11: Adhesion Molecules in Immunity and Inflammation

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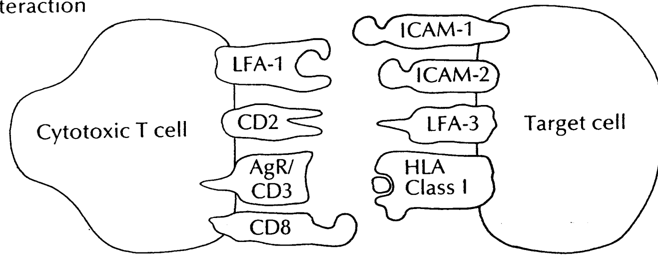
Cellular adhesion and recognition mechanisms are among the most basic requirements both for the evolution of multicellular organisms and for the immune response. Discovery of receptors on the cell surface involved in cell–cell and cell–substrate interactions has been a key factor in understanding the mechanisms underlying inflammatory and immune phenomena. Three protein families, the immunoglobulin (Ig) family (Williams and Barclay 1988; Hunkapiller and Hood 1989), the integrin family (Hynes 1987; Ruoslahti and Pierschbacher 1987; Kishimoto *et al.* 1989a; Larson and Springer 1989; Hemler 1990) and the recent selectin family (Stoolman 1989), have been described that are extensively involved in a network of cell–cell and cell–matrix interactions in the immune system. Engagement of these surface receptors can transduce a signal leading to cellular events that change the phenotype, gene expression or activation state of the cell. On the other hand, cytoplasmic signals regulate the functional activity and surface expression of these receptors. These receptors thus mediate a dialogue, transferring information in both directions across the membrane. Furthermore, adhesion receptors are important in regulating cell migration and localization within tissues. This chapter first discusses the role of adhesion receptors in antigen-specific responses, and concludes with an overview of the role of adhesion receptors in regulating lymphocyte recirculation and in controlling leucocyte extravasation and localization in inflammation.

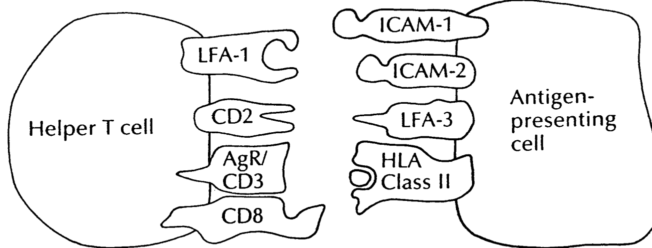
Antigen recognition by T lymphocytes

Antigen-specific responses of lymphocytes have been found to require not only the antigen receptor but also a set of adhesion molecules on the T lymphocyte and complementary structures on the target cell or antigen-presenting cell (Fig. 11.1).

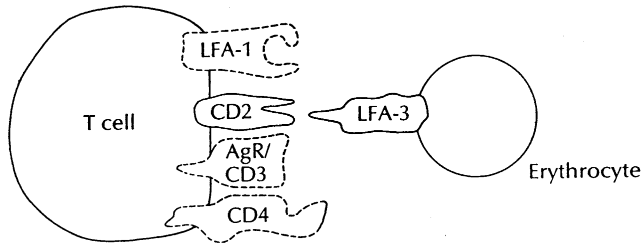
(a) Killer T cell interaction



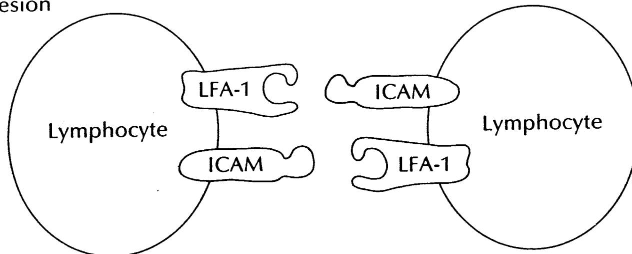
(b) Helper T cell interaction



(c) T cell erythrocyte rosetting



(d) Homotypic adhesion



(e) Adhesion to artificial membranes

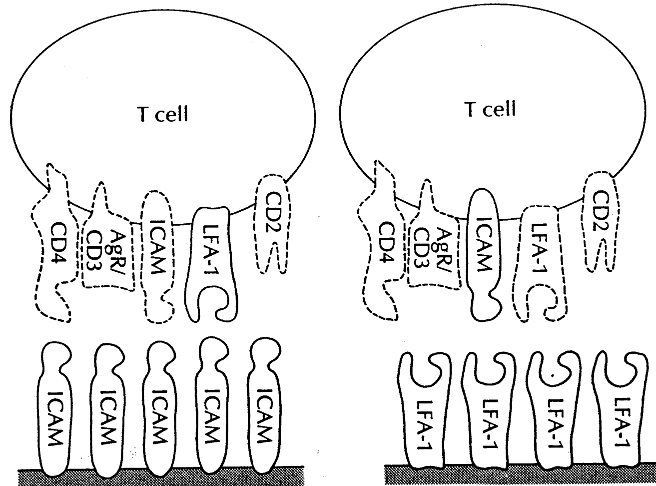


Fig. 11.1. Lymphocyte adhesion systems.

Monoclonal antibodies to any one of these surface molecules can inhibit T lymphocyte-mediated killing, showing that it is a highly complex process requiring co-operation between a number of different surface molecules. Subsequent studies have demonstrated that all of the molecules shown in Fig. 11.1 are involved in adhesion and many are also involved in the signalling events attendant upon antigen recognition. Thus, they are most properly referred to as adhesion receptors. The properties of these receptors, and the counter-receptors to which they bind, all of which are glycoproteins, are summarized in Table 11.1 and described in turn in the sections below.

The T cell antigen receptor and associative recognition by CD4 or CD8

T lymphocytes recognize foreign antigen in the form of short peptides, bound to Class I or Class II major histocompatibility complex (MHC) molecules (Fig. 11.1(a) and (b), respectively). The T cell receptor (TCR) and CD8 (or CD4) appear to be 'co-receptors' that 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. The specificity of the TCR is conferred by two subunits that contain variable and constant domains analogous to those of antibodies (Marrack and Kappler 1986; Brenner *et al.* 1988). These two chains under physiological conditions are always associated with CD3, a complex of five chains that mediate the intracellular signalling function of this seven-subunit receptor (Clevers *et al.* 1988; Weissman *et al.* 1989). This single structural unit is referred to here as the TCR. Monoclonal antibodies against the TCR can act as agonists that mimic interaction with antigen-bearing cells, stimulate Ca^{2+} mobilization and phosphatidyl inositol hydrolysis, and induce T cell proliferation and 'functional programmes' appropriate to killer or helper T cells.

Major histocompatibility complex molecules appear to be specialized for interaction with peptides derived from foreign antigen, binding them within a deep cleft formed by two α-helices of the MHC molecule. Class I MHC molecules bind to peptides derived from endogenously synthesized molecules such as viruses. Class I MHC–peptide complexes are primarily recognized by CD8 +ve killer T lymphocytes (Fig. 11.1(a)). Class II MHC molecules bind to peptides derived from endocytosed antigen, and are primarily recognized by CD4 +ve helper T lymphocytes (Fig. 11.1(b)).

