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Area Code Molecules of Lymphocytes

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The organization of animal cells in differentiated organs and tissues has long been postulated to depend on cell-surface interactions both with molecules on the surface of other cells and with the extracellular matrix [1, 2]. Structures on the surface of cells that control organization have been termed 'area code molecules' [3]. Since this paper is presented at an international meeting, 'international calling code molecules' might be more appropriate. One can imagine that one or two surface molecules, by binding to tissue-specific counter-receptors, could route a cell to a particular organ of the body, much as digits route a call to a particular country in the world. Further molecules could route the cell to a specific subregion of that organ, just as further digits route a call to a particular region in a country, and so on. Development involves both the movement and involution of cell sheets, and the migration of single cells as in the case of cells derived from the neural crest and the cells of the immune system. The international calling code or area code hypothesis is best suited to freely migrating cells that have many routes open to them, and can therefore be directed by the interactions of cell surface adhesion receptors. Chemoattractants or morphogenetic gradients can also guide cell migration. There are important interactions between these mechanisms, because adhesive interactions are required for cells to gain the traction required for migration toward an attractant; also, chemoattractants can alter gene expression and thus alter the display of area code molecules. Inflammation alters the distribution of leukocytes within the body; they congregate at sites of infection and in the lymph nodes draining the site of infection where antigen is trapped and immune responses are mounted. In the area code hypothesis, this may be termed 'call forwarding'. If one is away from home and wants to receive phone calls on someone else's phone, some telephone companies provide a service that

forwards calls to that number. This effectively adds a new phone number to that phone just as in inflammation new adhesion molecules are induced and displayed on the surface in addition to the preexisting ones.

Many mechanisms for regulating adhesion have been richly illustrated by studies on the area code molecules of the immune system. Rapid transition between adherent and nonadherent states is of key importance as the cells of the immune system patrol the body for infectious organisms. They must both circulate as nonadherent cells in the blood and lymph and migrate as adherent cells through tissues; in the presence of a foreign antigen they must be able to congregate in lymphoid organs, cross endothelial and basement membrane barriers to aggregate at sites of infection, and adhere to cells bearing foreign antigen [4]. Condensing on a recent review [5], I shall explain what is known of the adhesive interactions that take place when lymphocytes have been activated by a foreign antigen and that direct their localization and migration, before describing receptors that determine lymphocyte homing to different lymphoid organs and neutrophil localization in inflammation. Three families of adhesion receptors mediate these interactions (fig. 1–3): the immunoglobulin superfamily, which includes the antigen-specific receptors of T and B lymphocytes; the integrin family, which is important in dynamic regulation of adhesion and migration, and the selectins, which are prominent in lymphocyte and neutrophil interaction with vascular endothelium.

Antigen-Specific Recognition by T Lymphocytes

Most of what is known of the molecules regulating lymphocyte adhesion has come from the study of T lymphocytes whose functions in immunity depend upon close contact with other cells. The T-cell receptor (TCR) for antigen (fig. 1) recognizes antigen as a peptide fragment bound to cell-surface molecules encoded by the major histocompatibility complex (MHC; fig. 1, 4). The adhesion molecules now known to participate in the recognition by T cells of their targets were originally identified by monoclonal antibodies against cell-surface molecules of T lymphocytes. Two of the molecules so identified, CD8 and CD4, have since been shown to act as co-receptors for class-I and class-II MHC molecules, respectively [6, 7]. The TCR and the co-receptors diffuse independently in the plane of the T-cell membrane until they are brought together by co-recognition of the same peptide-MHC molecule complex (fig. 4a,b). At physiological densities on T

