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THE SENSATION AND REGULATION OF INTERACTIONS WITH THE EXTRACELLULAR ENVIRONMENT: THE CELL BIOLOGY OF LYMPHOCYTE ADHESION RECEPTORS

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INTRODUCTION

Adhesion in Developmental Biology and the Immune Response

The organization of metazoan animal cells in differentiated organs and tissues has long been postulated to depend on cell surface interactions. Cells interact both with molecules on the surface of other cells and with the extracellular matrix. The matrix includes proteoglycans and proteins such as fibronectin, collagens, and laminins, which are organized in fibrillar meshworks and basement membranes. Sperry proposed that a limited number of adhesion molecules in spatial gradients within tissues could generate a degree of specificity sufficient for organization of the nervous system (Sperry 1963). A basic assumption of this theory—that on substrates with adhesive gradients, cells can migrate towards regions of high adhesiveness—has been found to be correct (Carter 1967). Localization of cells can thus be driven by interactions of cell surface receptors with complementary molecules on the surface of other cells and in the matrix so that the most favorable interactions determine the hierarchy of cell and tissue organization. Cell migration can also be directed by chemoattractant gradients; however, there is interplay between these mechanisms because cells must gain a foothold and regulate adherence to other cells or to the matrix in order to migrate. Sensing of the environment has a dual role in regulating both cell localization and differentiation (Ruoslahti & Pierschbacher 1987), and thus can explain much of development. These principles operate throughout the biology of multicellular organisms (Trinkaus 1984), and the cells of the immune system, which provide many opportunities for studying the cell biology of cell-cell interactions, are no exception. The distinctive paradigms of antigen-specific interactions studied by immunologists and cell and tissue-selective adhesive interactions studied by cell biologists have historically found little common ground, however. This has changed of late. Studies of interactions of T lymphocytes with antigen-bearing cells have yielded not only the antigen-specific receptors sought by immunologists, but also a rich harvest of the type of cell adhesion molecules that have long been of interest to cell biologists.

What has been learned about adhesion molecules of the immune system may have wide application in cell biology. Some of the adhesion receptors

of the immune system transmit information in both directions across the membrane thereby mediating a dialogue between the inside and outside of the cell. Binding of cell surface adhesion receptors to molecules in the cellular environment can transduce signals that alter cellular responsiveness and differentiation and, conversely, signals from inside the cell can affect the way adhesion molecules interact with the extracellular environment. Homologous families of adhesion receptors reveal relationships between molecules in the immune system and those in the nervous system and many other tissues, between cell-cell and cell-matrix receptors, and between regulation of cell adhesion and de-adhesion and the regulation of cell migration. Several adhesion receptors of the immune system are also utilized as receptors by viruses.

Mechanisms for regulating adhesion, in which molecular alterations occur on time scales ranging from seconds to days, and are transient or permanent, are richly illustrated by the adhesion receptors of the immune system. Rapid transition between nonadherent and adherent states is of key importance to immune surveillance and responsiveness. On one hand, cells of the immune system must circulate as nonadherent cells in the blood and lymph, and on the other, they must be able to congregate in lymphoid organs, cross endothelial and basement membrane barriers, localize at sites where foreign antigens and microorganisms are present, and adhere to cells that bear foreign antigen (Parrott & Wilkinson 1981).

This review focuses on aspects of immune cell adhesion likely to be of most interest to the cell biologist and expands on a previous review (Springer 1990). For related topics, the reader is referred to recent reviews (Kishimoto et al 1989b; Makgoba et al 1989; Hemler 1990; Williams & Barclay 1988; Bierer & Burakoff 1989), to two volumes on the subject (Springer et al 1989; Moller 1990), and to earlier reviews (Springer et al 1987; Anderson & Springer 1987; Martz 1987).

Dissection of the Molecular Basis of T Lymphocyte Recognition with Monoclonal Antibodies

Antibodies have been key to the discovery of adhesion molecules. In early studies with the slime mold *Dictyostelium* and with nervous system cells (Garisch 1980; Edelman 1986), polyclonal antibodies were raised to these cells and Fab fragments of the antibodies were found to inhibit cell aggregation. Cell adhesion molecules were then purified by biochemical techniques, and their enrichment during purification was assayed by neutralization of the ability of Fab fragments to inhibit adhesion. The invention of a method for producing monoclonal antibodies (mAb) (Kohler & Milstein 1975) allowed a much more direct and powerful approach to dissecting the molecular basis of cell adhesion (Springer et al

1987; Martz 1987; Kishimoto et al 1989b). After immunization with whole cells, monoclonal antibodies were elicited to a wide array of surface molecules, and those mAb that could inhibit cell adhesion or other cell contact-dependent phenomena were selected for cloning and further characterization. The mAb could then be used on immunoabsorbent columns to purify the adhesion molecule, to determine its cellular distribution by immunofluorescence, and to probe its involvement in further cellular functions by inhibition. DNA clones could be obtained based on protein structure, or by using the mAb to screen expression libraries.

The long mysterious process of T lymphocyte adhesion (Figure 1 *a,b*) has yielded its secrets to this approach (Springer et al 1987; Martz 1987; Kishimoto et al 1989*b*; Bierer et al 1989). T (or thymus-derived) lymphocytes do not secrete antibodies like B (bone marrow-derived) lymphocytes. T lymphocytes must come into direct contact with cells bearing foreign antigen both for stimulation of proliferation of those T-cell clones that have appropriate antigen receptors and for stimulation of effector function. Two subsets of T lymphocytes have distinct effector functions. Helper T lymphocytes secrete cytokines that augment immune responses by other cells. Killer T lymphocytes lyse cells that bear foreign antigen and are important in the response to cells infected with virus.

T lymphocyte-mediated killing (Figure 1*a*) was particularly amenable to molecular dissection with mAb, largely because of the simplicity of the killing assay. The assay involves mixing T lymphocytes and target cells together, and measuring, by radioisotope release, the percentage of target cells that are lysed within four hr. Killing is antigen-specific and is absolutely dependent on adhesion of the T cell to the target cell, which is known as conjugate formation. Conjugate formation can be measured by microscopy or flow cytometry and thus assessed independently of target cell lysis. A large number of mAb were screened for their ability to inhibit T lymphocyte-mediated killing, and this led to the identification of surface molecules that were operationally called lymphocyte function-associated (LFA). Three distinct molecules called LFA-1 (CD11a/CD18), LFA-2 (identical to T11 and now called CD2), and LFA-3 (CD58) were thus defined (Table 1). At about the same time, the CD4 and CD8 molecules were identified with mAb that labeled mutually exclusive subsets of mature T cells, and the T-cell receptor for antigen (TCR) was identified with mAb that labeled one clone of T cells but not others. Later the counter-receptors for LFA-1, called intercellular adhesion molecules (ICAMs), were identified in adhesion assays described below (Springer et al 1987; Martz 1987; Kishimoto et al 1989b; Bierer et al 1989).

Monoclonal antibodies to any one of these surface molecules can inhibit T lymphocyte-mediated killing, thus showing that it is a highly complex

