SELECTINS IN T-CELL RECRUITMENT TO NON-LYMPHOID TISSUES AND SITES OF INFLAMMATION

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The ability to home to extralymphoid tissues, such as the skin, lungs and gut, is acquired by many effector and memory T cells after initial antigen encounter. This review discusses the role of E-, P- and L-selectin and their glycoprotein ligands in this process. During activation, some T cells differentiate to bind E-selectin through induced expression of fucosyltransferase-VII and to bind P- and L-selectin through additional expression of core 2 glucosaminyltransferase-I. This inducible expression of selectin ligands, together with the regulation of L-selectin expression, has a role in the recruitment of activated T cells to extralymphoid tissues and sites of inflammation.

TETHERING OR CAPTURE
The initial, transient adhesive contact of a T cell with the endothelium or an adherent leukocyte or platelet.

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T cells are found in almost all tissues and organs in the body. Naive T cells almost exclusively traffic through secondary lymphoid organs, including peripheral and mesenteric lymph nodes, Peyer's patches and the spleen, in which they are most likely to encounter antigen in a context that induces their activation and clonal expansion[1].

After antigen encounter, T cells leave the secondary lymphoid organs through efferent lymphatics and enter the systemic circulation through the thoracic duct (FIG. 1). Most activated T cells can home to extralymphoid sites in the presence or absence of inflammation. It is believed that activated T cells enter extralymphoid tissues from blood vessels, most probably postcapillary venules, through a cascade-like process that is similar to that used by neutrophils and monocytes. This cascade starts with a TETHERING OR CAPTURE process that establishes contact between the T cell and the endothelial cell, followed by rolling, arrest and eventually transmigration[2,3]. Although this is a plausible scenario, as there is good evidence that antigen-experienced T cells can enter the non-inflamed lungs[4], gut[5], skin[6] and central nervous system[7], there has been no direct visualization of T-cell recruitment to extralymphoid tissues.

This review explores the role of selectins and their ligands in the trafficking of T cells to extralymphoid sites. As more is known about the regulation of selectin binding in $CD4^+$ T cells than $CD8^+$ T cells, most of the presented material is derived from studies of $CD4^+$ T cells, with the understanding that many, but not all, findings might also hold true for $CD8^+$ T cells. In this article, we emphasize functions that are important in human disease processes, based on studies in cell lines, isolated T cells, and mouse models of T-cell trafficking and disease.

Selectins

The selectins are a family of three C-TYPE LECTINS expressed exclusively by bone-marrow-derived cells and endothelial cells. The selectins are identified by capital letters, L for leukocyte (L-selectin), E for endothelial cell (E-selectin), and P for platelet and endothelial-cell selectin (P-selectin). Their modular structure — an amino-terminal lectin domain, one EGF-like domain, several consensus repeats with homology to complement regulatory proteins, a single membrane-spanning domain and a carboxy-terminal cytoplasmic domain — has been reviewed elsewhere[8–10].

The main physiological function of all selectins is in mediating leukocyte adhesion under flow, but both selectins and their ligands also have signalling functions[11]. L-selectin is expressed by all myeloid cells, naive T cells, and some activated and memory T cells, whereas P-selectin is found in secretory granules of platelets and

C-TYPE LECTINS
Calcium-dependent animal lectins that are carbohydrate-binding proteins. The binding activity of C-type lectins is based on the structure of the carbohydrate-recognition domain (CRD), which is highly conserved among this family. Calcium is essential not only for the carbohydrate binding itself, but it also contributes to the structural maintenance of this domain.

LEUKOCYTE ROLLING ASSAYS
Assays in which T cells or other leukocytes are allowed to interact with endothelial cells or recombinant adhesion molecules in the presence of shear stress, usually induced by perfusing blood or cell-culture media. These assays are essential for testing physiologically relevant selectin-mediated interactions.

is expressed on the platelet surface after activation, and E-selectin is expressed by acutely inflamed endothelial cells in most organs and in non-inflamed skin microvessels (TABLE 1). P-selectin is also constitutively expressed on the endothelium of lung and choroid plexus microvessels[12], and is inducibly expressed by inflamed endothelial cells in many diseases including atherosclerosis[8,10]. Physiologically relevant selectin interactions with their ligands can be identified by testing the tethering and rolling of leukocytes on endothelial cells or recombinant molecules in flow chamber systems *in vitro*, or in microvessels *in vivo*.

Selectin ligands

Carbohydrate ligands for selectins. Selectin ligands are carbohydrate-containing molecules, and several glycosyltransferases that have a role in the biosynthesis of selectin ligands have been identified (TABLE 2). These include two α1,3-fucosyltransferases, FucT-VII and FucT-IV, the O-linked branching enzyme core 2 β1,6-glucosaminyltransferase-I (C2GlcNAcT-I), a β1,4-galactosyltransferase-I (β1,4GalT-I), and at least two sialyl transferases of the ST3Gal family that add sialic acid to galactose in a 2–3 linkage, one of which is ST3Gal-IV. A composite structure of a prototypic carbohydrate selectin ligand, resulting from the activity of these enzymes, is outlined in FIG. 2. In addition, at least one of two tyrosine sulphotransferases[13] must be active to produce high-affinity P-selectin binding, and the sulphated tyrosine residue(s) of P-selectin glycoprotein ligand 1 (PSGL1) directly participate in P-selectin binding[14].

Glycoprotein scaffolds for selectin ligands. To bind selectins effectively, ligand carbohydrate structures must be presented on specialized glycoprotein scaffolds (TABLE 3). PSGL1 is a 240 kDa homodimer that, if properly glycosylated, can bind all three selectins[15]. FucT-VII, C2GlcNAcT-I, β1,4GalT-I and at least one ST3Gal are essential for PSGL1 binding to P- and L-selectin, whereas PSGL1 binding to E-selectin seems to mainly require FucT-VII and ST3Gal activity, although there is evidence that the initiation of E-selectin-dependent rolling (tethering or capture) requires PSGL1 (REFS 16,17) and C2GlcNAcT-I (REF. 18). However, sustained E-selectin-mediated rolling is not PSGL1 or C2GlcNAcT-I dependent[18,19]. These distinct enzyme requirements for individual selectins binding to PSGL1 reflect the observation that although the P- and L-selectin binding epitope is at the extreme amino-terminus of PSGL1, it is different from the E-selectin binding epitope[20]. Indeed, commonly used PSGL1-specific antibodies, such as PL-1 or KPL-1 for human PSGL1, and 4RA10 or 2PH1 for the mouse molecule[21], do not block E-selectin binding. Although PSGL1 protein is expressed by all T cells, it is not always glycosylated correctly for selectin binding and this probably explains why naive and many other T cells cannot bind selectins. In activated T cells, glycosyltransferases are expressed that modify PSGL1 and enable its binding to all three selectins (FIG. 3). Investigation of Psgl1-deficient mice showed that P-selectin also binds ligands other than Psgl1, although Psgl1 activity accounts for more than 90% of P-selectin binding activity in LEUKOCYTE ROLLING ASSAYS[17,22–24]. The nature of these other P-selectin ligands[17] is unknown, and it is unclear whether they have any role in T-cell homing to extralymphoid tissues.

