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Ann. Rev. Immunol. 1987. 5: 223–52
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THE LYMPHOCYTE FUNCTION-ASSOCIATED LFA-1, CD2, and LFA-3 MOLECULES: Cell Adhesion Receptors of the Immune System

Timothy A. Springer, Michael L. Dustin, Takashi K. Kishimoto, and Steven D. Marlin

Department of Pathology, Harvard Medical School and Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Boston, MA 02115

INTRODUCTION

Cell adhesion molecules are thought to play an important role in guiding cell migration and localization in the development of the embryo and in organogenesis. In the immune system, cell adhesion molecules enhance the efficiency of specific receptor-dependent lymphocyte-accessory cell and lymphocyte-target cell interactions; they are also important in leukocyte-endothelial cell interactions and lymphocyte recirculation. Recent studies with monoclonal antibodies (MAb) that perturb antigen-receptor-dependent T-lymphocyte functions have defined a number of cell surface molecules that are associated with lymphocyte function (lymphocyte function–associated or LFA antigens) (Table 1). The antigens LFA-1, CD2, LFA-3, CD8, and CD4 appear to enhance antigen-specific functions by acting as cell adhesion molecules. Further studies have shown that the LFA-1, CD2, and LFA-3 molecules are also important in antigen-independent T-lymphocyte adherence and function and that the LFA-1 molecule is important in the adherence and function of essentially all leukocyte cell types.

This review focuses on LFA-1, CD2, and LFA-3. The role of CD4 and CD8 is reviewed by Littman in this volume. We discuss (*a*) the contributions of LFA-1, CD2, and LFA-3 to antigen-dependent and antigen-

0732–0582/87/0410–0223$02.00

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Table 1 Cell surface molecules that regulate T-cell interactions

T Cells			Other Cells		
Name	M_r	Cell distribution	Name	M_r	Cell distribution
LFA–1	$\alpha = 180K, \beta = 95K$	Thymocytes, T and B lymphocytes, LGL, monocytes, activated macrophages, neutrophils	ICAM-1	90–110K	Wide, regulated by IL-1, TNF, and IFN-γ
CD2 (LFA-2/T11)	50–58K	Thymocytes, T lymphocytes, LGL	LFA-3	55–70K	Wide
Antigen Receptor-CD3 (T3) Complex	$\alpha = 50K, \beta = 40K$ $\gamma = 28K$ $\delta = 22K$	Mature thymocytes T lymphocytes	Antigen in association with major histocompatibility complex (MHC) molecules		
CD8 (T8)	32K disulfide-linked dimer	Subset of thymocytes and T lymphocytes, LGL	MHC class I	$\alpha = 44K, \beta = 12K$	Wide, regulated by IFN-α, β, γ
CD4 (T4)	55K	Subset of thymocytes and T lymphocytes, monocytes/macrophages	MHC class II	$\alpha = 34K, \beta = 29K$	Wide, regulated by IFN-γ

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independent adhesiveness, (*b*) a putative ligand for LFA-1, designated intercellular adhesion molecule 1 (ICAM-1), (*c*) the direct molecular interaction of CD2 with its ligand, LFA-3, and (*d*) the dual function of CD2 in T lymphocyte adhesion and triggering. The reader is also referred to previous reviews (1–4), an excellent recent review by Martz on accessory (LFA) molecules (5), and a concurrent review by Anderson & Springer on inherited leukocyte adhesion deficiency disease (6).

LFA-1

Mouse and Human LFA-1

LFA-1 has been defined in the mouse with rat MAb (7, 8) and in the human with mouse MAb (9, 10). The tissue distribution, structure, and function of murine and human LFA-1 are highly similar. In hybrid cells, the α and β subunits of mouse and human LFA-1 promiscuously coassociate in interspecies $\alpha\beta$ complexes, further suggesting homology (11). Results from the mouse and human are described interchangeably below.

Tissue Distribution

LFA-1 is expressed by all leukocytes, with the exception of some macrophages (Table 1) (12, 13). There are 15,000 to 40,000 LFA-1 surface sites per peripheral lymphocyte, with more abundant expression on T than B lymphocytes and increased expression on T blasts (12, 14). LFA-1 is present on ~50% of bone marrow cells. In B and myeloid lineages, LFA-1 is first seen at the pre–B cell (cytoplasmic μ chain positive) and late myeloblast stages, respectively (15). LFA-1 is absent or low on myeloid and erythroid precursor cells (15, 16) and is absent from nonhematopoietic cells.

