The Role of Chemokines in the Recruitment of Lymphocytes to the Liver

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Chemokines direct leukocyte trafficking and positioning within tissues, thus playing critical roles in regulating immune responses and inflammation. The chemokine system is complex, involving interactions between multiple chemokines and their receptors that operate in combinatorial cascades with adhesion molecules. The involvement of multiple chemokines and chemokine receptors in these processes brings flexibility and specificity to recruitment. The hepatic vascular bed is a unique low-flow environment through which leukocytes are recruited to the liver during homeostatic immune surveillance and in response to infection or injury. The rate of leukocyte recruitment and the nature of cells recruited through the sinusoids in response to inflammatory signals will shape the severity of disease. At one end of the spectrum, fulminant liver failure results from a rapid recruitment of leukocytes that leads to hepatocyte destruction and liver failure; at the other end, diseases such as chronic hepatitis C infection may progress over many years from hepatitis to fibrosis and cirrhosis. Chronic hepatitis is characterized by a T lymphocyte-rich infiltrate and the nature and outcome of hepatitis will depend on the T cell subsets recruited, their activation and function within the liver. Different subsets of effector T cells have been described based on their secretion of cytokines and specific functions. These include Th1 and Th2 cells, and more recently Th17 and Th9 cells, which are associated with different types of immune response and which express distinct patterns of chemokine receptors that promote their recruitment under particular conditions. The effector function of these cells is balanced by the recruitment of regulatory T cells that are able to suppress antigen-specific effectors to allow resolution of immune responses and restoration of immune homeostasis. Understanding the signals that are responsible for recruiting different lymphocyte subsets to the liver will elucidate disease pathogenesis and open up new therapeutic approaches to modulate recruitment in favor of resolution rather than injury.

Key Words
Leukocytes · Chemokines · Hepatic inflammation

Abstract
Chemokines direct leukocyte trafficking and positioning within tissues, thus playing critical roles in regulating immune responses and inflammation. The chemokine system is complex, involving interactions between multiple chemokines and their receptors that operate in combinatorial cascades with adhesion molecules. The involvement of multiple chemokines and chemokine receptors in these processes brings flexibility and specificity to recruitment. The hepatic vascular bed is a unique low-flow environment through which leukocytes are recruited to the liver during homeostatic immune surveillance and in response to infection or injury. The rate of leukocyte recruitment and the nature of cells recruited through the sinusoids in response to inflammatory signals will shape the severity of disease. At one end of the spectrum, fulminant liver failure results from a rapid recruitment of leukocytes that leads to hepatocyte destruction and liver failure; at the other end, diseases such as chronic hepatitis C infection may progress over many years from hepatitis to fibrosis and cirrhosis. Chronic hepatitis is characterized by a T lymphocyte-rich infiltrate and the nature and outcome of hepatitis will depend on the T cell subsets recruited, their activation and function within the liver. Different subsets of effector T cells have been described based on their secretion of cytokines and specific functions. These include Th1 and Th2 cells, and more recently Th17 and Th9 cells, which are associated with different types of immune response and which express distinct patterns of chemokine receptors that promote their recruitment under particular conditions. The effector function of these cells is balanced by the recruitment of regulatory T cells that are able to suppress antigen-specific effectors to allow resolution of immune responses and restoration of immune homeostasis. Understanding the signals that are responsible for recruiting different lymphocyte subsets to the liver will elucidate disease pathogenesis and open up new therapeutic approaches to modulate recruitment in favor of resolution rather than injury.

Chemokines

Chemokines are 8- to 12-kDa heparin-binding cytokines with the ability to attract leukocyte subsets to specific sites and thereby shape the outcome of immune responses, including intrahepatic inflammation. An efficient immune reaction requires leukocytes to be at the
right place at the right time, and thus needs a system to regulate the migration and positioning of cells in lymphoid and nonlymphoid tissues [1]. The chemokine system provides cues for the recruitment of effector and regulatory subsets and is central to the pathogenesis of inflammatory diseases.

