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T cell adhesion to endothelium: the FRC conduit system and other anatomic and molecular features which facilitate the adhesion cascade in lymph node

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Since T cell surveillance depends on movement from blood into tissue and back again, rapid, efficient and selective T cell adhesion to vascular endothelium is essential. This adhesion involves a multistep cascade clarified by a recent consensus model: (1) initial tethering by selectin-mediated interactions; (2) triggering of adhesive function of T cell integrins by ligands at or near the endothelial surface; and (3) strong adhesion mediated by T cell integrins. We recapitulate this model, particularly as it pertains to the lymph node, and explore additional molecular and anatomic elements which contribute to the effectiveness of the adhesion cascades at that site: (1) importance of cytokines/soluble mediators as triggering ligands; (2) role of glycocalyx and proteoglycans on high endothelial venule (HEV) endothelium in capturing and presenting triggering cytokines; (3) remarkable function of what we designate the 'fibroblastic reticular cell (FRC) conduit system' in rapidly transporting cytokines to the HEV; (4) importance of the unique anatomy of the flap-valve junctions between HEV endothelium in enabling intravasation of cytokines and transmigration of lymphocytes. Taken together, these molecular mechanisms and these three anatomic features of lymph node facilitate extremely efficient lymphocyte traffic to this site critical for T cell-mediated immune responses. Analogous mechanisms contribute to T cell interaction with endothelium at other sites.

Key words: T lymphocyte / lymph node / endothelium / adhesion / cytokine / fibroblastic reticular cell

LEUKOCYTE MIGRATION from circulation into tissue is rapid, selective and efficient. It is rapid in two senses: first that recruitment can begin within seconds or minutes of the tissue insult; and second that the leukocyte decision to migrate into a particular tissue occurs in a second or two. It is selective both in an anatomic and a pathologic sense; particular subsets of leukocytes bind preferentially to endothelium (and transmigrate) at particular anatomic sites and under particular inflammatory conditions. Finally, it is efficient despite the constraints of rapidity and selectivity; leukocytes can bind the appropriate endothelia with efficiencies of at least 20% and perhaps approaching 100% of the relevant subset.[1] This remarkable triad has been achieved during evolution by a sophisticated set of molecular and anatomic strategies. Our brief commentary first reviews the molecular strategies of an adhesion cascade, whose importance is increasingly appreciated. It then develops concepts which are evolving regarding anatomic strategies which facilitate the cascade of T cell interaction with endothelium. Because of limitations in space we have depended heavily on citations to reviews to enable the reader to find supporting literature; furthermore, we have had to truncate some complicated discussions into simpler versions.

We believe that the concept of an adhesion cascade pertains to the interaction of every T cell with every kind of endothelium. However, the exact ensemble of molecules involved will necessarily differ, since the specificity of interaction of particular leukocyte subsets with endothelium in particular sites requires differences in details of molecular usage.[2,3] For the purposes of this review, we choose to emphasize the interactions of T lymphocytes with endothelium in LN. Nevertheless, the conclusions drawn are generally relevant to interactions of T cells with endothelium at other sites and we comment briefly on such parallels.

The rationale for choice of the lymph node (LN) for emphasis in this review is threefold. First, LNs (and other secondary lymphoid tissue, including Peyer's patches and organized lymphoid infiltrates) are the sites of the highest frequency T lymphocyte binding to endothelium (and transmigration) in the

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normal mammalian host.[4-6] Second, it is clear that binding/migration into LN is governed by processes distinct from those in non-lymphoid tissue, and that such differential recruitment is critical to normal immune function.[7-11] Specifically, naïve T cells are preferentially recruited in large numbers into LN. Antigen is brought to the LN in afferent lymph, presented on specialized antigen-presenting cells and stimulates the rare T cell clone able to recognize it. The specialized microenvironment in the LN cortex facilitates and regulates the activation of naïve cells and their differentiation into memory cells. Once they have become memory cells, they exit the LN and display the ensemble of molecules which enable them to efficiently bind to endothelium in non-lymphoid tissue such as skin. Third, it is our impression that concepts regarding T lymphocyte interaction with LN endothelium have not evolved as much recently as has understanding of other endothelial interactions. By integrating insights from past *in vivo* and anatomic studies[12-17] with current molecular paradigms, we formulate a more comprehensive model which we hope will facilitate progress in this area.

The distinctive anatomic features in LN are emphasized later in this review. However, at this point it needs to be mentioned that there is a specialized type of endothelium present in LNs which is the site of entry of most lymphocytes.[18-20] The specialized post capillary venules (PCV) in which they are found are called high endothelial venules (HEV) and the endothelial cells are called HEV endothelia. The term 'high' refers to their distinctive plump shape (with luminal surface high above the basal surface) contrasted to the flat morphology of most endothelium. Even in a 'resting' LN, there is an extraordinarily high rate of entry of lymphocytes (relative to non-lymphoid tissue); moreover, when it is 'stimulated', the rate of entry may increase 5- to 10-fold.[19,21-23] This high entry rate, does not reflect an unusual degree of vascularity or exceptionally high blood flow. Rather, it reflects special endothelium and a microanatomy to facilitate recruitment by that endothelium.

