The Future of GI and Liver Research: Editorial Perspectives
II. Modulating leukocyte recruitment to splanchnic organs to reduce inflammation

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Bonder, Claudine S., and Paul Kubes. The Future of GI and Liver Research: Editorial Perspectives. II. Modulating leukocyte recruitment to splanchnic organs to reduce inflammation. Am J Physiol Gastrointest Liver Physiol 284: G729–G733, 2003; 10.1152/ajpgi.00023.2003.—A hallmark feature of intestinal inflammation is the recruitment of various types of leukocytes to the afflicted site. Neutrophils are the predominant cell type in acute inflammation, whereas mononuclear cells are the major cells recruited in chronic inflammation. The key factor in the recruitment of these cells, regardless of the type of inflammation, is the expression of functional adhesion molecules on the surface of endothelium of small postcapillary venules. Figure 1 summarizes the cascade of events involved in leukocyte recruitment including a role for selectins (E-selectin, P-selectin, and L-selectin) and the α4-integrin (β1- or β7-integrin) as key molecules regulating the initial tethering and rolling step. Once the rolling is initiated, chemokines presented on the surface of endothelium engage their receptors on the surface of leukocytes and activate integrins (β1- or β7-integrin) to cause firm adhesion. The emigration process out of the vasculature then makes use of numerous additional proteins including PECAM-1, CD99, and JAMs. The driving force behind research in this area is the appreciation that interference of the recruitment process may very likely lead to therapeutic benefit. The major aim of this themes article is to very briefly summarize some of the milestones and highlights in adhesion and intestinal inflammation, to draw attention to some of the negative and controversial results, and to provide an opinion on directions that should be explored further. For details on adhesion and intestinal inflammation, we draw the readers attention to excellent reviews on the topic (22, 27).

TARGETING NEUTROPHILS

Granger and Parks (6) in the early 1980s first demonstrated that ischemia of the intestine followed by reperfusion was closely associated with impairment of both the microvascular barrier and the mucosal barrier and that this pathology was dependent on oxygen free radicals. Subsequent studies demonstrated that neutrophils, a major source of oxygen free radicals, were essential for the development of the postischemic microvascular dysfunction (9). Indeed, neutrophils were demonstrated to accumulate in the postischemic intestine, and depletion of circulating neutrophils resulted in a reduction in the ischemia-reperfusion-associated vascular dysfunction. Similar observations have since been made in other splanchnic organs including posts ischemic stomach, pancreas, liver, and mesentery.

Although the mesentery is structurally and functionally quite different from other gastrointestinal organs, we and others have used it as a prototype tissue to study the basic mechanisms of leukocyte recruitment in vivo. This is done primarily because the mesentery is a translucent tissue and permits transillumination. Therefore, intravital microscopy permits the direct visualization of postcapillary venules within this tissue. Intravital microscopy of the mesentery has clearly demonstrated that the rolling is indeed dependent on...
The Leukocyte Recruitment Cascade

Fig. 1. The leukocyte recruitment cascade. Fast-moving leukocytes in the bloodstream tether and roll on activated endothelium via interactions between selectins and their ligands or, in some cases, integrin cellular adhesion molecule (CAM) interactions. Chemokines or other proinflammatory mediators released by various sources within the tissue are presented on the endothelium to rolling leukocytes, resulting in integrin activation and firm adhesion. Firm adhesion permits leukocyte transmigration across the endothelium and entry into inflamed tissue via CAM and junction adhesion molecules (JAM). PSGL-1, P-selectin glycoprotein ligand-1.