The correlation between the type of MHC molecule recognized and CD8 or CD4 expression is very strong, and led to the proposal that CD8 and CD4 bind to determinants on Class I and Class II

Table 11.1. Characteristics of T cell adhesion receptors and counter-receptors

Receptor	Mass (kD)	Distribution	Counter-receptor	Mass (kD)	Distribution
LFA-1 (CD11a/CD18)	α 180, β 95	Thymocytes, T and B lymphocytes, LGL, monocytes, activated macrophages, neutrophils	ICAM-1 (CD54)	90–110	Restricted, widely inducible by IL-1, TNF, IFN-γ and LPS
			ICAM-2	45	Constitutive on endothelial cells
CD2 (LFA-2/T11)	50–58	Thymocytes, T lymphocytes, LGL	LFA-3 (CD58)	55–70	Wide
CD8	30–38, α–α or α–β dimer	Subset of thymocytes and T lymphocytes, LGL	Class I MHC	α 44, β 12	Wide, increased by IFN-α, β and γ
CD4	55	Subset of thymocytes and T lymphocytes, monocytes, macrophages	Class II MHC	α 34, β 29	Restricted, widely inducible by IFN-γ

LFA = lymphocyte function-associated antigen; LGL = large granular lymphocytes; ICAM = intercellular adhesion molecule; IL = interleukin; TNF = tumour necrosis factor; IFN = interferon; LPS = lipopolysaccharide; MHC = major histocompatibility complex.

MHC molecules, respectively (Swain 1983; Bierer *et al.* 1989). Indeed, expression of supraphysiological levels of CD4 molecules on transfected fibroblasts allowed detection of binding to cells bearing Class II MHC molecules (Doyle and Strominger 1987), and similar studies have shown specific interactions between transfected cells expressing high levels of CD8 and Class I MHC molecules (Norment *et al.* 1988). At physiological surface densities on T lymphocytes, however, CD4 and CD8 mediate little (Spits *et al.* 1986) or no (Shaw *et al.* 1986) antigen-independent adhesion; their primary physiological importance appears to be in signalling. When this signalling contribution is blocked, as with a monoclonal antibody to CD4 or CD8, T cells require 100-fold higher concentration of antigen to induce responsiveness; at high antigen concentrations (or with so-called high-avidity TCR) the contribution may not be apparent (Janeway 1988; von Boehmer 1988; Bierer *et al.* 1989; Kupfer and Singer 1989; Parnes 1989).

Many lines of evidence support the model that co-association of the TCR and CD4 (or CD8), induced by binding to the same MHC–peptide molecular complex, results in synergistic signalling. This has not been directly demonstrated, but has been inferred from the ability of TCR directed to peptide Class I and Class II MHC complexes to synergize only with CD8 and CD4, respectively (Janeway 1988; Bierer *et al.* 1989; Parnes 1989). Consistent with associative recognition, the sites on the Class I MHC molecule recognized by CD8 and TCR appear distinct. The TCR recognizes bound peptide and surrounding polymorphic residues in the most membrane-distal domains of the Class I MHC molecule (Bjorkman *et al.* 1987), whereas CD8 binds to monomorphic residues in a membrane-proximal domain of Class I MHC (Potter *et al.* 1989; Salter *et al.* 1989). When CD4 +ve helper T cell clones form conjugates with antigen-presenting B cells, both the TCR and CD4 redistribute to the site of adhesion (Kupfer and Singer 1989). There may be some tendency for self-association between the TCR and CD4, since this can be induced by certain monoclonal antibodies to the TCR and correlates with the ability of these antibodies to directly activate T cells (Janeway 1988; Kupfer and Singer 1989). Intriguingly, both CD4 and CD8 are associated with a lymphocyte-specific tyrosine kinase, lck. This association is mediated by specific amino acid residues in the cytoplasmic segments of CD4 and CD8 and in the N-terminal domain of lck (Shaw *et al.* 1989).

T cell precursors in the thymus co-express the CD4 and CD8 molecules. Co-association of one or other of these molecules with the TCR may help to signal whether the TCR recognizes Class I or Class II MHC, and may regulate subsequent differentiation into CD8 +ve cytotoxic or CD4 +ve helper T lymphocyte subsets (Janeway 1988; von Boehmer 1988).

The activation-regulated CD2–lymphocyte function associated antigen 3 and lymphocyte function associated antigen 1–intercellular adhesion molecule adhesion mechanisms

Stimulation of lymphocytes with specific antigen or mitogens is accompanied by greatly increased adhesiveness, and thus correlates with *in vivo* findings that lymphocytes, responding to specific antigen, temporarily cease recirculation and localize in lymph nodes where antigen is present (Butcher 1986). Studies on T cell lines maintained in culture by weekly stimulation with foreign antigen and addition of T cell growth factors show that they will conjugate with target cells even when the target cells do not express the antigen to which the T cells are immune (Spits *et al.* 1986; Shaw and Luce 1987; Springer *et al.* 1987). This antigen-independent adhesion is due to binding of the CD2 and lymphocyte function-associated antigen (LFA)-1 molecules on the T cell to the LFA-3 and intercellular adhesion molecules (ICAM) on the target cell, respectively (Fig. 11.1(a), (b)). Although not appreciated in the original studies, this antigen-independent adhesion is related to T cell activation, since it is not seen with resting T lymphocytes (Dustin and Springer 1989). On activated T lymphocytes and with typical target cells, the CD2 and LFA-1 molecules are much stronger adhesion molecules than the TCR and CD4 or CD8 molecules.

Regulation of lymphocyte adhesion by the CD2–lymphocyte function associated antigen 3 mechanism

CD2 and LFA-3 are receptors for one another. Early studies showed that monoclonal antibodies to CD2 and LFA-1 blocked T cell function by

binding to the T cell, whereas monoclonal antibodies to LFA-3 blocked by binding to the target cell (Krensky *et al.* 1983). Later evidence showed a lack of additive effects of monoclonal antibodies to CD2 and LFA-3, in contrast to additive effects of these monoclonal antibodies with those to LFA-1. Furthermore, CD2 and LFA-3 function in an adhesion mechanism at 4 °C and in the absence of Mg^{2+}, in contrast to LFA-1 (Shaw *et al.* 1986; Denning *et al.* 1987). Definitive evidence for interaction of CD2 with LFA-3 was provided by saturable binding of detergent-solubilized, purified CD2 to LFA-3 +ve cells, and blocking of this binding with monoclonal antibodies to LFA-3 (Selvaraj *et al.* 1987a). In subsequent studies, purified LFA-3 incorporated in artificial, glass-supported planar membranes was shown to specifically bind CD2 +ve cells (Dustin *et al.* 1987b). This observation was further corroborated by interaction of lipid vesicles containing purified LFA-3 and CD2, saturable binding of purified LFA-3 to CD2 +ve cells, and binding of LFA-3 +ve cells to transfected cells expressing CD2 (Springer *et al.* 1987; Bierer *et al.* 1989).

CD2 and LFA-3 are both members of the Ig superfamily, a large family of molecules that are related to Ig and are often expressed on cell surfaces (Williams and Barclay 1988). The structural unit that members of this superfamily share in common is the Ig domain, composed of 90–100 amino acids arranged in a sandwich of two sheets of antiparallel β strands, which are stabilized by a disulphide bond at their centre (Alzari *et al.* 1988; Williams and Barclay 1988). The Ig 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 (Harrelson and Goodman 1988).