Chemokines are generally classified into two functional groups, ‘inflammatory’ and ‘homeostatic/constitutive’, based on whether they are induced by inflammation or constitutively expressed and involved in homeostatic immune regulation (table 1). Inflammatory chemokines are expressed in inflamed tissues by resident and infiltrating cells on stimulation by pro-inflammatory cytokines or during contact with pathogenic agents. These chemokines are secreted early after infection in response to activation of pattern recognition receptors on epithelial, stromal and immune cells. They recruit the initial wave of innate immune effectors including neutrophils, monocytes, natural killer cells and natural killer T cells, all of which express inflammatory chemokine receptors and immature dendritic cells (DCs) that provide the link between innate and adaptive immunity. After antigen-specific activation of lymphocytes by activated DCs, inflammatory chemokines then attract antigen-specific effector T cells to the inflammatory site [2]. At the same time, regulatory T cells are also recruited and the balance between effector and regulatory cells recruitment determines the outcome of the local inflammation.

In contrast, homeostatic chemokines are produced in discrete microenvironments within lymphoid (bone marrow, thymus and secondary lymphoid organs) or nonlymphoid tissues such as the skin and mucosa. These constitutively produced chemokines are involved in physiological trafficking and positioning of cells, antigen sampling in secondary lymphoid tissue and immune surveillance. However, this distinction (although useful) is somewhat artificial because chemokines previously thought to be homeostatic, such as the CCR7 ligands CCL19 and CCL21 and the CXCR5 ligand CXCL13 (which are critical for the development and function of lymphoid tissues), are also induced at sites of chronic inflammation. Homeostatic chemokine receptors bind only one or two chemokines. For example, the chemokine that recruits hematopoietic progenitors to bone marrow has one main receptor CXCR4, and a monogamous pair, CXCR5 and CXCL13, recruit B cells to follicles in lymph nodes; however, receptors that recruit cells to inflammatory sites often have several chemokine ligands [3].

The Human Chemokine System

The human chemokine system includes more than 50 chemokines and 20 chemokine receptors (table 1) that can be divided into structural subsets based on the presence of NH2-terminal cysteine motifs [4, 5]. The large CC chemokine family consists of chemokines in which the first two cysteine residues are adjacent, whereas in CXC chemokines, they are separated by a single amino acid residue. Fractalkine (CX3CL1) is the only member of the CX3C chemokine family in which three amino acid residues separate the first two cysteines. Finally, two related chemokines, XCL1 and XCL2, both of which bind the XCR1 receptor, lack two adjacent cysteine residues. Chemokines and their receptors also undergo post-translational modifications which alter their function, allowing them to provide almost limitless potential receptor ligand pairs to bring exquisite specificity to the control of leukocyte homing and positioning in tissues [3].

Dysregulated expression of chemokines and their receptors is involved in the development of many human diseases, including autoimmune and chronic inflammatory diseases as well as immunodeficiency and cancer.

Chemokines exert their chemotactic functions by binding to specific G protein-coupled receptors, seven-transmembrane-spanning proteins coupled to heterotrimeric G proteins (fig. 1). Chemokine binding to chemokine receptors dissociates Goi and Gβ-γ subunits of the heterotrimeric G proteins, leading to calcium flux and activation of the phosphatidylinositol 3-kinase and the small Rho GTPases signaling pathways [6]. Consistent with Goi association, the majority of chemokine responses are inhibited by treatment with pertussis toxin [7], which blocks the global G protein receptor.

Chemokines and Leukocyte Trafficking

Chemokines regulate leukocyte extravasation into tissue [3, 8, 9] by providing signals that allow the leukocyte to respond to environmental cues. At least four stages take a free flowing cell from the blood via endothelium into tissue [10]. An initial carbohydrate-dependent ‘tether’ brings the cell into contact with the vessel wall and a second rolling step slows the leukocyte, allowing interactions with the endothelium. In the liver, rolling is greatly attenuated, probably as a consequence of the low levels of shear stress in hepatic sinusoids, and there is little role for selectins [11, 12]. In the subsequent ‘triggering’ stage, signaling from the leukocyte chemokine receptor triggers conformational activation of leu-
### Table 1. Chemokines and chemokine receptors in human immune system