selectins, and the adhesion is dependent on the β2-integrins (16). Moreover, inhibition of rolling indeed inhibits adhesion and inhibition of rolling, or adhesion will inhibit microvascular dysfunction (18). In addition to ischemia-reperfusion, the mesentery has been used to examine the molecular mechanisms of leukocyte recruitment associated with numerous other intestinal pathologies (22). For example nonsteroidal anti-inflammatory drugs induce ulceration and neutrophil recruitment in the stomach and small bowel. Radiation-induced enteritis is also associated with neutrophil recruitment. In addition, various bacterial toxins, including those isolated from H. pylori, C. difficile, and even lipopolysaccharide from E. coli, clearly induce intestinal inflammation. In each case, direct exposure of the mesentery to any of the aforementioned procedures or noxious stimuli also results in profound neutrophil recruitment in the mesenteric microcirculation. Although antiadhesion therapy reduces the neutrophil recruitment and vascular dysfunction within the mesentery, key experiments to demonstrate that this prevents inflammation in, for example, the intestine in many cases have not been completed. This is undoubtedly an issue that warrants further experimentation.

Clearly, the mesenteric microvasculature has functioned as an extremely important tool to elucidate molecular mechanisms associated with leukocyte recruitment. However, the limited amount of antiadhesion work completed in other splanchnic organs raises some concerns about the use of the mesentery. For example, Kurtel and colleagues (19) clearly demonstrated that the neutrophil recruitment response to ischemia-reperfusion appears to be very similar in various parts of the intestinal tract including the small and large bowel (muscularis layer, mucosa, and serosa), and the results are comparable with the mesentery. Moreover, the anti-β2-integrin antibody inhibited neutrophil recruitment into each of the aforementioned compartments, and microvascular dysfunction was clearly abrogated in the intestine with anti-β2-integrin therapy. By contrast, the same concentration of β2-integrin that inhibited neutrophil recruitment into the intestine did not reduce the mucosal barrier dysfunction, suggesting that the circulating neutrophils could not account for all pathology in the intestinal mucosal compartment (15).

Interestingly, an assessment of the intestine revealed that relative to other organs there was always a very significant number of neutrophils within the intestine. These cells were only transiently in the interstitium (passing from vasculature to lumen of the bowel), because treatment of animals with anti-β2-integrin antibody for 72 h resulted in abolition of all neutrophils within the mucosal compartment (presumably neutrophil migration into the intestinal lumen is a constitutive, homeostatic event). When the neutrophil-depleted intestine was subjected to ischemia-reperfusion, the intestine remained devoid of neutrophils and no mucosal barrier dysfunction occurred (15). Clearly, interstitial (tissue) neutrophils within the intestine contributed to the intestinal mucosal dysfunction associated with ischemia-reperfusion and must also be considered when designing novel therapy.

The leukocyte recruitment responses within the liver are also quite different from the mesentery, at least in part due to very significant architectural differences between the hepatic and mesenteric microvasculature. The liver has a dual blood supply that includes the venous blood from the intestinal tract via the portal (presinusoidal) venules and arterial blood entering via the hepatic arterioles. Convergence of these two blood supplies occurs at the sinusoids, which then drain the blood into the central (postsinusoidal) venules. It has been suggested that leukocyte recruitment in portal and central venules is very similar to that found in mesenteric and cremasteric venules, whereas the sinusoids use a very different mechanism (31, 31). Indeed, although the sinusoids are the major site for leukocyte recruitment in acute inflammation, leukocyte recruitment in inflamed sinusoids was not diminished in P-selectin-deficient mice, P/E-selectin double-deficient mice, and P/E-selectin double-deficient mice given L-selectin or α4-integrin antibody (5, 31). Essani et al. (3) and Jaeschke and colleagues (13, 14) have also reported that neither ICAM-1 nor β2-integrins were very important in leukocyte recruitment in liver in a sepsis model (13). In addition, the liver sinusoidal endothelial cells lack the capacity to express E-selectin, P-selectin, PECAM-1, CD34, and VE-cadherin, induction of VCAM-1 is lower than on other vessels, and yet ample adhesion occurs in sinusoids. One possibility is that the low blood flow rates through these vessels and the constitutively high levels of ICAM-1 (relative to other vascular beds) permit direct adhesion of leukocytes within sinusoids (28). However, ICAM-1 inhibition has
not always worked in the liver. Moreover, other less well-studied adhesion molecules such as vascular adhesion protein (VAP)-1, found expressed constitutively on sinusoidal endothelium, may potentially mediate the leukocyte recruitment in sinusoids (21). Because the leukocytes and sinusoids have similar diameters, an alternative possibility is that the physical constraints (the narrow sinusoids) rather than true adhesion may be important in leukocyte sequestration in sinusoids.