The Ig domain may have diversified and been adopted so widely in evolution because its stable disulphide-bonded β sheet 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.

Adhesion molecules that are members of the Ig superfamily include CD4, CD8, CD2, LFA-3, ICAM-1 and ICAM-2 (Fig. 11.2) in the immune system and neural cell adhesion molecules (NCAM) (Edelman 1986; Cunningham *et al.* 1987; Rutishauser *et al.* 1988) and fasciculin II (Harrelson and Goodman 1988) in the nervous system. CD2 and LFA-3 are more related to one another (21% identity of amino acid sequence) than to other Ig superfamily members. This raises the possibility that this heterophilic interaction (between unlike molecules) could have evolved from a homophilic (like–like) interaction of a common ancestral molecule (Seed 1987).

Interaction between cells bearing CD2 and LFA-3 is finely poised and is tipped toward adhesion by T cell activation. CD2 +ve cells do not

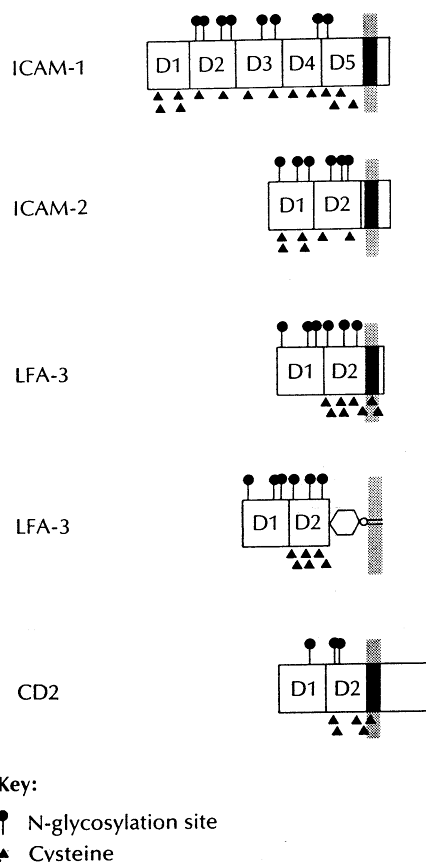


Fig. 11.2. Immunoglobulin superfamily adhesion receptors of the immune system. Structures are based on (Sewell *et al.* 1986; Seed 1987; Seed and Aruffo 1987; Wallner *et al.* 1987; Simmons *et al.* 1988; Staunton *et al.* 1988, 1989).

inherently bind to LFA-3 +ve cells, as demonstrated in studies of 'rosetting' (Fig. 11.1(c)). Activated T lymphocytes and resting T lymphocytes both express CD2, but only activated T cells rosette with erythrocytes. The physiological significance of this reaction is just beginning to be understood; it may prevent immune responses from occurring in the bloodstream. However, since human erythrocytes lack ICAM and MHC molecules but express LFA-3, rosetting provides a convenient system for studying CD2 interaction with LFA-3 in the absence of the LFA-1 and CD4/CD8 adhesion mechanisms (Fig. 11.1(c)).

The equilibrium governing interaction through CD2 and LFA-3 is very sensitive to receptor density. Rosetting is enhanced by incorporation of additional purified LFA-3 into erythrocyte membranes (Selvaraj *et al.* 1987c). Resting human T lymphocytes bind to sheep erythrocytes but not to human erythrocytes, apparently because there is a fourfold higher density of the LFA-3 homologue on sheep erythrocytes (Selvaraj *et al.* 1987b). The affinity for CD2 and the charge density of erythrocytes in both species are identical. The higher LFA-3 density on sheep erythrocytes is compensated for by a lower CD2 density on sheep peripheral blood T lymphocytes (Selvaraj *et al.* 1987b; Mackay *et al.* 1988). In both sheep and humans, T lymphocyte activation alters the equilibrium to allow rosetting with autologous erythrocytes.

Close cell–cell contact is opposed by repulsion between cells due to their net negative surface charge, and by the decrease in entropy that occurs when the glycocalyces interdigitate (Bell *et al.* 1984). A decrease in cell surface repulsion appears to occur after T lymphocyte activation. This may regulate all adhesion mechanisms, and is particularly well documented for the CD2–LFA-3 interaction. Addition of positive charge by chemical derivatization of erythrocytes, removal of negative charge by neuraminidase digestion of erythrocytes or T cells, and removal of negative charge and glycocalyx by protease digestion all allow resting T cells to form rosettes via CD2–LFA-3 interaction (Bentwich *et al.* 1973; Plunkett *et al.* 1987). Charge neutralization alters the morphology of the contact between T lymphocytes and sheep erythrocytes, converting small islands of close contact surrounded by larger areas of greater intermembrane distance to a single large area of close contact. Sialic acid is the major contributor to the net negative cell surface charge (Wigzell and Hayry 1974). Despite their larger surface area, T cell blasts and thymocytes have fivefold less sialic acid per cell than resting T cells (Despont *et al.* 1975) and are less negatively charged (Shortman *et al.* 1975), 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 are aggregating in germinal centres are greatly under-sialylated, while areas containing B and T cells that are in rapid transit between blood and lymph are normally sialylated (Butcher *et al.* 1982). In the nervous system as well, cell interactions are regulated by sialylation; polysialylation of NCAM antagonizes its ability to promote adhesion (Rutishauser *et al.* 1988).

Cell adhesion provides an opportunity for multiple receptor–counter-receptor interactions between two cells. This has hindered measurement of the affinities of individual molecules for one another. However, monovalent interaction of CD2 with LFA-3 was recently found to have a K_d of 1 μM (Selvaraj *et al.* 1987a; Dustin *et al.* 1989; Sayre *et al.* 1989). Lymphocyte function-associated antigen 3 with an intact phosphatidylinositol lipid anchor forms octameric protein micelles which bind to approximately four cell surface CD2 molecules/octamer; this multivalent interaction has a K_d of 1 nM (Dustin *et al.* 1987b). Human erythrocytes contain 4×10^3 LFA-3 molecules (Selvaraj *et al.* 1987b); it is not known what fraction of these would be involved in binding to an activated T lymphocyte, but 4×10^3 interactions with a univalent K_d of 1 μM would give a change in free energy of -2×10^{-9} erg, an upper limit of the energy expenditure required to stabilize adhesion between two cells.

CD2 may transduce a signal which augments or synergizes with signals from the TCR (Bierer *et al.* 1989). Certain pairs of CD2 monoclonal antibodies or a combination of one CD2 monoclonal antibody with multimeric LFA-3 can stimulate T cells, but CD2–LFA-3 interaction alone has no effect (Tiefenthaler *et al.* 1987; Dustin *et al.* 1989). Transfection of cells with CD2 and LFA-3 has confirmed early monoclonal antibody inhibition results (Springer *et al.* 1987) showing that CD2–LFA-3 interaction can contribute a 4- to 30-fold enhancement of the immune response (Bierer *et al.* 1988; Moingeon *et al.* 1989). The unusually basic,

histidine- and proline-rich 120 amino acid cytoplasmic region of CD2 is required for stimulation by pairs of monoclonal antibodies (He *et al.* 1988; Moingeon *et al.* 1989); 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 (Bierer *et al.* 1988; Moingeon *et al.* 1989).

