Cell Adhesion and Migration

III. Leukocyte adhesion and transmigration in the liver vasculature*

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Jaeschke, Hartmut, and C. Wayne Smith. Cell Adhesion and Migration. III. Leukocyte adhesion and transmigration in the liver vasculature. Am. J. Physiol. 273 (Gastro-intest. Liver Physiol. 36): G1169–G1173, 1997.—Leukocytes, i.e., neutrophils, monocytes, and lymphocytes, can accumulate in the hepatic vasculature and contribute to the pathophysiology of various liver diseases. Recently, significant progress has been made in the understanding of the basic mechanisms of neutrophil infiltration and cytotoxicity in the liver. However, there are a substantial number of unresolved issues. This article describes the current knowledge and the gaps in our understanding of mechanisms of neutrophil sequestration in sinusoids and venules, adhesion to endothelial cells, and transmigration and adherence to parenchymal cells. From these data, it is clear that assumptions regarding the roles of adhesion molecules in the liver may be misleading if drawn from studies of peripheral vascular beds. Greater insight into these mechanisms is critical for the development of selective therapeutic strategies that attenuate excessive inflammatory responses without compromising the vital host defense.

neutrophils; adhesion molecules; inflammation; ischemia-reperfusion; sepsis; endotoxin

Neutrophils are found in the liver in a variety of pathological conditions, including alcoholic hepatitis, endotoxemia, sepsis, hepatic ischemia and reperfusion, hemorrhagic shock, hepatocarcinoma, and remote organ injury (11). The accumulation of neutrophils in various tissues and tissue damage during acute inflammation are dependent on a cascade of adhesive interactions among leukocytes and endothelial cells, other leukocytes, extracellular matrix, and parenchymal cells (10). However, the individual steps in this cascade vary substantially among tissues, and relatively little is known about the specific interactions that allow neutrophils to damage liver parenchyma. The current status of knowledge regarding adhesion molecule regulation and expression in normal and diseased liver and the specific interactions and mechanisms of toxicity between neutrophils and hepatic parenchymal cells have been reviewed recently (11, 19). Numerous major questions remain to be answered. We wish to highlight some areas of particular interest.

Leukocyte localization in the hepatic vasculature

In general, leukocyte adhesion and transmigration in most organs take place in postcapillary venules (10). In the liver, neutrophils accumulate in sinusoids (capillaries) and in postsinusoidal venules (3, 31). Recently, the relative pathophysiological importance of neutrophil adherence in both types of vessels has been investigated in a model of galactosamine and endotoxin-induced liver injury (3). Despite neutrophil adherence in sinusoids and venules, only sinusoidal neutrophils actually extravasated and caused injury (3). In other models of neutrophil-dependent liver injury, e.g., ischemia-reperfusion (12, 16), the distribution of neutrophils is predominantly sinusoidal; an injury pattern of areas of focal necrosis further supports the hypothesis that mainly sinusoidal neutrophils are responsible for the damage. A limited number of neutrophils marginate in postsinusoidal venules under a variety of inflammatory conditions (3, 31). Intercellular adhesion molecule-1 (ICAM-1) is constitutively expressed on venular endothelial cells, and ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and selectins can be upregulated during an inflammatory response (11). Adhesion of neutrophils in venules could be attenuated by antibodies to ICAM-1 (31) and P-selectin (7). This suggests that neutrophil rolling and firm adherence in postsinusoidal venules include the interactions between selectins and selectin ligands and between β₂-integrins and ICAM-1, respectively. However, in the few experimental models in which venular leukocyte adhesion has been observed, there was no evidence that relevant numbers of neutrophils extravasated from that location. This does not rule out that under different pathophysiological conditions neutrophils may transmigrate in venules and contribute to the injury process. In most experimental models investigated, it is obvious that the majority of neutrophils sequestered in the hepatic vasculature are localized in sinusoids (3). Under inflammatory conditions, ICAM-1 and VCAM-1, but not P-selectin,
can be expressed in sinusoidal lining cells (11). However, the importance of these adhesion molecules and their counterparts on leukocytes for neutrophil accumulation in sinusoids is controversial. Antibodies to the α-chain (CD11a, CD11b) and the β-chain (CD18) of β2-integrins as well as to their counterreceptor ICAM-1 failed to prevent neutrophil accumulation in sinusoids (15). Furthermore, antibodies to P-selectin or the use of P-selectin knockout mice had no effect on sinusoidal neutrophil sequestration during endotoxemia (7). Wong et al. (33) observed that after superfusion of the liver with the chemotactic peptide N-formyl-Met-Leu-Phe, mice deficient in P-selectin exhibited normal levels of neutrophil sequestration in sinusoids, whereas mice deficient in both P-selectin and ICAM-1 had markedly reduced accumulation. Thus a role for ICAM-1 in neutrophil accumulation may vary with the inflammatory stimulus.