Although E-selectin can bind to PSGL1 (REFS 15,25), PSGL1 is not the main E-selectin ligand, and the other E-selectin ligand(s) remain poorly defined[19]. Several reports have indicated that human neutrophil L-selectin might be glycosylated such that it can serve as a ligand for human E-selectin[26,27], but the role of this interaction is controversial, even for neutrophils, and it is unlikely to have a role in T-cell homing to extralymphoid sites. In bovine γδ-T cells, a 250 kDa ligand for E-selectin has been described[28], but it is not known whether a homologous molecule exists in humans or mice. Although E-selectin ligand 1 (ESL1) has been shown to bind E-selectin by biochemical methods *in vitro*[29], its functional importance, if any, in T-cell or other leukocyte trafficking has not been described.

Antibodies specific for the tetrasaccharide SIALYL-LEWIS X (sLe^x) recognize most, but not all selectin-binding molecules[30], and also recognize other carbohydrate

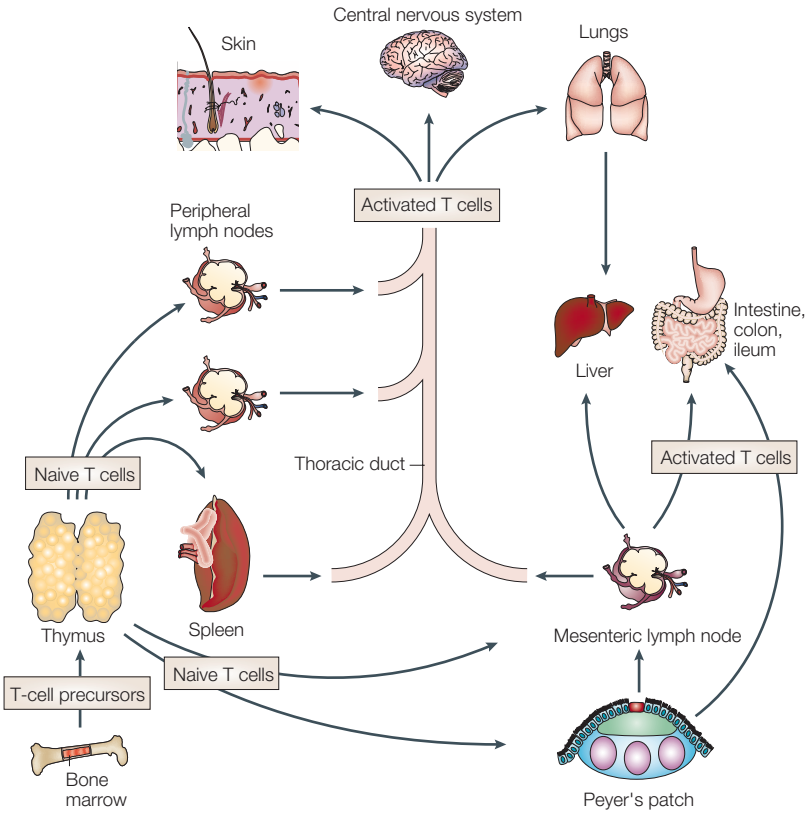

Figure 1 | **T-cell trafficking patterns.** T cells originate from bone-marrow precursors that mature into naive T cells in the thymus. Naive T cells traffic to secondary lymphatic organs, including peripheral lymph nodes, Peyer's patches, mesenteric lymph nodes and the spleen, where they might encounter antigen and become polarized into T helper 1 (T_H1), T_H2 and other effector T cells, which are collected in efferent lymphatics and enter the circulation through the thoracic duct. Activated T cells traffic to extralymphoid organs, including the uninflamed lungs, skin, central nervous system and gastrointestinal organs. Many activated T cells ultimately migrate to the liver to undergo apoptosis. Activated T cells can home to almost all inflamed organs and tissues (not shown).

Table 1 | **Selectin expression**

Selectin	Cell/tissue	Expression pattern	References
L-selectin	Myeloid cells	Constitutive	8
	Naive T cells	Constitutive	8
	Effector T cells	Low/negative	82
	Effector memory T cells	Absent	82
	Central memory T cells	Re-expressed or retained	82
E-selectin	Skin endothelium	Constitutive	33
	Inflamed endothelium	Inducible in most organs	8
P-selectin	Choroid plexus	Constitutive	12
	Lung endothelium	Constitutive	105
	Platelets	After activation	106
	Platelet-derived microparticles	Constitutive	107
	Peritoneal macrophages	Constitutive	108
	Inflamed endothelium	Inducible in most organs	8,10

SIALYL-LEWIS X (sLe^x). Sialylated and fucosylated tetrasaccharide (GlcNAc(α1,3Fuc)β1,4Galα2,3 Sia) related to selectin ligands.

HIGH ENDOTHELIAL VENULES (HEVs). The site of entry for lymphocytes from the blood stream into lymph nodes and Peyer's patches.

structures that are unrelated to selectin ligands. It is controversial whether sLe^x expression is actually required for selectin binding, because certain cell lines that lack sLe^x expression bind selectins, whereas others that express sLe^x do not[31], and in most assays, sLe^x-specific monoclonal antibodies do not block recognition of selectins. The monoclonal antibody HECA-452 recognizes the T-cell antigen cutaneous lymphocyte antigen (CLA), so called as HECA-452 reactivity is preferentially found in T cells that infiltrate the skin[32]. HECA-452 recognizes sLe^x and/or a closely related carbohydrate epitope, and HECA-452 reactivity correlates with the ability of T cells to bind to E-selectin, which is constitutively expressed in skin microvessels[33]. HECA-452 reactivity of leukocytes[34] and endothelial cells[35] also correlates with their ability to bind L-selectin. One report indicates that CLA is a specialized glycoform of PSGL1 (REF. 25), but the study did not show that the HECA-452-reactive glycoform of PSGL1 was actually required for E-selectin binding or skin homing. Therefore, although HECA-452-reactive cells are more likely to bind E-selectin and home to the skin, a possible causal relationship remains to be shown. HECA-452 reactivity is a good marker for FucT-VII activity in human T cells, because its expression level quantitatively correlates with enzyme activity and mRNA levels[36,37].

