Endothelial cell monolayers as a tool for studying microvascular pathophysiology

PETER R. KVIETYS1 AND D. NEIL GRANGER2

1London Health Sciences Centre Research, Inc., London, Ontario, Canada N6A 4G5; and 2Department of Molecular and Cellular Physiology, Louisiana State University Medical Center, Shreveport, Louisiana 70533-3932

Kviety, Peter R., and D. Neil Granger. Endothelial cell monolayers as a tool for studying microvascular pathophysiology. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1189–G1199, 1997.—Endothelial cells contribute to a variety of biological responses that facilitate organ function. This critical role of the endothelial cell has resulted in the development of different in vitro models that utilize monolayers of cultured cells to simulate conditions that exist in the intact animal. This review focuses on endothelial cell monolayers as a model system for research on certain pathophysiological conditions affecting the gastrointestinal tract. The advantages and limitations of endothelial cell monolayers are addressed, along with evolving technologies and strategies that hold promise for extending the utility of this in vitro model for studies of gastrointestinal function and disease.

ischemia-reperfusion; nonsteroidal anti-inflammatory drugs; nitric oxide; human umbilical vein endothelial cells; leukocyte migration; monolayer permeability; neutrophils

For well over a century, endothelial cells were generally thought to be merely a passive barrier lining the inner walls of blood vessels. At present, however, there is growing awareness that vascular endothelium is an active metabolic component of tissues that serves a number of important physiological functions (Table 1). The recognition that abnormal endothelial cell responses frequently accompany organ dysfunction and disease has led to an intense interest in developing both in situ and in vitro model systems to study endothelial cell function. Intravital videomicroscopic techniques have proven to be the most common and successful means of assessing endothelium-dependent responses in vivo; however, this approach often relies on indirect strategies to evaluate vascular responses that can be specifically attributed to changes in endothelial cell function. To circumvent the limitations of in vivo models, many investigators have chosen to use cultured endothelial cells. Endothelial cell monolayers have been widely used to characterize the responses of the microvasculature to different physiological stimuli and pathological stresses, as well as to elucidate the mechanisms that underlie the microvascular dysfunction and tissue injury that accompany several diseases and disorders of the gastrointestinal tract (Table 2). Enthusiasm for this approach has increased as a result of recently reported successes in isolating and culturing endothelial cells derived from normal and diseased human intestine (10, 11, 34).

This review briefly summarizes how endothelial cell monolayers have been used to study certain pathophysiological states in the gastrointestinal tract. This discussion is followed by a critical assessment of the advantages and limitations of endothelial cell monolayers as a model of events that occur in intact tissue. Finally, we describe the evolving technologies and strategies that hold potential for significantly extending the utility of cultured endothelial cells for studies of gastrointestinal function and disease.

Characteristics of Endothelial Cells in Culture

Endothelial cells have been harvested, grown, and studied in culture for roughly a quarter of a century (46). Technological and methodological advances have allowed for the harvesting of endothelial cells from a variety of organ systems of different species, including humans (1, 28, 37, 39, 48, 93, 107). Of interest in this regard is the current successful harvesting and use of human intestinal microvascular endothelial cells to study the contribution of these cells to intestinal inflammatory states (11, 34). Several different general approaches have been used to harvest endothelial cells, each with certain advantages and limitations.

The most common approach for isolation of endothelial cells is to enzymatically (e.g., collagenase) remove the cells from the internal lining of large blood vessels,
Table 1. Physiological functions of vascular endothelial cells

<table>
<thead>
<tr>
<th>Function</th>
<th>Endothelial Cell Response</th>
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<tbody>
<tr>
<td>Regulation of microvascular fluid and solute exchange</td>
<td>Cell contraction</td>
</tr>
<tr>
<td>Maintenance of antithrombogenic vessel surface</td>
<td>Production of PGI$_2$ and NO</td>
</tr>
<tr>
<td>Modulation of vessel proliferation (angiogenesis)</td>
<td>Production of adenosine</td>
</tr>
<tr>
<td>Control of leukocyte trafficking</td>
<td>Surface expression of adhesion molecules (e.g., ICAM-1)</td>
</tr>
<tr>
<td>Regulation of vascular tone and blood flow</td>
<td>Production of NO, adenosine, endothelin, etc.</td>
</tr>
<tr>
<td>Metabolism of circulating molecules</td>
<td>Surface expression of enzymes (e.g., lipoprotein lipase)</td>
</tr>
</tbody>
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PGI$_2$, prostaglandin I$_2$; NO, nitric oxide; ICAM-1, intercellular adhesion molecule-1.

such as human umbilical veins. The advantages to this approach include a good yield and a high purity (little contamination with nonendothelial cells). A disadvantage is that the enzymatic treatment may exert detrimental effects on certain surface proteins that are of relevance to the issues addressed by the in vitro model.