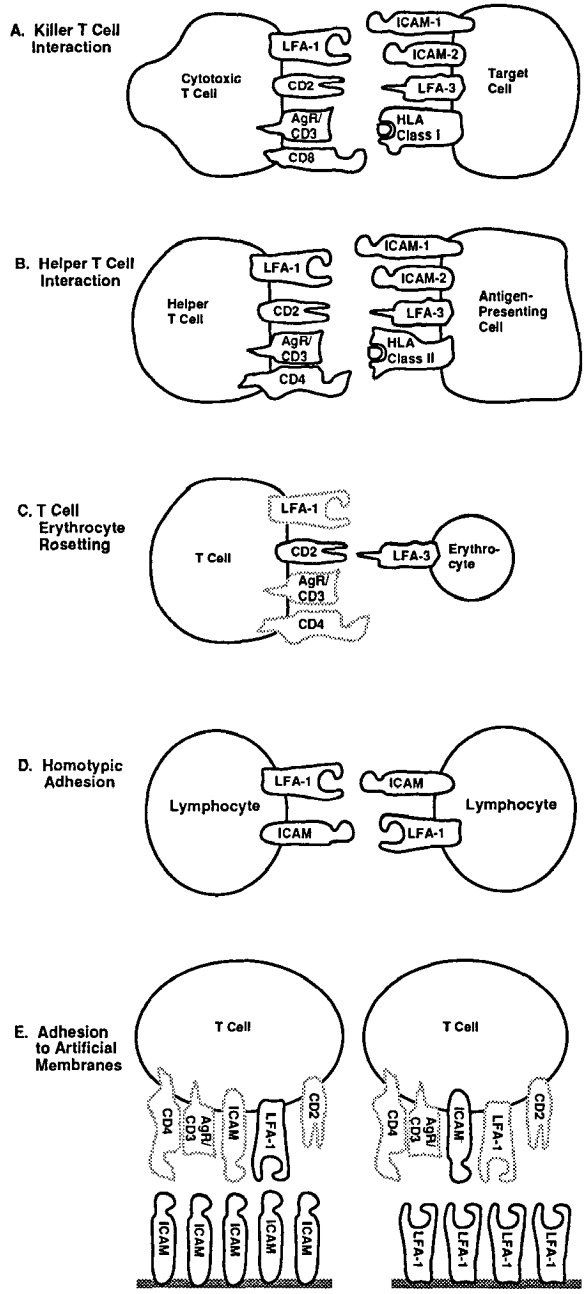


Figure 1 Lymphocyte adhesion systems.

Table 1 Characteristics of T-cell adhesion receptors and counter-receptors

Receptor	Mass (kd)	Distribution	Counter-receptor	Mass (kd)	Distribution
LFA-1 (CD11a/ CD18)	α180, β95	Thymocytes, T&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	Wide. 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	MHC Class I	α44, β12	Wide, increased by IFN-α, β, γ
CD4	55	Subset of thymocytes and T lymphocytes, monocytes, macrophages	MHC Class II	α34, β29	Restricted, widely inducible by IFN-γ

process requiring cooperation between a number of different surface molecules. Subsequent studies have demonstrated that all of the molecules shown in Figure 1 are involved in adhesion, and many are also involved in the signaling 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 1 and described in detail 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 major histocompatibility complex (MHC) class I or class II molecules (Figure 1*a* and *b*, respectively). The 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 & Kappler 1986; Brenner et al 1988). These two subunits, termed α and β or γ and δ depending on the class of TCR, under physiologic conditions are always associated with CD3, a complex of six chains that mediates the intracellular signaling function of this eight 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 programs appropriate to killer or helper T cells.

MHC 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. MHC class I molecules bind to peptides derived from endogenously synthesized molecules such as viruses. MHC class I-peptide complexes are primarily recognized by $CD8^+$, killer T lymphocytes (Figure 1*a*). MHC class II molecules bind to peptides derived from endocytosed antigen, and are primarily recognized by $CD4^+$ helper T lymphocytes (Figure 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 II molecules, respectively (Swain 1983; Bierer et al 1989). Indeed, expression of supraphysiologic levels of CD4 molecules on transfected fibroblasts allowed detection of binding to cells bearing MHC class II molecules (Doyle & Strominger 1987), and similar studies have shown specific interactions between transfected cells expressing high levels of CD8 and MHC class I molecules (Norment et al 1988). At physiologic 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 physiologic importance appears to be in signaling. When this signaling contribution is blocked, as with a mAb 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 (Bierer et al 1989; Janeway 1988; von Boehmer 1988; Parnes 1989; Kupfer & Singer 1989b).

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 signaling. This has not been directly demonstrated, but has been inferred from the ability of TCR directed to peptide MHC class I and class II complexes to synergize only

with CD8 and CD4, respectively (Bierer et al 1989; Janeway 1988; Parnes 1989). Consistent with associative recognition, the sites on the MHC class I 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 MHC class I molecule (Bjorkman et al 1987), whereas CD8 binds to monomorphic residues in a membrane-proximal domain of MHC class I (Potter et al 1989; Salter et al 1989). When $CD4^+$ helper T-cell clones form conjugates with antigen-presenting B cells, both the TCR and CD4 redistribute to the site of adhesion (Kupfer & Singer 1989). There may be some tendency for self-association between the TCR and CD4, since this can be induced by certain mAb to the TCR and correlates with the ability of these mAb to directly activate T cells (Janeway 1988; Kupfer & 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 the other of these molecules with the TCR may help to signal whether the TCR recognizes MHC class I or class II and may regulate subsequent differentiation into $CD8^+$ cytotoxic or $CD4^+$ helper T-lymphocyte subsets (Janeway 1988; von Boehmer 1988).

THE ACTIVATION-REGULATED CD2 : LFA-3 AND LFA-1 : ICAM ADHESION MECHANISMS

Introduction

Stimulation with specific antigen or mitogens causes resting lymphocytes to enlarge, divide, and become more motile and adhesive (Parrott & Wilkinson 1981). Lymphocytes responding to specific antigen in vivo temporarily localize in tissues where antigen is present and leave the circulation (Butcher 1986). The transition upon activation of lymphocytes from free circulating cells to cells that dwell in contact with other cells in lymphoid and non-lymphoid tissues appears to reflect an overall increase in lymphocyte adhesiveness. Although much remains to be learned about how activation regulates lymphocyte interactions in vivo, antigen-independent adhesion mechanisms recently defined in vitro are likely to be important. 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 (Springer et al 1987; Spits et al

1986; Shaw et al 1986). This antigen-independent adhesion results from binding of the CD2 and LFA-1 molecules on the T cell to the LFA-3 and ICAM molecules on the target cell, respectively (Figure 1*a,b*). 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. Although not appreciated in the original studies, antigen-independent adhesion is related to T-cell activation, since it is not seen with resting T lymphocytes (Dustin & Springer 1989).

Regulation of Lymphocyte Adhesion by the CD2 : LFA-3 Mechanism

CD2 AND LFA-3 ARE RECEPTORS FOR ONE ANOTHER Early studies showed that mAb to CD2 and LFA-1 blocked T-cell function by binding to the T cell, whereas mAb to LFA-3 blocked by binding to the target cell (Krensky et al 1983). Later evidence showed a lack of additive effects of mAb to CD2 and LFA-3, in contrast to additive effects of these mAb with mAb 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$^+$ cells and blocking of this binding with mAb to LFA-3 (Selvaraj et al 1987c). In subsequent studies, purified LFA-3 incorporated in artificial, glass-supported planar membranes was shown specifically to bind CD2$^+$ cells (Dustin et al 1987a). This observation was further corroborated by saturable binding of purified LFA-3 to CD2$^+$ cells, binding of LFA-3$^+$ cells to transfected cells expressing CD2, and interaction of lipid vesicles containing purified LFA-3 and CD2 (Springer et al 1987; Bierer et al 1989).

CD2, LFA-3, AND THE Ig SUPERFAMILY CD2 and LFA-3 are both members of the immunoglobulin (Ig) superfamily, a large family of molecules that are related to immunoglobulins and are often expressed on cell surfaces (Williams & Barclay 1988). The structural unit that members of this superfamily share in common is the Ig domain, composed of 90 to 100 amino acids arranged in a sandwich of two sheets of anti-parallel β strands, which are stabilized by a disulfide bond at their center (Williams & Barclay 1988; Alzari et al 1988). The immunoglobulins and TCR, which are specialized for antigen recognition, are the only known members of this family with variable regions that undergo somatic diversification. The function of molecules of the Ig superfamily in adhesion evolutionarily predates specialization for antigen recognition, which occurs only in vertebrates; Ig superfamily members are present in insects as nervous system adhesion

molecules involved in axon guidance and fasciculation (Harrelson & Goodman 1988).

Adhesion molecules that are members of the Ig superfamily include CD4, CD8, CD2, LFA-3, ICAM-1, and ICAM-2 (Figure 2) in the immune system, and NCAM (Edelman 1986; Cunningham et al 1987; Rutishauser et al 1988) and fasciculin II (Harrelson & Goodman 1988) in the nervous system. The Ig domain may have diversified and been adopted so widely in evolution because of its stable disulfide-bonded β strand structure, which is analogous to an automobile chassis on which many different styles of bodies and fenders may be hung. These bodies and fenders 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.