In addition to binding to PSGL1, L-selectin also binds to endothelial ligands, most of which are characterized by MECA-79 reactivity and are collectively known as peripheral node addressins (PNADs). MECA-79 is a monoclonal antibody that detects sulphated *N*-acetylglucosamine (GlcNAc-6-SO_4)[38]. The glycoprotein structure(s) that express the MECA-79 antigen are not completely known, but include CD34 (REFS 39,40). Luminal expression of the MECA-79 antigen requires the activity of a specific sulphotransferase known as high endothelial cell (HEC) -GlcNAc6ST, and mice lacking this enzyme have deficiencies in naive T-cell homing to peripheral lymph nodes[41]. In the context of T-cell trafficking to extralymphoid sites, it is important to realize that the MECA-79 antigen can also be expressed at sites of chronic inflammation. The organized lymphoid aggregates that form in chronic inflammatory settings generally contain venules with a morphology that is similar to the HIGH ENDOTHELIAL VENULES (HEVs) found in lymphoid tissues that express PNAD[42,43]. MECA-79$^+$ vessels — of a high-walled or flat phenotype — are also found in other inflammatory lesions where organized lymphoid structures are not present[38,44]. In several examples of chronic inflammation, the L-selectin–PNAD system has been confirmed to be functionally significant in lymphocyte recruitment[45–48]. Furthermore, in models of autoimmune diabetes, several studies have reported prominent pancreatic PNAD expression and an important role for L-selectin in T-cell homing to the pancreatic islets[49,50].

Finally, glycolipids have the ability to bind to selectins and support the rolling of leukocytes under flow[51], but their physiological importance for T-cell binding has not been established, and they are not discussed further.

Selectin-ligand biosynthesis in T cells

In contrast to myeloid cells such as neutrophils and monocytes, which constitutively express all of the enzymes required for selectin-ligand biosynthesis (TABLE 2), expression of at least some of these enzymes is inducible and regulated in T cells. Indeed, because there is no evidence that the activity of any glycosyltransferase is regulated by post-translational mechanisms, such as phosphorylation, regulation of expression of the genes that encode these enzymes is functionally equivalent to regulation of expression of selectin ligands. So, neutrophils, monocytes, dendritic cells and other myeloid cells constitutively express high levels E-, P- and L-selectin ligands on PSGL1 and possibly other scaffolds, whereas T cells express them in a heterogeneous and regulated manner (FIG. 3). The pathways by which expression of these enzymes is regulated in T cells are considered later.

Table 2 | **Glycosyl- and sulphotransferases relevant to selectin-ligand biosynthesis**

Enzyme	Abbreviation	Involved in biosynthesis of ligand for	Expression
Fucosyltransferase-VII	FucT-VII	L-, E- and P-selectin	Myeloid cells, activated T cells and HEVs
Fucosyltransferase-IV	FucT-IV	L-, E- and P-selectin*	Myeloid cells and some HEVs
Core 2 β1,6-glucosaminyltransferase	C2GlcNAcT-I	L- and P-selectin	Myeloid cells, activated T cells and B cells
Sialyl 3-galactosyltransferase	ST3Gal	L-, E- and P-selectin	Ubiquitous
β1,4-galactosyltransferase	β1,4GalT-I	P-selectin‡	Ubiquitous
Tyrosine protein sulphotransferases 1,2	TPST1,2	L- and P-selectin	Ubiquitous
HEC-glucosaminylsulphotransferase	HEC-GlcNAc6ST	L-selectin	HEVs and chronically inflamed endothelial cells

*Only in myeloid cells, not T cells. ‡Not yet tested for E- or L-selectin; probably involved in L-selectin-ligand biosynthesis. HEC, high endothelial cell; HEV, high endothelial venule.

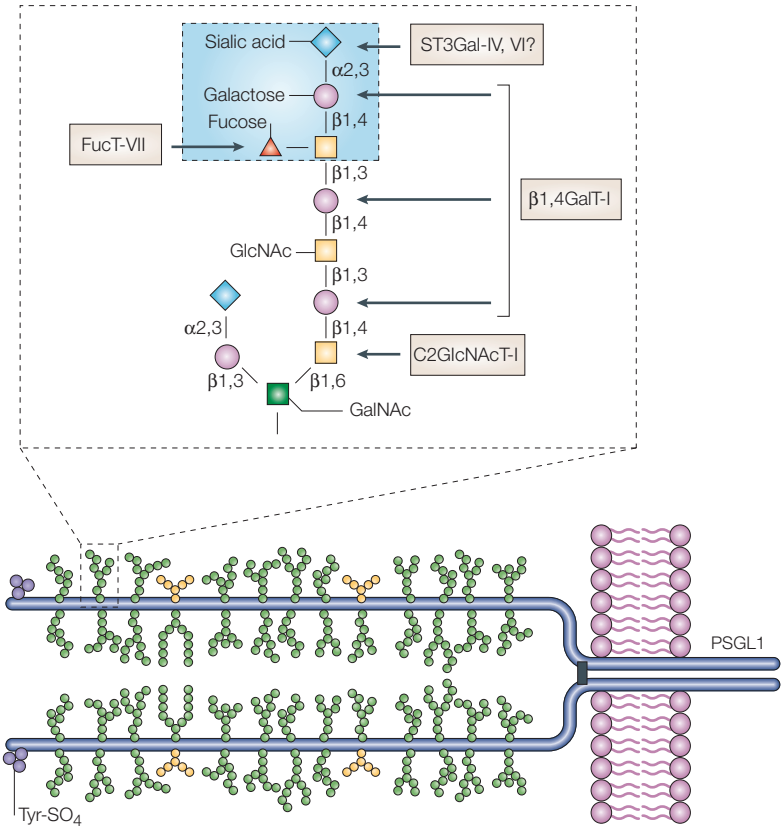

Figure 2 | **Prototypic selectin ligand.** The selectin-binding carbohydrate structure is O-linked through *N*-acetylgalactosamine (GalNAc) to a serine or threonine residue. The core 2 branching enzyme core 2 β1,6-glucosaminyltransferase-I (C2GlcNAcT-I) attaches a GlcNAc in β1,6. The chain is extended by a galactosyltransferase (β1,4GalT-I), and can contain variable numbers of repeating lactosamine units. The last *N*-acetylglucosamine is fucosylated in α1,3 linkage by fucosyltransferase-VII (FucT-VII), whereas FucT-IV would fucosylate the penultimate GlcNAc (not shown). The structure is terminated by a sialic acid attached by sialyl 3-galactosyltransferase (ST3Gal-IV) and possibly ST3Gal-VI. The blue box indicates sialyl-Lewis x structure. This, or a similar ligand structure, is found on threonine 57 of human P-selectin glycoprotein ligand 1 (PSGL1); protein backbone (blue), O-linked glycans (green), N-linked glycans (yellow) and sulphated tyrosines (purple). The L-selectin-binding glycan structure of peripheral node addressin (PNAD) (not shown) is different and contains GlcNAc-6-SO_4 and sialic acid.

