The observation that circulating leucocytes adhere to and migrate across the vascular endothelium was first made 70 yr ago; this was noted to occur without breach of the endothelial barrier, suggesting the presence of complex regulatory mechanisms [1]. More recently, in a series of classic experiments, Gowans and Knight observed that lymphocytes isolated from the rat thoracic duct homed rapidly back to lymph nodes and secondary lymphoid organs upon reinjection: furthermore, it was noted that this occurred across the distinctly shaped endothelial cells of the postcapillary venules [2]. Since then we have learnt much about the molecular basis of leucocyte extravasation and the regulatory mechanisms involved. In this review we will describe molecular interactions involved in the stages of leucocyte recruitment to inflammatory sites. Finally, we will emphasize the central role that adhesion molecules have in the development of the inflammatory response by drawing from examples of human disease, and describe recent progress in the therapeutic targeting of these molecules with particular reference to inflammatory arthritis.

Recruitment of leucocytes to inflammatory sites: the multistep model

The ‘multistep model’ of leucocyte migration (Fig. 1) was originally described a decade ago as the integration of a number of sequential, apparently discrete, stages beginning with the adhesion of white blood cells to the vascular lumen and culminating in migration through the endothelial cell layer into the extravascular space [3]. These steps are mediated by multiple molecules, and include: tethering and rolling, activation, firm adhesion, and finally transendothelial migration (TEM).

Rolling: selectins and their ligands

Tethering and subsequent rolling represent the ‘braking’ of leucocytes flowing at speeds of up to 4000 μm/s, resulting from initial contact between the leucocyte and the luminal endothelium. A series of relatively low-affinity interactions, mediated by several types of adhesion molecules (most importantly the selectins and their carbohydrate ligands) effects the slowing of the leucocytes.

The selectins (Table 1) are a group of structurally related transmembrane glycoproteins (reviewed in [4, 5]), each consisting of an NH2 calcium-dependent (c-type) lectin terminal domain repeated prior to a transmembrane domain and a short cytoplasmic tail [5]. They bind mucin ligands, proteins rich in serine and threonine residues with O-linked sugars, displaying specific carbohydrate epitopes containing sialylated/fucosylated residues typified by the tetrasaccharide sialyl-Lewisx [5]. Synthesis of these glycoproteins is mediated by enzymes of the large glycosyltransferase family, the expression of which regulates cellular synthesis of specific glycoprotein structures [6]. Three selectins, designated L-, P- and E-selectin, have been described in humans. L-selectin is constitutively expressed by most circulating leucocytes, although it is down-regulated after lymphocyte activation; the majority of cell-surface L-selectin is expressed on the tips of microvilli [7], which has been shown to enhance the initiation of rolling, particularly in larger-diameter vessels [8]. As will be discussed later, it is critical to the rolling of naïve lymphocytes on the high endothelial venules (HEVs) of the secondary lymphoid organs. L-selectin ligands are also expressed at sites of acute inflammation, although they are less well characterized than those in the lymphoid tissues; significant reduction of leucocyte infiltration was observed in L-selectin knockout mice in a model of acute inflammation [9] and reduced levels of leucocyte rolling in inflamed postcapillary venules have been demonstrated in these animals [10]. In vitro adhesion assays have demonstrated that L-selectin can bind P-selectin glycoprotein ligand 1 (PSGL-1) and that leucocyte-expressed PSGL-1 can act as a ligand for L-selectin [11]; such secondary tethering to endothelial-adherent leucocytes enhances subsequent primary P-selectin-mediated capture in these assays. This provides a mechanism of positive feedback whereby adherent leucocytes recruit more leucocytes exponentially; this amplification of recruitment is abolished when L-selectin is blocked [12]. In an in vivo model, leucocyte rolling was examined in inflamed cremaster endothelium by intravital microscopy; most of the L-selectin-mediated leucocyte adhesion was mediated by secondary tethering. Furthermore, in PSGL-1 knockout mice L-selectin-dependent leucocyte-rolling was abolished; a marked reduction was also seen after treatment of wild-type mice with a blocking anti-PSGL-1 antibody. L-selectin ligands can also be expressed by the vascular endothelium in sites of chronic inflammation. Ectopic lymphoneogenesis with the formation of lymphoid follicles has been described in a number of diseases, often in association with the adoption of an HEV-like morphology by endothelial cells [13], which can bind L-selectin [14]. Such ectopic lymphoneogenesis is well described in the inflamed rheumatoid synovium [15–18].

P-selectin is expressed by activated platelets and the endothelial cells of inflamed tissues, where it is stored in secretory granules (α-granules in platelets and Weibel–Palade bodies in endothelium) which are translocated to the plasma membrane upon activation...
This allows rapid up-regulation of surface expression in response to an inflammatory stimulus, often mediating early leucocyte recruitment. Its major ligand is the sialylated and fucosylated glycoprotein PSGL-1, which is expressed by circulating myeloid cells and T cells [19]. As seen with L-selectin, PSGL-1 is expressed on the tips of microvilli of leucocytes [20].

