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

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Key Words

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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 re-

cruited, 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.

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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 G α_1 and G β - 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 G_i 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

Receptor	Chemokine	Functions	Distribution of chemokine receptors
CC subgroup			
CCR1	CCL3, CCL5, CCL7, CCL8, CCL13–16	T cell and monocyte migration; hypersensitivity; innate and adaptive immunity; inflammation	monocytes, memory T cells, Th1, NK
CCR2	CCL2, CCL7, CCL8, CCL13	T cell and monocyte migration; innate and adaptive immunity; Th1 inflammation	monocytes, memory T cells, basophils, pDC
CCR3	CCL5, CCL7; CCL11, CCL15–16, CCL24, CCL26	eosinophil and basophil migration; allergic inflammation; Th2 response	eosinophils, basophils
CCR4	CCL17, CCL22	T cell and monocyte migration; allergic inflammation; T _{reg} retention; skin homing; expressed on CD4 Th2 cells	Th2 cells, T _{reg} eosinophils, basophils, DC, T _{reg}
CCR5	CCL3; CCL4; CCL5; CCL8	Th1 response, adaptive immunity; inflammation, HIV infection	monocytes, Th1 cells, NK
CCR6	CCL20	DC migration, memory T cells, Th17 cells at site of inflammation	memory T cells, B cells, Th17, immature mDC
CCR7	CCL19, CCL21	T cell and DCs homing to secondary lymphoid tissue; lymphoid development	naïve T, B, mature mDC, Th1, Th2, T _{reg}
CCR8	CCL1	T cell trafficking; Th2 response	monocytes, Th2, T _{reg} , NK
CCR9	CCL25	T cell homing to gut and thymus tolerogenic DCs	DC, memory T cells, thymocytes
CCR10	CCL27, CCL28	T cell homing to skin and bowel	memory T cells, T _{reg}
CXC subgroup			
CXCR1	CXCL6, CXCL7, CXCL8	neutrophil migration; innate immunity; acute inflammation	PMN, monocytes, mast cells
CXCR2	CXCL1–3; CXCL5–8	neutrophil migration; innate immunity; acute inflammation; angiogenesis	PMN, monocytes, mast cells
CXCR3	CXCL9, CXCL10, CXCL11	T cell recruitment; adaptive immunity; Th1, Th2, Th17, T _{reg} inflammation	memory T cells, Th1, Th2, Th17, T _{reg} , NKT
CXCR4	CXCL12	stem cell migration; B cell lymphopoiesis	T and B cells, monocytes, stem cells, NKT
CXCR5	CXCL13	B cell homing in lymphoid organ	B cells
CXCR6	CXCL16	T cell migration	memory T cells, Th1, NK, NKT
CXCR7			
CX3C and XC family			
CX3CR1	CX3CL1	T cell and NK cell trafficking and adhesion; innate and adaptive immunity; Th1 inflammation	monocytes, Th1, NK
XCR1	XCL1–2	NK cell recruitment	NK

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 consensus 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_{reg} = regulatory T cell.

Fig. 1. Schematic diagram of the G protein-coupled receptor. Chemokine receptors are seven-transmembrane molecules coupled to heterotrimeric G proteins. When the chemokine interacts with its receptor, the β - and γ -subunits are assembled into $\beta\gamma$ -dimers that act as functional units. The α -subunits bind guanine nucleotides, and are active when GTP is bound. The G proteins are usually classified into four classes, α_i , α_s , α_q and $\alpha_{12/13}$, by the nature of their α -subunit. Chemokine receptors can signal through different G α -protein families, leading to distinct transduction pathways and downstream biological effects. Adapted from Viola and Luster [89].

kocyte integrins that mediate firm adhesion to endothelial ligands, such as ICAM-1 and VCAM-1. This stage includes slow rolling, arrest, adhesion strengthening and intravascular crawling, in which the leukocyte migrates on the vessel wall looking for the signals that drive transmigration through endothelium into tissue. Arrest depends on rapid increases in integrin affinity in response to chemokines displayed on proteoglycans on the endothelial glycocalyx [13].

During diapedesis, leukocytes migrate across the endothelium and basement membrane to enter tissue [10, 14]. In most tissues, diapedesis is regulated by adhesion receptors that localize to endothelial junctions through which the majority of migrating leukocytes cross. However, hepatic sinusoids lack conventional tight junctions, and the signals required for transmigration may differ in the sinusoids [15]. Additionally, how cells traverse the space of Disse is poorly understood. Once in tissue, the leukocyte follows chemokine gradients to sites of injury or infection. In secondary lymphoid tissues leukocytes can migrate through tissues in the absence of integrins by using chemokine-mediated changes in the actin cytoskeleton to propel migration [16, 17].

Chemokines secreted by other cells in the microenvironment can be taken up and undergo transcytosis across endothelial cells for presentation on the endothelial glycocalyx, thereby localizing chemokines to specific sites in the vasculature [18] where they can undergo further post-translational activation or deactivation by ectoenzymes such as CD26 [3, 19]. A group of promiscuous nonsignal-

ing chemokine receptors, termed interceptors, act as a sump to remove chemokines, or in the case of D6, promote their disposal via lymphatics [20]. One such receptor, DARC, promotes chemokine transcytosis and retention when expressed on endothelium; however, on red cells it retains chemokines in the circulation, thereby preventing random activation of circulating leukocytes [21].

