

ORCHESTRATED INFORMATION TRANSFER UNDERLYING LEUKOCYTE ENDOTHELIAL INTERACTIONS¹

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KEY WORDS: leukocyte-endothelial adhesion, chemokines, information transfer, recruitment,
reticular network

ABSTRACT

The specificity and efficiency of leukocyte binding to endothelial cells (ECs) depends on coordinated information transfer from the underlying tissue to endothelium and from there to the leukocyte. We address three distinct information-transfer points in this system: 1. How does the leukocyte read information from the EC? This process is best accounted for by the paradigm of a multi-step adhesion cascade optimized for rapid information readout; it consists of primary adhesion (rolling/tethering), triggering, and strong adhesion. Recent studies with T cells, monocytes, and eosinophils confirm the generality of the paradigm. The concept of primary adhesion has been expanded to involve not only the selectins, but also certain integrins; furthermore, it depends on receptor concentration on leukocyte microvilli. 2. What information from the underlying tissue does the EC transform into signals for the leukocytes? And what rules govern that process? We illustrate the principles with chemokines, believed to participate in the triggering

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step. The endothelium displays chemokines either (*a*) directly by “posting” them from other cells or (*b*) by integrating a variety of tissue and environmental signals and “relaying” that information by producing its own chemokines and surface adhesion molecules. The rules for this endothelial transduction include specificity coupled with redundancy, amplification, synergy, and coordinated induction of ensembles of molecules. Finally, 3. How does the relevant information reach the endothelium? Simple diffusion is sufficient to deliver signals from cells close to the vessel. However, longer range soluble mediator transport appears to be facilitated by fiber bundles, particularly those ensheathed by fibroblastic reticular cells in the lymph node.

INTRODUCTION—THE INFORMATION PROBLEM

Leukocyte-endothelial interactions are a special case of cell sorting, in which the endothelium discriminates among circulating leukocytes in order to select cells for transmigration into surrounding tissue. Unlike cell sorting in embryogenesis or wound healing, where contact between cells lasts minutes to hours, sorting in the vascular system depends on contact that lasts much less than a second unless specific adhesive events prolong it. The biological objective of leukocyte-endothelial interactions is to direct circulating cells into their appropriate tissue sites with efficiency and specificity. Endothelial cells (ECs) play a singular role in this process, receiving information from the underlying tissue and transforming it into information that can be read rapidly by the passing leukocyte.

There is a high degree of specificity in the interaction of ECs with circulating cells. “Recirculation” is the process whereby lymphocytes undergo repetitive cycles of migration from the circulatory system into tissue and back into the vasculature. Regardless of where a foreign antigen enters the body, this process assures that it will be found by an immune cell with the relevant antigen-specific receptor (“immune surveillance”). Thus, T cells, B cells, and NK cells all undergo continuous recirculation, even in the absence of injury or inflammation. Recirculation is governed by a high degree of specificity, so that phenotypically distinct subsets of these cells migrate preferentially into certain anatomic sites. Superimposed on this is “recruitment” of immune cells to inflammatory sites. The anatomic location and the nature of the inflammatory stimulus determine which leukocytes migrate to an inflammatory site; usually recruitment includes cells that do not recirculate such as neutrophils, eosinophils, and monocytes. In addition to these commonly cited roles of mature cells in immune responses, specific migration occurs in other contexts, including odysseys of T cell and B cell precursors throughout their maturation, dispersal of γ - δ T cells to their

relevant tissues, and the seeding of stem cells. In short, we infer that there are dozens of distinct circulating cell types that are discriminated during endothelial interactions (1, 2). In this review we focus on the biological strategies of information transfer, from tissue to endothelium to leukocyte, which make possible this high level of specificity, and we refer readers to other reviews for detailed descriptions of which subsets migrate under particular pathophysiologic conditions, and for more background information on the molecules involved (1, 3–11). Transmigration into tissue subsequent to binding is a complex sequence of events (12) not addressed in this review.

CIRCULATING CELLS “READ” INFORMATION FROM ENDOTHELIUM

A Prototypic Adhesion Cascade

Understanding of the rapid binding events between leukocytes and endothelium has given rise to a conceptually powerful paradigm that views the interaction as a multistep process (1, 3–11). As a starting point, we present a prototypic adhesion cascade: an *in vitro* model of neutrophil binding to ischemia-injured endothelium described by Nash and coworkers (13). Cultured endothelium within a tube is transiently injured by ischemia and then perfused with a suspension of granulocytes. Granulocytes that flow adjacent to the ECs are captured and begin to roll along the surface. This constitutes the first step of the cascade, called “tethering” or “primary” adhesion. This interaction is mediated by P-selectin on the EC, previously shown to bind the granulocyte mucin PSGL-1 (14). This is consistent with the cascade model, in which selectins mediate tethering. From the point of view of information transfer, this step is arguably the most important. It slows the forward motion of the leukocyte and maintains its surface in close proximity to the surface of the endothelium (see discussion below).