Fucosyltransferase-VII. On the basis of analysis of mice engineered to be deficient in specific enzymes, it has been shown that FucT-VII is essential for the synthesis of all selectin ligands in T cells. FucT-VII-deficient T cells have undetectable expression of selectin ligands in all assays, including binding of soluble recombinant selectins, rolling on individual selectins *in vitro* and rolling on endothelial cells at sites of inflammation *in vivo*[37,52,53]. The absence of FucT-VII expression by naive T cells explains the lack of selectin ligands on these cells. Furthermore, T helper 1 (T_H1) cells, generated *in vitro* from naive T cells, have high levels of expression of FucT-VII and selectin ligands, whereas the low levels of FucT-VII in T_H2 cells, produced by the polarization of naive T cells *in vitro*, correlate with the low level of selectin ligands on these cells[54–56]. However, the levels of FucT-VII required for the generation of P- and E-selectin ligands differ, with E-selectin ligands requiring higher levels[36,37]. FucT-VII is therefore the limiting enzyme for E-selectin-ligand biosynthesis. In mouse neutrophils, FucT-IV also contributes to E-selectin-ligand biosynthesis[57], but FucT-IV is expressed at low levels by T cells and does not seem to contribute to the generation of functional selectin ligands in T cells.

The induction of FucT-VII expression by T cells seems to be controlled mainly by the T-cell receptor (TCR), and recent data indicate that marked RAS activation, leading to activation of mitogen-activated protein (MAP) kinases is an important pathway downstream of the TCR that induces FucT-V11 expression[58] (FIG. 4). Together with the observation that a subset of activated $CD4^+$ T cells or retrovirally infected Jurkat T cells express FucT-VII (REF. 58), this indicates that a threshold of TCR signal strength might govern the induction of FucT-VII expression by a population of activated T cells. Heterogeneity of expression levels of FucT-VII might therefore arise in a clonally derived T-cell population as a function of antigen dose, leading to specialization of daughter clones for distinct functions, including B-cell help, memory or homing to sites of inflammation. This is consistent with the observation that the dose of antigen can regulate whether a cell-mediated or humoral immune response is mounted[59].

In both mice and humans, the T_H1-type cytokine IL-12 enhances or maintains FucT-VII expression[54,55,60]. However, the relative importance of TCR- and IL-12-derived signals is apparently different in humans compared with mice, with IL-12 signals being more important than TCR signals in humans and *vice versa* in mice; although to what extent these apparent species differences are due to different populations of T cells under study is not clear. The mechanism by which IL-12 acts to regulate FucT-VII levels is also not clear, but it does not seem to require the transcription factor signal transducer and activator of transcription 4 (STAT4)[61]. By contrast, the T_H2-type cytokine IL-4 represses the induction of FucT-VII expression, at least *in vitro*, by naive $CD4^+$ T cells[54,55,62]. These opposing effects on FucT-VII levels explain why naive $CD4^+$ T cells polarized *in vitro* towards T_H1 or T_H2 have high or low levels, respectively, of FucT-VII mRNA and selectin ligands. High levels of FucT-VII enable the biosynthesis of E-selectin ligands, whereas low levels are sufficient for the biosynthesis of P-selectin ligands. The lower level of FucT-VII required for P-selectin-ligand biosynthesis indicates that during the course of T-cell activation, T cells will express functional P-selectin ligands earlier or more rapidly than E-selectin ligands, consistent with P-selectin having a more important role in early recruitment to sites of inflammation.

However, T_H2 cells from atopic individuals express FucT-VII mRNA and CLA — a marker for FucT-VII expression in humans[63–65]. If IL-4 represses the induction of FucT-VII expression, how can T_H2 cells express FucT-VII and selectin ligands? First, it should be emphasized that the repression of FucT-VII by IL-4 is so far largely an *in vitro* finding, and might not be relevant for the generation of T_H2 cells *in vivo*. Second, examination of $CD4^+$ T-cell clones that have been propagated extensively *in vitro* under polarizing conditions revealed that all of them, whether they are

Table 3 | **Selectin glycoprotein ligands involved in T-cell homing**

Selectin glycoprotein ligand	Selectins bound	Cell/tissue	Expression pattern and functionality	References
PSGL1	P-, L- and E-selectin	Myeloid cells	Constitutively functional	15
		Naive T cells	Not functional	15
		Effector T cells	Functional in some	5,76,77
		Monocyte-derived microparticles	Constitutive	107
PNAD (several)	L-selectin	High endothelial venules	Secondary lymphatic organs	38
		Inflamed endothelium	Pancreas, salivary glands and others	43

PNAD, peripheral node addressin; PSGL1, P-selectin glycoprotein ligand 1.

T_H1, T_H0 or T_H2 type, expressed high levels of FucT-VII (G.S.K., unpublished observations). This suggests a model by which naive T-cell induction of FucT-VII expression in response to TCR ligation can be inhibited by IL-4, but this ability to repress FucT-VII expression is gradually lost with further stimulation. How FucT-VII expression is regulated in T cells *in vivo* remains to be explored.

The physiological consequences of FucT-VII induction were shown in an adoptive transfer model, in which T_H1 cells polarized *in vitro* acquired the ability to bind to P-selectin and home to the intestinal lamina propria in a P-selectin-dependent manner[5]. As inducible regulated expression of FucT-VII by T cells is likely to involve distinct transcriptional mechanisms compared with constitutive expression by myeloid cells, this enzyme is an attractive target for pharmacological intervention in inflammatory disease.

Core 2 β1,6-glucosaminyltransferase-I. The core 2 transferase C2GlcNAcT-I — one of at least three such core 2 transferases — is required for the generation of P- and L-selectin ligands through modification of PSGL1, but is dispensable for the generation of most E-selectin ligands[18,66,67]. Neutrophils from C2GlcNAcT-I-deficient mice show only modest defects in binding to E-selectin at high shear stress[17,18], which indicates that C2GlcNAcT-I is not strictly required for most E-selectin binding. Established T-cell rolling on E-selectin, as distinct from tethering (initiation of rolling), is not affected, and even tethering is only partially affected by the absence of C2GlcNAcT-I. The quantitative importance of this E-selectin tethering function of PSGL1 in T-cell recruitment might be limited *in vivo*.

C2GlcNAcT-I expression levels are low in naive T cells, but are markedly upregulated *in vitro* in both T_H1 and T_H2 cells, as well as in T_H0 cells[54,55,61]. T_H1-cell induction of C2GlcNAcT-I in response to IL-12 depends on STAT4 (REFS 55,61). As many signals downstream of the IL-4 and IL-2 receptors are known to require STAT6 and STAT5, respectively[68], it has been suggested that C2GlcNAcT-I expression in response to IL-4 or IL-2 (particularly for $CD8^+$ T cells) might involve the activation of STAT6 and STAT5, providing a model by which many cytokines acting through many different STAT proteins can upregulate C2GlcNAcT-I expression. Whether this pattern of regulation is the same in $CD4^+$ and $CD8^+$ T cells is not yet clear, although IL-12 is a potent inducer of C2GlcNAcT-I expression in $CD8^+$ T cells, as is IL-2 (REF. 69). The crucial role for C2GlcNAcT-I in the generation of P-selectin ligands, coupled to a lower (albeit still absolute) requirement for FucT-VII, make C2GlcNAcT-I the limiting enzyme for P-selectin-ligand formation.