Overview of consensus model of adhesion cascade

Over the last several years, a 'consensus model' of leukocyte adhesion to endothelium has evolved from creative studies in a number of laboratories on diverse leukocyte types: granulocytes, lymphocytes and platelets. This model proposes a three step regulated cascade and is supported by strong data from varied approaches. Consequently, we believe that any given leukocyte interaction with endothelium would be expected to occur by such a three step process unless proven otherwise. The brief discussion of this model here should be supplemented by the many excellent recent reviews on this topic.[2,3,24,25]

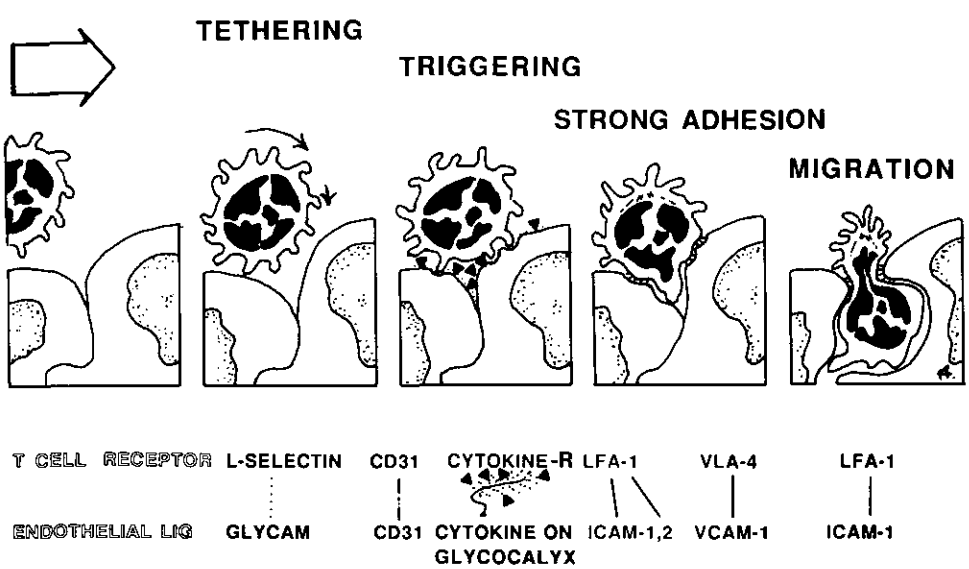

Figure 1. Consensus model of T cell adhesion cascade for binding to LN high endothelial venules. See details in text. Note the flap valve overlap of the HEV endothelial cells.

Our version of the consensus model, as it relates to T cell binding to HEV endothelia, is illustrated in Figure 1. We call the first step tethering, referring to the establishment of a loose and somewhat transient adhesive interaction between lymphocyte and endothelium. This may result in either lymphocyte rolling on the endothelium[26] or bouncing along with brief contacts of a second or less.[1] The selectin family of molecules participates in tethering for all leukocytes.[27-29] The importance of this tethering is to provide time and cell contact which is essential for the second step, namely triggering. During the second step, the lymphocyte responds to ligands on the endothelial surface or in close proximity in the lumen. When one or more ligands acts on complementary receptor(s) on the lymphocyte, then rapid activation of adhesivity occurs; we believe this must be a rapid process, requiring less than a second for onset. We suspect that a wide variety of lymphocyte surface receptors will be involved, including members of the 7-pass G-protein linked family and the Ig superfamily.[30,31] The objective of this 'triggering' is to turn on the adhesive function of integrins,[32-34] which are critical to the third step, namely strong adhesion. Although circulating lymphocytes express substantial amounts of a variety of integrin adhesion receptors, those receptors are NOT normally functionally competent. However, they can be rapidly 'turned on' to a functionally competent state by particular activation stimuli provided in the preceding step of the cascade. Once turned on, the integrins are very efficient in mediating the strong adhesion required to arrest a rolling lymphocyte.

As noted above, leukocyte binding to endothelium is rapid, selective and efficient. The rapidity reflects the speed of each of the individual steps in the cascade. The efficiency reflects the remarkable co-ordination of the specialized 'skills' of the collaborating molecules. The selectins are excellent in initiating adhesion during flow but cannot mediate sufficient adhesion for arrest; the integrins have the opposite skills, so together they work efficiently.[24] The selectivity reflects the combinatorial use of the unique ensembles of receptors/ligands in the three steps.[2,3,30]

Tethering

The distinctive biophysics of rolling in flow must be mediated by a special class of complementary receptor/counterreceptor on lymphocyte and endothelium reviewed by Rosen in this issue[27] and by others.[28,29] In the case of T lymphocyte binding to HEV, the major T lymphocyte receptor is L-selectin and the major HEV ligands are distinctive carbohydrate moieties on glycam and probably other proteins expressed on HEV.[10,27,35] The L-selectin/glycam interaction has all the required characteristics for a tether.

