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Review

Effector CD8 T cell trafficking within the liver

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ABSTRACT

CD8 T cells play a critical role in several pathological conditions affecting the liver, most notably viral hepatitis. Accordingly, understanding the mechanisms that modulate the intrahepatic recruitment of CD8 T cells is of paramount importance. Some of the rules governing the behavior of these cells in the liver have been characterized at the population level, or have been inferred by studying the intrahepatic behavior of other leukocyte subpopulations. In contrast to most microvascular beds where leukocyte adhesion is restricted to the endothelium of post-capillary venules, it is now becoming clear that in the liver leukocytes, including CD8 T cells, can efficiently interact with the endothelium of hepatic capillaries (i.e. the sinusoids). While physical trapping has been proposed to play an important role in leukocyte adhesion to hepatic sinusoids, there is mounting evidence that T cell recruitment to the liver is highly regulated and depends on recruitment signals that are either constitutive or induced by inflammation. We review here several specific adhesive mechanisms that have been shown to regulate CD8 T cell trafficking within the liver, as well as highlight recent data that establish platelets as key cellular regulators of intrahepatic CD8 T cell accumulation.

1. Introduction

CD8 T cells play a fundamental role in the pathogenesis of liver disease and viral clearance during acute, self-limited hepatitis B virus (HBV) and hepatitis C virus (HCV) infection (Guidotti and Chisari, 2006; Iannacone et al., 2006). Moreover, the pathogenesis of chronic HBV or HCV infection is thought to involve functionally inefficient CD8 T cells that do not eradicate the infection but sustain repetitive cycles of immune-mediated hepatocellular necrosis, hepatocellular regeneration and inflammation that are likely to precipitate random genetic damage and promote HCC development (Guidotti and Chisari, 2006). Both CD8 T cells' defensive and destructive functions are mediated by antigen (Ag)-experienced effector cells and depend on these cells' ability to migrate from the blood to the liver. Understanding the signals that modulate the intrahepatic recruitment of CD8 T cells is therefore critical to get insight into the pathogenesis of acute and chronic viral hepatitis.

The classic paradigm for leukocyte migration from blood vessels to interstitial tissues involves a multistep process that occurs in post-capillary venules (Springer, 1994) but not in arterioles or capillaries (where leukocyte adhesion may limit gas exchange and tissue perfusion, Andrian and Mackay, 2000). The initial weak rolling interactions between leukocytes and endothelial cells are mediated by a family of proteins called selectins (Kansas, 1996). There are three types of selectins: one expressed on leukocytes (L-selectin), one on endothelial cells (E-selectin), and one on platelets and on endothelial cells (P-selectin). The ligands for selectins are sialylated oligosaccharides bound to mucin-like glycoprotein backbones (Kansas, 1996). Firm adhesion of leukocytes to endothelial cells is mediated by a family of heterodimeric leukocyte surface proteins called integrins (Hynes, 1992; Springer, 1994). The combination of cytokine-induced endothelial expression of integrin ligands, mainly vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), and chemokine-mediated conversion of integrins to a high-affinity state on leukocytes (Hynes, 2002) results in firm adhesion of leukocytes to the endothelium at sites of inflammation.

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; Ag, antigen; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; fMLP, N-formyl-methionyl-leucyl-phenylalanine; LSECs, liver sinusoidal endothelial cells; LCMV, lymphocytic choriomeningitis virus; IgSF, immunoglobulin superfamily; VLA-4, very late antigen 4; MAdCAM-1, mucosal addressin cell adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; LAD-1, leukocyte adhesion deficiency type 1; TCR, T cell receptor; PECAM-1, platelet endothelial cell adhesion molecule 1; IFN, interferon; NK, natural killer; IL, interleukin; VAP, vascular adhesion protein; Th, T helper; PG, prostaglandin; TX, thromboxane; ADP, adenosine diphosphate; GP, glycoprotein; vWF, von Willebrand factor; PSGL-1, P-selectin glycoprotein ligand 1.

