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ROLE OF LYMPHOCYTE ADHESION RECEPTORS IN TRANSIENT INTERACTIONS AND CELL LOCOMOTION

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Abstract

Lymphocytes adhere to other cells and extracellular matrix in the process of immunological recognition and lymphocyte recirculation. This review focuses on regulation of lymphocyte adhesion and the use of adhesion mechanisms by lymphocytes to obtain information about their immediate environment. The CD2 and LFA-1 adhesion receptors appear to have distinct roles in the regulation of adhesion and modulation of T lymphocyte activation. Adhesion mediated by interaction of CD2 with LFA-3 is dramatically altered by surface charge and adhesion receptor density in such a way that this pathway is latent in resting T lymphocytes but becomes active over a period of hours following T-cell activation. CD2 ligation can mediate or enhance T-cell activation, suggesting that signals from CD2/LFA-3 adhesive interactions are integrated with signals from the T-

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cell antigen receptor during immunological recognition. A model for the role of LFA-3 lateral diffusion in adhesion is presented, based on the lateral diffusion of different LFA-3 forms in glass supported planar membranes. Interaction of LFA-1 with ICAMs is also regulated by cell activation but in a different way than in interaction of CD2 with LFA-3. LFA-1 avidity for ICAMs is transiently increased by T-cell activation over a period of minutes. Cycles of avidity change are also observed for other T lymphocyte integrins which bind to extracellular matrix components. We propose that integrin avidity cycles may have an important role in the interconnected phenomena of locomotion, initial cell-cell adhesion, and cell-cell de-adhesion. Recent observations on recirculation of T lymphocyte sub-populations are discussed in the context of general lessons learned from study of the CD2/LFA-3 and LFA-1/ICAM adhesion mechanisms.

INTRODUCTION

The interaction of lymphocytes with other cell types is critical for immune function (1–4) and provides excellent opportunities to study the cell biology of dynamic cell-cell and cell-extracellular matrix interactions. The most striking characteristic of lymphocyte adhesion is its regulation. Lymphocytes rapidly interconvert between a nonadherent state in circulation and an adherent and highly motile state in lymphoid and other tissues. This cycle is repeated many times over the life span of a lymphocyte.

This review focuses on the rich topic of lymphocyte adhesion in the context of immune responses and lymphocyte migration. Two adhesion mechanisms that are widely utilized in lymphocyte interactions with other cells are emphasized. Several themes generally applicable to other types of dynamic cell-cell and cell-extracellular matrix interactions are addressed. Our thesis is that the mechanisms of lymphocyte adhesion have a dual function—to provide a foothold for cell interactions and migration, and to transmit information across the cell membrane. Sensation through adhesion receptors operates in two directions. Transmembrane signalling from the extracellular to the intracellular environment of the cell shares features with classical transmembrane signalling by hormone receptors. Conversely, the ability to transduce signals from the intracellular to the extracellular environment allows cells to rapidly regulate adhesion by "inside-out" signalling mechanisms. Anchorage and two-way chemoreception appear to be critical for explaining the role of adhesion mechanisms in the immune response, and more broadly, in morphogenesis, inflammation, and wound healing. In our discussion below, we refer to interacting components of lymphocyte adhesion mechanisms as adhesion receptors (5, 6).

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DEFINITION OF LYMPHOCYTE ADHESION MECHANISMS

Dissection of complex, multistep T lymphocyte function with inhibitory monoclonal antibodies (mAb) yielded a surprisingly rich harvest of adhesion receptors. Two major types of T lymphocyte responses are routinely assayed in vitro to test for inhibitory mAb from panels of anti-T lymphocyte mAb. The most accessible system is T lymphocyte–mediated lysis of cells bearing foreign antigens that can be assayed by following release of cytoplasmic labels from dying target cells (Figure 1*A*). Helper T lymphocytes recognize foreign antigens expressed on a restricted range of antigen presenting cells, but assays for helper T lymphocyte function are of much longer duration than assays for cytotoxic T lymphocyte (CTL) function (Figure 1*B*). Another advantage of T lymphocyte–mediated lysis is that the killing process can be resolved into a number of discrete steps: Mg^{2+} dependent adhesion/recognition, Ca^{2+} and CTL-dependent "programming for lysis," and CTL-independent target cell death (7). Knowledge of the stage inhibited by an mAb facilitates rapid definition of the recognized molecule's function.

Strikingly, many of the mAb selected to inhibit function of CTL identify adhesion receptors. These include LFA-1, CD2 (then LFA-2), and LFA-3 (Table 1) (8). LFA-1 and CD2 are used by the CTL; and LFA-3, but not LFA-1, is used by the target cell (Figure 1*A*) (8–12). In contrast, functional interaction of helper T lymphocytes and antigen presenting cells requires use of LFA-1 by both cell types (Figure 2*B*) (13, 14). Parallel adhesion mechanisms may be resolved from each other by comparing partial inhibition of adhesion by each mAb added singly with the level of inhibition obtained with distinct pair-wise combinations of the same mAb (15). Inhibition with pairs of mAb to LFA-1 and CD2, or to LFA-1 and LFA-3, is additive, while inhibition by combinations of mAb to CD2 and LFA-3 is not greater than inhibition by either of these mAb alone. This suggests that CD2 and LFA-3 are components of the same adhesion mechanism, while LFA-1 is a component of a distinct adhesion mechanism (Figure 1*A*).

Inhibition of T lymphocyte adhesion to target cells by mAb suggests an interaction between CD2 and LFA-3, but much more satisfactory evidence for this interaction is provided by studies with a model system for the CD2/LFA-3 interaction and experiments with immunoaffinity purified CD2 and LFA-3. Sheep erythrocytes adhere avidly to human T lymphocytes and this assay has been used for years as a clinical test for T lymphocytes (Figure 1*C*) (16). Rosetting with sheep or human erythrocytes is blocked by mAb binding to CD2 on the T lymphocyte (17–21), and mAb

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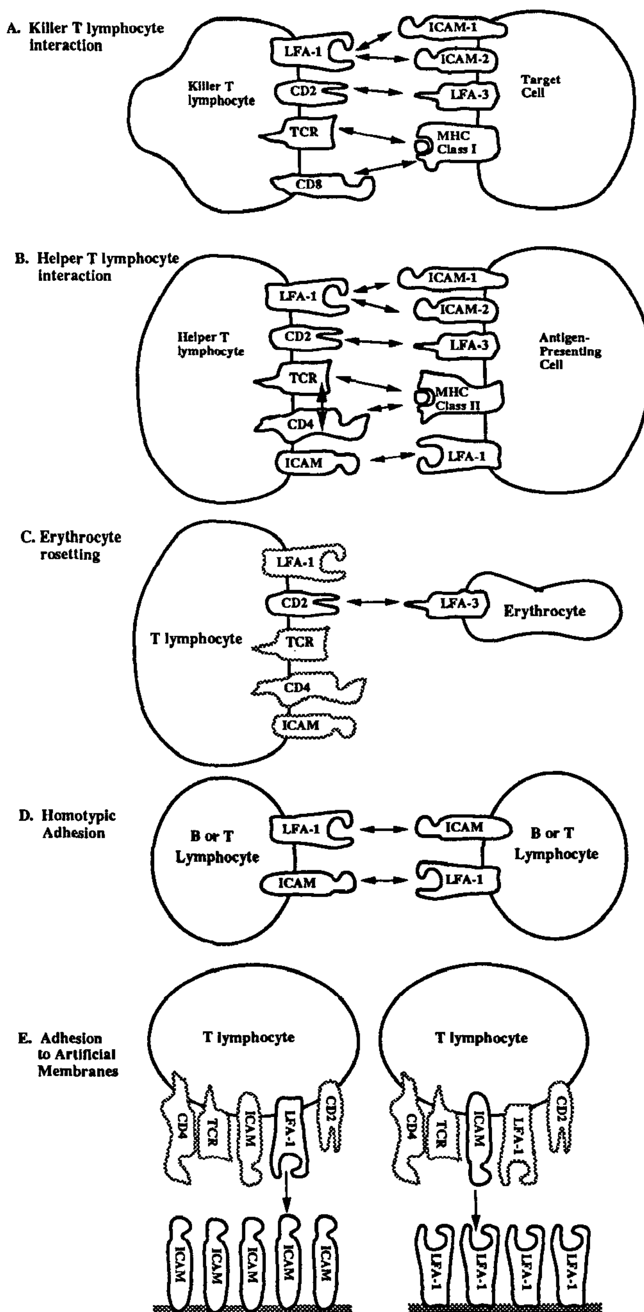

Figure 1 Receptors in lymphocyte adhesion and model systems.