Another approach is to mince and enzymatically digest the entire organ system and then, through the use of specific media and/or other means (e.g., paramagnetic beads armed with endothelium-specific monoclonal antibodies [MAb]), select out endothelial cells from the other cell populations. This approach can yield microvascular endothelial cells, which may be more useful for studies of events that occur during inflammation. One disadvantage to this approach is the potential for contamination of the endothelial cells with other cell types. A notable example of this is the early report of successful culture of human microvascular endothelial cells from adipose tissue (48). A subsequent study provided evidence that cells isolated are likely to be mesothelial cells (105), which are difficult to distinguish from endothelial cells. This problem has been circumvented in one organ system, the lung, by first removing the visceral pleura (mesothelial cells) and then using selective growth media to encourage endothelial cell growth while discouraging the growth of other cell types (e.g., fibroblasts). Similar strategies have been applied to harvest endothelial cells from organ systems that do not have a mesothelial cell component, such as mammary glands (39).

A third approach for isolating endothelial cells involves either mechanically scraping the cells from the walls of large blood vessels or using microcarrier beads to remove endothelial cells from the venular microvasculature (89). These approaches avoid problems with enzymatic damage to endothelial cells and contamination with other cell types. Although the bead technique can be used to obtain venular microvascular cells, it produces a low yield and it has been successfully applied only to the lung and heart (89, 93).

Regardless of the method of isolation, endothelial cells in culture display certain characteristic features that can be used to confirm their identity after harvesting and plating the isolates. Endothelial cells form confluent monolayers that restrict the movement of macromolecules, albeit not to the degree observed in vivo (see below). They have a typical cobblestone appearance, which can be modified by shear stress to a more elongated shape oriented in the direction of flow (68). Endothelial cell monolayers can also be identified by the presence of angiotensin-convverting enzyme on the cell surface, uptake of acetylated low-density lipoprotein (LDL), and expression of factor 8 (105). Some of these markers may not be unique to endothelial cells, since mesothelial cell monolayers also take up acetylated LDL. The ability of endothelial cells under certain culture conditions to form tubes, much like embryonic blood vessels or those undergoing angiogenesis after tissue injury, was believed to be a unique characteristic of endothelial cells (67). However, epithelial cells have also been reported to form tubelike structures in culture (73). Hence, this criterion for identifying endothelial cells is also of questionable specificity. In general, most investigators use the most expedient method of harvesting cells of high purity and yield and subsequently use several criteria to confirm their identity as endothelial cells.

Extracellular matrix components can affect endothelial cell growth and phenotype (46, 63, 93). In general, endothelial cells adhere better to surfaces coated with extracellular matrix components such as collagen, fibronectin, and laminin or more complex matrices such as Matrigel (93) or amnion (40). Endothelial cells in culture can also synthesize their own matrix components (46). Although a variety of extracellular materials are used to facilitate culture of endothelial cells, simple approaches (one extracellular component, such as fibronectin) are preferred over more complex systems (amnion).

Two basic difficulties have emerged from this effort to obtain and study endothelial cells in culture: 1) endothelial cells from different organs and/or species behave differently in culture (25, 46), and 2) endothelial cells derived from the microvasculature behave differently from endothelial cells harvested from large blood vessels (25, 121). The first problem has a rather obvious
resolution, i.e., use endothelial cells derived from the organ and species relevant to the experimental issues under investigation. The second difficulty transcends organ and/or species differences and is central to the inflammatory response. Because inflammation is generally manifested by leukocyte-endothelial cell interactions and endothelial barrier dysfunction at the microvascular (venular) level, differences in responsiveness between micro- and macrovascular endothelial cells may be of paramount importance. However, despite studies indicating that these differences may exist (27), the absence of well-designed experiments that more definitively address the issue (micro- and macrovascular endothelial cells from the same organ system, from the same passage, and under the same culture conditions) precludes a final resolution of this argument. Currently, the cell preparation most often used for in vitro studies of inflammation is endothelial cells that have been enzymatically removed from human umbilical veins (HUVEC).

ENDOTHELIAL CELL MONOLAYERS: RELEVANCE TO GASTROINTESTINAL MUCOSAL INJURY

Endothelial cell monolayers have been used to simulate some of the microvascular responses associated with certain pathological conditions of relevance to the gastrointestinal tract (Table 2). Although endothelial cell responses such as angiogenesis, thrombogenesis, and vasodilator production have received some attention (5, 25, 46), most of the published work in this area has focused on models of acute and chronic inflammation (56).

