Hepatic leukocyte recruitment in a model of acute colitis

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Scott, Jeffrey R., and Alison E. Fox-Robichaud. Hepatic leukocyte recruitment in a model of acute colitis. Am J Physiol Gastrointest Liver Physiol 283: G561–G566, 2002. First published April 10, 2002; 10.1152/ajpgi.00462.2001.—There is a close relationship between inflammatory bowel disease (IBD) and various hepatobiliary disorders. The objective of this study was to determine whether hepatic leukocyte recruitment occurs in experimental colitis. We used the murine model of colitis induced by 2,4-dinitrobenezesulfonic acid (DNBS). Male C57Bl/6 mice received an intrarectal injection of 4 mg DNBS in 100 μl 50% ethanol. Controls received 100 μl 50% ethanol. The hepatic microcirculation was examined at 3 and 14 days post-DNBS by intravital video microscopy. Three days post-DNBS, when mice had developed acute colitis, there was associated hepatic leukocyte recruitment. Within the postsinusoidal venules there was a fourfold increase in the flux of rolling leukocytes that was P-selectin dependent but not α4-integrin dependent. There was also an increase in stationary leukocytes within the sinusoids, although this was not associated with an increase in serum alanine transaminase. By 14 days post-DNBS when macroscopic evidence of colonic inflammation was resolved, rolling within the postsinusoidal venules had returned to control levels. In this murine model of colitis, we describe a link between acute colonic inflammation and remote hepatic leukocyte recruitment that is P-selectin dependent. Active IBD may lead to remote hepatic inflammation.

Inflammatory bowel disease; intravital microscopy; microcirculation; P-selectin; integrins

The hallmark of any inflammatory process is the tissue infiltration of leukocytes. This requires leukocyte recruitment, which proceeds via a classic four-step process of leukocyte-endothelial cell interaction (16, 21). Recruitment involves the initial tethering and rolling of leukocytes along the endothelium, followed by firm adhesion and subsequent emigration into the tissue (16, 21). This is mediated by the expression of specific adhesion molecules located on the surface of endothelial cells and circulating leukocytes. These include the selectins, integrins, and members of the immunoglobulin superfamily (12). It has been well established that the selectins are responsible for mediating the observed rolling phenomenon. Rolling can lead to the activation of integrins on the surface of leukocytes that interact with members of the immunoglobulin superfamily (i.e., intercellular adhesion molecule-1) on the endothelium. These molecules are responsible for leukocyte adhesion and tissue emigration (12). Leukocyte recruitment is an appropriate response to fighting infections and healing wounds, although this may also lead to an exaggerated inflammatory response as seen in inflammatory bowel disease (IBD).

IBD encompasses Crohn’s disease (CD) and ulcerative colitis (UC), both chronic idiopathic inflammatory conditions. UC primarily affects the colon and is characterized by chronic ulceration limited to the mucosal layer. CD may be found throughout the entire gastrointestinal tract, with deep chronic ulceration involving the mucosa and surrounding layers. Approximately 5–10% of adult patients with IBD have significant hepatobiliary disease (19). The gastrointestinal tract and hepatobiliary system are closely linked anatomically, with all mesenteric venous drainage ascending via the portal vein into the liver. Once blood enters the liver it encounters the unique hepatic microcirculation consisting of perportal, sinusoidal, and pericentral regions. This makes the liver and biliary system a direct target for remote leukocyte recruitment during the exaggerated colonic inflammatory response seen in IBD. Additionally, previous studies involving murine models of hepatic inflammation [i.e., endotoxin administration, ischemia-reperfusion (I/R)] have shown that the majority of leukocyte recruitment occurs within the sinusoids, while rolling is limited to the pre- and post-sinusoidal venules (2, 4, 9).

Therefore, the aims of this study were to use intravital video microscopy to directly visualize the hepatic microcirculation to determine whether colitis is associated with an induction of hepatic leukocyte recruitment, and 2) to investigate the molecular mechanisms that mediate this inflammatory process.