(1) It mediates activation-independent adhesion. In contrast to the integrins discussed below, this adhesion must occur on unperturbed lymphocytes in circulation. That this is the case is demonstrated by Stamper-Woodruff type assays of lymphocyte binding to HEV on frozen sections of LN. This interaction is inhibited by: antibodies against L-selectin on lymphocytes; by antibodies against the distinctive carbohydrate on glycam and by soluble L-selectin molecules.[10,27,36]
(2) The molecules must be physically available for interaction on initial cell contact. This means that the binding domains of receptor and counterreceptor must be at the outer fringe of the glycocalyces of the lymphocyte and endothelium, implying that they are long and extended molecules. The selectins are inferred to be extended, as is glycam by virtue of its extensive O-linked glycosylation.[35] It is of special note that L-selectin is preferentially expressed on the tips of microvilli in granulocytes,[37] since the initial interaction of the lymphocyte is understood to be also via its microvilli.[38-40]
(3) The molecular interaction must have unique reaction properties to facilitate rapid initiation during flow and subsequent shear-dependent release to allow rolling.[41]

Triggering

There is much less certainty about the molecules involved in the triggering step in LN, than about the tethering step. Therefore, we outline the principles and make working hypotheses about the details. Many aspects of adhesion regulation are covered in the accompanying review by Mobley *et al.*[34] From *in vitro* studies, it is apparent that a substantial number of receptors on the surface of resting T cells can turn on integrin adhesive function.[30,31,42] We believe these effects are biologically relevant and that this diversity reflects the pivotal importance of

adhesion regulation in many aspects of T cell function. The question then becomes, which of these are involved in T cell interaction with HEV? We single out two which we think are most likely and which illustrate distinct structural families and issues: CD31 and the MIP-1β receptor.

CD31 is an integral membrane protein, composed of six extracellular Ig domains, which belongs in the family of adhesion-triggering receptors on T cells.[43] Several aspects of CD31 distinguish it from other adhesion triggers on T cells and make it our leading candidate for an Ig family molecule involved as a trigger in T cell binding to HEV. First, other known triggers require high order cross-linking whereas CD31 induction of adhesion requires only bivalent Ig. This suggests to us that CD31 is poised to act very early in cell contact whereas other molecules contribute when more extensive contact and mutual co-capping has occurred.[44] Second, CD31 preferentially activates VLA-4 adhesion compared with LFA-1 adhesion, which fits with the concept of a pre-eminent role of VLA-4 in T cell binding to endothelium.[43] Third, CD31 has a unique distribution on subsets of T cells, with more frequent expression on CD8 cells and naïve cells. The preferential expression on naïve cells fits with preferential migration of naïve cells into LN. Fourth, CD31 has a structure consistent with a long molecule which could extend to the tip of the glycocalyx and therefore be involved early in a sequence of interactions between T cell and endothelium. Fifth, CD31 is widely expressed on endothelium and has been inferred to be involved in leukocyte interactions with endothelium;[45] indeed the possibility of CD31 on T cells interacting with CD31 on endothelium is consistent with the concept that CD31 participates in homophilic as well as heterophilic interactions.[46]

Our other strong candidate for the triggering step during T cell binding to HEV is the receptor for MIP-1β (interacting with its cytokine ligand MIP-1β).[47] By screening a fairly broad panel of cytokines, we identified the cytokine MIP-1β as an inducer of T cell adhesion via VLA-4 to VCAM-1.[48] This cytokine is a member of the β subfamily of the recently identified and growing chemokine family. Many members of this family,[49,50] including its best known member IL8, are able to induce chemotaxis *in vitro* and leukocyte recruitment *in vivo*; although the specificity of action is often complex, the actions are usually selective for some leukocyte subsets but not others. Although the T cell receptor for MIP-1β is not known, by analogy to receptors for other chemokine family members it is likely to be a seven pass G-protein linked receptor.

For strong theoretical reasons, we originally disliked the idea of soluble mediators participating directly in the adhesion cascade.[47] We overcame these conceptual problems by introducing the concept that cytokines such as MIP-1β are retained/presented on the endothelial surface. Antal Rot[51] has independently developed this concept for IL8. IL8, MIP-1β and other chemokines have a prototypic sequence for binding to glycosaminoglycans, and several members have been shown to bind to proteoglycan. Proteoglycans are present on the luminal surface of endothelium and are known to facilitate localization of other proteins such as protease inhibitors at the vessel wall.[47,52] We believe that endothelial proteoglycans bind MIP-1β or IL8 and retain it for 'presentation' to passing leukocytes. We have been able to simulate this situation *in vitro*[47] and Rot has shown selective binding of chemokine to endothelial surface *in vitro*.[51]

Of particular importance for this discussion, immunohistologic analysis demonstrates that MIP-1β is present in and immediately around the HEV in reactive LN and tonsil.[48] Thus, MIP-1β is present in the right place and has the right 'pro-adhesive' function to contribute to triggering of T cell adhesion. Although MIP-1β is the only cytokine we have yet identified with such striking effects on T cell adhesion, we expect that many more cytokines will prove to have selective pro-adhesive effects. Later in this review, we describe features of LN architecture which we think are designed to facilitate delivery of soluble factors to HEV and their presentation by HEV.

