Lymphocyte interactions with endothelial cells

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Adhesion of lymphocytes to endothelium is vital to lymphocyte migration into lymphoid tissue and into inflammatory sites. In this review, Yoji Shimizu and colleagues identify the molecules that mediate lymphocyte–endothelial cell adhesion, describe the underlying principles of lymphocyte migration, and discuss a model of the sequence of events that allow a lymphocyte to successfully attach to endothelium and migrate into the surrounding tissue.

Lymphocytes circulate throughout the body in the ongoing process of immune surveillance by traveling through the bloodstream, moving into tissue and then returning to the circulatory system via the lymphatics[1–4]. Since lymphocyte recognition of, and response to, foreign antigen typically occurs in lymphoid organs or in nonlymphoid tissue, the principles and mechanisms that regulate lymphocyte movement into tissue are critical to the generation of an immune response. The interaction of lymphocytes with endothelial cells lining blood vessel walls represents the first critical step in lymphocyte movement into tissue. The role of endothelium as the gatekeeper regulating lymphocyte interactions with tissue is more complex than for other cells such as neutrophils and platelets, which bind to endothelium under an inflammatory crisis situation. Lymphocytes not only adhere strongly to inflamed endothelium and play a critical role in the inflammatory response[5], but they also interact in a precisely regulated fashion with normal endothelium and thereby migrate into lymphoid and nonlymphoid tissue[1–4]. Here, the various molecules that mediate lymphocyte interactions with endothelial cells, and the underlying principles and mechanisms used by the immune system that allow this generally overlapping set of molecules to mediate both normal lymphocyte migration and influx into inflammatory sites, are reviewed. Although the focus of recent studies has been on T-cell interactions with endothelium, earlier seminal work suggests that similar considerations apply to B cells[6].

Lymphocyte and endothelial cell adhesion molecules

The complexity of T-cell interactions with endothelium is illustrated by the multiplicity of molecules that mediate this cell–cell interaction. The large number of molecules that play some role in lymphocyte adhesion to either specialized endothelium in lymphoid organs (designated high endothelial venules (HEV)) or activated endothelial cells (Table 1) can be divided into four groups. The list is limited to those molecules that have already been cloned and characterized at the molecular level.

Selectins

The three members of the selectin family of adhesion molecules (L-selectin, E-selectin and P-selectin) appear to play particularly important roles in mediating cell–cell adhesion in the vasculature[7]. Selectins have a characteristic extracellular structural motif consisting of a lectin domain, a domain with homology to epidermal growth factor and a variable number of complement regulatory protein repeat sequences[7]. Both L-selectin and E-selectin have been shown to be involved in T-cell adhesion to endothelium. L-selectin[8,9] (also designated LECAM-1, MEL-14 and LAM-1) is expressed on a subset of T cells and has been extensively studied as the lymphocyte molecule mediating homing to peripheral lymph nodes (see below). However, L-selectin also appears to be involved in both neutrophil and lymphocyte adhesion to activated endothelial cells[10–15]. E-selectin (also designated ELAM-1) is an inducible endothelial cell surface molecule first described as mediating the adhesion of neutrophils to activated endothelium[16]; however, recent studies have shown that E-selectin also mediates the adhesion of a subpopulation of resting $CD4^+$ memory T cells to activated endothelium[17,18].

Both E- and L-selectin bind to specific sialylated carbohydrates[19]. Depending on the labeling conditions, the L-selectin ligand can be recognized on molecules of 50 and 100 kDa (and several other less predominant species) by an L-selectin chimeric protein and a functionally inhibitory monoclonal antibody (mAb), MECA-79, that specifically stains peripheral lymph node HEV[20,21]. The HEV ligands for L-selectin and other putative homing receptors have been referred to as 'vascular addressins', signifying their role in mediating the tissue-specific adhesion of lymphocytes expressing the appropriate homing receptors. The ligands for E-selectin include sialyl Lewis X (sLeX), present on neutrophils and macrophages, and a similar if not identical carbohydrate on a subset of memory T cells[13,19].