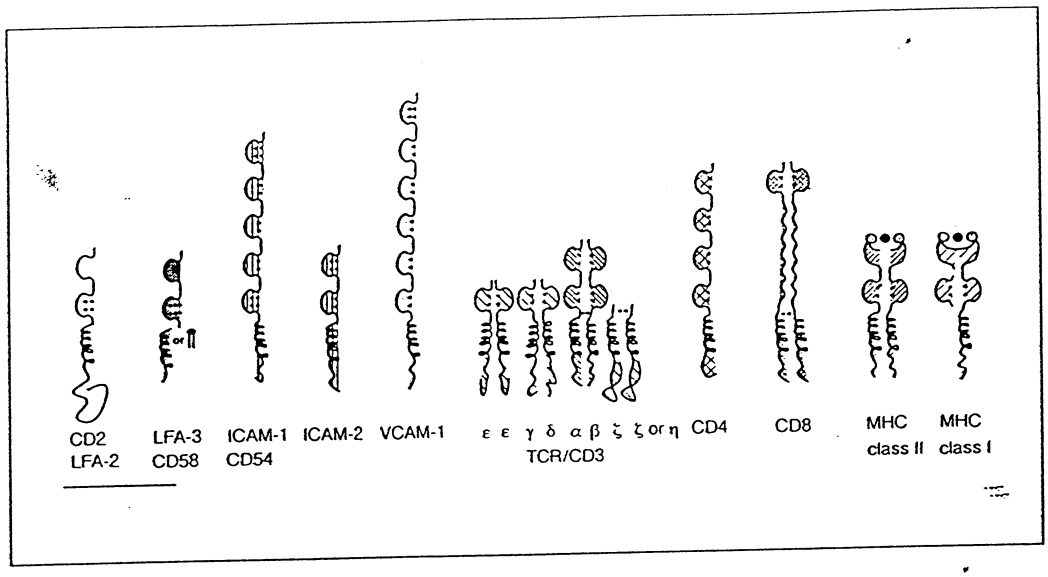
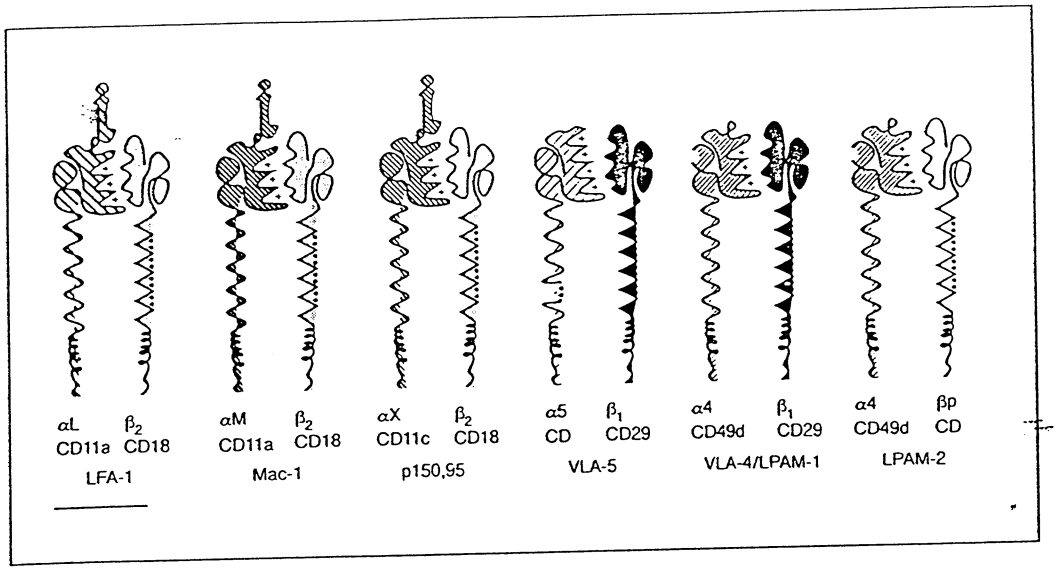


Fig. 1. Ig superfamily adhesion receptors. Members of the Ig superfamily share in common the Ig domain, composed of 90 to 100 amino acids arranged in a sandwich of two sheets of anti-parallel β strands, which is usually stabilized by a disulfide bond at its center [103, 104]. The immunoglobulins and TCR, which are specialized for antigen recognition, are the only known members of this family with variable regions that undergo somatic diversification. The function of molecules of the Ig superfamily in adhesion evolutionarily predates specialization for antigen recognition, which occurs only in vertebrates; Ig superfamily members are present in insects as nervous system adhesion molecules involved in axon guidance and fasciculation [105]. The Ig domain may have diversified and been adopted so widely in evolution because its stable disulfide-bonded β-strand structure is analogous to an automobile chassis on which many different styles of bodies and fenders may be hung. These latter may be analogous to the loops connecting the β strands, and also to the alternating residues in the β strands that point outward away from the interior of the domain.

An interesting feature of adhesion molecule structure is that, by contrast to immunoglobulins and MHC molecules, which have paired Ig domains, the Ig domains of ICAM-1 [106] and NCAM [107] are unpaired. Ig domains known by X-ray crystallography are ellipsoids with a dimension of 4 nm parallel to the β strands and 2.5 nm in the two perpendicular dimensions [18, 104]. Electron micrographs of ICAM-1 show that it is a bent rod about 17 nm long. This is only compatible with a model in which its 5 Ig domains are unpaired, and are arranged end to end at a slight angle to the β strands.

Overall size and shape of molecules are shown to scale (bar = 10 nm), although the Ig domains are schematized; dots denote disulfide-bonded cysteines. Dimensions are based



on the following information: MHC class-I molecule, X-ray crystallography [18]; MHC class-II, approximation as MHC class I, TCR, approximation of αβ heterodimer as an Fab fragment [108]; ICAM-1, electron microscopy [106]; ICAM-2 [30], CD2, LFA-3 [103], CD4 [14] and VCAM-1 [60] approximation to two, four, or six unpaired Ig domains as in ICAM-1; CD8, a disulfide-linked α–α or α–β dimer of one Ig domain, the length of the connecting or hinge-like peptide [14] of 40 or 65 amino acid for α or β, respectively, is arbitrary and is consistent with a spacing of 1.5–3.3 Å/residue [109], note that the distance the connecting peptide is shown to extend in CD8 is similar to what would be predicted for 3 of the 4 IG domains in CD4, allowing the N-terminal Ig domain of CD4 to occupy a position similar to that shown for the Ig domain of CD8.

Fig. 2. Representative integrin family adhesion receptors. Integrins contain α and β subunits of approximately 1,100 and 750 amino acids, respectively, which are noncovalently associated. The α subunits are 25–65% identical in amino acid sequence and the β subunits are 37–45% identical; the structural and functional similarities are so strong that integrins should be considered a protein family rather than a superfamily [22, 55]. Although cartoons, the overall size and shape of the integrins is shown to scale, based on electron microscopy of VLA-5 [110] and CD41/CD61 [111] (bar = 10 nm). The I domain is shown sticking out for emphasis, but it may fold up with the rest of the globular head. The dots denote cysteine-rich regions in the β subunit and a disulfide bridging a cleavage site in some α subunits. Divalent cation binding sites are symbolized by '+'. Structures are cited in [22, 57].