The next *visible* step in the cascade is arrest, in which the rolling granulocyte abruptly becomes immobile. Antibody inhibition studies in the ischemia model demonstrate that arrest is mediated by granulocyte $\beta 2$ integrins, presumably by binding to endothelial ligands ICAM-1 and ICAM-2. This step of the cascade, known as “secondary” or “strong” adhesion, is always mediated by integrins, which efficiently and rapidly regulate adhesion (15).

A Triggering Step Precedes Strong Adhesion

This step is undetected unless correct strategies are used to demonstrate it, and thus its importance is often overlooked. In the ischemia model, antibody to the chemokine IL-8 inhibits arrest by more than 85% (13). Thus, IL-8 produced

by ischemic endothelium is as critical to the process as are either the primary or secondary adhesion pathways. The time of action of IL-8 lies after the rolling (which anti-IL-8 does not inhibit) and before the arrest. Conceptually, triggering is needed to “activate” the integrins; despite high levels of expression on circulating cells, integrins are generally in a functionally inactive state that can be rapidly converted to an active one (15).

The cascade has the potential for encoding an enormous amount of information, as clearly articulated by Butcher (1). Each of the three prototypic steps occurs via specific receptor-ligand combinations. According to the paradigm, strong adhesion will only result efficiently when a leukocyte expressing one set of three receptors meets an EC displaying the complementary set of three counter-receptors. Since each of the receptors and ligands can be selectively expressed on leukocytes and ECs, the number of possible combinations is very large.

Primary Adhesion and Its Roles in Information Readout

The paradigm of the adhesion cascade has been solidified and refined in the last several years. Originally derived from observations in granulocytes (1, 16), it was rapidly generalized to all leukocytes, based on fragmentary information. Validation is now forthcoming for other important circulating cell types. Many studies have distinguished primary and secondary adhesion events for T cell binding to endothelium, either in vitro (17–26) or in vivo (24, 27), as has one study of monocytes in vitro (23) and another of eosinophils in vivo (28).

The findings for each of these are remarkably consistent with the cascade paradigm—although they necessitate a few refinements. Each cell type studied can use selectins for its primary adhesion (rolling). For example, under different experimental conditions, each of the three selectins can mediate rolling of T cells. Those subsets of T cells that roll are those possessing the relevant selectin ligand, including CLA for E-selectin, PSGL-1 for P-selectin, and L-selectin for PNad (peripheral lymph node addressin) (17, 22). However, one notable broadening of the paradigm is that molecules other than selectins can mediate rolling. The integrin molecules $\alpha 4\beta 1$ and $\alpha 4\beta 7$ mediate both rolling (characteristic of primary adhesion) and arrest (21, 24, 28). Indeed, integrins bind to the same ligand for both rolling and arrest (VCAM-1 for $\alpha 4\beta 1$ and principally MAdCAM-1 for $\alpha 4\beta 7$). Integrin-mediated rolling occurs both in vitro, with purified ligand, and in vivo.

There appear to be additional molecular pathways that mediate leukocyte rolling on endothelium. Rolling of human T cells in vitro on IL-1-activated ECs is not inhibitable by anti-selectin or anti-integrin antibodies, suggesting additional receptors and or ligands (20). The binding of CD44 to hyaluronic

acid can mediate rolling and thus may represent another class of receptor-ligand interaction in primary adhesion. For example, the BW5147 cell line rolls either on purified hyaluronate or cultured ECs; in each case the binding is dependent on both CD44 and hyaluronate (29). Although the biologic relevance of this result remains uncertain, it may help explain the involvement of CD44 and hyaluronate in lymph node binding and homing (30, 31).

A second extension of the concept of primary adhesion is that rolling can occur between cells other than leukocytes and EC. Platelets roll along on P-selectin present on activated endothelium (D Wagner, personal communication). Trauma-induced histamine release from mast cells, with resultant endothelial P-selectin externalization, may allow platelet rolling in order to effectively "search out" areas of vessel wall damage. In addition, granulocytes participate in selectin-mediated rolling on immobilized platelets (32) and on granulocytes that have recently adhered to endothelium (33). Thus, at areas of acute inflammation/damage, platelets and granulocytes can act as partial surrogates for the endothelial surface in primary adhesion. Finally, certain carcinoma cells can roll on endothelium (34, 35), although the relevance of this *in vivo* is unknown. Thus, rolling occurs in a wider range of intravascular binding events than was previously appreciated.

The third extension is the importance of microvilli in primary adhesion. Based on scanning EM studies in the 1970s (36, 37), leukocyte binding to endothelium was postulated to occur via microvilli. The theoretical argument was that close contact over broad membrane areas would be prevented by the electrostatic repulsion of the negatively charged surface glycocalyx present on each cell. Thus, microvilli—thin, long (up to 0.5 μm) protrusions—would more easily make contact because of dramatically reduced net charge. Molecular evidence came from studies demonstrating that L-selectin is preferentially expressed on the tips of granulocyte microvilli (38). The $\alpha 4\beta 7$ integrin that mediates primary adhesion is likewise concentrated on the tips of lymphocyte microvilli (24); in contrast, the LFA-1 integrin does not mediate primary adhesion and is not selectively expressed on microvilli. Compelling validation of this concept comes from studies of the functional effects of changing the spatial localization of L-selectin at the cell surface (132). When the cytoplasmic tail of L-selectin is exchanged for that of CD44, the chimeric molecule now localizes primarily to the cell body rather than to microvilli; such cells no longer roll efficiently on L-selectin ligand under flow, although they still bind under static conditions.