Integrins

Integrins are a large family of $\alpha\beta$ heterodimeric cell surface proteins that are expressed on a wide variety of

Table 1. Molecules/pathways involved in lymphocyte adhesion to endothelial cells

T-cell receptor		Endothelial cell counter-receptor			Role in			
Molecule	Family	Molecule	Family	Increased by inflammation?	Traffic	Inflammation	Comments	Refs
L-selectin	SEL	Sgp50, Sgp100, MECA-79	CHO	Yes?	Yes[a]	Yes	Peripheral lymph node homing receptor, mediates neutrophil rolling	8,10
sLeX-related structure	CHO	E-selectin	LEC	Yes	?[b]	Yes?[c]	Mediates binding of resting $CD4^+$ memory T-cell subset	17,18
LFA-1	INT	ICAM-1	Ig	Yes	Yes?	Yes	Inflammation-induced ligand for strong adhesion	22,24,26
LFA-1	INT	ICAM-2	Ig	No	Yes?	Yes?	Constitutive ligand for strong adhesion	22,24,33
VLA-4	INT	VCAM-1	Ig	Yes	Yes?	Yes?	Peyer's patch homing receptor in the mouse (designated LPAM-2)	23,28,29
α4βp/ LPAM-1	INT	?	?	?	Yes?	?	Peyer's patch homing receptor	28
CD44	CL	HA, MECA-367?	CHO	?	Yes?	Yes?	–	38–41
$HEBF_{LN}$	?	?	?	?	Yes?	?	Blocks binding to peripheral lymph node HEVs in rat	42
Human HEBF	?	?	?	?	Yes?	?	Blocks binding to peripheral lymph node HEVs in human	43
$HEBF_{PP}$	?	?	?	?	Yes?	?	Blocks binding to mucosal HEVs in rat	44

[a]'Yes' indicates *in vivo* functional evidence using either mAbs or soluble adhesion molecules; [b]'?' indicates a current lack of existing *in vitro* or *in vivo* data implicating the interaction in the indicated adhesive function; [c]'Yes?' indicates a putative functional role based on *in vitro* mAb blocking studies; SEL: selectin; INT: integrin; Ig: immunoglobulin supergene family; CHO: carbohydrate; CL: cartilage link proteins; HA: hyaluronic acid.

cell types and mediate adhesion to other cells and to components of the extracellular matrix (ECM)[7]. Both the leukocyte function-associated antigen 1 (LFA-1) and very late antigen 4 (VLA-4) integrins play major roles in T-cell adhesion to activated endothelium by binding their respective cell surface ligands, intercellular adhesion molecule 1 (ICAM-1) and ICAM-2 (for LFA-1) and vascular cell adhesion molecule 1 (VCAM-1) (for VLA-4)[22–24]. LFA-1 is expressed by all T cells, but at one- to twofold higher levels by memory cells than by naive cells[25]. LFA-1 is involved in lymphocyte trafficking, but its role appears to be one of general non-organ-specific strengthening of adhesion[26]. VLA-4 shows a much more heterogeneous pattern, with low expression on most naive cells and very heterogeneous expression on memory T cells (Ref. 27 and K.J. Horgan *et al.*, unpublished). In the mouse, two integrin molecules, lymphocyte Peyer's patch HEV adhesion molecule 1 (LPAM-1) and LPAM-2, each composed of the VLA-4 α chain but with distinct β chains, have been implicated as receptors mediating lymphocyte migration to mucosal lymphoid organs, such as Peyer's patches[28]: LPAM-2 is homologous to human VLA-4; LPAM-1 consists of the VLA-4 α chain associated with a distinct β chain, designated βp[28]. While VLA-4 binds to VCAM-1 on cultured activated endothelial cells[23,29], the relationship of the LPAM-1/2 ligand on Peyer's patch HEVs to VCAM-1 is not known; one study in humans has failed to detect VCAM-1 on mucosal HEVs[30]. VLA-4 is also one of two integrin fibronectin receptors on T cells[25,31] and inhibition of lymphocyte adhesion to rat high endothelial cells by peptides containing the VLA-4 recognition sequence on fibronectin suggests a possible role for ECM molecules in lymphocyte–endothelial cell adhesion[32].