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Table 1 Guide to lymphocyte adhesion molecules

			Adhesion receptors/counter-receptors		
Name[a]	Synonyms	Size[b] (kd)	Name	Synonyms	Size (kd)
LFA-1	Integrin α_L/β_2 CD11a/CD18	α, 180 β, 95	ICAM-1 ICAM-2	CD54	74–114 70
CD2	E rosette receptor, T11, leu 5, LFA-2	50	LFA-3	CD58	55–70
CD4	T4, leu 3	55	MHC class II		α, 34 β, 29
CD8	T8, leu 2	α-α & α-β 30–38	MHC class I		α, 44 β, 12
CD44	ECMR-III, Pgp-1, Hermes	90 & 200	(?) (collagen)		
VLA-4	Integrin α_4/β_1 CD49d/CD29 LPAM-1	α, 150 β, 110	VCAM-1 Fibronectin CS-1 region	INCAM-110	110
LPAM-2	Integrin α_4/β_P CD49d/CD-	α, 150 β, 110	(?)		
(?)			Mucosal addressin (MECA-367)		58–66
Mel-14	LAM-1, leu 8 TQ1	90	Phosphorylated oligosaccharides		(?)

[a] Name used in this review.
[b] Size in kilodaltons estimated by SDS-PAGE under reducing conditions.

binding to LFA-3 on erythrocytes also blocks rosetting (20–22). An mAb to sheep erythrocytes that completely abrogates rosetting identifies a homologue of LFA-3 on sheep erythrocytes (22). Both sheep and human LFA-3 have been purified and shown to block binding of CD2 mAb to human T lymphocytes, suggesting that they can act as ligands for human CD2 (22–25). In parallel, CD2 interaction with LFA-3 was implicated in the developmentally important interaction of thymocytes with thymic epithelial cells (26, 27).

Saturable binding of purified CD2 to cells expressing LFA-3 demonstrates directly that CD2 can interact autonomously with cells, and inhibition of this binding by mAb to LFA-3 strongly suggests that LFA-3 is the counter-receptor (20, 24, 28). Purified, intact CD2 binds to LFA-3 expressing cells with a K_d of $1–5 \times 10^{-8}$ M. Experiments with purified LFA-3 reciprocal to those with purified CD2 confirm that LFA-3 is the counter-receptor for CD2 (23). In addition, $CD2^+$ cells adhere to the artificial membranes containing purified LFA-3 (23, 29). Finally, binding

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between purified CD2 and purified LFA-3, both in liposomes, confirms the results of the reciprocal cell binding studies (30).

In contrast to CD2 which has only one known counter-receptor, LFA-1 has at least two counter-receptors identified by specifically tailored strategies. Phorbol ester–stimulated aggregation of lymphocytes is a simply assayed, robust response that is inhibited completely by mAb to LFA-1 (Figure 1*D*) (31, 32). Selection for mAb which block phorbol ester–stimulated aggregation, excluding mAb to LFA-1, resulted in the identification of mAb to intercellular adhesion molecule-1 (ICAM-1) (33). Lysis of some targets by CTLs is inhibited by such mAb; in these instances ICAM-1 is required on the target cell (34). Interaction of LFA-1 and ICAM-1 is confirmed by adhesion of LFA-1 expressing cells to immunoaffinity purified ICAM-1 on solid substrata (Figure 1*E*). This adhesion is inhibited by mAb binding to LFA-1 or ICAM-1 (35, 36). However, failure of mAb to ICAM-1 to inhibit some interactions that are inhibited by mAb to LFA-1 suggested the presence of other LFA-1 counter-receptors (33, 37, 38), and a cDNA encoding a second LFA-1 ligand, ICAM-2, was isolated by expression cloning in COS cells (39).

The distribution of CD2, LFA-3, LFA-1, and ICAMs suggests that CD2 and LFA-1 are specialized for use by leukocytes, while LFA-3 and ICAMs are distributed to facilitate T lymphocyte interactions with any cell in the body under appropriate conditions. CD2 is expressed only on T lymphocytes and their progenitors (17). LFA-1 is expressed only on leukocytes (9). In contrast, LFA-3 is expressed on virtually all cells. Expression of ICAM-1 is restricted in noninflamed tissues but is found on diverse cell types in response to inflammatory mediators or to activation (40). ICAM-1 expression is also closely coordinated with the progression of immune responses. ICAM-1 and ICAM-2 are expressed on a partially overlapping subset of cells based on mRNA content (39), and this has been confirmed with mAb to ICAM-1 and ICAM-2 (A. DeFougerolles and T. A. Springer, unpublished).

Adhesions mediated by LFA-1 interaction with ICAMs and by CD2 interaction with LFA-3 have distinct physical requirements. While CD2 interaction with LFA-3 is independent of divalent cations interaction of LFA-1 with ICAMs requires Mg^{2+} ions (15, 35). Once contact between cells is established, the CD2/LFA-3 mechanism appears to be significantly more efficient in mediating adhesion at 4°C than at 37°C, suggesting that cell motility or other active processes may work against stable adhesion mediated by this mechanism (41) (P.-Y. Chan, M. L. Dustin, and T. A. Springer, unpublished). However, establishment of CD2/LFA-3 mediated adhesion between CTLs and target cells at 4°C requires the cells to be cosedimented at 10–50 × g (15); when the CTLs and target cells are allowed to cosediment at 1 × g, CD2/LFA-3 dependent adhesion does

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not occur at 4°C (42). In contrast, LFA-1/ICAM–dependent adhesion is strongest at 37°C and is not observed at 0°C, even when close contact between cells is established by co-centrifugation (15, 35). Similarly, adhesion of cells to purified ICAM-1 in planar membranes is strongest at 37°C and decreases at lower temperatures until no adhesion is observed at 4°C (35). In contrast, ICAM-1$^+$ lymphocytes show significant adhesion to purified LFA-1 in planar membranes at 4°C, as well as 37°C (5). Thus, there is evidence for sidedness of temperature requirements for the LFA-1/ICAM mechanism.

ADHESION RECEPTOR FAMILIES

All of the adhesion receptors we discuss are related to other interactive molecules by sequence homology and are thus considered to be members of protein "families." Similarities between family members include not only structural features, but functional characteristics and an understanding of these relationships form the basis for our subsequent discussion of adhesion receptors.

CD2, LFA-3, and ICAMs in the Immunoglobulin Superfamily

The primary structures of CD2, LFA-3, and both ICAMs resemble those of the immunoglobulin superfamily, a functionally diverse family of molecules many of which are expressed on the cell surface. Members of the Ig-superfamily share variable numbers of 90–100 amino acid domains with similar structures: a sandwich of two antiparallel β-pleated sheets usually held together by a disulfide bond (43, 44). Several other adhesion receptors are members of the Ig-superfamily including CD4, CD8, the T cell antigen receptor (TCR), VCAM-1 in the immune system (see below), and a number of adhesion receptors in the nervous system (45). Antibodies are the best characterized members of the Ig-superfamily and provide prototypes for interactions of other Ig-like molecules. The hypervariable regions of antibodies are situated in the loops connecting the β-strands at one end of a domain; and the combining site is formed by six of these loops from two domains (46).

LFA-1 and the Integrin Family

Integrins are a family of cell surface heterodimers which participate in diverse cell-cell and cell-extracellular matrix interactions (47, 48). LFA-1 is a member of this family. The name *integrin* was based on the concept that these proteins formed an integral membrane protein linkage between the extracellular matrix and the cytoskeleton (47). Integrins show strong conservation of a basic structural plan. Large α subunits contain three or

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four divalent cation binding repeats (49–53). This α subunit is non-covalently associated with a smaller β subunit containing a large proportion of cysteine residues in a conserved arrangement (48, 54, 55). The integrin family is loosely organized into three subfamilies based on three distinct β subunits: $\beta1$ (CD29, VLA proteins), $\beta2$ (CD18, leukocyte integrins), and $\beta3$ (CD61, cytoadhesins), each of which associates predominantly with its own complement of α subunits (Table 2) (56). Exceptions to this organization are increasingly recognized as additional β subunits are described, and novel combinations of known subunits are observed (Table 2). LFA-1 ($\alpha L\beta2$) is a member of the $\beta2$ subfamily along with the leukocyte adhesion receptors Mac-1 ($\alpha M\beta2$) and p150,95 ($\alpha X\beta2$).

LEUKOCYTE ADHESION DEFICIENCY (LAD) LAD is a recessive inherited disorder in which defects in the $\beta2$ genes result in loss of leukocyte integrin expression and profound defects in adhesion (57). Defects in neutrophil adhesion and extravasation predispose LAD patients to life threatening bacterial infections and poor wound healing. Lymphocyte function is relatively intact in vivo, although in vitro lymphocyte functions are impaired (58). This relative sparing of lymphocyte function in LAD may be due to the expression of $\beta1$ integrins that can adequately perform migratory and cell interaction functions normally carried out by LFA-1. In contrast, neutrophils do not appear to express $\beta1$ integrins in significant amounts (59). T lymphocytes in LAD patients, alternatively, may be selected to function in the absence of LFA-1. Mature T lymphocytes are selected in the thymus for recognition of self-MHC plus foreign antigens from large numbers of thymocyte clones expressing different TCR (60). Thymic epithelial cells and other components of the thymic stroma express ICAM-1, and interaction between normal thymocytes and thymic epithelial cells is mediated in part by LFA-1/ICAM-1 interaction (38, 61, 62). Absence of LFA-1 may lead to selection of thymocytes possessing TCR with higher avidity for self-MHC.