At least three biophysical strategies make efficient rolling possible. First, there are rapid on-rates between receptor and ligand (3, 39). Second, the complementary receptors are spatially distributed to optimize encounter. This

is achieved both by the placement of receptors on the tips of leukocyte microvilli and by using receptors (both selectins and mucins) that are long and stiff, thereby exposing their binding sites beyond the bulk of the glycocalyx (40). Third, local concentrations of receptor/ligand are very high. L-selectin is expressed at a high uniform level of approximately 300,000 molecules per cell on most circulating leukocytes (41, 42), which makes it one of their most abundant surface molecules; when it is concentrated on the tips of microvilli, its effective local concentration will be exceptionally high. The mucin counterreceptors likewise can be present at high concentrations, and their repeating carbohydrate side chains represent a high-density array of ligand; they may even be localized on filopodia of ECs (43). The combination of the foregoing strategies makes possible efficient establishment and maintenance of rolling.

Thus, several strategies are utilized to achieve efficient rolling, suggesting that rolling is pivotal to subsequent information transfer between endothelium and leukocyte. Rolling provides two essential elements: time and proximity. First, it prolongs what would otherwise be a repulsive encounter of a few milliseconds into a period of contact as long as several seconds. Second, it maintains contact between the leukocyte and the information source (the endothelium) for the subsequent events in the cascade. Furthermore, the molar concentrations of cell surface molecules are extremely high because they are constrained to diffuse in the plane of the membrane (44); rolling presumably establishes an optimal separation distance between the cells, thereby increasing the frequency of receptor-ligand encounters.

Insights and Controversies on the Triggering Step

Unlike the general consensus that exists on most issues relating to primary adhesion and secondary adhesion, controversy surrounds the triggering step: First, what agents are proposed to mediate triggering in the cascade? Second, is triggering a necessary step?

There are two classes of molecular interactions proposed to provide triggering in the cascade. The first class consists of soluble factors present at or near the endothelial surface. These include primarily the classical chemoattractants for myeloid cells (fMLP, C5a, LTB₄, and PAF) (45) and the family of chemokines, which includes more than 25 members (46–49); all of these signal via serpentine G-protein-linked receptors on the leukocyte (50). An additional soluble factor proposed to trigger the adhesion cascade is HGF, a differentiation factor, capable of acting on a T-lymphocyte tyrosine kinase receptor (51). Of these, so far only IL-8, PAF, and LTB₄ have met the rigorous test of triggering leukocyte endothelial adhesion under conditions of flow. Yet the other candidate molecules share many of the properties of the proven mediators—the ability

to induce integrin-mediated adhesion, for example—and therefore are strongly suspected of also mediating triggering. This has been demonstrated for several chemokines and classic chemotactic factors (52–54) as well as for HGF (51). Many also induce chemotaxis, cell polarization, transmigration, and similar responses, which appear closely linked biologically to adhesion triggering in the cascade (46–48). All the foregoing are therefore reasonable candidates for contributing to triggering in the cascade.

Surface molecules are another class of molecules with possible triggering function. L-selectin has been proposed as a trigger, since cross-linking on neutrophils leads to a calcium flux (55, 56), and L-selectin-mediated contact with cultured high endothelial venule (HEV) endothelium triggers motility (57). Although these studies demonstrate that L-selectin transduces some signals, it is unclear whether these are the signals critical for integrin activation since: 1. the calcium flux is relatively slow (40–60; 55); 2. the response is not blocked by pertussis toxin (56; and see below); and 3. L-selectin binding to PNAd does not augment LFA-1 binding (17). Activation of leukocyte integrins has also been reported after binding to E-selectin (58, 59) or to CD31 antibody (60, 61).

Is triggering a necessary step in adhesion cascades? Three general kinds of evidence indicate that it is. The most compelling are experiments that identify a required triggering signal in a flow-based study. The ischemia model outlined above (13) provides a particularly good example by identifying IL-8 in granulocyte arrest. PAF likewise has been convincingly shown to contribute to triggering arrest: a PAF inhibitor decreases arrest without influencing rolling in both a cat ischemia reperfusion model (62) and superoxide-induced vessel injury (63). Models whose readout is recruitment into tissue, although suggestive, cannot distinguish triggering from effects on transmigration. For example, demonstration that anti-IL-8 mAbs inhibit granulocyte influx in rabbit endotoxin-induced pleurisy (64) reveals a critical role for IL-8 in the process as a whole, but does not establish it as a trigger for granulocyte arrest.

A second compelling kind of evidence comes from use of pertussis toxin to inhibit the function of the G-proteins on which the leukocyte serpentine receptors depend for much of their downstream signaling events. Extending earlier observations on pertussis toxin inhibition of lymphocyte homing (65), Bargatze et al (66) demonstrated that it inhibits lymphocyte arrest, but not rolling. Thus, a G-protein-mediated step must precede arrest.

