

The lymphocyte homing receptors: gatekeepers of the multistep paradigm

Robert Sackstein

Purpose of review

Lymphocyte trafficking is regulated by adhesion molecules mediating the initial shear-resistant binding of circulating cells to target tissue endothelium (Step 1). This review focuses on the current and emerging perspectives of the biology of these 'homing receptors.'

Recent findings

The conventional multistep paradigm holds that leukocyte migration represents a cascade of events, initiated by tethering and rolling interactions of leukocytes on the endothelial surface (Step 1). These interactions are indispensable, required to dampen velocities sufficiently to allow cells to sense the local chemokines and/or other inflammatory signals resulting in activation of integrin adhesiveness (Step 2), with ensuing firm adherence on the vessel wall (Step 3) followed by endothelial transmigration (Step 4). Mechanistic studies now suggest that some effectors of Step 1 interactions themselves activate integrin adhesiveness and trigger transmigration – in some cases by forming complexes with integrins – thus bypassing the need for chemokine signaling. These findings force a reconsideration of the multistep paradigm, and shift focus now to identifying all relevant effectors of Step 1 interactions using adherence assays performed under shear stress to mimic the dynamic conditions of blood flow.

Summary

Recent findings suggest that homing receptors are not merely molecular brakes. The cross-talk among the homing receptors and integrins opens a new 'avenue' to lymphocyte migration, suggesting that homing receptors may be sufficient alone, in some cases, to perform the function the name implies.

Keywords

CD44, cutaneous lymphocyte antigen, hematopoietic cell E-/L-selectin ligand, homing receptor, lymphocyte, lymphocyte migration, lymphocyte trafficking, multistep paradigm, P-selectin glycoprotein ligand-1, selectin, selectin ligand

Abbreviations

CLA	cutaneous lymphocyte antigen
GALT	gut-associated lymphoid tissues
GPCRs	G-protein coupled receptors
HCELL	hematopoietic cell E-/L-selectin ligand
HEV	high endothelial venules
mAb	monoclonal antibody
PNAd	peripheral lymph node addressins
PSGL-1	P-selectin glycoprotein ligand-1

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Introduction

Lymphocyte migration from vascular to extravascular sites is a tightly controlled process critical to immune surveillance and host defense. Site-specific endothelial transmigration is the culmination of a cascade of events, starting with the binding of circulating lymphocytes to the endothelium of target tissue(s). Most commonly, cells in blood flow exit the vasculature at postcapillary venules, where shear stress ranges from 1 to 4 dynes/cm² [1]. The well-established multistep paradigm of leukocyte trafficking holds that circulating cells must first make contact with the endothelial surface through receptors specialized to engage respective ligands under hydrodynamic flow stress, thereby mediating adhesive interactions capable of overcoming the shear forces generated by blood flow [2]. In this recruitment stage (otherwise known as 'Step 1'), cells initially loosely attach (tether) and then roll directly on endothelial cells at velocities below the prevailing fluid stream. Cells can thus be exposed to chemical signals (principally chemokines, but also cytokines and other pro-inflammatory mediators) in the local milieu ('Step 2'), consequently leading to activation-dependent up-regulation of integrin adhesive capabilities resulting in firm arrest ('Step 3'). Firm arrest is then followed by transmigration ('Step 4') (Fig. 1). Although conventionally envisioned in multiple steps, only those lymphocytes in blood flow that are capable of participating in tethering/rolling interactions will become tissue residents. Thus, the critical gatekeeper for all emigration stands at the initiation of the cascade through adhesive interactions mediated by Step 1 effector molecules, known as the 'homing receptors.' These molecules control physiologic tissue-specific lymphocyte migration (e.g. to lymphoid tissues, gut, and skin) as well as pathobiologic lymphocyte trafficking in acute and chronic inflammatory conditions and in lymphoma metastasis. This article reviews our current understanding of the biology of these molecules, and the implications of emerging evidence for coassociations between homing receptors and integrins.