There are two isoforms of LFA-3, derived by differential messenger ribonucleic acid (mRNA) splicing, which differ in membrane anchor (Dustin *et al.* 1987a; Seed 1987; Wallner *et al.* 1987). One isoform has a glycophosphatidylinositol (GPI) anchor, which replaces a C-terminal hydrophobic polypeptide in the precursor, whereas the other isoform has a classical C-terminal polypeptide with a transmembrane hydrophobic segment and a 12 amino acid cytoplasmic segment. Both the polypeptide-anchored isoform (Hollander *et al.* 1988) and the GPI-anchored isoform (Bierer *et al.* 1988; Moingeon *et al.* 1989) are fully active in mediating CD2-dependent adhesion and in promoting T cell effector function. The functional significance of the isoforms remains obscure, although it is intriguing that NCAM also has anchor isoforms (Cunningham *et al.* 1987; Rutishauser *et al.* 1988).

Intercellular adhesion molecules 1 and 2, counter-receptors for lymphocyte function associated antigen 1

Subsequent to the identification of LFA-1 as one of the molecules recognized by monoclonal antibodies that inhibited T cell-mediated killing, it was found that LFA-1 is required for the adhesion step in cytotoxic T lymphocyte (CTL)-mediated killing, as well as for a broad range of leucocyte functions involving adhesion, including T helper and B lymphocyte responses, natural killing, antibody-dependent cytotoxicity mediated by monocytes and granulocytes, and adherence of leucocytes to endothelial cells, fibroblasts and epithelial cells (Springer *et al.* 1987; Kishimoto *et al.* 1989a). A counter-receptor for LFA-1, ICAM-1 was identified using a simple assay called homotypic adhesion (Fig. 11.1(d)), in which homogeneous cell populations such as B or T cell lines adhere to one another to form multicellular clusters (Springer *et al.* 1987; Dustin *et al.* 1988). Although resting lymphocytes do not form homotypic aggregates, they do so when stimulated with phorbol esters; transformed lymphoid cell lines aggregate weakly or, if stimulated, strongly. Homotypic adhesion is completely inhibited by LFA-1 monoclonal antibodies and is not observed with cell lines established from patients genetically deficient in LFA-1 (see below). The ability of LFA-1 +ve cells to co-aggregate with LFA-1 −ve cells in the homotypic adhesion assay showed that LFA-1 is not a homophilic receptor which binds to itself, but rather is a heterophilic receptor which binds to a distinct counter-receptor. A counter-receptor was defined by immunizing mice with LFA-1 −ve cells and selecting monoclonal antibodies that would inhibit LFA-1-dependent homotypic adhesion. This counter-receptor was designated ICAM-1 (Table 11.1). Confirming the receptor–counter-receptor relationship, lymphocyte binding to purified ICAM-1 is inhibited with LFA-1 monoclonal antibodies (Kishimoto *et al.* 1989a), and purified LFA-1 protein micelles bind to purified ICAM-1 on artificial substrates (Dustin and Springer 1989). Intercellular adhesion molecule 1 is a member of the Ig superfamily with five Ig domains (Dustin *et al.* 1988) (Fig. 11.2).

Induction of ICAM-1 in inflammation is one important means of regulating the LFA-1–ICAM interaction (Dustin *et al.* 1988; Kishimoto *et al.* 1989a). In contrast to LFA-1, which is restricted to leucocytes, ICAM-1 can be expressed on a wide variety of cells. In the absence of an inflammatory response, however, ICAM-1 is expressed on only a few cell types (Dustin *et al.* 1986). Consistent with its importance in *in vitro* immune responses (Makgoba *et al.* 1988; Altmann *et al.* 1989), *in vivo* ICAM-1 is well expressed in germinal centres, both on follicular dendritic cells and on the activated B lymphocytes which congregate in these centres (Dustin *et al.* 1986). Germinal centres are formed in lymphoid tissue during immune responses to specific antigen, and homotypic adhesion involving LFA-1 and ICAM-1 may contribute to their formation. Inflammatory mediators, including lipopolysaccharide, interferon γ (IFN-γ), 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 (Springer *et al.* 1987; Dustin

and Springer 1988; Dustin *et al.* 1988; Kishimoto *et al.* 1989a). Plots of lymphocyte binding to purified ICAM-1 reconstituted in planar lipid bilayers are sigmoidal, with no adhesion below a threshold value of 100 ICAM-1 molecules/μm^2 and rising sharply to a plateau at 1000 molecules/μm^2 (Dustin and Springer 1988). 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 leucocytes to endothelium is the first step in localization of circulating cells at an inflammatory site. *In vivo*, ICAM-1 induction accompanies T cell-mediated hypersensitivity reactions in the skin (Wantzin *et al.* 1989), and, after administration of IFN-γ and IL-1, appearance of ICAM-1 on endothelial cells correlates with sites of mononuclear cell infiltration (Munro *et al.* 1989). Intercellular adhesion molecule 1 induction is largely regulated at the mRNA level. Increased surface expression is first seen after 4 hours and is usually maximal by 24 hours (Dustin *et al.* 1988).

A second LFA-1 ligand, differing in tissue distribution from ICAM-1, was originally defined by the ability of LFA-1 monoclonal antibody but not ICAM-1 monoclonal antibody to inhibit certain cell adhesion assays. Based on this functional property, an ICAM-2 complementary deoxyribonucleic acid (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. Intercellular adhesion molecule 2 is a transmembrane protein that binds to LFA-1 and shares no antigenic determinants with ICAM-1 (Staunton *et al.* 1989). It has two Ig-like domains, in contrast to ICAM-1, which has five (Fig. 11.2), and these are 35% identical to the N-terminal two domains of ICAM-1. Intercellular adhesion molecules 1 and 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. A family of LFA-1 counter-receptors emphasizes the importance of this adhesion mechanism and may be a means of imparting fine specificity and functional diversity. Unlike ICAM-1, ICAM-2 is well expressed basally on endothelial cells and its mRNA is not increased by inflammatory mediators. Whether further ICAMs exist is an open question.

Lymphocyte function associated antigen 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. Increased expression of ICAM-1 after cytokine induction is detectable *in vitro* or *in vivo* after 4–6 hours, and is maximal by 9–24 hours (Dustin *et al.* 1988; Kishimoto *et al.* 1989a; Munro *et al.* 1989). This time course is typical of regulation at the mRNA 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 (Reichner *et al.* 1988) and glycoprotein turnover, which is in the order of 12–24 hours. Yet T cells can regulate adhesion over a much shorter time-scale, adhering to target cells, delivering a lethal hit, de-adhering and engaging in repeated target cell interactions, with a cycle time as short as 15–30 minutes (Martz 1977). This requires a mechanism for regulating adhesion over a shorter time-scale. Moreover, in contrast to cytotoxic cells stimulated *in vitro*, which show a general increase in adhesiveness, cells primed *in vivo* adhere only to those cells bearing the antigen to which they were primed (Martz 1987; Springer *et al.* 1987). It has recently been found that cross-linking of the TCR on resting T lymphocytes stimulates adhesiveness through LFA-1. This adhesiveness is transient, allowing regulation of adhesion and de-adhesion over a time-scale of minutes (Dustin and Springer 1989).