Strong adhesion

Integrins are a ubiquitous family of adhesion molecules which are involved in virtually all strong lymphocyte adhesion; their characteristics and regulation are explored in detail in the accompanying article by Mobley *et al.*[34] Of the six known integrin chain pairs on resting T lymphocytes, only VLA-4 ($\alpha 4\beta 1$) and LFA-1 ($\alpha L\beta 2$) have been demonstrated to participate in T cell interaction with endothelium; however, others including LPAM-1 ($\alpha 4\beta 7$) may also do so. Antibody inhibition studies of *in vivo* migration to LN and *in vitro* binding to HEV document contributions of both LFA-1 and VLA-4.[53-55] The

known endothelial ligands for VLA-4 are the inflammation-induced molecule VCAM-1[56] and potentially CS-1.[57] The endothelial ligands for LFA-1 are the basally expressed ICAM-2 and the inflammation-induced ICAM-1.[10,58,59]

Combinatorial specificity of T cell adhesion to endothelium

Whether or not a given T cell will bind efficiently to a given HEV will depend on the T cell having an ensemble of receptors complementary to the ligands displayed on the endothelial cell. This results in a complex combinatorial determination of specificity by multiple molecules expressed both on the HEV endothelium and the T cell.[2,3] Given the number of molecules involved, and the complex regulation of their expression, it is impossible to review this in detail. We emphasize several conceptual issues here.

First, the expression of the receptors on T cells is regulated during T cell differentiation and therefore different T cell subsets express different ensembles of receptors. Both L-selectin and CD31 are preferentially expressed on naïve cells.[43,60] Although we expect that these will contribute to the movement of naïve cells into LN, details of expression of CD31 and L-selectin illustrate that the rules are not simple. For example, L-selectin is expressed not only on all naïve cells but also a subset of memory cells. Indeed, it is expressed on granulocytes, which do not migrate through HEV. How can specificity be achieved when such widely expressed molecules are involved? In large part, the specificity seems to be achieved by the distinct combinations of such molecules on specific subsets. In addition, there are probably structural variations which are not yet fully appreciated; although L-selectin is not selectively expressed on T cells, differences in L-selectin structure between T cells and granulocytes[37] suggest that the T cell form is structurally/functionally unique.

Even low levels of expression of T cell receptors are likely important. MIP-1β stimulates adhesion even though there are less than 1000 MIP-β receptors/cell on resting T cells (D. Taub, personal communication). Although VLA-4 and LFA-1 are preferentially expressed on memory cells, they are also expressed on naïve cells and mediate endothelial binding by them.[9,59]

Second, the ligands on the endothelial cell have carefully regulated expression, depending both on anatomic site and on inflammatory stimuli. This is illustrated particularly well by glycam. Glycam and its unique carbohydrate are expressed almost exclusively on LN HEV.[35] Furthermore, the increased influx of lymphocytes into a reactive LN is probably enhanced by increased expression of glycam (or other sulfated L-selectin ligands), since reactive LN show increased sulfate incorporation.[27,61] However, other endothelial ligands, such as VCAM-1 and pro-adhesive cytokines, have much more complex distributions. The VLA-4 ligand VCAM-1 is best known as an inflammation-induced ligand on diverse endothelium but can also be present on HEV. Although VCAM-1 has been detected on some resting HEV (A. Ager, personal communication), in other situations it appears to be induced only in reactive LNs.[62,63] This fits with the expectation that HEV in a reactive LN will be bathed in pro-inflammatory cytokines (see below) and respond by increased VCAM-1 expression, as well as increased glycocalyx[64] and presumably increased ICAM-1.[63] In addition, the cytokines will 'decorate' endothelium. MIP-1β (and MIP-1α) is found at endothelium not only in secondary lymphoid tissue, but also in other tissues both on vascular endothelium and other cell types in complex patterns (S. Hubscher, D. Adams, personal communication).

Both T cells and endothelial cells are lineages of cells within which there is great specialization. Particular lymphocytes interact with particular endothelial cells only when they each have the right combination of receptors and ligands. Currently we do not know enough to account for the entire specificity—partly because of the complex rules regulating expression of these molecules, and partly because there are molecules, structural complexities and regulatory interactions which we do not yet understand.