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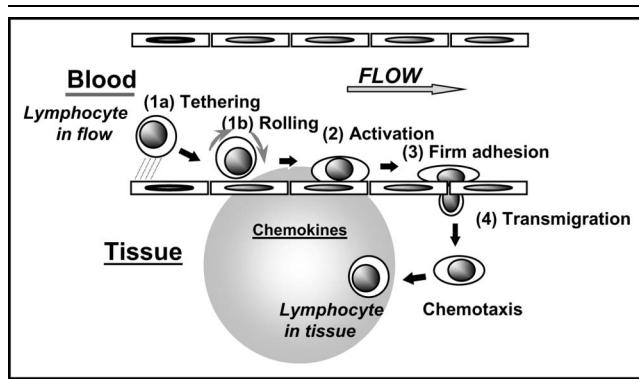
Departments of Dermatology and Medicine, Brigham & Women's Hospital, Harvard Skin Disease Research Center, Harvard Medical School; and Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Correspondence to Robert Sackstein, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Suite 671, Boston, MA 02115, USA
Tel: 617 525 5604; fax: 617 525 5571;
e-mail: rsackstein@rics.bwh.harvard.edu

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Figure 1. The multistep model of lymphocyte-endothelial interactions.



Schematic representation of the multiple steps involved in lymphocyte migration from vascular to tissue compartments. Steps 1a and 1b are mediated by the 'homing receptors.' Activation of integrins (Step 2) results in firm adhesion (Step 3), then transendothelial migration (Step 4).

The definition of 'homing receptor'

The origins of the term 'homing receptor' date back to seminal studies of Gowans [3] on the recirculation of lymphocytes (from blood to lymph nodes to thoracic duct and back to blood) in the 1950s. Early studies showed that lymphocyte trafficking to lymphoid tissues was conspicuously nonrandom, and critically involved the binding of lymphocytes to specialized postcapillary endothelial structures in lymph nodes and Peyer's patches known as 'high endothelial venules' (HEV). Yet despite abounding physiologic and histologic evidence that homing to lymphoid tissues depended on lymphocyte-HEV interactions, it was not until the mid-1970s that the adhesive interactions between lymphocytes and lymph node HEV were first identified *in vitro* by Stamper and Woodruff [4]. Importantly, these investigators correctly reasoned that the lymphocyte-HEV interaction was occurring under hydrodynamic stresses of blood flow, and thus they performed their adherence assays under shear delivered by a rotatory platform. This shear-based assay then allowed for the development of monoclonal antibodies (mAb) that recognized discrete surface structures of lymphocytes and of the HEV of peripheral lymph node and of Peyer's patches, respectively, that mediated high-affinity binding interactions [5–7]. These mAb blocked migration of lymphocytes to respective lymphoid tissues, confirming the central role of these membrane molecules in lymphoid organ-specific homing. For migration to peripheral lymph nodes, the principal homing receptor was identified as L-selectin, whereas the homing receptor for Peyer's patches (gut-associated lymphoid tissues (GALT)) was the integrin $\alpha_4\beta_7$ (also known as LPAM-1). It thus appeared – at least initially – that the mystery of tissue-specific lymphocyte homing had been solved.

However, it was soon clear from multiple converging studies that homing involves chemoattractants typically operating through G-protein coupled receptors (GPCRs). The most important of these chemoattractants are chemokines, a superfamily of small proteins that function as potent chemotactic agents, some of which are widely distributed and some of which have a tissue-specific or inflammation-specific distribution (reviewed in [8]). Additional complexity evolves from the fact that chemokine GPCRs have a cell-specific distribution. Where relevant, triggering of these GPCRs results in activation of leukocyte integrin adhesiveness to their pertinent endothelial ligands, such as VLA-4 and LFA-1 to their ligands, VCAM-1 and ICAM-1, respectively (reviewed in [9]). Thus, a generalized model was proposed, whereby tissue-specific homing was defined by a sequence of overlapping steps with combinatorial diversity allowing for an 'address' or 'code' for leukocyte homing. This model was repeatedly tested and confirmed, providing insight into a variety of previously unexplained paradoxes. For example, though it was well known that neutrophils bind to lymph node HEV in *in vitro* adherence assays and that they, too, express L-selectin [10], curiously, these cells do not routinely migrate to lymph nodes. This conundrum was solved with the discovery that lymph node HEV constitutively express the chemokine SLC (CCL21) that binds the lymphocyte GPCR known as CCR7 [11–13]. Neutrophils lack CCR7, and, therefore, they can roll on HEV but cannot convert these interactions into firm adherence [14].

Rolling interactions of lymphocyte(s) onto the endothelial surface allow relevant cell(s) bearing the proper 'taste bud(s)' (chemokine receptors) to taste local condiment(s) (chemokines); therefore, the homing receptors are obligatory for lymphocyte trafficking. Given that expression of certain homing receptors is not exclusive to lymphocytes and their subsets, how are they defined? Implicitly, homing receptors mediate lymphocyte migratory patterns, but operational biophysics offers a better definition: the homing receptors are molecules that possess the requisite chemical characteristics to achieve fast on-off binding kinetics with their respective endothelial counterreceptors; in the setting of fluid shear forces, these adhesive interactions are translated into cellular torque, resulting in rolling interactions at velocities below the prevailing hydrodynamic flow [15]. Simply stated, homing receptors are the effectors of Step 1.