ADDITIONAL T LYMPHOCYTE INTEGRINS The extracellular matrix components collagen, fibronectin, laminin, vitronectin, and others are deposited and assembled into basement membranes and into three-dimensional fibrillar networks forming roads, boundaries and signposts in tissues that are probably used to guide lymphocyte migration. T lymphocytes express a number of integrins besides LFA-1, most of which are known to interact with different extracellular matrix components. Resting T lymphocytes express VLA-4 ($\alpha4\beta1$), and T lymphocyte subpopulations express high levels of VLA-5 ($\alpha5\beta1$) and VLA-6 ($\alpha6\beta1$) (63) (Table 2). The ability of VLA-4 to bind a recently identified cell surface counter-receptor, VCAM-1 (64), in addition to an alternative cell-binding domain of fibronectin (65,

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Table 2 The integrin family[a]

Subunit	Name	CD[b]	Ligand/counter-receptor[c]	I-domain[d]	RGD[e]	Cleaved α
$\alpha 1\beta 1$	VLA-1	—/29	LM, CO	yes[f]	no	no
$\alpha 2\beta 1$	VLA-2, gpIaIIa, ECMRII	49b/29	LM, CO	yes	no	no
$\alpha 3\beta 1$	VLA-3, ECMRI	—/29	FN, LM, CO	no	yes	yes
$\alpha 4\beta 1$	VLA-4, LPAM-1	49d/29	FN, VCAM-1	no	no	yes[g]
$\alpha 5\beta 1$	VLA-5, FNR, gpIcIIa, ECMRVI	—/29	FN	no	yes	yes
$\alpha 6\beta 1$	VLA-6, gpIcIIa	49f/29	LM		no	yes
$\alpha 4\beta P$	LPAM-2	49d/—	?	no		yes[g]
$\alpha 6\beta 4$	$\alpha E\beta 4$	49f/—	?		no	yes
$\alpha V\beta 1$		51/29	FN	no	yes	yes
$\alpha L\beta 2$	LFA-1	11a/18	ICAM-1, ICAM-2, others?	yes	no	no
$\alpha M\beta 2$	Mac-1	11b/18	ICAM-1, C3bi, FX, FB, others	yes	?	no
$\alpha X\beta 2$	p150,95	11c/18	C3bi?, others	yes	?	no
$\alpha IIb\beta 3$	gpIIbIIIa	41/61	FB, FN, vWF, FB	no	yes	yes
$\alpha V\beta 3$	VNR	51/61	VN, FB, vWF, TSP	no	yes	yes
$\alpha V\beta 5$	$\alpha V\beta S$	51/—	VN, FN	no	yes	yes

[a] This table is modified from a table in a recent review by Springer (6).
[b] International cluster of differentiation nomenclature, e.g. 11a/18 means α = CD11a, β = CD18.
[c] LM, laminin; CO, collagen; FN, fibronectin; FX, factor X; VN, vitronectin; vWF, von Willebrand factor; TS, thrombospondin.
[d] Contains immunoglobulin homology sequence(s).
[e] Contains arginine-glycine-aspartic acid sequence.
[f] Rat $\alpha 1$ homologue has an I-domain (M. J. Ignatious, T. H. Large, M. Houde, A. Barton, F. Esch, S. Carbonetto, L. F. Reichardt, in preparation).
[g] The $\alpha 4$ subunit cleavage occurs in a different location than that in other cleaved integrin α subunits.

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66), may contribute to the relatively intact lymphocyte migration in LAD patients. Integrin function appears to be required for invasion of the thymus by thymocyte progenitors during early development (67). A novel lymphocyte β subunit has been found to associate with $\alpha4$ to form LPAM-2 ($\alpha4\beta$P), a receptor for Peyer's patch endothelial cells (68). In addition, in vitro activation of lymphocytes over a period of weeks results in expression of the very late activation antigens $\alpha1\beta1$, $\alpha2\beta1$, and a molecule similar or identical to $\alpha V\beta3$ (56, 69).

Selectin and Link Families in T Lymphocyte Migration

Lymphocyte migration requires members of at least two additional adhesion receptor families which mediate adhesion by binding to uncharacterized counter-receptors on endothelial cells (Table 1). Selectins are a family of surface molecules possessing three different structural motifs. Selectins possess a single N-terminal (extracellular) lectin motif followed by a single epidermal growth factor repeat and a varying number of short consensus repeat homology units as found in complement-binding proteins (70–75). Mel 14 is a selectin on lymphocytes that has a critical role in interaction of lymphocytes with peripheral lymph node endothelial cells (71, 73–76). Other known members of this family are involved in the interaction of leukocytes and platelets with each other and with endothelial cells (72, 77). Therefore, selectins may be specialized for mediating adhesion in the presence of shear forces associated with blood flow. Several selectins appear to be regulated by selective loss from the cell surface. In a dramatic reaction, granulocytes lose Mel 14 surface expression within minutes after activation (78); Mel 14 is also lost from the surface of activated T lymphocytes (74). Endothelial leukocyte adhesion molecule-1 (ELAM-1) and GMP-140 are transiently expressed on the surface of monokine or thrombin-activated endothelial cells, respectively (70, 79). Transience in these cases may involve a proteolytic susceptibility built into selectins. Consistent with the presence in selectins of a domain with homology to lectins, there is evidence that Mel 14 binds to phosphorylated oligosaccharides (80).

"Link" family members can function as proteoglycan core proteins although proteoglycan is not always added (81–83). The N-terminal region of CD44 is similar to a repeat within cartilage link protein. Link forms a complex with hyaluronic acid and proteoglycan monomers. Thus CD44 may mediate cell interactions by binding either to protein or glycan components. A subpopulation of CD44 molecules bears a chondroitin sulfate glycan moiety, which could also have ligand binding specificity (84). CD44 is discussed here both as a potential regulator of the interaction between CD2 and LFA-3, and as an adhesion receptor for lymph node endothelial

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cells. CD44-transfected 3T3 cells aggregate by a mechanism that appears to involve CD44 interaction with an unknown counter-receptor (82).

ANTIGEN RECOGNITION, ANTIGEN-DEPENDENT ADHESION, AND KINETICS OF INTERACTIONS

Understanding the nature of T lymphocyte interactions with other cells during immune responses is important for understanding the context for regulation of lymphocyte adhesion. A brief description of these interactions is presented here.

The T cell antigen receptor (TCR) carries out recognition of foreign antigens bound to molecules of the major histocompatibility complex (MHC) on other cells (85, 86) (Figures 1*A* and *B*). In general CTLs recognize foreign antigens associated with MHC class-I molecules, while helper T lymphocytes recognize foreign antigens associated with MHC class-II molecules (Figures 1*A*,*B*). Each type of TCR recognizes a single foreign antigen when it is associated with only one of several MHC class-I or -II alleles expressed in each individual. Foreign antigens bind to a deep cleft in MHC class-I molecules formed by two α helices with a β sheet floor (87). Since T lymphocytes can only recognize foreign antigen displayed on the surface of other cells, antigen recognition requires adhesion. Inhibition of interactions involving TCR by mAb to adhesion receptors suggests that antigen recognition itself does not mediate adhesion. However, TCR is an efficient signal transducing machine. Ligation of TCR leads to increases in phosphatidylinositol lipid turnover, Ca^{2+} mobilization, and protein phosphorylation (88). These signals can initiate long-term (hours to days) differentiation of nondividing resting T lymphocytes to proliferating T lymphocyte blasts, as well as the rapid (seconds to minutes) release of cytokines and mediators required for lytic and helper functions.

The function of two other adhesion mechanisms appears to be closely linked to antigen recognition. MAbs to CD8 on CTLs or CD4 on helper T lymphocytes inhibit responses or dramatically decrease the efficiency of antigen recognition (89). CD8 on CTLs may mediate adhesion by interaction with MHC class-I molecules on target cells (90) (Figure 1*A*). Insights into the biological role of CD8/MHC class-I molecule interaction are provided by CTL recognition of target cells in which the MHC class-I allele recognized by TCR bears a mutation that blocks binding of CD8 (91, 92). No contribution of CD8 to recognition of these mutant alleles is observed despite the fact that greater than 80% of the MHC class-I alleles on the target cell can still bind CD8, but not TCR (93). The TCR and CD8 must interact with the same MHC class-I molecule on target cells to

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boost recognition (94). CTLs can be CD8 independent, and this may reflect greater affinity of their TCR for the foreign antigen/MHC class-I complex, or a difference in the requirement for signals transduced by CD8 (95, 96). Evidence that CD8 interaction with "bystander" MHC class-I molecules contributes to the efficiency of recognition by CTLs has been presented, but the effect is relatively small (97). CD4 on helper T lymphocytes interacts with MHC class-II antigens on other cells, and CD4 can be induced to form a weak complex with TCR in the plane of the membrane (98, 99a). CD8 interaction with MHC class-I molecules appears to be regulated by T-cell activation (99b; see below). CD4 and CD8 both associate with tyrosine kinase. This association may have a role in outside-in and inside-out signalling through CD4 and CD8.