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Chemokine</th>
<th>Functions</th>
<th>Distribution of chemokine receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CC subgroup</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CCR1</strong></td>
<td>CCL3, CCL5, CCL7, CCL8, CCL13–16</td>
<td>T cell and monocyte migration; hypersensitivity; innate and adaptive immunity; inflammation</td>
<td>monocytes, memory T cells, Th1, NK</td>
</tr>
<tr>
<td><strong>CCR2</strong></td>
<td>CCL5, CCL7, CCL8, CCL13</td>
<td>T cell and monocyte migration; innate and adaptive immunity; Th1 inflammation</td>
<td>monocytes, memory T cells, basophils, pDC</td>
</tr>
<tr>
<td><strong>CCR3</strong></td>
<td>CCL5, CCL7; CCL11, CCL15–16, CCL24, CCL26</td>
<td>eosinophil and basophil migration; allergic inflammation; Th2 response</td>
<td>eosinophils, basophils</td>
</tr>
<tr>
<td><strong>CCR4</strong></td>
<td>CCL17, CCL22</td>
<td>T cell and monocyte migration; allergic inflammation; T&lt;sub&gt;reg&lt;/sub&gt; retention; skin homing; expressed on CD4 Th2 cells</td>
<td>Th2 cells, T&lt;sub&gt;reg&lt;/sub&gt;, eosinophils, basophils, DC, T&lt;sub&gt;reg&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>CCR5</strong></td>
<td>CCL3; CCL4; CCL5; CCL8</td>
<td>Th1 response, adaptive immunity; inflammation, HIV infection</td>
<td>monocytes, Th1 cells, NK</td>
</tr>
<tr>
<td><strong>CCR6</strong></td>
<td>CCL20</td>
<td>DC migration, memory T cells, Th17 cells at site of inflammation</td>
<td>memory T cells, B cells, Th17, immature mDC</td>
</tr>
<tr>
<td><strong>CCR7</strong></td>
<td>CCL19, CCL21</td>
<td>T cell and DCs homing to secondary lymphoid tissue; lymphoid development</td>
<td>naive T, B, mature mDC, Th1, Th2, T&lt;sub&gt;reg&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>CCR8</strong></td>
<td>CCL1</td>
<td>T cell trafficking; Th2 response</td>
<td>monocytes, Th2, T&lt;sub&gt;reg&lt;/sub&gt;, NK</td>
</tr>
<tr>
<td><strong>CCR9</strong></td>
<td>CCL25</td>
<td>T cell homing to gut and thymus tolerogenic DCs</td>
<td>DC, memory T cells, thymocytes</td>
</tr>
<tr>
<td><strong>CCR10</strong></td>
<td>CCL27, CCL28</td>
<td>T cell homing to skin and bowel</td>
<td>memory T cells, T&lt;sub&gt;reg&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>CXC subgroup</strong></td>
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<tr>
<td><strong>CXCR1</strong></td>
<td>CXCL6, CXCL7, CXCL8</td>
<td>neutrophil migration; innate immunity; acute inflammation</td>
<td>PMN, monocytes, mast cells</td>
</tr>
<tr>
<td><strong>CXCR2</strong></td>
<td>CXCL1–3; CXCL5–8</td>
<td>neutrophil migration; innate immunity; acute inflammation; angiogenesis</td>
<td>PMN, monocytes, mast cells</td>
</tr>
<tr>
<td><strong>CXCR3</strong></td>
<td>CXCL9, CXCL10, CXCL11</td>
<td>T cell recruitment; adaptive immunity; Th1, Th2, Th17, T&lt;sub&gt;reg&lt;/sub&gt;, inflammation</td>
<td>memory T cells, Th1, Th2, Th17, T&lt;sub&gt;reg&lt;/sub&gt;, NKT</td>
</tr>
<tr>
<td><strong>CXCR4</strong></td>
<td>CXCL12</td>
<td>stem cell migration; B cell lymphopoiesis</td>
<td>T and B cells, monocytes, stem cells, NKT</td>
</tr>
<tr>
<td><strong>CXCR5</strong></td>
<td>CXCL13</td>
<td>B cell homing in lymphoid organ</td>
<td>B cells</td>
</tr>
<tr>
<td><strong>CXCR6</strong></td>
<td>CXCL16</td>
<td>T cell migration</td>
<td>memory T cells, Th1, NK, NKT</td>
</tr>
<tr>
<td><strong>CXCR7</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>CX3C and XC family</strong></td>
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<tr>
<td><strong>CX3CR1</strong></td>
<td>CX3CL1</td>
<td>T cell and NK cell trafficking and adhesion; innate and adaptive immunity; Th1 inflammation</td>
<td>monocytes, Th1, NK</td>
</tr>
<tr>
<td><strong>XCR1</strong></td>
<td>XCL1–2</td>
<td>NK cell recruitment</td>
<td>NK</td>
</tr>
</tbody>
</table>