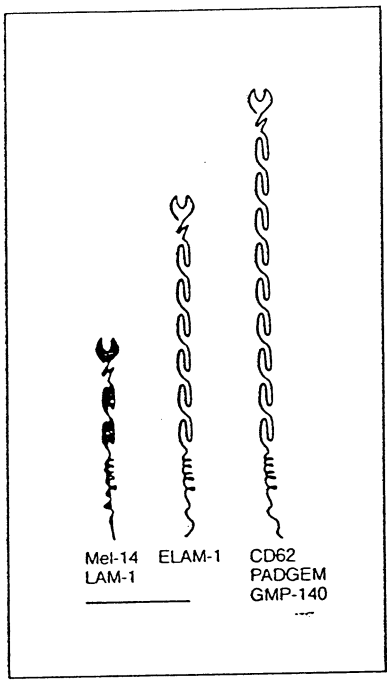


Fig. 3. Selectins have an N-terminal domain of 117–120 amino acids which is homologous to a variety of Ca^{2+}-dependent animal lectins [112] including hepatic galactose receptors, soluble mannose-binding lectins, and invertebrate lectins, as well as to proteins known to bind ligands independent of carbohydrate, including the low affinity receptor for IgE (CD23). Following the N-terminal lectin domain is a single epidermal growth factor motif of 34–40 amino acids, and then short consensus repeat motifs of 62 amino acids, as found in many proteins involved in regulating complement activation. Structures are from [92–95]. Although cartoons, selectin domains are shown approximately to scale (bar = 10 nm): the short consensus repeats extend 4.1 nm each, based on a length of 33 nm [113] for 8 short consensus repeats [114], epidermal growth factor repeats extend about 2.3 nm [115], and the lectin-like N-terminal domain is modeled as a globular sphere [112].

lymphocytes and in the absence of antigen, CD4 and CD8 mediate little or no adhesion to MHC [8, 9] although overexpression in transfected fibroblasts has demonstrated binding by CD4 to class II and CD8 to class I [10, 11]. The main physiological importance of these molecules is in signalling: when their contribution is blocked by antibodies, T cells require 100-fold higher concentrations of antigen to induce responsiveness [6, 12–15].

Synergistic signalling by the association of the TCR with its co-receptor has been directly demonstrated for CD8 [16], which has been shown to bind to the membrane-proximal domain of the MHC class-I molecule which is not polymorphic [16, 17], while the TCR binds to the polymorphic membrane-distal domains [18]. The evidence on CD4 is less direct; but when $CD4^+$ helper cells form conjugates with antigen-presenting B cells, both the TCR and CD4 redistribute to the site of adhesion [15], and antibodies that induce the association of CD4 with the TCR also activate T cells [12, 15]. The signalling function of CD4 and CD8 may depend upon the association of their cytoplasmic segments with a lymphocyte-specific tyrosine kinase, *lck* [19].

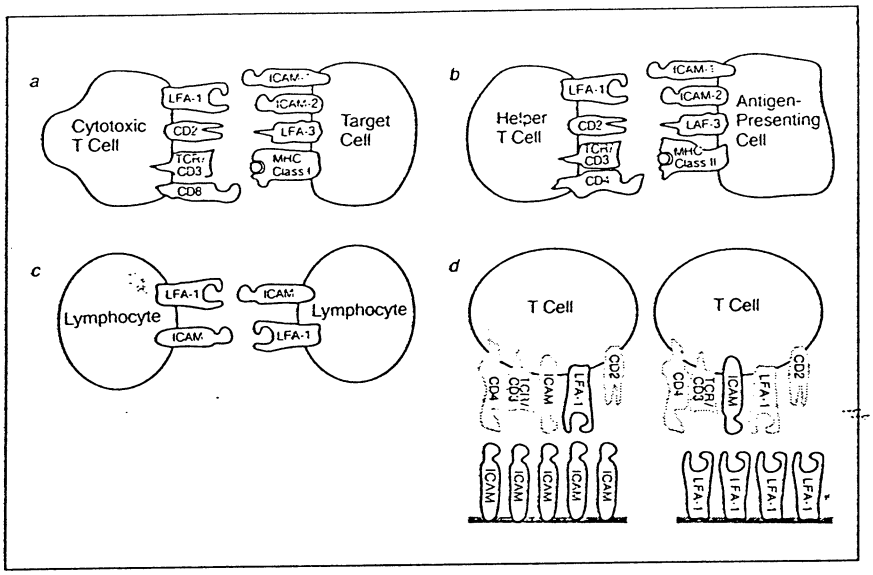


Fig. 4. Adhesion molecules in cell interactions. *a, b* Antigen-specific cytolytic and helper T-cell interactions. *c* Homotypic adhesion. *d* Adhesion to purified molecules in artificial membranes.

Activation-Dependent Adhesion Mechanisms

The definition of other cell-surface molecules essential for the interaction of T cells with their targets has been focused principally on killer T-cell interactions, largely because of the simplicity of the killing assay [6, 20–22]. Thus three molecules have been identified by screening monoclonal antibodies for their ability to inhibit killing and were operationally termed lymphocyte-function associated: LFA-1, LFA-2 (CD2) and LFA-3 (fig. 4a, b). These molecules are now known to account for the antigen-independent adhesion that is induced by prolonged antigenic stimulation of T cells in vitro [8, 20, 23, 24] and presumably help localize activated T cells to sites of antigen accumulation in the lymph nodes in vivo [25].