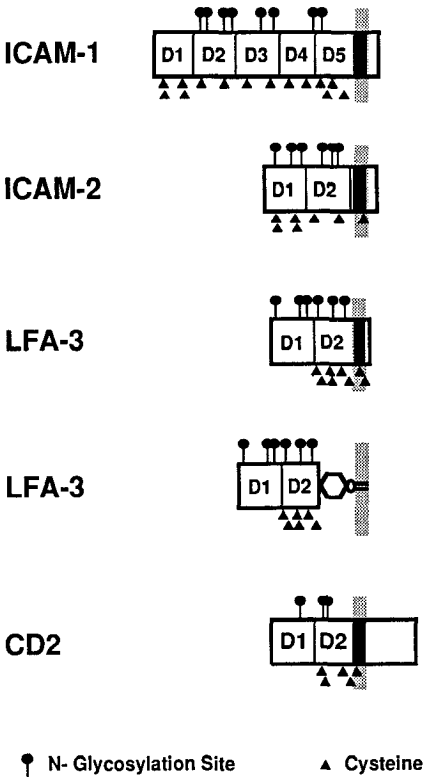


Figure 2 Ig Superfamily adhesion receptors of the immune system. Structures are based on (Simmons et al 1988; Staunton et al 1988, 1989; Wallner et al 1987; Seed 1987; Sewell et al 1986; Seed & Aruffo 1987).

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). The genes for CD2 and LFA-3 map to the same band on chromosome 1 (Sewell et al 1988). Sites on CD2, which bind to LFA-3, have been localized to the first Ig domain (Peterson & Seed 1987; Richardson et al 1988).

REGULATION OF ADHESION BY T-CELL ACTIVATION Interaction between cells bearing CD2 and LFA-3 is finely poised and is tipped toward adhesion by T-cell activation. $CD2^+$ cells do not inherently bind to LFA-3^+ cells, as demonstrated in studies of "rosetting" with LFA-3^+ erythrocytes (Figure 1*c*). Activated T lymphocytes and resting T lymphocytes both express CD2, but only activated T cells adhere to erythrocytes to form rosettes. The physiologic significance of adherence of activated T lymphocytes to erythrocytes is just beginning to be understood; it may prevent immune responses from occurring in the blood stream. Interactions between $CD2^+$ T lymphocytes and LFA-3^+ dendritic cells are seen in lymph, where no erythrocytes are present. Dendritic cells, which are released from the skin into the lymph from where they drain to the local lymph node, are important in taking up and presenting foreign antigen to T cells. Dendritic cells in the lymph are found in rosettes with $CD2^+$ TCR $\alpha\beta^+$, but not $CD2^-$ TCR $\gamma\delta^+$ T lymphocytes. The CD2 on these rosetting T lymphocytes is redistributed to the site of adherence to the LFA-3^+ dendritic cell (Mackay et al 1988).

REGULATION OF ADHESION BY RECEPTOR DENSITY Since human erythrocytes lack ICAMs 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 (Figure 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 1987b). Resting human T lymphocytes bind to sheep erythrocytes, but not to human erythrocytes, because there is a fourfold higher density of the LFA-3 homologue on sheep erythrocytes. The affinity for CD2 and the charge density of erythrocytes in both species are identical (Selvaraj et al 1987a). The cell surface density of CD2 and LFA-3 appears to be reciprocally adjusted to a threshold that does not give rosetting of erythrocytes with resting T lymphocytes of the same species; thus, 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 1987a; Mackay

et al 1988). In sheep, humans, and many other species, T-lymphocyte activation alters the equilibrium to allow rosetting with autologous erythrocytes.

REGULATION OF ADHESION BY SURFACE CHARGE Close cell-cell contact is opposed by repulsion between cells because of their net negative surface charge and by the decrease in entropy that occurs when the glycocalyxes 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 (Plunkett et al 1987; Bentwich et al 1973). Charge neutralization alters the morphology of the contact between T lymphocytes and sheep erythrocytes by converting small islands of close contact surrounded by larger areas of greater intermembrane distance to a single large area of close contact (Bentwich et al 1973). Sialic acid is the major contributor to the net negative cell surface charge (Wigzell & Hayry 1974). Despite their larger surface area, T lymphoblasts 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). The sialic acid density on the cell surface may be a primary factor determining whether the CD2 : LFA-3 mechanism and other adhesion mechanisms are active or latent. In lymph nodes, the activated antigen-responsive lymphocytes that aggregate in germinal centers are greatly undersialylated, while areas containing B and T cells 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).

THE EQUILIBRIA OF RECEPTOR AND CELLULAR INTERACTIONS 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. Monovalent interaction of CD2 with LFA-3, however, was recently found to have a K_d of 1 μM (Selvaraj et al 1987c; Dustin et al 1989; Sayre et al 1989). LFA-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 1987a).

The energetics of adhesion molecule binding and electrostatic repulsion can be calculated to test the prediction that they should be nearly equal in

a system that is finely regulative. Human erythrocytes contain 4×10^3 LFA-3 molecules (Selvaraj et al 1987a); it is not known what fraction of these would be involved in binding to a human 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. Analogous to assuming that all of the erythrocyte's LFA-3 molecules are engaged for calculation of the binding energy, for calculation of charge repulsion, all its surface area can be assumed to come in contact with the lymphocyte, and since the charge density of erythrocytes (see Selvaraj et al 1987a) but not lymphocytes is known, the interaction can be approximated as between two erythrocytes. The electrostatic work (Lerche 1983) required to bring two planar surfaces with area and charge density of a human erythrocyte initially uniformly distributed in glycocalyxes 10 nm thick from infinite separation to within 16 nm of one another (approximately as predicted for CD2/LFA-3 interaction, see below), using a Debye-Huckel parameter for saline, is 9.1×10^{-9} erg (M. Lawrence, T. Springer, unpublished). The resulting free energy change would be $+7.1 \times 10^{-9}$ erg, which is unfavorable for adhesion. By contrast, sheep erythrocytes have more LFA-3 molecules ($\sim 10^4$/cell) and only 37% the surface area of human erythrocytes; therefore, the calculated energies for adhesion and electrostatic work are -5.2×10^{-9} erg and 3.4×10^{-9} erg, respectively. This gives a favorable free energy change for the interaction of -1.8×10^{-9} erg. Although these calculations neglect changes in entropy caused by glycocalyx comparison, interdigitation, and redistribution of receptors to sites of close cell-cell contact, the similarity in the magnitude of the binding and electrostatic energies, and correct prediction of resting human T-cell interaction with sheep, but not human erythrocytes, affirms the importance of counterbalancing adhesive and repulsive forces in regulating cell-cell interactions.

SIGNAL TRANSDUCTION CD2 may transduce a signal that augments or synergizes with signals from the TCR (Bierer et al 1989). Certain pairs of CD2 mAb or combination of one CD2 mAb with multimeric LFA-3 can stimulate T cells, an effect that requires the presence of the TCR (Moingeon et al 1989a; Bockenstedt et al 1988). CD2-LFA-3 interaction alone, however, has no effect (Dustin et al 1989; Tiefenthaler et al 1987). Transfection of cells with CD2 and LFA-3 has confirmed early mAb inhibition results (Springer et al 1987) showing CD2/LFA-3 interaction can contribute a 4- to 30-fold enhancement of the immune response (Bierer et al 1988; Moingeon et al 1989b). The unusually basic, histidine and proline-rich 120 amino acid cytoplasmic region of CD2 is required for stimulation by pairs of mAb (He et al 1988; Moingeon et al 1989b); however, in antigen-specific responses, truncation of the cytoplasmic domain of CD2

has given ambiguous results, leaving unclear the relative contributions of adhesion and signaling to enhancement of the immune response by CD2 : LFA-3 interaction (Bierer et al 1988; Moingeon et al 1989b).

ISOFORMS OF LFA-3 There are two isoforms of LFA-3, derived by differential mRNA splicing, that differ in membrane anchor (Dustin et al 1987; Wallner et al 1987; Seed 1987). One isoform has a glycophosphatidyl inositol 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 (Figure 2). Both the polypeptide-anchored isoform (Hollander et al 1988) and the GPI-anchored isoform (Bierer et al 1988; Moingeon et al 1989b) 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 the neural cell adhesion molecule (NCAM) also has anchor isoforms (Cunningham et al 1987; Rutishauser et al 1988).