Chemokine receptors can be divided into subfamilies on the basis of the position of conserved cysteine residues within a conserved tetracysteine motif. In CC chemokines, the first two consensuses cysteines are next to each other; in CXC chemokines, they are separated by a nonconserved amino acid. These two subfamilies account for all but three of the known chemokines, the others being CX3CL1 (three intervening amino acids between the first cysteines) and XCL1 and XCL2, which lack two of four canonical cysteines. Data have been drawn from Viola and Luster [89], and Heydtmann and Adams [120]. Human chemokines and chemokine receptors could also be classified according to their function: inflammatory (italics) and homeostatic (underlined). Chemokines and their receptors belonging to both subfamilies are shown in bold. CCL = Chemokine (C-C motif) ligand; CCR = chemokine (C-C motif) receptor; CXCL = chemokine (C-X-C motif) ligand; CXCR = chemokine (C-X-C motif) receptor; CX3CL = chemokine (C-X3-C motif) ligand; CX3CR = chemokine (C-X3-C motif) receptor; mDC = myeloid dendritic cell; NK = natural killer; NKT = natural killer T; pDC = plasmacytoid dendritic cell; PMN = polymorphs; Th1 = T helper 1; Th2 = T helper 2; Th17 = T helper 17; T<sub>reg</sub> = regulatory T cell.
The liver has an important role in the removal of pathogens and particulate antigens entering from the systemic circulation via the hepatic artery and from the gut via the portal vein. Blood enters the hepatic parenchyma via sinusoids, which are low-flow vascular channels that are supplied by both the hepatic artery and portal veins and lined by specialized liver sinusoidal endothelial cells, which are interspersed with the liver-resident macrophage population of Kupffer cells (KCs). The sinusoids drain into the central veins and from there blood returns to the systemic circulation. Liver sinusoidal endothelial cells have open fenestrae arranged in sieve plates which allow the passage of solutes between blood and underlying cells and which also allow contact between hepatocytes and leukocytes within the sinusoids. The space of Disse lies beneath the endothelium and contains hepatic stellate cells and DCs (fig. 2, 4). The liver is constantly exposed to food antigens and low levels of endotoxin from the gut and has evolved mecha-

**Hepatic Inflammation and Chemokines**

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Chemokines and Lymphocyte Recruitment

Lymphocytes: Regulators vs. Effectors

In chronic hepatitis, both effector subsets and regulatory lymphocytes are found at the site of inflammation, and the balance of the cells recruited will determine the nature of the hepatitis and liver disease [24]. The naive CD4 T cell is a multipotential precursor with defined antigen specificity, but substantial plasticity, for development down distinct effector or regulatory lineages, contingent upon signals from cells of the innate immune system. The most important lineages are Th1, Th2, Th17 and Th9 effector cells and anti-inflammatory regulatory T cells (T_{reg}; fig. 3). Th1 cells differentiate in response to...
interleukin (IL)-12 via signaling through signal transduction and activator of transcription (STAT)-4 and the transcription factor T-bet. Interferon (IFN)-γ, tumor necrosis factor (TNF)-α and lymphotoxin are involved in cell-mediated immunity and the clearance of intracellular pathogens including viruses. The differentiation of Th2 cells is driven by IL-4, STAT-6 and Gata-3. They secrete IL-4, IL-5, IL-10, IL-13 and IL-21 and are involved in humoral immunity and the clearance of extracellular organisms and parasites. The recently described IL-17 secreting CD4 T cells mediate defense against extracellular bacteria and fungi and drive inflammatory and autoimmune disease [25, 26]. Th17 differentiation is programmed during T cell activation in the presence of IL-6, transforming growth factor (TGF)-β, IL-1β and IL-21 [125–128], leading to expression of the transcription factor ROR-γt via activation of STAT-3 and the aryl hydrocarbon receptor (AHR) [27, 28]. Their
expansion and pathogenicity is maintained by IL-23 [29, 30]. Th17 cells secrete IL-17A, IL-17F and CCL20, and depending on local signals IL-22, IFN-γ or IL-10. They express high levels of CCR6 and CCR4, but little is known about the signals responsible for their recruitment to inflamed tissues [31].