Whereas most studies have failed to define adhesive mechanisms for the sinusoidal localization of neutrophils, there are several factors under inflammatory conditions that might favor physical trapping of neutrophils. Swelling of Kupffer and endothelial cells has been reported during exposure to inflammatory mediators (24). An imbalance of vasoconstrictor (e.g., endothelin-1) and vasodilator (e.g., nitric oxide) production may narrow the vessel diameter (1). Inflammatory stimuli such as activated complement fragments have been shown to reduce the deformability of neutrophils (34), thereby increasing the likelihood of trapping in small-diameter vessels. Combinations of these factors may be more significant in the initial localization of neutrophils in liver sinusoids during inflammation than specific adhesive mechanisms. Thus, although the accumulation of neutrophils in the sinusoids is evident in models of liver inflammation, the exact mechanisms involved remain to be exactly defined. The conditions that induce neutrophil accumulation in the liver also induce the expression of adhesion molecules. It is becoming more apparent that assumptions regarding the roles of these molecules may be misleading if drawn from studies of peripheral vascular beds such as the mesenteric or cremasteric venules.

Although extensive accumulation of neutrophils occurs in the sinusoids during ischemia-reperfusion (16), endotoxemia (17, 21), and excessive chemokine production (22, 29) and after complement activation (13), injury caused by these cells appears to be limited. In fact, endothelial and parenchymal cell damage during the early phase appears to be caused largely by Kupffer cells (12, 21). Marked stimulation is required for neutrophils simply sequestered in the sinusoids to induce liver injury (36). This may occur when animals are subjected to different insults in which the first insult acts as a priming event and the second further activates the cells (21). However, even under these conditions, the damaging effect of activated neutrophils may be limited to the sinusoidal lining cells and may not affect parenchymal cells. Because neutrophils are also less likely to simply plug sinusoids (36), the mechanism of injury requires neutrophil transmigration.

**TRANSENDOTHELIAL MIGRATION**

There are several lines of evidence that support the hypothesis that transmigration of neutrophils is critical for parenchymal cell injury in the liver. First, comparison of the responses between animals treated with endotoxin and animals treated with galactosamine and endotoxin indicated that the inflammatory response, including sinusoidal neutrophil sequestration, is similar in both models. However, only in galactosamine and endotoxin-treated animals did neutrophils actually transmigrate and cause injury (3). Furthermore, inhibition of transmigration with antibodies to ICAM-1 (6) and VCAM-1 (5) protected against the injury. Second, general overexpression of the neutrophil chemotactic factor interleukin-8 (IL-8) induced neutrophil accumulation in the liver but did not cause injury (29). However, in animals that generated excessive amounts of cytokine-induced neutrophil chemoattractant (CINC) selectively in parenchymal cells, i.e., established a chemotactic gradient, neutrophils extravasated and caused liver cell injury (22). These data strongly suggest that transmigration of neutrophils is essential for parenchymal cell damage, at least in livers with intact sinusoidal lining. In the event of substantial endothelial cell injury and potential denudation of the sinusoidal lining cells, e.g., during extensive Kupffer cell-induced damage, neutrophils can have direct access to parenchymal cells without transmigration.

The fact that transmigration is critical for leukocyte-induced injury raises questions regarding the involvement of adhesion molecules and the driving force behind the movement of leukocytes. In general, transmigration of neutrophils requires interaction between β2-integrins and ICAM-1 (8). Consistent with this concept, antibodies to ICAM-1 prevented transmigration and protected against galactosamine and endotoxin-induced liver injury (6). Furthermore, antibodies to β2-integrins (17) proved to be beneficial in this model. On the other hand, a recent study indicated that antibodies to VCAM-1 can also inhibit liver injury by preventing neutrophil extravasation (5). This indicates that neutrophils may be able to use interactions between β2-integrin and VCAM-1 in addition to between β2-integrin and ICAM-1 for transmigration. In contrast to these observations with antibodies, animals with a targeted deletion of ICAM-1 (disruption of exon 4) were as susceptible to galactosamine and endotoxin-induced liver damage as wild-type mice (35). These apparent discrepancies have not been resolved, but there is evidence that ICAM-1 knockouts based on deletion of the extracellular domains of ICAM-1 may not be null mutations, since alternatively spliced forms of ICAM-1 may exist in mice (20). Currently, the majority of evidence supports the hypothesis that leukocytes can use integrins and their respective counterreceptors to extravasate from the sinusoids. The fact that ICAM-1 and VCAM-1 are not only upregulated in experimental models of liver inflammation but also in human livers during allograft rejection, alcoholic liver cirrhosis, and viral hepatitis (see Ref. 11 for review) supports this concept.
Why do leukocytes extravasate from the hepatic vasculature? In general, there has to be a signal, i.e., a chemotactic gradient, toward the extravascular space. Chemokines are one of the most potent chemotactic factors. Members of this class of mediators can have selective chemotactic properties for neutrophils, lymphocytes, monocytes, or eosinophils. Human IL-8, rat CINC, human epithelial neutrophil-activating protein-78 (ENA-78), human Gro, and mouse Kupffer cell Gro are C-X-C chemokines that are highly chemotactic for neutrophils. Cytokines can induce formation of these mediators in hepatocytes in vitro and vivo. Antibodies to ENA-78 protected against the neutrophil-induced injury phase in hepatic ischemia-reperfusion injury. Moreover, selective overexpression of CINC in hepatocytes induced neutrophil sequestration, transmigration, and injury in the liver. These data support the hypothesis that C-X-C chemokines can be a potent signal for transendothelial migration. In addition, general cell injury may produce other chemotactic factors. Recent evidence indicates that apoptotic cell death can signal neutrophil emigration in endotoxin shock and that blocking apoptosis with an inhibitor of caspases can prevent neutrophil emigration and hepatocyte necrosis. Because the toxicity of neutrophils and potentially other leukocytes appears to depend on extravasation, the identification of chemotactic factors that induce the migration out of the vasculature may provide new possibilities for therapeutic interventions.