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

ICAM-1 Subsequent to the identification of LFA-1 as one of the molecules recognized by mAb that inhibited T cell-mediated killing, it was found that LFA-1 is required for the adhesion step in CTL-mediated killing, as well as for a broad range of leukocyte functions involving adhesion, 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 (Springer et al 1987; Kishimoto et al 1989b). A counter-receptor for LFA-1, ICAM-1 was identified, using a simple assay called homotypic adhesion (Figure 1*d*), in which homogenous cell populations such as B- or T-cell lines adhere to one another to form multicellular clusters (Rothlein et al 1986; Dustin et al 1988; Springer et al 1987). 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 mAb and is not observed with cell lines established from patients genetically deficient in LFA-1 (see below). The ability of LFA-1$^+$ cells to co-aggregate with LFA-1$^-$ cells in the homotypic adhesion assay showed that LFA-1 is not a homophilic receptor that binds to itself, but rather is a heterophilic receptor that binds to a distinct counter-receptor (Rothlein & Springer 1986). A counter-receptor was defined by immunizing mice with LFA-1$^-$ cells and selecting mAb that would inhibit LFA-1-dependent homotypic adhesion. This counter-receptor was designated an intercellular adhesion

molecule (ICAM-1) (Table 1). Confirming the receptor/counter-receptor relationship, lymphocyte binding to purified ICAM-1 is inhibited with LFA-1 mAb (Marlin & Springer 1987), and purified LFA-1 protein micelles bind to purified ICAM-1 on artificial substrates (Dustin & Springer 1989). ICAM-1 is a member of the Ig superfamily with five Ig domains (Simmons et al 1988; Staunton et al 1988) (Figure 2).

REGULATION OF ICAM-1 EXPRESSION Induction of ICAM-1 in inflammation is one important means of regulating the LFA-1/ICAM interaction (Dustin et al 1986, 1988; Kishimoto et al 1989b). In contrast to LFA-1, which is restricted to leukocytes, 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 centers, both on follicular dendritic cells and on activated B lymphocytes, which congregate in these centers (Dustin et al 1986). Germinal centers 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, IL-1, and 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 (Dustin et al 1986, 1988; Kishimoto et al 1989b; Dustin & Springer 1988). Expression of ICAM-1 can reach $> 10^6$ sites/cell. 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 rise sharply to a plateau at 1000 molecules/μm^2 (Dustin & 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 different 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. In vivo, ICAM-1 induction accompanies T-cell-mediated hypersensitivity reactions in the skin (Wantzin et al 1989) and, after administration of γ-IFN and IL-1, appearance of ICAM-1 on endothelial cells correlates with sites of mononuclear cell infiltration (Munro et al 1989). ICAM-1 induction is largely regulated at the mRNA level. Increased surface expression is first seen after 4 hr and is usually maximal by 24 hr (Dustin et al 1988). In monocytes, by contrast, ICAM-1 is stored in an intracellular pool that can be mobilized to the cell surface (Dougherty et al 1988).

ICAM-2 A second LFA-1 ligand, differing in tissue distribution from

ICAM-1, was originally defined by the ability of LFA-1 mAb, but not ICAM-1 mAb, to inhibit the adhesion of certain cell types. Based on this functional property, an ICAM-2 cDNA was isolated from an expression library by screening for binding of transfected cells, in the presence of ICAM-1 mAb, to purified LFA-1 coated on Petri dishes. ICAM-2 is a transmembrane protein that binds to LFA-1 and shares no antigenic determinants with ICAM-1 (Staunton et al 1989). ICAM-2 has two Ig-like domains, in contrast to ICAM-1 which has five (Figure 2), and these are 35% identical to the N-terminal two domains of ICAM-1. ICAM-1 and ICAM-2 are much more similar to one another than to other members of the Ig superfamily and thus represent an Ig subfamily specialized to interact with LFA-1. 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.

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

The mechanisms discussed so far for regulating adhesive interactions operate on a relatively long time scale. Increased expression of ICAM-1 after cytokine induction is detectable in vitro or in vivo after 4–6 hr and is maximal by 9–24 hr (Dustin et al 1988; Munro et al 1989; Kishimoto et al 1989b). 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 on the order of 12–24 hr. Yet, T cells can regulate adhesion over a much shorter time scale, adhere to target cells, deliver a lethal hit, de-adhere and engage in repeated target cell interactions, with a cycle time as short as 5-30 min (Martz 1977; Poenie et al 1987). This requires a mechanism for regulating adhesion over a shorter time scale. Moreover, by contrast with cytotoxic cells stimulated in vitro, which show a general increase in adhesiveness, cells primed in vivo adhere only to those cells bearing the antigen for which they were primed, i.e. adherence is antigen-specific (Springer et al 1987; Martz 1987).

It has recently been found that cross-linking of the T-cell receptor on resting T lymphocytes stimulates adhesiveness through LFA-1. Thus LFA-1 adhesiveness can be antigen-specific. Adhesiveness is transient, allowing regulation of adhesion and de-adhesion over a time scale of minutes (Dustin & Springer 1989). Homotypic adhesion of leukocytes (Figure 1*d*),

which is dependent on LFA-1 and ICAM-1, is stimulated by 1 hr treatment with phorbol ester and yet is accompanied by no increase in LFA-1 or ICAM-1 surface expression (Rothlein & Springer 1986; Rothlein et al 1986). This suggests 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 T-cell receptor (Rothlein & 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 (Figure 1*e*), regulation of the avidity of cellular LFA-1 and of cellular ICAM-1 could be separately tested (Dustin & Springer 1989). Stimulating resting T lymphocytes with phorbol esters or crosslinking the TCR with mAb converts cellular LFA-1 from a low to high avidity state, whereas cellular ICAM-1 is constitutively avid. There is no change in LFA-1 surface density. Similar conclusions have been reached (van Kooyk et al 1989), measuring T-cell clone homotypic adhesion. In contrast to T-cell clones, resting peripheral blood T lymphocytes do not conjugate with B-lymphocyte target cells (Dustin & Springer 1989). TCR stimulation, however, induces conjugate formation that is inhibited completely by LFA-1 mAb and only marginally by CD2 mAb, which shows the preeminent role of LFA-1 in regulating the avidity of cell-cell interactions. The high avidity state peaks 5–10 min after TCR stimulation and returns to the low avidity state by 30 min; kinetics are influenced by the amount of TCR crosslinking. Subsequent addition of phorbol ester returns LFA-1 to the high avidity state, thereby demonstrating that the adhesion machinery is still intact. After phorbol ester stimulation, LFA-1 does not return to the low avidity state. Pharmacologic agents have a dramatic effect on the high avidity state of LFA-1, either by stimulating it directly or inhibiting its stimulation by TCR crosslinking. This suggests that the TCR controls LFA-1 through intracellular signaling pathways.

A model based on these findings for cooperation between the TCR and adhesion molecules to mediate antigen specific recognition (Dustin & Springer 1989) is as follows. LFA-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 ATP (Marlin & Springer 1987). Thus, in the absence of antigen, the equilibrium governing adherence of T lymphocytes to other cells favors 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 favor 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 redis-

tribution, or some other mechanism, is controlled by the TCR. LFA-1 is an adhesion servomotor operated by the TCR. Cellular energy expended in converting LFA-1 to a high avidity state helps drive the adherence/nonadherence equilibrium toward stable adherence, analogous to the use of ATP to favor an otherwise energetically unfavorable 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 (Dustin & Springer 1989). The transience of the high avidity state provides a mechanism for regulating lymphocyte de-adhesion. Antigen density and hence the number of TCR molecules engaged may influence the kinetics of the signaling cascade and thus the kinetics of avidity regulation. Other T-cell adhesion molecules may also influence kinetics because the high avidity state is more prolonged after stimulation through CD2 (van Kooyk et al 1989). 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 & T. Springer, unpublished). Duration of adhesion may also be influenced by the level of ICAM expression and whether or not 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