T-bet is a recently described transcription factor that has a crucial role in T_H1-cell differentiation, in part by controlling expression of the receptor for IL-12, specifically the IL-12Rβ2 chain[70–73]. Unlike $CD4^+$ T cells from *Stat4*$^{-/-}$ mice, which show an absence of functional P-selectin ligands when cultured under T_H1-polarizing conditions but only a modest decrease in functional E-selectin ligands, *T-bet*$^{-/-}$ $CD4^+$ T cells cultured under T_H1-polarizing conditions show severe defects in both E- and P-selectin ligands (G.S.K., unpublished observations). Because C2GlcNAcT-I is not required for E-selectin-ligand biosynthesis under these *in vitro* conditions[67], this implies that STAT4 and T-bet directly or indirectly control the expression of other enzymes, possibly sialyl transferases of the ST3Gal family[74], which are important for selectin-ligand biosynthesis.

Core 2 β1,6-glucosaminyltransferase-I and fucosyltransferase-VII are independently regulated. As both C2GlcNAcT-I and FucT-VII regulate selectin binding, it is important to understand how their expression is regulated. Independent regulation of C2GlcNAcT-I and FucT-VII indicates that independent control of the expression of ligands for E-selectin versus P-selectin is possible. One example of such independent control was shown by comparing primary plasma cells with *in vitro* generated T_H1 and T_H2 cells. Unlike T_H1 cells, which express high levels of both FucT-VII and C2GlcNAcT-I and high levels of ligands for both P- and E-selectin, or T_H2 cells, which express high levels of C2GlcNAcT-I but little FucT-VII, IgG-secreting plasma cells express high levels of FucT-VII but low levels of C2GlcNAcT-I, and bind well to E-selectin but poorly to P-selectin[75]. Independent control of ligands for E- versus P-selectin could be important for precise control of T-cell homing to distinct vascular beds that differ in their expression of these endothelial selectins. For example, gut-associated lymphoid tissue, including the lamina propria, is uniquely dependent on P-selectin for homing of activated T cells[5], whereas both E- and P-selectin participate in the recruitment of $CD4^+$ and $CD8^+$ T cells to the inflamed peritoneum[76] and skin[77].

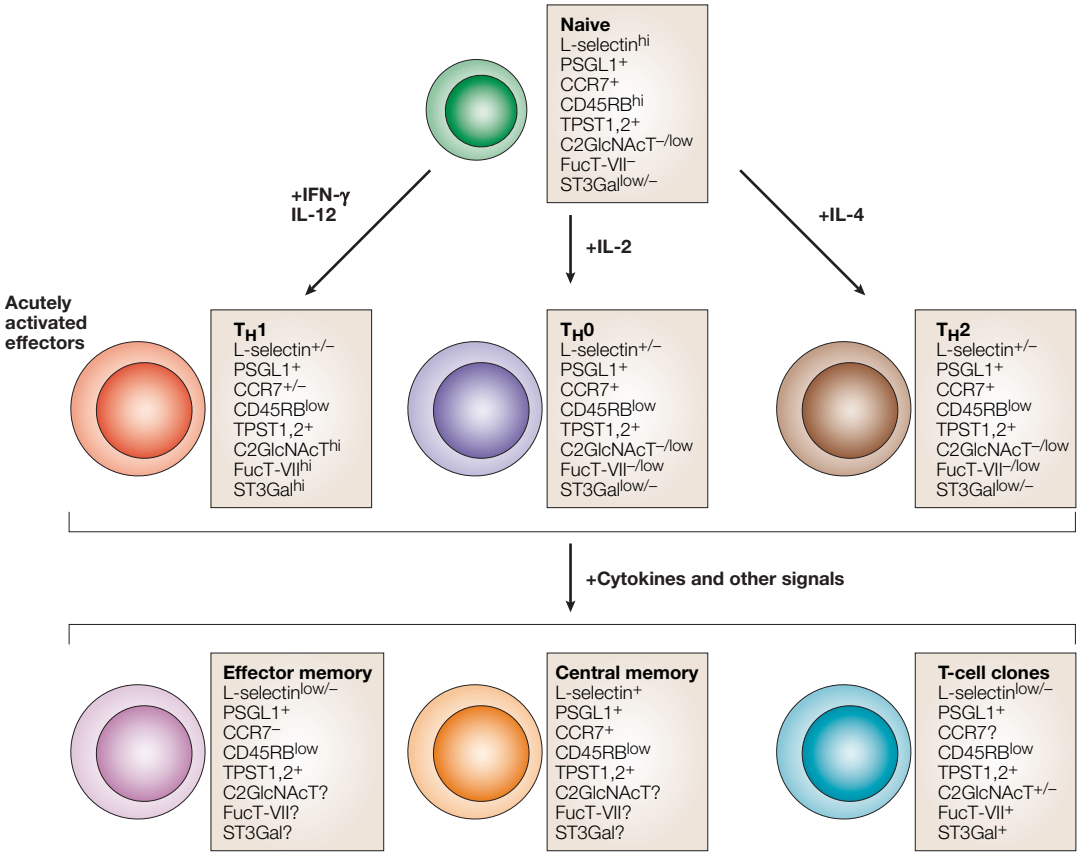

Figure 3 | **Selectin and selectin-ligand expression by CD4^{+} T cells.** Naive T cells (CD45RB^{+}) express L-selectin, but no functional P-selectin glycoprotein ligand 1 (PSGL1) or E-selectin ligands, because they lack fucosyltransferase-VII (FucT-VII) and core 2 β1,6-glucosaminyltransferase-I (C2GlcNAcT-I). The PSGL1 protein and the two tyrosine sulphotransferases are constitutively expressed and required, but not sufficient, for P-selectin-ligand activity. Sialyltransferases (such as ST3Gal-IV) are expressed at low levels. After activation (CD45RB^{low}), T cells acquire FucT-VII and C2GlcNAcT-I activity. FucT-VII and sialyltransferases are present at higher levels in T helper 1 (T_H1)-polarized cells than T_H2-polarized cells. After activation and under the influence of cytokines, both central memory T cells that re-express L-selectin and CC-chemokine receptor 7 (CCR7), and effector memory T cells, which do not express L-selectin or CCR7, emerge. T-cell clones *in vitro* retain FucT-VII and C2GlcNAcT-I activity. IFN-γ, interferon-γ; IL, interleukin; TPST, tyrosine protein sulphotransferase.

Regional regulation of selectin ligands. Another level of control of expression of both E- and P-selectin ligands and L-selectin is the microenvironment in which the T cells are activated. Activation of T cells in distinct anatomic sites leads to differences in the expression of homing receptors, including E- and P-selectin ligands and L-selectin[65,78,79], possibly through the action of dendritic cells; for example, T cells that encounter antigen presented by dendritic cells in the gastrointestinal tract acquire the ability to home preferentially to gastrointestinal organs[80,81]. Activation of T cells in the peripheral lymph nodes leads to the retention of L-selectin expression by at least some activated T cells, thereby allowing them to return to the lymph node, which represents the site of initial antigen encounter. Expression of E- and P-selectin ligands, as inferred from CLA expression, might be regulated in a similar organ-specific manner. Therefore, T-cell trafficking is effectively regulated by the expression of all three selectins and their ligands on T cells, endothelial cells and other blood cells.