The third line of evidence is that addition of exogenous triggering agents enhances arrest. For example, studies with PAF and LTB₄ *in vivo* demonstrate augmentation of neutrophil adherence and transmigration (7). *In vitro*, the addition of known triggering agents also increases the frequency of arrest.

The agents used, such as manganese or an integrin-activating antibody, bypass serpentine receptors and act directly on the integrin. Studies in the highly controlled system of lymphocytes that use $\alpha 4\beta 1$ or $\alpha 4\beta 7$ for both primary and secondary adhesion show that such exogenous activation dramatically augments arrest, with minimal effect on rolling (17, 21, 24, 67). Are there circumstances in which acute triggering is not required? Yes—arrest without acute triggering may occur for cells in circulation whose integrins are in the active state, rather than the normal inactive state. This may explain why cells cultured *in vitro* that are infused back into the host show atypical homing patterns. For example, cultured tumor-infiltrating lymphocytes or IL-2-activated lymphocytes have integrins in an active conformation and bind to endothelium *in vitro* without exogenous triggers (68, 69); although some cells do migrate into tumor when infused, the overall pattern is clearly disregulated (69, 70). Similarly, animal models of disease that involve infusion of cultured T cells (such as EAE) may include inappropriate access of cells to tissue compartments due to elimination of the need for a triggering step (and other alternations in their adhesion phenotype). In some experimental model systems, leukocyte arrest occurs with little or no rolling (20, 71). This may reflect either prior triggering or very efficient triggering on contact.

RETENTION OF CHEMOKINE AT THE ENDOTHELIAL SURFACE In order for soluble triggering factors to convey information rapidly during leukocyte-endothelial interactions, they must achieve a threshold local concentration. Most will be rapidly cleared by blood flow (72, 73), although theory predicts limited accumulation in a “concentration boundary layer” close to the vessel wall (74), which might be enhanced by endothelial glycocalyx (75). To circumvent this washout, several strategies may be used by ECs to concentrate factors at or near the endothelial surface. First, the heparin-binding capacity shared by all chemokines (76) may specifically retain them on the proteoglycan-rich glycocalyx of ECs (72). Second, recent IL-8 studies of Middleton et al (77) suggest that chemokine may be concentrated on endothelial protrusions, either in vesicles or at the plasma membrane. Third, the Duffy antigen receptor for chemokines (DARC) is strongly and very selectively expressed on postcapillary venules, the site of virtually all leukocyte emigration (78). DARC is a “promiscuous” chemokine receptor that binds many but not all chemokines; no signaling function has yet been demonstrated. Its functional importance on postcapillary venules has not been proven, but it has the potential to hold chemokines at the endothelial surface and thereby regulate their presentation to leukocytes (either positively or negatively). Furthermore, since transfection studies indicate that DARC can be endocytosed (79), it could play a role in transendothelial transport (described below).

CHEMOKINE RECEPTORS Functional chemokine receptors are selectively expressed on subsets of leukocytes (50). Accordingly, each chemokine acts on a restricted subset of cell types to induce chemotaxis, activation, or adhesion (46–48). Undoubtedly additional chemokine receptors will be identified, adding to the complexity of, and thereby the potential for, information throughput via these receptors. For example, the recently described BLR1-chemokine family receptor is expressed not only on B cells, but also on small specific subsets of CD4 and CD8 T cells (80). Thus, T cells may be as heterogeneous for chemokine receptor expression (and responsiveness) as they are for adhesion molecule expression (2). Both increase the combinatorial sorting capacity of the adhesion cascade.

Triggering during the adhesion cascade must occur on a second or sub-second time scale (66, 71). Although this is extremely rapid compared to most immunologic responses, this is routine for sensory responses. The serpentine receptor family is used for time-critical sensory functions, such as sight and neural transmission (50). When a chemokine binds to its serpentine receptor, it typically generates a calcium flux and phosphoinositide hydrolysis via G-protein coupling (50). The subsequent events must likewise be rapid but are not well understood. We suspect a major role for cytoskeleton given the importance of cytoskeleton to regulating integrin-mediated adhesion (15, 81), the sensitivity of cytoskeleton to calcium and phospholipid mediators (82), and the extreme rapidity of many cytoskeletal events (83, 84).

Issues in Secondary Adhesion

The concepts of secondary adhesion have remained relatively stable over the last several years, with ICAM-1, ICAM-2, and VCAM-1 as the predominant endothelial ligands for strong adhesion. A major solidification has come from the cloning of MAdCAM and demonstration that it is the primary ligand for $\alpha 4\beta 7$ (3, 6). This now substantially explains in molecular terms the preferential lymphocyte migration to the gut. One provocative feature of MAdCAM is its incorporation of both an integrin-ligand and a mucin-ligand for L-selectin (26) in a single molecule. Although it seems not to be a common strategy in the adhesion cascade, it is conceptually a very parsimonious one.

However, surprises probably await. The alternatively spliced form of fibronectin (CS-1) is found on the luminal aspect of vessels in rheumatoid joints and is a dominant ligand for VLA-4 *in vitro* (85). Neutrophils can bind to endothelium via a bridge of fibrinogen which in turn is bound to ICAM-1 (86). Likewise, pro-thymocytes show VLA-6-dependent homing to the thymus, which appears, surprisingly, to involve the VLA-6 integrin on the endothelial side of the interaction (10).