Ischemia-Reperfusion

The phenomenon of ischemia followed by reperfusion (I/R) has been implicated in the microvascular and parenchymal cell injury associated with several pathological conditions, including gastric ulcer formation, multiple organ failure, nonocclusive mesenteric ischemia, and organ transplantation (21, 29, 30). Intestinal I/R affects the microvasculature, particularly postcapillary venules, in a manner consistent with an acute inflammatory response, that is, it results in enhanced production of reactive oxygen species (via xanthine oxidase) and lipid mediators (leukotriene B4 (LTB4) and platelet-activating factor (PAF)), a reduction in nitric oxide (NO) generation, the rolling, firm adherence, and emigration of leukocytes, and an increased microvascular permeability (21, 29, 30, 56).

Several investigators have exposed endothelial cell monolayers to hypoxia followed by reoxygenation (H/R) in an effort to mimic the microvascular dysfunction normally elicited by I/R. With this approach, it has been demonstrated that H/R results in the following alterations in endothelial cell function: 1) xanthine oxidase activation (44, 83, 100), 2) enhanced formation of superoxide and hydrogen peroxide (102, 123), 3) activation of nuclear transcription factors NF-κB and AP-1 (42), 4) increased expression of endothelial cell adhesion molecules (42), 5) increased adhesivity to neutrophils (119), and 6) a reduction in endothelial barrier function (45). Mechanistic studies on H/R-treated monolayers have revealed that endothelial cells produce and liberate hydrogen peroxide, which results in a PAF-dependent upregulation of neutrophil adhesion molecules (CD11/CD18) and the subsequent engagement of these adhesion receptors to their counterparts on the endothelial cell surface (42, 99, 100, 119). These in vitro models have also provided unique insight into the role of proteases and oxidants to H/R-induced, neutrophil-mediated endothelial cell injury (43, 44). Moreover, endothelial cell monolayers have allowed for a detailed characterization of the contribution of different leukocyte and endothelial cell adhesion glycoproteins to the elevated neutrophil-endothelial cell adhesion elicited by H/R (42, 119).

Although H/R-exposed endothelial cell monolayers have proven to be remarkably accurate at simulating several of the microvascular alterations elicited in vivo by I/R, there are some responses that cannot be mimicked in the in vitro model. For example, leukotriene biosynthesis inhibitors and LTB4 receptor antagonists are highly effective in blunting the recruitment of leukocytes into mesenteric postcapillary venules exposed to I/R (21, 29, 30); however, these same reagents do not affect H/R-induced neutrophil adhesion to endothelial cell monolayers (119). Another difference between the in vitro and in vivo models relates to the contribution of different selectins to the recruitment of leukocytes that is observed after reperfusion (reoxygenation). Although P-selectin-specific MAbs are effective in attenuating leukocyte adhesion in postischemic venules (21, 29, 30), these MAbs are often ineffective in blunting the neutrophil adhesion to endothelial monolayers exposed to H/R (29).

The few inconsistent responses of intact vs. cultured endothelial cells to I/R (H/R) may be attributed to a variety of factors. The inability to demonstrate a role for P-selectin in some in vitro models of H/R may simply result from the use of late-passaged endothelial cells, or it may reflect the absence of applied shear forces in many in vitro studies of neutrophil-endothelial cell adhesion (see below). Alternatively, it may reflect the absence of mast cells, which are known to degranulate and promote P-selectin-dependent leukocyte adhesion in postcapillary venules (50). A similar explanation may be invoked concerning the inability of leukotriene-targeted reagents to attenuate neutrophil adhesion in vitro. Endothelial cells appear to have a limited capacity to generate leukotrienes. However, there is evidence for transcellular cooperation in the biosynthesis of leukotrienes when endothelial cells are cocultured with platelets or neutrophils (14), i.e., endothelial cells gain the capacity to generate leukotrienes when appropriate substrates are provided by other cell types.

NSAID-Induced Inflammation

There is a large body of evidence that implicates neutrophils as mediators of the gastrointestinal muco-
sal injury associated with ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) (109). Animals that are either rendered neutropenic (111) or that receive MAb directed against leukocyte or endothelial cell adhesion molecules (109, 112) exhibit a markedly reduced level of NSAID-induced injury. Furthermore, it has been demonstrated that direct exposure of mesenteric venules to either aspirin or indomethacin (at concentrations expected to be experienced by gastric venules after ingestion of the respective NSAID) leads to the recruitment of adherent and emigrated leukocytes and an enhanced leakage (extravasation) of albumin (6, 7, 55). Additional studies have revealed that the NSAID-induced leukocyte adhesion in mesenteric venules is mediated by the engagement of \( \beta_2 \)-integrins (CD11b/CD18) on leukocytes with constitutively expressed intercellular adhesion molecule-1 (ICAM-1) on endothelial cells (55). LTB\(_4\) (but not PAF) appears to mediate the NSAID-induced upregulation of CD11/CD18 on neutrophils, since LTB\(_4\) antagonists and 5-lipoxygenase inhibitors profoundly blunt the NSAID-induced inflammatory responses (6, 7). The critical role of lipoxygenase products in mediating NSAID-induced leukocyte adhesion in postcapillary venules is exemplified by the observation that NSAIDs that possess both cyclooxygenase and 5-lipoxygenase activity (e.g., Tenidap) do not exhibit the in vivo proinflammatory properties of pure cyclooxygenase inhibitors such as aspirin or indomethacin (77).