Immunoglobulin supergene family

Three members of the immunoglobulin supergene family are involved in T-cell–endothelial-cell interactions, namely ICAM-1, ICAM-2 and VCAM-1. They serve as endothelial cell surface ligands for the LFA-1 and VLA-4 integrins. Differential regulation of ICAM-1, ICAM-2 and VCAM-1 expression plays a critical role in the use of these various adhesion pathways by T cells. ICAM-2 is constitutively expressed at a high level on resting endothelial cells and its expression is not augmented by activation[33]. In contrast, ICAM-1 is weakly expressed and VCAM-1 is absent on resting endothelium, but the expression of each is rapidly increased by endothelial cell activation[22,24,34].

CD31, another Ig supergene family member, is postulated to mediate platelet–endothelial-cell adhesion[35]. CD31 is strongly expressed, not only on HEVs, but also on a subset of T cells that are predominantly naive T cells (Y. Tanaka *et al.*, submitted). CD31 may mediate adhesion through a homophilic interaction[36], and recent data suggest a critical role for CD31 in regulating adhesion of unique T-cell subsets to endothelium (Y. Tanaka *et al.*, submitted and see below).

Other molecules

The CD44 molecule is a widely expressed cell surface protein with structural homology to cartilage link proteins[37]. CD44-specific mAbs have been shown to inhibit lymphocyte binding to HEVs[38] and activated endothelial

cells[39]. CD44 binds to the glycosaminoglycan, hyaluronic acid, and CD44 binding to some cultured endothelial cells can be blocked by treatment of these cells with hyaluronidase[40]. However, other studies have shown that binding to HEVs is not inhibited by soluble hyaluronic acid or hyaluronidase treatment of HEVs[41], suggesting that CD44 may bind to additional ligands, such as the mucosal vascular addressin defined by the MECA-367 mAb[4]. The other molecules listed in Table 1 define antigens recognized by mAbs that block adhesion to HEVs in various species[42–44]. The relationship of these antigens to the more well-defined molecules discussed above remains to be determined.

Fundamental principles of T-cell migration

The functions of T cells require their incessant movement. The simplest traffic pattern would be random migration of all T cells through all tissues. Instead, evolution has made the process more efficient by routing T cells in different ways according to multiple cues. For the purposes of this discussion, the differential migration of T cells in normal, healthy individuals is termed trafficking or 'homing'. Four governing principles regulate T-cell trafficking: (1) each lineage of immune cells will have distinct rules governing its migration; (2) naive T cells migrate into lymph nodes, while memory T cells migrate primarily into nonlymphoid tissue; (3) memory cells become biased to preferentially home to tissues related to the one in which they were previously stimulated; and (4) inflammation augments the influx of T cells and reduces the selectivity that governs normal homing.

Cell-lineage specific migration

Cells of different lineages (for example T cells versus neutrophils versus platelets) differ in their interactions with endothelial cells; this results in distinct patterns of migration[6]. In addition, subsets within each lineage, such as $CD4^+$ and $CD8^+$ T cells, also exhibit differences in their movement through the body[45]. Further phenotypic differences between $CD4^+$ and $CD8^+$ T cells that would be expected to influence interactions with endothelium also exist. For example, a higher proportion of $CD8^+$ than $CD4^+$ circulating T cells express CD45RA (thought to be a marker of naive T cells), LFA-1, VLA-4 and CD31 (Ref. 46 and Y. Tanaka *et al.*, submitted).