Anatomic strategies which facilitate the adhesion cascade

The adhesion cascade described above is very efficient both in LN and in inflamed non-lymphoid tissue. What accounts for its extraordinary efficiency in recruiting lymphocytes to LN? Although part of it will probably be due to ligands expressed uniquely in LN, part of it is due to the unique anatomy which facilitates the function of the cascade. As described above, we expect cytokines/soluble factors to be very important in recruitment. Indeed, maintenance of

HEV activity requires continuous supply of afferent lymph.[65] We single out three remarkable anatomic features in LN which we propose maximize the role in lymphocyte recruitment of cytokines from afferent lymph: a transport system to bring soluble factors to the base of the HEV, specialized junctions between HEV to allow factors to reach the lumen and a rich glycocalyx potentially able to bind and/or trap factors.

The fibroblastic reticular cell and its 'FRC conduit system' in cytokine transport to the HEV

We believe that there is a specialized transport system within the LN for rapid and efficient delivery of cytokines and other soluble molecules directly to the HEV; we refer to this transport system as the 'fibroblastic reticular cells (FRC) conduit system'. For convenience in the following discussion we will refer to cytokines but mean this to be taken far more generally and to include a range of soluble mediators including: lipids, neuropeptides, shed cell surface molecules, products of damaged cells and microbial byproducts. Cytokines have two major effects on lymphocyte recruitment. First, some cytokines induce marked changes in the endothelial cell; for example, cytokines such as IL1, TNF and IFNγ induce expression of ICAM-1 and VCAM-1 which are important ligands for T lymphocyte adhesion. Interleukins also induce endothelial cells to produce glycosaminoglycan-rich extracellular matrix.[64] Second, some cytokines released into HEV will directly affect T lymphocyte recruitment by inducing/promoting lymphocyte binding/transmigration. Cytokines like MIP-1β and IL8 (and possibly many more) will have their effectiveness magnified by immobilization on the luminal surface of endothelium.

FRC and the interconnected system of fibers associated with them have been widely recognized in many tissues.[14,15,66,67] The major function ascribed to the FRC has been structural, namely supporting the tissue, filtering the lymph and co-ordinating the dilation and permeability of lymphatics when tissue fluid pressure increases. A second function has been suggested in LN, namely transport of antigen from lymphatics to antigen presenting cells.[12,14,15,17,67] We propose a distinct role for the FRC conduit system in transport, namely bathing the HEV in cytokines which influence lymphocyte recruitment. As outlined below, we believe that this system transports cytokines to the HEV from afferent lymph and from specific cellular sources within LN. Our concept of the FRC conduit system is based on its microanatomical configuration and experimental evidence of directional molecular transfer along it.

There is strong functional evidence that there must be a rapid transport mechanism for transferring molecules from afferent lymph to HEV. Subcutaneous inoculation of cytokines into the lymphatic drainage bed results in augmented lymphocyte migration into the draining LN within 3 min; the maximum effect is observed in 30 min. This has been demonstrated for IL8,[68,69] RANTES, MIP-1β, LD-78 and ACT II (A.O. Anderson, unpublished observations). This rapid effect is virtually impossible to explain by conventional understanding of lymph flow and diffusion through tissue.

The details of rapid transport can be directly investigated by studies of non-particulate tracers such as horseradish peroxidase (HRPO), heparin or soluble dyes. When HRPO is injected into afferent lymphatics in the gut wall it rapidly progresses to the LN and within minutes is found bathing the HEV—beneath HEV, between adjacent HEV and leaking out into the HEV lumen (Figure 2). At that early time point, there is characteristic intense HRPO staining of the fibroblastic reticulum (described in detail below), while most of the interstitium is not stained.[12,38,70] This suggests that the HRPO underwent facilitated transport along the reticular fibers. Since the injections were made at low pressure in a small volume, the transport was not due to abnormal intralymphatic pressure.

What anatomic features enable such transport via the FRC conduit system? Simply stated, we envision the FRC conduit system as a meshwork of conduits which run from the site of entry of afferent lymph (the subcapsular sinus) to HEV. Some details of its structure are illustrated in Figures 3, 4.

(a) Each reticular fiber has a tubular structure suggestive of a channel for fluid flow (Figure 3): Each is composed of a parallel bundle of evenly spaced collagen fibrils ensheathed within the cytoplasmic processes of reticular cells. The integrity of the sheath is generally maintained by junctional complexes at borders where the FRC sheaths meet. In some sense, the strongest analogy to this sheath is that of a myelin sheath which electrically isolates the axon. The fibrils are held in parallel array by fine cross-bridges. The characteristic microstructure of this sheathed array is consistent with a functional role in rapid movement

