Adhesion receptors of the immune system

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The adhesive interactions of cells with other cells and with the extracellular matrix are crucial to all developmental processes, but have a central role in the functions of the immune system throughout life. Three families of cell-surface molecules regulate the migration of lymphocytes and the interactions of activated cells during immune responses.

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]. Localization of cells can thus in principle be driven by the interplay between interactions with cell-surface and matrix molecules that regulate adherence, and chemoattractant gradients that direct cell migration. These principles operate throughout the biology of multicellular organisms[2,3], and the cells of the immune system in particular depend upon regulated interactions with other cells to activate and direct the response to infection. The distinctive antigen-specific interactions studied by immunologists, and the cell and tissue-selective adhesive interactions studied by cell biologists, have however traditionally found little common ground. This has changed recently as studies of the interactions of T lymphocytes with antigen-bearing cells have yielded not only the antigen-specific receptors, but also a harvest of cell adhesion molecules. Homologous families of adhesion receptors reveal relationships between molecules in the immune system and many other tissues, and participate in the control not only of cell-cell interactions but also in the regulation of cell migration.

The mechanisms for regulating adhesion, and their functional importance, are particularly richly illustrated by the adhesion receptors of the immune system. To patrol the body effectively for infectious organisms, the cells of the immune system 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]. Rapid transition between adherent and nonadherent states is of key importance to the dual functions of immune surveillance and responsiveness. In this review, I shall explain what is known of the adhesive interactions that take place when lymphocytes have been activated by 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 (Figs 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. Many adhesive molecules play a part in more than one of these functions, and some of them are exploited as receptors by viruses.

Antigen-specific recognition by T lymphocytes

Most of our knowledge about the molecules regulating lymphocyte adhesion has come from the study of T lymphocytes, whose functions in immunity depend on close contact with other cells. The T-cell receptor (TCR; Fig. 1) recognizes antigen as a peptide fragment bound to cell-surface molecules encoded by the major histocompatibility complex (MHC; Fig. 1). T lymphocytes are activated by specific binding of their receptors to peptide-MHC complexes on the surface of antigen-presenting cells such as macrophages. This stimulates proliferation and differentiation of two types of functional T cells: helper cells, which promote the proliferation and maturation of antibody-producing B lymphocytes; and killer cells, which lyse cells infected with intracellular parasites such as viruses. Helper T cells recognize MHC-antigen complexes on the surface of the B cells whose proliferation they induce; killer T cells recognize complexes of viral peptides with MHC on the surface of the infected cells they kill.

The MHC molecules that present peptide antigens for recognition by TCRs fall into two structurally distinct classes (Fig. 1). MHC class I molecules bind to peptides derived from endogenously synthesized molecules such as viral proteins in infected cells and are primarily recognized by killer cells (Fig. 4*a*). MHC class II molecules bind to peptides derived from endocytosed antigen, and are recognized primarily by helper T lymphocytes (Fig. 4*b*).

All of the 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 respectively for class I and class II MHC molecules[5,6]. The T-cell receptor 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. 4*a*, *b*). At physiological densities on T lymphocytes and in the absence of antigen, CD4 and CD8 mediate little or no adhesion to MHC[7,8] although overexpression in transfected fibroblasts has demonstrated binding by CD4 to class II and CD8 to class I[9,10]. 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[5,11-14].

Synergistic signalling by the association of the TCR with its co-receptor has been directly demonstrated for CD8 (ref. 15), which has been shown to bind to the membrane-proximal domain of the MHC class I molecule that is not polymorphic[15,16], whereas the TCR binds to the polymorphic membrane-distal domains[17]. 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[14], and antibodies that induce the association of CD4 with the TCR also activate T cells[11,14]. The signalling function of CD4 and CD8 may depend on the association of their cytoplasmic segments with a lymphocyte-specific tyrosine kinase encoded by *lck* (ref. 18).

CD4 and CD8 may play a critical part in the differentiation of T-cell precursors in the thymus. Early in thymic ontogeny, immature T cells express both CD4 and CD8; coassociation of one or the other of these molecules with the T-cell receptor may signal to the thymocyte whether its TCR recognizes class I or class II MHC, and thereby regulate subsequent differentiation into $CD8^+$ cytotoxic or $CD4^+$ helper T-lymphocyte subsets[11,12].

Activation-dependent adhesion mechanisms

The definition of other cell-surface molecules essential for the

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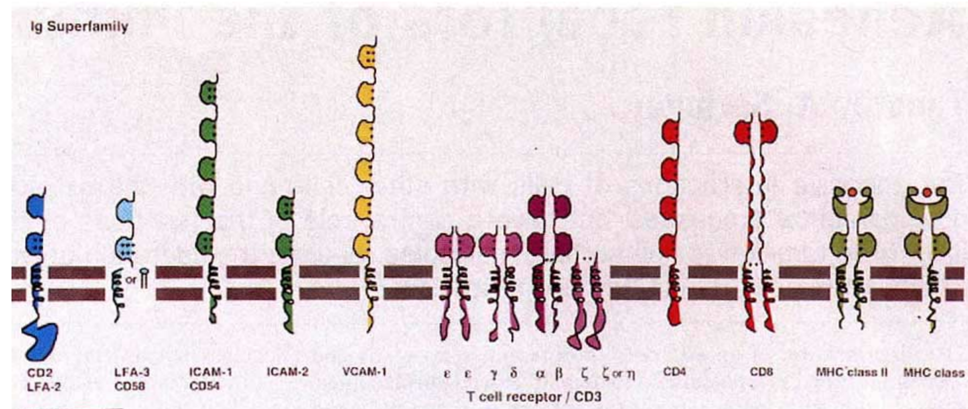