Differential migration of naive and memory T cells

Within the $CD4^+$ T-cell lineage, the most fundamental distinction among subsets is between naive cells (which have not been stimulated by antigen after export from the thymus) and memory cells[24,25,47–49]; CD45RA is currently the best marker of naive cells and CD45RO of memory cells. Although the body of experimental data is much less complete, CD45RA/CD45RO also subdivides $CD8^+$ T cells and may reflect a similar functional dichotomy[48,50,51]. Naive and memory T cells have radically different trafficking patterns[47]. Virtually all the T cells found in tissues such as skin[52], gut lamina propria[53] and on bronchial surfaces[54] are of the memory phenotype. Thus, memory cells preferentially migrate into both normal and inflamed nonlymphoid tissue. Conversely, naive T cells account for most of the torrent of cells entering lymph nodes[45]. This well-known concept of selective lymphocyte trafficking[6,47,55] makes good sense, since the lymph node serves as a specialized site that brings together the rare relevant naive T cells, specialized antigen-presenting cells (APCs), and the antigen load drained from local tissue in a microenvironment particularly suited to T-cell stimulation.

Dramatic progress has been made in understanding the molecular basis for this fundamental difference in migration pattern. Memory $CD4^+$ T cells express higher levels of several adhesion molecules than do naive $CD4^+$ T cells[25]; the enhanced expression of LFA-1, VLA-4, VLA-5 and VLA-6 on memory cells is associated with their increased capacity to bind to the relevant ligands ICAM-1, VCAM-1, fibronectin and laminin[25]. More recently, expression of a carbohydrate epitope expressed on a memory T-cell subset that binds to E-selectin has been described (Ref. 18 and Y. Shimizu, unpublished). More memory cells than naive cells bind to endothelial cells *in vitro*[24,56–58]; this binding is mediated by VLA-4, LFA-1 and the E-selectin-binding carbohydrate present on a subset of memory cells[17,18,24]. Thus, there is a striking correlation between *in vitro* studies of T-cell binding to cultured endothelial cells and *in vivo* findings regarding memory T-cell interaction with endothelium from nonlymphoid organs.

Which molecules mediate the preferential migration of naive T cells into lymph nodes by interaction with peripheral lymph node HEVs? In the mouse, assays of T-cell binding to HEVs in frozen lymph node sections and mAb blocking of *in vivo* migration of T cells[1–4] have implicated L-selectin, which is expressed on all naive T cells and a subset of memory T cells[27]. We suggest that CD31 may also be involved in preferential naive and $CD8^+$ T-cell interaction with HEVs, since CD31 can potently induce integrin-mediated adhesion. Like L-selectin, CD31 is preferentially expressed on naive T cells (Y. Tanaka *et al.*, submitted).

There's no place like home

The third principle governing T-cell interaction with endothelium is that memory T cells are further subdivided into subpopulations that preferentially migrate into particular anatomic sites. Memory T cells are biased to return to the tissue in which they were originally stimulated; this makes sense as an evolutionary strategy since T cells are most likely to re-encounter an antigenic threat in the same (or similar) anatomic location[27,47]. It is considered that there is a substantial number of anatomical compartments served by specialized T-cell subsets, including gut[28], synovium[59] and skin[18]. The experimental basis for this hypothesis comes from studies in which the fate of tagged lymphocytes derived from different anatomic sites was monitored following re-introduction into the host. These data, complemented by the recent understanding of naive and memory cells, clearly establish tissue-specific migratory patterns[1–4]. Such homing patterns are, presumably, the result of specific pairs of molecules on T cells and endothelial cells that confer regional specificity. For gut homing, the integrins LPAM-1 and LPAM-2 are thought to interact with an undefined ligand on Peyer's patch HEVs; this inference is based on functional inhibition by relevant mAbs of tissue-specific binding of selective T-cells with Peyer's

patch endothelium[28]. Similar studies have implicated other endothelial molecules in homing to peripheral lymph nodes (via L-selectin) and mucosal sites (possibly via CD44). Molecules that might mediate homing under normal, noninflammatory conditions to other anatomic sites, such as the skin and lung, remain poorly defined.