Clearly in most acute liver inflammation, selectins, and even integrins, are not essential for leukocyte recruitment. By contrast, an important role for selectins in liver in ischemia-reperfusion has been reported, and substantial numbers of leukocytes adhere in both the post-sinusoidal and the sinusoidal venules (17). Because the majority of blood that perfuses the liver initially drains the intestine, ischemia-reperfusion of the liver would also have induced ischemia-reperfusion in the intestine. Therefore, if the antiselectin therapy reduced injury in the intestine, this may have reduced the injury downstream in the liver. Indeed, Horie et al. (10) reported that ischemia-reperfusion of the intestine caused significant liver damage and that this was eliminated with antiselectin approaches. When ischemia-reperfusion was performed exclusively in the liver (intestine was removed), the results suggested that the liver was quite resistant to this insult. Only a small amount of leukocyte recruitment was noted in postischemic liver, and antiselectin therapy had minimal effects in sinusoids (17).

Finally, different inflammatory responses in liver may differ in the localization of leukocyte recruitment, i.e., sinusoids vs. postsinusoidal vessels. Indeed, administration of adenovirus vectors causes profound liver injury, but neutrophils were recruited almost exclusively to post-sinusoidal venules, not to sinusoids (20). In this model of liver inflammation, neutrophil recruitment was amenable to antiadhension therapy. Thus it appears that in certain inflammatory responses, leukocytes can be recruited in the postsinusoidal venules but not sinusoids, and under these conditions, antiadhension therapy may be beneficial. These data as a whole clearly highlight the importance of exploring leukocyte recruitment in individual organs and in different inflammatory processes.

Therefore, despite the initial view that antiadhension would be the panacea for inflammatory diseases of the splanchnic organs, there is a growing body of evidence that the adhesion processes may differ among organs, among different compartments of the same organ, and even in different acute inflammatory conditions. This is further complicated in chronic intestinal inflammation. In the intestine, it is clear that there exists a very delicate balance between a homeostatic, appropriate, and even essential host response against intestinal flora vs. an overexuberant inappropriate immune response. However, modulation of the immune balance, with the intent of dampening immune response, has often led to initiation or exacerbation of intestinal inflammation. This is best exemplified by spontaneous development of inflammatory bowel disease (IBD) in at least eight different mutant mouse systems (see Table 1). Along the same

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lines, the absence of selectins has resulted in exacerbation of intestinal inflammation in some models of colitis. Surprisingly, neutrophil recruitment was either not depressed or even enhanced in some of these models, suggesting the induction of other mechanisms of neutrophil recruitment. Indeed, the possibility that α4-integrin may be upregulated on neutrophils in chronic inflammatory diseases or even in acute systemic diseases (sepsis) raises the possibility that this adhesion molecule could contribute to neutrophil recruitment in IBD. This is important, because α4-integrin can support rolling and adhesion and can bypass the necessity for selectins as well as β2-integrins (see Fig. 1). Indeed, monoclonal antibodies against the α4-integrin attenuated the spontaneous colitis in the cotton-top tamarin (24). Whether this is due to inhibition of mononuclear cell recruitment or mononuclear cell and neutrophil recruitment remains unclear but does require some assessment.