Cultured endothelial cells have provided novel information concerning the cell population (e.g., endothelial cells vs. leukocytes) that is directly affected by NSAIDs. Exposure of human neutrophils to aspirin and indomethacin results in an increased adhesion to endothelial cell (HUVEC) monolayers (23, 118, 120). Pretreatment of HUVEC alone with NSAIDs does not promote adhesion (120). Flow cytometric data have revealed increased surface expression of CD11b/CD18 on neutrophils exposed to aspirin or indomethacin (23, 120). Furthermore, NSAID-induced neutrophil adhesion to HUVEC is greatly reduced by MAb to either CD11b, CD18, or ICAM-1 (23, 120), as well as LTB\(_4\) receptor antagonists and 5-lipoxygenase inhibitors (120). Hence, the responses of the in vitro model of NSAID-induced neutrophil adhesion are largely consistent with the in vivo results derived from mesenteric venules. In addition, the in vitro studies appear to exclude a direct action of NSAIDs on endothelial cells per se.

The HUVEC model of aspirin-induced neutrophil adhesion has also provided valuable insight into the potential mechanisms that underlie the increased albumin leakage elicited by NSAIDs in postcapillary venules (55). It has been noted that aspirin-activated neutrophils induce endothelial cell retraction (without cell lysis) followed by detachment from the underlying matrix (118). This cell retraction and detachment (which likely relates to the increased vascular permeability noted in vivo) does not occur if neutrophil adhesion is prevented with CD18-specific antibodies. Inhibitors of neutrophilic elastase, but not of superoxide dismutase or catalase, also prevented the endothelial cell retraction and detachment responses to aspirin. Hence, these in vitro studies not only support the in vivo observation that leukocyte adhesion is required for aspirin-mediated vascular protein leakage but also extend the in vivo work to invoke a major role for elastase in this adhesion-dependent vascular permeability response.

**LIMITATIONS OF ENDOTHELIAL CELL MONOLAYERS AS A MODEL OF THE INTACT MICROVASCULATURE**

Although the rather remarkable ability of endothelial cell monolayers to mimic events that are elicited in vivo by I/R or NSAIDs underscores the physiological relevance and utility of this in vitro methodology, the few but rather substantial differences noted between the in vivo and in vitro models also raises the need for caution when relating and extrapolating data from the in vitro to the in vivo situation. This section discusses several of the known characteristics and responses of cultured endothelial cells that may explain some of the discordant results generated from this model system.

**Adhesion Molecules**

Endothelial cells in culture, like their in vivo counterparts, express adhesion molecules when activated by inflammatory mediators or cytokines (18, 34, 37, 76). The basal level of expression and kinetics of lipopolysaccharide (LPS)-stimulated expression of ICAM-1 on HUVEC are qualitatively similar to those observed in various tissues of the rat (76). When ICAM-1 density estimates are corrected for endothelial surface area, the level observed in unstimulated intestinal microvasculature is very similar to that detected on HUVEC monolayers (76). However, there are some rather substantial quantitative differences in ICAM-1 expression between the muscle (heart and skeletal) vasculature and HUVEC (76). Other studies have revealed that the level of expression of certain adhesion molecules can differ substantially between cultured endothelial cells derived from different sources. Human brain microvascular endothelial cells increase E-selectin expression in response to cytokines, which peaks at 4 h and returns to negligible levels by 24 h, a temporal response that is very similar to that observed on HUVEC (37). However, the intensity of cytokine-mediated E-selectin expression on brain endothelium is much less than that observed on HUVEC. Cytokine-induced expression of E-selectin, vascular adhesion molecule-1 (VCAM-1), and ICAM-1 on monolayers of human intestinal microvascular endothelial cells (HIMEC) also follows a time course similar to that noted in HUVEC (34). However, with a few exceptions, the magnitude of the adhesion molecule upregulation is far greater on HUVEC than on HIMEC. Furthermore, although interleukin-4 (IL-4) induces VCAM-1 expression on HUVEC, it does not induce VCAM-1 expression on HIMEC. Hence, the available data indicate that HUVEC does not always provide a valid reflection of the intensity of adhesion molecule expression that can occur in vivo or on endothelial cells derived from other tissues. These differ-
ences may reflect organ-to-organ variability and/or differences between endothelial cells derived from large and small blood vessels.