METHODS
This study was approved by the Animal Research Ethics Board of McMaster University and met the guidelines of the Canadian Council on Animal Care. Male C57Bl/6 mice were

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Control mice received 100 mg of DNBS in 100 μl 50% ethanol was injected intrarectally. Control mice received 100 μl 50% ethanol. Mice were then partially suspended for 30 s to avoid rectal drainage. After DNBS administration, mice received a 1-ml subcutaneous injection of 0.9% NaCl and drinking water consisting of 8% sucrose and 0.2% NaCl to avoid excessive dehydration.

**Induction of Colitis**

Mice (20–30 g) were initially anesthetized with 5% enflurane and maintained at 2% throughout the procedure. A polyethylene catheter was inserted 4 cm into the colon, and 4 mg of DNBS in 100 μl 50% ethanol was injected intrarectally. Control mice received 100 μl 50% ethanol. Mice were then partially suspended for 30 s to avoid rectal drainage. After DNBS administration, mice received a 1-ml subcutaneous injection of 0.9% NaCl and drinking water consisting of 8% sucrose and 0.2% NaCl to avoid excessive dehydration.

**Intrarectal Temperature Maintenance**

An intravenous catheter was inserted into the right jugular vein for the maintenance of anesthesia and antibody (Ab) injection. The abdomen was opened via a midline incision that extended laterally to the subcostal margins. Mice were transferred to the microscope stage and placed in a left lateral position. The left lobe of the liver was gently reflected onto a slide and covered with Glad wrap plastic to prevent dehydration. Animal temperature was monitored with an intrarectal temperature probe (Traceable) and maintained at 2% throughout the procedure. A circulating water jacket was performed in the clinical chemistry lab using standard techniques.

**Microscopy**

Immediately after microscopy a blood sample was obtained via cardiac puncture. Serum alanine transaminase levels were measured to assess hepatocellular damage. This assay was performed in the clinical chemistry lab using standard techniques.

**Statistics**

Data are presented as means ± SE. ANOVA and Student’s t-test with Bonferroni’s correction were used for multiple comparisons. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

**DNBS Induces Acute Colitis 3 Days After Intrarectal Administration**

To determine the effect that colitis has on remote hepatic leukocyte recruitment, it was essential to assess the adequacy of our murine model. The distal colon was examined and assessed with macroscopic and microscopic analysis. Three days after DNBS administration, mice developed acute colitis with visible macroscopic evidence (score 5 ± 1) of bowel wall thickening, hyperemia, and mucosal ulceration. This colonic inflammation was not associated with an increase in leukocyte count [2.3 ± 0.4 × 10⁶ cells/ml control vs. 1.3 ± 0.2 × 10⁶ cells/ml DNBS; not significant (NS)]. Microscopic analysis revealed mucosal structural modification with goblet cell depletion, polymorphonuclear (PMN) cell infiltrate, and submucosal edema (Fig. 1).

**DNBS Induces Hepatic Leukocyte Recruitment 3 Days After Administration**

Three days post-DNBS there was a fourfold increase in the flux of rolling leukocytes within the postsinusoidal venules compared with controls (Fig. 2A). This was not associated with a significant change in postsinusoidal venular adhesion (data not shown). There was also a significant increase in sinusoidal leukocyte sequestration (Fig. 2B). However, this was not associated with a significant increase in hepatocellular injury as determined by biochemical serum analysis of alanine transaminase (Fig. 3).

**Hepatic Leukocyte Recruitment Induced by DNBS is Not Associated with Bile Duct Inflammation 3 Days After Administration**

The most commonly reported hepatic lesions associated with IBD are seen within the periportal region.

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**Assessment of Colitis Severity**

**Macroscopic damage assessment.** Immediately after microscopy, the colon was removed and opened via a longitudinal antimesenteric incision. This allowed for an immediate macroscopic damage assessment based on an accepted gross morphological scoring system (26).

**Microscopic damage assessment.** Portions of the colon immediately adjacent to ulcers were removed and fixed in 10% formalin followed by hematoxylin and eosin staining. An arbitrary quantified damage assessment was performed based on a previously published scoring system (26).

**Serum Chemistry**

Immediately after microscopy a blood sample was obtained via cardiac puncture. Serum alanine transaminase levels were measured to assess hepatocellular damage. This assay was performed in the clinical chemistry lab using standard techniques.