LFA-1, which is a member of the integrin family (fig. 3), is expressed on T lymphocytes; its counter-receptor on the target cell is ICAM-1 or ICAM-2, both members of the immunoglobulin (Ig) family (fig. 1) [20, 22, 24, 26–30]. LFA-2 or CD2, another member of the Ig family, is expressed on the T lymphocyte: its counter-receptor on the target cell, LFA-3, is also a member of the Ig family [6, 20, 31, 32] (fig. 1). The LFA-1–ICAM-1

interaction and the CD2–LFA-3 interactions can be shown by the effects on adhesion of monoclonal antibodies against the individual molecules to be independent; monoclonal antibodies to any of them, or to the TCR or CD8, can inhibit T-lymphocyte killing, showing that it is a highly complex process requiring cooperation between a number of different surface molecules [6, 21, 22, 33]. Indeed, we shall see later that binding of the TCR can modulate the affinity of LFA-1 for its counter-receptor. Adhesive interactions between CD2 and LFA-3 are also regulated by activation of T cells, though by a different mechanism.

Regulated Adhesion through the CD2/LFA-3 Interaction

The interaction between cells bearing CD2 and LFA-3 is finely poised, and is tipped toward adhesion by T-cell activation. The increase in adhesion between CD2 and LFA-3 on T-cell activation may be largely due to the regulation of the negative charge on the T-cell surface, which is mainly due to sialic acid [34]. Close cell–cell contact between circulating cells is opposed by the charge repulsion and by the decrease in entropy required for interdigitation of the surface glycocalyces [35]: these repulsive interactions seem to be reduced in activated T lymphocytes. Despite their larger surface area, T-cell blasts and thymocytes have 5-fold less sialic acid per cell than resting T cells [36] and are less negatively charged [37], and this may be a primary factor determining whether the CD2: LFA-3 mechanism and other adhesion mechanisms are active or latent. In lymph nodes, the activated antigen-responsive lymphocytes that aggregate in germinal centers are greatly undersialylated, while areas containing B and T cells in rapid transit between blood and lymph are normally sialylated [38].

CD2 may transduce a signal which augments or synergizes with signals from the TCR [6]. Certain pairs of antibodies against CD2, or combination of one such antibody with multimeric LFA-3, can stimulate T cells, but CD2–LFA-3 interaction alone has no effect [39, 40]. Transfection of cells with CD2 and LFA-3 has confirmed early antibody inhibition results [20] showing CD2–LFA-3 interaction can contribute a 4- to 30-fold enhancement of the immune response [41, 42]. The unusually basic, histidine and proline-rich 120 amino acid cytoplasmic region of CD2 is required for stimulation by pairs of monoclonal antibodies [42, 43]; however, in antigen-specific responses, truncation of the cytoplasmic domain of CD2 has

given ambiguous results, leaving unclear the relative contributions of adhesion and signalling to enhancement of the immune response by CD2–LFA-3 interaction [41, 42].

ICAM-1 and ICAM-2, Counter-Receptors for LFA-1

LFA-1, although it was originally identified by monoclonal antibodies that inhibit T-cell-mediated killing, is also required for a broad range of other leukocyte functions, including T-helper and B-lymphocyte responses, natural killing, antibody-dependent cytotoxicity mediated by monocytes and granulocytes, and adherence of leukocytes to endothelial cells, fibroblasts, and epithelial cells [20, 22]. A counter-receptor for LFA-1, ICAM-1, was identified using a simple assay called homotypic adhesion (fig. 4c), in which homogeneous cell populations such as B- or T-cell lines adhere to one another to form multicellular clusters [20, 44]. Aggregates form in this assay only if the lymphocytes have been stimulated with phorbol esters, and adhesion is completely inhibited by monoclonal antibodies against LFA-1 and is not observed with cell lines established from patients genetically deficient in LFA-1 (see below). LFA-1$^+$ cells can, however, coaggregate with LFA-1$^-$ cells [45]; and an LFA-1 counter-receptor, ICAM-1, was defined by immunizing mice with LFA-1$^-$ cells, and selecting monoclonal antibodies that would inhibit LFA-1-dependent homotypic adhesion [46]. ICAM-1 is a member of the Ig superfamily with five Ig domains (fig. 1).

In contrast to LFA-1, which is restricted to leukocytes, ICAM-1 can be expressed on a wide variety of cells and its induction in inflammation is an important means of regulating LFA-1–ICAM-1 interactions [22, 44] and thereby presumably inflammatory responses. In the absence of an inflammatory response, ICAM-1 is expressed on only a few cell types [47]. Its importance has been demonstrated in vitro by blocking T-cell killing with antibody to ICAM-1 [48], and by transfection experiments in which fibroblasts expressing sub-optimal levels of MHC molecules can be enabled to activate T-helper cells by co-transfection with the gene encoding ICAM-1 [49].