A number of aspects of homing into tissue warrant comment. First, tissue specificity is undoubtedly relative, not absolute; for example, putative skin-homing cells can almost certainly migrate elsewhere, particularly under conditions of inflammation. Second, the specificities for different anatomic sites are probably determined in a combinatorial fashion by multiple receptors, not exclusively by a single receptor. Third, we predict that there is much more diversity among memory T cells than is currently appreciated and that they are in effect an 'army of specialists' that interact well with a limited number of the large range of specialized microenvironments[51]. Fourth, early models of T-cell differentiation proposed that naive cells have a full complement of homing receptors, with loss of the irrelevant ones during differentiation. The data now suggest that, in some cases, the receptor on the relevant memory cell subset, such as the carbohydrate-mediating binding to E-selectin, is not used/expressed by naive cells[27].

Inflammation increases adhesion and reduces the selectivity of trafficking

The fourth principle governing T-cell interaction with endothelium is that it is dramatically modified by inflammation. The fundamental change is in the phenotype of the endothelial cell. The change can result from a very wide range of stimuli, including 'alarm cytokines', such as interleukin 1 (IL-1) and tumor necrosis factor (TNF), that are released/secreted by a wide variety of cell types, and lymphokines, such as IL-4 and gamma-interferon, derived from T cells that have encountered their specific antigen[7,22,23,34,60,61]. The expression of endothelial ligands for T-cell binding is either increased (typically the case for ICAM-1) or induced *de novo* (typically the case for VCAM-1 and E-selectin). The specific patterns of endothelial cell ligand expression are complex and depend on: (1) the inducing cytokine or combination of cytokines; (2) the time after cytokine exposure; and (3) the type of endothelial cell and its environmental cues from neighboring cells and the ECM. Such ligand induction by cytokines occurs *in vitro*, and can be observed in a wide variety of natural and experimental inflammatory lesions *in vivo*[60,61]. The major consequence of this change in endothelial cell phenotype is increased efficiency of T-cell binding, particularly of memory T cells. It is noteworthy that T-cell adhesion molecules that are thought to mediate tissue-specific homing under normal conditions, such as L-selectin and VLA-4, also contribute to T-cell adhesion to inflamed endothelium[5,12,23,24,62]. This suggests that expression of many of the ligands that contribute to normal lymphocyte homing can be induced by inflammation at sites where they are not normally expressed. We surmise that the immune system can afford refined regional strategies under conditions of normal surveillance, but mobilizes many of these same molecular interactions for widespread use when a state of emergency exists in a tissue. Conversely, other molecules that are currently considered to function only under inflammatory conditions, such as E-selectin, may also play a role in normal lymphocyte trafficking (W. Newman, unpublished). Inflammation not only influences the influx of memory cells into nonlymphoid tissue, but also dramatically increases lymphocyte (presumably naive T cells) entry into lymph nodes[63].

The adhesion cascade

Lymphocyte adhesion to endothelial cells clearly involves multiple receptor–ligand interactions. For example, at least five distinct receptor–ligand interactions have been implicated in the binding of $CD4^+$ T cells to activated endothelial cells: LFA-1 (to ICAM-1 and ICAM-2), VLA-4 (to VCAM-1), E-selectin and CD44 (Refs 22–24,39 and Table 1). The potential advantages of the use of multiple adhesion interactions are clear when it is realized that the goal at an inflammatory site is to rapidly and efficiently capture a large number of lymphocytes from the blood. The overall strength of adhesion can be amplified with the use of multiple receptor–ligand interactions, each of which individually may provide only minimal adhesive strength under physiological conditions of flow in a venule.

An additional functional rationale for the use of multiple adhesion molecules in lymphocyte–endothelial-cell interactions is that each receptor–ligand pair may provide a distinct and unique function that is necessary for adhesion to, and migration through, endothelium. Thus, lymphocyte adhesion to endothelium may involve an 'adhesion cascade', defined as the sequential, temporal use of various adhesion molecules during the entire process, from initial attachment to the final entry of lymphocytes into the surrounding tissue. The best known enzymatic cascades, clotting and complement, demonstrate amplification and regulation. Recent studies of platelets and neutrophils (Refs 14,15,64–66 and G. Zimmerman, this issue), coupled with the existing data on T cells, suggest an adhesion cascade in T-cell interactions with endothelium. We propose a model involving the following sequence of events: (1) multiple random collisions with the endothelium and establishment of a tenuous, unstable interaction, (2) delivery of a triggering signal that activates T-cell integrin function, (3) establishment of strong adhesion to the endothelium and (4) subsequent migration to endothelial junctions and transmigration into the surrounding tissue (Fig. 1).