Several observations implicate Th17 cells in inflammatory liver disease [32–36]:

- Hepatic IL-17 is increased in autoimmune/inflammatory diseases and the IL-6-, TGF-β-rich hepatic environment contributes to IL-17 induction [32].
- IL-22, a signature Th17 cytokine, is increased in liver disease and induces hepatocyte proliferation [35, 37].
- Endogenous ligands for the aryl hydrocarbon receptor [38, 39] include bilirubin which has an EC50 for aryl hydrocarbon receptor activation of 30 µM, a level seen in clinical jaundice [40].

- Increased numbers of IL-17+ cells are found in human liver disease. We detected IL17+ CD4 (Th17) and CD8 (Tc17) cells in liver from patients with autoimmune liver disease or steatohepatitis and in collaboration with Paul Klenerman (Oxford) showed that intrahepatic Tc17 cells predict outcome in chronic hepatitis C [129]. Th17 and Tc17 cells from the liver express CD161 [41], IL-23R and RORc and high levels of CCR5, CCR6, CXCR3 and CXCR6. They include subpopulations that secrete IL-22 and IFN-γ [35]. Th17 cells are associated with many types of hepatitis, but may be particularly important in steatohepatitis where they are associated with a neutrophil-rich lymphocytic infiltrate and extensive tissue destruction.

- Hepatic endothelium and epithelium express IL-17 receptors and respond to IL-17 by secreting chemokines (fig. 2) [35].
It has recently been shown that another subset of Th9 cells that secrete IL-9 can develop from Th2 cells stimulated with IL-4 and TGF-β. On the regulatory arm of the immune system, CD4+ T<sub>reg</sub> develop in response to retinoic acid and TGF-β and activation of STAT-5 and the transcription factor FOXP3 [124]. T<sub>reg</sub> suppress immune response against self-antigens and also damp down effector responses to allow resolution and the restoration of immune homeostasis, thereby preventing autoimmunity and uncontrolled inflammation and tissue injury.

A critical factor in regulating the involvement of specific subsets at sites of inflammation is the differential expression of chemokine receptors between these different subsets of T cells. In human blood, effector CD8 T cells express CXCR3, CCR5 and CXCR6 as do Th1 cells, whereas Th2 cells express CCR4 and CCR8, Th17 cells express CCR4, CCR6, CXCR3 and CXCR6 [31, 42–45] (fig. 3) and T<sub>reg</sub> express CCR4 and CXCR3 [130]. Thus the combination of T cells recruited will be shaped by the expression of local chemokines that can attract specific subsets of cells [46, 47].

**Roles of Chemokines in the Context of Hepatic Inflammation**

Immune surveillance in the liver is provided by both resident and blood-borne lymphocytes and macrophages [48]. During liver inflammation, the rate of lymphocyte recruitment via sinusoidal endothelium increases and retention within the liver by localization at sites of inflammation or at epithelial surfaces results in chronic hepatitis and persistent inflammation [49]. Chronic hepatitis C virus (HCV) infection and primary biliary cirrhosis are both dominated by Th1 immune responses [50–52], whereas there is some evidence for Th2 involvement in primary sclerosing cholangitis (PSC). Recent studies suggest that alcoholic and non-alcoholic steatohepatitis (from our own unpublished data) involve Th-17 responses and it is likely that overlapping mechanisms are involved in most situations. Most effector T cells infiltrating the chronically inflamed human liver express high levels of CXCR3, CCR6, CR1 and CCR5 consistent with a Th1 predominance and a tissue-infiltrating phenotype [51, 53–56]; the roles played by these different receptors in recruitment is beginning to be understood. In addition to recruiting different subsets of lymphocytes, there is evidence for compartmentalization of recruitment as well. CCR5 may have a particular role in recruitment to portal tracts, whereas CXCR3 appears to be essential for recruitment into the parenchyma (table 2) [57–60].