Although most endothelial cell adhesion molecules can be readily detected on stimulated monolayers of late-passaged endothelial cells, this is not observed for P-selectin. The expression of this adhesion molecule on activated HUVEC appears to wane with cell passage (60). This phenomenon may be due to the fact trypsin (an enzyme that is commonly used to passage cells) stimulates P-selectin expression (18), and consequently multiple passages may reduce the ability of HUVEC to respond appropriately to stimuli.

**Inflammatory Modulators**

NO. NO has been shown to modulate leukocyte-endothelial cell-adhesive interactions in vivo and in vitro (54, 70). NO synthase (NOS) inhibitors rapidly (within 30 min) elicit oxidant stress and promote leukocyte adhesion and emigration in postcapillary venules (54). Cultured endothelial cells derived from animal tissue contain both the constitutive (eNOS) and inducible (iNOS) isoforms of NOS (94). Although it has been shown that NO is generated by endothelial cells in intact human umbilical vessels (104), it is very difficult to demonstrate a biological role for NO using HUVEC in culture (61, 70). Only when HUVEC monolayers are pretreated for long periods (2–4 h) with NOS inhibitors do these endothelial cells exhibit an oxidant stress and become proadhesive for neutrophils (70). Furthermore, cytokine stimulation of endothelial cells derived from animal sources results in increased calcium-independent NO production, indicating that iNOS activity is increased by cytokines (32, 82, 113). By contrast, cytokine-stimulated HUVEC produce NO via a calcium-dependent pathway, indicating that eNOS, not iNOS, activity is increased (87). The latter effect appears to be due to cytokine-enhanced synthesis of tetrahydrobipterin, an important cofactor for NOS activity (84, 87). Apparently, tetrahydrobipterin is present in HUVEC in vivo but rapidly disappears under culture conditions (87). The loss of tetrahydrobipterin from HUVEC after harvesting may also explain the difficulty in demonstrating a role for NO in neutrophil-HUVEC adhesive interactions in the absence of cytokine stimulation. These studies underscore the differences in responses between endothelial cells derived from different species and the metabolic alterations that can result when cells are grown in culture.

Oxidants. Unstimulated endothelial cells in culture can generate superoxide (85, 102) and hydrogen peroxide (98). This observation suggests that cultured endothelial cells may be experiencing oxidant stress. In vivo endothelial cells are exposed to a maximum PO2 of 80–90 mmHg (arterial) and a minimum PO2 of 40 mmHg (venules). By contrast, cultured endothelial cells are routinely maintained at ambient PO2 of 140 mmHg. The magnitude of this oxidant stress may vary with culture conditions. For example, the endothelial cell content of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) varies as a function of passage number (12, 36, 106). This variation in the antioxidant status of endothelial cells with age is unpredictable, i.e., some enzymes decrease with age whereas others increase. Intracellular iron, which facilitates the generation of different oxidant species, also decreases in HUVEC with age (106), thus rendering the cells resistant to oxidant-induced cytotoxicity. Hence, if oxidant stress is an important component of an experimental model under study, then the antioxidant status of the endothelial cells should be considered.

An important source of oxidants in endothelial cells is xanthine oxidase (102). Endothelial cells in culture contain xanthine dehydrogenase, which reduces NAD, and xanthine oxidase, which reduces molecular oxygen to superoxide (75, 78, 81). Xanthine dehydrogenase can be converted to the oxidant-generating form, xanthine oxidase, by stresses such as hypoxia and cytokines (75). In addition, the conversion of xanthine dehydrogenase to xanthine oxidase can be elicited by activated neutrophils via a mechanism not involving oxidants (81). Recently, endogenous NO has been implicated in inhibiting xanthine oxidase activity in rat pulmonary microvascular cells (19).

Endothelial cells can also release xanthine oxidase into extracellular fluid (79) under basal conditions. This release of xanthine oxidase can be augmented when endothelial cells are exposed to certain stresses (hypoxia). The released xanthine oxidase can bind to the surface of other endothelial cells in a heparin-inhibitable fashion, indicating that the enzyme tethers to proteoglycans on the endothelial cell membrane (2). If the appropriate substrates (hypoxanthine or xanthine) are present, xanthine oxidase-derived oxidants can be generated immediately adjacent to the endothelial cell membrane to cause damage. Recently, it has been shown that extracellular xanthine oxidase can render endothelial cells more proadhesive to neutrophils (101). Pretreatment of endothelial cells with xanthine oxidase increased endothelial cell surface expression of P-selectin and neutrophil adhesion to the endothelium. This effect was inhibited by heparin and mediated by hydrogen peroxide and PAF. These observations may have significant implications for in vitro models of inflammation, inasmuch as the level of membrane-bound xanthine oxidase on endothelial cells may determine the nature and/or magnitude of the inflammatory responses.