FIG. 1 Immunoglobulin superfamily adhesion receptors. Members of the superfamily share the immunoglobulin domain, composed of 90–100 amino acids arranged in a sandwich of two sheets of anti-parallel β-strands, which is usually stabilized by a disulphide bond at its centre[136,137]. The immunoglobulins 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 superfamily in adhesion evolutionarily predates specialization for antigen recognition, which occurs only in vertebrates; immunoglobulin superfamily members are present in insects as nervous system adhesion molecules involved in axon guidance and fasciculation[138]. The immunoglobulin domain may have diversified and been adopted so widely in evolution because its stable disulphide-bonded β-strand structure is analogous to a car chassis on which many different styles of bodies and bumpers 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, in contrast to immunoglobulins and MHC molecules, which have paired immunoglobulin domains, the domains of ICAM-1 (ref. 130) and NCAM (ref. 139) are unpaired. Immunoglobulin domains studied 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[17,137]. Electron micrographs of ICAM-1 show that it is a bent rod 18.7 nm long. This is only compatible with a model in which its five domains are unpaired, and arranged end-to-end at a slight angle to the β-strands. Overall size and shape of the immunoglobulin domains are shown to scale (bar, 10 nm), although schematized, see Fig. 8 for a more accurate scale drawing; dots denote disulphide-bonded cysteines. Dimensions are based on the following information: MHC class I molecule, X-ray crystallography[17]; MHC class II, approximation as MHC class I; TCR, approximation of $\alpha\beta$ heterodimer as an Fab fragment[140]; ICAM-1, electron microscopy[130]; ICAM-2 (ref. 29), CD2, LFA-3 (ref. 136), CD4 (ref. 13) and VCAM-1 (ref. 70) approximation to two, four or six unpaired domains as in ICAM-1; CD8, a disulphide-linked α–α or α–β dimer of one immunoglobulin domain, the length of the connecting or hinge-like peptide[13] of 40 or 65 amino acids for α or β, respectively, is arbitrary and is consistent with a spacing of 1.5–3.3 Å per residue[141]; note that the distance the connecting peptide is shown to extend in CD8 is similar to that which would be predicted for three of the four immunoglobulin domains in CD4, allowing the N-terminal domain of CD4 to occupy a position similar to that shown for the CD8 domain.

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[5,19-21]. Thus, three molecules have been identified by screening monoclonal antibodies for their ability to inhibit killing and were operationally termed lymphocyte function-related antigens: LFA-1, LFA-2 (CD2) and LFA-3 (Fig. 4*a*, *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*[7,8,19,22,23] and presumably help localize activated T cells to sites of antigen accumulation in the lymph nodes *in vivo*[24].

LFA-1, which is a member of the integrin family (Fig. 2), is expressed on T lymphocytes; its counter-receptor on the target cell is ICAM-1 or ICAM-2 (for intercellular cell adhesion molecule), both members of the immunoglobulin family (Fig. 1)[19,21,23,25-29]. LFA-2 or CD2, another member of the immunoglobulin family, is expressed on the T lymphocyte: its counter-receptor on the target cell, LFA-3, is also a member of the immunoglobulin family[5,19,30,31] (Fig. 1). The LFA-1/ICAM and 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 several different surface molecules[5,20,21,32]. Indeed, we shall see later that binding of the T-cell receptor can modulate

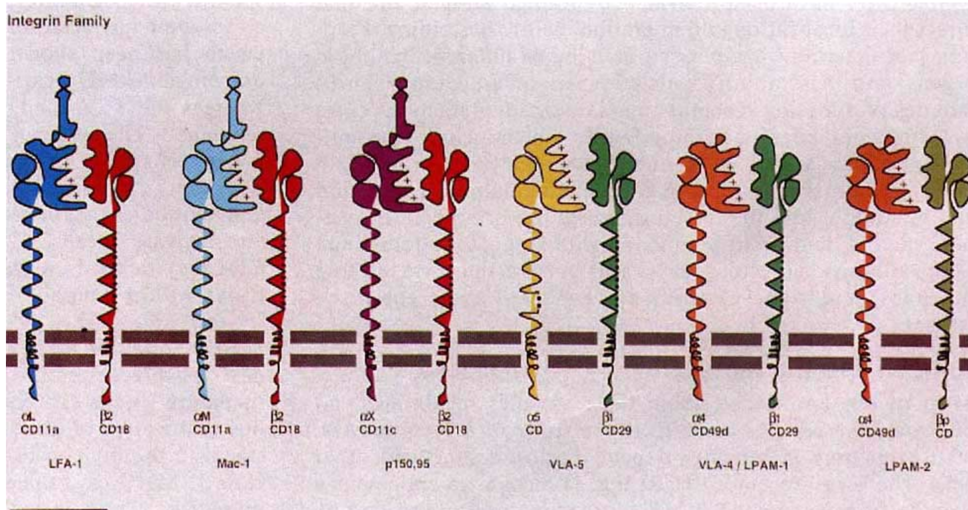

FIG. 2 Representative integrin family adhesion receptors. Integrins contain α- and β-subunits of approximately 1,100 and 750 amino acids, respectively, which are noncovalently associated. The α-subunits are 25–65% identical in amino-acid sequence and the β-subunits are 37–45% identical; the structural and functional similarities are so strong that integrins should be considered a protein family rather than a superfamily[21,65]. Although schematic, the overall size and shape of the integrins is shown to scale, based on electron microscopy of VLA-5 (ref. 142) and CD41/CD61 (ref. 143) (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 disulphide bridging a cleavage site in some α-subunits. Divalent cation binding sites are symbolized by '+'. Structures are cited in refs 21, 67 and Table I.

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the affinity of LFA-1 for its counter-receptor. Adhesive interactions between CD2 and LFA-3 are also regulated by activation of T cells, although by a different mechanism.

Regulated adhesion by 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. This is reflected in 'rosetting' (Fig. 5), in which erythrocytes, which express LFA-3, adhere *in vitro* to activated T cells expressing CD2: resting T cells do not form rosettes with autologous erythrocytes.