Structure and Biosynthesis of LFA-1

LFA-1 is a heterodimer consisting of an α subunit of 180 kd and a noncovalently associated β subunit of 95 kd (17, 18). Crosslinking experiments show the presence of only one α and one β subunit per complex. The α and β subunits are synthesized as separate precursors of 170 kd and 87 kd respectively (18). The precursors contain N-glycoside high mannose carbohydrate groups linked to polypeptide chain backbones of 130 kd (α) and 72 kd (β) (19). The α and β precursors must associate intracellularly before conversion of the high mannose carbohydrates to a complex form occurs in the Golgi apparatus (18–20). The $\alpha\beta$ complex is then expressed on the cell surface. The N-linked carbohydrates of LFA-1 are sulfated in

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thymocytes and splenic T cells but not on macrophages, splenic B cells, or bone marrow cells (21).

Functional Studies

LFA-1 was initially defined on human and murine lymphocytes by monoclonal antibody–mediated inhibition of killing by cytotoxic T lymphocytes (CTL) and natural killer cells (7–10). Since then, MAb, $F(ab')_2$, and Fab fragments to both the α and β subunits of LFA-1 have been shown to inhibit a wide variety of adhesion-dependent leukocyte functions. The pattern of inhibition is highly discreet, and MAb binding to many other antigens present at higher density on the cell surface has no effect (1, 2, 22, 23). Furthermore, normal cells treated with LFA-1 MAb exhibit the same pattern of defects as genetically LFA-1-deficient cells (see below).

LFA-1 MAb inhibit CTL-mediated lysis of allogeneic (2, 7–9, 13, 24–30), xenogeneic (7, 31–33), virus-infected (10), and hapten modified targets (25) by both cloned CTL and bulk populations. In addition to T cell-mediated cytotoxicity, LFA-1 is involved in natural killer (NK) cell–mediated cytotoxicity and antibody-dependent cytotoxicity mediated by granulocytes or peripheral blood mononuclear cells (10, 13, 24, 26, 34–39).

Two steps in cytolytic T lymphocyte–mediated killing have clearly been distinguished: adhesion and lethal hit delivery (40). These steps are Mg^{+2} and Ca^{+2} dependent, respectively. Cytolytic T lymphocytes can be distinguished from target cells by size or by means of fluorescent dyes. Adhesion of CTL to target cells (conjugate formation) can be quantitated microscopically or with flow microfluorometry, while killing can be measured as the release of label from the target cell. Adhesion to target cells clearly precedes and is required for lethal hit delivery. LFA-1 MAb block CTL-mediated killing by acting at the Mg^{+2}-dependent adhesion stage rather than the Ca^{+2}-dependent lethal hit delivery step (1). LFA-1 MAb inhibit conjugate formation between CTL and target cell, and preformed conjugates are reversed (1, 25, 29, 41, 42).

LFA-1 is also involved in helper-T-cell functions. Anti-LFA-1 MAb inhibit the proliferation of T cells in response to soluble antigens, viruses, alloantigen, xenoantigen, and mitogens (8, 13, 24–26, 43–45). LFA-1 MAb block only if added before or within the first few hours of initiation of these assays, before proliferation begins. Thus, induction of proliferation rather than proliferation itself is inhibited. Responses of cell lines such as CTLL2 that require only IL-2 for proliferation are not inhibited. These results suggest that the T cell–antigen presenting cell interaction is blocked, but this remains to be demonstrated.

In contrast to conventional LFA-1 MAb, a MAb reactive with an

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activation determinant present on LFA-1 and additional surface molecules stimulates proliferation and IFN-γ release by T-cell clones and inhibits cytolysis by the same cells (46).

Antibody responses by B cells are inhibited by anti-LFA-1 MAb, apparently by affecting interactions with T cells or antigen-presenting cells. T cell–dependent antibody responses to antigen or mitogens are inhibited, while T-independent responses are unaffected (8, 25, 35, 37, 44, 45). Pretreatment of either T cells or monocytes, but not B cells, inhibits the in vitro antibody response to influenza virus (43).