**Intravascular and Extravascular Factors**

Plasma. It is generally accepted that plasma can inhibit neutrophil-mediated proteolysis of various substrates, an effect attributed to the antiproteases present in plasma (114). Plasma can also inhibit neutrophil-endothelial cell adhesive interactions. A recent study indicates that soluble adhesion molecules (sP- and sE-selectins and sCAM-1) are partially responsible for the inhibitory effect of platelet-poor plasma on neutrophil adhesion to endothelial monolayers and on neutrophil transendothelial migration (71).

Platelets. Platelets have also been shown to modify neutrophil-endothelial cell adhesive interactions (115).

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For example, activated platelets can enhance neutrophil adhesion to endothelial cell monolayers, as well as neutrophil-mediated endothelial cell injury (13). Others have reported that platelets per se produce inhibitors of neutrophil adhesion to endothelial cells (20). This issue appears worthy of attention in that intravascular platelet-leukocyte aggregates are a common feature of acute inflammation in vivo.

Shear stress. Under in vivo conditions, leukocyte-endothelial interactions occur in the presence of a shear stress imposed by flowing blood. Although a majority of the in vitro studies dealing with leukocyte-endothelial adhesive interactions are performed under static conditions (absence of shear stress), there is a growing interest in examining these interactions in the presence of shear stress. The development of parallel plate flow chambers allows for the controlled application of various shear stresses on endothelial cell monolayers or other substrates (65). These studies have helped unravel the molecular determinants of the initial weak adhesive interaction of leukocytes with endothelium, termed “rolling” (mediated by the selectins), as well as the stronger adhesive interactions of activated leukocytes to endothelium (mediated by CD11/CD18 and ICAM-1), which eventually lead to emigration of the leukocytes into the interstitium.

One interesting difference between in vivo and in vitro studies of leukocyte-endothelial cell adhesion has become apparent. Under in vitro conditions, leukocyte adhesion to endothelial cell monolayers is completely abolished at acute shear stresses of 2–4 dynes/cm² (58, 59, 120). By contrast, leukocyte adhesion to endothelium in postcapillary venules occurs at shear stresses as high as 35 dynes/cm² and, on average, is abolished only when shear stress is reduced to 18 dynes/cm² (80). This discrepancy in the ability of adherent leukocytes to tolerate shear forces on endothelium of HUVEC monolayers vs. postcapillary venules remains to be resolved.

The application of chronic (hours) shear stress at physiological levels (10–25 dynes/cm²) to endothelial cell monolayers provides some interesting insights concerning the effects of shear stress on endothelial cell expression of adhesion molecules. For example, chronic shear stress appears to upregulate surface expression of ICAM-1 on HUVEC in a biphasic manner, i.e., there is an initial increase in ICAM-1 expression at 4–12 h followed by a return to basal levels by 24 h (69, 92, 103). The expression of VCAM-1 and E-selectin mRNA is decreased (92), and surface levels of VCAM-1 are unchanged (103). Shear stress can also increase endothelial cell NO production by increasing eNOS gene expression and NOS protein (94). These findings may explain why endothelial cells exposed to chronic levels of high shear (e.g., in postcapillary venules) do not sustain the same level of leukocyte adhesion as endothelial cells that are not exposed to chronic shear (e.g., HUVEC monolayers).

Interstitial matrix components can also influence neutrophil-endothelial cell interactions (22, 49, 64, 74). For example, certain matrix components may facilitate transendothelial leukocyte migration (22) yet inhibit leukocyte activation during the migration process (64).

Mast cells. Mast cells have been shown to play an important role in modulating the inflammatory response in mesenteric venules in vivo (50). However, there do not appear to be any in vitro studies that have employed coculture approaches to evaluate the modulatory role of mast cells in neutrophil-endothelial cell adhesive interactions. This is presumably because mucosal mast cells are very difficult to isolate and the available mast cell lines (e.g., rat basophilic leukemic cell line) are difficult to degranulate. One approach that has been taken is to use mast cell sonicates to simulate degranulation (57). These mast cell sonicates promote neutrophil transendothelial migration, an effect that is prevented by an LTβ receptor antagonist but not by a PAF receptor antagonist. These observations support the need to develop coculture techniques for assessing the role of mast cell products on endothelial cell function during inflammation.

Controversial Issues

Transendothelial leukocyte migration. In vivo studies indicate that during acute inflammation, leukocytes emigrate across venules into the interstitium, a process that is largely prevented by elastase inhibitors (16, 117, 122). However, in vitro studies have yielded conflicting results regarding the role of elastase in modulating transendothelial leukocyte migration. When transendothelial leukocyte migration is studied in reductionist models (HUVEC monolayers on fibronectin-coated filters), elastase inhibitors can prevent the increased transmigration of activated neutrophils (17, 118). However, when more complex in vitro models are used (HUVEC monolayers grown on artificial constructs of blood vessel walls consisting of basement membrane generated on a collagen matrix or HUVEC cultured on amnion) the transendothelial migration of activated leukocytes is not prevented by elastase inhibitors, or for that matter a broad spectrum of antiproteases (24, 41). The reason for the discrepancy is not readily apparent, but differences between simple reductionist approaches and more complex systems should be kept in mind when addressing the issue of transendothelial neutrophil migration.