Structure and Ligand Specificity

LFA-1 is a member of the integrin family (Table 2). Integrins are perhaps the most sophisticated of the adhesion molecule families, both in terms of 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 noncovalently associated (Figure 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 (Kishimoto et al 1989b, 1987; Hynes 1987). Three subfamilies of integrins may be dis-

Table 2 The integrin family of cell-cell and cell-matrix receptors

Subunits	Names	α Subunit[a]		Ligands[b]	RGD tripeptide role	Distribution		References[e,f]
		I	C			Non-leukocyte[c]	Leukocyte[d]	
αLβ2	CD11a/CD18, LFA-1	+	–	ICAM-1, 2	–	—	B, T, M, G	(1)
αMβ2	CD11b/CD18, Mac-1, CR3	+	–	C3bi, FX, FB	+?	—	M, G	(1)
αXβ2	CD11c/CD18, p150,95	+	–	?	?	—	M, G	(1)
α1β1	CD-/CD29, VLA-1	?	–	LM, CO	–	F, BM	B*, T*	(2)
α2β1	CD49b/CD29, VLA-2, gpIaIIa, ECMRII	+	–	LM, CO	–	P, F, EN, EP	T*	(3–6)
α3β1	CD-/CD29, VLA-3, ECMRI	–	+	FN, LM, CO	–	EP, F	—	(4)
α4β1	CD49d/CD29, VLA-4, LPAM-1	–	*	FN, VCAM1	–	NC, F	B, T, M, LGL	(7–11)
α5β1	CD-/CD29, VLA-5, FNR, gpIc/IIa, ECMRVI	–	+	FN	+	F, EP, EN, P	Th, T	(4)
α6β1	CD49f/CD29, VLA-6, gpIc/IIa	–	+	LM	–	P	T	(12)
α4βp	CD49d/CD-, LPAM-2	–	*	?	?	—	T	(13)
α6β4	CD49f/CD-, αEβ4	–	+	?	–	E	—	(14–16)
αIIbβ3	CD41/CD61, gpIIbIIIa	–	+	FB, FN, vWF, FB	+	P	—	(17)
αVβ3	CD51/CD61, VNR	–	+	VN, FB, vWF, TSP	+	EN	B*, M	(17–19)
αVβ1	CD51/CD29	–	+	FN	+	NC, F	—	(20)
αVβ5	CD51/CD-, αVβS	–	+	VN, FN	+	C, F, EP	M	(19, 21, 22)

[a] I, I domain; C, cleavage; +, cleavage to disulfide linked heavy and light chains at amino acid 853–860; *, cleavage to nondisulfide linked chains at ~ amino acid 573.

[b] LM, laminin; CO, collagen; FN, fibronectin; FB, fibrinogen; FX, factor X; VN, vitronectin; vWF, von Willebrand factor; TS, thrombospondin.

[c] EN, endothelial cells; EP, epithelial cells; F, fibroblasts or other connective tissue; NC, neural crest, melanocytes; P, platelets; C, carcinomas; BM, basement membrane-associated.

[d] 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 IV (reference 23).

[e] General references: (1, 2, 24, 25).

[f] 1. Kishimoto et al 1989b; 2. Hemler 1990; 3. Kunicki et al 1988; 4. Wayner et al 1988; 5. Languino et al 1989; 6. Takada & Hemler 1989; 7. Holzmann et al 1989; 8. Takada et al 1989; 9. Elices et al 1990; 10. Wayner et al 1989; 11. Guan & Hynes 1990; 12. Sonnenberg et al 1988; 13. Holzmann & Weissman 1989; 14. Sonnenberg et al 1988; 15. Kajiji et al 1989; 16. Hemler et al 1989; 17. Plow & Ginsberg 1989; 18. Savill et al 1990; 19. Krissansen et al 1990; 20. Vogel et al 1990; 21. Cheresh et al 1989; 22. Freed et al 1989; 23. Gilks 1989; 24. Ruoslahti & Pierschbacher 1986, 1987; 25. Hynes 1987.

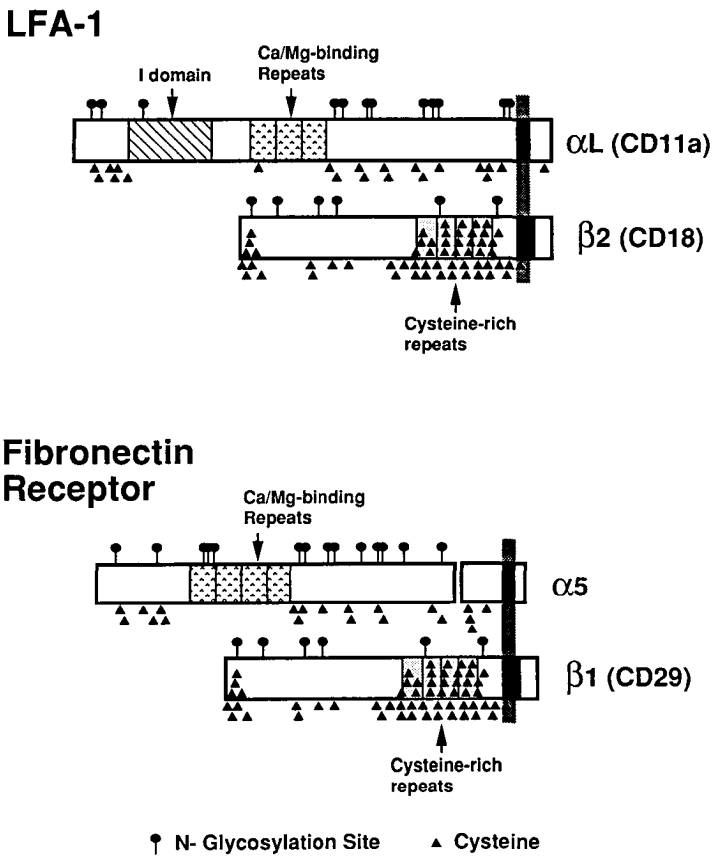


Figure 3 Representative integrin family adhesion receptors. Structures are based on (Kishimoto et al 1987; Larson et al 1989; Tamkun et al 1986; Argraves et al 1987).

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

LFA-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 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 (Kishimoto et al 1989b) (Table 2). The important role of the leukocyte integrins is illustrated in congenital leukocyte adhesion deficiency (LAD) in which all three leukocyte integrin $\alpha\beta$ complexes are deficient because of mutations

in the common β2 subunit (Anderson & Springer 1987; Kishimoto et al 1989b). 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 leukocyte localization in vivo. Administration of mAb to the β2 subunit in vivo mimics defects in LAD and appears to be clinically useful in inhibiting leukocye extravasation and neutrophil-mediated tissue injury in myocardial infarction and ischemic shock (Kishimoto et al 1989b).

The β1 integrin subfamily includes receptors for the extracellular matrix components fibronectin, laminin, and collagen (Table 2). These ligands show interesting patterns of expression in the fibrillar meshworks found throughout many tissues and in the basement membranes found in muscle, the nervous system, and underlying the epithelium and endothelium (Ruoslahti & Pierschbacher 1987, 1986; Hynes 1987). β1 integrins are thus likely to be involved in controlling the organization within tissues of the many nonhematopoietic and leukocyte cell types on which they are expressed (Table 2). β1 integrins have been designated very late activation (VLA) antigens because of the appearance of VLA-1 and VLA-2 on lymphocytes 2–4 weeks after antigen stimulation in vitro (Hemler 1990). VLA is not an apt acronym, however, because some VLA molecules are basally expressed on leukocytes, and their expression on nonhematopoietic cells does not require activation (Table 2). 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 that is expressed on resting lymphocytes, monocytes, and neural crest-derived cells and functions as both a matrix and 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 & Hynes 1990). As a cell receptor, it binds to a molecule recently described as VCAM-1 that is a member of the Ig superfamily (Osborn et al 1989; Elices et al 1990) and appears identical to INCAM110 (Rice & Bevilacqua 1989). The binding sites on VLA-4 for VCAM-1 and fibronectin appear to be distinct (Elices et al 1990). VCAM-1 is induced by inflammatory mediators on endothelium with kinetics similar to ICAM-1. Previous studies on lymphocyte binding to endothelium demonstrated a second adhesion mechanism distinct from the LFA-1 : ICAM interaction (Haskard et al 1986; Dustin & Springer 1988), and this second mechanism now appears to be 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 & Springer 1987). This appears to be 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 & McIntyre 1990; Campanero et al 1990) suggests some functional redundancy with LFA-1. VLA-4 also helps mediate lymphocyte recirculation (Holzmann et al 1989) as described below.