Homotypic adhesion of leucocytes (Fig. 11.1(d)), which is dependent on LFA-1 and ICAM-1, is stimulated by 1 hour's treatment with phorbol ester and yet is accompanied by no increase in LFA-1 or ICAM-1 surface expression (Rothlein and Springer 1986; Rothlein *et al.* 1986). This suggested that the activation of protein kinase C might increase the avidity of LFA-1 for its counter-receptor, and that this might mimic events triggered by the TCR (Rothlein and Springer 1986). By testing the binding of cells co-expressing LFA-1 and ICAMs to plastic substrates coated with either purified ICAM-1 or purified LFA-1 (Fig. 11.1(e)), regulation of the avidity of cellular LFA-1 and of cellular ICAM-1 could be separately tested (Dustin and Springer 1989). Stimulating resting T lymphocytes with phorbol esters or cross-linking the TCR

with monoclonal antibody converts cellular LFA-1 from a low- to a high-avidity state, whereas cellular ICAM-1 is constitutively avid. There is no change in LFA-1 surface density. In contrast to T cell clones, resting peripheral blood T lymphocytes do not conjugate with B lymphocyte target cells. However, TCR stimulation induces conjugate formation that is inhibited completely by LFA-1 monoclonal antibody and only marginally by CD2 monoclonal antibody, showing the pre-eminent role of LFA-1 in regulating the avidity of cell–cell interactions. The high-avidity state peaks 5–10 minutes after TCR stimulation and returns to the low-avidity state by 30 minutes; kinetics are influenced by the amount of TCR cross-linking. Subsequent addition of phorbol ester returns LFA-1 to the high-avidity state, demonstrating that the adhesion machinery is still intact. After phorbol ester stimulation, LFA-1 does not return to the low-avidity state. Pharmacological agents have a dramatic effect on the high-avidity state of LFA-1, either by stimulating it directly or by inhibiting its stimulation by TCR cross-linking. This suggests that the TCR controls LFA-1 through intracellular signalling pathways.

A model based on these findings for co-operation between the TCR and adhesion molecules to mediate antigen-specific recognition (Dustin and Springer 1989) is as follows. Lymphocyte function-associated antigen 1 on unactivated cells such as resting T lymphocytes is in a low-avidity state, which may be equivalent to the inactive state of LFA-1 on cells depleted of adenosine triphosphate (ATP) (Marlin and Springer 1987). Thus, in the absence of antigen, the equilibrium governing adherence of T lymphocytes to other cells favours free, mobile T lymphocytes leading to efficient immune surveillance. On contact with cells bearing specific antigen, TCR ligation generates intracellular signals that lead to energy-dependent conversion of LFA-1 to a high-avidity state and favour LFA-1/ICAM-dependent adhesion. Antigen specificity is maintained because the input of energy to convert LFA-1 to the high-avidity state, whether or not this energy is used to fuel protein phosphorylation, LFA-1 redistribution or some other mechanism, is controlled by the TCR. Lymphocyte function-associated antigen 1 is an adhesion servo-motor operated by the TCR. Cellular energy expended in converting LFA-1 to a high-avidity state helps drives the adherence/non-adherence equilibrium toward stable adherence, analogous to the use of ATP to favour an otherwise energetically unfavourable reaction in intermediary metabolism. Because TCR binding to peptide–MHC does not have to stabilize cell–cell adhesion but instead triggers adhesion amplification, the sensitivity of T cells can be increased by lowering the number of TCR–ligand interactions required for antigen recognition.

The transience of the TCR-stimulated increase in LFA-1 avidity could be explained if TCR triggers a cascade of phosphorylation events or second messengers such that early events lead to an increase in LFA-1 avidity, whereas later events are responsible for lowering LFA-1 avidity. The transience of the high-avidity state provides a mechanism for regulating lymphocyte de-adhesion. Antigen density and hence the number of TCR engaged may influence the kinetics of the signalling cascade and thus the kinetics of avidity regulation. The high-avidity state of LFA-1 appears to be stabilized by binding to ICAM-1, because transience is more marked for cells in suspension than for cells bound to ICAM-1 (M. Lawrence, M. Dustin and T. Springer, unpublished). Duration of adhesion may also be influenced by the level of ICAM expression and whether ICAM-1 or ICAM-2 is the ligand. It is important to remember that, since ICAM-1 is inducible by cytokines (Springer *et al.* 1987; Dustin *et al.* 1988), T cell stimulation could lead to induction of ICAM-1 on antigen-presenting cells and secondarily alter the kinetics of T cell interactions.

The integrin family

Lymphocyte function associated antigen 1 is a member of the integrin family (Table 11.2). Integrins are perhaps the most sophisticated of the adhesion molecule families, in terms of both versatility in ligand recognition and ability to transmit information in both directions across the membrane. This is reflected in their large size, with α and β subunits of approximately 1100 and 750 amino acids, respectively, which are non-covalently associated (Fig. 11.3). 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 (Hynes 1987; Kishimoto *et al.* 1987, 1989a). Three subfamilies of integrins

Table 11.2. The integrin family of cell–cell and cell–matrix receptors[a]

Subunits	Names[b]	α Subunit[c]		Ligands[d]	RGD role[e]	Distribution	
		I	C			Non-leuc[f]	Leuc[g]
αLβ2	CD11a/CD18, LFA-1	+	−	ICAM-1, 2	−		B, T, M, G
αMβ2	CD11b/CD18, Mac-1, CR3	+	−	C3bi, FX, FB	+?		M, G
αXβ2	CD11c/CD18, p150,95	+	−	?	?		M, G
α1β1	CD−/CD29, VLA-1	?	−	LM, CO	−	F, BM	B*, T*
α2β1	CD49b/CD29, VLA-2, GPIaIIa, ECMRII	+	−	LM, CO	−	P, F, EN, EP	T*
α3β1	CD−/CD29, VLA-3, ECMRI		+	FN, LM, CO	−	EP, F	
α4β1	CD49d/CD29, VLA-4, LPAM-1	−	*	FN, VCAM-1	−	NC, F	B, T, M, LGL
α5β1	CD−/CD29, VLA-5, FNR, GPIc/IIa, ECMRVI	−	+	FN	+	F, EP, EN, P	Th, T*
α6β1	CD49f/CD29, VLA-6, GPIc/IIa		+	LM	−	P	
α4βp	CD49d/CD−, LPAM-2	−	*	?	?		T
α6β4	CD49f/CD−, αEβ4,		+	?	−	EP	
αIIbβ3	CD41/CD61, GPIIbIIIa	−	+	FB, FN, vWF	+	P	
αVβ3	CD51/CD61, VNR	−	+	VN, FB, vWF, TS	+	EN	
αVβ5	CD51/CD−	−	+	VN, FN	+	C	
αVβs	CD51/CD−	−	+	?	+	F	

a References: Ruoslahti and Pierschbacher 1986, 1987; Hynes 1987; Kishimoto *et al.* 1989a; Hemler 1990; Springer 1990.
b LFA, lymphocyte function-associated antigen; CR, complement receptor; VLA, very late activation; GP, glycoprotein; ECMR, extracellular matrix receptor; FNR, fibronectin receptor; VNR, vitronectin receptor.
c I, I domain; C, +: cleavage to disulphide-linked heavy and light chains at amino acids 853–860, *: cleavage to non-disulphide-linked chains at ~amino acid 573; −, no cleavage.
d ICAM, intercellular adhesion molecule; FX, factor X; FB, fibrinogen; LM, laminin; CO, collagen; FN, fibronectin; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor; VN, vitronectin; TS, thrombospondin.
e RGD, arginine-glycine-aspartic acid.
f F, fibroblasts or other connective tissue; BM, basement membrane-associated; P, platelets; EN, endothelial cells; EP, epithelial cells; NC, neural crest, melanocytes; C, carcinomas.
g B, B lymphocytes; T, T lymphocytes; M, monocytes; G, granulocytes; *, activated lymphocytes only; LGL, large granular lymphocytes; Th, thymocytes. Some data from *Leukocyte Typing Database*, vol. III (Gilks *et al.* 1988) and vol. IV.