E-selectin is also expressed by inflamed endothelium but, unlike P-selectin, it is regulated by increased transcription in response to inflammatory stimuli, peak expression occurring at around 4 h [21]. The best characterized ligand for E-selectin is the cutaneous lymphocyte antigen (CLA), so called because it is expressed by up to 90% of T cells at sites of chronic cutaneous inflammation compared with only 10–25% in the circulation [22]. CLA is a sialylated carbohydrate epitope originally defined by reactivity with the HECA452 antibody; the precise structure is unknown, although it has been shown that PSGL-1 can act as the core molecule [23, 24]. As discussed above, E-selectin can also bind L-selectin and recent in vivo evidence suggests that it can contribute to PSGL-1-mediated \( \alpha_{HI} \) cell rolling [25].

Although selectins are the best characterized mediators of lymphocyte rolling, they are not always required and it may be mediated by other cell adhesion molecules (CAMs). CD44 is a type I transmembrane glycoprotein that is expressed in an activated form by subgroups of activated lymphocytes [reviewed in [26]] and can mediate selectin-independent lymphocyte rolling on inflamed endothelium [27]. Its endothelial ligand is hyaluronic acid, a polysaccharide component of the extracellular matrix, expression of which can be up-regulated by pro-inflammatory stimuli [28]; a recent report has suggested that E-selectin may also be a ligand for CD44 [29]. Slow lymphocyte rolling can also be mediated by the interaction of the \( \alpha_{v} \) integrins with their endothelial ligands. It appears, therefore, that a number of molecular interactions can be involved in the progressive slowing of a lymphocyte as it encounters the endothelial surface. In addition to L-, P- and E-selectins [30], the \( \alpha_{v} \)-integrins (which are also expressed on the tips of microvilli [31]) can support slow rolling [32], as can the \( \beta_{2} \)-integrins in synergy with E- and L-selectin [30, 33].

Rolling brings leucocytes into contact and allows them to sample the local microenvironment at the endothelial surface. In the absence of stimuli, leucocytes detach and remain in the circulation; however, in the presence of luminally expressed activating molecules (principally chemokines), they progress to firm adhesion.

### Chemokines and leucocyte activation

Chemokines (CKs; chemoattractant cytokines) are secreted polypeptides of 67–127 amino acids with molecular weights of 8–12 kDa (reviewed in [34–36]). Structurally, in most CKs, the short NH\(_{2}\)-terminus region precedes a central core, with a COOH-terminal \( \alpha \)-helix [37]. Chemokines have been implicated in a variety of functions, including angiogenesis, organogenesis and tumour metastasis as well as chemotaxis [35]. They are classified into families according to the spacing of four highly conserved cysteine residues near the N-terminus; the largest of these are the CC family (i.e. with the two cysteine residues adjacent) and CXC (in which they are separated by one amino acid) family. The XC chemokines (XCL1 and XCL2), containing only one conserved cysteine, and the CX-C chemokine CX5CL1, containing three inter-cysteine amino acids, have also been described [34, 35].

The original nomenclature, whereby CKs were classified according to their (apparent) primary function, has been replaced by a systematic classification by family (Table 2) [38]. Their receptors are serpentine seven-transmembrane G-protein-coupled receptors which are classified according to the family of their CK ligand(s), i.e. CCR1-9, CXCR1-5, CXR1 and CX2CR1 [36]. There is considerable overlap between receptor and ligand specificity, some receptors binding only one CK and others having affinity for several. Equally, CKs may have one or more cognate receptors [36]. Ligation of the leucocyte-expressed CK receptor results in firm adhesion to the vascular lumen, a process mediated predominantly by leucocyte integrins and their endothelial ligands (see next section).

Chemokine expression is up-regulated by a diverse range of stimuli in a large number of cell types. Expression is largely
transcriptionally regulated, although CK may also possibly be stored in secretory granules. For instance, storage of CXCL8 (IL-8) in Weibel–Palade bodies has been demonstrated in human endothelial cells after prolonged stimulation [39]. CKs can also be broadly divided into inflammatory and homeostatic (Table 2) types. Inflammatory CKs mediate leucocyte recruitment to sites of inflammation, whereas homeostatic CKs mediate immunosurveillance of secondary lymphoid organs and the peripheral tissues [35]. Some CKs do not fall neatly into either category and are known as having dual function.

Chemokines expressed by lymph node HEVs are not necessarily synthesized locally, as evidenced by the lack of local expression of, for instance, CCL19 mRNA [40], and it has been shown that CKs synthesized elsewhere within the LN or even arriving in the lymph can be transported to the luminal surface of the HEV [40, 41]; transcytosis of inflammatory CKs to the luminal endothelium from extravascular sources has also been demonstrated [42]. After secretion, CKs would simply be washed away by the blood flow if mechanisms for anchoring to the endothelial surface were not present: glycosaminoglycans (GAGs) are polysaccharides attached to a protein core (proteoglycans); they are expressed by endothelial cells, heparan sulphate being the most abundant [43]. They can bind multiple CKs and hence present them to rolling leucocytes; GAGs bind CKs with variable affinity and therefore provide a framework for the formation of haptotactic gradients [44]. Furthermore, variations in patterns of GAG expression are demonstrable between the endothelia of normal and diseased tissues [35]; some GAGs bind multiple CKs and hence present them to rolling leucocytes; TECK, thymus-expressed chemokines. aChemokines with dual function.