Inflammatory mediators, including lipopolysaccharide, interferon-γ, interleukin-1, and tumor necrosis factor cause strong induction of ICAM-1 in a wide variety of tissues and greatly increase binding of lymphocytes and monocytes through their cell surface LFA-1 [20, 22, 44, 50]. Endothelial, fibroblastic, and epithelial cells vary as to which cytokines are capable of inducing ICAM-1 expression, and the types of mediators released may

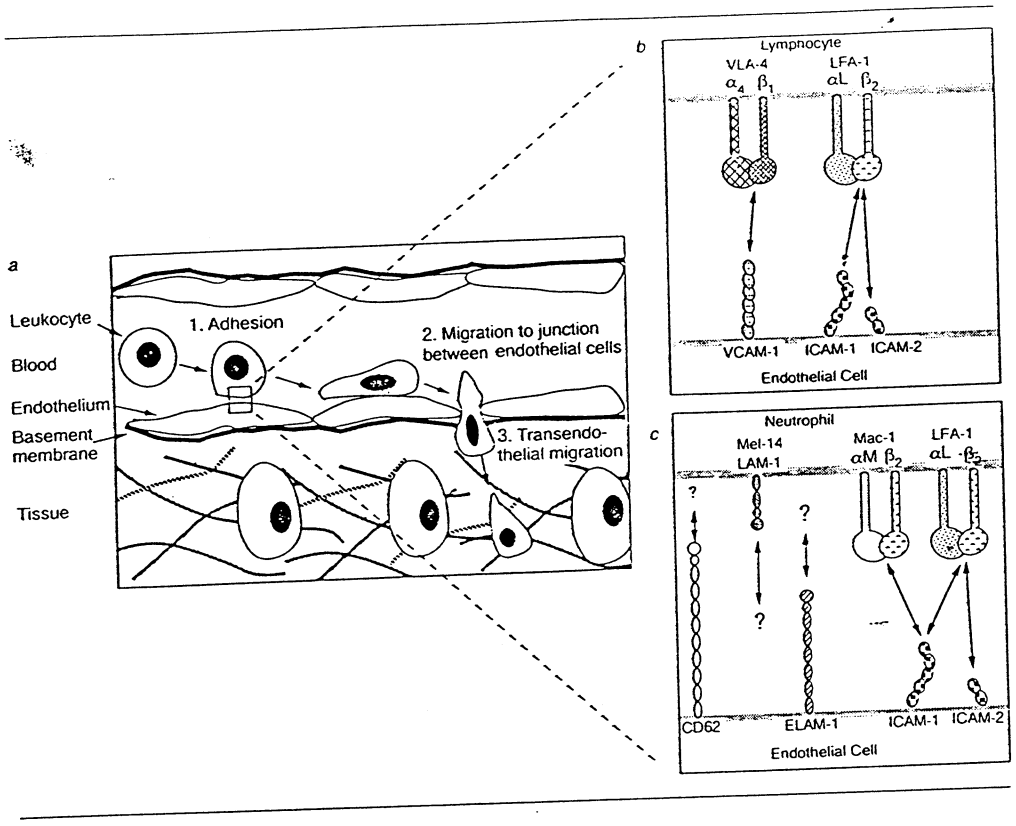


Fig. 5. Leukocyte extravasation.

therefore help regulate differing patterns of cell localization induced by inflammatory stimuli. Binding of leukocytes to endothelium is the first step in localization of circulating cells at an inflammatory site (fig. 5). In vivo, ICAM-1 induction accompanies T-cell-mediated hypersensitivity reactions in the skin [51] and, after administration of interferon-γ and interleukin-1, the appearance of ICAM-1 on endothelial cells correlates with sites of mononuclear cell infiltration [52]. ICAM-1 induction is largely regulated at the mRNA level [28, 29]. Increased surface expression is first seen after 4 h and is usually maximal by 24 h [44].

A second LFA-1 ligand, differing in tissue distribution from ICAM-1, was originally defined by the ability of antibodies against LFA-1, but not those against ICAM-1, to inhibit certain cell adhesion assays. On the basis

of this functional property, an ICAM-2 cDNA was isolated from an expression library by screening for binding of transfected cells, in the presence of ICAM-1 monoclonal antibody, to purified LFA-1 coated on Petri dishes [30]. ICAM-2 has two Ig-like domains, in contrast to ICAM-1 which has five (fig. 1), and these are 35% identical to the N-terminal two domains of ICAM-1. ICAM-1 and ICAM-2 are much more similar to one another than to other members of the Ig superfamily, and thus represent an Ig subfamily specialized to interact with LFA-1. Unlike ICAM-1, ICAM-2 is well expressed basally on endothelial cells and its mRNA is not increased by inflammatory mediators.

LFA-1 Avidity: A Dynamic Mechanism for Regulating Lymphocyte Adhesion and De-Adhesion

The mechanisms discussed so far for regulating adhesive interactions operate on a relatively long time scale. Regulation at the mRNA level of surface adhesion receptor density requires hours. Alteration of cell surface charge requires de novo glycoprotein biosynthesis and glycoprotein turnover, which is on the order of 12–24 h. Yet, adhesion by cytotoxic T cells can be regulated over a much shorter time scale; they can adhere to target cells, deliver a lethal hit, de-adhere, and engage with another target cell, with a cycle time as short as 1–5 min [53]. Moreover, while cytotoxic cells stimulated in vitro show a general increase in adhesiveness, cells stimulated in vivo adhere only to those cells bearing the antigen to which they were primed [20, 21]. This can be explained by the finding that crosslinking of the T-cell receptor on resting T lymphocytes transiently stimulates adhesiveness through LFA-1, allowing regulation of adhesion and de-adhesion over a time scale of minutes [24].