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[33]. 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[34]; these repulsive interactions seem to be reduced in activated T lymphocytes. The effect of activation on the CD2/LFA-3 interaction can be mimicked by the addition of positive charge through chemical derivatization of erythrocytes or removal of negative charge from erythrocytes or T cells by digestion of the glycocalyx[35,36]. Charge neutralization alters the morphology of the contact between T lymphocytes and erythrocytes, converting small islands of close contact surrounded by larger areas of greater intermembrane distance to a single large area of close contact[36]. Despite their larger surface area, T-cell blasts and thymocytes have fivefold less sialic acid per cell than resting T cells[37] and are less negatively charged[38]; 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 centres are greatly undersialylated, whereas areas containing B and T cells in rapid transit between blood and lymph are normally sialylated[39]. In the nervous system as well, cell interactions are regulated by sialylation; polysialylation of NCAM antagonizes its ability to promote adhesion[40]. Theoretical calculations[41] confirm that the attractive and repulsive energies in rosetting are of a similar order of magnitude and thus finely balanced, based on the affinity of the CD2/LFA-3 interaction of 10^6 M^{-1} (refs 30, 42, 43), adhesive receptor surface density and charge density[163].

CD2 may transduce a signal which augments or synergizes with signals from the TCR[5]. 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[42,44]. Transfection of cells with CD2 and LFA-3 has confirmed early antibody inhibition results[19] showing CD2/LFA-3 interaction can contribute a 4- to 30-fold enhancement of the immune response[45,46]. The unusually basic, histidine- and proline-rich 120-amino-acid cytoplasmic region of CD2 is required for stimulation by pairs of monclonal antibodies[46,47]; in antigen-specific responses, however, 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[45,46].

There are two isoforms of LFA-3, derived by differential messenger RNA splicing: one is anchored in the membrane by a glycophosphatidyl inositol tail, whereas the other has a classical transmembrane hydrophobic segment and a 12-amino-acid cytoplasmic segment[48-50] (Fig. 1). Both are fully active in mediating CD2-dependent adhesion and in promoting T-cell function[42,45,46,51]. The functional significance of the isoforms remains obscure, although it is intriguing that the neural cell adhesion molecule (NCAM) also has anchor isoforms[40,52].

ICAM-1 and ICAM-2 counter-receptors for LFA-1

LFA-1, although 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[19,21]. A counter-receptor for LFA-1, ICAM-1, was identified using a simple assay called homotypic adhesion (Fig. 6*a*), in which homogeneous cell populations such as B- or T-cell lines adhere to one another to form multicellular clusters[19,53]. 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[54]; 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[55]. ICAM-1 is a member of the immunoglobulin superfamily[27,28], with five immunoglobulin 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[21,53] and thereby presumably inflammatory responses. In the absence of an inflammatory response, ICAM-1 is expressed on only a few cell types[56]. Its importance has been demonstrated *in vitro* by blocking T-cell killing with antibody to ICAM-1 (ref. 57), and by transfection experiments in which fibroblasts expressing suboptimal levels of MHC molecules can be enabled to activate T-helper cells by cotransfection with the gene encoding ICAM-1[58]. This is consistent with its natural expression during the response to a specific antigen. ICAM-1 is expressed both on the follicular dendritic cells and on the

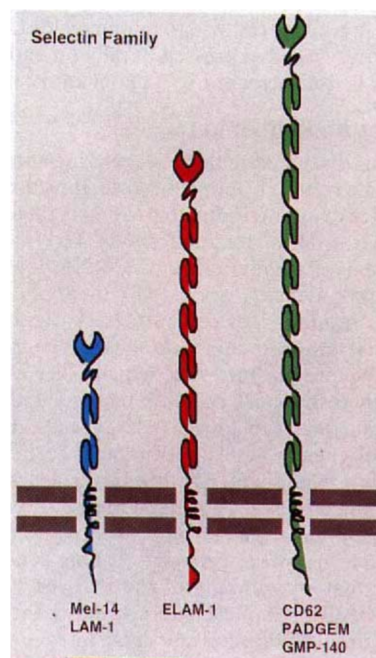

FIG. 3 Selectins. Selectins have an N-terminal domain of 117–120 amino acids which is homologous to a variety of Ca^{2+}-dependent animal lectins[144] including hepatic galactose receptors, soluble mannose-binding lectins, and invertebrate lectins, as well as to proteins known to bind ligands independently of carbohydrate, including the low-affinity receptor for IgE (CD23). After the N-terminal lectin domain is an EGF (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 refs 110, 112–115. Selectin domains are shown roughly to scale (bar, 10 nm): the short consensus repeats (SCR) extend 4.1 nm each, based on a length of 33 nm (ref. 145) for 8 SCR (ref. 146); EGF repeats extend about 2.3 nm (ref. 147), and the lectin-like N-terminal domain is modelled as a globular sphere[144].

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activated B lymphocytes found in germinal centres[56], and may contribute through homotypic aggregation to formation of germinal centres.

Inflammatory mediators, including lipopolysaccharide, γ-interferon (γ-IFN), interleukin-1 (IL-1) and tumour necrosis factor (TNF) 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 (refs 19, 21, 53, 59). Endothelial, fibroblastic and epithelial cells vary as to which cytokines are capable of inducing ICAM-1 expression, and the types of mediators released may 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. 7*a*). *In vivo*, ICAM-1 induction accompanies T cell-mediated hypersensitivity reactions in the skin[60], and, after administration of γ-IFN and IL-1, appearance of ICAM-1 on endothelial cells correlates with sites of mononuclear cell infiltration[61]. ICAM-1 induction is largely regulated at the mRNA level[27,28]. Increased surface expression is first seen after 4 h, and is usually maximal by 24 h (ref. 53).

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 this basis, an ICAM-2 complementary DNA was isolated from an expression library by screening for binding of transfected cells, in the presence of monoclonal antibody against ICAM-1, to purified LFA-1 coated on Petri dishes[29]. ICAM-2 has two immunoglobulin-like domains, in contrast to ICAM-1 which has five (Fig. 1), and these are 35% identical to the two N-terminal domains of ICAM-1. ICAM-1 and ICAM-2 are much more similar to one another than to other members of the immunoglobulin superfamily, and thus represent a 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 and adhesion

The mechanisms discussed so far for regulating adhesive interactions operate on a relatively long timescale. Increased expression of ICAM-1 after cytokine induction is detectable *in vitro* or *in vivo* after 4–6 h, and is maximal by 9–24 h[21,53,61]. This time course is typical of regulation at the RNA level of surface adhesion receptor density, and seems to apply to CD2 and LFA-3 as well. Alteration of cell surface charge by changes in glycoprotein sialylation requires *de novo* glycoprotein biosynthesis[62] and glycoprotein turnover, which takes about 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, detach and engage with another target cell, with a cycle time as short as 1–5 min (ref. 63). Moreover, whereas 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[19,20]. This can be explained by the finding that crosslinking of the TCR on resting T lymphocytes transiently stimulates adhesiveness through LFA-1, allowing regulation of adhesion and detachment over a timescale of minutes[23].