Hepatic Inflammation and Chemokines

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-

Fig. 2. Leukocyte adhesion cascades in hepatic sinusoid. Chemokines are critical factors in regulating lymphocyte recruitment from the blood into the liver. Leukocyte extravasation into tissue involves multiple steps. First, lymphocytes are captured, bringing the flowing cell into contact with the vessel wall, to interact with the endothelium. Second, triggering involves chemokines which are immobilized on the endothelial glycocalyx that activate chemokine receptors on leukocytes, resulting in integrin binding to endothelial ligands such as ICAM-1 and VCAM-1. During this

stage, the leukocyte adheres on the vessel wall. Finally transendothelial migration into tissue occurs, which involves chemokines. Once in tissue, the cell follows chemokine gradients to sites of inflammation, using chemokine-mediated changes in the actin cytoskeleton to propel migration. CXCR = Chemokine (C-X-C motif) receptor; ICAM-1 = intercellular cell adhesion molecule 1; VCAM-1 = vascular cell adhesion molecule 1; VAP-1 = vascular adhesion protein-1; CXCR3 ligands = MIG, IP-10, I-TAC.

nisms to suppress immune responses to harmless antigens whilst maintaining the capacity to respond to infections, such as hepatitis viruses [22, 23]. In chronic inflammatory and autoimmune liver diseases, such responses are generated and persist, leading to chronic hepatitis, fibrogenesis and eventually cirrhosis. Vigorous intrahepatic immune responses probably depend on activation of T lymphocytes by mature, fully activated DCs within secondary lymphoid tissues (fig. 4). Whether the final result of immune recognition is immune tolerance or an effector response, the initial step is the recruitment of lymphocytes from blood via interactions with liver sinusoidal endothelial cells (fig. 4).

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

Fig. 3. T cell lineage and balance between T_{reg} and Th1, Th2, Th17 and Th9. Cytokine-mediated amplification of effector CD4⁺ Th1, Th2, Th17 and Th9 cells. After being activated, naive T cells differentiate into distinct CD4⁺ helper T cell subsets depending on the cytokine milieu, which drives a program of differentiation that includes the induction of key transcription factors as well as specific chemokine receptors. Th1 cells express inflammatory

type chemokine receptors CCR5 and CXCR3, Th2 cells express CCR4 and CCR8, and the recently discovered Th17 cells express CCR4, CCR6 and CXCR3. Th9 subsets were demonstrated recently from Th2 cells lineage when they were stimulated by IL-4 and TGF-β. Naturally occurring, thymic-derived T_{reg} maintain the immune tolerance and prevent excessive damage during infection and inflammation by controlling these T effector cells.

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 ex-

tracellular 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

Fig. 4. Regulatory and effector T cell recruitment to hepatic inflammation. During hepatic inflammation, liver-resident DCs sample foreign antigen and carry it to local portal lymph nodes for presentation to naive T cells recruited via high endothelial venules of lymph nodes. Following antigen presentation, different types of T effector and T regulatory cells leave the nodes and drain back to systemic circulation before being recruited into the liver

and the site of injury or inflammation via low-flow hepatic sinusoidal endothelial vascular bed. Local antigen presentation by epithelial and endothelial cells to naive T cells in the liver usually results in immunological tolerance, although microenvironmental signals form cytokines, and APCs are important in maintaining inflammation and a polarized local immune response [121–123].

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 μ M, 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_{reg} develop in response to retinoic acid and TGF- β and activation of STAT-5 and the transcription factor FOXP3 [124]. T_{reg} 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_{reg} 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 which may explain the characteristic neutrophil-rich infiltrate [33]. However, unlike some murine models, human chronic inflammatory diseases cannot be so easily assigned to Th1- versus Th2- versus Th17-dominated 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, CXCR6, CCR1 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

Table 2. Chemokines and receptors involved in the pathogenesis of liver disease

Location in the liver	Chemokines	Chemokine receptor
Portal vessels	CCL3, 4, 5	CCR5
Liver sinusoids	CXCL9, 10, 11	CXCR3
	CXCL16	CXCR6
Biliary epithelium	CCL28	CCR10
	CXCL16	CXCR6
	CX3CL1	CX3CR1
	CXCR4	CXCL12
Portal-associated lymphoid tissue	CCL19, 21	CCR7

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.

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].

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 effector 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 NF κ B-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 T_{reg} 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 β 1 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 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 T_{reg} in blood the CCR10⁺, liver-derived T_{reg} 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-

endothelium [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 CD62L^{low} and LFA-1^{high}, 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 groups 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 dis-

ease. 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 $\alpha_4\beta_7+$ 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 $\alpha_4\beta_7$ and that the hepatic endothelium expresses CCL25 and MAdCAM-1, providing a mechanism to explain the recruitment of $\alpha_4\beta_7$ +CCR9+ mucosal lymphocytes to the liver [70]. The unique CCR9+/ $\alpha_4\beta_7$ + 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+ $\alpha_4\beta_7$ 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 T_{reg} 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 T_{reg} will depend upon the cytokine milieu in which they are activated. Inducible T_{reg} can express patterns of homing receptors that overlap with T effector cells, Th1 cells (CXCR3, CCR5 and CXCR6), Th2 cells (CCR4 and CCR8) and Th17 cells (CCR4, CCR6, CCR2 and CXCR3), allowing T_{reg} to co-localize together with diverse effector T cells to suppress a wide range of inflammatory conditions [112]. We have found that T_{reg} in the inflamed human liver express CXCR3 and use this 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-localise 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.

Disclosure Statement

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