This rapid modulation of adhesion is due to qualitative rather than quantitative changes in the cell-surface expression of adhesive molecules. Homotypic adhesion of leukocytes (fig. 4c), which is dependent on LFA-1 and ICAM-1, is stimulated by treatment for 1 h with phorbol ester but is accompanied by no increase in LFA-1 or ICAM-1 surface expression [45, 46]. By testing the binding of cells expressing LFA-1 and ICAM to plastic substrates coated with either purified ICAM-1 or purified LFA-1 (fig. 4d), however, it can be shown that stimulating resting T lymphocytes with phorbol esters or crosslinking the TCR with monoclonal antibodies converts cellular LFA-1 from a low- to a high-avidity state, with no change in

surface density. Cellular ICAM-1 by contrast is constitutively avid. These changes can moreover be shown to influence the ability of stimulated peripheral blood lymphocytes to form conjugates with target cells. Conjugate formation is inhibited completely by LFA-1 and only marginally by CD2 monoclonal antibodies, showing that LFA-1 is primarily responsible for regulating the avidity of cell–cell interactions. Avidity peaks 5–10 min after stimulation of the TCR and returns to resting values by 30 min. Thus, contact TCRs with cells bearing specific antigen generates intracellular signals that lead to the conversion of LFA-1 to a high-avidity state and regulates LFA-1–ICAM-1-dependent adhesion in an antigen-specific manner. The transience of the high-avidity state provides a mechanism for regulating lymphocyte de-adhesion.

Integrin Family

LFA-1 is a member of the integrin family, perhaps the most versatile of the adhesion molecule families. Each integrin molecule comprises an α and a β subunit (fig. 2) and three subfamilies of integrins can be distinguished by their subunits: these are known as the β1 (CD29), β2 (CD18) and β3 (CD61) integrins. Both α and β subunits affect ligand specificity.

LFA-1 belongs to the β2 subfamily and is most closely related to two other integrins, Mac-1 and p150,95 with which it shares the β2 subunit [26] (fig. 2). These three β2 integrins are also known as the leukocyte integrins because their expression is limited to white blood cells. Mac-1 and p150,95 are particularly important in the adhesion of myeloid cells to other cells and to ligands that become insolubilized during activation of the complement and clotting cascades [22]. The importance of the leukocyte integrins is illustrated in congenital leukocyte adhesion deficiency (LAD) in which they are deficient because of mutations in the common β2 subunit [22, 54]. Patients have recurring infections, often fatal in childhood unless they are corrected by bone marrow transplantation. Neutrophils from these patients fail to orient and migrate in response to chemoattractants and are unable to bind to and cross the endothelium at sites of infection, so that pus fails to form. This is a most striking example of the role of adhesion molecules in leukocyte localization in vivo.

The β1 integrin subfamily includes receptors that bind to the extracellular matrix components fibronectin, laminin, and collagen and that are expressed on many nonhematopoietic and leukocyte cell types. These

receptors are likely to play a general part in tissue organization by binding to molecules in the extracellular matrix within many tissues and in the basement membranes found in muscle, the nervous system, and underlying the epithelium and endothelium [2, 55, 56]. The β1 family molecules have been designated very late activation (VLA) because two of them, VLA-1 and VLA-2, appear on lymphocytes 2–4 weeks after antigen stimulation in vitro [57]. In fact, however, some VLA molecules are basally expressed on leukocytes, and their expression on nonhematopoietic cells does not require activation.

VLA-4 (CD49d/CD29) is an unusual β1 integrin that is expressed on resting lymphocytes, monocytes, and neural crest-derived cells, and functions as both a matrix and cell receptor [57]. As a matrix receptor, it binds to an alternatively spliced domain of fibronectin distinct from the classical cell-binding site recognized by VLA-5 [58, 59]. As a cell receptor, it binds to a molecule recently described as VCAM-1 or INCAM-110 that is a member of the Ig superfamily [60–62]. This molecule is induced by inflammatory mediators on endothelium with kinetics similar to ICAM-1 and its interaction with VLA-4 provides an explanation for earlier evidence of a second lymphocyte-endothelium adhesion mechanism distinct from the LFA-1–ICAM interaction [50, 63] (fig. 5). In congenital deficiency of the β2 integrins, which does not affect VLA-4, lymphocytes retain the ability to emigrate across the endothelium at inflammatory sites [54]. This seems related to expression of VLA-4 by lymphocytes and not by neutrophils (fig. 5). Involvement of VLA-4 in T-cell-mediated killing [64] and in homotypic adhesion [65] suggests some functional redundancy with LFA-1. VLA-4 also helps mediate lymphocyte recirculation [66] as described below.

Structure and Regulation of Integrins

The structural domains of integrins (fig. 3) have been correlated with ligand binding by crosslinking to peptides containing the sequence arginine-glycine-aspartic acid (RGD), a ligand recognition motif for several but not all integrins. On the β3 subunit, ligand peptides are crosslinked within residues 109–171 [67]. This is the most highly conserved region among the β1, β2, and β3 subunits, and in LAD single amino acid substitutions in this region of β2 prevent association with α [68]; thus close association of α with this region of β may form a ligand-binding pocket. Integrin α subunits have three or four tandem repeats of a putative diva-

lent cation-binding site motif (fig. 3), and require Ca^{2+} or Mg^{2+} for function [22]. LFA-1α has three such repeats and has been shown to bind Mg^{2+}, and this correlates with the requirement for Mg^{2+} in T-cell adhesion and in binding of purified LFA-1 to purified ICAM-1 [24]. A ligand is crosslinked to amino acids 294–314 of the α subunit of αIIbβ3, which define the second divalent cation-binding site [69].