ENDOTHELIUM “REPORTS” INFORMATION FROM ITS ENVIRONMENT

Virtually all the information accessible to the passing leukocyte is managed by the EC. There is a fundamental dichotomy between two kinds of information. In one case, for soluble factors such as MIP-1 β or IL-8 from other cells, the endothelium can facilitate access of the tissue-derived factor to the luminal surface, perhaps retaining it there; we refer to this as “posting” (as occurs with endothelial retention described above). Alternatively, the endothelium can integrate incoming signals and “relay” that information by creating a new set of signals for the leukocyte, for example, by regulating synthesis of VCAM-1, E-selectin, or IL-8.

Regulation of expression of adhesion molecules on endothelium has been frequently reviewed (3, 4, 6, 10, 11). Based on the foregoing discussion, we believe that chemokine production by ECs is likewise an important element in the adhesion cascade, and therefore we review the principles and specifics of endothelial production of chemokines. Marked similarities between the rules governing expression of chemokines and adhesion molecules suggest that their regulation has coevolved to optimize the binding of leukocytes.

Chemokine Production by Endothelium

Among the more than 25 chemokines described, at least five of these are expressed by ECs *in vivo*. MCP-1 and IL-8 appear to be the most “promiscuous” EC-expressed chemokines and have been described extensively in both models, *in vivo* and *in vitro* (46, 49). Both chemokines are constitutively expressed in cultured endothelium (87, 88) as well as in apparently normal endothelium in certain tissues *in vivo* (89–91). Failure to detect IL-8 message in normal heart and brain endothelium may reflect tissue-specific differences in IL-8 transcription in resting endothelium, or simply differences in assay sensitivity (92, 93). It is no more surprising that IL-8 and MCP-1 are transcribed at a basal level in some tissue endothelium, than that ICAM-1 is as well. Their low basal levels may contribute to low frequency T cell binding during recirculation and/or may facilitate rapid induction in inflammatory conditions. They are strongly upregulated by various stimuli [(46), and see below].

In addition to IL-8 and MCP-1, two chemokines have been demonstrated to be produced by endothelium *in vivo* (IP-10 and RANTES), and three are strong candidates (gro- α /MGSA, Mig, and ENA-78). IP-10 mRNA expression is induced in liver and kidney after intravenous administration of IFN- γ , particularly in the microvasculature (94). RANTES was found to be expressed in ECs surrounding granulomas during DTH reactions in lymph nodes (95).

Gro- α expression has been described in cultured endothelium where it can be induced by a variety of stimuli (96–98). Mig was found recently to be expressed in cultured EC where its regulation was most similar to that of IP-10 (88). Finally, ENA-78 was found at the surface of ECs in synovial tissue (99); it should be noted that finding chemokines localized around endothelium does not necessarily indicate production of the chemokine by that endothelium (100, and see below). Taken together, these findings indicate that ECs can be induced to express members of both the CC- (RANTES, MCP-1) and the CXC-subfamilies (IL-8, Gro- α , IP-10, Mig) of chemokines.

Chemokine Inducers

Many of the agents that induce chemokine expression in EC also induce adhesion molecules relevant to the adhesion cascade. This is consistent with a concept of coordinated display of all required elements for information transfer to the leukocyte. Among the strongest stimuli are the classical inflammatory cytokines TNF- α , IL-1, and IFN- γ (46). Many other soluble mediators can do so also. For example, growth factors such as monocyte-colony-stimulating factor (101) and immune-derived cytokines such as IL-4 and IL-10 (102, 103) induce MCP-1 expression. Likewise, histamine is a potent inducer of IL-8 (87), consistent with a critical role for mast cells (see below). Finally, thrombin can very rapidly induce MCP-1 expression (104), linking chemokine induction to the coagulation cascade.

ECs produce chemokines in response to other important classes of stimuli that are rather different in character from the host protein mediators described above. Microbial products, ischemia, and shear influence chemokine as well as adhesion molecule expression by endothelium. Endotoxin (LPS) from many Gram-negative bacteria is a strong stimulus for chemokine production (46). In addition, capsular polysaccharide from the Gram-positive *Staphylococcus aureus* and outer surface proteins from the spirochete *Borrelia burgdorferi* act as inducers as well (105; and K Ebnet, MM Simon, S Shaw, manuscript in preparation).

Ischemia acts as a potent inducer of MCP-1 and IL-8 production by EC, just as it induces adhesion ligands (92, 93, 106, 107). Such activation of the adhesion cascade by ischemia presumably evolved to deal with wounds but often creates damage in ischemia-reperfusion injuries such as myocardial infarction (108). Finally, mechanical forces such as shear stress generated by blood flow induce MCP-1 mRNA expression in ECs (109), and shear stress-responsive *cis*-acting elements have been identified in the promoters of shear-regulated genes in EC (110). The local expression of chemokines induced by turbulent flow in the vascular tree might therefore contribute to the generation of atherosclerotic lesions (108).