To date, 18 human chemokines have now been described, many of which have been shown to activate specific leucocyte subtypes in vitro [48], although definite in vivo evidence is only available for a fraction of these [49]. CK ligation results in up-regulation of integrin-mediated firm adhesion and TEM; this effect is both rapid and transient, and is dependent on levels of receptor occupancy. Interestingly, however, the kinetics of CK-mediated firm adhesion and TEM may differ [50]. Clearly, the expression of multiple CK receptors by particular leucocyte subgroups in combination with variable expression of CKs within tissues has enormous potential for the differential tissue localization of leucocytes, and it has been suggested that differential endothelial CK expression is responsible for the bulk of the variability in homing pattern [51]. Furthermore, a number of features of CK/CK receptor interaction can further contribute to the diversity of leucocyte response. It has recently been shown that some CKs can antagonize particular CK receptors; agonists for CXCR3, for instance, can antagonize CCR3 [37]. Moreover, ligation of different CK receptors in the same cell can initiate different signalling pathways [52]; indeed the same level of occupancy of a particular CK receptor by different CKs can have differing effects [53].

Fractalkine (CX3CL1) is structurally distinct from most chemokines (reviewed in [54]); it can be soluble or membrane-bound and its expression is up-regulated by pro-inflammatory mediators. It has an extracellular membrane-proximal mucin-like region [54]. Due to the presence of its mucin domain, fractalkine is unique amongst the chemokines in that it combines adhesion (tethering) as well as signalling functions (G-protein stimulation) [55]. Its receptor, CX3CR1, is expressed on circulating NK cells, monocytes and a subpopulation of activated T cells, and hence it may have important functions in inflammation.

Chemokines expressed by lymph node HEVs are not necessarily synthesized locally, as evidenced by the lack of local expression of, for instance, CCL19 mRNA [40], and it has been shown that CKs synthesized elsewhere within the LN or even arriving in the lymph can be transported to the luminal surface of the HEV [40, 41]; transcytosis of inflammatory CKs to the luminal endothelium from extravascular sources has also been demonstrated [42]. After secretion, CKs would simply be washed away by the blood flow if mechanisms for anchoring to the endothelial surface were not present: glycosaminoglycans (GAGs) are polysaccharides attached to a protein core (proteoglycans); they are expressed by endothelial cells, heparan sulphate being the most abundant [43]. They can bind multiple CKs and hence present them to rolling leucocytes; GAGs bind CKs with variable affinity and therefore provide a framework for the formation of haptotactic gradients [44]. Furthermore, variations in patterns of GAG expression are demonstrable between the endothelia of normal and diseased tissues [35]; some GAGs bind multiple CKs and hence present them to rolling leucocytes; TECK, thymus-expressed chemokines. aChemokines with dual function.

Table 2. Chemokines and receptors involved in leucocyte homing

<table>
<thead>
<tr>
<th>Systematic name</th>
<th>Functional name</th>
<th>Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2</td>
<td>MCP-1</td>
<td>CCR2</td>
</tr>
<tr>
<td>CCL3</td>
<td>MIP-1α</td>
<td>CCR1, CCR5</td>
</tr>
<tr>
<td>CCL4</td>
<td>MIP-1β</td>
<td>CCR5</td>
</tr>
<tr>
<td>CCL5</td>
<td>RANTES</td>
<td>CCR1, CCR3, CCR5</td>
</tr>
<tr>
<td>CCL11</td>
<td>Eotaxin</td>
<td>CCR3</td>
</tr>
<tr>
<td>CCL27</td>
<td>CTACK</td>
<td>CCR10</td>
</tr>
<tr>
<td>CXCL8</td>
<td>IL-8</td>
<td>CXCR1, CXCR2</td>
</tr>
<tr>
<td>CX3CL1</td>
<td>Fractalkine</td>
<td>CX3CR1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systematic name</th>
<th>Functional name</th>
<th>Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL1*</td>
<td>I-309</td>
<td>CCR8</td>
</tr>
<tr>
<td>CCL17a</td>
<td>TARC</td>
<td>CCR4</td>
</tr>
<tr>
<td>CCL19</td>
<td>MIP-3β</td>
<td>CCR7</td>
</tr>
<tr>
<td>CCL21</td>
<td>SLC</td>
<td>CCR7</td>
</tr>
<tr>
<td>CCL22</td>
<td>MDC</td>
<td>CCR4</td>
</tr>
<tr>
<td>CCL25a</td>
<td>TECK</td>
<td>CCR9</td>
</tr>
<tr>
<td>CXCL12a</td>
<td>SDF-1α/β</td>
<td>CXCR4</td>
</tr>
<tr>
<td>CXCL13</td>
<td>BCA-1</td>
<td>CCR5</td>
</tr>
</tbody>
</table>

BCCA-1, B-cell-attracting chemokines-1; CTACK, cutaneous T-cell-attracting chemokine; ELC, EBI1 ligand chemokines; MCP-1, monocyte chemotactic protein-1; MDC, macrophage-derived chemokine; MIP-1α, macrophage inflammation protein-1α; RANTES, regulated on activation normal T-cell expressed and secreted; SDF-1α, stromal-cell-derived factor-1α; SLC, secondary lymphoid tissue chemokines; TARC, thymus- and activation-regulated chemokine; TECK, thymus-expressed chemokines. aChemokines with dual function.