Step 1 – initial interaction between T cell and endothelium

There are two points to consider here. First, in contrast to many cartoons of endothelial cells that show a smooth surface of flattened cells, endothelial cells, particularly HEVs, have a rough surface morphology that is characterized by clefts and interfacing networks among the cells[67,68]. This endothelial morphology has two important consequences: it increases the overall endothelial cell surface area, and it modifies the flow from laminar to tubular. Under normal circumstances, the slowest flow rates in the circulatory tree occur in the post-capillary venules; these would therefore be expected to be (and are) the normal site for lymphocyte interaction with endothelium. Furthermore, at an inflammatory site, the blood flow rate is reduced owing to vessel dilation. All of

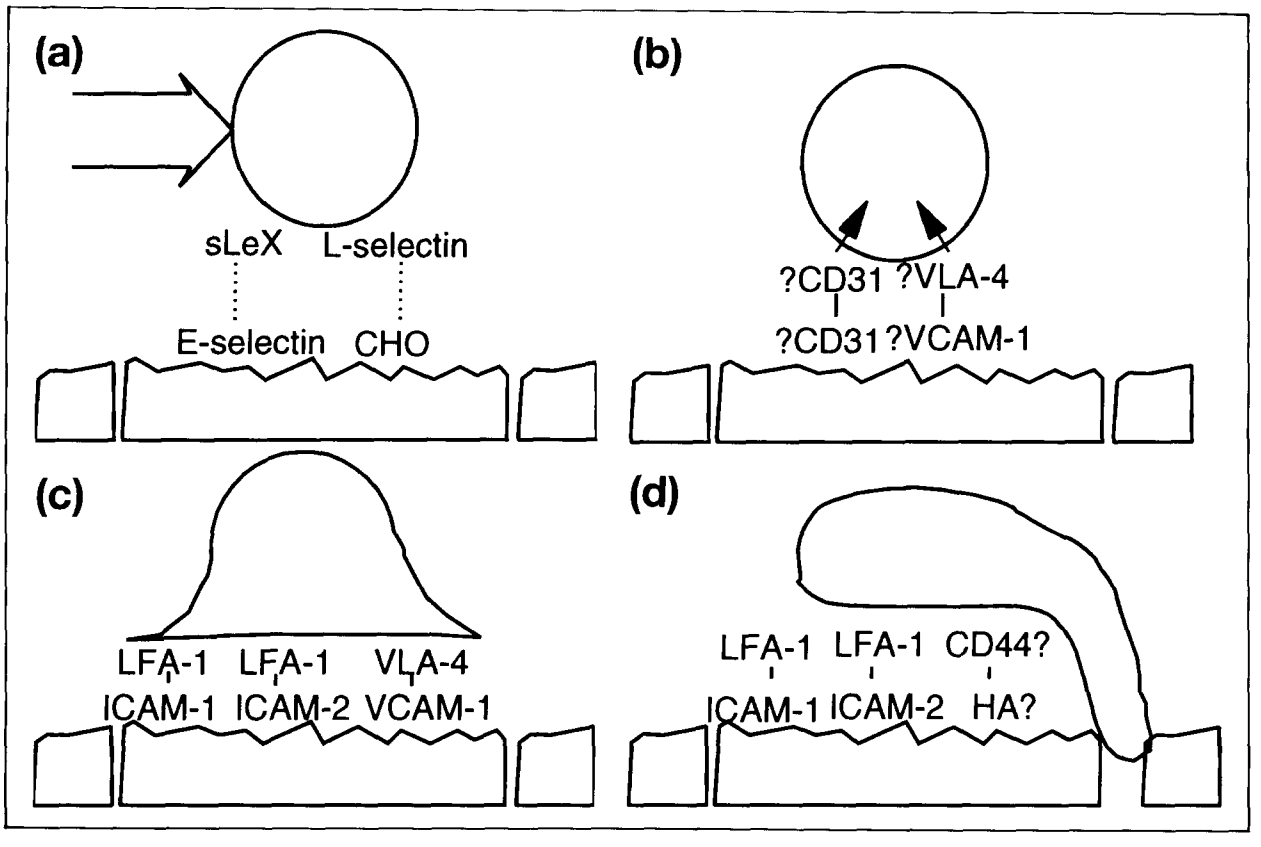

Fig. 1. *The proposed sequence of events in an adhesion cascade mediating T-cell adhesion to endothelium is shown. The cascade involves: (a) tenuous adhesion or tethering of a flowing T cell via selectin-mediated interactions; (b) delivery of a triggering signal to upregulate integrin function; (c) strong integrin-mediated adhesion; and (d) migration of the T cell through the endothelium into the surrounding tissue. Potential candidate receptor–ligand interactions involved in each step of the cascade are indicated; however, additional molecules may also be involved at each step and the use of specific adhesion pathways is probably dependent on the type of T cell and endothelial cell involved.*

these factors result in increased opportunities for a passing lymphocyte to interact with endothelium. At least in the one tissue studied, lymphocytes, unlike neutrophils, do not exhibit rolling behavior; analysis of lymphocyte interactions with Peyer's patch HEVs show that many of the lymphocytes entering the postcapillary venule collide more than once with the endothelium and rapidly stop[69].

Second, the majority of lymphocytes in the circulation are in a resting state. Thus, it is unlikely that integrins such as LFA-1 and VLA-4 play a major role in the initial adhesion, since T-cell integrins require an activation signal to mediate strong binding[7,25,70,71]. This is further supported by findings that integrins on activated T cells do not bind well to ICAM-1 and fibronectin under physiological