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The liver represents an exception to this leukocyte migration paradigm in several respects (Lee and Kubes, 2008). First, leukocyte adhesion is not restricted to the endothelium of post-capillary venules, but it also occurs in sinusoids (Lee and Kubes, 2008); indeed, in response to a chemotactic stimulus such as N-formyl-methionyl-leucyl-phenylalanine (fMLP), the majority of leukocytes have been shown to adhere to the sinusoidal bed, with only a small fraction of leukocytes adhering to post-sinusoidal venules (Wong et al., 1997). It is of note, however, that the quantitative importance of sinusoidal adhesion is less established for CD8 T cells, particularly in the context of intrahepatic Ag recognition. Second, visualization of leukocyte behavior in the liver microvasculature revealed that while in post-sinusoidal vessels rolling precedes adhesion, leukocyte adhesion to liver sinusoidal endothelial cells (LSECs) often occurs independent of any notable rolling (Lee and Kubes, 2008). It is also of note that LSEC are morphologically unique and characterized by the absence of tight junctions between cells and the lack of a basal membrane (Wisse et al., 1985). This is in contrast to most vascular beds in other tissues and organs, where a continuous endothelial cell layer and a basement membrane physically separate parenchymal cells from circulating leukocytes (Wisse et al., 1985). Moreover, hepatocyte membranes often protrude from the fenestrated endothelial barrier of sinusoids, thus providing the opportunity for direct interaction of circulating cells with the underlying hepatocytes (Warren et al., 2006). For all these reasons, the molecular mechanisms leading to leukocyte adhesion to LSEC appear to be somewhat different from those occurring in post-capillary venules of other vascular districts (Lee and Kubes, 2008). We will review below our current understanding of the molecular and cellular mechanisms mediating CD8 T cell homing to the liver, focusing, when possible, on effector CD8 T cell trafficking in the context of Ag recognition.

2. Selectins

A mentioned earlier, the selectin family has three members: L-selectin (CD62L), E-selectin (CD62E) and P-selectin (CD62P). Selectins are the quintessential adhesion molecules: they are highly efficient mediators of tethering and rolling (Kansas, 1996), and they do so constitutively, i.e. they do not require an activating stimulus to bind to a carbohydrate ligand through their N-terminal, Ca^{2+}-dependent lectin domain. The role of selectins in leukocyte recruitment into organs such as lymph nodes, peritoneal cavity, mesentery, muscle and skin has been extremely well characterized (Lee and Kubes, 2008).

Consistently with the idea that leukocyte adhesion to LSEC occurs independently of any notable rolling (see above), selectins were shown to be dispensable for leukocyte adhesion in liver sinusoids (Wong et al., 1997; Essani et al., 1998; Fox-Robichaud and Kubes, 2000; Bowen et al., 2004). Similarly, migration of virus-specific CD8 T cells to lymphocytic choriomeningitis virus (LCMV)-infected liver was shown to occur in the absence of endothelial (E/P) selectins (Bartholdy et al., 2000).

3. Integrins

Integrins are a large family of heterodimeric glycoproteins (Hynes, 1992, 2002; Springer, 1994) that are found on most cell types. Two subfamilies are most important for leukocyte migration: the α_4-(CD49) and the β_2-(CD18) integrins. Endothelial ligands for these molecules are members of the immunoglobulin superfamily (IgSF). Arguably, the most important ligand for β_2-integrins is the IgSF member ICAM-1. In most vascular districts ICAM-1 is constitutively expressed on post-capillary venules and only minimally on capillaries; by contrast, the density of ICAM-1 in hepatic sinusoids is comparable to that of central venules (Iigo et al., 1997). The IgSF member VCAM-1 (the ligand for the α_4-integrin VLA-4) is not expressed in normal liver tissue but it is markedly upregulated on sinusoidal endothelium when inflammation is present (Volpes et al., 1992). During some inflammatory conditions the hepatic endothelium can be induced to also express the mucosal addressin cell adhesion molecule-1 (MAdCAM-1; the ligand for $\alpha_4\beta_7$), which is normally confined to mucosal endothelium in the bowel (see later).