TARGETING LYMPHOCYTES

To date, a significant emphasis has been on targeting the more overt inflammatory processes including neutrophil recruitment. However, it is well documented that a plethora of cells infiltrate the inflamed mucosa. In fact, many studies of enterocolitis have implicated T lymphocytes as the primary mediator in IBD based on 1) increased T cell numbers in affected tissue, 2) the state of activation of T cells at these sites, and 3) their production of numerous proinflammatory cytokines [see recent review by Strober et al. (29)]. Bacterial flora stimulate the development of the intestinal immune system, and, under normal conditions, tolerance to this enteric bacteria is generated. Once the balance of regulatory and proinflammatory T cells is perturbed, pathogenic intestinal inflammation develops and is self-reinforcing due to the proinflammatory cytokine milieu generated via a feedback loop between T cells and antigen-presenting cells. The direct demonstration that effector T lymphocytes are reactive to conventional antigens of the enteric bacteria was described by Powrie and coworkers (25). In 1993, they observed that adoptive transfer of CD4+CD45RBhigh T cells to severe combined immunodeficient (SCID) recipients induced intestinal inflammation. In addition, Sundberg and colleagues (30) observed that within weeks of life, C3H/HeJBir mice develop a spontaneous colitis that coincided with 1) the time of bacterial colonization and 2) the presence of T cells producing IL-2 and IFN-γ [α T helper (Th) type 1 (Th1) cell
response]. An adhesive mechanism of action has now been identified, because blocking studies showed that α4β7 helps direct the migration (homing) of CD4+CD45RB<sup>high</sup> lymphocytes to the intestine (23). However, this is a very specific controlled mouse model, wherein specific lymphocytes home to the intestine via a very specific adhesive mechanism. Whether such a specific mechanism exists in IBD remains unclear.

**T CELL DICHOTOMY IN IBD**

By way of mouse models, it is becoming increasingly evident that mucosal inflammation reflects a remarkably wide variety of causes (as exemplified in Table 1). It is also clear that 1) the driving force of the inflammation is the mucosal microflora and 2) the inflammation is predominantly mediated by either an excessive Th1 or Th2 T cell response. Indeed, in Crohn’s disease (CD) patients, an overproduction of Th1 cytokines, such as IFN-γ, TNF-α, and IL-12, by CD4<sup>+</sup> T cells has been implicated in disease development, and anti-TNF-α therapy is one of the most effective treatments to date. In contrast, the amelioration of ulcerative colitis (UC) severity, with antibodies to Th2 cytokines, e.g., IL-4, implies that this is a Th2-mediated disease. These observations imply that both CD4<sup>+</sup> Th1 and Th2 cells can function as effector cells in IBD. Therefore, studying the effector T lymphocyte populations that home to the chronically inflamed intestine and the adhesive mechanisms used (particularly if they are different) has important implications for understanding IBD.

As previously stated, therapeutic strategies targeting adhesion molecules (i.e., selectins and CAMs) have limited efficacy in different models of colitis. Furthermore, whether this form of therapy is effective long term in chronic disease states is still not known. An alternative approach may be to encourage the recruitment of counterreactive cells to the inflamed site. The Th1-Th2 paradigm suggests that Th1 and Th2 cells counterbalance each other. For example, although Th1 cells promote a Th1-type inflammation, it has been proposed that Th2 cells, which secrete IL-4, protect disease development by dampening the activity of Th1 cells. Therefore, simply inhibiting Th1 but not Th2 cell recruitment could alleviate Th1-mediated disease. However, an increasing number of studies using well-defined, phenotypically committed Th1 and Th2 cells expressing identical T cell receptors does not support this hypothesis. Recently Iqbal and coworkers (12) observed that mice colonized with *E. coli*, producing ovalbumin, before injection of either Th1- or Th2-polarized lymphocytes (activated in vitro with the same ovalbumin), developed intestinal inflammation. In this study, both Th1 and Th2 cells induced IBD, which developed at the same rate and to equivalent severity but with a distinct pattern of inflammation. Th1-induced lesions contained predominantly lymphocyte, monocyte, and macrophage infiltrates. By contrast, in Th2-induced lesions, >40% of the infiltrating cells were eosinophils. This study showed that either effector T cell population could induce a pathogenic response to the same antigenic stimulus. Furthermore, whether the combination of Th1 and Th2 cells would decrease disease progression or severity is not known. Other studies have also shown that Th1 and Th2 cells, or their mediators, are not counterreactive. First, Th1-type colonic inflammation was shown to be exacerbated by treatment with IL-4 (4), and second, the introduction of Th1 cells into Th2 cell-induced asthma significantly increased airway inflammation (8). These observations suggest that the Th1-Th2 paradigm, which predicts that Th2 cells can suppress Th1-mediated effects and vice versa, may be more complex in IBD than initially appreciated. Regulation of T cell-mediated inflammation may require other cells.