VLA-4 may be important in metastasis. Induction of INCAM110/VCAM-1 on endothelium enables binding of melanoma cells (Rice & Bevilacqua 1989), which richly express VLA-4 (Humphries et al 1987; Wayner et al 1989). Since VLA-4 binds to both endothelium and fibronectin, it could be important in metastasis at two different steps: binding of circulating tumor cells to the endothelium and subsequent migration through the subendothelial basement membrane.

The complexity of the integrin family has recently been increased by the discovery of novel β subunits that can associate with the $\alpha 4$, $\alpha 6$, and αV subunits alternatively to the previously described $\beta 1$ and $\beta 3$ subunits (Kajiji et al 1989; Sonnenberg et al 1988a; Hemler et al 1989; Cheresh et al 1989; Freed et al 1989; Holzmann & Weissman 1989) (Table 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 (Figure 3) have been correlated with ligand binding by crosslinking to peptides containing the sequence arginine-glycine-aspartic acid (RGD), a ligand recognition motif for several but not all integrins (Table 2). On the $\beta 3$ subunit ligand peptides are crosslinked within residues 109–171 (D'Souza et al 1988). This is the most highly conserved region among the $\beta 1$, $\beta 2$, and $\beta 3$ subunits, and in LAD single amino acid substitutions in this region of $\beta 2$ prevent association with α (Wardlaw et al 1990); 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 an EF hand-like putative divalent cation binding site motif (Figure 3) and require Ca^{2+} or Mg^{2+} for function (Kishimoto et al 1989b). LFA-1 α has three such repeats and has been shown to bind 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 (Dustin & Springer 1989). 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 aspartic acid residue (D) in RGD

binds to the metal held in the alpha subunit divalent cation binding pocket, and forms a sixth coordination 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 leukocyte integrin α subunits and the VLA α2 subunit have a domain of 200 amino acids, termed the inserted or I domain, not present in other integrin α subunits. 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 (Kishimoto et al 1989b). Cysteines are notably few in the putative ligand binding regions of the α and β subunits (Figure 3), thus permitting conformational changes that regulate ligand binding.

Regulation of Ligand Binding and Intracellular Signals

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 proximal 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 α5β1 (Horwitz et al 1986). Talin redistributes with LFA-1 to sites of antigen-specific adhesion and cocaps with LFA-1 after phorbol ester stimulation (Kupfer & Singer 1989a); talin association may be a wide-spread 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 are affected by their substrates, largely through integrins (Ruoslahti & Pierschbacher 1986, 1987; Hynes 1987). Examples within the immune system include regulation of T-cell proliferation by LFA-1 (van Noesel et al 1988; Pircher et al 1986) and VLA-5 (Matsuyama et al 1989).

Integrins are novel receptors with respect to the type of inside-out signaling described 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 besides LFA-1 appear to undergo avidity regulation. On unactivated platelets, the integrin gpIIbIIIa does not bind fibrinogen, but upon activation it binds soluble fibrinogen with a kd of

29–45 nM (Plow & Ginsberg 1989). The high avidity state of gpIIbIIIa appears to be permanent rather than transient. Upon stimulation with chemoattractants, neutrophils show transient adhesion to other neutrophils, endothelial cells and to C3bi-coated cells. There is evidence that this may be caused by a transient change in avidity of Mac-1 (Wright & 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 & Springer 1989). Avidity regulation may be a unifying feature in the cell biology of integrins. Recently, regulation of avidity has been extended to integrins on lymphocytes besides LFA-1. TCR crosslinking stimulates the avidity for fibronectin and laminin of $\beta 1$ (CD29) integrins on lymphocytes including VLA-5 and VLA-6 (Shimizu et al 1990).

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 & Ginsberg 1989) in sites distinct from the ligand binding site suggests that conformational changes in the ligand binding site may be possible. The antibody to the activation determinant on LFA-1 can induce adhesion that is LFA-1-dependent as shown by inhibition with mAb to other epitopes on LFA-1 (Keizer et al 1988). Analogous results have been obtained with mAb to VLA-4 (Bednarczyk & McIntyre 1990; Campanero et al 1990). An activation epitope on LFA-1 (Keizer et al 1988) appears after stimulation of resting T lymphocytes with kinetics similar to the conversion of LFA-1 from the low to high avidity state; however, the activation epitope remains expressed after 30 min and thus, in contrast to the high avidity state, is not transient (Larson et al 1990). If the changes in avidity and epitopes reflect conformational alterations, these findings suggest at least three distinct conformational states for LFA-1.

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 $\beta 2$) 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 (M. Hibbs et al, unpublished 1990). Truncation of the chicken integrin $\beta 1$ cytoplasmic domain prevents localization at focal contacts of chimeric mouse α/chicken β integrins in mouse fibroblasts (Solowska et al 1989). This could be the result of either an effect on cytoskeletal association or affinity for ligand. Another study showed that deletion of 16 amino acids could prevent localization at focal contacts without affecting ligand binding (Hayashi et al 1990). More extensive deletion affected ligand binding, but the size of the α subunit with which the truncated β subunit associated was altered, which allows

the possibility of trivial explanations for the loss of binding, such as association with a distinct α subunit. Although phosphorylation of a tyrosine residue in the β1 subunit cytoplasmic domain has been suggested to regulate ligand binding and cytoskeleton association, mutation at this residue had no effect (Hayashi et al 1990).

Cell Migration

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 orient to target cells (Poenie et al 1987; Kupfer & Singer 1989b). 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 neighboring 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 (Weiss 1961; Abercrombie 1961). Neutrophils from patients that are deficient in the leukocyte integrins fail to orient and migrate in response to chemoattractants (Anderson & 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).

Studies on LFA-1 demonstrate that it can mediate lymphocyte migration, in contrast to adhesion molecules of the Ig superfamily found on lymphocytes. Lymphoblastoid cells adhering to substrates bearing purified ICAM-1 have a spread, bipolar morphology, whereas the same cells adhering to phosphatidyl-inositol anchored LFA-3 are rounded and non-motile (Dustin & Springer 1988). B lymphoblastoid cells crawl over planar membranes containing ICAM-1 at a speed of 10–30 μm/min. Cells do not crawl on ICAM-1 membranes in the presence of LFA-1 mAb, or on planar membranes lacking proteins. By contrast, on LFA-1 planar membranes B lymphoblastoid cells are immobile. They become firmly attached to the planar membrane through their uropod. The cell body suspended above the substrate by the uropod undergoes vigorous ruffling but no locomotion occurs (M. Dustin, O. Carpen, T. Springer, unpublished). These findings

correlate with the observation that ICAM-1 can be found preferentially distributed on the uropod of cells in suspension (Dougherty & Hogg 1987).

ADHESION RECEPTOR PHENOTYPE AND LYMPHOCYTE BEHAVIOR

Education of Lymphocytes Stably Alters Adhesion Receptor Phenotype

Lymphocytes newly emigrated from the thymus are considered "naive" and remain so until they encounter and are stimulated by specific antigen. They then become "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 naive to memory phenotype. Shortly after antigen stimulation, naive T lymphocytes of both $CD4^+$ and $CD8^+$ subsets acquire surface LFA-3 and increased levels of a cohort of surface molecules including the adhesion receptors CD2, LFA-1, and CD29 (Cerottini & MacDonald 1989; Sanders et al 1988) (Table 3). Increased expression of these surface molecules persists after the stimulated lymphocytes have reverted to the resting state and probably lasts for the life of the memory cell. The changes in surface phenotype of memory T

Table 3 Conversion of naive to memory T lymphocytes alters surface molecule phenotype[a]

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 (UCHL1)	↑29
CD45 RA (2H4)	+ → −
CD4	1.0
CD8	1.0
TCR (CD3)	1.0

[a] Modified from Sanders et al 1988.

cells may have important consequences for their localization since they occupy distinct microenvironments within lymphoid organs (Janossy et al 1989) and have different recirculation routes (Mackay et al 1990).