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. 6*a*), 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[54,55]. 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. 6*b*), 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[23]. Cellular ICAM, by contrast, is constitutively avid. These changes 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. The transience of the high-avidity state provides a mechanism for regulating lymphocyte deadhesion.

Thus, in the absence of antigen, the equilibrium governing adherence favours free, mobile T lymphocytes and efficient immune surveillance. Contact of 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. LFA-1 is an adhesion servomotor controlled by the TCR.

The integrin family

LFA-1 is a member of the integrin family (Table 1), 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 β subunits: these are known as the β1 (CD29), β2 (CD18) and β3 (CD61) integrins.

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[25] (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 adhesion of myeloid cells to other cells and to ligands that become insolubilized during activation of the complement and clotting cascades[21] (Table 1). 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[21,64]. 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 (Table 1) and they are expressed on many non-haematopoietic 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,

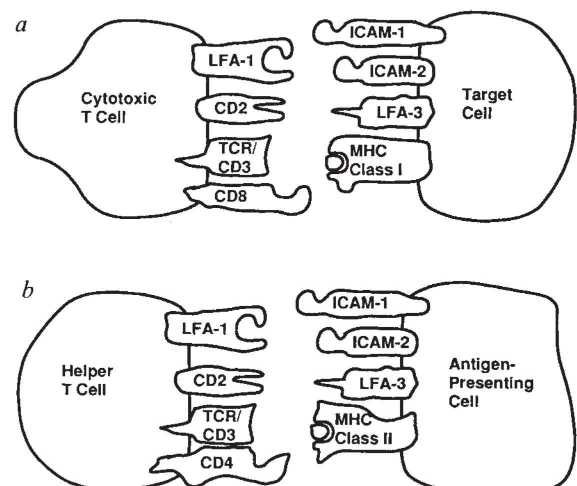

FIG. 4 Antigen-specific T-cell interactions.

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TABLE 1 Integrin family of cell-cell and cell-matrix receptors

Subunits	Names	α-subunit* I	C	Ligands†	RGD role	Distribution Non-leukocyte‡	Leukocyte§	Ref.‖
αLβ2	CD11a/CD18, LFA-1	+	−	ICAM-1,2	−		B, T, M, G	21
αMβ2	CD11b/CD18, Mac-1, CR3	+	−	C3bi, FX, FB	+?		M, G	21
αXβ2	CD11c/CD18, p150, 95	+	−	?	?		M, G	21
α1β1	CD-/CD29, VLA-1	?	−	LM, CO	−	F, BM	B′, T′	67
α2β1	CD49b/CD29, VLA-2, gpIaIIa, ECMRII	+	−	LM, CO	−	P, F, EN, EP	T′	154–157
α3β1	CD-/CD29, VLA-3, ECMRI		+	FN, LM, CO	−	EP, F		155
α4β1	CD49d/CD29, VLA-4, LPAM-1	−	!	FN, VCAM-1	−	NC, F	B, T, M, LGL	68, 69, 71, 74, 76
α5β1	CD-/CD29, VLA-5, FNR, gpIc/IIa, ECMRVI	−	+	FN	+	F, EP, EN, P	Th, T	155
α6β1	CD49f/CD29, VLA-6, gpIc/IIa		+	LM	−	P	T	94, 158
α4βp	CD49d/CD-, LPAM-2	−	!	?	?		T	82
α6β4	CD49f/CD-, αEβ4		+	?	−	E		77–79
αIIbβ3	CD41/CD61, gpIIbIIIa	−	+	FB, FN, vWF, FB	+	P		95
αVβ3	CD51/CD61, VNR	−	+	VN, FB, vWF, TSP	+	EN	B′, M	95, 159, 160
αVβ1	CD51/CD29	−	+	FN	+	NC, F		161
αVβ5	CD51/CD-, αVβS	−	+	VN, FN	+	C, F, EP	M	80, 81, 160

*I, I domain; C +, cleavage to disulphide-linked heavy and light chains at amino acid 853–860; !, cleavage to nondisulphide-linked chains at roughly amino acid 573.
† LM, laminin; CO, collagen; FN, fibronectin, FB, fibrinogen; FX, factor X; VN, vitronectin; vWF, von Willebrand factor; TS, thrombospondin.
‡ EN, endothelial cells; EP, epithelial cells; F, fibroblasts or other connective tissue; NC, neural crest, melanocytes; P, platelets; C, carcinomas; BM, basement membrane-associated.
§ B, B lymphocytes; T, T lymphocytes; ′, activated lymphocytes only; Th, thymocytes; M, monocytes; G, granulocytes; LGL, large granular lymphocytes. Some data from Leukocyte Typing Database III (ref. 162) and IV.
‖ General refs 2, 21, 65–67.

and underlying the epithelium and endothelium[2,65,66]. The β1 family molecules have been designated VLA (very late activation) because two of them, VLA-1 and VLA-2, appear on lymphocytes 2–4 weeks after antigen stimulation *in vitro*[67]. In fact, some VLA molecules are basally expressed on leukocytes, and their expression on nonhaematopoetic cells does not require activation (Table 1). Induction of VLA-1, -2, -3 and -5 expression after leukocytes cross the endothelial barrier may be of great importance in controlling leukocyte localization in inflammation.