Adhesion of helper T lymphocytes and CTLs to other cells can be more efficient when the appropriate antigen : MHC complex is recognized, despite the apparent inability of antigen recognition to mediate adhesion directly (7, 101). When adhesion of T lymphocytes is greater to cells with appropriate foreign antigen than without, this difference is referred to as antigen-dependent adhesion. The majority of studies showing antigen-dependent adhesion have been done with freshly isolated T lymphocytes (7). In contrast, T lymphocytes that have been stimulated to proliferate in culture show strong adhesion to nonantigen bearing cells. This antigen-independent adhesion is due to CD2 and LFA-1 on T lymphocytes interacting with LFA-3 and ICAMs on target cells, respectively (15, 28, 35, 36, 102). Although it was not clear in the original studies, antigen-independent adhesion requires prior activation of T lymphocytes since this type of spontaneous adhesion is low or absent when resting T lymphocytes are tested (5).

Antigen-dependent interactions of helper and cytolytic T lymphocytes show a dramatic spatial coordination of antigen recognition, cytoskeletal organization, and adhesion mechanisms. Contact of T lymphocytes with antigen-bearing cells is followed by a rapid reorientation of the T lymphocyte's Golgi apparatus and microtubule organizing center toward the interface between these cells (103). Interaction of helper T lymphocytes with antigen-bearing cells also results in redistribution to the area of cell-cell contact of TCR, CD4, LFA-1, and the cytoskeletal protein talin (104). Another feature of antigen-dependent T lymphocyte adhesion is very close membrane apposition between T lymphocytes and antigen bearing cells (13, 101, 105). In one study on natural killer cells, a ring of close membrane apposition was observed to surround the site of exocytosis, possibly generating a microenvironment in which secreted agents would be kept at a high local concentration (106). Formation of this structure is blocked by mAb to LFA-1 even when adhesion is mediated by interaction of CD2

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with LFA-3. These observations suggest that T lymphocytes may only "focus" on and adhere strongly to one target or antigen-presenting cell at a time. This is supported by observations of antigen-dependent interaction between CTLs and target cells in suspension (107). Under conditions where target cell lysis is blocked by chelation of extracellular Ca^{2+}, CTL adhesion to labeled targets is stable for hours, but addition of unlabeled target cells results in exchange of unlabeled for labeled target cells (net de-adhesion from labeled target cells). It is likely that binding of the unlabeled target cells causes some CTLs to reorient toward the unlabeled target cell, thus weakening adhesion to the labeled target cell.

The kinetics of helper and killer T lymphocyte interaction with cells expressing different antigens can vary. Helper T lymphocytes show antigen dependent interaction with antigen presenting cells for hours to days (108). In contrast, highly active CTLs interact strongly with target cells only briefly (on the order of minutes), followed by de-adhesion and adhesion to other targets (109, 110). However, quantitative study of spontaneous de-adhesion of T lymphocytes from antigen bearing cells has been incomplete, possibly because all the required ingredients for spontaneous de-adhesion have not been present in most in vitro experiments (see below). However, it is clear that CTL can "recycle" and kill many target cells with intervening migration (109–112), and that clusters of helper T lymphocytes with antigen-presenting cells disperse upon decay of antigen (108). The strength of adhesion between CTLs and target cells during the adhesion/recognition stage is so great that when adherent cells are pulled apart the cells are dramatically deformed before they separate (113). Therefore the strength of adhesion must be dramatically reduced to allow de-adhesion. Differing adhesion kinetics between helper T lymphocytes and CTLs may be related to the kinetics of their distinct functions. Helper T lymphocytes are activated to secrete lymphokines which may require hours for synthesis and even longer for action on the antigen-presenting cells. On the other hand, CTLs deliver lytic agents to the target cell within minutes and can then go on to other targets leaving a trail of dying cells (110).

CD2 AND LFA-3

Regulation of CD2 Interaction with LFA-3

A general concept which applies to the CD2/LFA-3, LFA-1/ICAM, and other adhesion mechanisms is that possession by two cells of the appropriate complementary adhesion receptors does not necessarily mean that these cells will adhere to each other. Mechanisms for regulation of lymphocyte adhesion fit into two general groups: (*a*) nonspecific changes in cellular

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properties that influence adhesion mediated by any adhesion mechanism, and (*b*) changes in adhesion receptors that alter the effectiveness of specific adhesion mechanisms.

The affinity of the interaction between CD2 and LFA-3 is a key parameter for understanding the nature of this adhesion mechanism. The interaction of CD2 and LFA-3 appears to have a relatively low affinity with a K_d on the order of 1 μM. Hydrophilic, recombinant CD2 binds LFA-3 on B lymphoblastoid cells with a $K_d = 4 \times 10^{-7}$ M (114). Similarly, a water-soluble monomeric form of LFA-3 generated by enzymatic cleavage blocks adhesion with an IC(50) = 10^{-6} M (115). In contrast to these hydrophilic forms, in the absence of detergent, purified LFA-3 with an intact hydrophobic anchor aggregates to form octameric protein micelles (115). Purified intact CD2 also probably aggregates in solution, although the form taken by CD2 in solution has not been characterized (20, 28). Intact CD2 binds LFA-3 on B lymphoblastoid cells with a K_d of 5×10^{-8} M, almost 10-fold higher than binding of monomeric CD2 (28). Octameric LFA-3 binds T lymphoma cells with a $K_d = 2 \times 10^{-9}$ M (115). The LFA-3 octamers interact with cells through an average of four sites/octamer. Thus, interaction through an average of four sites increases the avidity of interaction 200-fold over monovalent interaction.

The driving force in regulation of cell-cell adhesion mediated by the CD2/LFA-3 mechanism is T lymphocyte activation (Figure 1*C*). The resting T lymphocytes fail to adhere to B lymphoblastoid target cells or to human erythrocytes, both of which express LFA-3 (5, 20). In contrast, activated T lymphocytes adhere avidly to B lymphoblastoid target cells and human erythrocytes (15, 20). It is notable that thymocytes also have the ability to form rosettes with human erythrocytes. From 12 to 24 hr are required to develop these differences between resting and activated T lymphocytes during the activation process (116).

Two factors that contribute to this activation-dependent regulation have been deduced from experiments with erythrocyte rosetting: surface charge/glycocalyx density and adhesion receptor concentration. Removal of negative surface charges from erythrocytes or T lymphocytes with neuraminidase, removal of glycocalyx from T lymphocytes with proteases which spare CD2, or introduction of positive charges to the surface of erythrocytes, all increase the efficiency of rosetting (16, 20, 116). Negative surface charge of cells, mostly borne on sialic acid residues of various glycans, combined with the bulk of surface glycoproteins and proteoglycans together make the close approach of cell surfaces energetically unfavorable (117). Differences in surface charge may contribute to different levels of adhesion mediated by the CD2/LFA-3 mechanism because thymocytes have five-fold less surface sialic acid than do resting T lympho-

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cytes, and according to histochemical studies activated lymphocytes in germinal centers possess low levels of siliac acid (118, 119).

Rosetting is also sensitive to the level of LFA-3 expression on erythrocytes. Little difference appears in either the surface charge density or binding avidity for purified CD2 between sheep and human erythrocytes. Sheep erythrocytes, however, have a several-fold higher density of LFA-3 molecules than do human erythrocytes, and this appears to account for the difference in rosetting (24). The only caveat to this interpretation is that sheep erythrocytes are much smaller than human erythrocytes, and this may make sheep erythrocyte rosettes more stable during resuspension and counting than are human erythrocyte rosettes. However, insertion of exogenous purified LFA-3 into the membranes of human erythrocytes increases rosetting consistent with the sensitivity of the CD2/LFA-3 mechanism to LFA-3 density (120). Although expression of certain CD2 epitopes increases dramatically during T lymphocyte activation, these epitopes do not appear to be directly involved in interaction with LFA-3, and the density of CD2 does not appear to increase (116, 121). The availability of CD2 and LFA-3 on resting T lymphocytes for interaction with other cells appears to be balanced by the surface charge/glycocalyx repulsion. Resting T lymphocytes have less than the threshold level of CD2/LFA-3 required for adhesion to target cells or autologous erythrocytes (24). By decreasing the surface charge/glycocalyx density on the T lymphocytes, activation allows the CD2/LFA-3 mechanism to mediate adhesion.

The expression of CD2 and LFA-3 may be balanced in different species to prevent CD2/LFA-3–mediated adhesion of resting T lymphocytes, while allowing use of the CD2/LFA-3 mechanism by activated T lymphocytes. This is supported by the observation that while the density of sheep LFA-3 is higher than that of human LFA-3, sheep CD2 expression is considerably lower and more restricted than human CD2 expression (122, 123). Thus, in sheep also, autologous rosetting only occurs with activated, but not resting, T lymphocytes (22).