**Firm adhesion: integrins and their ligands**

Integrins (reviewed in [57, 58]) are glycosylated transmembrane proteins which exist as non-covalently associated dimers consisting of one α- and one β-chain: they have a large extracellular domain consisting of 70–1100 residues and a short cytoplasmic domain of 30–50 residues (with the exception of β7, which has 1000 residues) [57]. To date, 18 α and eight β subunits have been described in humans, which form 24 known heterodimers. The integrins bind to components of the extracellular matrix or specific counter-receptors and have a diverse array of roles in mediating both within the immune system and in tissue organization and cellular signalling [58]. Five integrins have been identified as being particularly important to leucocyte migration (Table 3) [5]. The endothelial counter-ligands for leucocyte integrins are members...
of the immunoglobulin superfamily and include (ICAM)-1–5, (VCAM)-1 and mucosal addressin cell adhesion molecule-1 (MAdCAM-1). They are type I transmembrane glycoproteins, consisting of a short cytoplasmic tail, a single transmembrane region and a variable number of extracellular immunoglobulin domains [59]. ICAM-1 and endothelium ICAM-2 are ligands for lymphocyte function-associated antigen-1 (LFA-1) and Mac-1; ICAM-1 appears to be the dominant ligand in inflammation as it is up-regulated by pro-inflammatory stimuli [59], a process that is transcriptionally regulated [60]. Unlike ICAM-1, ICAM-2 is expressed constitutively by the vascular and lymphatic endothelium and is not induced by inflammatory mediators [61, 62]. Both ICAM-1 and ICAM-2 are important LFA-1 ligands mediating leucocyte migration into peripheral lymph nodes. There is significant redundancy with both: in vivo lymphocyte homing to peripheral lymph nodes (PLNs) was affected little by blockade of either ICAM-1 or ICAM-2, whereas blocking both resulted in significant inhibition of homing [63]. ICAM-2 has been shown to act as an endothelial counter-receptor for DC-SIGN, a C-type lectin expressed by circulating dendritic cell precursor; in an in vitro assay this interaction could support rolling under flow conditions and transendothelial migration [62].

ICAMs also appear to have a signalling function; ligation of ICAM-1, for instance, has been shown to activate kinase-dependent signalling pathways with consequent up-regulation of secreted cytokine and membrane-bound protein expression [60]. VCAM-1, like ICAM-1, is expressed at low levels by resting endothelial cells and is up-regulated by pro-inflammatory stimuli: it is the ligand for the α4β1 integrin (very late antigen (VLA)-4), which is expressed by most leucocyte subtypes and can also bind α5β1 [64]. As well as supporting firm adhesion, VCAM-1 can also support rolling mediated by VLA-4, and this can progress to firm adhesion in the absence of cytokine stimulation [32].

Up-regulation of integrin binding affinity has been shown to occur by two mechanisms: conformational change to a high-affinity state and increased lateral mobility leading to cell-surface clustering (increased avidity) and polarization. This up-regulation is transient [65] and can be extremely rapid; cell-surface clustering has been shown to occur in less than 0.1 s [66]. These processes are dependent on the ‘inside-out’ signalling that follows the engagement of cell surface receptors by external stimulants and leads to the triggering of various intracellular signalling pathways involved in cytoskeleton reorganization [66–68]. Multiple signalling pathways have been implicated in inside-out signalling by integrins following ligation of CK receptors (reviewed in [48, 69]). The G-protein-dependent activation of the small RAS family GTPases results in the formation of integrin-binding complexes, including RAP1/RAPL, which bind the integrin α-chain; activated talin binds the β-chain, and these interactions result in a conformational change in the integrin into a higher-affinity state, an event also dependent on activation of the RAS-family protein RHOA. Activation of the atypical protein kinase C-ξ by phosphatidylinositol-3-kinase and RHOA, and the RAP1-dependent activation of protein tyrosine kinase 2β results in their translocation to the plasma membrane and an increase in integrin lateral mobility and clustering [69]. The same CK can induce up-regulation of the binding affinity of different integrins through separate pathways—in eosinophils conformational changes in Mac-1 and clustering of VLA-4 were both effected by CCL5 and CCL7, although it is unclear whether this is mediated by different CK receptors [68]. Interestingly, it was shown that rapid clustering of VLA-4 was stimulated only by localized CK receptor ligation and not by saturating levels of soluble CK, emphasizing the importance of localized signalling for adhesion and subsequent migration [66]. Moreover, recent work has shown that only immobilized CK can induce LFA-1/ICAM-1-mediated lymphocyte arrest [70]; this report showed that induction of an intermediate affinity state in the integrin was essential to ICAM-1 binding in flow conditions. Binding to ICAM-1 induced a further conformational change to the high-affinity state; this process was shown to be very rapid and not to require progressive integration of CK signal [70]. This effect also appears to depend on the density of ICAM-1 expression and was not seen with soluble ICAM-1 [71]. Integrins can also initiate internal signalling pathways following external stimuli, the so-called outside-in signalling. This follows receptor clustering and formation of the focal adhesion plaque, with subsequent signalling dependent upon the GTPase RhoA and the mitogen-activated protein kinase pathway [72]. Outside-in signalling has diverse functions in the regulation cell function, including proliferation and apoptosis. Up-regulation of integrin binding results in firm adhesion, bringing the adherent cell to a halt and allowing progression to transendothelial migration.