An important feature of integrins is their "tunability" (Hynes, 2002). While selectins are always active, integrins must first assume an activated state to mediate adhesion and their affinity and/or avidity towards the respective ligands can be rapidly modified in response to stimuli such as chemokines (see later). We will review below our current understanding of the role that specific integrins play in the recruitment of CD8 T cells to both uninflamed and inflamed liver microvasculature.

3.1. β_2-Integrins

The leucocyte-restricted β_2-integrins comprise four members, namely $\alpha_L\beta_2$ (LFA-1), $\alpha_M\beta_2$ (Mac-1), $\alpha_x\beta_2$ (p150, 95) and $\alpha_D\beta_2$. Each of the four known β_2-integrin heterodimers has a different cellular distribution, with LFA-1 expressed on all leukocytes, including CD8 T cells (Luo et al., 2007; Tan, 2012). The absence of β_2-integrins in humans results in leukocyte adhesion deficiency type 1 (LAD-1), a syndrome that manifests itself with increased susceptibility to infections and impaired capacity of wound healing (Anderson and Springer, 1987). β_2-Integrin knockout mice have a phenotype similar to that of LAD-1 patients, and leukocytes derived from these animals show diminished ability to extravasate at sites of infection or injury (Grabbe et al., 2002).

Although it is now well appreciated that β_2-integrin mediates firm adhesion of leukocytes in many tissues, the evidence for a role for this integrin in the liver is less compelling (Lee and Kubes, 2008). LFA-1 has been proposed to mediate both naïve (Bertolino et al., 2005) and effector (John and Crispe, 2004; Sato et al., 2006) CD8 T cell adhesion to LSEC, but this may occur only in the context of antigen presentation in the liver, possibly because of a TCR-mediated increase in LFA-1 affinity for ICAM-1.

3.2. α_4-Integrins

The α_4-integrin family includes $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$, two molecules that are expressed on lymphocytes and monocytes (Springer, 1994). VLA-4 and $\alpha_4\beta_7$ bind to VCAM-1 and MAdCAM-1, respectively, on endothelial cells, and this process regulates the trafficking of different leukocyte subsets in mucosal tissues, especially the gut (Springer, 1994).

Antigen non-specific adhesion of activated CD8 T cells to LSEC has been proposed to occur via VCAM-1/α_4-integrin (John and Crispe, 2004). In a graft versus host disease model, however, α_4-integrin was found to be dispensable for the recruitment of activated CD8+ T cells into the liver (Sato et al., 2006). Also, naïve CD8 T cells appear not to need α_4-integrin to be recruited to the uninflamed liver (Bertolino et al., 2005).

As mentioned earlier, when the hepatic vasculature is inflamed, additional adhesion molecules are expressed (Crispe, 2012). Of particular interest is MAdCAM-1, which engages the $\alpha_4\beta_7$ integrin that is prominently expressed on intestinal lymphocytes. This interaction might account for the hepatic trapping of activated gut-derived T cells in inflammatory bowel disease (Grant et al., 2001).

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3.3. $\alpha_V\beta_3$

Activated lymphocytes express the integrin $\alpha_V\beta_3$, which binds several extracellular matrix molecules and PECAM-1 (CD31), an IgSF molecule that has been implicated in leukocyte migration across endothelial cells (Muller et al., 1993). Although the role of $\alpha_V\beta_3$ in CD8 T cell trafficking to the liver has never been tested, in vivo blockade of this molecule via specific antibodies had no effect on hepatic neutrophil accumulation (Chosay et al., 1998). This is consistent with the observation that LSEC express little or no PECAM-1 (Chosay et al., 1998).

4. Chemokines

Chemokines (*chemo*tactic cyto*kines*) are secreted polypeptides that bind to surface receptors and transmit signals through $G\alpha_i$ proteins (Rot and Andrian, 2004). Just like adhesion molecules, chemokine receptors can be upregulated or lost as cells differentiate, allowing leukocytes to coordinate migratory routes and biological function (Rot and Andrian, 2004). After secretion into extracellular spaces, chemokines bind to heparin-like glycosaminoglycans on cell surfaces and in the extracellular matrix; leukocytes can track down these immobilized chemokines, which may persist in tissues longer and at higher concentrations than freely diffusible molecules. Since lymphocytes must be positioned correctly to interact with other cells, the pattern of chemokine receptors and the type and distribution of chemokines in tissues critically influence immune responses (Rot and Andrian, 2004).