Selectivity in Chemokine Production

There is redundancy of chemokine production by ECs in two respects: Multiple agents induce the same chemokine (e.g. IL-1, LPS, and ischemia all induce IL-8); and a single agent induces multiple chemokines (e.g. TNF induces IL-8, MCP-1, RANTES, and others). However, this does not mean absence of specificity; redundancy in information systems serves to maximize error-free information transmission, and it is often characteristic of biologic cytokine networks (111). Superimposed on this redundancy are elements of specificity, some of which are obvious and some more subtle. The most obvious example of specificity is that endothelium produces only a limited subset of the known chemokines; for example, we know of no evidence for MIP-1 α or MIP-1 β production by endothelium. Another clear example, as noted above, is that upon intravenous administration of IFN- γ in mice, there is striking preferential induction of IP-10 message in liver and kidney microvascular endothelium; expression on endothelium at other sites is weak or non-existent (94). Thus, in the intact organism, chemokine production by endothelium can be highly specific.

For the most part we do not understand the tissue-derived regulatory elements that confer this kind of specificity *in vivo*. However, studies *in vitro* provide some clues. First, we find partial selectivity when looking systematically at patterns of induction of chemokines on cultured endothelium by classic inflammatory mediators. For example, IP-10 and Mig are better induced than MCP-1 by high doses of IFN- γ , while the converse is true for induction by IL-1 (88). Second, additivity/synergy is often seen with the proinflammatory cytokines; endothelial cultures give strong chemokine responses to mixes of TNF- α , IL-1 β , and IFN- γ , which when given individually induce minimal response (88, 112). Third, responses to the strong primary inducers such as TNF- α , IL-1 β , IFN- γ , or LPS are regulated by other soluble factors. IL-4 and IL-13 partially inhibit the TNF- α /IFN- γ -induced RANTES expression in human umbilical vein endothelial cells (HUVEC) (112). IL-4, however, has also been described as amplifying the IL-1- or LPS-induced production of MCP-1 or IL-8 in HUVEC (113), indicating that the same cytokine can have opposite effects on different chemokines. IL-3 acts cooperatively with TNF- α in the induction of IL-8 and E-selectin expression (114). Finally histamine potentiates IL-8 production induced by TNF- α (87), suggesting that mast cells enhance leukocyte infiltration via chemokine induction.

“Relay” chemokine production by the endothelium itself has theoretical advantages over “posting” of chemokines made by other cells, notably amplification and integration. The foregoing paragraph illustrates the principle of integration of multiple signals. Amplification can be a powerful feature of the relay when the inducing factors are present at low concentration (e.g. early

in the response). For example, less than 1U/ml of TNF- α , IL-1 β , and IFN- γ is sufficient to induce more than half-maximal response of IL-8 in cultured endothelium; the lag time in this process is quite modest, since peak levels of IL-8 message are achieved within an hour (88).

HOW INFORMATION REACHES THE ENDOTHELIUM

Short-Range vs Long-Range Information Transfer

Whether the endothelium uses “posting” or “relay” mechanisms to display proadhesive molecules on its luminal surface, there remains the fundamental problem of how the signal is transported from its source to the vessel. Passive diffusion is of primary value in situations where vessels are in extremely close proximity to the source of the activating signal. Postcapillary venules are within a few cell diameters of various tissue cells including pericytes, stromal cells, and mast cells. Rapid intercellular communication between these cells is illustrated by mast cells; trauma-induced degranulation causes release of small, highly diffusible mediators, including histamine and PAF, that quickly and dramatically initiate endothelial changes (115). Furthermore, inflammatory cells often accumulate in the perivascular region; cytokines generated within these infiltrates have relatively easy access to the adjacent endothelium. Indeed, the extreme example of short-range signaling is that leukocyte membrane-bound IL-1 can induce endothelial IL-8 production via juxtacrine mechanism (116).

In other situations, the signal may need to traverse substantial distances to reach its target vessel. It apparently can do so rapidly: Subcutaneous injection of IL-8 in rats causes a detectable increase in transendothelial migration of lymphocytes in draining lymph nodes within three minutes, peaking at three times normal levels in half an hour (117). This implies extremely rapid transport to lymph node vessels via multiple steps: from inoculation site to dermal lymphatics, to lymph node subcapsular sinus, to paracortical postcapillary venules (see below). Omnidirectional passive diffusion of soluble factors is dissatisfying as an explanation for long-range information transport on several grounds: It is slow, it dilutes the concentration signal exponentially in proportion to the radius of diffusion, and it may be impeded by physical barriers and biochemical interactions within tissues (118). The rules for movement of molecules through tissue are complex and only partially understood (119, and references therein). But as an example, passive diffusion of the growth factor activin appears to occur at an estimated speed of around 55 μm per hour (120). In simplified model systems in which a factor such as bFGF is migrating through agarose (with which it has charge interactions), diffusion occurs at about 70 μm per hour. Carrier molecules may increase the speed and radius of diffusion: bFGF bound

to heparin diffuses roughly four times more rapidly through agarose, about 3 mm in 10 h (121). Even so, the speed of passive diffusion is insufficient to account for the rapidity of the lymphocyte recruitment into lymph node described above. Rather, there is evidence for anatomic structures that transport material through tissues and to vessels (122). Of special importance is the concept of fiber bundles in facilitating such transport, which will be illustrated in the lymph node and extended to other tissues.