### Table 3. Lymphocyte integrins and their endothelial ligands

<table>
<thead>
<tr>
<th>Integrin</th>
<th>Subunits</th>
<th>Expression</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFA-1, CD11a/CD18</td>
<td>α4β2</td>
<td>Monocytes neutrophils T cells</td>
<td>ICAM-1, -2, -3</td>
</tr>
<tr>
<td>Mac-1, CD11b/CD18</td>
<td>αMβ2</td>
<td>Monocytes Neutrophils</td>
<td>ICAM-1 iC3b, factor X</td>
</tr>
<tr>
<td>p150, 95, CR4 CD11c/CD18</td>
<td>αβ2</td>
<td>Monocytes Neutrophils NK cells</td>
<td>ICAM-1 Fibrinogen</td>
</tr>
<tr>
<td>VLA-4, CD 49d/CD29</td>
<td>αβ1</td>
<td>B and T cells monocytes</td>
<td>MAdCAM-1, Fibronectin</td>
</tr>
<tr>
<td>LPAM-1</td>
<td>αβ7</td>
<td>B and T cells</td>
<td>VCAM-1, MAdCAM-1, Fibronectin</td>
</tr>
</tbody>
</table>

ICAM, intercellular adhesion molecule; LFA-1, leucocyte functional antigen-1; LPAM-1, lymphocyte Peyers patch adhesion molecule-1; MAdCAM-1, mucosal addressin adhesion molecule-1; VCAM, vascular cell adhesion molecule, VLA-4, very late antigen-4.
The JAMs also act as integrin ligands: JAM-A, -B and -C bind LFA-1, Mac-1 and VLA-4, respectively [74]. TEM is mediated by the binding of leucocyte integrins to PECAM-1 (through homotypic adhesion) and endothelial CAMs to CD99 (which binds VLA-4). It involves the activation of multiple signalling pathways mediated by the ligation of integrins on the leucocyte and endothelial cell CAMs. Intracellular signalling results in the breakage and formation of leucocyte-endothelial bonds and the opening of the endothelial barrier, as well as mediating the trailing edge retraction and leading edge protrusion of the leucocyte with cell-surface polarization of CK receptors [75]. It has been demonstrated in vitro that leucocytes can migrate through increasing concentration gradients towards the source of a CK [76]. Diverse patterns of CK are expressed at inflammatory sites; this, coupled with the expression of multiple CK receptors by leucocytes, allows precise navigation and localization of leucocytes. Leucocytes are able to respond to sequential CK gradients, thus migrating in a step-wise fashion; this is likely to be due in part to CK receptor desensitization at high/saturating concentrations, when the orientation of CK receptor ligation will also be lost [76]. Furthermore, it is apparent that some chemoattractants can augment the response to others, whilst for some cross-desensitization of CK receptor occurs in a hierarchical manner, providing a potential mechanism for the step-wise response seen [77]. Another mechanism for this effect appears to be the ability of leucocytes to prioritize their response to newly encountered chemoattractants, i.e. they can ‘memorize’ previous components of the chemoattract cascade [78].

The final obstacle to the extravasating leucocyte is the perivascular basement membrane (PBM). The mechanisms by which leucocytes cross the PBM remain incompletely defined; recent work has demonstrated a role for the laminin-binding integrin α5β1 [79]. Laminin is a component of the PBM and it has been shown that its surface expression on neutrophils is up-regulated by the homophilic interaction of leucocyte and endothelial PECAM-1; the passage of neutrophils across the PBM was inhibited by a α5β1-blocking antibody in vitro [79]. In vitro studies have suggested a role for leucocyte proteases in trans-PBM migration (reviewed in [80]), although their role in vivo remains unproven.

**Cooperation between families of adhesion molecules: refinement of the multistep paradigm**

It is now apparent that there is considerable overlap between the phases included in the multistep model, and that there is also substantial cooperation between the CAMs involved. As discussed previously, there appears to be sequential selectin and integrin involvement at progressively lower rolling velocities, allowing the controlled adhesion of circulating leucocytes to the endothelium. It has also been suggested that there is sequential involvement of integrins; firm adhesion is initially dependent on α4 integrins, the β2 integrins mediating subsequent spreading and transmigration [81].

It is clear that there is considerable communication between families of adhesion molecules, many of which have well-characterized signal transduction properties (reviewed in [82, 83]). For example, ligation of L-selectin by GlyCAM-1 can up-regulate β1-integrin-mediated adhesion to fibronectin [84], and β2-mediated binding to ICAM-1 with LFA-1 [85] and fibrinogen (with Mac-1) [86]. It has also been shown that ligation of PSGL-1 can activate LFA-1-mediated adhesion via cell-surface clustering [87]. Crosstalk between integrins has also been demonstrated; ligation of high-affinity VLA-4 by VCAM-1 can up-regulate LFA-1 binding to ICAM-1, probably via cell-surface clustering [88]. Conversely, LFA-1 binding to ICAM-1 results in down-regulation of VLA-4 binding to VCAM-1 and fibronectin and enhancement of cell migration via α6β1/fibronectin [89]. A recent report described a physical association between CD44 and VLA-4, which are co-expressed on lymphocyte microvilli [90].