The chemokine molecular signature includes four conserved cysteine residues that form two disulfide bonds pairing the first with the third and the second with the fourth cysteines (Zlotnik and Yoshie, 2012). Based on the arrangement of the N-terminal two cysteine residues, chemokines are grouped into four subfamilies: CXC, CC, (X)C, and CX3C. In the CXC chemokines, one amino acid separates the first two cysteines, whereas in CC chemokines, these two cysteines are adjacent. A single member of the CX3C subfamily, CX3CL1 or fractalkine, has three amino acids between the two cysteines, whereas the first and third cysteines are missing in the (X)C subfamily (Zlotnik and Yoshie, 2012).

Several lines of evidence from human studies suggest that the intrahepatic recruitment of CD8 T cells and other inflammatory cells may be promoted by chemokines. Most effector T cells infiltrating the chronically inflamed human liver express high levels of CXCR3, CXCR6, CCR1 and CCR5 (Shields et al., 1999; Kunkel et al., 2002; Boisvert et al., 2003; Leroy et al., 2003; Heydtmann et al., 2006; Dumoulin et al., 1997; Apolinario et al., 2002; Arai et al., 2002; Diago et al., 2006; Larrubia et al., 2007), with CCR5-bearing T cells preferentially accumulating around portal tracts and CXCR3-bearing T cells distributing more evenly throughout the liver lobule (Murai et al., 1999; Harvey et al., 2003; Curbishley et al., 2005). We review below examples of specific chemokine/chemokine receptor interactions that appear to be predominant at mediating CD8 T cell recruitment into the liver.

4.1. CXCR3 and its ligands CXCL9, CXCL10 and CXCL11

Using flow-based adhesion assays it has been shown that the CXCR3 ligands CXCL9, CXCL10 and CXCL11 are important not only in adhesion, but also in transmigration of human effector T lymphocytes through the hepatic endothelium (Curbishley et al., 2005). Additional evidence for a role of CXCR3 and its ligands in liver T cell trafficking comes from studies in which HBV-replication competent transgenic mice were used as recipients of HBV-specific effector CD8 T cells. In those studies it was shown that CXCL9 and CXCL10 are rapidly and strongly induced in the liver after T cell transfer (Kakimi et al., 2001); the transferred T cells produce neither chemokine but, rather, they activate (via the secretion of IFN-γ) liver non-parenchymal cells and especially hepatocytes to produce them (Kakimi et al., 2001); importantly, blocking CXCL9 and CXCL10 in vivo reduces the recruitment of host-derived mononuclear cells into the liver, particularly those subsets that are known to express CXCR3 (NK cells, myeloid dendritic cells and effector CD8 T cells) (Kakimi et al., 2001). It is also important to note that CXCL9 and CXCL10 neutralization only partially reduces the recruitment of virus-specific effector CD8 T cells (Kakimi et al., 2001), suggesting that other receptor/ligand pairs play a role in the intrahepatic recruitment of these cells.

4.2. CXCR6 and its ligand CXCL16

Both human (Heydtmann et al., 2005) and mouse (Sato et al., 2005) studies have shown that CXCR6/CXCL16 can regulate the recruitment of activated CD8 T cells to the inflamed liver. Along these lines, Klenerman and colleagues have recently reported a unique subset of HCV-specific CXCR6+ liver-inflitrating CD8 T cells that express the C-type lectin CD161 and secrete IL-17 and IFN-γ (Northfield et al., 2008). Of note, CXCR6 was also shown to be required for the hepatic homing of NK and NKT cells (Geissmann et al., 2005).