CENTRAL MEMORY T CELLS
Memory T cells that express L-selectin and CC-chemokine receptor 7, and can be found in the blood and lymphoid organs.

EFFECTOR MEMORY T CELLS
Memory T cells that do not express either L-selectin or CC-chemokine receptor 7 and can be found in extralymphoid sites.

L-selectin in extralymphoid T-cell trafficking

A discrete subset of T cells, distinct from the cells that acquire selectin ligands by expressing glycosyltransferases, can enter some extralymphoid sites by expressing L-selectin. These are CENTRAL MEMORY T CELLS, which are a subset of memory cells in both CD4^{+} and CD8^{+} T-cell lineages[82]. As classically defined[82], central memory T cells differ from the other defined memory subset, known as EFFECTOR MEMORY T CELLS, by expression of both L-selectin and CC-chemokine receptor 7 (CCR7) — a chemokine receptor that is required for passage of cells from the bloodstream across lymph node HEVs. This CCR7^{+}L-selectin^{+} phenotype is identical to that of naive T cells. Unlike naive T cells, however, central memory T cells express many inflammatory chemokine receptors, which in combination with L-selectin, enable these cells to enter sites of chronic inflammation expressing L-selectin ligands such as PNAD. In contrast to central memory T cells, which recirculate and are mainly found in the blood and lymphoid organs, effector memory T cells are mainly found in non-lymphoid tissues[83].

This location in extralymphoid tissues, such as the lungs, leaves these cells poised for rapid responses, and obviates the need for post-infection homing to these sites. Whether effector memory T cells express selectin ligands and whether these are involved in their localization to extralymphoid tissues is not known.

It is important to emphasize that the L-selectin phenotype of a T cell says little about the history of that cell. As L-selectin is expressed by all naive T cells, but only by a subset of antigen-experienced/memory T cells, and is lost from most activated T cells, it is not possible to infer the history of a cell from its expression of L-selectin alone. In particular, it is impossible to determine whether an activated or memory T cell retained or re-expressed L-selectin. The factors that determine loss or retention of L-selectin expression *in vivo* are unknown and the loss of L-selectin expression by T-cell clones, or after activation *in vitro*, is unlikely to mimic the *in vivo* situation. The identification of central memory and effector memory T cells by differential L-selectin expression is complicated, and might not conform precisely to the classic phenotypes, particularly in the case of $CD8^+$ T cells[82,84]. Nevertheless, the expression of L-selectin by a subset of memory T cells that also express chemokine receptors that are not expressed by naive T cells, is a second pathway, independent of expression of E-/P-selectin ligands, by which these cells can home to sites of inflammation.

Selectin ligands on regulatory T cells

Recently, a unique lineage of $CD4^+$ T cells has been defined that has the capacity to inhibit T-cell activation and function. Known as regulatory T (T_{Reg}) cells, these cells seem able to inhibit T-cell activation in various settings, including autoimmunity and graft rejection[85]. Subsets of T_{Reg} cells have been defined by differential expression of cell-surface molecules, and, in some cases, discrepancies have been observed between suppressive capacity *in vitro* and *in vivo*. It is therefore of interest to consider their migratory properties.

Studies by Hamann and colleagues[86] have shown that subsets of T_{Reg} cells can be defined by differential expression of $\alpha_E\beta_7$ integrin[86]. Relevant to this review, expression of ligands for E- and P-selectin and the ability to home to inflamed skin is limited to the $\alpha_E\beta_7^+$ subset of T_{Reg} cells, which contains a marked proportion of cells that are L-selectin negative and have higher levels of β_1-, β_2- and β_7-integrin expression[87]. Expression of receptors for inflammatory chemokines is also largely limited to this subset. By contrast, T_{Reg} cells that are $\alpha_E\beta_7^-$ are almost all L-selectin$^+$, and home preferentially through secondary lymphoid organs[87]. So, to a significant degree, $\alpha_E\beta_7^-$ and $\alpha_E\beta_7^+$ subsets of T_{Reg} cells correspond to naive and effector/memory regulatory T cells in their expression of molecules that govern T-cell homing, as well as in their expression of other markers that traditionally define naive and effector/memory T-cell subsets.

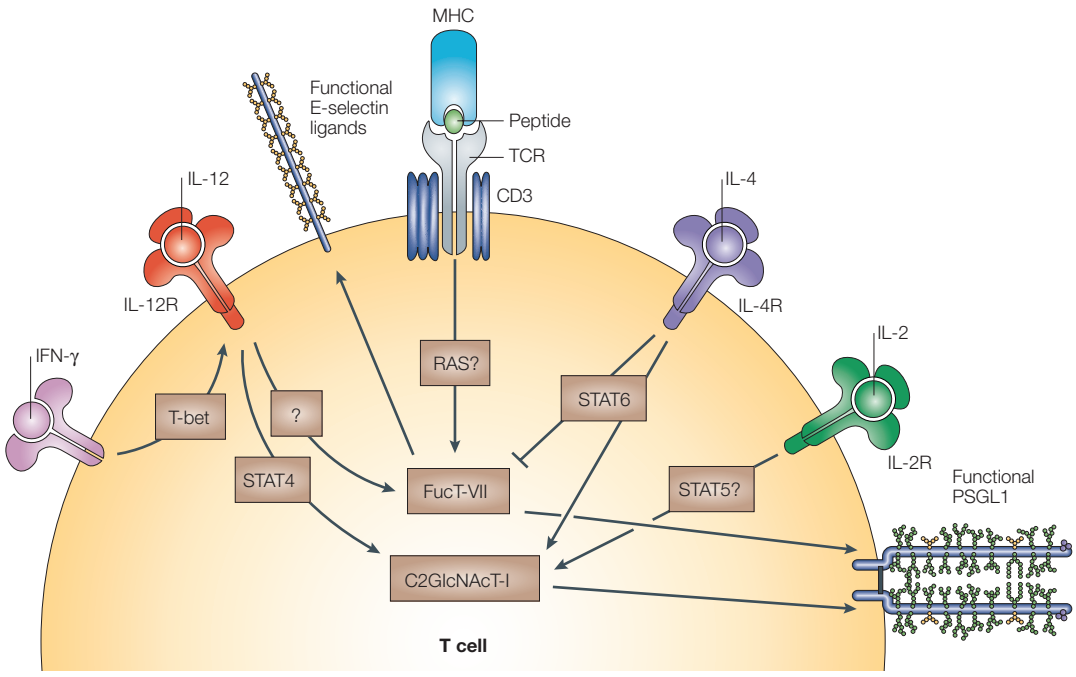

Figure 4 | **Regulation of glycosyltransferases that determine selectin-ligand activity.** The expression of fucosyltransferase-VII (FucT-VII) is upregulated through T-cell receptor (TCR) ligation, possibly through RAS and by ligation of the interleukin-12 receptor (IL-12R), but this is inhibited by ligation of the IL-4R. Activation of T cells in the presence of interferon-γ (IFN-γ) increases IL-12R expression through T-bet. Core 2 β1,6-glucosaminyltransferase-I (C2GlcNAcT-I) expression is upregulated by IL-12 through signal transducer and activator of transcription 4 (STAT4), and by IL-2 and IL-4, possibly through STAT5 and STAT6, respectively. High levels of FucT-VII expression regulate E-selectin-ligand activity, whereas some FucT-VII and C2GlcNAcT-I expression is required for P-selectin-ligand activity. Regulation of sialyltransferases and sulphotransferases is not shown. PSGL1, P-selectin glycoprotein ligand 1.