**Adhesion Molecule Ab Injection**

**P-selectin Ab.** A separate group of mice was treated with anti-P-selectin Ab (RB40.34) 3 days after DNBS administration. Baseline rolling was recorded, followed by intravenous Ab injection (20 μg/animal; BD Pharmingen). After a 5-min delay, leukocyte rolling was examined in the postsinusoidal venules.

**α₁-Integrin Ab.** A separate group of mice were given an intravenous injection of an α₁-integrin Ab (R1–2, 75 μg/animal; BD Pharmingen) after establishing baseline rolling in the postsinusoidal venules. Twenty minutes after Ab injection, leukocyte rolling was examined. As previously demonstrated, this time was required for effective blockade by the Ab (14).
Using the transillumination technique of hepatic intravital microscopy, we were unable to consistently view the portal venules in the transverse plane. In addition, intravitral video microscopy does not allow one to identify the bile ducts; therefore, histological analysis was performed to evaluate periportal and biliary leukocyte infiltration. Three days post-DNBS, microscopic analysis revealed an increase in circulating leukocytes within the hepatic portal venules, but no increase in inflammatory cell infiltrate around the bile ducts (Fig. 4).

**Hepatic Leukocyte Recruitment Was Reduced by 14 Days Post-DNBS**

Fourteen days after DNBS treatment, macroscopic colonic inflammation was resolved (macroscopic score = 0). There was again no significant difference in leukocyte count (2.0 ± 0.4 × 10^6 cells/ml for control vs. 2.9 ± 0.8 × 10^6 cells/ml in the DNBS treated). As shown in Fig. 5, the flux of rolling leukocytes in the post sinusoidal venules had returned to control levels.

**DNBS-Induced Leukocyte Rolling in the Post sinusoidal Venules is P-selectin but Not α4-Integrin Dependent**

In a separate group of experiments, a baseline leukocyte rolling flux was established within the post sinusoidal venules 3 days post-DNBS. Five minutes after intravenous injection of anti-P-selectin Ab (RB40.34), leukocyte rolling was reduced to baseline (Fig. 6). A separate group of mice were given an intravenous injection of α4-integrin Ab (R1–2); 20 min after Ab injection, leukocyte rolling remained at baseline level (Fig. 6).

**DISCUSSION**

It has been well established that there is an association between IBD and various hepatobiliary disorders, including pericholangitis, primary sclerosing cholangitis, cirrhosis, and cholangiocarcinoma (19), suggesting that the liver and biliary system may be susceptible targets for remote inflammation in patients with active IBD. Various rodent models have been used to examine the link between tissue injury and remote organ inflammation. We have used this model concept to investigate the clinically known link between IBD and the liver. In this study, we describe a link between the colonic inflammatory response and hepatic leukocyte recruitment.

Trinitrobenzenesulfonic acid (TNBS) has been used extensively in rodents as a model of intestinal inflammation. However, intracolonic administration of the hapten DNBS leads to the development of acute colitis indistinguishable from that induced by TNBS (27).
DNBS represents a murine model of IBD that mimics symptoms seen in the clinical setting during exacerbation. The colonic microcirculation plays a key role in mediating the inflammatory response associated with the trauma induced by hapten administration. Intravital microscopy of the colon has shown that 3 days after intrarectal administration of TNBS there was a significant reduction in colonic capillary blood flow accompanied by histological evidence of inflammation (8). Hydration of the TNBS-treated animals improved blood flow but did not alter inflammation. Three days post-DNBS, we observed both macroscopic and microscopic evidence of acute colitis in the distal colon with extensive tissue inflammation and cellular necrosis. Macroscopic analysis revealed bowel wall thickening, hyperemia, and ulceration. Further microscopic analysis indicated that there was mucosal structural modification, with goblet cell depletion, submucosal PMN infiltration, and tissue edema. After establishing that mice treated with DNBS develop acute colitis 3 days after administration, our attention was directed toward determining the potential effect on the hepatic microcirculation.