4.3. CCR5 and its ligand CCL3

In murine models of graft-versus-host disease CCR5 and CCL3 have been shown to support effector CD8 T cell recruitment to portal tracts (Murai et al., 1999, 2003).

4.4. CCR9 and its ligand CCL25

During primary sclerosing cholangitis, a chronic inflammatory liver disease characterized by progressive bile duct destruction and developing as an extra-intestinal complication of inflammatory bowel disease, liver-infiltrating lymphocytes include CCR9+ mucosal T cells (Eksteen et al., 2004). It has been suggested that the hepatic recruitment of CCR9+ T cells depends on the aberrant liver expression of the gut-specific chemokine CCL25 (Eksteen et al., 2004).

5. Other adhesion molecules (VAP-1, CD44)

5.1. VAP-1

VAP-1 is a 170-kDa homodimeric glycoprotein that is expressed by endothelial cells and mediates lymphocyte binding to high endothelial venules under shear conditions (Salmi and Jalkanen, 1996; Salmi et al., 1997). VAP-1 is expressed at high levels in the human liver and it promotes lymphocyte adhesion and transmigration across hepatic sinusoidal endothelial cells in vitro (Lalor et al., 2002). In humans, LSEC have been shown to constitutively express VAP-1 and to up-regulate this protein during inflammatory responses (McNab et al., 1996). In mice, LSEC express very little VAP-1 under basal conditions, but they do significantly express it when inflammation is present (Bonder et al., 2005).

Although CD4 T cells polarized to a Th2 phenotype have been shown to require VAP-1 for efficient homing to the inflamed liver (Bonder et al., 2005), CD8 T cell recruitment into the liver has been shown to be independent of VAP-1 (Bertolino et al., 2005). Of note, the ligand for VAP-1 remains to be identified, but it has been postulated that, upon activation, this glycoprotein leads to the upregulation of E-selectin, ICAM-1 and VCAM-1 on LSEC and the secretion of CXCL8, thus supporting leukocyte homing indirectly (Lalor et al., 2007).

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5.2. CD44

Interaction of CD44 with sinusoid-expressed hyaluronan has been recently proposed to be the dominant mechanism for neutrophil sequestration in inflamed liver sinusoids (Mcdonald et al., 2008). Antigen-experienced CD8 T cells are known to express high levels of CD44, but whether this molecule can also support lymphocyte adhesion to hepatic sinusoids is yet to be defined.

6. Kupffer cells and platelets

So far we discussed molecular interactions between CD8 T cells and the sinusoidal endothelium that have been shown to regulate T cell trafficking to the liver. Lymphocytes and endothelial cells, however, exist in a complex multi-cellular microenvironment where other cells types might influence their behavior via paracrine interaction. One example of this is provided by Kupffer cells, liver-resident intravascular macrophages. Although Kupffer cells can transiently interact with T cells (Bertolino et al., 2002), their contribution to the intrahepatic accumulation of effector CD8 T cells was shown to be negligible (Sitia et al., 2011). Another example is provided by platelets, anucleated blood cells that have been shown to interact with leukocytes and modulate their function (Ruggeri, 2009; Vieira-de-Abreu et al., 2012).

