Cell Adhesion and Migration
II. Leukocyte-endothelial cell adhesion in the digestive system

D. NEIL GRANGER
Department of Molecular and Cellular Physiology, Louisiana State University Medical Center, Shreveport, Louisiana 71130-3932

Granger, D. Neil. Cell Adhesion and Migration. II. Leukocyte-endothelial cell adhesion in the digestive system. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G982–G986, 1997.—The adhesion of leukocytes to vascular endothelial cells is a highly coordinated process that is governed by a number of factors, including the expression of specific adhesion glycoproteins, physical forces generated within the microcirculation, and inflammatory mediators released by a variety of activated cells. The digestive system, with its large resident population of immune cells and its tremendous capacity to generate inflammatory mediators, has proven to be a valuable source of information on the mechanisms involved in the regulation of leukocyte-endothelial cell adhesion. This article considers some of the evolving issues that have surfaced as a consequence of the rapidly growing body of literature on this topic. Particular emphasis is devoted to unresolved issues related to the expression and shedding of endothelial cell adhesion molecules, the contribution of capillaries to the inflammatory response, and the role of mast cells, macrophages, and lymphocytes in the modulation of leukocyte-endothelial cell adhesion.

THERE IS a large body of evidence that implicates leukocyte-endothelial cell adhesion as a rate-limiting component of the injury process associated with different experimental models of gastrointestinal and liver disease (5, 12, 25). The recognition that leukocytes must firmly bind to endothelial cells before tissue injury and organ dysfunction are manifested in these models has led to an intensive effort directed toward defining the factors that regulate the adhesion of leukocytes within the microvasculature. The application of technology that allows for real-time imaging of leukocyte-endothelial interactions in the intact microcirculation has resulted in an explosion of novel information related to the inflammatory response and has led to an increased awareness of the many different factors that can influence the recruitment of leukocytes in experimental models of gastrointestinal and liver disease.

Many important questions have been addressed over the past decade concerning the contribution of specific adhesion glycoproteins, shear forces, and the products of endothelial cell activation (e.g., nitric oxide) to leukocyte adhesion within the microcirculation. However, the results of these studies have brought to light many new issues and raise additional compelling questions for future investigation. The intent of this article is to identify and discuss some of these new and evolving areas of investigation (Table 1), including regional differences in the expression and regulation of endothelial cell adhesion molecules (CAMs), the influence of leukocyte-endothelial cell adhesion in postcapillary venules on other segments of the microvasculature (capillaries, arterioles), and the role of auxiliary cells in the modulation of leukocyte-endothelial cell adhesion.

ENDOTHELIAL CELL ADHESION MOLECULES ARE SUBJECT TO REGULATION AND SHEDDING

The current paradigm used to explain the recruitment of leukocytes into inflamed tissues invokes a role for selectins in the initial capture (rolling) of leukocytes, while the engagement of β2-integrins on leukocytes with intercellular adhesion molecule-1 (ICAM-1) on endothelial cells mediates the firm adhesion and emigration of leukocytes (6). The pivotal role of endothelial CAMs in regulating leukocyte recruitment has been demonstrated in different models of gastrointestinal and liver inflammation with the use of either blocking monoclonal antibodies (MAbs) directed against specific CAMs or mice that are genetically deficient in one or more endothelial CAMs (6). Immunohistochemical staining studies have revealed the expression of a number of adhesion molecules (e.g., ICAM-1, vascular cell adhesion molecule 1, and P-selectin) on stimulated (inflamed) endothelial cells (and some other cell types) of the digestive system (e.g., see Ref. 2). These observations have been corroborated by quantitative measurements of endothelial CAM expression that are based on the tissue accumulation of radiolabeled CAM-specific MAbs relative to nonbinding, isotype-matched MAbs (3, 8, 20). The data obtained with this method indicate that there may be substantial differences in basal and stimulated endothelial expression of CAMs between organs in the digestive system, even after the expression values are corrected for differences in vascular surface area between tissues. For example, experiments performed in wild-type mice reveal significant basal expression of ICAM-1 and P-selectin in the...
Table 1. The outstanding questions