VLA-4 (CD49d/CD29) is an unusual β1 integrin expressed on resting lymphocytes, monocytes and neural crest-derived cells, and functions as both a matrix and cell receptor[67]. As a matrix receptor, it binds to an alternatively spliced domain of fibronectin distinct from the classical cell binding site recognized by VLA-5[68,69]. As a cell receptor, it binds to a molecule recently described as VCAM-1 or INCAM110 that is a member of the immunoglobulin superfamily[70–72]. 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[59,73] (Fig. 7*b*). In congenital deficiency of the β2 integrins, which does not affect VLA-4, lymphocytes retain the ability to migrate across the endothelium at inflammatory sites[64]. This seems related to expression of VLA-4 by lymphocytes and not by neutrophils (Fig. 7*c*). Involvement of VLA-4 in T cell-mediated killing[74] and in homotypic adhesion[75] suggests some functional redundancy with LFA-1. VLA-4 also helps mediate lymphocyte recirculation[76], as described below.

The complexity of the integrin family has recently been increased by the discovery of novel β-subunits that can associate with the α4, α6 and αV subunits alternatively to the previously described β1 and β3 subunits[77–82] (Table 2). Both α- and β-subunits affect ligand specificity. Their combinatorial use confers considerable diversity in ligand recognition, and differences in transmembrane and cytoplasmic domains may also help regulate communication between the inside and outside of the cell.

Structure and regulation of integrins

The structural domains of integrins (Fig. 2) have been correlated with ligand binding by crosslinking to peptides containing the sequence Arg-Gly-Asp (RGD in the single-letter amino-acid code), a ligand recognition motif for several but not all integrins (Table 1). On the β3 subunit, ligand peptides are crosslinked within residues 109–171 (ref. 83). 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 the α-subunit (ref. 84); thus close association of the α-subunit with this region of the β-subunit may form a ligand-binding pocket. Integrin α-subunits have three or four tandem repeats of a putative divalent cation-binding motif (Fig. 2), and require Ca^{2+} or Mg^{2+} for function[21]. LFA-1 α-subunit has three such repeats and binds Mg^{2+}; this correlates with the requirement for Mg^{2+} in T-cell adhesion and in binding of purified LFA-1 to purified ICAM-1 (ref. 23). The divalent metal-binding motif in integrins has only five of six predicted metal coordination sites. It has been proposed that in integrin binding to RGD-containing ligands, the Asp residue (D) in RGD binds to the metal held in the divalent cation-binding pocket of the α-subunit, forming a sixth coordination site[85]. Consistent with this, a ligand is crosslinked to amino acids 294–314 of the α-subunit of αIIbβ3, which define the second divalent cation-binding site[86].

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[21]. 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[87]. Talin seems to interact with the cytoplasmic domain of α5β1 (ref. 88). Talin redistributes with LFA-1 to sites of antigen-specific adhesion and cocaps with LFA-1 after stimulation with phorbol ester[14]; talin association may be a widespread feature of integrins. It is intriguing that redistribution of LFA-1 and talin is highly sensitive to low antigen concentrations and may correlate with the high-avidity state of LFA-1[89].

Like many receptors, integrins transduce information from the outside to the inside of the cell. The growth and differentiation of many connective tissue and nervous system cells is affected by their substrates, largely through integrins[2,65,66]. Examples in the immune system include regulation of T-cell proliferation by LFA-1 (refs 90–92), VLA-5 (ref. 93), VLA-4 and VLA-6 (ref. 92).

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Integrins are novel receptors with respect to the type of inside-out signalling described for LFA-1, in which signals from the cytosol are transduced across the membrane to generate changes in extracellular functions such as adhesion. The avidity of other integrins besides LFA-1 seems to be regulated. On lymphocytes, the avidity of the $\beta1$ integrins VLA-4 and VLA-5 for fibronectin and VLA-6 for laminin is increased analogously to LFA-1 after TCR crosslinking[94]. On unactivated platelets, the integrin gpIIbIIIa does not bind fibrinogen, but on activation binds soluble fibrinogen with a dissociation constant K_d of 29–45 nM (ref. 95). The high-avidity state of gpIIbIIIa seems 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 (ref. 96) and in gpIIbIIIa (ref. 95) in sites distinct from the ligand-binding site suggests conformational changes in the ligand binding site may also occur. On stimulation with chemoattractants, neutrophils transiently adhere 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 (refs 97–99). 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-1 β-subunit (integrin $\beta2$) 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 (ref. 100, and M. L. Hibbs, H. Xu, S. A. Stacker and T.A.S., unpublished data).

Lymphocyte education

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 receptors CD2, LFA-1 and VLA family members[94,101,102] (Table 2). 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 because they occupy distinct microenvironments within lymphoid organs[103] and have different recirculation routes[104].

TABLE 2 Conversion of naive to memory T lymphocytes alters surface molecule phenotype*

Molecule	Difference in expression (-fold increase)
CD2	2.8
LFA-1	2.4
LFA-3	− to +
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)	+ to −
CD4	1.0
CD8	1.0
TCR (CD3)	1.0

* Modified from refs 94, 102.

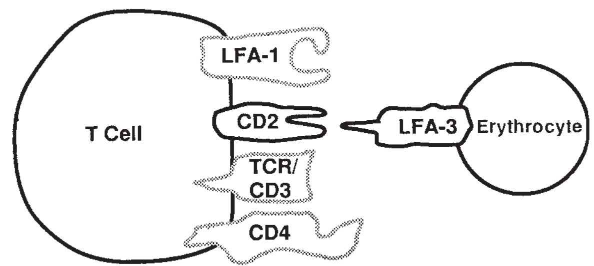

FIG. 5 T-cell rosetting with erythrocytes.