<table>
<thead>
<tr>
<th>Location in the liver</th>
<th>Chemokines</th>
<th>Chemokine receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal vessels</td>
<td>CCL3, 4, 5</td>
<td>CCR5</td>
</tr>
<tr>
<td>Liver sinusoids</td>
<td>CXCL9, 10, 11</td>
<td>CXCR3, CCR6</td>
</tr>
<tr>
<td>Biliary epithelium</td>
<td>CCL28, CCL16, CXCR3</td>
<td>CCR10, CCR6, CX3CR1</td>
</tr>
<tr>
<td>Portal-associated lymphoid tissue</td>
<td>CCL19, 21</td>
<td>CCR7</td>
</tr>
</tbody>
</table>

Lymphocytes can be recruited across the portal endothelium, which is mediated by the chemokines CCL3–5, ligands for CCR5. Recruitment across liver sinusoidal endothelium is mediated by the chemokines CXCL9–11 as well as CXCL16. CCL28 expression on biliary epithelial cells has been associated with the recruitment of CCR10 expressing regulatory T cells. In some forms of chronic hepatic inflammation, there is neovessel formation within the portal tracts which has characteristics of lymphoid tissue, which is termed portal-associated lymphoid tissue and has been shown to be associated with the chemokines CCL19 and CCL21, which are ligands for CCR7.

**CXCR3 and Its Ligands CXCL9, CXCL10 and CXCL11 in Lymphocyte Recruitment**

Expression of CXCR3 on T cells is closely linked to Th1 function, and its ligands CXCL9, CXCL10 and CXCL11 are induced by the Th1 cytokines IFN-γ and TNF-α. Studies dating back 10 years report increased levels of CXCR3 ligands in chronic hepatitis and of increased CXCR3 on CD4 and CD8 effector cells within the liver [61–65]. Increased levels of hepatic CXCR3 ligands are characteristic of many chronic inflammatory liver diseases, suggesting they play a generic role in effector cell recruitment to the inflamed liver [60, 66, 67]. The sources of CXCR3 ligands in liver inflammation include hepatocytes, stellate cells, sinusoidal endothelial cells and infiltrating leukocytes. The expression of CXCR3 ligands requires stimulation with IFN-γ and TNF-α, which are released by activated hepatic macrophages, KCs and the initial wave of infiltrating innate immune cells [59, 61, 68]. An additional contri-
bution comes from activated CD4+ T cells themselves, which release CXCR3 ligands after activation within the liver [69], thereby providing a feedback loop in which antigen-specific cells maintain the expression of the chemokines required for effector cell recruitment.

Our group has previously demonstrated by using flow-based adhesion assays that chemokines CXCL9, CXCL10 and CXCL11 are important not only in adhesion, but also in transmigration of effectors T lymphocytes through hepatic endothelium under physiological conditions of blood flow [60, 70]. CXCR3 ligands on the sinusoidal wall can originate from neighboring cells and then be taken up and transcytosed to the endothelial surface [71]. In addition, chemokines secreted ‘upstream’ by other cell types including cholangiocytes in portal tracts can be captured from the slow-flowing sinusoidal blood by proteoglycans within the endothelial cell glycocalyx and presented at the endothelial surface [60]. Thus, local presentation of chemokines on the sinusoids is the result of cross-talk between sinusoidal endothelial cells and stellate cells, KCs, cholangiocytes and hepatocytes, all of which secrete chemokines upon appropriate stimulation [72–74]. Hepatocytes not only secrete chemokines, but also sensitize the endothelium to respond to low levels of TNF-α by increasing chemokine secretion and adhesion molecule expression [24, 75].

In chronic hepatitis, many of the infiltrating cells are probably not antigen-specific, but rather bystander lymphocytes recruited as part of a broad immune response. Both antigen-dependent and independent infiltrating effector cells express high levels of CD154, whose ligand, CD40, is expressed on hepatocytes, cholangiocytes and KCs. Activation of CD40 on these liver cells triggers NFκB-dependent secretion of CXCR3 ligands [76, 77], demonstrating another mechanism by which local chemokine gradients can be amplified during the evolution of the anti-viral immune response. The situation is further complicated by the recent evidence that Treg also use CXCR3 to enter liver tissue, although other signals may determine where they migrate to within the inflamed liver and, hence, where they mediate their anti-inflammatory effects [78].

**CXCR6, CXCL16 and Immune Cell Localization at Epithelial Surfaces in the Liver**

In human blood, CXCR6 is expressed on Th1 cells and effector CD8 T cells. We and others have reported high levels of CXCR6 on CD4 and CD8 T cells within the inflamed human liver. The CXCR6 ligand CXCL16 is one of only two chemokines that exist in a transmembrane form [79–81]. CXCL16 is upregulated on inflamed bile ducts and hepatocytes and is also expressed by sinusoidal endothelium. The engagement of CXCR6 on T cells by CXCL16 on epithelial cells promotes β1 integrin-dependent adhesion, which we believe is important for the positioning, retention and survival of effector cells in the inflamed liver [82]. Klenerman and colleagues [41] have recently reported a unique subset of HCV-specific CXCR6+ liver-infiltrating CD8 T cells which express the C-type lectin CD161 and secrete IL-17 and IFN-γ. Defining these cells may represent an important liver-specific subsets of effector cells.