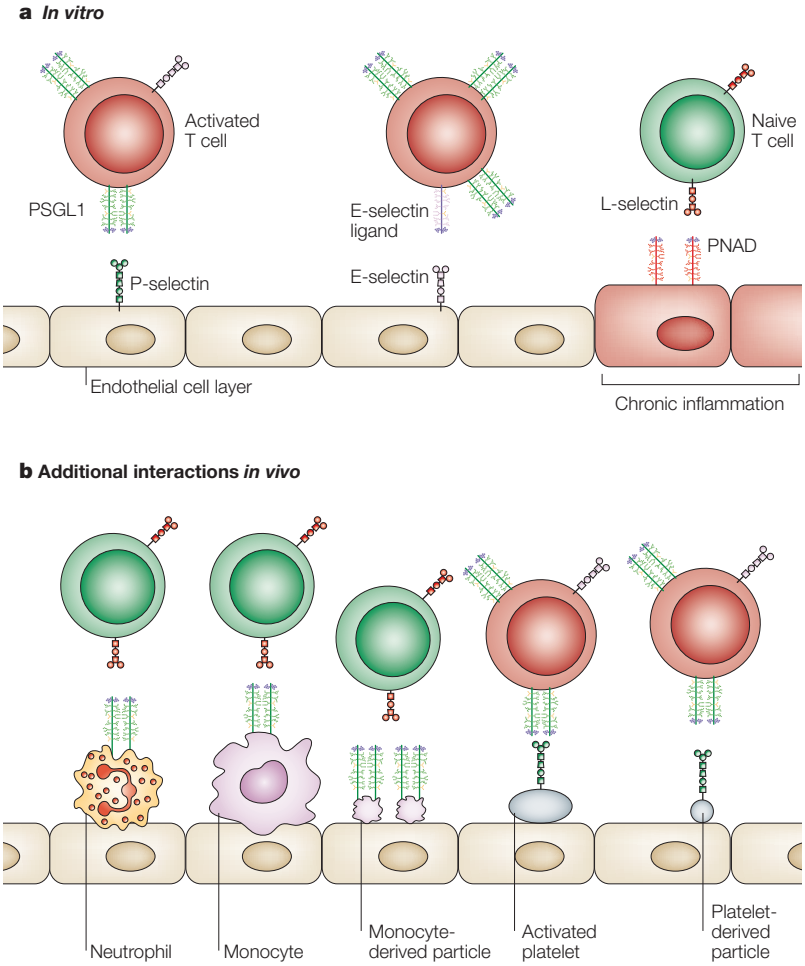

Figure 5 | **Selectin-dependent T-cell interactions in extralymphoid blood vessels.** **a** | T cell–endothelial-cell interactions as studied in flow chamber systems *in vitro*. P-selectin-dependent interactions require functional P-selectin glycoprotein ligand 1 (PSGL1); E-selectin interactions require poorly defined E-selectin ligand(s) on activated T cells. L-selectin on T cells interacts with peripheral node addressin (PNAD). At sites of inflammation, endothelial cells express P- and E-selectin and during chronic inflammation PNAD is also expressed by endothelial cells. **b** | At sites of inflammation, neutrophils and monocytes, or neutrophil- or monocyte-derived microparticles[107], interact with the inflamed endothelium and present functional PSGL1 to T cells. This PSGL1 can interact with L-selectin on naive or central memory T cells. Activated platelets and platelet-derived microparticles are also known to interact with the vascular endothelium[109] and can present P-selectin to T cells. Note that for simplicity, not all domains of the selectins are depicted, although they are represented according to their relative sizes.

In vivo T-cell homing

On the basis of these *in vitro* findings, the rules for extralymphoid T-cell homing seem deceptively simple: once activated, T cells acquire P- and E-selectin ligands, more so if polarized towards a T_H1-type phenotype than a T_H2-type phenotype, and therefore, can home to sites of inflammation and tissue injury, where endothelial P- and E-selectin are inducibly expressed. This process is mainly driven by regulated expression of the FucT-VII and C2GlcNAcT-I glycosyltransferases. In addition, some activated T cells can re-express or retain L-selectin expression through unknown mechanisms, and can reach sites of chronic inflammation, where endothelial L-selectin ligands are expressed. However, this picture is incomplete, because the *in vivo* situation is much more complex (FIG. 5). In addition to interacting with selectins and selectin ligands on endothelial cells, T cells can also interact with selectins and selectin ligands presented by adherent monocytes, neutrophils, platelets or their microparticle fragments, which are all found at sites of inflammation (BOX 1). This means that observations of altered T-cell trafficking in selectin- and selectin ligand-deficient mice must be discussed in light of altered selectin and selectin-ligand expression not only by endothelial cells, but also by myeloid cells and platelets.

The best *in vivo* evidence for E-selectin involvement in T-cell trafficking is available for T_H1-cell homing to the inflamed skin[56,88]. In addition, CLA-expressing human T_H2 cells have also been shown to home to human skin transplanted into immunodeficient mice, and this was inhibited by blocking E-selectin[89]. In another study in humans, herpes-virus-specific skin-tropic $CD8^+$ T cells were found to express CLA and home to the skin[90]. Similar findings were reported in mice[16], although a possible role for $CD8^+$ T-cell interactions with neutrophils or other adherent blood cells was not tested in either study. By contrast, in a patient with leukocyte-adhesion deficiency type II — a disease involving a defective fucose transporter[91] that leads to impaired selectin-ligand biosynthesis — a T-cell-dependent cutaneous hypersensitivity response was found to be largely unaltered, indicating that CLA and fucosylated selectin ligands are not strictly required for T-cell homing to sites of skin inflammation[92]. In an allograft model of skin transplantation, rejection was reduced in L-selectin-deficient mice[93]. It is unclear whether this effect was related to ineffective activation of T cells or to impaired T-cell trafficking, although cytotoxic T lymphocyte responses were found to be equivalent in L-selectin-deficient mice compared with controls.