Monolayer permeability. In general, in vitro studies indicate that the permeability of endothelial cell monolayers to macromolecules is far greater than that measured in vivo. In one study, the endothelial cell monolayers failed to demonstrate charge selectivity or restricted diffusion to macromolecules, which was attributed to gaps in the monolayer (4). Other studies have provided evidence that endothelial cell monolayers do indeed exhibit restricted diffusion (33, 88, 96). Thus direct quantitative comparisons of permeability characteristics between in vivo and in vitro studies should be approached with caution. Nonetheless, it should be noted that endothelial cell monolayers do respond to agonists such as histamine (95), thrombin (96), tumor necrosis factor (88), PAF (17), and IL-1 (15, 88) by
increasing transendothelial albumin movement. Hence, it would appear that some basic mechanisms involved in transendothelial protein movement can be addressed using endothelial cell monolayers. For example, a role for phosphorylation of the light chain of myosin has been proposed in histamine-induced increases in HUVEC permeability (95). Another study has provided evidence that a shear stress of 10 dynes/cm² elicits a 10-fold increase in the permeability of bovine aortic endothelial cell monolayers to albumin (47). Monolayer permeability returned to control levels when the shear stress was terminated, indicating that this effect is reversible. Data have also been presented to indicate that NO stabilizes endothelial monolayers to such an extent that sucrose permeation is decreased (72).

Relationship between transendothelial leukocyte migration and protein movement. In vivo studies of acute inflammation indicate that vascular protein leakage appears to be coupled to leukocyte emigration (51–53). In vitro studies using a reductionist approach (HUVEC grown on filters) indicate that enhanced neutrophil migration is also associated with increased protein leakage (86). Indeed, recent in vitro studies indicate that the extent of transendothelial protein movement is directly correlated to the rate of transendothelial neutrophil migration (17). However, in vitro studies in which more complex systems are used (HUVEC monolayers on amnion) indicate that transendothelial neutrophil migration can occur in the absence of enhanced transendothelial protein movement (40).

Two issues concerning the relationship between transendothelial leukocyte migration and transendothelial protein exchange warrant further attention. First, the discrepancy between the different in vitro studies may be due to the complexity of the models used. The reductionist approach offers only one significant barrier to protein movement, the endothelial monolayer (17), whereas the more complex model offers two restrictive barriers, the endothelial cell monolayer and the amnion (40). The more complex model may present difficulties for studies that address the relationship between transendothelial neutrophil migration (assessed by counting neutrophils entering the amnion and transendothelial protein movement (as measured after protein has passed through the amnion). In this system, transendothelial neutrophil migration can be assessed within minutes of neutrophil activation, whereas an assessment of protein movement may require hours because of the diffusional barrier imposed by the amnion (40). Thus correlations between neutrophil transendothelial migration and transendothelial protein movement may not be readily apparent in more complex systems in which the two events cannot be measured simultaneously.

Many inflammatory mediators (e.g., PAF) can directly affect endothelial monolayer permeability (17). Consequently, transendothelial protein movement may occur independently of transendothelial migration. This complication may explain the in vivo observation that during acute inflammation vascular protein leakage occurs before any detectable changes in leukocyte emigration (52, 53). Thus careful consideration should be given to the dose of inflammatory mediators used for in vitro studies of inflammation-induced alterations in vascular permeability.

Neutrophil-mediated endothelial cell injury. In vitro, neutrophil-mediated endothelial cell injury is nonlytic and is manifested as endothelial cell detachment from the underlying matrix (35, 43, 44, 66, 116, 118). This endothelial cell detachment occurs 3–6 h after neutrophils react with the endothelial cell monolayer and is mediated by neutrophil-derived elastase (43, 116, 118). In vivo, endothelial cell detachment from microvessels across which substantial neutrophil emigration occurs is rarely observed. One reason why neutrophil-mediated endothelial cell injury is noted in vitro but not in vivo models of inflammation is due to the artificial experimental conditions imposed in vitro. In vivo, activated neutrophils use elastase to facilitate their migration across the endothelium to a specific target site within the interstitium. In most in vitro models neutrophil transendothelial migration is largely prevented because the endothelial monolayers are established on nonporous surfaces, i.e., plastic. Under these conditions, rather than simply passing across and away from the monolayers, the activated neutrophils continuously degrade the endothelial cell surface and/or junctional proteins. This prolonged (up to several hours) proteolytic attack leads to endothelial cell retraction and subsequent detachment. This contention is supported by the observation that activated neutrophils do not cause cell retraction and detachment if they are allowed to migrate across endothelial monolayers grown on porous surfaces (118).