Further integrin domains may be involved in ligand binding. All three leukocyte integrin α subunits and the VLA α2 subunit have a domain of 200 amino acids not present in other integrin α subunits, and hence termed the 'inserted' or I domain. The I domains are homologous to ligand-binding repeats in von Willebrand factor and other proteins, and may confer modes of ligand recognition in addition to those shared by all integrins [22]. Cysteines are notably few in the putative ligand-binding regions of the α and β subunits, permitting conformational changes that regulate ligand binding.

Interactions of integrins with the cytoskeleton may be regulated by binding to ligands, and conversely, may help regulate ligand binding, thus mediating a bidirectional dialogue across the membrane. Several of the integrins can localize near to focal contacts, areas where the cell membrane is closely opposed to the extracellular matrix substrate and where actin bundles terminate, surrounded by a ring of vinculin and talin [70]. Talin appears to interact with the cytoplasmic domain of α5β1 [71]. Talin redistributes with LFA-1 to sites of antigen-specific adhesion and cocaps with LFA-1 after phorbol ester stimulation [15]; talin association may be a widespread feature of integrins. It is intriguing that redistribution of LFA-1 and talin has been shown to be highly sensitive to low antigen concentrations and may correlate with the high avidity state of LFA-1 [72].

Other integrins beside LFA-1 appear to undergo avidity regulation. On lymphocytes, the avidity of the β1 integrins VLA-4 and VLA-5 for fibronectin and VLA-6 for laminin is increased analogously to LFA-1 after TCR crosslinking [73]. On unactivated platelets, the integrin gpIIbIIIa does not bind fibrinogen, but upon activation binds soluble fibrinogen with kD of 29–45 n*M* [74]. The high avidity state of gpIIbIIIa appears to be permanent rather than transient. The mechanism of avidity regulation is unclear, but the ability of antibodies to detect conformational changes in LFA-1 [75] and in gpIIbIIIa [74] in sites distinct from the ligand-binding site suggests conformational changes in the ligand-binding site may also occur. Upon stimulation with chemoattractants, neutrophils show transient adhesion to other neutrophils, endothelial cells and to C3bi-coated

cells and there is some evidence that this may be due to a transient change in avidity of Mac-1 [76–78]. The cytoplasmic domains of integrins, perhaps by interaction with other cellular proteins, may signal the changes in the extracellular domains that regulate avidity. The LFA-1β subunit (integrin β2) cytoplasmic domain is required for functional activity in binding to ICAM-1, as shown by transfection of patient cell lines that genetically lack the β subunit with intact and truncated β subunit cDNAs [79] (and unpublished data).

Integrins are known to be essential for the migration of many cell types. Localized changes in integrin avidity, with high avidity at the leading edge of the cell and low avidity at the trailing edge may help regulate and drive cell migration [24], in conjunction with tension generated by the cytoskeleton. These two mechanisms for regulating cell migration may be coordinated by interactions between the cytoplasmic domains of integrins and the cytoskeleton.

Education of Lymphocytes Stably Alters Adhesion Receptor Phenotype

Lymphocytes newly emigrated from the thymus are considered 'naive', and remain so until they encounter and are stimulated by specific antigen. They then become longer-lived 'memory' lymphocytes. As discussed above, transient alterations in adhesion mechanisms lasting for minutes to days accompany lymphocyte activation. But permanent alterations in surface density also occur, as a result of the transition from the naive to memory phenotype. Shortly after antigen stimulation, naive T lymphocytes of both $CD4^+$ and $CD8^+$ subsets acquire increased levels of a cohort of surface molecules including the adhesion recepors CD2, LFA-1, and VLA family members [73, 80, 81] (table 1). Increased expression of these surface molecules persists after the stimulated lymphocytes have reverted to the resting state, probably lasting for the life of the memory cell. The changes in surface phenotype of memory T cells may have important consequences for their localization since they occupy distinct microenvironments within lymphoid organs [82] and have different recirculation routes [83].

Naive and memory T-cell subsets differ in lymphokine secretion, in some functional assays [80, 81, 84, 85], and in other important respects. Memory T cells seem to be more sensitive to antigen, because they are responsive to stimulation by much lower concentrations of TCR monoclonal antibody, although they have quantities of TCR, CD8, and CD4

Table 1. Conversion of naive to memory T lymphocytes alters surface molecule phenotype[1]

Molecule	Difference in expression[2]
CD2	↑2.8
LFA-1	↑2.4
LFA-3	− → +
CD49d (VLA-4)	↑2.7
VLA-5	↑3.6
CD49f (VLA-6)	↑3.4
CD29 (VLA-β, 4B4)	↑3.7
CD44 (Hermes Pgp-1)	↑2.1
CD45RO (UCHL1)	↑29
CD45 RA (2H4)	+ → −
CD4	1.0
CD8	1.0
TCR (CD3)	1.0

[1] Modified from Shimizu et al. [73] and Sanders et al. [81].
[2] Fold increase or decrease.

identical to naive T cells [86]. Increased expression of the LFA-1 and CD2 molecules should also enhance their sensitivity to antigen by facilitating interactions with antigen-presenting cells.