Naive and memory T-cell subsets differ in lymphokine secretion, in some functional assays[101,102,105,106], 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 monoclonal antibody against TCR, although they have quantities of TCR, CD8 and CD4 identical to naive T cells[107]. 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 collected from specific lymph nodes, they show a twofold preference for recirculation to lymph nodes of the type from which they came[24,108]. 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. Lymphocyte 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[24,108,109]. Molecules, termed 'addressins', selectively expressed on specialized high endothelium in different types of lymph nodes, are good candidates for the homing receptor ligands[24,108,109]. Binding and immigration to lymph nodes may be a cooperative process involving several receptors on the lymphocyte and counter-receptors on the endothelium, analogous to antigen-specific interactions (Fig. 4) because CD44, LFA-1, VLA-4 and Mel-14/LAM-1 on the lymphocyte have all been implicated in binding to high endothelial venules (HEV). The more interesting candidates for specific receptors are Mel-14/LAM-1 as a peripheral lymph node receptor, and the VLA-4 α-subunit associated with either of two integrin β-subunits as a Peyer's patch receptor[24,82,108–110]. But 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 (ref. 104). 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 show increased expression on the memory T-lymphocyte subset (Table 2) and LAM-1 (Leu8/TQ1) is preferentially expressed

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on a distinctive but overlapping memory lymphocyte subset[111]. Differences of several fold in surface density of these receptors may be adequate to give rise to selectivity in recirculation.

The role of selectins

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[109,110,112-115] (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[108-110,113]. The number of short consensus repeats, which varies from two to nine in the three different selectins discovered so far, 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. 7*c*). The selectin Mel-14 not only functions as a lymphocyte recirculation receptor, but also contributes to neutrophil emigration at inflammatory sites[24,116]. After stimulation of neutrophils with chemoattractants, Mel-14/LAM-1 is rapidly shed from the cell surface[117]. The endothelial leukocyte adhesion molecule, ELAM-1, is a selectin that is transiently expressed on endothelial cells 2–8 h after stimulation with IL-1 and other inflammatory agents, and mediates a neutrophil adhesion pathway distinct from that mediated by ICAMs and leukocyte integrins[112,118]. The neutrophil chemoattractant IL-8, which is secreted by activated endothelial cells, acts on neutrophils to inhibit binding to ELAM-1 (ref. 119). 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 function in an early step in neutrophil binding to the endothelium, before 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[64,117]. 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[120,121] (Fig. 7*a*). This may explain why congenital deficiency of the leukocyte integrins so completely inhibits neutrophil emigration from the blood[64].

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[115,122]. Thus, selectins function in a wide range of cell interactions in the vasculature and are expressed both on leukocytes and endothelial cells.

Virus receptors

Several cell adhesion receptors are subverted as virus receptors[123]. The human immunodeficiency virus (HIV), which causes AIDS, binds to CD4. Although rhinoviruses, which cause common colds, have evolved more than 100 non-crossreactive antigenic variants in an attempt to evade the immune response, 90% of them bind to the same receptor, ICAM-1.

The use of cell-adhesion receptors by viruses may be more than coincidental. First, virus–cell and cell–cell adhesion are in principle very similar. Both require accessible cognate sites on the receptor, both involve multivalent interactions and both may be facilitated by receptor redistribution to the site of adhesion. Second, binding to molecules that participate in the immune response has important consequences for the host–virus relationship. Human immunodeficiency virus (HIV) infects and kills or renders anergic $CD4^+$ T-helper cells, and downregulates CD4 expression[124]. Binding of HIV or its shed surface glycoprotein gp120 may transduce signals through CD4 which interfere with normal responsiveness[125]. HIV can spread by fusion of infected cells with uninfected cells to form syncytia. This requires CD4 and gp120 but also LFA-1 (ref. 126). Rhinoviruses, by contrast, rather than thwarting the immune response, use it to their own ends. Mucous secretions and sneezing induced by the immune response facilitate infection of other individuals. If ICAM-1 has a signal transducing function on antigen-presenting cells, this may be mimicked by the virus and may serve its ends by stimulating production of cytokines which increase nasal secretions carrying the virus.

In view of the rationales discussed above, it is of considerable interest that viruses have evolved to bind to the same regions of CD4 and ICAM-1 as do their cell adhesion counter-structures. HIV binds to the first and part of the second immunoglobulin-like domains of CD4 (refs 127–129); the same overall region of CD4 binds to MHC class II molecules[129]. LFA-1 and rhinoviruses bind to overlapping but distinct regions of the first immunoglobulin-like domain of ICAM-1 (ref. 130). The binding site for ICAM-1 is hypothesized to be in a 'canyon' on the rhinovirus surface which is too narrow to admit an antibody[164], consisting of paired immunoglobulin domains, but is of the right dimensions to fit unpaired domains as exist in ICAM-1 (ref. 130).

Close encounters at the membrane

We are just beginning to understand adhesion on a molecular scale (Fig. 8). The size and shape of several immune system adhesion molecules are known by electron microscopy or X-ray crystallography, and others may be modelled from the structure of homologous molecules or constituent domains (Figs 1, 2 and 3 legends). The binding sites for several of the adhesion receptors and their counter-receptors are known (Fig. 8 legend), allowing prediction of how close together two cell membranes, or a cell membrane and the extracellular matrix, would have to come to allow interaction. A model of the distances over which adhesion receptors would mediate cellular interactions can then be constructed (Fig. 8). This model can be compared with measurements of distances made in micrographic studies[131,165]. Furthermore, the sizes of two major components of the leukocyte glycocalyx that bear most of the cell-surface sialic acid[132], CD43

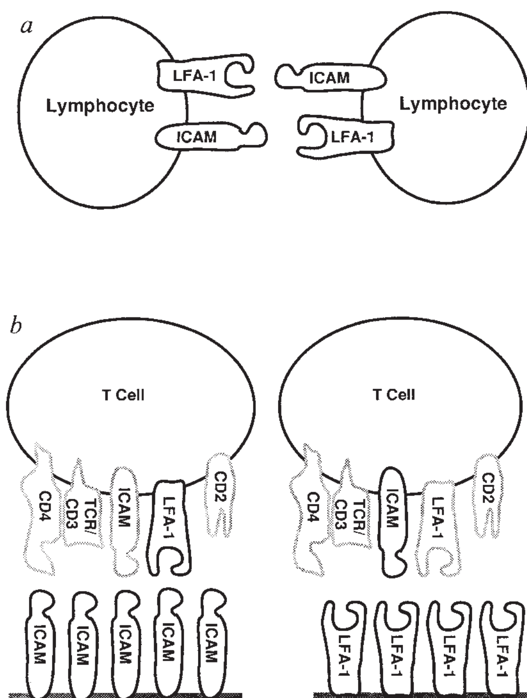

FIG. 6 Homotypic adhesion (*a*) and adhesion to purified molecules in artificial membranes (*b*).