There is also evidence that cell migration in itself can regulate leucocyte adhesion; for instance, monocyte and T-lymphocyte adhesion can stimulate up-regulation of endothelial adhesion molecule expression, possibly acting as an amplification mechanism for leucocyte recruitment [91, 92]. In contrast to this, a recent report has described inhibition of lymphocyte adhesion following neutrophil interaction with endothelium in vitro due to down-regulation of VCAM-1 expression [93]. Neutrophil-derived proteases have been implicated in the regulation of cell adhesion and migration and have been shown in vitro, for instance, to cleave PSGL-1 [94], and to inactivate CXCL12 and its receptor CXCR4 [95]. In an in vitro adhesion assay, it was shown that CXCL12-dependent lymphocyte migration was inhibited by pre-incubation with neutrophils or purified neutrophil elastase [96].

**CAMs in human disease: the leucocyte adhesion deficiencies**

The leucocyte adhesion deficiency (LAD) syndromes are a group of rare, genetically determined disorders characterized by aberrant adhesion molecule expression; these disorders underline the pivotal role of the CAMs on the propagation of the immune response. In all three syndromes patients have raised peripheral leucocyte counts and recurrent bacterial infections. LAD-I is caused by expression of a mutant β2 chain [97]; as would be expected, neutrophils isolated from these patients show normal rolling in inflamed venules but impaired firm adhesion and extravasation [98]. LAD-II—one of a group of syndromes caused the congenital disorders of glycosylation (CDG) and also known as CDG-IIC [99]—is caused by the deficiency of fucosylation with resultant hypofucosylation of glycoproteins, including loss of expression of SLTs; affected patients have more widespread developmental abnormalities as well as the adhesion defect [100]. Neutrophils from these patients show poor rolling on inflamed endothelium, with absence of firm adhesion [98]. The recently described LAD-III is caused by a defect in CK receptor G-protein signalling with loss of consequent rapid leucocyte integrin activation [101]. In addition to these syndromes, a variety of CAM polymorphisms have been associated with inflammatory disease [102].

Thus, cell migration plays a crucial role in the inflammatory process. In addition, the diversity of usage of CAMs and CK receptors allows the further specialization of cellular responses in relation to the type and site of inflammation. The mechanisms involved in tissue-specific leucocyte migration will be discussed in the next section.

**Organ-specific lymphocyte homing**

Lymphocytes circulate through to the peripheral tissues in a non-random manner, allowing optimization of the immune system’s resources. Recirculation of naive lymphocytes through lymphoid tissue maximizes their chances of encountering antigen, and activation into the effector phenotype is accompanied by the acquisition of homing properties for peripheral tissues where antigen may be re-encountered [103]. Studies of lymphocyte adhesion to frozen sections of tissue have demonstrated that lymphocytes isolated from peripheral lymph nodes, gut mucosa and synovium exhibit enhanced binding properties to the tissue of origin [104], and it can be shown that lymphocytes isolated from draining lymph at different sites have differential surface adhesion molecule expression [105].

**Tissue ‘area codes’: addressins and homing receptors**

Organ-specific lymphocyte homing is a complex process dependent on the presence of specific ligand/receptor interactions at each
stage of the adhesion process. Although many aspects still remain to be defined, for some tissues at least some of the homing mechanisms have been described. Tissue-specific ligands expressed by vascular endothelial cells are known as ‘addressins’ and bind homing receptors expressed by subpopulations of leucocytes.

For example, the homing receptor/addressin pair L-selectin/ peripheral lymph node addressin (PNAd) mediates lymphocyte adhesion to peripheral lymph node HEV [14]. PNAd is a complex of sialomucins (defined by reactivity with the MECA-79 antibody) consisting, in humans, of CD34 podocalyxin and endomucin (for an excellent review of L-selectin ligands see [14]); sulphation by a specific sulphotransferase is required for the recognition of these epitopes [106]. GlyCAM-1, another component of the PNAd family of ligands, is a secreted molecule [107] and may therefore have a role in the regulation of selectin-mediated leucocyte binding [4]. This observation is supported by studies in knockout mice; in an L-selectin knockout there was no leucocyte adhesion to PLN HEV, and PLNs were smaller in size due to reduced numbers of intra-PLN lymphocytes [10]. These homing receptors are, however, neither sufficient nor always necessary for organ-specific homing. Endothelial vascular adhesion protein 1 (VAP-1), for instance, may contribute to the adhesion of naive lymphocytes to PLN HEV; this may be co-dependent on L-selectin or, in some cases, L-selectin-independent [108]. Another level of homing specificity is conferred by CKs: CCL19 and CCL21, ligands for the CK receptor CCR7, are transcytosed to lymphoid organ HEV and mediate lymphocyte extravasation to these sites [40, 109]. CCR7 is required for the homing of naive lymphocytes to lymphoid tissue; mice expressing a mutant form of CCR7 have disordered PLN architecture [110]. Furthermore, expression of L-selectin and CCR7 defines a subset of memory T cells (central memory T cells) which retain homing affinity for lymphoid organs [111]. All PLN-naive cells express CCR7, as do most peripheral tissue T cells; this may be necessary for re-entry of these cells into the lymphatics expressing CCL21 [112].