Other surface molecules besides CD2 and LFA-3 appear to regulate rosetting directly or indirectly. MAb to sheep erythrocytes have identified two such molecules (124). One is a surface protein on sheep erythrocytes, referred to as S14. Its human homologue H19 is involved in human T lymphocyte activation (125). A second structure identified on sheep erythrocytes is called S110-220, which may, based on its size, be equivalent to CD44 in humans. CD44 mAb partially inhibit adhesion between human erythrocytes and T lymphocytes, which process is completely inhibited by CD2 mAb or LFA-3 mAb (126, 127). This was surprising since CD44 had been implicated as a lymphocyte recirculation receptor and a collagen receptor (81, 128, 129). Two lines of evidence suggest that CD44

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does not contribute to this adhesion by binding to a counter-receptor. First, T lymphocytes do not adhere to purified CD44 (126). Second, rosetting still occurs with erythrocytes genetically deficient in CD44 (127). These data suggest instead that CD44 is not required for rosetting but can interfere with CD2/LFA-3 dependent adhesion in a heterologous manner when ligated by mAb. CD44 is associated with ankyrin in murine lymphocytes and may therefore associate with the erythrocyte membrane skeleton (130). In one scenario, cross-linking CD44 may decrease the deformability of erythrocytes which could inhibit adhesion by limiting the area of contact between T lymphocyte and erythrocyte. Finally, mAb to a glycoprotein of 32 kd partially inhibit human thymocyte rosetting with sheep or human erythrocytes by binding to thymocytes (131).

Signalling Through CD2

In contrast to the use of mAb as antagonists of adhesion, mAb have also been used as agonists for signalling through numerous surface structures. This approach has been used to suggest a signal transducing function for several T lymphocyte surface molecules. Pairs of non-cross blocking CD2 mAb trigger T lymphocyte to proliferate (121). Cross-linking CD2 in this way triggers phosphatidylinositol turnover and Ca^{2+} mobilization. This observation has a precedent since sheep erythrocytes bearing LFA-3 increase the responsiveness of lymphocytes to mitogenic stimulation (132). Neither sheep erythrocytes nor octameric LFA-3 induce T lymphocyte proliferation alone. However, sheep erythrocytes or octameric LFA-3 synergize with suboptimal concentrations of mitogenic mAb to CD2 (115, 133, 134). Furthermore, four observations support the hypothesis that CD2 cooperates with TCR in signalling. One observation is that a single mAb to CD2 cooperates with a single mAB to TCR (CD3) in activation of T lymphocytes (135). A second is that activation through CD2 requires expression of TCR (136–138). A third is that L cells expressing human LFA-3 synergize with nonmitogenic mAb to CD3 to stimulate T lymphocyte proliferation (139). Finally, a lateral association between CD2 and TCR has recently been suggested by coprecipitation of CD2 with a particular mAb to CD3 that may induce this association (140). However, as with CD4 and CD8, CD2 is not generally part of the TCR complex.

Consistent with a role in signalling, CD2 function appears to transcend adhesion in at least two in vitro systems: CTL lysis of endothelial cells and Burkitt's lymphoma cells. Adhesion of T lymphocytes to cultured endothelial cells is mediated partially by LFA-1 interaction with ICAMs, but adhesion is unaffected under diverse conditions by mAb to CD2 or LFA-3 (141–143). In contrast, mAb to CD2 and LFA-3 significantly inhibit killer T lymphocyte and helper T lymphocyte functional responses

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to endothelial cells, apparently dissociating the requirements for adhesion and for functional responses (144, 145). However, conjugate formation was not measured in these latter studies. Therefore, failure of CD2/LFA-3 interaction to mediate adhesion, while contributing to function, has not been demonstrated in a single set of experiments. In the second system, an unusual ICAM-1$^+$, LFA-3$^-$, EBV$^+$ Burkitt's lymphoma cell line was not lysed by EBV-specific CTLs despite adhesion mediated by LFA-1 interaction with ICAMs (146). However, the conclusive experiment of transfecting LFA-3 into this cell line to determine if this is the required element was not performed. In both of these systems there is a suggestion that the interaction of CD2 with LFA-3 may deliver a co-signal with TCR that increases the efficiency of CTL-mediated lysis.

LFA-3 Membrane Anchorage Mechanisms

LFA-3 is anchored to the membrane by two distinct mechanisms. Biosynthesis and glycosidase experiments reveal two LFA-3 precursors corresponding to two distinct polypeptides of 29 and 25.5 kd (147). The two polypeptides are present on diverse nucleated cells, but erythrocytes express only the low M_r form of LFA-3. The release of the low M_r form from nucleated cells with phosphatidylinositol specific phospholipase C (PIPLC) indicates that it is anchored to the membrane by a glycosylphosphatidylinositol (GPI)-moiety. The higher M_r polypeptide corresponds to a transmembrane form of LFA-3. LFA-3 is absent on erythrocytes from patients with an acquired defect in the ability of hematopoietic stem cells to synthesize GPI-anchored proteins, a condition referred to as paroxysmal nocturnal hemoglobinuria (PNH) (120). Results of the biosynthesis, PIPLC digestion, and PNH studies were further confirmed by isolation of two different LFA-3 cDNAs clones. One encodes a transmembrane form with a 12 amino acid cytoplasmic domain (148). The other is a classical GPI-anchored protein precursor, which yields GPI-anchored LFA-3 on expression in COS cells, and is identical to the first mRNA in the transmembrane and extracellular regions (149). There is only one LFA-3 gene, and the similarity of the messages suggests that alternative splicing of a single transcript generates the two mRNAs although this has not been formally demonstrated.

To test whether the GPI-anchored and transmembrane forms of LFA-3 possess unique properties, GPI-anchor deficient cells analogous to PNH hematopoietic progenitor cells were created and tested for susceptibility to CTL-mediated lysis (150). GPI-anchor deficient cell lines express the transmembrane form of LFA-3 but degrade the precursor for the GPI-anchored form. The GPI-anchor deficient cell lines and the parental cell line behaved identically as targets for CTL adhesion and lysis. In this

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context, the transmembrane form is sufficient, and these experiments do not reveal a distinct function of the GPI-anchored LFA-3 form. The function of alternative transmembrane and GPI-anchored forms of NCAM also remains mysterious (151).

LFA-3 Lateral Mobility and Adhesion

On a different tack, it should be possible to take advantage of an artificial system, in which GPI-anchored LFA-3 is laterally mobile and the transmembrane form is immobilized, to ask basic questions about the requirement for adhesion receptor lateral diffusion for adhesion. McConnell and colleagues demonstrated that phospholipids in glass-supported planar membrane bilayers are laterally mobile, with diffusion coefficients of 10^{-8} $cm^2\ sec^{-1}$. While transmembrane proteins can diffuse laterally in cell membranes, they are laterally immobile in glass-supported membranes ($< 10^{-12}\ cm^2\ sec^{-1}$). Immobility may result from interaction of the proteins with the glass underlying the artificial membrane. Consistent with this speculation, fluorescence recovery after photobleaching using FITC-labeled mAb to LFA-3 (TS2/9 IgG) indicates that the GPI-anchored LFA-3 diffuses laterally in planar membranes ($2 \times 10^{-9}\ cm^2\ sec^{-1}$; 70–90% recovery), while the transmembrane form is immobile (M. L. Dustin, P.-Y. Chan, T. A. Springer, L. Ferguson, and D. Golan, unpublished). GPI-anchored LFA-3 in planar membranes is eight-fold more potent than transmembrane LFA-3 in planar membranes for adhesion of the Jurkat T lymphoma line under gentle wash conditions, and the difference is even more dramatic under high shear conditions (P.-Y. Chan, M. Lawrence, M. L. Dustin, and T. A. Springer, unpublished). However, IL-2–activated natural killer cells show surprisingly poor adhesion to planar membranes bearing GPI-anchored LFA-3 (O. Carpen, J. Caulfield, M. L. Dustin, and T. A. Springer, in preparation). The basis of the difference between T lymphocytes and natural killer cells in binding to the GPI-anchored form of LFA-3 is not understood. However, CD2 is associated with different signal transducing mechanisms in natural killer cells than in T lymphocytes (153, 154). Whether lateral immobilization of LFA-3 is ever used as a regulatory mechanism by cells remains to be seen. Regardless, this model system demonstrates the importance of lateral mobility for mechanisms of cell adhesion.

LFA-1 AND ICAMS

Regulation of LFA-1/ICAM Adhesion Mechanism

T lymphocyte adhesion shows regulation on both short and long time-scales. Long time-scale regulation is apparent for the CD2/LFA-3 mech-

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anism since changes in cell surface charge occur over hours to days. The LFA-1/ICAM mechanism shows both short and long time-scale regulation through LFA-1 and ICAM-1, respectively. For some time it has been suspected that LFA-1 might hold the key to regulation of T lymphocyte adhesion to antigen-bearing cells (7, 32, 37). Evidence that the LFA-1/ICAM-1 adhesion mechanism has the potential to regulate both antigen dependent adhesion and entry of lymphocyte into sites of inflammation, is presented below.

LFA-1 Avidity for ICAMs

Regulation of LFA-1/ICAM interaction has been probed using phorbol ester–stimulated aggregation as a model (Figure 1*D*). Phorbol esters stimulate a rapid increase in lymphocyte aggregation mediated by the LFA-1/ICAM mechanism (31–33, 155). Aggregation occurs rapidly (≤ 1 hr) and does not involve changes in LFA-1 or ICAM-1 surface density (33). These results fueled speculation about regulation of the LFA-1/ICAM adhesion mechanism, but this work was not extended due to limitations of the aggregation technique. LFA-1/ICAM interaction can be studied in more detail using purified LFA-1 or ICAM-1 immobilized on solid substrates (Figure 1*E*). Such studies demonstrate that phorbol esters increase the avidity of cell surface LFA-1 for purified ICAM-1 but do not significantly increase avidity of cell surface ICAMs for purified LFA-1 (5). This is consistent with the sidedness of the temperature dependence noted above and further suggests a relationship between the temperature and energy requirements of cell surface LFA-1 and regulation of LFA-1 avidity by activation of protein kinase C.