The contributions of complement activation are of potential interest as well but are poorly defined. During endotoxemia, complement C3a appears to activate neutrophils and may contribute to the reduced deformability with attendant physical trapping or priming of the neutrophils for enhanced secretory activity with subsequent chemotactic stimulation, or C5a may function to stimulate neutrophil motility necessary for emigration. Of potential interest is the recent observation that C5a receptors (C5aR) are not limited to myeloid cells as previously thought but are found on tissue cells, including endothelial cells and hepatocytes. The fact that ligation of the C5aR on monocytes stimulates production of IL-8 and on hepatocytes stimulates production of acute phase proteins implicates a potential role for C5a beyond simply direct activation of neutrophil sequestration or motility. Although a role for C5a in endotoxin shock and hepatic ischemia-reperfusion injury has been recognized for sometime, the exact definition remains to be defined.

ADHERENCE-DEPENDENT CYTOTOXICITY

Mechanisms of neutrophil-mediated killing of hepatocytes remain poorly defined. Two major mechanisms have been the principal focus of most investigations: reactive oxygen and proteases. Marked generation of reactive oxygen species and hepatic lipid peroxidation during neutrophil-induced injury have been readily demonstrated (see Ref. 19 for review). Antioxidants and other interventions directed toward detoxification of reactive oxygen attenuate inflammatory liver injury. Regarding the contribution of proteases, it has been shown that protease inhibitors attenuate neutrophil-induced liver injury. Thus it appears that both mechanisms are operative in vivo. Studies in vitro to define more exactly the mechanisms by which reactive oxygen and proteases contribute to hepatocyte killing have generally revealed only the involvement of proteases. Thus much remains to be determined regarding the exact contributions of reactive oxygen.

Fig. 1. General mechanisms of neutrophil (PMN) accumulation in sinusoids and venules of the liver. EC, endothelial cells; PC, parenchymal cells/hepatocytes; TNF-α, tumor necrosis factor-α; IL-1, interleukin-1; IL-8, interleukin-8 and related neutrophil chemotactic C-X-C chemokines; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; ROS, reactive oxygen species; NF-κB, nuclear factor-κB.
roles may involve neutralization of antiproteases, activation of transcription factors that signal expression of proinflammatory genes, depletion of nitric oxide, and generation of peroxynitrite, among others.

The necessity for neutrophil adhesion to hepatocytes is uncertain. Supernatants from high concentrations of maximally stimulated neutrophils are able to cause parenchymal cell injury in vitro (9). However, in coculture conditions and in vivo, adhesion may provide the proximity needed for high cytotoxic efficiency. Direct interactions of neutrophils and hepatocytes have been observed at the electron microscopic level in vivo (27), and adhesive interactions have been demonstrated in vitro after cytokine stimulation of the hepatocytes and chemotactic stimulation of the neutrophils (26). In addition, adhesion significantly increases neutrophil secretory activity, principally through Mac-1. All of the mediators used to activate neutrophils in vitro and those known to be present in vivo upregulate the function of Mac-1 (25, 26, 32), and antibodies to Mac-1 reduce adherence as well as reduce cytotoxic effects of neutrophils both in vivo (14, 17) and in vitro (23). These data support the potential importance of direct adhesion between neutrophils and hepatocytes, though much remains to be defined regarding specific mechanisms of adhesion and signaling. Recent evidence indicates that Mac-1 may interact with a ligand other than ICAM-1 on the hepatocyte and that a significant portion of adhesion is not blocked by antibodies to either ICAM-1 or the CD18 integrins (26), indicating that unde ned adhesion molecules may also be of importance.

In summary, significant progress has been made in our understanding of how leukocytes, especially neutrophils, accumulate in the hepatic vasculature, migrate across the sinusoidal endothelial cell layer, and attack parenchymal cells (Fig. 1). However, as outlined in this brief overview, there are many unresolved issues at every step that need further clarification. Moreover, our knowledge regarding adhesive interactions of leukocytes other than neutrophils with the liver vasculature is very limited. A more detailed understanding of these mechanisms is critical for the development of selective therapeutic strategies that attenuate excessive inflammatory responses without compromising the vital host defense.

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REFERENCES