Another example of a homing receptor/addressin pair is the integrin α4β7. This binds to MadCAM-1, is expressed specifically by gut mucosal venular endothelium and is critical for lymphocyte recruitment to gut-associated lymphoid tissue (GALT) [113]. MadCAM-1 is expressed by the HEVs of Peyer’s patches in the gut; it contains both immunoglobulin and mucin domains [114] and can also support L-selectin-mediated rolling when decorated by MECA-79-reactive epitopes [115]. Antibodies to the α4β7 subunits inhibit lymphocyte recruitment to the gut [116] and mice deficient in α4 or β7 integrin subunits have markedly underdeveloped GALT [117, 118]. The CK CCL25, which is chemotactic for cells expressing CCR9, is expressed preferentially by regions of the small intestine, where a majority of infiltrating lymphocytes express CCR9, suggesting a further level of homing specificity for the gut [119]; interestingly, CCL25 was not expressed in the large intestine.

As already discussed, CLA, a glycosylation variant of PSGL-1, is a ligand for E-selectin and is expressed by a majority of infiltrating lymphocytes in inflamed skin [22] and in patients with contact dermatitis; a proliferative response to antigen is confined to cells expressing CLA [120]. Moreover, infiltrating T cells from a series of patients with cutaneous T-cell lymphomas were preferentially shown to express CLA [121]. Further evidence that CLA+ cells have homing specificity for the inflamed skin comes from studies of patients with psoriatic arthritis: despite having inflammatory lesions at both the joints and the skin, CLA+ lymphocytes are confined to the skin [122]. However, E-selectin is widely expressed in inflammation and, as with gut-homing cells, further components of the ‘area code’ are necessary for specific recruitment. Most CLA+ skin-infiltrating lymphocytes express high levels of CCR4, the receptor for CCL17, expression of which is up-regulated in inflamed skin [123] (although not exclusively); in this study CCR4 expression by α4β7+ cells was low or negative. CCL27 is expressed preferentially by resting and inflamed skin and is chemotactic for a subset of CLA+ cells [124]. In addition, CCR10 is the receptor for CCL27 and is expressed by most infiltrating lymphocytes in inflamed skin [125]. Furthermore, E-selectin, CCL17 and ICAM-1 co-localize in some dermal vessels from non-inflamed skin, providing a molecular framework enabling immunosurveillance [126]. It was recently reported that CCR8 is expressed by a majority of T cells in normal skin, although rarely in peripheral blood; CCL1, the only ligand for CCR8, is expressed in normal skin and it is therefore likely that this CK has a role in cutaneous immunosurveillance [127].

Whilst there may be evidence of specific lymphocyte infiltration at other sites, descriptions of specific addressin/homing receptor pairs for the synovium are lacking. Nonetheless, distinct endothelial cell recognition systems for the synovium have been described [128] and a number of leucocyte-expressed adhesion molecules have been implicated, including CD44, L-selectin and α4β7, integrins on leucocytes and PNAd, and VAP-1, P-selectin and E-selectin in the synovium [129, 130]. Homeostatic chemokines have been associated with the formation of ectopic lymphoid tissue in the rheumatoid synovium and a number of inflammatory chemokines are up-regulated in inflamed RA tissue [131]. Indeed, infusion of CXCL8, the prototypical pro-inflammatory cytokine, into the knee joints of rabbits produces clinical arthritis [132]. The CK receptors CXCR3, CXCR6 and CCR5 are preferentially expressed by lymphocytes in inflamed synovial tissue [133]; this pattern of expression is associated with the T-memory phenotype [134]. It has also been shown that leucocytes isolated from normal and inflamed gut adhere preferentially to inflamed synovium in vitro, suggesting a mechanism for the recirculation of effector cells between the synovium and gut, and a possible pathogenic link for the known clinical association between gut and synovial inflammation [135, 136]. Specific adhesion molecule expression in the synovium may not be the only explanation for specific leucocyte homing; CS1, a splice variant of fibronectin and a counter-receptor for α4β7, is expressed by synovial endothelial cells and up-regulated in inflammation [137]. The description of unique peptide sequences, identified by in vitro phage display, with binding specificity for human synovium also lends credence to the existence of a synovial addressin [138].

Until recently it was unclear what drove the acquisition of a particular pattern of CAM and CK receptor expression by lymphocytes. More is now understood about the mechanisms of such imprinting, and this will be discussed in the next section.