INSIDE-OUT SIGNALLING AS A MECHANISM FOR ANTIGEN-DEPENDENT ADHESION Earlier, the paradox of antigen dependent adhesion was presented: The TCR does not appear to mediate substantial adhesion, yet adhesion is frequently dependent on foreign antigen recognition. This puzzle would be solved if evidence for regulation of adhesion mechanisms by TCR were obtained. We have found that a striking increase in the avidity of LFA-1 for ICAMs is triggered by cross-linking TCR on resting T lymphocytes (5). This provides a plausible mechanism for antigen-dependent adhesion. We suggest that the interaction of TCR with foreign antigen : MHC directs the use of metabolic energy to drive an increase in LFA-1 avidity and thus leads to stronger adhesion.

In contrast to the phorbol ester–stimulated increase in LFA-1 avidity which persists for several hours, the increase in LFA-1 avidity triggered by cross-linking TCR is transient, peaking at 10–20 min and decreasing to basal levels by 40 min. Transient increases in LFA-1 avidity provide

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a mechanism for de-adhesion from foreign antigen bearing cells after communication is complete. Since helper T lymphocytes interact with antigen bearing cells for hours to days, there must be mechanisms to prolong the increase in LFA-1 avidity beyond 30 min. Cross-linking of CD2 with pairs of mAb appears to result in a longer term increase in LFA-1 avidity (156). Therefore, cross-linking of TCR with other adhesion molecules may allow longer interactions. Alternatively, distinct regulation of LFA-1 on antigen-presenting cells may have a profound effect on the kinetics of interaction with helper T lymphocytes. In fact, the avidity of LFA-1 on B lymphocytes, a major antigen presenting cell type, is increased by ligation of MHC class-II antigens (see Figure 1*B*) (157). The kinetics of the LFA-1 avidity increase that is stimulated by TCR cross-linking are consistent with the kinetics of active CTL interaction with target cells (4).

The TCR-stimulated increase in LFA-1 avidity is blocked by increasing cytoplasmic cyclicAMP or by inhibiting protein kinase C. These results indicate that cytoplasmic signals connect the TCR cross-linking event to the increase in LFA-1 avidity. Regulation of LFA-1 avidity, therefore, appears to be a true example of inside-out signalling. Deletion of the cytoplasmic domain of the LFA-1 β subunit but not the α subunit eliminates binding to ICAM-1 and renders LFA-1 anergic to phorbol ester stimulation, supporting the inside-out signalling model (M. L. Hibbs, H. Xu, S. A. Stacker, and T. A. Springer, unpublished).

It is not known whether inside-out signalling stimulates LFA-1 avidity by increasing LFA-1 affinity for ICAMs or by some other mechanism which increases the number or strength of LFA-1/ICAM interactions. LFA-1 is phosphorylated on both the α and β subunit, but these phosphorylations have not been correlated with function (158–160). The presence of conformational flexibility in LFA-1 consistent with a change in affinity for ICAMs is detected by two mAb to LFA-1 (161, 162). Interestingly, binding of both mAb is divalent cation dependent, and binding of one of these mAb appears to drive LFA-1 into a high LFA-1 avidity state independent of cellular activation or energy levels, apparently bypassing the inside-out signalling mechanism (161).

Is the observation of avidity regulation for LFA-1 generalizable to other integrins that mediate diverse cell-matrix and cell-cell interactions? There is evidence for avidity regulation in two other systems: binding of fibrinogen by platelets and binding of multiple ligands and counter-receptors by Mac-1, a close relative of LFA-1 (163–165). Conformational changes in gpIIbIIIa on activation and ligand binding are indicated by distinct mAb which may be sensitive to conformation or the environment of the receptors (166–168).

Regulation of integrin avidity appears to be dependent on cell type, in

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addition to integrin type. In contrast to LFA-1 on lymphocytes which is regulated by activation, LFA-1 expressed in COS cells is constitutively active, and avidity was not further increased by phorbol ester treatment (169). Similarly, VLA-5 (fibronectin receptor) is constitutively active on nonmitotic fibroblasts, but when expressed on resting T lymphocytes, VLA-5 and VLA-6 have low constitutive avidity for fibronectin and laminin, respectively, which can be increased by treatment with phorbol esters or TCR cross-linking (63). The conclusion of greatest significance for immune cell interactions and migration is that β_1 integrins on resting T lymphocytes may be regulated in parallel with LFA-1.

AVIDITY CYCLES DRIVE MIGRATION? Migration of cells over substrates requires adhesion to the substrate at the leading edge and de-adhesion at the trailing edge. A spatial gradient of LFA-1 avidity with high avidity at the leading edge of the cell and low avidity at the trailing edge offers a mechanism for required adhesion and de-adhesion that does not generate large amounts of tension across the length of the migrating lymphocyte (5, 170). A gradient of integrin avidity over the length of the cell may drive migration, analogous to the ability of gradients of adhesive materials on inert substrates to drive cell migration (171). Lymphocytes adhering to surfaces bearing purified ICAM-1 have a spread, and frequently bipolar morphology, consistent with a migrating cell, while the same cells adhering to the GPI-anchored form of LFA-3 appear rounded and nonmotile (142). Time-lapse microscopy of B lymphoblastoid cells adhering to ICAM-1 on planar membranes shows that these cells crawl randomly over the planar membranes at a rate of 10–20 μm/min. Strikingly similar rates are observed with unstimulated or PMA stimulated cells. Cells do not crawl on planar membranes without proteins. In contrast, B lymphoblastoid cells become anchored to planar membranes containing LFA-1, and despite dramatic motility of the cell body, no locomotion is observed over 12 hr of observation. The ability of cell surface LFA-1 and other integrins to undergo cycles of avidity regulation may thus be critical for cell migration over the surface of other cells or through the extracellular matrix. One context for LFA-1–dependent lymphocyte migration is during extravasation in which lymphocytes crawl over the surface of endothelial cells expressing ICAM-1 (Figure 2). In fact, transendothelial migration of lymphocytes and granulocytes in vitro is inhibited by mAb to LFA-1 (172, 173).

Signal Transduction Through LFA-1

LFA-1 transduces a signal from the inside of the cell to the outside in the process of avidity regulation. On the other hand, outside-in signal

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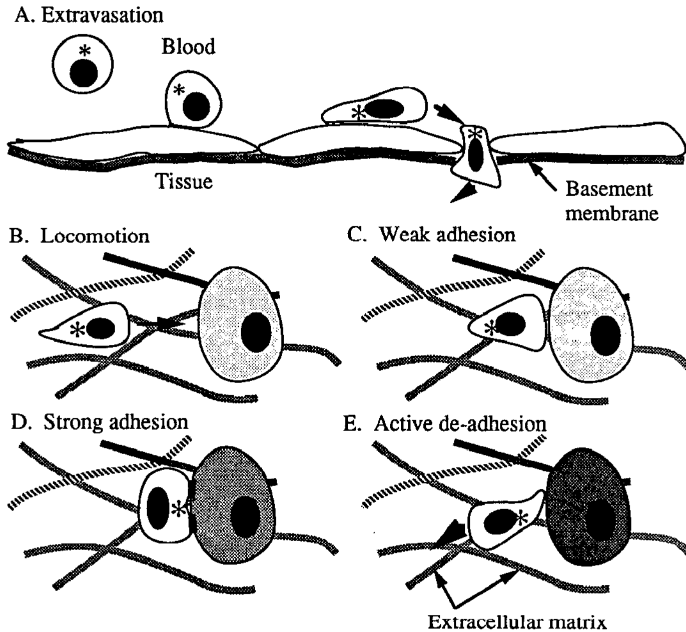

Figure 2 A model for T lymphocyte interactions in tissues. The polarity of the T lymphocyte is indicated by the likely position of the microtubule organizing center (*) relative to the nucleus (103) (M. L. Dustin and T. A. Springer, unpublished).

transduction by LFA-1 may inform T lymphocytes of their environment and influence T lymphocyte activation and differentiation. Since LFA-1 mAb block many T lymphocyte responses by interfering with reception of signals through TCR and other surface molecules, it is necessary to use systems in which this kind of effect is absent in order to examine signalling through LFA-1 itself. MAb to the LFA-1 α chain enhance responses to anti-TCR mAb immobilized on tissue culture plastic (174). Furthermore, when LFA-1 is cross-linked both with mAb to LFA-1 α chain and with a second antibody to Ig, phosphatidylinositol turnover and Ca^{2+} fluxes are stimulated. (175). On the other hand, mAb to LFA-1 β chain generally inhibited responses to solid phase TCR mAb and did not stimulate increases in cytosolic Ca^{2+} when cross-linked. One study found that LFA-1 α and β subunit mAb augmented CD3 mAb-stimulated Ca^{2+} fluxes (176), but there have been no reports of LFA-1 mAb alone stimulating T lymphocyte proliferation as is seen with CD2 mAb combinations. It is intriguing that cross-linking LFA-1 with mAb generates signals implicated in triggering an increase in LFA-1 avidity for ICAMs. This suggests a

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positive feedback loop favoring maintenance of high LFA-1 avidity when LFA-1 is ligated following or concurrent with ligation of TCR. Physiological integrin ligands also augment responses to immobilized anti-TCR mAb. Purified ICAM-1, fibronectin, and laminin adsorbed to tissue culture plastic synergize with nonmitogenic anti-TCR mAb adsorbed to the same surface to stimulate proliferation of $CD4^+$ T lymphocytes (177, 178). These observations suggest that both LFA-1 and β_1 integrins may provide "co-stimulatory" signals for T lymphocyte activation.