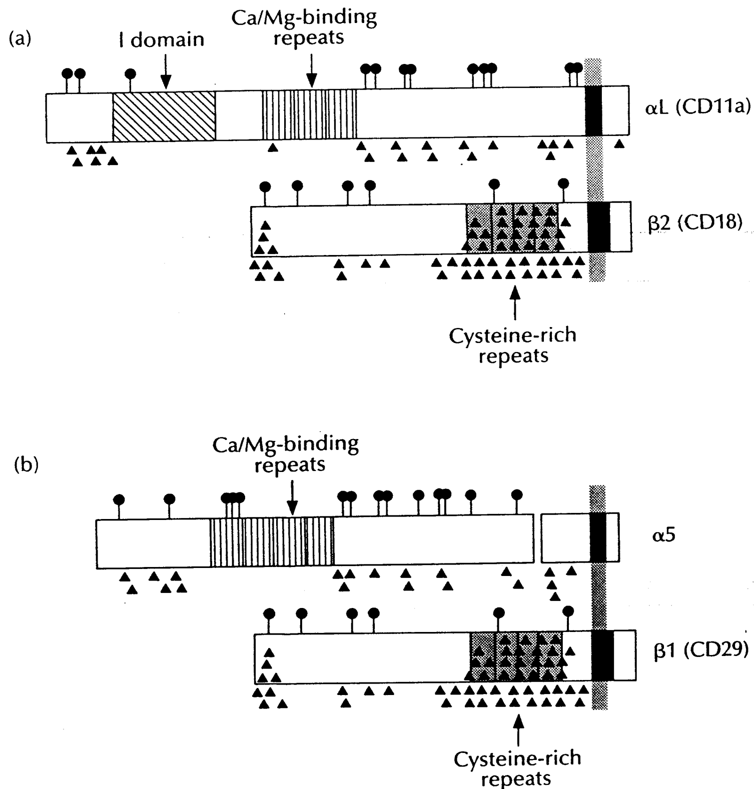


Fig. 11.3. Representative integrin family adhesion receptors: (a) LFA-1; and (b) fibronectin receptor. Structures are based on (Tamkun *et al.* 1986; Argraves *et al.* 1987; Kishimoto *et al.* 1987; Larson *et al.* 1989).

may be distinguished by their β subunits, known as the β1 (CD29), β2 (CD18) and β3 (CD61) integrins.

Lymphocyte function associated antigen 1 is most closely related to two other integrins, Mac-1 and p150,95; they share the β2 subunit (Kishimoto *et al.* 1987). These three β2 integrins are also known as the leucocyte 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 (Kishimoto *et al.* 1989a) (Table 11.2). (An account of these two integrins as complement receptors is given in Chapter 13.) The important role of the leucocyte integrins is illustrated in congenital leucocyte adhesion deficiency (LAD), in which all are deficient because of mutations in the common β2 subunit (Anderson and Springer 1987; Kishimoto *et al.* 1989a). Patients have recurring infections, which are often fatal in childhood unless they are corrected by bone marrow transplantation. Patient monocytes and neutrophils 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 leucocyte localization *in vivo*. Administration of monoclonal antibody to the β2 subunit *in vivo* mimics defects in LAD, and appears to be clinically useful in inhibiting leucocyte extravasation and neutrophil-mediated tissue injury in myocardial infarction and ischaemic shock (Kishimoto *et al.* 1989a).

The β1 integrin subfamily includes receptors for the extracellular matrix components fibronectin, laminin and collagen (Table 11.2). These ligands show interesting patterns of expression in the fibrillar meshworks found throughout many tissues and in the basement membranes found in muscle, in the nervous system and underlying the epithelium and endothelium (Ruoslahti and Pierschbacher 1986, 1987; Hynes 1987). β1 integrins are thus likely to be involved in controlling the organization within tissues of the many

non-haemopoietic and leucocyte cell types on which they are expressed (Table 11.2). The designation VLA (very late activation) denotes the appearance of VLA-1 and VLA-2 on lymphocytes 2–4 weeks after antigen stimulation *in vitro* (Hemler 1990). However, VLA is not an apt acronym because some VLA molecules are basally expressed on leucocytes, and their expression on non-haemopoietic cells does not require activation (Table 11.2). Induction of VLA-1, 2, 3 and 5 expression after leucocytes cross the endothelial barrier may be of great importance in controlling leucocyte localization in inflammation.

Very late activation molecule 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 a cell receptor (Hemler 1990). As a matrix receptor, it binds to an alternatively spliced domain of fibronectin distinct from the classical cell binding site recognized by VLA-5 (Wayner *et al.* 1989; Guan and Hynes 1990). As a cell receptor, it binds to a molecule recently described as VCAM-1 or INCAM-110, which is a member of the Ig superfamily (Osborn *et al.* 1989; Rice and Bevilacqua 1989; Elices *et al.* 1990). This molecule is induced by inflammatory mediators on endothelium with kinetics similar to ICAM-1. Previous studies on lymphocyte binding to endothelium had demonstrated a second adhesion mechanism distinct from the LFA-1–ICAM interaction (Haskard *et al.* 1986; Dustin and Springer 1988), and this second mechanism now appears due to the VLA-4–VCAM-1 interaction (Elices *et al.* 1990). In congenital deficiency of the β2 integrins, which does not affect VLA-4, lymphocyte function is less severely affected than neutrophil function, and, in contrast to neutrophils, lymphocytes emigrate across the endothelium at inflammatory sites (Anderson and Springer 1987). This seems related to expression of VLA-4 by lymphocytes and not by neutrophils. Involvement of VLA-4 in T cell-mediated killing (Takada *et al.* 1989) and in homotypic adhesion (Bednarczyk and McIntyre 1990) suggests some functional redundancy with LFA-1. Very late activation molecule 4 also helps mediate lymphocyte recirculation (Holzmann *et al.* 1989), 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 (Sonnenberg *et al.* 1988; Cheresh *et al.* 1989; Freed *et al.* 1989; Hemler *et al.* 1989; Holzmann and Weissman 1989; Kajiji *et al.* 1989) (Table 11.2). Both α and β subunits affect ligand specificity. Their combinatorial use creates greater diversity in ligand recognition capability, and differences in transmembrane and cytoplasmic domains may also help regulate communication between the inside and outside of the cell.