P- and E-selectin are constitutively expressed in microvessels of the choroid plexus and have been suggested to be responsible for T-cell trafficking into the brain and cerebrospinal fluid[12]. This conclusion was based on the finding that PSGL1 was expressed by T cells in cerebrospinal fluid, but, as explained earlier, PSGL1 expression is not sufficient to demonstrate selectin-binding activity; so the function of these selectins in T-cell trafficking remains to be determined. Furthermore, the role of P-selectin in T-cell recruitment to the brain in the experimental autoimmune encephalitis (EAE) model of multiple sclerosis is controversial. Whereas one group reported a marked contribution to leukocyte rolling and adhesion[94], another group failed to find a role for P-selectin[95]. In both studies, the type of leukocyte interacting with the brain and meningeal microvasculature was not identified, but EAE is thought to be a $CD4^+$ T-cell-dependent process[96].

Several reports have indicated that E- and P-selectins are responsible for T-cell recruitment to inflamed lungs[97,98]. In a model of antigen-induced allergic pneumonitis, T-cell accumulation was reduced in E- and P-selectin double-knockout mice[63,99]. The authors show normal expression of several chemokines in these mice; however, the possibility that altered neutrophil

Box 1 | *In vivo* analysis of selectin-dependent T-cell trafficking

***In vivo*, T-cell–endothelial-cell interactions take place in whole blood in the presence of plasma proteins and other blood cells, their products and fragments. Platelets express P-selectin when activated and bind to endothelial cells, thereby changing T-cell homing properties. Platelets, monocytes, neutrophils and other cells produce microparticles, which circulate in the blood and provide a source for additional selectins and selectin ligands. Neutrophils and neutrophil-derived particles are deposited on inflamed endothelial cells[110], presenting functional P-selectin glycoprotein ligand 1 (PSGL1) to circulating T cells (FIG. 5). Platelets and many leukocyte subsets can be selectively labelled by fluorescent dyes or by cell-type-specific expression of fluorescent proteins[111–114] to detect such interactions.**

The interpretation of *in vivo* experiments is complicated by the dysregulation of expression of many genes in various organs and tissues in response to deletion of a single gene. For example, mice that lack E- and P-selectin have 10- to 50-fold increased neutrophil counts, altered T-cell populations and increased levels of granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage CSF, and interleukin-17 (REFS 115–117). These and other inflammatory cytokines are likely to affect T-cell homing to extralymphoid sites.

The inflammatory response associated with T-cell trafficking to extralymphoid sites changes tissue perfusion, wall shear stress (the force per unit area acting on the endothelium in the direction of blood flow), expression of chemokines and adhesion molecules, endothelial-cell permeability and other factors. *In vivo* assays that address T-cell homing to extralymphoid tissues often involve an inflammatory response. Therefore, the demonstration that T-cell homing to a certain extralymphoid tissue is reduced in mice in which a selectin was blocked or deleted does not mean that the manipulated selectin is directly responsible for T-cell adhesion. The interplay between inflammatory-cell and T-cell trafficking is ignored in all but a few studies. The best and most reproducible *in vivo* homing assays compare the recruitment of two populations of T cells injected into the same mouse, but labelled with different fluorochromes or other markers. These competitive homing assays largely eliminate confounding influences of blood flow, tissue perfusion and inter-individual variations in vascular selectin or selectin-ligand expression.

adhesion in E- and P-selectin-deficient mice might have secondary effects on T-cell recruitment was not explored. There is also evidence supporting a role for L-selectin in T-cell trafficking in a model of allergen-induced lung inflammation[100].

There is mixed evidence for the role of selectins in T-cell homing to the liver and kidney. The liver contains a large number of T cells, but homing mechanisms are incompletely understood. In concanavalin A-induced liver injury, P-selectin, which might be presented on platelets, seems to be required for the accumulation of T cells[101]. Platelet binding to T cells has been demonstrated and is suggested to influence T-cell homing to lymph nodes[102].

Insulitis — chronic inflammation of the pancreatic islets — leads to diabetes mellitus in humans and several mouse models of type I diabetes. In non-obese diabetic (NOD) mice, insulitis seems to be induced and maintained by L-selectin-dependent T-cell infiltration. Pancreatic islet venules differentiate into HEV-like vessels and express PNAD[50]. Treatment with L-selectin-specific antibodies has been reported to prevent the onset of diabetes in this model[49], indicating that L-selectin-dependent T-cell interactions with PNAD-expressing microvessels in the diabetic pancreas might be important in the islet-destructive immune response. However, a more recent study[103] did not find a difference in the onset or frequency of diabetes in L-selectin-deficient NOD mice, leaving the true role of L-selectin in this model unresolved. In alternative models of disease, insulitis and diabetes mellitus can be induced by expressing lymphotoxin-α and -β under control of the rat insulin promoter. This induces expression of PNAD and binding of L-selectin-expressing T cells in pancreatic microvessels[43]. PNAD expression was also described in lymphoid hyperplasia of the thymic medulla — a condition found in the AKR mouse strain[45]. Inflamed LACRIMAR GLANDS were found to express PNAD in NOD mice — a model that has some similarity to SJOGREN'S SYNDROME[48]. Some evidence of L-selectin-ligand expression in rejecting transplanted kidneys was provided by immunostaining for HECA-452 or MECA-79 (PNAD), but the functional consequences of this, if any, were not investigated[104]. Taken together, these data indicate that PNAD expression and L-selectin-dependent T-cell recruitment to extralymphoid sites is most important in inflammatory diseases of exocrine and endocrine glands.

LACRIMAR GLANDS
Tear-producing glands in and around the eyelids.

SJOGREN'S SYNDROME
An inflammatory autoimmune disease of the salivary and lacrimar glands that leads to dryness of the mouth and eyes.

Conclusions

Selectin-dependent T-cell homing to extralymphoid sites is mediated by two fundamentally different processes: the inducible acquisition of expression of selectin ligands and L-selectin. Some activated and effector T cells acquire the ability to bind vascular selectins through the induction of expression of glycosyltransferases that capacitate selectin ligands. The induction of FucT-VII expression seems to be sufficient for most E-selectin binding, whereas P-selectin binding requires additional expression of C2GlcNAcT-I. Expression of these two enzymes is independently regulated. The signalling pathways that control these and other glycosyl- and sulphotransferases are under investigation. The second mechanism involves the induction of expression of L-selectin ligands at sites of chronic inflammation. These ligands seem to be similar to those found in HEVs of lymph nodes, because they express the MECA-79 antigen PNAD, which is largely controlled by the expression of HEC-GlcNAc6ST. Successful T-cell homing to PNAD-expressing extralymphoid sites requires the expression of L-selectin by naive T cells or re-expression of L-selectin, which is initially lost after T-cell activation. Both the induction of glycosyltransferase expression and the regulation of L-selectin are incompletely understood; yet, both seem to be clinically important in disease states, and specific drug-like molecules could potentially be developed to modulate glycosyltransferase activity and L-selectin expression for therapeutic purposes. Taken together, there is compelling evidence that selectin-mediated adhesion events are important for the homing of $CD4^+$ and $CD8^+$ T cells with T_H1-type phenotypes, with some evidence that other T-cell subsets might also use selectin-dependent mechanisms for homing to extralymphoid sites.

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Competing interests statement
The authors declare that they have no competing financial interests.

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