Model for T Lymphocyte Interactions

An area of current controversy is the order of events in immune recognition. Does TCR ligation come first (7) or does weak antigen-independent adhesion mediated by moderately active adhesion mechanisms precede TCR interaction and subsequent adhesion strengthening (102, 179)? It is unlikely that strong adhesion precedes antigen recognition (102) on the grounds that it would impair the efficiency of immune surveillance and that it is inconsistent with the behavior of freshly isolated T lymphocytes (5). A drawback of models suggesting that weak adhesive interactions precede antigen recognition is that no evidence for weak adhesion is provided with resting T lymphocytes (179). Another question involves deadhesion. Why is this clearly critical event so elusive in vitro? The answers to both questions may be found by proposing a role for a component not considered in most discussions of T lymphocyte interactions with other cells: the surrounding extracellular matrix and other cells (Figure 2).

For many years it has been known that lymphocytes actively migrate through the stroma of lymph nodes and tissues (180). This migration is probably an ongoing process which is only temporarily suspended when lymphocytes are circulating in the blood and lymph, or possibly when they are involved in localized immune responses. This prior observation provides evidence that lymphocytes in tissues have active adhesion mechanisms since it is very likely that adhesion is intrinsic to migration. Furthermore, the hypothesis that integrin avidity cycles drive migration predicts that migrating lymphocytes have a small number of high avidity integrins, including LFA-1, at their leading edge. The manner in which T lymphocytes are activated to begin migrating is unclear.

We propose the following sequence of events in leukocyte migration. The initial transformation from a nonadherent cell in the blood to an adherent and polarized cell attached to the blood vessel wall may represent an activation step initiating integrin cycling (Figure 2*A*). The possibility that endothelial cells may provide activating signals to lymphocytes can be inferred from the involvement of LFA-1 in adhesion of lymphocytes to "high" endothelial cells of lymph nodes and sites of chronic inflammation

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(181). In addition to this putative activating function, endothelial cells bear counter-receptors that reveal the location and/or immunological state of the surrounding tissue (6). In the tissues T lymphocytes remain polarized and locomote by forming transient bonds to the extracellular matrix and cell surfaces (2B). The direction of migration may be dictated by chemotactic factors produced at a focus of inflammation (182) or may be guided by extracellular matrix components or counter-receptors such as ICAM-1 induced in inflammatory sites (38). When lymphocytes encounter cells at their leading edge, weak adhesive interactions form which allow TCR time to encounter and bind appropriate foreign antigen : MHC complexes if present (2C). TCR ligation leads to strengthening of adhesion (2D). These last two steps are similar to those proposed earlier (5, 147, 179), except that initial adhesion can be seen as an extension of T lymphocyte migration. If no relevant foreign antigen is present on the encountered cell, then migration continues over and past this cell with minimal loss of precious time (110, 183). This hypothesis suggests an alternate interpretation of the co-stimulatory effect of ICAM-1, fibronectin, and laminin coimmobilized with TCR/CD3 mAb on substrate (177, 178). The integrin ligands immobilized on the substrate could facilitate cell migration and thus allow cells that are optimally suited for interaction to find one another and mount a response.

In vivo, the conjugate of a T lymphocyte and antigen-bearing cell is surrounded by other cells and extracellular matrix; interaction with these cellular and extracellular components may facilitate de-adhesion. The absence of such components from in vitro assays, apart from sparse deposition of serum adhesive glycoproteins on the culture surface, may account for difficulty in observing de-adhesion in vitro. To continue the sequence of events described above, after antigen-dependent communication between the T lymphocyte and antigen-bearing cell is complete (as indicated by a change in the antigen bearing cell) the avidity of integrins probably decreases to the basal migrating levels. However, the T lymphocyte and antigen-bearing cell do not fall apart. Instead, it seems more likely that the polarity of the T lymphocyte becomes random, and the T lymphocyte actively crawls away using interactions with neighboring extracellular matrix (or cells) to undermine remaining adhesive interactions (E). This active, almost competitive, aspect of de-adhesion is a second novel aspect of this model. This active process is likely to be essential for extrication of cells from interactions mediated by the CD2/LFA-3 adhesion mechanism which may only have a single avidity state.

This model does not take into account the potential role of LFA-1 on cells with which T lymphocytes interact. T lymphocytes frequently interact with target cells and antigen-presenting cells expressing LFA-1; little is

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known about LFA-1 avidity regulation by physiological factors on potential target cells and antigen presenting cells. LFA-1 on B lymphocyte blast target cells did not play a major role in antigen-dependent interactions with CTLs (10). However, a role for monocyte LFA-1 in initial interactions with resting T lymphocytes (Figure 2*C*) has been proposed (14). Furthermore, a prominent role for B lymphoblast LFA-1 in interactions with helper T lymphocytes was detected in one study (13). Results of a second study with LFA-1^- B lymphoblasts appear to support a role for B lymphocyte LFA-1 in interactions with helper T lymphocytes, but it was also proposed that the LAD patients which supplied the B lymphocytes might be deficient in memory B lymphocytes primed specifically for the recall antigen utilized (184). A possible mechanism for recruitment of B lymphocyte LFA-1 into antigen-dependent interactions is suggested by the observation that B lymphoblast aggregation that is dependent on LFA-1 is triggered by ligation of MHC class-II molecules with mAb or Staphylococcal exotoxin B (a superantigen which binds MHC class-II molecules) (157).

Regulation of ICAM-1 Expression: Localization of Immune Responses

The LFA-1/ICAM mechanism is regulated through changes in counter-receptor expression in addition to changes in LFA-1 avidity. Regulation of ICAM expression allows control of the spectrum of cells to which activated T lymphocytes can adhere and in some cases coordinates the ability to adhere with the presence of MHC molecules required for foreign antigen presentation. In most cases, ICAM-1 expression is regulated by cytokine receptors coupled to mechanisms for altering gene expression.

Resting lymphocytes lack ICAM-1 expression, but ICAM-1 expression increases over a period of days during T and B lymphocyte activation (38, 185, 186); activated lymphocytes in germinal centers and at sites of inflammation are strongly positive (38, 187). In contrast, monocytes possess an intracellular store of ICAM-1 which can be mobilized to the cell surface by adherence to fibronectin (188). Neither the nature of the compartment in which ICAM-1 is sequestered in monocytes nor the relationship of this compartment to the peroxidase negative granules containing rapidly mobilizable Mac-1 is known (189).

Regulation of ICAM-1 expression on nonleukocytes is even more dramatic. ICAM-1 is absent from most cells in normal, nonlymphoid tissues, except for expression of low levels on endothelial cells. Local immune responses result in a rapid increase in ICAM-1 expression on endothelial cells and induction of ICAM-1 on epithelial and mesenchymal cells (38, 142, 187, 190). These in vivo results are correlated with the ability of

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products of activated lymphocytes (lymphokines) and monocytes (monokines) to increase ICAM-1 expression on cultured fibroblasts, endothelial cells, epithelial cells, and astrocytes (38, 40, 142, 191, 192). The increase in ICAM-1 expression on malignant melanomas and carcinomas may be secondary to local immune reactions generating cytokines (193–195). Cytokine stimulated increases in ICAM-1 require transcription and translation and take 4–48 hr to reach maximal levels, after which the expression remains elevated in the presence of cytokines.

Expression of ICAM-1 and ICAM-2 overlap, and both contribute to LFA-1 dependent adhesion. ICAM-1 regulation on epidermal keratinocytes is the major determinant for the ability of activated T lymphocytes to adhere to these cells (196). On the other hand, adhesion of activated T lymphocytes to unstimulated endothelial cells or some leukocyte lines ICAM-2 (39, 142; A. de Fouge-rolles and T. Springer, unpublished). The relative roles of these adhesion mechanisms can be dissected out using mAb to block certain pathways; in the absence of appropriate mAb, lymphocyte cell lines which lack certain adhesion receptors can be used to isolate particular mechanisms for study (142).