Other chemokines may also be involved in retaining T cells within the liver. These include CXCL12 [83, 84] and the other transmembrane chemokine CX3CL1 (fractalkine), both of which are expressed on inflamed bile ducts [85, 86]. The fractalkine receptor CX3CR1 is expressed by Th1 cells and may help to retain these cells at sites of epithelial inflammation. We detected increased expression of another chemokine, CCL28, on cholangiocytes in a variety of liver diseases, including HCV, and found increased numbers of T cells expressing the CCL28 receptor, CCR10, in the inflamed human liver [78]. A high proportion of these cells were FOXP3+ CD4 T cells that secrete IL-10 and suppress T cell activation. Thus, CCL28 appears to recruit regulatory rather than effector cells to bile ducts. When compared with Treg in blood the CCR10+, liver-derived Treg express high levels of CXCR3 and low levels of CCR7 consistent with a tissue-infiltrating phenotype leading us to propose that they use CXCR3 to enter the liver but then localize to inflamed bile ducts using CCR10. CCL28 was originally isolated from the gut, but is widely expressed at mucosal surfaces throughout the body [87, 88].

**CCR5 and CCR1/CCL5 and Liver Inflammation**

CCR1, CCR2 and CCR5 have all been implicated in hepatic inflammation. CCR2 and CCR5 expressing CD8 T cells are enriched in the inflamed human liver, and CCR1 is important in the regulation of hepatic inflammation in murine models [51, 55]. The three receptors share chemokine ligands: CCR5 interacts with CCL3, CCL4, CCL5 and CCL8; CCR2 with CCL2, CCL13, CCL7 and CCL8; and CCR1 with CCL3, CCL5, CCL7, CCL23 and CCL14–16 [3, 89]. All of these chemokines have been detected in the liver [51, 56, 90–92]. CCR1, CCR2 and CCR5 expression is characteristic of memory T cells and CCR5 is expressed on Th1 cells [89, 93], with particularly high frequencies detected in the human liver [51, 55, 90]. CCR5 ligands are strongly expressed on portal and vascular en-
dothelium [94] and in murine models of graft-versus-host disease CCR5 and CCL3 support effector cell recruitment to portal tracts [57, 95]. Although other murine models of liver inflammation are characterized by intrahepatic CCR5+ lymphocytes [96, 97], mice that lack CCR5 are more susceptible to Con A-induced hepatitis and exhibit extensive inflammation mediated by CCR1+ effectors [58, 97]. This emphasizes the complexity of chemokine networks and suggests that under some conditions CCR5 recruits anti-inflammatory as well as effector cells [96].

**Homeostatic Chemokines and Lymphocyte Egress from the Liver**

Homeostatic chemokines can be upregulated at sites of inflammation where they play important roles in regulating leukocyte trafficking, particularly through the formation of tertiary neolymphoid structures [98, 99]. Neolymphoid follicles that express CCL19 and CCL21 are a feature of many chronic inflammatory liver diseases, particularly PBC, PSC and HCV infection [54]. They may be sites for ongoing lymphocyte recruitment and provide survival signals to maintain the chronic inflammatory infiltrate within the liver [83, 84, 100, 101]. The CCR7 receptor is expressed by naive T cells and a subset of central memory cells to promote their recirculation through secondary lymphoid tissues. However, we detected CCR7+ T cells in livers from patients with chronic autoimmune liver disease and chronic HCV infection [100]. However, most (if not all) of these cells were memory rather than naive cells [54, 102], thus CCR7+ CD8 T cells in the HCV-infected liver were CD62Llow and LFA-1high, which is characteristic of memory cells. Because CCL19 and CCL21 are expressed on sinusoids and lymphatic vessels in portal tracts [54, 98], we suggest that these cells use CCR7 to migrate out of the liver via afferent lymphatics to drain lymph nodes where they are either removed during the resolution of infection or re-stimulated to maintain chronic hepatitis. This pathway may be defective in HCV infection because the numbers of intrahepatic CCR7+ memory T cells are reduced. Others have reported an important role for CCR7 in promoting resolution of inflammation, and a lack of CCR7 in mice leads to enhanced inflammation [103–105].