There is increasing evidence that another lymphocyte subset is involved in T cell homeostasis. These CD4<sup>+</sup>CD25<sup>+</sup> T regulatory (Treg) cells are defined by their unique profile of cytokine production and make high levels of transforming growth factor-β and IL-10 but no IFN-γ, TNF-α, IL-4, or IL-2 (7). Furthermore, experimental systems have revealed a functional interplay between the CD45RB<sup>high</sup> and CD45RB<sup>low</sup> CD4<sup>+</sup> T cells. As previously mentioned, transfer of CD45RB<sup>high</sup> CD4<sup>+</sup> T cells to SCID mice led to the development of a Th1-mediated intestinal inflammation. Notably, cotransfer of the CD4<sup>+</sup>CD45RB<sup>low</sup> T cell population prevented disease induction by the CD45RB<sup>high</sup> cells (25). Annunziato and coworkers (1) recently published that Treg cells showed poor or no proliferation in mixed lymphocyte culture and suppressed in a dose-dependent fashion the proliferation response to allogeneic stimulation of CD4<sup>+</sup>CD25<sup>+</sup> T cells. In essence, these CD4<sup>+</sup>CD25<sup>+</sup> cells are a phenotypically and functionally distinct population of regulatory T cells capable of controlling inflammation via their production of IL-10 and TGF-β. Treg cells have therefore been suggested to play a pivotal role in intestinal homeostasis, and it is conceivable that increased recruitment of Treg cells to sites of inflammation might ameliorate chronic diseases such as IBD.

**TARGETING CHEMOKINES**

Although chemokines and their receptors are considered to be mediators of inflammation and tissue injury in inflammatory diseases, their precise role in the pathophysiology of gastrointestinal diseases remains incompletely understood. Just as CD and UC are characterized by a differential expression of polarized inflammatory cytokines, their T cells are also characterized by different chemokine receptors. CD and the mouse models that are likened to it (e.g., IL-10 knock-out and IL-2 knockout) contain, in their inflammatory milieu, Th1 cells expressing the chemokine receptors CXCR3, CCR5, and CCR7. In contrast, UC patients and their correlative mouse models (exemplified by TCRα<sup>−/−</sup> and oxazolone) contain Th2 cells that express specific chemokine receptors (CCR4, CCR3, CCR8, and CXCR4) that differ from those expressed by the Th1 cells (2). Treg cells also have a particular chemokine receptor profile. They express the chemokine receptors...
CCR8 and CCR4 and chemokaxis to their respective ligands CCL22, CCL1, and CCL17 (11). Notably, CCR4 and CCR8 are also expressed on Th2 cells, implying that further investigation is required to better characterize these cells before we can encourage only Treg recruitment to sites of inflammation. The observation that both UC and CD were partly ameliorated with the treatment of IFN-α (which promotes Treg cell proliferation) supports the notion that Treg cells can indeed suppress intestinal inflammation and that this, as a therapeutic approach, is worth pursuing (26).

CONCLUDING REMARKS

It is unclear whether traditional antiadhesion approaches (e.g., anti-selectins and anti-integrins) will work. However, as we gain a better understanding of cellular recruitment in splanchic organs, traditional antiadhesion therapy may become useful. Alternatively, encouraging the recruitment of Treg cells to the inflamed intestine while negatively targeting other populations of leukocytes could potentially be used in therapy to modulate immune responses in vivo. Clearly, the sooner we identify distinct adhesive differences between the effector and regulatory CD4+ T cells (for either increased subset proliferation or homing), the sooner we may be able to develop these new therapeutic strategies.

REFERENCES