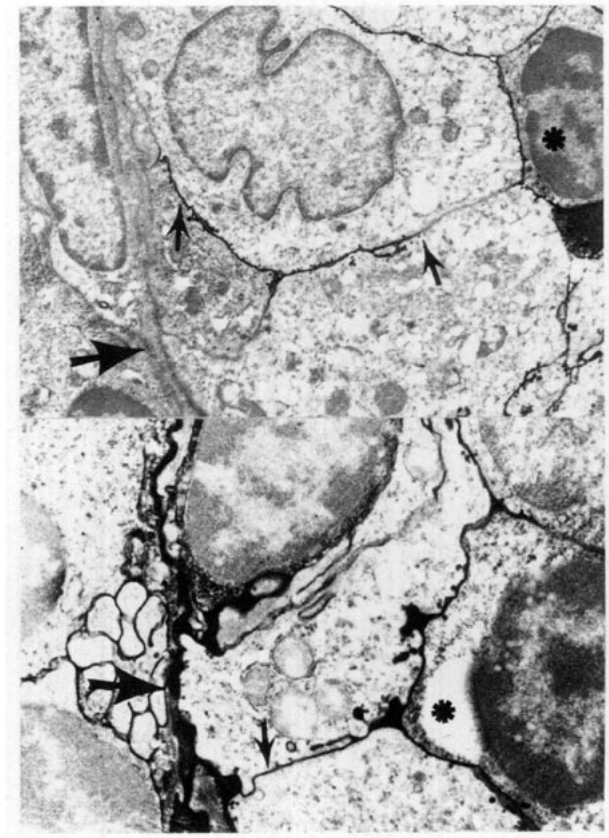

Figure 2. Contrast between distribution of extracellular tracer HRPO when injected intra-arterially (top) versus via lymphatics (bottom). Within the first minute of infusion, the tracer given intra-arterially penetrates from the lumen (asterisk) most of the way between HEV (small arrows) but fails to reach the abluminal surface and reticular cell sheath (large arrow). In contrast the intralymphatic tracer stains the reticular sheath (large arrow), the full course of the inter-HEV space (small arrow) and the lumen (asterisk).

of solute that occurs with minimal fluid volume and without dispersion due to fluid turbulence.

(b) Transport of molecules from afferent lymph to HEV would be most direct via a system which ran vertically within the cortical lobules of the LN from the floor of the subcapsular sinus (at the periphery of the LN) to HEV (which often lie deep within the lobule). This is the primary orientation of the fibers within the FRC conduit system (Figure 4).

(c) The only gaping 'hole' in the continuity of the sheath of the FRC conduit system is at the wall of the HEV. At that site, reticular fibers spread out to surround the abluminal wall of the HEV; there is no visible barrier to the movement of fluid out of the FRC conduit system into the (potential) space

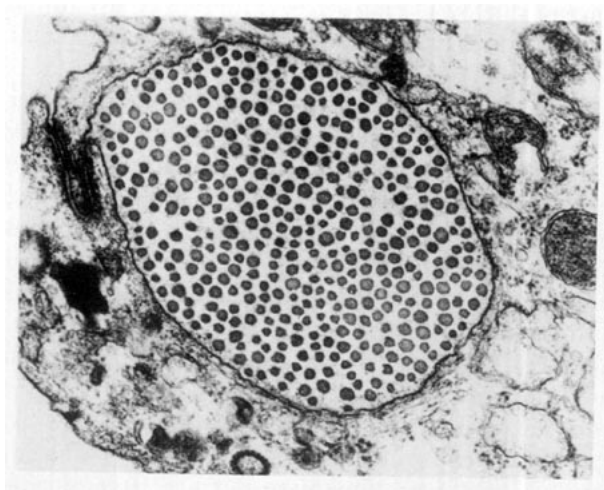

Figure 3. Cross section through reticular fiber showing characteristic bundle of fibrils sheathed in processes of the FRC. Note the junctional complexes at borders where the FRC sheaths meet to the left of the fiber in this photomicrograph.

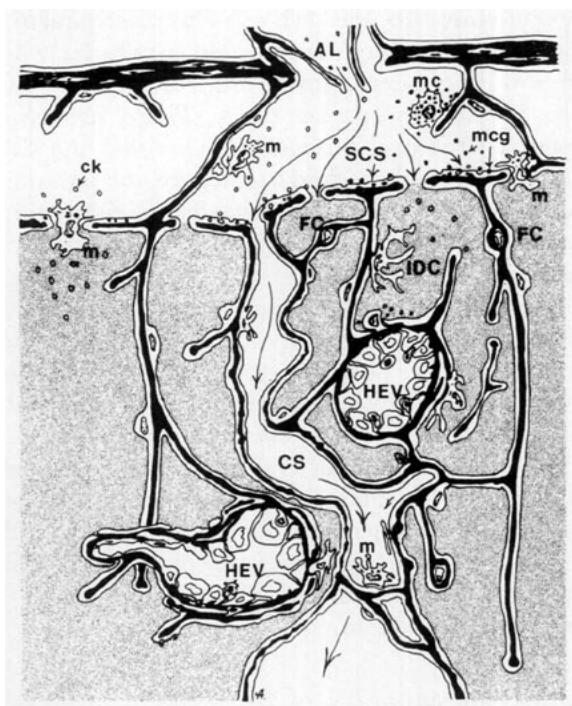

Figure 4. Schematic of the FRC conduit system in LN. Abbreviations are as follows: A, artery; AL, afferent lymph; ck, cytokine; CS, cortical sinus; FC, fenestrated capillary; HEV, high endothelial venule; IDC, interdigitating reticular cell; m, macrophage; mc, mast cell; mcg, mast cell granule; SCS, subcapsular sinus.

between HEV (see section on interendothelial junctions below). Therefore, fluids which are in the FRC conduit system appear to have a natural exit at the HEV.