**CCR9 and the Gut-Liver Interface in PSC**

PSC, a chronic inflammatory liver disease characterized by progressive bile duct destruction, develops as an extra-intestinal complication of inflammatory bowel disease. The existence of a population of long-lived memory T cells capable of homing both to the liver and the gut could explain the link between inflammatory bowel disease and liver disease. Evidence suggests that a proportion of the lymphocytic infiltrate in PSC consists of cells generated in the gut during episodes of inflammation which enter the liver in response to aberrantly expressed gut-homing molecules and chemokines [46]. The gut-associated chemokine CCL25 regulates the recruitment of T cells to the bowel by [46, 106, 107] activating its receptor CCR9, which is largely restricted to mucosal T cells and which promotes adhesion of α4β7 to MAdCAM-1 expressed on gut vessels [108]. We have found that in PSC, 20% of effector T cells in the liver express CCR9 and α4β7 and that the hepatic endothelium expresses CCL25 and MADCAM-1, providing a mechanism to explain the recruitment of α4β7/CCR9+ mucosal lymphocytes to the liver [70]. The unique CCR9+/α4β7+ phenotype is imprinted on T cells during activation by gut DCs [109, 110], but we have been unable to reproduce this phenotype using liver-derived DCs, suggesting that the cells must originally have been activated in the gut [111]. The liver-infiltrating CCR9+ α4β7 cells are primed to secrete IFN-γ on stimulation, suggesting that they can be rapidly expanded into effector cells in the liver, thereby mediating inflammatory damage, although the triggering antigen is still unknown.

**Regulatory T Cells, Chemokines and Human Liver**

Expression of distinct chemokine receptors by Treg is critical for their recruitment and localization in inflamed nonlymphoid sites for the suppression of effector T cell-mediated inflammatory responses. The chemokine receptors expressed by Treg will depend upon the cytokine milieu in which they are activated. Inducible Treg can express patterns of homing receptors that overlap with Th1 effector cells, Th1 cells (CXCR3, CCR5 and CXCR6), Th2 cells (CCR4 and CCR8) and Th17 cells (CCR4, CCR6, CCR2 and CXCR3), allowing Treg to co-localize together with diverse effector T cells to suppress a wide range of inflammatory conditions [112]. We have found that Treg in the inflamed human liver express CXCR3 and use these receptors to migrate through sinusoidal endothelium under flow, whereas they co-localise within the liver using different receptors: CCR10 for recruitment to biliary epithelium and CCR4 to co-localize with liver infiltrating dendritic cells within inflammatory infiltrates [78, 130].
Interstitial Migration and Position of Recruited Lymphocytes

Once they have crossed the endothelial barrier, infiltrating lymphocytes need cues to position themselves in tissue and chemokines can provide such coordinated directed migration signals [113], particularly when acting as a surface-bound gradient in the extracellular matrix and stromal cells [114, 115]. The stroma can also modulate the function of the overlying endothelium, emphasizing the importance of the tissue microenvironment in shaping recruitment [116]. Leukocyte migration within interstitial tissue in vitro and in vivo is only partially integrin-dependent, being directed instead by chemokine-dependent migration along the confining ECM scaffold. In the setting of hepatic inflammation, once the lymphocytes are recruited into the inflamed tissue, the liver stromal cell network, characterized by hepatic stellate cells and activated liver myofibroblasts, direct the post-endothelial migration (fig. 2) [117, 118]. This is partly chemokine-dependent, but also involves chemokine-independent mechanisms [119].

Conclusions

Chemokines are a family of structurally related proteins that share the ability to induce migration of specific subsets of leukocytes, both effectors and regulators and are critical regulators of immunity and inflammation in human liver diseases. These specialized chemotactic cytokines play a central role in the generation of cellular inflammation, both in the protective responses to invading pathogens and in the pathological processes associated with infection and immune-mediated diseases. Understanding the role of specific chemokines might allow them to be targeted for therapeutic intervention in a wide range of diseases. However, such approaches are complex because although blocking chemokines that drive inflammation and fibrogenesis may be beneficial, such approaches run the risk of inhibiting the anti-inflammatory regulatory immune response.

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Chemokines and Lymphocyte Recruitment

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41
Chemokines and Lymphocyte Recruitment