Naive and memory T-cell subsets differ in lymphokine secretion in some functional assays (Cerottini & MacDonald 1989; Sanders et al 1988; Morimoto et al 1985; Tedder et al 1985) 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 mAb, although they have quantities of TCR (CD3), CD8, and CD4 identical to naive T cells (Sanders et al 1989). Their increased expression of the LFA-1 and CD2 molecules should also enhance sensitivity to antigen by facilitating interactions of memory T cells with antigen-presenting cells. Although 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 & Gray 1986), this does not occur for TCR variable regions (Davis & Bjorkman 1988). The above-mentioned alterations, however, 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 there 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 newborns in that, when harvested from specific lymph nodes, they show a two-fold preference for recirculation to lymph nodes of the type from which they came (Butcher 1986; Yednock & Rosen 1989). These findings have been taken to suggest 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 overlayed on sections of lymph nodes bind to these specialized venules; recirculation or homing receptors on lymphocytes have been defined by mAb that block binding to the high endothelial cells of specific types of lymph nodes (Butcher 1986; Yednock & Rosen 1989; Stoolman 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; Yednock & Rosen 1989; Stool-

man 1989). Binding and immigration into lymph nodes may be a cooperative process involving multiple receptors on the lymphocyte and counter-receptors on the endothelium, analogous to antigen-specific interactions (Figure 1*a,b*) since CD44, LFA-1, VLA-4 and Mel-14/LAM-1 on the lymphocyte have all been implicated in binding to high endothelial venules. 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; Yednock & Rosen 1989; Stoolman 1989; Tedder et al 1989). The function of these molecules in adhesion hardly seems limited to lymphocyte recirculation, as discussed above for VLA-4. In view of the idea that lymphocyte education alters homing, it is encouraging that the putative homing receptors CD44 and VLA-4 (CD29) show increased expression on the memory T-lymphocyte subset (Table 3) and that Mel-14/LAM-1 (Leu8/TQ1) is preferentially expressed on a distinctive but overlapping lymphocyte subset (Tedder et al 1990). Differences of several-fold in surface density of these receptors would appear adequate to give rise to the two-fold selectivity seen in recirculation to different types of lymph nodes, although this remains to be tested.

In addition to emigrating through specialized endothelium in lymphoid tissues, lymphocytes also emigrate through nonspecialized endothelium into nonlymphoid tissues. Recently it was found that memory T lymphocytes almost exclusively emigrate from blood through tissue endothelium, whereas naive T lymphocytes exclusively emigrate through lymph node endothelium (Mackay et al 1990). This correlates with the increased expression of adhesion receptors on memory lymphocytes. Previous findings that lymphocyte priming allows recirculation to specialized lymphoid organs may thus result from selective emigration through specialized endothelium in tissues rather than in lymph nodes. The endothelia of skin and gut may bear specialized receptors that allow selective recirculation through these tissues, from whence lymphocytes arrive in regional lymph nodes through the afferent lymph.

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; Lasky et al 1989; Siegelman et al 1989; Johnston et al 1989; Stoolman 1989; Tedder et al 1989) termed selectins (Figure 4) (M. Bevilacqua, personal communication). All have an N-terminal domain of 117–120 amino acids,

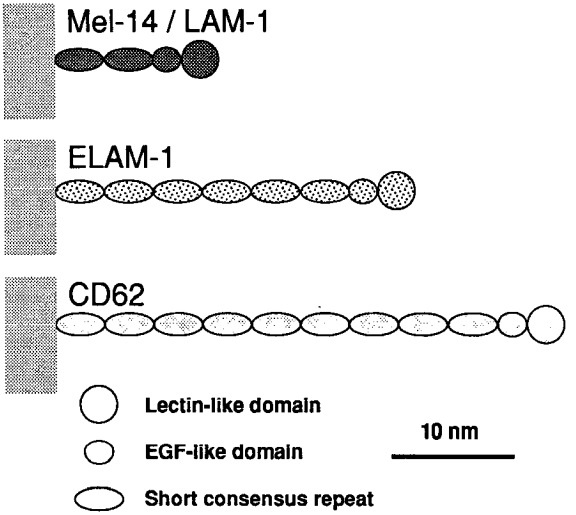


Figure 4 Selectins. Scale models of selectins (Bevilacqua et al 1989; Lasky et al 1989; Siegelman et al 1989; Johnston et al 1989) are proposed. The short consensus repeats (SCR) extend 4.1 nm each, based on a length of 33 nm (Dahlback et al 1983) for 8 SCR (Chung et al 1988), EGF repeats extend about 2.3 nm (Taylor et al 1989), and the lectin-like N-terminal domain is modeled as a globular sphere (Drickamer 1988).

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 that is found in many proteins involved in regulating complement activation (Figure 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 (Stoolman 1989; Yednock & Rosen 1989; Lasky et al 1989). 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 (Figure 4).

Although the importance of the leukocyte integrins for neutrophil binding to endothelium and emigration has been amply demonstrated in vitro (Kishimoto et al 1989b; Lo et al 1989; Smith et al 1988, 1989) and by their

congenital deficiency in vivo (Anderson & Springer 1987), three different selectins are involved in additional mechanisms for regulating neutrophil binding to endothelium. Cooperation 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 of neutrophils to the endothelium, the leukocyte integrins are still required for the subsequent event of transendothelial migration (Smith et al 1989; Lawrence et al 1989). The selectin Mel-14 functions not only as a lymphocyte recirculation receptor, but also contributes to neutrophil emigration at inflammatory sites (Jutila et al 1989; Butcher 1986). With similar kinetics, but in contrast to leukocyte integrins, which are increased on the cell surface by mobilization from granule compartments within minutes after stimulation of neutrophils with chemoattractants (Kishimoto et al 1989b), Mel-14 is released from the cell surface by proteases (Kishimoto et al 1989a). It may function in an early step of neutrophil adhesion to the endothelium and is shed before transendothelial migration (Kishimoto et al 1989a). The endothelial leukocyte adhesion molecule (ELAM)-1 is a selectin that is transiently expressed on endothelial cells 2–8 hr after stimulation with IL-1 and other inflammatory agents and mediates a neutrophil adhesion pathway distinct from that mediated by ICAMs and leukocyte integrins (Bevilacqua et al 1989; Luscinskas et al 1989). 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 (Johnston et al 1989; Larsen et al 1989). Thus selectins function in a wide range of cell interactions in the immune system and in clotting, and they are expressed both on leukocytes and endothelial cells.

VIRUS RECEPTORS

Several cell adhesion receptors are subverted as virus receptors (White & Littman 1989). 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. A molecule with an Ig domain structure similar to ICAM-1 is a receptor for poliovirus; both rhino and polio viruses are small, protein-encapsulated RNA virus (picornaviruses). It should be noted that receptor expression is only one of many factors affecting the pattern of

virus infection in vivo; rhinovirus infection, for example, is limited to the nasal epithelium, whereas ICAM-1 expression is broader.

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. HIV infects and kills or renders anergic $CD4^+$ T helper cells, and down-regulates CD4 expression (Sattentau & Weiss 1988). Binding of HIV, or its shed surface glycoprotein gp120, may transduce signals through CD4 that interfere with normal responsiveness (Linette et al 1988). HIV can spread by fusion of infected cells with uninfected cells to form syncytia. This requires CD4 and gp120, but also LFA-1 (Hildreth & Orentas 1989). 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 either of these rationales, 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 regions between amino acid 37 and 131 of CD4, spanning the first Ig-like domain and part of the second domain (Landau et al 1988; Peterson & Seed 1988; Clayton et al 1989). The same overall region of CD4 binds to MHC class II (Clayton et al 1989). LFA-1 and rhinoviruses bind to overlapping but distinct regions of the N-terminal Ig-like domain of ICAM-1 (Staunton et al 1990). Truncation of ICAM-1 by deleting one, two, or three of the more C-terminal domains of ICAM-1 decreases the binding of rhinovirus and, to a lesser extent, LFA-1 by decreasing the accessibility of the N-terminal Ig domain. 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 (Rossmann 1989), consisting of paired Ig domains, but is of the right dimensions to fit unpaired Ig domains as exist in ICAM-1 (Staunton et al 1990).