- What factors account for the heterogeneity of expression of endothelial cellular adhesion molecules (CAMs) between vascular beds in the digestive system? Does this heterogeneity of endothelial CAM expression translate into regional differences in the recruitment of specific leukocyte populations? Do other factors such as vascular shear forces and the level of chemokine or nitric oxide production also contribute to these regional differences?
- Do circulating soluble isoforms of endothelial CAMs exert physiologically relevant anti-inflammatory properties?
- What are the contributions of steric hindrance, homotypic and heterotypic cell aggregation, and leukocyte-endothelial cell adhesion to the sequestration of leukocytes in capillaries?
- Can leukocytes that are adherent to endothelial cells in post-capillary venules affect endothelial cell function in upstream microvessels (capillaries and arterioles) via an ascending propagation mechanism? What endothelial cell signaling pathways could allow for sensing and communicating this propagated response?
- When and to what extent do mast cells, macrophages, and other auxiliary cells contribute to leukocyte rolling, adherence, and emigration in postcapillary venules?
- What cell products released by these auxiliary cells account for their regulatory influence on leukocyte-endothelial cell adhesion?

It is not clear whether the apparent organ-to-organ differences in the kinetics of endothelial CAM expression translate into a comparable regional heterogeneity of leukocyte recruitment after exposure to a given inflammatory stimulus. Furthermore, it remains unresolved whether or not the quantitative differences in endothelial CAM expression that have been noted between tissues (e.g., mesentery and intestinal mucosa) yield comparable quantitative differences in the number of rolling and/or adherent leukocytes that traffic through the same vascular bed. The answer to this question will provide some insight not only into the merits of using the technically advantageous mesentery to study events assumed to take place in the bowel wall but also on the physiological relevance of 50% vs. 5- to 10-fold increases in endothelial CAM expression. Although organ-based measurements of endothelial CAM expression using radiolabeled MABs now make it possible to study the correlation between adhesion molecule density and leukocyte recruitment, a more definitive resolution of this issue must await the development of on-line videomicroscopic imaging methods that allow for simultaneous quantification of endothelial CAM expression and leukocyte-endothelial cell adhesion within discrete regions of the microvasculature.

Circulating soluble forms of endothelial CAMs (sCAMs) have been demonstrated in healthy humans and experimental animals, with substantially elevated levels reported in various disease states, including inflammation (4). These soluble isoforms contain most of the extracellular portion of the endothelial CAMs and are generally thought to represent shed fragments of their membrane-bound counterparts. The circulating level of these soluble isoforms is considered to reflect the level of expression of their membrane-bound counterparts on the surface of endothelial cells; consequently, the soluble CAMs are extensively used to monitor inflammatory disease activity in the clinical setting. Because the splanchic circulation is likely to represent the second largest (after the lung) source of soluble circulating CAMs, it may be proposed that the plasma level of sICAM-1 and other soluble CAMs is a useful predictor of inflammation intensity within the digestive system. However, there are published reports demonstrating that both human and murine endothelial cells possess mRNA that specifically encodes sICAM-1 (15, 25). Furthermore, it appears that the kinetics of appearance and disappearance of sICAM-1 in mouse plasma after tumor necrosis factor-α (TNF-α) administration are dissociated from the ICAM-1 expressed on endothelial cells of lung, intestine, and other organs (16). These observations do not negate the potential use of circulating sCAM levels as qualitative markers of endothelial cell activation; however, they do raise a concern about the value of these measurements in mechanistic studies of gastrointestinal inflammation.