Several recent studies have demonstrated that intrahepatic recruitment of antigen-specific effector CD8 T cells is critically dependent on platelets (Iannacone et al., 2005, 2007a,b; Lang et al., 2008; Iannacone et al., 2009; Sitia et al., 2012). Indeed, in mouse models of CD8 T cell-mediated acute viral hepatitis, we recently showed that platelet depletion is associated with a profound reduction in the intrahepatic accumulation of virus-specific effector CD8 T cells and a proportional reduction in liver disease severity, both of which are restored upon reconstitution with normal platelets, but not upon reconstitution with platelets treated with prostaglandin (PG)E_1, a known inhibitor of platelet activation (Iannacone et al., 2005). In vitro findings also indicate that, under the low shear flow conditions likely occurring in the venous circulation of the liver, antigen-specific effector CD8 T cells tightly interact with platelets and, again, this process is inhibited when platelets are treated with PGE_1 (Iannacone et al., 2005). In the ongoing effort to explain mechanistically why platelets are required to support CD8+ T cell-induced liver pathology, we also found that this process is influenced by two specific inhibitors of platelet activation pathways, aspirin that blocks thromboxane (TX) A2 production and clopidogrel that blocks the P2Y12 ADP receptor (Cattaneo, 2004). Indeed, treating mice with aspirin, clopidogrel, or a combination of the two, attenuates acute liver injury by reducing the hepatic accumulation of antigen-specific CD8+ T cells and antigen-nonspecific inflammatory cells (Iannacone et al., 2007a). Of note, platelet activation follows adhesion to activated endothelium and/or exposed subendothelial matrix and is mediated primarily by two receptors, GPIb-α and GPVI, which bind to von Willebrand factor (vWF) and collagen, respectively (Ruggeri, 2002). Platelet activation induces cytoskeletal assembly and shape changes, secretion of agonists promoting further activation and aggregation, and functional expression of molecules such as P-selectin or GPIIbIIIa (Weyrich and Zimmerman, 2004) that could be involved in the interaction with effector CD8 T cells. Pertinent to this, platelet P-selectin has been shown to interact with PSGL-1 on leukocytes (including T cells) and promote their rolling along the endothelium of lymph nodes (Diacovo et al., 1996). Upon interaction with platelets, leukocytes are also thought to roll on the endothelium of cutaneous post-capillary venules thanks to platelet expression of GPIIbIIIa, which may secondarily interact with endothelial ICAM-1 (Ludwig et al., 2004). Along these lines, intravital microscopy studies in mesenteric venules have recently suggested that, after directly supporting an initial rolling of leukocytes in a P-selectin-dependent manner, platelets stimulate endothelial cells to become activated, express P-selectin themselves, and further sustain leukocyte rolling (Dole et al., 2005). Based on the aforementioned evidence, it is possible that the expression of P-selectin or GPIIbIIIa on platelets and PSGL-1 on effector CD8 T cells (Borges et al., 1997) may promote interaction between these cell types.

If a functional connection between platelets and T cells depends on direct and/or indirect intercellular interactions within the liver remains to be demonstrated. We have proposed that the activation-dependent expression of platelet CD40 ligand contributes to the expansion phase of virus-specific CD8+ T cells, resulting in their accumulation at sites of infection (Iannacone et al., 2008); this effect may reflect direct interaction of activated platelets with CD8+ T cells that express CD40 (Bourgeois et al., 2002; Meunier et al., 2012). Others have indicated that platelet CD40 ligand has the potential to enhance virus-specific CD8+ T cell responses indirectly, mostly by promoting the maturation of dendritic cells (Elzey et al., 2003; Li, 2008).

While the exact molecular mechanisms by which platelets support CD8 T cell-mediated liver immunopathology remains ill-defined, we recently adapted a mouse model of chronic immune-mediated hepatitis B that progresses to HCC (Nakamoto et al., 1998, 2004) to evaluate whether aspirin and clopidogrel may also blunt the hepatic accumulation of pathogenic effector CD8 T cells under conditions of sustained liver injury. We were able to show that anti-platelet therapy suppresses hepatic immunopathology overtime, thus preventing/delaying the development of HCC and improving overall survival (Sitia et al., 2012).

7. Conclusions and future directions

Significant advances have been made in our comprehension of hepatic CD8 T cell recruitment, how it differs from the recruitment of these cells to other tissues or organs and how the process is modulated by inflammation. While some of the rules that govern CD8 T cell homing to the liver have started to be clarified at the population level, we still have limited knowledge of the precise dynamics of intrahepatic CD8 T cell migration and interaction with other cell types at the single-cell level, particularly in the context of intrahepatic antigen recognition. We believe that recent advances in the field of live imaging, coupled with animal models that express viral antigens in the hepatocyte, will provide the opportunity to tackle some of these questions directly in the living animal. This will not only greatly improve our understanding of CD8 T cell trafficking within the liver but it may also provide tools for the design of new immune therapeutic strategies for the treatment of chronic viral hepatitis and liver cancer.

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