CLOSE ENCOUNTERS AT THE MEMBRANE

Scale Models of Cell Adhesion Receptors

We are just at the beginning stages in our understanding of adhesion on a molecular scale (Figure 5). The size and shape of a number of the immune

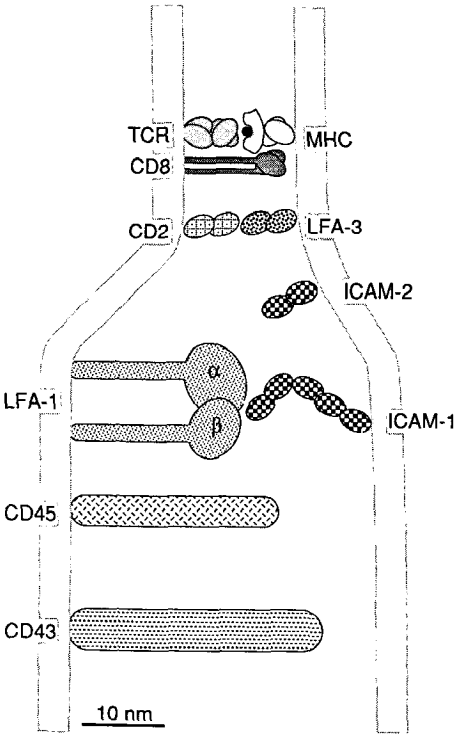
system adhesion molecules are known by electron microscopy or X-ray crystallography, and others may be modeled from the structure of homologous molecules or constituent domains (Figure 5 legend). An interesting feature of adhesion molecule structure is that, by contrast to immunoglobulins and MHC molecules, which have paired Ig domains, the Ig domains of ICAM-1 (Staunton et al 1990) and NCAM (Becker et al 1989) are unpaired. Ig domains known by X-ray crystallography are ellipsoids with a dimension of 4 nm parallel to the β strands and 2.5 nm in the two perpendicular dimensions (Alzari et al 1988; Bjorkman et al 1987). Electron

Figure 5 Scale models of interacting adhesion receptors. Molecules are shown to scale, based on the following information: MHC class I molecule, X-ray crystallography (Bjorkman et al 1987); TCR, approximation as a Fab fragment (Davis & Bjorkman 1988); CD3 chains, some of which have one Ig domain, are not shown; ICAM-1, electron microscopy (Staunton et al 1990); ICAM-2, CD2, and LFA-3, approximation to two unpaired Ig domains as in ICAM-1; CD8, a disulfide-linked α-α or α-β dimer of one Ig domain, the length of the connecting or hinge-like peptide (Parnes 1989) of 40 or 65 amino acid for α or β, respectively, is arbitrary and is consistent with a spacing of 1.5 to 3.3 Å/residue (Schulz & Schirmer 1979). Note the distance that the connecting peptide is shown extending in CD8 is similar to what would be predicted for three of the four Ig domains in CD4, allowing the N-terminal Ig domain of CD4 to occupy a position similar to that shown for the Ig domain of CD8. LFA-1, electron microscopy of two homologous integrins, gpIIbIIIa (Carrell et al 1985) and the fibronectin receptor (Nermut et al 1988); CD43, the 235 residue extracellular domain (Kileen et al 1987; Shelley et al 1989) with one in five O-linked amino acids (Williams & Barclay 1986) is modeled as 235/420 the length of glycocalicin (CD42b), which was 420 amino acids with a similar O-linked carbohydrate content (Clemetson 1983; Lopez et al 1987); CD45, electron micrographs (Woollett et al 1985). 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. Connecting regions, however, 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 microscopic studies 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 (Davis & Bjorkman 1988). The binding site in MHC class I for CD8 is in the membrane proximal α3 domain (Potter et al 1989; Salter et al 1989); the binding site for MHC is assumed to be in the CD8 Ig domain analogously to the MHC binding site in CD4 (Clayton et al 1989). ICAM-1 binds to LFA-1 through its N-terminal Ig domain (Staunton et al 1990); the binding site in ICAM-2 is modeled in its homologous N-terminal Ig domain. The ICAM binding site in LFA-1 is assumed to be in the membrane-distal globular domain of integrins as predicted (Nermut et al 1988); the exact location within this domain will not affect overall conclusions. The binding site for LFA-3 in CD2 is in amino acids 37–52 and 82–94 of the N-terminal Ig domain, aligning with complementarity-determining residues of a K V region (Peterson & Seed 1987), and the binding site in LFA-3 is assumed to be in a homologous position.

micrographs of ICAM-1 show that it is a bent rod about 19 nm long. This is only compatible with a model in which its five Ig domains are unpaired, and are arranged end to end at a slight angle to the β strands (Figure 5).

The binding sites for several of the adhesion receptors and their counter-receptors are known (Figure 5 legend) and allow the prediction of how close together two cell membranes would have to come, or how close a cell membrane would have to come to the extracellular matrix, to allow interaction. A model of the distances over which adhesion receptors would mediate cellular interactions can then be constructed (Figure 5). This model can be compared to actual measurements of distances made in micrographic studies (Biberfeld & Johansson 1975; Verschueren 1985). Furthermore, the sizes of two major components of the leukocyte glycocalyx that bear the great bulk of cell surface sialic acid (Williams & Barclay 1986), CD43 and CD45, are included in Figure 5 as a reminder of the scale of the glycocalyx, which will oppose cell-cell adhesion because of negative charge repulsion and the loss of entropy involved in com-



pressing or interdigitating the glycocalyces of two contacting cells (Bell et al 1984).

The model (Figure 5) 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 approximately 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 (Biberfeld & Johansson 1975); however, antigen-specific T-cell interactions have not yet been studied with similarly accurate techniques.

By contrast to the TCR : MHC and CD2 : LFA-3 interactions, the interactions of LFA-1 with ICAM-2 and ICAM-1 are predicted to occur within an intermembrane distance approximately equal to or less than 27 or 36 nm, respectively (Figure 5). 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 : MHC and CD2 : LFA-3 interaction is predicted to require extensive interdigitation of the membrane glycocalyces of the two adhering cells, hinder the lateral mobility of surface glycoproteins, and force major glycocalyx components such as CD43 and CD45 either to be excluded from the zone or to assume a less extended conformation or a less perpendicular orientation to the membrane. Since the cytoplasmic domain of CD45 is a tyrosine phosphatase that acts on the tyrosine kinase associated with CD4 and CD8 (Ostergaard et al 1989), such considerations could affect signaling.

Implications for Cell Migration and Orientation

The distinct membrane distances predicted for the interactions of LFA-1 : ICAM compared to TCR : MHC and CD2 : LFA-3 may have important implications for the dynamics of cell migration. 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 to 15 nm, but instead have close contacts with a cell to substrate distance of $\sim$ 30 nm (Verschueren 1985), 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 (Lee et al 1990) and membrane proteins (Sheetz et al

1989) 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. How can integrins participate in cell migration, and could similar lateral lymphocyte membrane movements occur during orientation of the microtubule organizing center and Golgi apparatus of T lymphocytes toward target cells (Kupfer & Singer 1989a)? 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 to cell or cell to substrate distance that allows nearby 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.

The ideas that high integrin affinity at the leading edge of the cell and low affinity at the trailing edge can contribute to cell migration (Dustin & Springer 1989), and that integrins serve as transiently fixed points that connect the cytoskeleton to the extracellular matrix to generate the tension required to force the leading edge of the cell forward, can be unified into a single model of cell migration. This model requires that the high avidity state of the integrin and its attachment to the cytoskeleton are coordinated. The simplest idea would be that association with the cytoskeleton directly induces the high avidity state, an idea that receives some support from, but is far from proven by, observations described above that deletion of integrin β subunit cytoplasmic domains can eliminate both binding to extracellular ligands and localization at focal contacts.

CONCLUDING PERSPECTIVE

The dynamic role of lymphocyte adhesion receptors in regulating lymphocyte antigen-specific interactions, localization in lymphoid and nonlymphoid organs, and in bidirectionally transmitting information that 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 signaling pathways and interactions with gene expression. Further studies on three-dimensional structure and interactions with signaling 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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