What molecules comprise the 'homing receptors'?

A number of well-characterized adhesion molecules (with their ligands) serve as mediators of Step 1 rolling interactions: the three members of the selectin family (E-, P- and L-selectin, also known as CD62E, CD62P and CD62L, respectively), the 'hyaluronan receptor' CD44, and a small subset of the integrin superfamily: principally $\alpha_4\beta_7$, VLA-4,

and LFA-1 [15–20] (Table 1). Of all of these, the selectin receptor/ligand interactions are the most effective in promoting leukocyte tethering and rolling on endothelium in the hydrodynamic conditions of blood flow and are capable of maintaining rolling at higher fluid shear stresses than any other structures [15,21]. Among the integrins, $\alpha_4\beta_7$ mediates tethering and rolling with relatively high efficiency [21], LFA-1 has limited capacity [22], and VLA-4 tethering and rolling is optimal only after prior activation (e.g. by chemokines [23]). Generally, VLA-4 and LFA-1 are principally involved in firm adhesion of leukocytes (Step 3), and both are broadly expressed on all subsets of normal human lymphocytes.

The selectins are a family of integral membrane glycoproteins that function as Ca^{2+} -dependent lectins in binding to carbohydrate determinants expressed on their respective ligands, the prototypes of which are sialyl Lewis x (sLex) and sialyl Lewis a (sLea) [24]. L-selectin is expressed on leukocytes, whereas E-selectin and P-selectin are typically inducible endothelial molecules, and P-selectin is also expressed on activated platelets. Importantly, in humans, there is permanent expression of E-selectin (and, in mice, both E- and P-selectin) on the microvasculature of two tissue beds, the skin and the bone marrow [reviewed in 25*]; as will be described below, this pattern of expression has great implications for tissue-specific homing. The selectins bear the unique property of binding optimally to their ligands under physiologic shear conditions [15,26, 27]. Indeed, L-selectin binding to its ligands does not occur under static conditions [26]. Thus, Stamper and Woodruff would have never observed lymphocyte-HEV interactions in the first place had they performed their assays without shear.

To date, three tissue-specific lymphocyte homing receptors have been identified: L-selectin for homing to lymph nodes, $\alpha_4\beta_7$ for homing to GALT and gastrointestinal tract, and cutaneous lymphocyte antigen (CLA) for homing to skin (reviewed in [28*]). L-selectin is characteristically expressed at high levels among lymphocytes that recirculate continuously through lymph nodes, such as naïve

lymphocytes and central memory lymphocytes. However, L-selectin expression is typically low on effector and effector memory lymphocytes; instead, these cells bear high-level expression of tissue-specific homing receptors, such as those directing trafficking to gut and skin. Selectivity in L-selectin mediated homing to lymph node is due to constitutive expression of L-selectin ligands on HEV, known collectively as peripheral lymph node addressins (PNAd), which are reactive with a mAb known as MECA 79 [29]. PNAd comprises a family of sialomucins, one of which is a glycoform of CD34 expressed exclusively on HEV [30]. Although the expression of PNAd may be induced at certain inflammatory sites, their permanent expression on HEV (and that of CCL21) ensures that L-selectin+/CCR7+ lymphocytes home to lymph nodes. Gut-seeking effector and memory lymphocytes have high-level expression of $\alpha_4\beta_7$, which recognizes another ligand known as MAdCAM-1, which is permanently expressed on HEV of GALT and the microvascular endothelium of the intestinal lamina propria. In addition to $\alpha_4\beta_7$, L-selectin also binds MAdCAM-1 on HEV that bears specialized sialic acid modifications [31]; thus, L-selectin may also contribute to lymphocyte migration to GALT, but the principal homing receptor is $\alpha_4\beta_7$. In turn, lymphocytes homing to small intestine bear chemokine receptors for a chemokine, TECK (CCL25), which is constitutively expressed (and increased by inflammation) on lamina propria endothelium (reviewed in [8]).