The existence of circulating soluble isoforms of endothelial CAMs also raises questions about their role as endogenous anti-inflammatory agents. The putative anti-inflammatory properties of sCAMs are generally

intestinal vasculature, but only ICAM-1 is constitutively expressed in the gastric vasculature. Neither ICAM-1 nor P-selectin can be detected in the intestinal vasculature of mice that are genetically deficient in ICAM-1 or P-selectin, respectively, which suggests that the predicted basal expression of these endothelial CAMs in the intestine is not an artifact of the technique. The fact that P-selectin and ICAM-1 are basally expressed in the gut supports the view that this tissue is normally in a state of controlled inflammation, since these constitutively expressed leukocyte rolling (P-selectin) and adhesion/emigration (ICAM-1) receptors would allow for a constant turnover of phagocytic cells that appear to enter and reside in the normal gut mucosa.

Studies performed using the radiolabeled MAb technique have revealed that inflammatory stimuli (e.g., cytokines, endotoxin) elicit a time-dependent increase in the expression of all endothelial CAMs in different tissues of the digestive system (3, 8, 20). This observation is supported by immunohistochemical staining experiments that reveal a time-dependent increase in the number of postcapillary venules that stain positively for ICAM-1 after an inflammatory challenge (2). The latter observation suggests that cytokines and other inducers of endothelial CAM expression act to increase the number of endothelial cells (or segments of the venous microcirculation) that express ICAM-1 rather than eliciting a uniform increase in CAM density on the surface of all endothelial cells within a vascular bed. This clustering of endothelial CAMs under basal and stimulated conditions may account for the patchy distribution of adherent leukocytes that is noted in postcapillary venules examined by intravital video microscopy (1).
attributed to competition with their membrane-bound counterparts for relevant counterreceptors expressed on activated leukocytes. In human plasma, sICAM-1 concentration is ~200 ng/ml, and it increases up to 600 ng/ml in inflammatory conditions (4). In contrast, mice normally exhibit a resting sICAM-1 concentration between 10 and 20 µg/ml, which rises to >100 µg/ml after cytokine administration (16). The potential physiological relevance of these levels is supported by in vitro studies that reveal a concentration-dependent inhibition of lymphocyte adhesion to monolayers of cultured endothelial cells at sICAM-1 levels >200 ng/ml (21). It remains unclear whether this anti-adhesive effect of sICAM-1 is unique to human lymphocytes and whether the murine form of sICAM-1 is as effective in retarding adhesion as the human isoform. Studies designed to assess the ability of sCAMs to alter leukocyte-endothelial cell adhesion in postcapillary venules as well as the migration of leukocytes in the interstitium are also warranted, particularly in view of recently published reports that show a remarkable ability of soluble CAM ligands to attenuate leukocyte-dependent tissue injury (24).

**LEUKOCYTE-DEPENDENT VASCULAR DYSFUNCTION CAN OCCUR UPSTREAM FROM POSTCAPILLARY VENULES**

Most of the published work dealing with leukocyte-endothelial cell adhesion and the vascular consequences of these adhesive interactions (e.g., endothelial barrier dysfunction) has focused on postcapillary venules, because this is the primary site of leukocyte adhesion and transendothelial migration in inflamed tissue. However, it is increasingly evident that upstream segments of the microcirculation (capillaries and arterioles) can participate in the sequestration of circulating leukocytes within inflamed tissue and that these upstream vascular elements may be responsive to adhesion-dependent leukocyte-endothelial cell interactions that occur within postcapillary venules. Unlike postcapillary venules, which capture circulating leukocytes through adhesion receptor-counterreceptor interactions, capillaries can also sequester leukocytes by steric hindrance (23). The distinctive mechanisms of leukocyte capture that exist in the two regions of the microcirculation can be attributed to differences in internal vessel diameter as well as the density of endothelial CAMs.