The Lymph Node Reticular Network

Several studies indicate that soluble tracers arriving via afferent lymphatics penetrate the lymph node parenchyma in a defined and organized pattern (illustrated in Figure 1A). The peroxidase tracer arrives at the subcapsular sinus (S), from where it would be expected to diffuse into the upper cortex (C), producing a smooth concentration gradient. Instead, one observes a linear, web-like pattern of accumulation (F) (123). There is prominent accumulation of tracer around and even in the lumen of the high endothelial venule (HEV). This is of particular importance because HEV are the postcapillary venules specialized for the high volume recirculation of lymphocytes into the node. The formation of this pattern reflects a rapid transport mechanism since the micrograph shows tissue fixed 1 min after the tracer entered the sinus. These results were confirmed by studies of Sainte-Marie et al, using FITC-BSA injected into skin of rats (124). Although the route of administration was slightly different—the tracer had to reach lymphatics in the skin before entering the afferent lymphatic—the pattern and sequence of lymph node staining was identical: Tracer entered the subcapsular sinus, streaked downward into the cortex, and reached the HEV. These staining patterns correspond anatomically to the reticular network of the lymph node.

The reticular network has long been thought to be primarily a structural component of the lymph node. It is a network of collagen fibers that extends from the subcapsular sinus region into the deeper cortex and medullary regions. An unusual feature of these fibers is that they are ensheathed throughout most of their course by fibroblastic reticular cells (FRC, Figure 1B) (125, 126). As a result, the fibers comprise an extracellular space, effectively insulated from the surrounding lymph node parenchyma that contains lymphocytes and antigen presenting cells. Based on the unique ultrastructural anatomy of the reticular network, Moe proposed in 1963, that “it seems reasonable to hypothesize that the entire reticular interstitium may provide pathways for movement . . . for secretory products” (126). Many of these fibers end by insertion onto the outer surface of the HEV, forming a lace-like sleeve around the vessel (127). Thus, the reticular network is anatomically positioned for the transport of soluble information from the afferent lymph to the HEV. In support of this concept,