shear forces[64]. The selectins, including L-selectin and E-selectin, are the best candidates for initiating adhesion since both L-selectin and E-selectin mediate adhesion of resting T cells to endothelium[12,17], and selectins have been shown to mediate adhesive interactions of neutrophils under flow conditions[14,15,64]. The apparently unique ability of selectins to initiate adhesion may relate to the rapid reaction rate of the carbohydrate–lectin interactions that they exploit[72]. We do not discount the potential involvement of other adhesion molecules, such as CD44. Furthermore, under conditions of disease or inflammation, additional molecules may also contribute to this initial interaction because of disease-associated activation events that result in changes in T-cell adhesion molecule phenotype and/or function[73].

Step 2 – triggering

Integrins on resting T cells mediate strong adhesion only when the cell receives the right activating stimulus. Recent studies suggest that a number of T-cell surface molecules can induce signals that activate T-cell integrin function: the CD3–T-cell receptor, CD2, CD7, CD28, CD31 and VLA-4 (Refs 7,25,70,71,74,75). Which of these (or other) molecules might act as a trigger during T-cell interaction with endothelium? CD31 is a promising candidate to provide this trigger function for $CD31^+$ T cells, which are predominantly naive T cells. Anti-CD31 mAb perturbation of $CD8^+$ naive T cells results in a rapid induction of integrin function, particularly VLA-4 (Y. Tanaka *et al.*, submitted). Since CD31 is considered to participate in homophilic (and potentially heterophilic) interactions[36], CD31 (and potentially other ligands) on endothelial cells would engage CD31 on T cells and, thereby, trigger integrin-mediated adhesion; this is consistent with *in vitro* studies that show T-cell adhesion to purified CD31 (Y. Tanaka *et al.*, submitted). VLA-4 is also a candidate to amplify its own adhesion, since mAb ligation induces functional activation (Refs 76,77). Thus, weak binding of VLA-4 to VCAM-1 on the endothelium could provide a feedback loop to augment integrin adhesion. Such integrin autoregulation by ligand has recently been reported for the platelet integrin gpIIb–IIIa[78]. VLA-4, like CD31, is well expressed only on some T cells and therefore its postulated triggering function would, likewise, be subset specific.