The fact that the liver is second only to the lung as a site for leukocyte margination has led investigators to assume that the low driving pressures for leukocyte passage through these capillary networks account for their unique ability to entrap leukocytes (10). However, there are relatively few reports that specifically address the role of the capillary network in the retention of leukocytes by the liver (13). Recent studies using mice that are genetically deficient in either ICAM-1, P-selectin, E-/P-selectin, or CD11/CD18 (the counter-receptor for ICAM-1) have shown an attenuated accumulation of leukocytes in hepatic sinusoids after induction of liver inflammation with some stimuli (e.g., ischemia-reperfusion) but not others (e.g., N-formyl-Met-Leu-Phe) (11, 27). Although it is tempting to speculate, on the basis of these observations, that the entrapment of leukocytes in liver sinusoids can be mediated by specific endothelial CAMs, other plausible explanations should be considered in view of the existing controversy concerning the level of expression of some CAMs on the surface of the sinusoidal endothelium. For example, although P-selectin is not expressed on sinusoidal endothelium (13), the density of ICAM-1 expression in rat liver sinusoids (~0.8 µM) is comparable to that estimated for the central venules (12). Adhesion molecules such as P-selectin and CD11/CD18 can participate in the formation of platelet-leukocyte or leukocyte-leukocyte aggregates and thereby contribute to the retention (steric hindrance) of leukocytes in sinusoids. Studies in other tissues (e.g., skeletal muscle) suggest that it is also possible for adherent leukocytes in postcapillary venules to promote leukostasis in upstream capillaries via a CAM-mediated, leukocyte-dependent enhancement of fluid and protein filtration across venular endothelium (14). The resulting interstitial edema raises interstitial fluid pressure to a level sufficient to partially occlude the capillary lumen and thereby facilitate the trapping of leukocytes. Understanding the mechanisms that underlie the ability of CAMs to promote the retention of leukocytes in liver sinusoids and gastrointestinal capillaries is an important issue that deserves further attention because of the potential for this phenomenon to reduce tissue perfusion and result in cellular hypoxia. The profound differences in the density of ICAM-1 expression between mesenteric capillaries (1/10th that of postcapillary venules) and hepatic sinusoids (density equal to postcapillary venules) also justifies the need for additional work (12).

The results of a few recently published studies suggest that CAM-directed MAbs can preserve the function of endothelial cells in regions of the microvasculature that do not experience detectable adhesive interactions with circulating leukocytes. For example, it has been shown that the platelet-activating factor (PAF)-induced enhancement of fluid filtration across mesenteric capillaries can be largely abolished when rats are either rendered neutropenic or when leukocyte adhesion in downstream venules is prevented with CAM-specific MAbs (7). Because PAF did not promote leukocyte adhesion within capillaries, it was proposed that the leukocyte-endothelial cell adhesion in venules may elicit an ascending propagation (via cell-cell communication) of a signal that causes endothelial cell contraction in upstream capillaries. Such a retrograde propagated response has also been invoked to explain the ability of vasoactive agents to change the diameter of upstream arterioles (22). Endothelial cells are the likely route for conduction of the remote vascular responses, since they are electrically coupled via gap junctions and because capillaries are devoid of smooth muscle. Although the distances over which such a propagated response can be sustained have not been clearly defined, it is tempting to invoke this phenom-
enon as a possible explanation for the observation that CAM-specific MABs can ablate the defective endotheliometer-dependent vasodilation in tissues exposed to ischemia and reperfusion (18). Arteries and arterioles rarely sustain leukocyte-endothelial cell adhesion. Nonetheless, these segments of the vasculature could be influenced by inflammatory events that take place in postcapillary venules, if the engagement of leukocyte adhesion molecules (or a product of leukocyte activation) to endothelial receptors can elicit propagated impulses that are electrotonically transmitted along the entire length (and beyond) of the capillaries. Additional work is needed to determine if a mechanism that empowers the leukocyte to influence all segments of the microcirculation does indeed exist.