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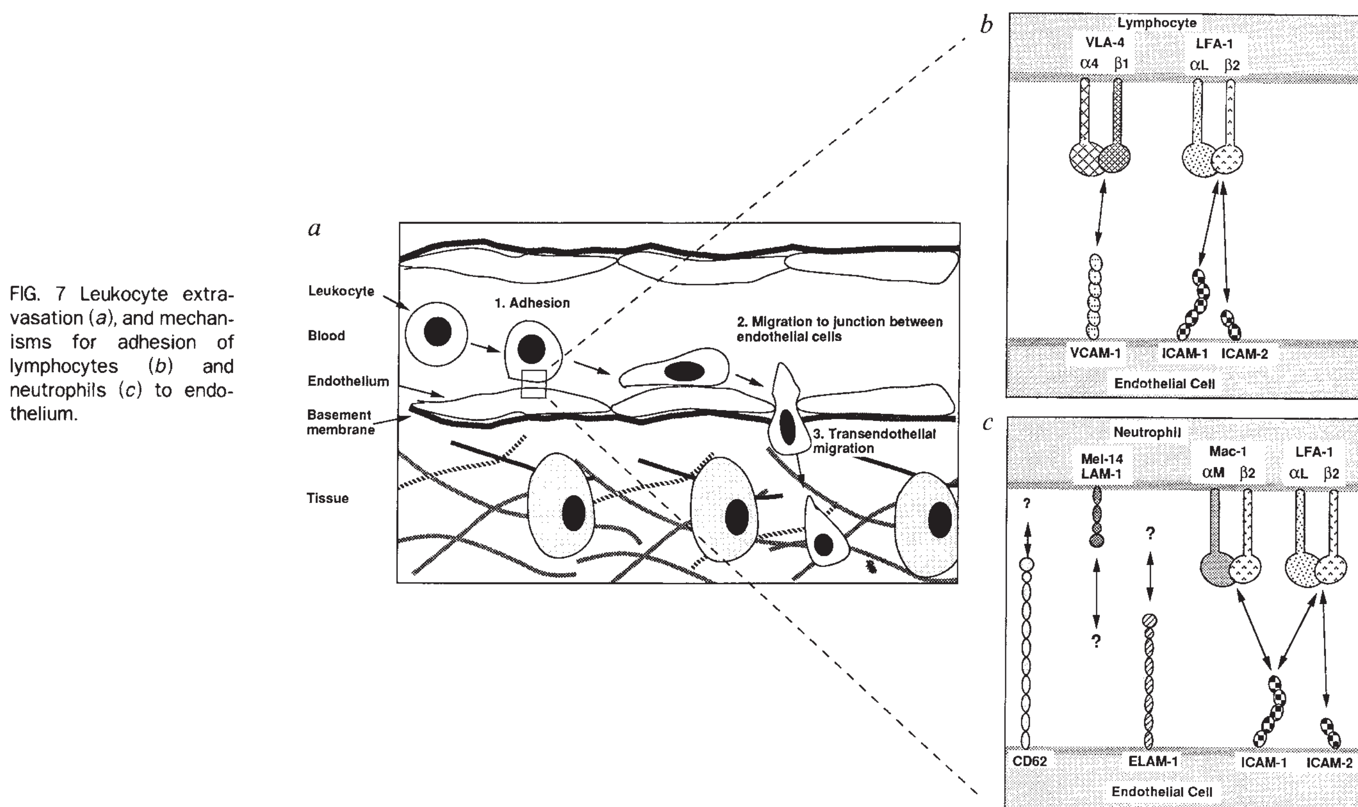

FIG. 7 Leukocyte extravasation (*a*), and mechanisms for adhesion of lymphocytes (*b*) and neutrophils (*c*) to endothelium.

and CD45, are included in Fig. 8 as a reminder of the scale of the glycocalyx, which will oppose cell-cell adhesion due to negative-charge repulsion and the loss of entropy involved in compressing or interdigitating the glycocalyces of two contacting cells[34].

The model (Fig. 8) predicts two classes of adhesion receptor interactions that differ significantly in the distance between the plasma membranes of the two closely apposed cells. The TCR-MHC and CD2/LFA-3 interactions are predicted to occur within an intermembrane distance of ~13 nm or less. This is compatible with electron microscopic measurements of intermembrane distances of 7.5 nm for point contacts of microvilli (0.2-μm wide) and 17 nm for broad areas several μm wide of membrane apposition in interactions of mitogen-stimulated T cells with target cells[165].

By contrast, the interactions of LFA-1 with ICAM-2 and ICAM-1 are predicted to occur within an intermembrane distance roughly equal to or less than 27 or 36 nm, respectively (Fig. 8). There may be important qualitative differences between these two types of membrane contacts, and they could occur in different membrane domains within the zone of adhesion between two cells. Very close contact mediated by the TCR-