The principal leukocyte counter-receptor for the vascular selectins is P-selectin glycoprotein ligand-1 (PSGL-1) (reviewed in [32]). PSGL-1 is a cell surface mucin-like glycoprotein that can serve as a ligand for all three selectins; however, specialized post-translational modifications on the core PSGL-1 protein backbone are necessary for ligand activity for each of the selectins. In particular, PSGL-1 E-selectin ligand activity depends on critical sialic acid and fucose modifications that are recognized by the rat IgM mAb HECA452 [33], which is directed against a sialyl Lewis x-like epitope [34]. On human lymphocytes, the expression of the HECA452 determinant on PSGL-1 is known as CLA. Early studies showed that most lymphocytes in skin display HECA452 reactivity, that this CLA determinant was found on a subset of skin-homing memory T cells, and that the CLA epitope itself was involved in binding to E-selectin [34]. Later biochemical studies showed that the CLA epitope is located on PSGL-1 [35], and more recent studies have provided direct evidence that only CLA(+)PSGL-1 functions as an E-selectin ligand whereas both CLA(+) and CLA(–) glycoforms of PSGL-1 can bind P-selectin [36]. The association between HECA452-reactivity and lymphocyte trafficking to skin was then operationally linked: just as L-selectin on lymphocytes mediates homing to lymph nodes that permanently express L-selectin ligands (PNAd/MECA79 antigens) on HEV, CLA(+)PSGL-1 on effector memory

Table 1. List of currently known homing receptors and ligands.

Lymphocyte homing receptor	Endothelial ligand(s)
L-selectin	PNAd (CD34 & other MECA79 antigens), MAdCAM-1
PSGL-1	P-selectin
CLA	E-selectin, also P-selectin
CD44 [HCELL]	Hyaluronic acid, E-selectin
$\alpha_4\beta_7$ (LPAM-1)	MAdCAM-1, also VCAM-1
$\alpha_4\beta_1$ (VLA-4)	VCAM-1
$\alpha_L\beta_2$ (LFA-1)	ICAM-1, ICAM-2
?	VAP-1
?	LOX-1

CLA, cutaneous lymphocyte antigen; HCELL, hematopoietic cell E-/L-selectin ligand; PNAd, peripheral lymph node addressins; PSGL-1, P-selectin glycoprotein ligand-1.

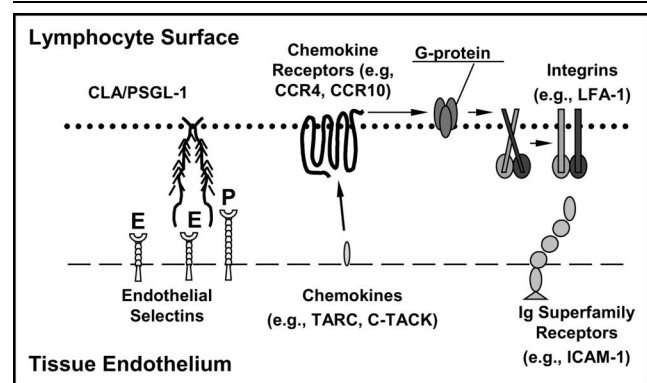
T cells engage E-selectin (and P-selectin), which is constitutively expressed on dermal microvasculature [37]. Notably, expression of the CLA carbohydrate determinants is tightly regulated by glycosyltransferases, induced by interleukins [38–40], providing primary control of lymphocyte trafficking into skin.

We have exploited this requirement for de-novo glycan synthesis in the elaboration of the CLA epitope to block dermatotropism of lymphocytes. Using a novel fluorinated analog of *N*-acetylglucosamine (2-acetamido-1,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro- β -D-glucopyranose, otherwise known as '4-F-GlcNAc') that prevents the synthesis of the polylactosamine carbohydrate chain presenting the CLA epitope on PSGL-1, both *in vitro* [41] and *in vivo* [42] experiments have shown a dramatic decrease in CLA synthesis and in lymphocyte homing to skin in a model of contact hypersensitivity. Notably, administration of this agent *in vivo* only prevented effector cell migration to skin without compromising the sensitization phase, thus achieving a profound anti-inflammatory effect without blunting immune responses critical to host defense. Independently of changes in lymphocyte CLA levels, dermal inflammatory reactions are associated with up-regulation of both E- and P-selectin on skin vessels, thus facilitating recruitment of CLA+ T cells to inflamed skin [37,43]. Normal and inflamed skin microvascular endothelium displays chemokines TARC (CCL17) and C-TACK (CCL27), which are recognized by receptors CCR4 and CCR10, respectively, expressed on CLA+ lymphocytes [reviewed in 28] (Fig. 2). Therefore, just as with lymphocyte migration to lymphoid tissues and gut, the combined expression of appropriate receptor/ligands for Step 1 effectors and chemokines directs trafficking to skin.