How do soluble factors from afferent lymph enter the FRC conduit system if it is so effectively ensheathed? One important mechanism appears to be *pinocytosis* (Figure 4). Afferent lymph enters the subcapsular sinus, the floor of which is composed of a pavement of flattened fibroblastic reticular cells; these cells encase reticular fibers that are continuous with the network of the deep FRC conduit system. Although there are no gaping holes in the sheath here, the presence of prominent pinocytotic vesicles by electron microscopy (EM) demonstrates that the sheath is continuously sampling the afferent lymph.[12,71] Given the huge surface area of the sheath at the floor of the subcapsular sinus, a substantial amount of lymph-borne cytokines may undergo uptake from the subcapsular sinus into the FRC conduit system.

There is an additional mechanism for fluid entry into the FRC conduit system which we propose provides increased effective fluid volume to deliver the cytokines to the HEV. This mechanism is transudation through fenestrated capillaries which are at their greatest density immediately below the floor of the subcapsular sinus. These specialized vascular structures are embedded within the FRC sheath. The ability of fluid to exit here and pass down the sheath has been demonstrated in time course studies of soluble tracers injected intra-arterially.[12] Our working hypothesis is that the primary role of this transudate is to provide volume to flush the pinocytosed lymph rapidly to the HEV. Another, more radical, possibility is that the limited myofilaments in the subcapsular sheath enable peristaltic waves to facilitate fluid movement.[14]

For the sake of simplicity, we have so far discussed only the subcapsular sinus. Most reticular fibers branch at least once, ending at the HEV and the cortical sinus nearby. Therefore, cortical sinuses also rapidly receive cytokine-rich fluid delivered via the FRC conduit system.

Endocytic capacity of the FRC conduit system

The foregoing discussion focuses on the cardinal issue for us, namely transport of lymph-borne cytokines to the HEV. However, there are two additional features which make the concept of the FRC conduit system even more powerful in bringing cytokines to HEV. The first special feature is the endocytic capacity of the FRC sheath. This is most dramatically apparent under conditions which induce mast cell degranulation in the subcapsular sinus and elsewhere (Figure 4). Within minutes of release, proteoglycan-rich mast cell granules can be observed undergoing endocytosis by coated pits in the membranes of reticular cells.[5,19,72] Subsequently, electron-dense metachromatic material can be found in the FRC conduit system, which is then distributed 'downstream' to the HEV. Endocytosis (and potentially other mechanisms of regulated uptake) may be a powerful process by which the FRC preferentially delivers certain relevant soluble materials into the FRC conduit system. We speculate that cytokines bound to proteoglycan carriers may thereby be delivered especially well by the FRC conduit system. Indeed, heparin proteoglycans administered exogenously rapidly move down the FRC conduit system.[12]

Specialized connections of certain cell types to FRC conduit system

Exhaustive EM studies suggest there may be local discontinuity of the sheath at some sites, such as points of contact with lymphoblasts, activated macrophages, interdigitating dendritic cells and follicular dendritic cells (Figure 4). At these points, the fiber continues to be sealed from the extracellular space by the apposing cell. These appear to be sites of privileged communication between the FRC conduit system and these cells; this might enable uptake of materials from the FRC conduit system (for example antigen) or distribution of cytokines from these cells to the HEV. The FRC itself is also known to produce and secrete cytokines which are essential for conditioning the lymphoid microenvironment.[73]

Special junction of HEV

The HEV endothelial barrier is functionally different from other endothelium in two critical respects. Rather than totally preventing fluid transfer, it facilitates delivery of cytokines from the FRC conduit system and the surrounding interstitial space to the luminal surface of the HEV. Rather than making cellular entry difficult, it provides an avenue of

relatively easy access for recruited lymphocytes. Understanding the unique anatomic characteristics of the junction between HEV is critical to these two functional properties. Two features are key: discontinuous closure and flap-valve relationship. The functional result is a regulated movement of fluid and cells in opposing directions. Remarkably, these can occur without gross vessel leakiness.

The inter-HEV junction differs from that characteristic of 'standard' capillary and arterial endothelium in that the junctions are not 'occluding' junctions ('zonula occludentes'). Unlike the typical band of attachment between endothelial cells, attachment between HEV is mediated by discontinuous 'spot welds' separated by areas of unattached membrane.[20,38] It would be virtually impossible for net fluid movement through an occluding junction and cells could move across only by gross disruption of the junction. In contrast, either cells or fluid could move through the spot-weld junction of HEV with minimal disruption.