Although there is no evidence yet to implicate CD2, CD7 or CD28 in triggering endothelial adhesion, antigen recognition via the CD3–T-cell receptor, in triggering integrin function, may play a role in the context of allogeneic organ transplantation[79]. E-selectin is also a candidate triggering molecule, since E-selectin appears to regulate Mac-1 function on neutrophils[65,66]; similar regulation of integrin function on T cells by E-selectin has not been reported. Although chemoattractants present at endothelial cell junctions have been proposed to result in upregulation of integrin function on neutrophils[65], soluble mediators such as cytokines have not been found to modulate integrin function on T cells (Y. Shimizu and G.A. van Seventer, unpublished).

Step 3 – strong adhesion to endothelium

Integrins, once their function is induced, undoubtedly play a major role as the main adhesive force or 'glue' that T cells use to stick to endothelium. It is presumably this strong adhesion that can rapidly bring flowing T cells to a halt[69]. The predominant pathways are thought to be VLA-4–VCAM-1, LFA-1–ICAM-1 and LFA-1–ICAM-2 (Refs 22–24,39). It is likely that other integrins, such as LPAM-1, also contribute in interactions with Peyer's patch HEVs[28].

Step 4 – migration to the endothelial cell junction and transmigration

Migration of the T cell into tissue requires arrest of the T cell by the endothelium and transendothelial migration. This requires a reduction in adhesion, followed by orderly migration. Several mechanisms may contribute to reduction in adhesion. The first is the transience of augmented integrin function. Signals via molecules such as CD3 (Refs 7,25,70) and CD31 (Y. Tanaka, unpublished) induce integrin function which then decays rapidly. Although the mechanism that accounts for this transience is not known, the end result will be to allow the T cell to move[70]. The second mechanism demonstrated for T cells is shedding; L-selectin is shed following T-cell activation[80], and this may occur during interaction with endothelial cells to release the T cell to migrate. Although *in vitro* migration assays have demonstrated inhibition of T-cell migration through endothelial monolayers by LFA-1 and CD44 mAbs[39] and decreased migratory capacity of T-cell clones deficient in LFA-1 expression[81], the precise role that these and other molecules play in the complex process of migration awaits further investigation.

Several features of this model warrant comment. First, since delivery of a partial activation signal is essential to the cascade, the T cell entering the surrounding tissue may be uniquely suited to respond vigorously to foreign antigen encountered on antigen-presenting cells. The ability of purified ligands, such as ICAM-1 and VCAM-1, to facilitate T-cell proliferative responses *in vitro* is consistent with this interpretation[82,83]. Second, we consider that the overall sequence of events will be similar, regardless of whether interaction with normal HEVs or inflamed endothelium is occurring. Third, the specificity of the T-cell interaction with endothelium is conferred not by a single molecule but by the entire cascade. Many of the T-cell surface molecules involved are present only on a subset of T cells and the ligands are restricted to certain endothelia. Thus, a $CD8^+$ naive T cell interacting with lymphoid tissue HEVs may use L-selectin for the first step, CD31 for the second and VLA-4 for the third; in contrast, a $CD4^+$ memory T cell interacting with inflamed endothelium might use E-selectin for the first, VLA-4 for the second and VLA-4 and LFA-1 for the third. Fourth, this model has relevance to the current interest in developing therapeutic reagents that modulate T-cell–endothelial cell adhesion, since it predicts that inhibition of any step in the cascade should result in inhibition of migration into tissue. Furthermore, the use of a generally overlapping set of molecules in trafficking and inflammation suggests that the therapeutic value of any of these reagents must be weighed against their effects on normal lymphocyte homing. Although many questions regarding the validity of this model remain to be answered, it nevertheless provides an important framework for the development of hypotheses that can be tested. The continued interest in lymphocyte–endothelial-cell interactions will undoubtedly lead to a further understanding of the molecules and mechanisms that mediate this critical cell–cell interaction.

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