In addition to its function in regulating homing to skin as CLA, experiments employing parabiosis and short-term homing assays have recently shown that PSGL-1 may mediate recruitment of prothymocytes into thymus via interactions with constitutively expressed P-selectin on thymic endothelium. Consistent with the known requirement for specialized carbohydrate modifications on PSGL-1 to bind P-selectin, prothymocytes deficient in expression of the key O-glycan branching enzyme (known as core 2 β 1,6-glucosaminyltransferase-I) showed a profound decrease in the capacity to seed the thymus [44•]. These data suggest that the PSGL-1/P-selectin axis functions as the thymus homing receptor in the mouse, but it is presently unclear whether this holds true for homing to human thymus.

A variety of different experimental results now indicate that the biology of CD44 receptor/ligand interactions in Step 1 is far more complicated than originally perceived. The CD44 molecule is widely distributed among hematopoietic and nonhematopoietic cells and is known best for

Figure 2. Molecular components of lymphocyte–dermal endothelial interactions mediating trafficking to skin.



The lymphocyte membrane molecules are shown on the dotted line, with relevant endothelial counterreceptors shown on dashed line. See text for details.

its role in binding extracellular matrix elements, principally hyaluronic acid, for which it is known as the 'hyaluronic acid receptor.' CD44 is an extremely heterogeneous and pleiotropic protein, because of alternative splicing of 10 encoding exons and a variety of post-translational modifications. Lymphocytes express predominantly the 'standard' unspliced CD44 isoform (core m.w. ~37,000) that migrates as a glycoprotein of ~98,000 m.w. on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, but lymphocyte activation is associated with expression of CD44 isoforms [45–47]. Over the past few years, a number of studies have shown that CD44/hyaluronic acid interactions mediate tethering and rolling of lymphocytes on endothelium under physiologic shear stress [18,48,49], and its role in lymphocyte migration to inflammatory sites is now well established [49,50]. Conversely, although the conventional view holds that hyaluronic acid is an inert, viscoelastic substance that functions as a 'space filler,' there is emerging evidence that specialized deposits of hyaluronic acid occur at inflammatory sites that promote engagement of CD44 [reviewed in 51•]. Indeed, it has been thought that native CD44 on resting lymphocytes has relatively low affinity for hyaluronic acid [50,52,53], and binding to hyaluronic acid requires prior activation of the cells. However, using Stamper-Woodruff assay conditions, we recently found that resting human lymphocytes avidly bind hyaluronic acid deposited on skin papillary endothelium of patients with acute cutaneous graft-versus-host disease [54••]. These findings highlight a previously unrecognized role of the regulation of hyaluronic acid structure/expression in promoting lymphocyte adhesive interactions under shear conditions. Thus, qualitative changes in CD44 and hyaluronic acid may singly, or together, result in profound effects on lymphocyte homing patterns. In fact, apart from splice variations and activation-induced modulation, post-translational changes such as glycosylations critically affect CD44 affinity for hyaluronic

acid [55,56] and confer new biology on CD44. For example, among human hematopoietic progenitor cells, a specialized sialofucosylated glycoform of CD44 known as hematopoietic cell E-/L-selectin ligand (HCELL) functions as the most potent E- and L-selectin ligand expressed on any cell type [reviewed in 25[•]]. Importantly, HCELL confers greater E-selectin ligand activity over a wider range of physiologic shear stresses compared with CLA [57]. This fact, combined with the high-level permanent expression of E-selectin on human (and murine) bone marrow endothelium, suggests that HCELL functions as the 'bone marrow homing receptor' for hematopoietic stem cells. Although HCELL expression is thought to be restricted to hematopoietic stem cells and not yet observed on lymphocytes, the fact that HCELL is a glycoform of CD44 created by expression of appropriate glycosyltransferases raises the hypothesis that transient up-regulation of these glycosyltransferases (in parallel fashion to the production of CLA on the PSGL-1 backbone) may contribute to CD44-specific selectin binding capabilities on subsets of lymphocytes.