LFA-1/ICAM in Lymphocyte Binding to Endothelial Cells

T lymphocytes adhere to endothelial cells to gain entry to tissues. Entry into tissues is critical for lymphocytes to encounter rare cells expressing foreign antigens complementary to their TCR. Lymphocytes recirculate continuously between lymph nodes, tissues, and blood; inflammation enhances lymphocyte extravasation in diverse tissues. The observations that ICAM-1 expression is dramatically upregulated on cultured endothelial cells treated with monokines and on endothelial cells at sites of inflammation in situ, and that the kinetics of ICAM-1 upregulation are similar to the kinetics of lymphocyte entry into sites of inflammation together suggest that the LFA-1/ICAM-1 interaction may have a role in regulation of T lymphocyte binding to endothelial cells.

Adhesion of T lymphocytes to cultured endothelial cells involves several adhesion mechanisms which until recently have been difficult to disentangle. Adhesion of resting or activated T lymphocytes to cultured endothelial cells is mostly inhibited by mAb to LFA-1 (141). The LFA-1–dependent adhesion to endothelial cell occurs one third by means of interaction with ICAM-1 and two thirds by interaction with ICAM-2. Stimulation of endothelial cells with monokines increases total adhesion and the ICAM-1 component but does not affect the ICAM-2 component (142). Monokine stimulation of cultured endothelial cells also increases an LFA-1–independent mechanism of comparable strength to the LFA-1/ICAM mechanism. This LFA-1–independent adhesion appears to be

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based on interaction of the integrin VLA-4 with the inducible endothelial cell counter-receptor VCAM-1 (64, 197). Experiments on adhesion of resting T lymphocytes to monokine-stimulated endothelial cells have not yet been done to determine if the LFA-1/ICAM and VLA-4/VCAM-1 interactions are the only adhesion mechanisms utilized for T lymphocyte adhesion to cultured endothelial cells.

The generality of the role of LFA-1 in lymphocyte interactions also extends to interactions with high endothelial postcapillary venules (HEV). HEV of lymphoid organs are the most efficient sites of lymphocyte extravasation. Lymphocyte adhesion to HEV can be modelled in vitro by examining the binding of lymphocytes to frozen sections of lymphoid tissues and counting lymphocytes bound per length of morphologically defined HEV (198). HEV also occur at sites of chronic inflammation, but postcapillary venules can become adhesive for lymphocytes in acute inflammation without acquiring the high phenotype (181, 199, 200). Binding studies with lymphocytes and lymphoid tumor cell lines suggest that separate recognition systems are involved in homing to peripheral lymph nodes, Peyer's patch (gut), and inflamed lymph nodes (201). Lymphocyte adhesion to peripheral and mucosal lymph node and to synovial HEV is inhibited 50–60% by mAb to LFA-1 (181, 202, 203). Consistent with expression of ICAM-2 on endothelial cells in vitro, mAb to ICAM-1 does not inhibit lymphocyte binding to HEV, suggesting that ICAM-2 is also expressed on endothelial cells in vivo (181). A more organ specific mechanism for lymphocyte adhesion to peripheral lymph node HEV is inhibited by mAb to the selectin Mel 14 (76). Adhesion to Peyer's patch HEV is inhibited by mAb to LPAM-1 and -2 ($\alpha4\beta$P and $\alpha4\beta1$) and CD44 (204, 205). However, a polyclonal antisera to CD44 inhibits lymphocyte adhesion to both peripheral lymph node and Peyer's patch HEV, calling into question the organ specificity of the CD44 mechanism (205). The major adhesion mechanisms in chronic inflammation may be VLA-4 interaction with VCAM-1 and LFA-1 interaction with ICAMs, but this has only been confirmed for LFA-1/ICAM (181).

ICAM-1: A Rhinovirus Receptor

Viruses enter cells by binding to surface components including specific glycoproteins that have normal cellular functions (206). Fortuitously or by evolutionary design, 90% of rhinoviruses, causative agents in 50% of common colds, utilize ICAM-1 as their cellular receptor. MAb that block binding of 90% of rhinovirus serotypes to HeLa cells immunoprecipitate a 90-kd glycoprotein similar in biochemical properties to ICAM-1 (207). Peptide sequencing and isolation of cDNA clones demonstrate that the major group rhinovirus receptor is ICAM-1 (208). Major

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group rhinoviruses bind to purified ICAM-1 and ICAM-1 transfected COS cells (209). In contrast to LFA-1/ICAM-1 interaction, the interaction of rhinovirus with purified ICAM-1 does not require divalent cations (210). Recombinant, secreted ICAM-1 blocks binding, entry, and replication of rhinovirus types that use ICAM-1 as a receptor with 50% maximal inhibition at 2×10^{-8} M (211). Rhinovirus and LFA-1 bind to overlapping but distinct sites on the N-terminal Ig domain of ICAM-1 (210).

MEMORY AND RECIRCULATION

Phenotype of Naive and Memory T Lymphocytes

An essential property of the immune system is its ability to remember previously encountered foreign antigens. Memory T lymphocytes are T lymphocytes or their clonal progeny activated in an animal's encounter(s) with a foreign antigen that survive and revert to a resting T lymphocyte morphology. Naive T lymphocytes are mature T lymphocytes not previously activated in an immune response. Memory and naive lymphocytes may recirculate differently, based on differences in recirculation patterns in neonatal versus adult animals. Some studies suggest that memory lymphocytes home preferentially to the lymphoid tissue in which they originally encountered foreign antigens (180). The recent ability to distinguish naive from memory cells based on their respective surface phenotypes allows more direct study of this question.

Naive and memory T lymphocytes are morphologically indistinguishable, but stable changes in the expression of a battery of surface molecules appear to record the prior activation of T lymphocytes; these changes are likely to be be functionally important for these subpopulations. One of the best markers for memory T lymphocytes is the CD45 molecule which is encoded by differentially spliced mRNAs (212). Two of these differential splicing products are distinguished by mAb. Naive cells express the CD45RA epitope, while memory cells express the CD45RO epitope (213, 214). Interestingly, a number of adhesion receptors are also increased on memory T lymphocytes. LFA-3 is negative on naive and positive on memory T lymphocytes. Several other adhesion receptors are two- to four-fold higher on the memory T lymphocytes than naive T lymphocytes, including LFA-1, CD2, β_1 integrin (CD29), and CD44. In general, the naive/memory division cuts across the CD4/CD8 subpopulations in peripheral T lymphocytes, although all CD8 cells appear to have the higher LFA-1 level (175, 215).

Dramatic differences in recirculation of naive and memory cells are observed using these markers. It appears that only naive cells enter lymph

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nodes through HEV, while cells with the memory phenotype leave the blood at other sites and enter lymph nodes through lymphatic drainage from these tissues (215). This is consistent with recent observations that T lymphocytes with the memory phenotype are highly enriched in inflammatory sites (216). It is not clear whether the failure of memory cells to enter tissues at lymph node HEV is due to more efficient binding to other postcapillary venules or less efficient binding to lymph node HEV. In agreement with the former explanation, memory T lymphocytes show stronger adhesion than do naive T lymphocytes to cultured endothelial cells stimulated with monokines (216). Activated T lymphocytes adherent to purified ICAM-1 are also enriched for cells expressing higher LFA-1, suggesting that when the relative activation state of lymphocytes is similar, differences in LFA-1 expression on the order of 2.5-fold are functionally significant (196).

Diverse T lymphocyte adhesion receptors are involved in interactions with endothelial cells, and some time may be required to resolve the adhesion mechanisms primarily involved in regional specificity from those involved in more general adhesion like LFA-1 and probably CD44. An alternative to this working hypothesis is that no single molecule will be primarily responsible for localization, but that combinations of diverse adhesion and recognition molecules may define where cells adhere and migrate (217). A fine balance involving multiple adhesion processes may determine the site at which T lymphocytes bind to endothelial cells and extravasate, in a way similar to the threshold effect observed for regulation of CD2/LFA-3 interaction. The increase in LFA-1, β_1 integrins, and CD44 expression on memory cells versus naive T lymphocytes may determine whether T lymphocytes adhere to the post capillary venules of other tissues that are encountered in every cycle through the circulatory system, prior to adhering to lymph node HEV, which are encountered in less than 1% of passages through the circulatory system. Thus, by virtue of a small increase in adhesiveness, memory T lymphocytes may be intercepted before encountering lymph node HEV.

CONCLUSIONS

Adhesion mechanisms utilized in the immune response are related to molecules used in morphogenesis and structural homeostasis in all organ systems. Lymphocytes draw on at least three families of adhesion molecules: the integrins, the immunoglobulin superfamily, and selectins. Each adhesion mechanism appears to be regulated at several levels to allow dynamic cell interactions. The CD2/LFA-3 mechanism is regulated by receptor expression, surface charge density, association with other surface

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structures, and possibly by the way LFA-3 is anchored in the membrane. The LFA-1/ICAM mechanism is regulated at the level of LFA-1 activity, ICAM-1 expression and multiple overlapping ligands. Intermeshed with the regulation of adhesion is the ability of adhesion receptors to contribute to the cell's image of its environment. The mechanism of transmembrane signalling, outside-in or inside-out, remains undefined, and adhesion is an appealing system for approaching these problems. In the process of addressing these questions, it is likely that a great deal will be learned about the integrated activities of the cell surface and cytoskeleton in regulating adhesion and the feedback of adhesion on cell growth and differentiation.

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