APPLICATION OF MOLECULAR BIOLOGICAL APPROACHES TO ENDOTHELIAL CELL MONOLAYERS

A number of molecular biological strategies have been devised that should greatly extend the utility of endothelial cell monolayers as models of human disease. Several methods have been described for delivering recombinant genetic material into vascular endothelial cells (62). Although some viral vectors have proven to be effective gene-delivery systems, most published viral transduction strategies continue to suffer from low transduction frequencies (e.g., <10% of endothelial cells) and the often transient nature of vector gene expression (62). Nonetheless, these approaches hold great potential for genetic manipulation of the production of different enzymes and proteins within cultured endothelial cells. Another approach that is gaining widespread application to cultured cell models is manipulation of gene function via antisense oligonucleotides (9). This strategy allows for highly specific and effective inhibition of protein synthesis in cultured cells. Efficiency of uptake of the larger oligonucleotides by endothelial cells may represent a limitation of this experimental strategy. Hence, gene-delivery systems and antisense oligonucleotides represent exciting and possibly more specific alternatives to synthetic ago-
ists, antagonists, and inhibitors for addressing mechanisms of endothelial cell dysfunction.

Gene-targeted mice with altered surface levels of adhesion molecules have been used in vivo to unravel the molecular mechanisms involved in leukocyte rolling, adhesion, and emigration in postcapillary venules (31). Recently, several investigators have successfully isolated and cultured microvascular endothelial cells from different tissues of mice genetically deficient in specific endothelial cell adhesion molecules, i.e., P- or E-selectin (8, 26). This unique approach represents a useful means for extending gene-knockout and transgenic mouse technology to in vitro models. Areas of endothelial cell biology related to inflammation may particularly benefit from this approach because of the large number of different mutants that have been developed for this general field. For example, knockout mice are now available for leukotrienes, specific cytokines, all isoforms of NOS, cyclooxygenase-2, and most leukocyte adhesion receptors on endothelial cells (90). Another approach used to study endothelial cell function in vitro is to harvest and culture endothelial cells from hemangiomas of virally transfected mice (91, 108).

Endothelial cell biologists have also used viral transfection techniques to create immortalized cell lines. These immortalized cell lines offer a number of investigational advantages, including decreased need for time-consuming isolation of endothelial cells from animal tissues, faster growth rates, lower possibility of nondenstrial cell contamination, and a diminished risk for infection with HIV or hepatitis B (3, 10, 97). Immortalized endothelial cells are particularly useful for enhancing the availability of cells derived from human tissues, such as the intestine (10). However, results derived from some immortalized cells created by viral transfection as well as from a spontaneously transformed HUVEC cell line (ECV304) suggest that the transformed cells either fail to express the same array of proteins and receptors as their nontransformed counterparts (97) or that the responsiveness of transformed cells to physiological stimuli is diminished compared with primary cultures (10). These limitations are likely to be eliminated as the procedures for immortalization are improved.

Epilogue

Evidence suggesting that endothelial pathology contributes to conditions such as inflammatory bowel disease, gastroduodenal ulceration, portal hypertension, and organ transplantation has increased the interest of gastrointestinal researchers in the science of endothelial cell biology. Cultured endothelial cells are proving to be a useful adjunct to in vivo studies for defining the contribution of these vascular cells to different experimental models of human disease. Endothelial cell monolayers have yielded inflammatory responses to conditions such as H/R or NSAID exposure that are remarkably similar to those observed in intact microvessels subjected to comparable perturbations. Nonetheless, some key events observed in the intact microcirculation cannot be mimicked with the cell monolayers, which indicates that important elements of the in vivo situation are not adequately represented in the in vitro models. The inadequacies of endothelial cell monolayers may result from factors such as the origin (species, tissue, and/or vessel) of the cultured cells, absence of physical forces (shear stress) or nonendothelial cell types (mast cells, smooth muscle, macrophages) to which the vessels are normally exposed, or subtle transformations in cell phenotype that result from culture procedures. An important challenge for future investigations in this field is to increase the complexity of the in vitro models in an effort to more adequately simulate the natural environment of endothelial cells within blood vessels. Recent technological advances that allow for the growth of endothelial cells under conditions of shear, the coculturing of endothelial cells with other relevant cell types, and the genetic manipulation of endothelial cell function should enable researchers to achieve this worthwhile objective.

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Address for reprint requests: P. R. Kvietys, London Health Sciences Centre Research, Inc., 375 South St., London, Ontario, Canada N6A 4G5.

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