Acquisition of homing properties by lymphocytes

It is well established that differentiation in secondary lymphoid tissues of naive lymphocytes into the effector/memory cells leads to changes in surface CAM and CK receptor expression associated with the acquisition of specific homing properties. Experiments using adoptive transfer of T cells have shown that such acquisition of tissue-specific homing properties occurs within 2 days of antigenic stimulation according to the lymphoid tissue in which antigen is encountered [139]. Furthermore, cytokine-dependent L-selectin and CLA expression during T-cell maturation has also been demonstrated in vitro [140, 141]. More recently it has become clearer that dendritic cells (DCs) are essential for the differentiation of lymphocytes into populations with tissue-homing specificity. For instance, culture of naive T cells with antigen-loaded DCs from mesenteric lymph nodes induced sub-stantially more expression of α4β7 and CCR9 than was seen with splenic DCs[142]. Furthermore, DCs isolated from Peyer’s patches have also been shown to induce α4β7 and CCR9 in lymphocytes that could migrate along a CCL25 gradient in vitro and demonstrated tropism for the small intestine in vivo [143]. In contrast, down-regulation of L-selectin, a typical phenomenon associated with lymphocyte activation, was seen after incubation with DCs from both sites [142]. Taken together, these data clearly indicate

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that DCs from different lymphoid stations can induce the repertoire of CAMs and CK receptors associated with tissue specific homing as well as antigen specificity associated with immunological competence. The importance of the lymphoid tissue local microenvironment for the regulation of homing in vivo is further emphasized by the fact that that antigen-primed DCs can induce skin- or gut-homing properties when given by intracutaneous or intraperitoneal injection, respectively, an effect not seen after intravenous administration [144]. This group also demonstrated differentiation into a skin-homing phenotype of T cells cultured with Langerhans cells isolated from the skin [144]. An important question is whether, once activated, T cells are permanently committed to a particular homing phenotype or can be re-educated. Recent work has shown that T cells which have acquired skin or gut-homing properties can alter their surface CAM and CK receptor expression according to their most recent encounter with DCs [145]. The mechanisms involved in T-cell imprinting by DCs are unclear, although T-cell activation is a necessary component [145]. However, as far as the gut is concerned, vitamin A appears to be a critical moiety in the induction of the gut homing repertoire. In a recent landmark paper, Iwata and colleagues showed that exposure of naive T cells to retinoic acid under stimulatory conditions resulted in the expression of the gut-homing phenotype α4β7/CCR9; expression of CLA was suppressed [146]. These cells showed chemotaxis to CCL25 and homed preferentially to the gut after adoptive transfer. They also showed that enzymes necessary for the oxidative metabolism of retinol to retinoic acid are expressed by DCs from mesenteric lymph nodes and Peyer’s patches as well as the intestinal epithelium, whilst they are only expressed at low levels by DCs from PLNs. Furthermore, it was demonstrated that inhibition of these enzymes suppressed α4β7 and CCR9 expression, as did blockade of the nuclear retinoic acid receptor. A wider understanding of these processes with particular reference to the joint could have significant therapeutic implications for the manipulation of the immune response (for a more detailed review of this topic see [147]) and for targeting specific treatments to rheumatic diseases.

**Therapeutic targeting of adhesion molecules**

Adhesion molecules are crucial to the orchestration of the immune response, and are therefore attractive therapeutic targets. Levels of soluble adhesion molecules can be correlated with disease activity in a number of inflammatory conditions, including RA [148, 149], although it is often debatable whether this confers any advantage over conventional inflammatory markers [102]. There has also been some success reported with the targeting of adhesion molecules for imaging; a radiolabelled anti-E-selectin antibody in RA was particularly promising [150]. The therapeutic targeting of a number of adhesion molecules have been investigated in animal models and, despite disappointing results with some agents, there have been encouraging results in some human studies. Perhaps the most successful to date are the glycoprotein IIb/IIIa (αIIbβ3) antagonists, which inhibit platelet aggregation and are used clinically in acute coronary syndromes and following angioplasty [151]. There are also encouraging data from human studies for α4-antagonists in multiple sclerosis and Crohn’s disease, although natalizumab, a humanized monoclonal antibody against the α4 subunit, has recently been associated with progressive multifocal leucoencephalopathy secondary to reactivation of latent JC polyomavirus [152]. Blockade of CXCL8 [102] and the α4 integrin subunit [153] have been effective in cutaneous psoriasis. In a phase I/II trial, RA patients given a single intravenous dose of an anti-ICAM-1 monoclonal antibody sustained a clinical improvement [154], and a preliminary study with an oral CCR1 (the ligand for the inflammatory CKs CCL3 and CCL5) antagonist showed a reduction in infiltrating macrophages and lymphocytes in the synovium of RA patients and a trend towards clinical improvement [155]. However, non-selective targeting of adhesion mechanisms may be associated with the iatrogenic development of some of the clinical problems highlighted by the LAD syndromes. Therefore, as mentioned above, better understanding of the mechanisms involved in tissue-specific lymphocyte recirculation is likely to bring more fruitful results.

**Conclusions**

Much is now known about the complex mechanisms involved in the extravasation of circulating leucocytes to inflammatory sites. Despite this there are still many unanswered questions, particularly the nature of unique molecular interactions enabling recruitment of subpopulations of cells to specific sites. It is clear, however, that there is substantial potential to exploit the interaction between circulating leucocytes and the endothelium to therapeutic advantage. In this respect there are already promising results, and it is certain that future work will continue to produce novel agents for the treatment of inflammatory disease principally localized in certain tissues.

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