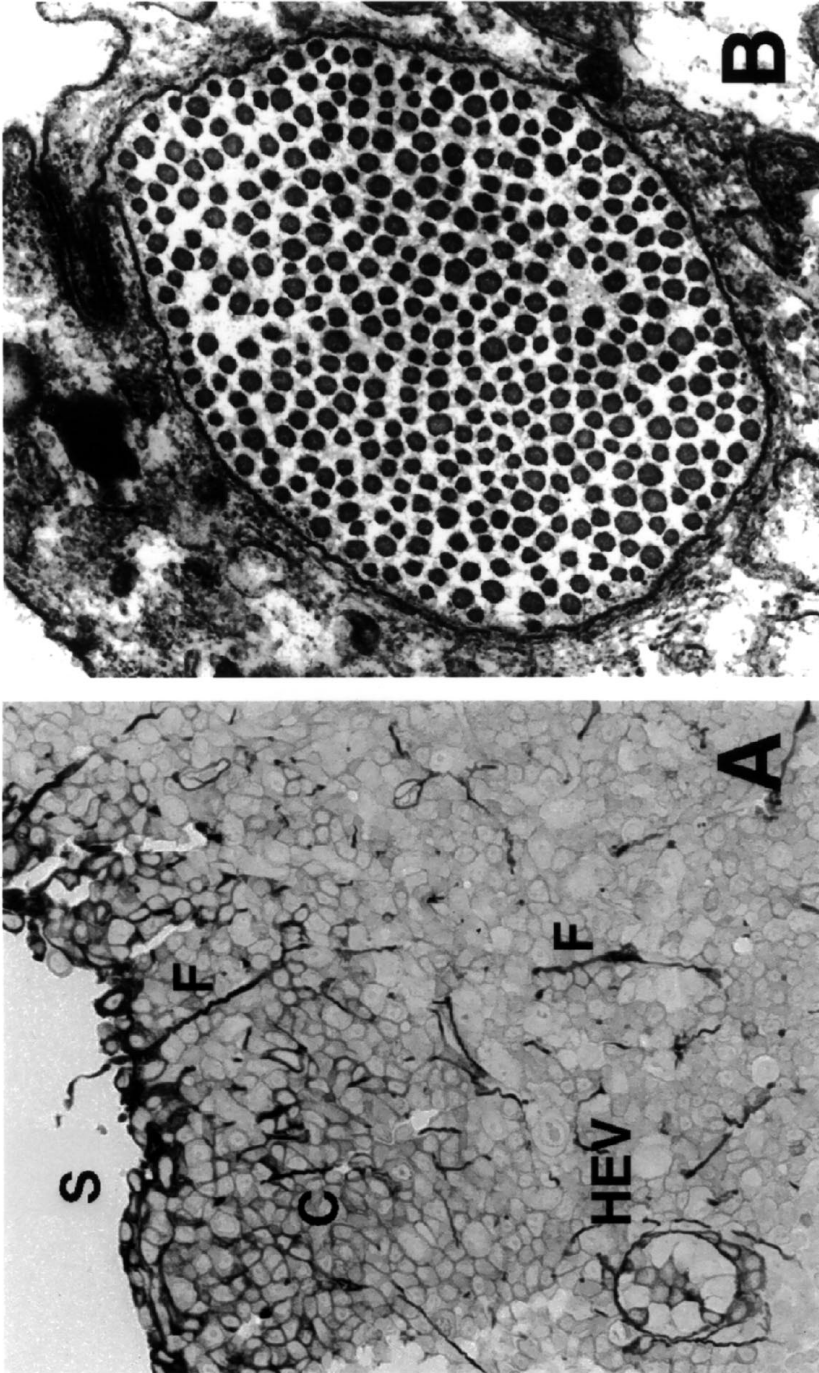


Figure 1 Reticular network of the rat lymph node. A. Horseradish peroxidase tracer movement with the reticular network. Tracer was injected into afferent lymphatic. Floor of subcapsular sinus (S), reticular fibers (F), and outer sheath of high endothelial venule (HEV) are stained. Cortex (C) containing lymphocytes is minimally stained. B. Sheathing of fiber. Electron micrograph of reticular fiber cross-section showing collagen fibrils enclosed by cytoplasmic processes that are joined at the top of the figure by a junctional complex. Adapted from (75, 123).

immunohistochemical studies of reactive lymph node have localized MIP-1 β to the reticular network (EP Kaldjian, ES Jaffe, S Shaw, manuscript in preparation). Although details of the transport mechanism are incomplete, three previously discussed features may contribute (75): solute transport into the reticular network via pinocytosis of lymph by sinus-lining cells; bulk fluid flow along the reticular network facilitated by fluid from fenestrated capillaries within the network; directionality of flow determined by enclosure of the reticular network by its sheath at all but defined locations, particularly HEV.

Fibers as Low-Resistance Pathways in Other Tissues

Fibers also appear to play a role in information transport in other tissues as well. In skin, the anatomic arrangement of elastic fibers and limited experimental data support this concept. The dermal lymphatics are associated with elastic fibers, which radiate perpendicularly from the lymphatic into the surrounding dermis. These fibers are continuous with a network of finer fibers that extend into the upper dermis and between keratinocytes into the epidermis (128). Colloidal carbon particles injected into the dermis localize preferentially to the elastic fibers as they migrate toward initial lymphatics (118, and references therein). Transport along fibers has also been observed in the rabbit mesentery, where low molecular weight fluorescent dyes injected into the mesenteric terminal vascular system move relatively rapidly out from the vessel and along connective tissue fibers in a pattern similar to the reticular pattern of elastic fibers within the tissue (122). Consequently, elastic fibers have been termed a "low resistance" pathway for fluid transport, in contrast to the much slower seepage through surrounding matrix. Sheathed reticular fibers can be found in other lymphoid organs, including spleen, tonsil, and Peyer's patch of the gut (129). Unsheathed fibers are present in many tissues, including liver sinusoids, neural adventitia, smooth muscle, and tendon. Their role in transport has not yet been evaluated.

Issues in Transendothelial Transport

How do soluble signals such as chemokines gain access to the luminal surface after movement through surrounding tissue? There are two possibilities and evidence for both. The first is endothelial transport of factors from the abluminal to the luminal side. Specific uptake and subsequent release of fMLP from cultured ECs suggest this could be a mechanism for its transport (130). Intracutaneous injection of IL-8 results in appearance of large endothelial vesicles whose morphology and kinetics suggested IL-8 transport into the lumen (131). Recently, Rot and colleagues have used immunoelectron microscopy and electron microscopic autoradiography to directly identify IL-8 within such vesicles (77). Time course studies demonstrated progressive accumulation of IL-8 at the luminal surface, with enrichment on the tips of endothelial protrusions induced

by the IL-8 administration. Thus, endothelial transport of triggering factors to the lumen via an endocytotic mechanism is strongly suggested. Alternatively, soluble factors can move between ECs and into the lumen (36), at least in HEV as shown in Figure 1A. Maintenance of the integrity of the vascular permeability barrier is accomplished by the overlapping of adjacent ECs that effectively form a flap valve (75). Regulated movement of fluid and cells across this specialized region is possible because the adjoining endothelial surfaces are held in apposition by discontinuous “spot welds” rather than the occluding junctions characteristic of most endothelia.

CONCLUDING REMARKS

Coordinated flow of information is pivotal to efficient and specific interactions of circulating cells with specialized endothelium: the transport of signals along fiber bundles to vessels, the relay or posting of signals at the endothelial surface, and the ultimate reading of the luminal display by the leukocyte. The adhesion cascade model has transformed our understanding of the binding itself. Much remains to be elucidated regarding the flow of information that dictates the specificity of that binding.

ACKNOWLEDGMENTS

We thank colleagues for generous contribution of pre-publication information and M. Bartlett, E. Gretz, P. Henkart, and E. Jaffe for constructive comments.

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