The liver has a unique microcirculation, consisting of periportal, sinusoidal, and pericentral regions. Blood entering in the periportal region flows through the sinusoids and drains via a central postsinusoidal venule. The hepatic microcirculation receives the majority of blood flow via the hepatic portal vein, with all mesenteric venous drainage flowing directly from the gastrointestinal tract into the liver for detoxification. Intravital microscopy has been used to investigate various rodent models of hepatic inflammation to determine the hepatic inflammatory response associated with different inflammatory stimuli. Models that have
been used to induce hepatic inflammation include endotoxin administration (5, 7, 24), cecal ligation/perforation (30), direct and remote I/R (22, 25), and administration of various inflammatory mediators [i.e., tumor necrosis factor (TNF-α) (9)]. Direct hepatic I/R resulting in focal tissue hypoxia (22), as well as endotoxin and TNF-α administration, have all been shown to induce leukocyte rolling and adhesion in the postsinusoidal venules and are associated with a significant increase in sinusoidal leukocyte sequestration. Bilateral hindlimb I/R (1) and intestinal I/R (10) have also been used to assess the remote effects on the hepatic microcirculation. The reperfusion of these previously ischemic organs is associated with the activation of hepatic endothelial cells and circulating leukocytes, which also leads to sinusoidal leukocyte sequestration, with rolling and adhesion occurring in the postsinusoidal venules. Our model of DNBS colitis reflects a similar trend, with a fourfold increase in the flux of rolling leukocytes occurring within the postsinusoidal venules 3 days after DNBS administration, accompanied by a significant increase in sinusoidal leukocyte sequestration. It should be noted that compared with other models of hepatic inflammation, the colitis induced at this concentration of DNBS represents a relatively mild insult to the liver with significantly less leukocyte recruitment. In fact, 3 days post-DNBS, when there was evidence of remote hepatic leukocyte recruitment, no biochemical evidence of hepato cellular injury was observed as indicated by normal serum alanine transaminase. It has been shown that hepatic histological changes can occur in association with colitis in the presence of normal biochemical liver function tests (28).

With the use of intravital microscopy, leukocyte-endothelial cell interactions have been well described in transparent tissues, such as the mesentery (18), cremaster muscle (17), and dermis (20). However, there is a growing body of evidence suggesting that the hepatic microcirculation does not possess the same generic characteristics (3, 9, 29). It is recognized that the adhesion molecule P-selectin is partially responsible for leukocyte rolling, which is considered a necessary step for eventual emigration into the tissue (15, 23). In contrast, sinusoidal leukocyte sequestration occurs in the absence of rolling, although this region still represents an area of extensive hepatic cellular injury in severe hepatic inflammatory models (12). P-selectin has been shown to be transcribed and present on the surface of endothelial cells within the postsinusoidal venules in response to endotoxin or cytokine stimulation (24), but it has not been found on the sinusoidal endothelium (6). This corresponds with the DNBS-induced rolling in the postsinusoidal venules observed in this study, which was reduced to baseline by intravenous injection of anti-P-selectin Ab (RB40.34). This suggests that the increase in rolling flux seen within the postsinusoidal venules at 3 days post-DNBS is P-selectin dependent and results from signals that upregulate P-selectin expression.

The α1-integrin is expressed on the surface of mononuclear cells and on neutrophils in rodents (11). It has been shown that it can support leukocyte rolling in chronic inflammation in the presence of selectins (13), as well as independently of selectins after TNF-α injection (9). Interestingly, 3 days post-DNBS the α1-integrin Ab (R1–2) did not reduce leukocyte rolling in the postsinusoidal venules, suggesting that systemic expression of TNF-α may not be the cytokine responsible for our observations. Other candidate cytokines for this response may include interferon-γ. The hepatic response to this cytokine has yet to be explored.

UC is characterized by multiple exacerbations and remissions, in which the colonic mucosa is frequently damaged, followed by tissue repair. This study has provided direct evidence that suggests that remote hepatic leukocyte recruitment occurs in association with acute colitis. Recurring episodes of active colitis may contribute to a further exaggerated colonic inflammatory response, leading to ultimate hepatic inflammation and resultant hepatocellular injury.

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