The structural domains of integrins (Fig. 11.3) have been correlated with ligand binding by cross-linking to peptides containing the sequence arginine–glycine–aspartic acid (RGD), a ligand recognition motif for several but not all integrins (Table 11.2). On the β3 subunit, ligand peptides are cross-linked within residues 109–171 (D'Souza *et al.* 1988). 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 (Wardlaw *et al.* 1989); 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 divalent cation-binding site motif (Fig. 11.3), and require Ca^{2+} or Mg^{2+} for function (Kishimoto *et al.* 1989a). Lymphocyte function-associated antigen 1 α 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 (Dustin and Springer 1989). The divalent metal-binding motif in integrins has only five of six predicted metal co-ordination sites. It has been proposed that, in integrin binding to RGD-containing ligands, the aspartic acid residue (D) in RGD binds to the metal held in the α subunit divalent cation-binding pocket, forming a sixth co-ordination site (Corbi *et al.* 1987). Consistent with this, a ligand is cross-linked to amino acids 294–314 of the α subunit of αIIbβ3, which define the second divalent cation-binding site (D'Souza *et al.* 1990).

Further integrin domains might be involved in ligand binding. All three leucocyte 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 recog-

nition in addition to those shared by all integrins (Kishimoto *et al.* 1989a). Cysteines are notably few in the putative ligand-binding regions of the α and β subunits (Fig. 11.3), 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 (Burridge *et al.* 1988). Talin appears to interact with the cytoplasmic domain of $\alpha5\beta1$ (Horwitz *et al.* 1986). Talin redistributes with LFA-1 to sites of antigen-specific adhesion and co-caps with LFA-1 after phorbol ester stimulation (Kupfer and Singer 1989); 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.

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 (Ruoslahti and Pierschbacher 1986, 1987; Hynes 1987). Examples within the immune system include regulation of T cell proliferation by LFA-1 (Pircher *et al.* 1986; van Noesel *et al.* 1988) and VLA-5 (Matsuyama *et al.* 1989).

Integrins are novel receptors with respect to the type of inside-out signalling describe for LFA-1 in which signals from the cytosol are transduced across the membrane to generate changes in extracellular functions such as adhesion. Other integrins beside LFA-1 appear to undergo avidity regulation. On unactivated platelets, the integrin GPIIbIIIa does not bind fibrinogen, but upon activation binds soluble fibrinogen with a K_d of 29–45 nM (Plow and Ginsberg 1989). 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 (Keizer *et al.* 1988) and in GPIIbIIIa (Plow and Ginsberg 1989) in sites distinct from the ligand-binding site suggests that conformational changes in the ligand-binding site may be possible. Upon stimulation with chemoattractants, neutrophils show transient adhesion to other neutrophils, to endothelial cells and to C3bi-coated cells. There is evidence that this may be due to a transient change in avidity of Mac-1 (Wright and Meyer 1986; Buyon *et al.* 1988; Lo *et al.* 1989), although other mechanisms may explain the transience and the studies are not as rigorous as for LFA-1 or GPIIbIIIa (Dustin and Springer 1989).

A model may be proposed in which localized changes in integrin avidity play a key role in regulating cell orientation and migration. In T cell interactions with target cells, increases in LFA-1 avidity may be confined to the area where TCR engagement generates localized signals, thus generating a spatial gradient that would help killer T cells orientate to target cells (Poenie *et al.* 1987; Kupfer and Singer 1989). Cell migration is dependent both on the cytoskeleton and on adhesion. Tension in the cytoplasm to force the leading edge of the cell forward may be generated by the cytoskeleton (Sheetz *et al.* 1989). The cytoskeleton must be anchored at sites where the cell is attached to the substrate or to the neighbouring cell over which it is moving, a function that appears to be subserved by integrins. In models of active cell translocation, it is generally appreciated that a mechanism for de-adhesion is required at the trailing edge of the cell (Abercrombie 1961; Weiss 1961). Neutrophils from patients who are deficient in the leucocyte integrins fail to orientate and migrate in response to chemoattractants (Anderson and Springer 1987). Chemoattractants may induce spatial gradients, with high integrin avidity at the leading edge of the cell and low integrin avidity at the trailing edge. This could provide a mechanism for de-adhesion at the trailing edge and differential adhesiveness at the leading and trailing edges could help drive cell migration. This would be analogous to haptotaxis, the ability of gradients of substrate adhesiveness to promote directed migration of cells (Carter 1967).

Education of lymphocytes alters adhesion receptor phenotype

Lymphocytes newly emigrated from the thymus are considered 'naïve', and remain so until they encounter and are stimulated by specific antigen. They then become 'memory' lymphocytes which

are longer-lived; some live for the lifetime of the animal. As discussed above transient alterations in adhesion mechanisms lasting for minutes to days accompany lymphocyte activation. In addition, permanent alterations in surface density occur as a result of the transition from the naïve to memory phenotype. Shortly after antigen stimulation, naïve T lymphocytes of both CD4+ve and CD8+ve subsets acquire surface LFA-3 and increased levels of a cohort of surface molecules, including the adhesion receptors CD2 and LFA-1 (Sanders *et al*. 1988; Cerottini and MacDonald 1989) (Table 11.3). 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 (Janossy *et al*. 1989).

Naïve and memory T cell subsets differ in lymphokine secretion, in some functional assays (Morimoto *et al*. 1985; Tedder *et al*. 1985; Sanders *et al*. 1988; Cerottini and MacDonald 1989) 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 (CD3), CD8 and CD4 identical to naïve T cells (Sanders *et al*. 1989). Their increased expression of the LFA-1 and CD2 molecules should also enhance sensitivity to

Table 11.3. Conversion of naïve to memory T lymphocytes alters surface molecule phenotype. Modified from Sanders *et al*. (1988)

Molecule	Difference in expression (fold increase or decrease)
CD2	↑2.8
LFA-1	↑2.4
LFA-3	– → +
CD29 (VLA-β, *4B4*)	↑3.7
CD44 (*Hermes*, *Pgp-1*)	↑2.1
CD45RO (*UCHL-1*)	↑29
CD45RA (*2H4*)	+ → –
CD4	1.0
CD8	1.0
TCR (CD3)	1.0

LFA = lymphocyte function-associated antigen; VLA = very late activation; TCR = T cell receptor. The names of monoclonal antibodies are shown in italic type.

antigen by facilitating interactions of memory T cells with antigen-presenting cells. Athough somatic mutations within antibody variable regions of B cells may allow for selection of higher-affinity B cell clones during the course of the immune response (MacLennan and Gray 1986), this does not occur for TCR variable regions (Davis and Bjorkman 1988). However, the above-mentioned alterations may equip memory T cells with a different battery of mechanisms for making sensitive and robust secondary responses.

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, from where 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 the gut differ in the types of antigens to which lymphocytes are exposed. Lymphocytes from adult animals differ from those in new-borns in that, when harvested from specific lymph nodes, they show a twofold preference for recirculation to lymph nodes of the type from which they came (Butcher 1986; Yednock and Rosen 1989). 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 frozen 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 (Butcher 1986; Stoolman 1989; Yednock and Rosen 1989). Molecules termed 'addressins', selectively expressed on specialized high endothelium in different types of lymph nodes, are good candidates to be the molecules to which lymphocytes bind (Butcher 1986; Stoolman 1989; Yednock and Rosen 1989). Binding and immigration into lymph nodes may be a co-operative process involving multiple receptors on the lymphocyte and counter-receptors on the endothelium, analogous to antigen-specific interactions (Fig. 11.1(a), (b)) since CD44 (Hermes),

LFA-1, VLA-4 and Mel-14 leucocyte adhesion molecule (LAM)-1 on the lymphocyte have all been implicated in binding to high endothelial venules (HEV). The more interesting candidates for specific receptors are VLA-4 as a Peyer's patch receptor, and Mel-14/LAM-1 as a peripheral lymph node receptor (Butcher 1986; Stoolman, 1989; Tedder *et al.* 1989a; Yednock and Rosen 1989). However, the function of these molecules in adhesion hardly seems limited to lymphocyte recirculation, as discussed above for VLA-4. It is encouraging that CD44 and VLA-4 (CD29) show increased expression on the memory T lymphocyte subset (Table 11.3) and that LAM-1 (Leu8/TQ1) is preferentially expressed on a distinctive but overlapping lymphocyte subset (Tedder *et al.* 1989b). Differences of several-fold in surface density of these receptors would appear adequate to give rise to the twofold selectivity seen in recirculation to different types of lymph nodes, although this remains to be tested.