New perspectives on homing receptors

At present, we cannot be confident that we know the identity of all homing receptors. In addition to the above-mentioned molecules, two endothelial structures known as LOX-1 [58] and VAP-1 [59] have also been implicated as mediators of Step 1 adhesive interactions (Table 1). Both are inducible molecules, and, at least for the case of VAP-1, there appears to be an obligatory contribution of other adhesive receptor/ligand interactions for engagement of this axis. Moreover, their importance in controlling lymphocyte trafficking is relatively uncertain at present because the relevant counterreceptors for these structures are unidentified. It may be particularly challenging to define the pertinent ligand(s) if they, just as with selectin receptor/ligand interactions, display shear-dependent binding. We had encountered this requirement for dynamic mechanical strain earlier in our attempts to identify the HCELL molecule, and we developed a new technique called the 'blot rolling assay' to address this feature [36,60]. This assay examines, under defined shear conditions, interactions between cells or particles in flow and adhesion molecules bound to blotting membranes. Relevant membrane molecules are resolved into component bands by gel electrophoresis and then transferred to a membrane (e.g. by Western blot). The membrane is then rendered semitransparent, incorporated into a parallel-plate flow chamber apparatus, and mounted on an inverted microscope. Cells or particles bearing known adhesion molecules (e.g. selectins) are introduced under controlled flow conditions, and tethering and rolling interactions on substrate band(s) are observed by video microscopy. The pertinent substrate molecule(s) can then be identified by immunostaining, or, if the protein is unknown/unrecognized, it can be extracted for mass spectrometry or

microsequencing. The blot rolling assay thus allows reproducible identification of both novel and known adhesion molecules that mediate binding under shear stress. Importantly, homing receptors can be identified by functional criteria within a complex mixture without requiring prior isolation or enrichment of the constituent molecules.

New data suggest that rolling interactions may be more complex than previously imagined, adding a new dimension of mechanosignaling to the multistep paradigm. The Step 3 effectors LFA-1 and VLA-4 are characteristically expressed on all subsets of lymphocytes, but binding to their respective endothelial ligands requires that they assume an 'activated' conformation [reviewed in 9]. A number of reports have indicated that engagement of homing receptors on leukocytes and other (nonhematopoietic) cells can be sufficient to activate integrins and result in firm adhesion in the absence of exogenous chemoattractants [61–67,68^{••},69^{••}]. Notably, for CD44, cross-linking not only directly results in activation of integrin adhesiveness, but also results in transendothelial migration in the absence of chemokines [67,68^{••}]. The molecular basis of this short-circuit effect may reflect a unique coassociation between CD44 and VLA-4. A recent report [70^{••}] demonstrates that the cytoplasmic tail of CD44 promotes the assembly of a bimolecular complex with VLA-4 on T cells, which results in direct Step 1-to-Step 3 cooperative interactions (i.e. direct transition of lymphocyte rolling to firm adhesion) after engagement of CD44, bypassing the requirement for chemokines. Clearly, further studies are warranted to determine whether the observed synergism between CD44 and VLA-4 extends to other Step 1 and Step 3 effectors. These observations raise the possibility that, dependent on site densities of relevant endothelial ligands and the expression/clustering of membrane homing receptor(s), engagement of homing receptors may promote activation of integrins, either eliminating the need for chemokine-induced activation or greatly reducing the threshold needed to prime integrins. This nuance was not originally predicted in the formulation of the multistep paradigm, yet it has remarkable implications: homing receptors may be sufficient alone, in some cases, to perform the function the name implies.

Conclusion

Over the past decade, somewhat overlooked by the intense scientific inquiry into the extraordinary diversity of chemokines and chemokine receptors is the fact that chemoattractant molecules are ineffective in lymphocyte recruitment without prior engagement of homing receptors. Furthermore, although the multistep paradigm has been validated by abundant *in vitro* and *in vivo* (e.g. intravital microscopy) studies, an emerging body of data is now suggesting that homing receptors can trigger integrin adhesiveness and transmigration in the absence of chemokines. This evolving cross-talk concept is not a challenge to the

significance of chemokines in lymphocyte migration, but merely serves to point out that dynamic overlap/redundancy exists throughout the recruitment cascade. Future work will be needed to determine the circumstances *in vivo* under which homing receptors can trigger integrins without chemokines, establish just how many homing receptors exist and on what lymphocyte subset(s), and elucidate the full spectrum of known (or yet unrecognized) homing receptor(s) whose engagement can result directly in integrin activation and transendothelial migration. Answers to these questions will no doubt help resolve many current mysteries regarding lymphocyte recruitment and will unravel the 'rolls' of the homing receptors in the multistep paradigm.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 513–514).

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