However, if the endothelial cell junction were inadequately sealed, then there would be fluid loss into the LN tissue space. One mechanism which appears to prevent this is extensive overlap between adjacent endothelial cells (depicted in Figure 1); this overlap is oriented in such a way that intraluminal pressure (and flow) closes the gap between endothelial cells. The effectiveness of this seal is demonstrated by EM studies showing failure of intraluminal tracers to penetrate to the basement membrane (Figure 2). It is noteworthy that a puff of tracer accompanies transmigrating lymphocytes as they breach the limited barriers between HEV. In short, lymphocytes cross these unusual blood vessels 'like ships in canal locks'.[74]

Special glycocalyx of HEV

A remarkable feature of HEV is the thick glycocalyx which coats the luminal surface. Historically, light microscopic studies first showed that the lumen and cytoplasm of HEV endothelial cells stain strongly for complex carbohydrates. The extent of this glycocalyx was often not appreciated on EM because it was destroyed by the solvents used for processing.[20] However, when preserved with fixatives designed to stabilize such carbohydrates, the HEV display an exceptional thickness of glycocalyx (Figure 5). Studies of S^{35} incorporation demonstrate that HEV have uniquely high rates of sulfate incorporation into

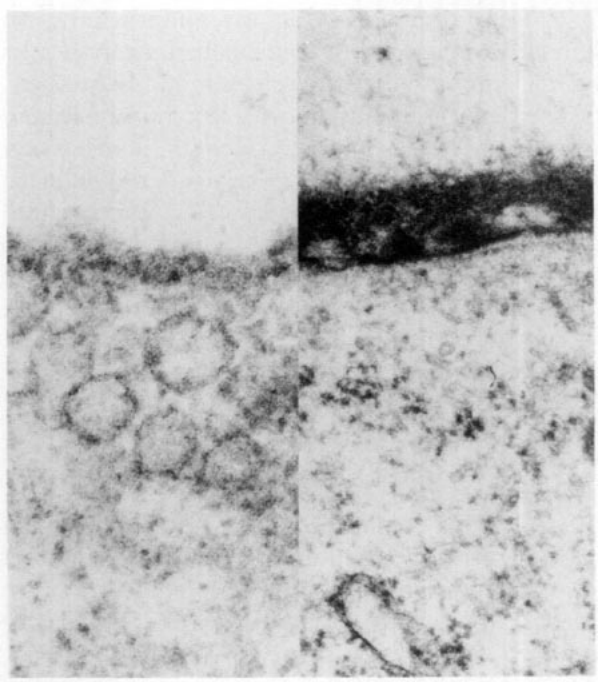

Figure 5. Contrast between the thin glycocalyx of a capillary (left) and the voluminous glycocalyx of an HEV (right).

complex carbohydrates which were secreted and/or shed.[61] A large part of that sulfation is due to glycam, which is highly sulfated.[75] It remains to be determined how much of this sulfation is contributed by proteoglycans.

Apart from the role of glycam as the ligand for L-selectin, no function has been proven for this glycocalyx. We propose that one of the ways in which it is likely to be important is in capturing soluble factors to present to passing lymphocytes. As described above, factors like MIP-1β or IL8 can be critical triggers in the adhesion cascade. We expect that the rich glycocalyx will provide binding sites for many factors, either on proteoglycan or potentially even on glycam. Given the abundance of glycocalyx, even weak interactions could facilitate retention.[47,68] In addition, simple trapping of cytokines in the glycocalyx may hold them there for readout by a penetrating microvillus on a T cell.

Generalizing to other tissues

Because the model can be illustrated most clearly in LN, we have focused our review on T cell interactions with HEV. However, we believe it is more

general. The adhesion cascade is viewed as a general model for interaction with any endothelium. In addition, we believe that the microanatomic principles which we propose here are relevant in other tissues. The site of entry of lymphocytes into non-lymphoid tissue is primarily through PCV. PCV share critical characteristics with HEV: flap-valve inter-endothelial anatomy;[76,77] connections to an FRC system within the tissue;[76,77] and capacity to rapidly differentiate into HEV-like morphology under conditions of inflammation.[21,78,79] Given space limitations, we will continue to extend this concept in subsequent publications.

Conclusions

We have explored the adhesion cascade model as it relates to T cell interaction with LN HEV. Enough molecular components have been identified to achieve a fair understanding of the three steps in this cascade. The triggering step is least well understood; however, in this review we have brought together a number of powerful concepts which we believe will be of fundamental importance in triggering: (1) cytokines as adhesion triggers; (2) endothelial presentation of cytokines by luminal proteoglycans; (3) rapid delivery of cytokines to the HEV by the FRC conduit system; and (4) movement of the cytokines to the luminal surface by the flap-valve anatomy of the HEV. Although, for the sake of clarity and integration we have illustrated these principles for the understanding of T cell interaction with LN HEV, we believe that similar principles apply to other leukocyte interactions with endothelium.

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