects (n = 6) were stained. Central and effector memory subsets were gated initially using CCR7 and CD45RO markers, and then expression of CXCR3 and CXCR6 was evaluated within these subsets. The histograms illustrate the expression of CXCR3 and CXCR6 on central and effector memory subsets. B, The percentage (mean and SD) expression of CCR2, CCR5, CXCR3, and CXCR6 on central and effector memory HCV-specific CD8^+ T cells. Statistical analyses were performed using Mann-Whitney t test.
the presence of both receptors may allow combined responses to shared ligands, such as to CCL3 and CCL5. The possible in vivo consequences of these chemokine ligands and CCR coexpression range from sequential action to synergy and even antagonism [26]. Interestingly, multivariate analysis in the previous study [11], suggested that T-cell recruitment to the liver was mediated by 2 independent pathways: 1 via CXCR3 and the other via CCR5, with a degree of coexpression of CXCR6. The complexities of these combined effects in vivo are illustrated in CXCR3- and/or CCR5-deficient mice challenged with influenza, in which CXCR3 knockout had no effect or mortality but rescued CCR5−/− mice from hyperlethal primary challenge and allowed protection from secondary challenge [27].

The current study has extended these findings to examination of antigen-specific CD8+ T cells, revealing preferential expression of CXCR3 on HCV-specific CD8+ T cells in both chronic and acute HCV infection. Preferential coexpression of CXCR3 and CXCR6 was found on effector CD8+ T lymphocytes. Interestingly, our previous immunohistochemical analysis in samples from this cohort showed that expression of CXCL10 correlated with accumulation of CD8+ T cells in both lobules and portal tracts [28], whereas CXCL16 was found to be preferentially associated with portal tracts [29]. In combination, these data suggest that the CXCR3-CXCL10 axis is particularly important for HCV-specific effector CD8+ T-cell recruitment into the liver lobules to mediate cytotoxicity.

Although antigen-specific effector CD8+ T cells are critical to initial viral control, the memory subset elicited during primary infection is also associated with ultimate control of viral replication and outcome of infection, as well as potential protection against reinfection [30]. In both acute and chronic infection, circulating HCV-specific CD8+ T cells were enriched for both effector memory (CCR7+/CD45RO+) and central memory (CCR7−/CD45RA−) phenotypes, with the former being predominant in acute phase samples. One previous study has shown that circulating antigen-specific effector memory T cells (defined by CCR7+CD45RA−) were the predominant population in acute and chronic HCV [31].

In the peripheral blood, CCR2 and CXCR3 expression was higher on HCV-specific central memory CD8+ T cells compared to effector memory CD8+ T cells. This finding suggests that CXCR3 and CCR2 (and their cognate ligands) may influence CD8+ T-cell differentiation commitment by controlling localization of new activated T cells within lymphoid organs during the early phase of infection. This finding is consistent with studies in lymphocytic choriomeningitis virus-infected mice, which suggest that induction of CXCR3 and CCR2 may be important in vaccination strategies in order to generate higher numbers of antigen-specific memory CD8+ T cells via activation of CXCR3-mediated T-cell migration to the site of infection. By contrast, CXCR6 expression on CD8+ T cells in the liver was shown to be critical to establish a long-lived memory T-cell population in murine malaria infection [32].

A potential caveat of the current study is the possibility of immune escape by the virus at HCV-specific CD8+ T-cell epitopes and thence altered CR expression. Interestingly, next-generation viral sequencing analysis in the acute cohort has revealed no escape in the subjects who cleared primary infection, and both escape and conserved epitopes in those who became chronically infected (manuscript in preparation). The CD8+ T-cell responses against the mutated (escape) epitopes measured by interferon-gamma enzyme-linked immunospot (ELISpot) were generally retained, albeit at a lower magnitude, indicating that the process of escape is often incomplete. Another limitation is the need for further investigation of the role of CRs in optimal formation of tissue-resident memory cells within the liver by examination of coexpression of CD69 and CD103 on LIMCs, as well as functional studies including proliferation in response to interleukin-7 (IL-7) and IL-15.

Further studies are warranted to explore the dynamics of coexpression of CXCR3, CXCR6, as well as CCR5 and CCR2 on HCV-specific effector and memory CD8+ T cells in longitudinally collected samples from subjects with acute HCV resulting in either chronic infection or clearance outcomes.

**SUPPLEMENTARY DATA**

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

**Acknowledgments.** We are grateful to the participants in the Hepatitis C Incidence and Transmission Study in prisons cohort who volunteered to participate in the research, and to Chris Brownlee and the Flow Facility of the Mark Wainwright Analytical Centre, University of New South Wales for helpful technical assistance.

**Financial support.** This work was supported by the Australian National Health and Medical Research Council (grant number 1043067 Practitioner Fellowship to A. R. L.); the Australian Centre for HIV and Hepatitis Virology Research; and M. R. P. and N. N. were supported by the Australian Government Post Graduate Awards.

**Potential conflicts of interest.** All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
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