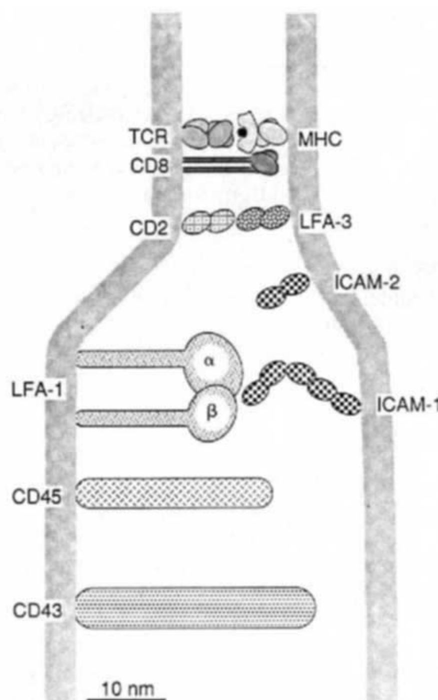

FIG. 8 Scale models of interacting adhesion receptors. Immunoglobulin superfamily members and integrins are drawn to scale (Figs 1, 2 legends). Glycocalyx components: CD43, the 235-residue extracellular domain[148,149] with one in five *O*-linked amino acids[132] is modelled as 235/420 the length of glycocalicin (CD42b), which has 420 amino acids with a similar *O*-linked carbohydrate content[150,151]; CD45 is based on electron micrographs[152]. The lipid bilayer is drawn to scale as 4–5-nm thick; protein transmembrane and cytoplasmic domains are omitted. The presence of hinge or connecting regions between globular domains and the membrane-spanning domain introduces uncertainty into how far some of these molecules extend from the membrane, because an extended polypeptide would extend much farther than globular regions with the same number of amino acids. But connecting regions are absent or short except for CD8, and are already included in the lengths determined by electron microscopy for integrins and CD45. Carbohydrate side chains are not shown; the sizes of molecules based on electron microscopy would take into account both protein and carbohydrate. The MHC class I peptide-binding site is assumed to interact with the complementarity-determining region of the TCR[140]. The binding site in MHC class I for CD8 is in the membrane proximal $\alpha 3$ domain[15,16]; the binding site for MHC is assumed to be in the CD8 immunoglobulin domain, analogously to the MHC binding site in CD4 (ref. 129). ICAM-1 binds to LFA-1 through its N-terminal immunoglobulin domain[130]; the binding site in ICAM-2 is modelled in its homologous N-terminal domain. The ICAM binding site in LFA-1 is assumed to be in the membrane-distal globular domain of integrins as predicted[142]; the exact location within this domain will not affect overall conclusions. The binding site for LFA-3 in CD2 is in the N-terminal immunoglobulin domain[153], and the binding site in LFA-3 is assumed to be in a homologous position.

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MHC and CD2/LFA-3 interaction would be expected to require extensive interdigitation of the membrane glycocalyces of the two adhering cells, hinder the lateral mobility of surface glycoproteins, and either exclude major glycocalyx components such as CD43 and CD45 from the zone altogether or require that they assume a less extended conformation or change their orientation to the membrane. Because the cytoplasmic domain of CD45 is a tyrosine phosphatase that acts on the tyrosine kinase associated with CD4 and CD8 (ref. 133), such considerations could affect signalling.

The longer range contacts mediated by the LFA-1 interaction with ICAM-1 and ICAM-2 are predicted to involve much less glycocalyx interdigitation. This may be compatible with lateral movement of one membrane surface relative to the other, without significant frictional drag imposed by interlocking of the apposed glycocalyces. Migrating cells do not possess focal contacts, which have a cell to substrate distance of 10–15 nm, but instead have close contacts with a cell to substrate distance of ~30 nm (ref. 131), in good agreement with the distance predicted for LFA-1/ICAM interaction, and analogously, for integrin-matrix interaction. There is now good evidence that lipids[134] and membrane proteins[135] are drawn forward by extension of the leading edge of the cell. Although evidence is best for the dorsal surface, results suggest that the ventral surface of the membrane also translates forward with respect to the substrate. LFA-1 and other integrins could serve as transiently fixed points in the membrane that connect the cytoskeleton to the extracellular environment, with a cell–cell or cell–substrate distance that allows membrane lipid and other proteins on the same cell surface to flow laterally in a tide drawn by membrane extension in another area of the cell. 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[23], 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.

Concluding remarks

I have emphasized the dynamic role of lymphocyte adhesion receptors in regulating lymphocyte antigen-specific interactions, localization in lymphoid and nonlymphoid organs, and in bidirectionally transmitting information which affects cellular differentiation and responsiveness and interaction with the environment. Adhesion receptors modulate interactions on different temporal scales and at different distances from the cell surface. There are important interactions between antigen receptors and adhesion molecules involving signalling pathways and interactions with gene expression. Further studies on three-dimensional structure and interactions with signalling pathways and the cytoskeleton promise to provide exciting insights into the mechanism of function of adhesion receptors. The role of these molecules *in vivo* in guiding cell interactions and localization in the complex microarchitecture of lymphoid organs, as well as in immune responses in other tissues, is another area that promises to yield rich insights. □

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ARTICLES

A 1,400-year tree-ring record of summer temperatures in Fennoscandia

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Tree-ring data have been used to reconstruct the mean summer (April–August) temperature of northern Fennoscandia for each year from AD 500 to the present. Summer temperatures have fluctuated markedly on annual, decadal and century timescales. There is little evidence for the existence of a Medieval Warm Epoch, and the Little Ice Age seems to be confined to the relatively short period between 1570 and 1650. This challenges the popular idea that these events were the major climate excursions of the first millennium, occurring synchronously throughout Europe in all seasons. An analysis of past warming trends suggests that any summer warming induced by greenhouse gases may not be detectable in this region until after 2030.

THE collection of living and remnant Scots pine (*Pinus sylvestris* L.) from the Torneträsk region in northern Sweden has been reported previously[1]. This material has yielded absolutely dated series of raw mean ring widths[2] and maximum latewood densities[3] extending unbroken from the present back to AD 436 and AD 443, respectively. At high latitudes, both of these variables are known to correlate well with climate data (specifically, summer temperatures[4–7]), but only qualitative inferences based on these particular long ring-width and density chronologies have been made to date[3,8]. Here we describe the reprocessing of the raw ring-width and density data to produce new chronologies and how they have been used to derive yearly estimates of mean Fennoscandian temperatures for a statistically defined 'summer' (April–August) season, reaching back to AD 500. These are the longest annually resolved climate reconstructions from tree rings yet published.

Torneträsk tree-ring chronologies

Standardization in dendroclimatology is a method for removing variability in tree-ring series that is not related to climate. It involves removing part of the low-frequency variability from the series of raw measurements[9]. The rationale behind this is a need to account for differences in the general growth rate or vitality of individual trees and to remove trends in individual tree-ring series that arise as a consequence of non-climate-

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