Lymphocyte Recirculation Receptors

Patrolling the body in search of foreign antigen, lymphocytes leave the blood, migrate through lymphoid organs and other tissues, and enter the lymphatics, whence they return to the blood through the thoracic duct. The peripheral lymph nodes draining the skin and the Peyer's patch and gut-associated lymph nodes draining mucosal surfaces differ in the types of antigens to which lymphocytes are exposed. Lymphocytes from adult animals differ from those in newborns in that, when harvested from specific lymph nodes, they show a 2-fold preference for recirculation to lymph nodes of the type from which they came [25, 87]. This suggests that priming by specific antigen may alter surface phenotype to enable selective recirculation to the type of secondary lymphoid organ where specific antigen was first encountered.

Lymphocytes in the blood enter lymph nodes by binding to specialized 'high' endothelial cells. Lyphocyte suspensions overlaid on sections of lymph nodes bind to these specialized venules, and 'recirculation' or 'homing' receptors on lymphocytes have been defined by monoclonal antibodies that block binding to the high endothelial cells of specific types of lymph nodes [25, 87, 88]. Molecules, termed 'addressins', selectively expressed on specialized high endothelium in different types of lymph nodes, are good candidates for the homing receptor ligands [25, 87, 88]. Binding and immigration into lymph nodes may be a cooperative process involving multiple receptors on the lymphocyte and counter-receptors on the endothelium, analogous to antigen-specific interactions (fig. 4a, b) since CD44, LFA-1, VLA-4, and Mel-14/LAM-1 on the lymphocyte have all been implicated in binding to high endothelial venule (HEV). The more interesting candidates for specific receptors are Mel-14/LAM-1, as a peripheral lymph node receptor, and the VLA-4α subunit associated with either of two integrin β subunits [25, 87–90]. However, the function of these molecules in adhesion hardly seems limited to lymphocyte recirculation, as discussed above for VLA-4.

Alternatively to emigration through HEV, lymphocytes may emigrate through endothelium within a tissue such as skin, and enter a lymph node through the afferent lymph. Memory T lymphocytes almost exclusively emigrate from blood through tissue endothelium, whereas naive lymphocytes emigrate through HEV [83]. It is thus possible that the endothelia of skin and mucosa may differ in expression of ligands that enable selective recirculation through these tissues. Congruent with this idea, the candidate homing receptors CD44 and VLA-4 (CD29) show increased expression on the memory T-lymphocyte subset (table 1) and LAM-1 (Leu8/TQ1) is preferentially expressed on a distinctive but overlapping memory lymphocyte subset [91]. Differences of several fold in surface density of these receptors may be adequate to give rise selectivity in recirculation.

Selectins and Their Role in Further Mechanisms for Neutrophil–Endothelial Interaction

The Mel-14/LAM-1 molecule is a representative of a novel class of molecules termed 'selectins' with an N-terminal lectin domain and diverse roles in adhesion [88, 89, 92–95] (fig. 3). The finding of the lectin-like

domain in Mel-14/LAM-1 correlates with the Ca^{2+} requirement for lymphocyte binding to peripheral lymph node endothelium and evidence that the counter-receptor is carbohydrate-like [87, 88, 93]. The number of short consensus repeats, which varies from two to nine in the three different selectins discovered to date, may serve to position their N-terminal lectin-like putative binding sites at varying distances from the plasma membrane (fig. 3).

All three selectins help regulate leukocyte binding to endothelium at inflammatory sites (fig. 5). The selectin Mel-14 not only functions as a lymphocyte recirculation receptor, but also contributes to neutrophil emigration at inflammatory site [25, 96]. After stimulation of neutrophils with chemoattractants, Mel-14 is rapidly shed from the cell surface [97]. The endothelial leukocyte adhesion molecule (ELAM)-1 is a selectin that is transiently expressed on endothelial cells 2–8 h after stimulation with interleukin-1 and other inflammatory agents, and mediates a neutrophil adhesion pathway distinct from that mediated by ICAMs and leukocyte integrins [92, 98]. The neutrophil chemoattractant interleukin-8, which is secreted by activated endothelial cells, acts on neutrophils to inhibit binding to ELAM-1 [99]. The proteolytic release of Mel-14 from the cell surface upon neutrophil activation, and the similar inactivation of the ELAM-1 counter-structure on the neutrophil, suggests that Mel-14 and ELAM-1 may function in an early step in neutrophil binding to the endothelium, prior to transendothelial migration. This contrasts with integrins, which are increased on the neutrophil surface by mobilization from granule compartments within minutes after stimulation of neutrophils with chemoattractants, and then remain permanently upregulated [54, 97]. Although both selectins and integrins can regulate neutrophil adhesion to endothelium, when selectins mediate adhesion, integrins are still required for the subsequent event of transendothelial migration [100, 101] (fig. 5). This may explain why congenital deficiency of the leukocyte integrins so completely inhibits neutrophil emigration from the blood [54].

A third selectin called PADGEM, GMP-140, or CD62 is stored in α-granules of platelets and Weibel-Palade bodies of endothelial cells and is rapidly mobilized to the surface of these cells after stimulation by products of the clotting cascade such as thrombin, where it mediates adhesion of neutrophils and monocytes [95, 102]. Thus selectins function in a wide range of cell interactions in the vasculature, and are expressed both on leukocytes and endothelial cells.

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