**AUXILIARY CELLS CAN MODULATE LEUKOCYTE-ENDOTHELIAL CELL ADHESION**

Although endothelial cells and leukocytes have served as major focal points in the search for the source(s) of chemical mediators that elicit and amplify leukocyte-endothelial cell adhesion in postcapillary venules, there is mounting evidence that other cell types can contribute significantly to this component of the inflammatory response. The mast cell is an excellent example of an auxiliary cell that can profoundly influence the process of leukocyte recruitment. Mast cell products have been invoked as mediators of the leukocyte-endothelial cell adhesion observed in several models of gastrointestinal inflammation, including ischemia-reperfusion, immunoglobulin E-mediated hypersensitivity reactions, Helicobacter pylori, and Clostridium difficile (17). Several experimental strategies have been employed to invoke a role for mast cell products in these models of leukocyte recruitment, including quantification of the number of activated and/or degranulated mast cells in proximity to postcapillary venules and treatment with agents that either degranulate (compound 48/80) or stabilize (ketotifen) mast cells or that antagonize mediators that are relatively unique to mast cells (e.g., antihistamines) (17).

The findings from studies using the aforementioned experimental strategies generally support a role for mast cell products in the recruitment of leukocytes within the liver, the mechanisms that account for the apparent ability of these macrophages to promote leukocyte-endothelial cell adhesion remain poorly defined. The rare earth metal gadolinium chloride (GdCl₃) has been widely used to assess the involvement of KCs in different experimental models. Aggregates of GdCl₃ are phagocytosed by KCs, which eventually blocks further phagocytosis and enhances their rate of apoptosis. Hence, by using GdCl₃ to eliminate KCs from the liver, it could be determined whether activated KCs affect leukocyte-endothelial cell adhesion simply by altering shear forces within the hepatic microcirculation or by promoting the increased expression of endothelial cell and/or leukocyte adhesion molecules via the release of inflammatory mediators. However, interpretation of these results may prove difficult if the actions of GdCl₃ are not confined to the KCs.

Assessment of the role of intestinal macrophages in the recruitment of leukocytes appears more difficult. The resident macrophages in this tissue lie outside of the vasculature (lamina propria), and agents used to deplete this cell population (e.g., dexamethasone) have the potential to profoundly influence the function of endothelial cells, mast cells, and leukocytes directly. However, the intestinal (or mesenteric) and hepatic microvasculature may prove more useful for assessing the contribution of other cell populations to the process of leukocyte-endothelial cell adhesion. Mice that are genetically deficient in some or all lymphocyte populations, such as SCID, nude, or Rag-1 mutant mice, hold much potential as tools for assessing the contribution of lymphocyte-derived cytokines in the modulation of leukocyte-endothelial cell adhesion in the gastrointestinal tract and liver. Reconstitution experiments can be
performed in these mutants that employ splenocytes (lymphocytes) derived from other mutant mice (e.g., TNF-α or interferon-γ knockouts) unable to generate specific cytokines. The utility of these strategies for assessment of lymphocyte involvement in hepatic ischemia-reperfusion-induced liver injury has recently been demonstrated (28). Indeed, of all the auxiliary cells that may play a major role in the modulation of leukocyte-endothelial cell adhesion, the lymphocyte appears most amenable to detailed investigations that capitalize on the technological advancements associated with genetic engineering.

The wealth of information that has resulted from the application of intravital microscopic techniques and molecular biological tools to the problem of leukocyte-endothelial cell adhesion has opened a number of new and important avenues of investigation in the field of gastrointestinal and liver inflammation. This article has focused on only a few of the outstanding questions that are evolving as a consequence of recent investigations in this area. Whether these questions hold promise for extension of the therapeutic potential of agents that interfere with the process of leukocyte-endothelial cell adhesion is purely speculative. Nevertheless, additional work on these and related problems is needed before the multiple factors that serve to modulate inflammatory responses in the digestive system become clear.

This work was supported by National Institutes of Health Grants HL-26441 and DK-43785. Address reprint requests to Dept. of Molecular and Cellular Physiology, Louisiana State Univ. Medical Center, 1501 Kings Hwy, Shreveport, Louisiana 71130-3932 (E-mail: dgrayang@mail.shlsumc.edu).

REFERENCES