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 with diverse roles in adhesion (Bevilacqua *et al.* 1989; Johnston *et al.* 1989; Lasky *et al.* 1989; Siegelman *et al.* 1989; Stoolman 1989; Tedder *et al.* 1989a) termed 'selectins' (Fig. 11.4) (M. Bevilacqua, pers. comm.). All have an N-terminal domain of 117–120 amino acids which is homologous to a variety of Ca^{2+}-dependent animal lectins (Drickamer 1988), 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). Following the N-terminal lectin domain is a single EGF (epidermal growth factor) motif of 34–40 amino acids, and then short consensus repeats of 62 amino acids, a motif found in many proteins involved in regulating complement activation (Fig. 11.4). 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 (Lasky *et al.* 1989; Stoolman 1989; Yednock and Rosen 1989).

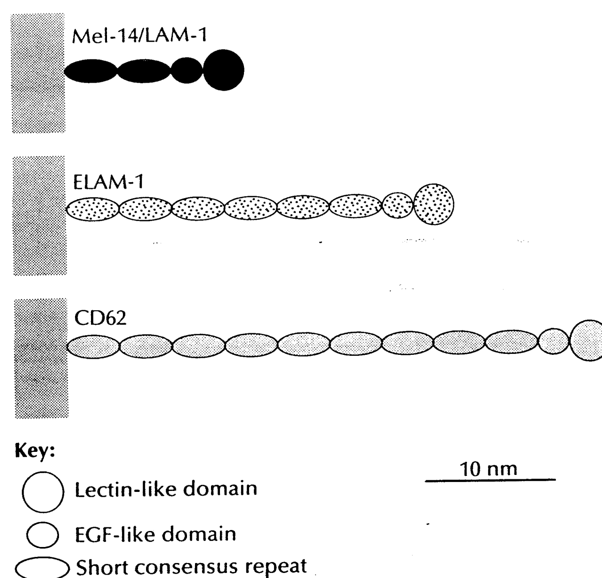


Fig. 11.4. Selectins. Scale models of selectins (Bevilacqua *et al.* 1989; Johnston *et al.* 1989; Lasky *et al.* 1989; Siegelman *et al.* 1989) are proposed. The short consensus repeats (SCR) extend 4.1 nm each, based on a length of 33 nm (Dahlback *et al.* 1983) for 8 SCR (Chung *et al.* 1988), epidermal growth factor (EGF) repeats extend about 2.3 nm (Taylor *et al.* 1989), and the lectin-like N-terminal domain is modelled as a globular (Drickamer 1988) sphere.

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. 11.4).

Although the importance of the leucocyte integrins for neutrophil binding to endothelium and emigration has been amply demonstrated *in vitro* (Smith *et al.* 1988, 1989; Kishimoto *et al.* 1989a; Lo *et al.* 1989), and by their congenital deficiency *in vivo* (Anderson and Springer 1987), three different selectins are involved in additional mechanisms for regulating neutrophil binding to endothelium. Co-operation between these mechanisms is likely to explain the absolute requirement for integrins in the emigration of neutrophils from the blood; even when other mechanisms can mediate the initial event of binding neutrophils to the endothelium, the leucocyte integrins are still required for the subsequent event of transendothelial migration (Lawrence *et al.* 1989; Smith *et al.* 1989). The selectin Mel-14 not only functions as a lymphocyte recirculation receptor, but also contributes to neutrophil emigration at inflammatory

sites (Butcher 1986; Jutila *et al.* 1989). With similar kinetics, but in contrast to leucocyte integrins, which are increased on the cell surface by mobilization from granule compartments within minutes after stimulation of neutrophils with chemoattractants (Kishimoto *et al.* 1989a), Mel-14 is released from the cell surface by proteases (Kishimoto *et al.* 1989b). It may function in an early step of neutrophil adhesion to the endothelium, and is shed before transendothelial migration (Kishimoto *et al.* 1989b). The endothelial leucocyte adhesion molecule (ELAM)-1 is a selectin that is transiently expressed on endothelial cells 2–8 hours after stimulation with IL-1 and other inflammatory agents, and mediates a neutrophil adhesion pathway distinct from that mediated by ICAMs and leucocyte integrins (Bevilacqua *et al.* 1989; Luscinskas *et al.* 1989). A third selectin called platelet activation dependent granule to external membrane (PADGEM), granule membrane protein (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 (Johnston *et al.* 1989; Larsen *et al.* 1989). The carbohydrate ligands for CD62 and ELAM-1 are closely related or identical to sialylated Lewis X, a tetrasaccharide that is expressed at the terminus of N and O-linked glycans and on glycolipids of neutrophils and monocytes (reviewed in Polley *et al.* 1991; Springer and Lasky 1991).

Selectins, by contrast to integrins and Ig family molecules, are expressed only on cells of the vascular system: leukocytes, platelets, and endothelial cells. Recent studies show that selectins are specialized to mediate adhesion in flow, a condition that occurs only in the vasculature. Selectins mediate the initial step of leukocyte attachment to the vessel wall and rolling, whereas integrins mediate subsequent arrest of the rolling cell and adhesion strengthening, followed by transendothelial migration. Neutrophil attachment and rolling at physiological shear stresses can be reproduced *in vitro* on artificial lipid bilayers containing the purified selectin CD62 (Lawrence and Springer 1991). Adhesion of resting or activated neutrophils through the integrins LFA-1 and Mac-1 to ICAM-1 in a lipid bilayer does not occur at physiologic shear stresses; however, static incubation of activated neutrophils on ICAM-1 bilayers allows development of adhesion that is greater than 100-fold more shear resistant than found on CD62. Addition of a chemoattractant to neutrophils rolling on bilayers containing CD62 and ICAM-1 results in arrest and adhesion strengthening of the rolling neutrophils through activation of interaction of LFA-1 and Mac-1 with ICAM-1. Similarly, LAM-1 has been found to be important in attachment of neutrophils at physiological shear stress to activated endothelial cell monolayers *in vitro* (Smith *et al.* 1991), and to mediate rolling of neutrophils in post capillary venules *in vivo* (Ley *et al.* 1991; von Andrian *et al.* 1991).

Summary

The dynamic role of lymphocyte adhesion receptors in lymphocyte antigen-specific interactions, in localization in lymphoid and non-lymphoid organs, and in bidirectionally transmitting information which affects cellular differentiation and responsiveness and interaction with the environment has been emphasized in this review. 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 micro-architecture 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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