Inhibition of adhesion in helper-T-lymphocyte and B-cell responses is consistent with results cited above but has thus far been assessed in only one report which showed that LFA-1 MAb inhibit conjugate-formation of hapten-specific B cells with carrier-specific T cells (47). Other reports show a differential effect of LFA-1 MAb on cell-cell interactions. Anti-LFA-1 MAb block IL-2 production by T-cell hybrids when stimulated by antigen-presenting or allogeneic cells (48–50) but not when stimulated with anti-T cell receptor antibody linked to Sepharose (48). Anti-CD3 induced cytolysis (51) and lysis by human CTL of murine hybridomas bearing surface membrane anti-CD3 immunoglobulin (32) are inhibited.

LFA-1 is absent from resident or thioglycollate-elicited peritoneal macrophages but is present on activated, tumoricidal macrophages. LPS and IFN-γ induce LFA-1 on thioglycollate-elicited macrophages in vitro (52). Pretreatment with LFA-1 $F(ab')_2$ inhibits selective binding of activated murine macrophages to tumor cells and prevents development of weak into strong binding as shown by the centrifugal force required for dissociation (53).

Inherited LFA-1, Mac-1, and p150,95 Deficiency

A novel immunodeficiency disease has been defined in which expression of LFA-1 and the related Mac-1 and p150,95 glycoproteins is selectively defective (54–57). Mac-1, p150,95, and LFA-1 are $\alpha\beta$ heterodimers that have identical β subunits. The α subunits are distinct but are 33–50% identical in amino acid sequence (58) (L. J. Miller, M. Wiebe, T. A. Springer, submitted). LFA-1, Mac-1, and p150,95 thus constitute a family of related $\alpha\beta$ complexes. Mac-1 (and p150,95) mediate 'nonspecific' adhesion of granulocytes and monocytes to endothelial cells and other substrates and also function as the complement receptor type 3 (CR3), binding to the complement component iC3b. In common with LFA-1, adhesion reactions mediated by Mac-1 and p150,95 require Mg^{+2}. Mac-1 and p150,95 are stored in intracellular pools in circulating monocytes and granulocytes; binding of chemoattractants to specific receptors results in translocation of Mac-1 and p150,95 to the cell surface. Adhesiveness of

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these cells is thereby increased, and this appears to mediate binding to endothelial cells and localization in inflammatory sites. A detailed discussion and pertinent references to the Mac-1 and p150,95 glycoproteins and the inherited disease is presented in a review by Anderson & Springer (6).

Patients deficient in LFA-1, Mac-1, and p150,95 are characterized by recurrent life-threatening bacterial and fungal infections, progressive periodontitis, lack of pus formation, and leukocytosis. Granulocytes, monocytes, and lymphocytes from patients display profound defects in both in vivo and in vitro adherence-dependent immune functions. We have suggested the designation leukocyte adhesion deficiency (LAD) for this disease, which has now been characterized in 30 patients worldwide. Quantitative analysis of Mac-1 and LFA-1 surface expression by flow cytometry indicates that all leukocytes are affected. There are two patient phenotypes, designated severe (<0.3% of normal expression) and moderate (5–10% of normal expression) deficiency (20, 54). For a given patient, the three α subunits and the common β subunit are deficient to similar extents, as shown with subunit-specific MAbs. The severity of the clinical complications correlates directly with the degree of LFA-1 deficiency. That patients with severe deficiency rarely survive beyond childhood underscores the importance of this family of molecules in vivo.

The deficiency of LFA-1, Mac-1, and p150,95 appears due to a defect in the common β subunit. Biosynthetic labeling of LFA 1–deficient T-cell blasts and EBV-transformed B cells shows that a normal amount of the LFA-1 α-chain precursor is made, but little is processed or transported to the cell surface (20, 59). Patient cells lack both α and β subunits on the surface. In mouse × human hybrids formed with patient cells (11), the patient LFA-1 α subunit complexes with the mouse β subunit, and the interspecies complex is expressed on the cell surface. Hybrids expressing the human β subunit complexed with the mouse α subunit can be derived from healthy human but not from patient cells. These results show that the α subunit is competent for surface expression if a functional β subunit is present and imply that the β subunit is defective. Immunoprecipitation with a rabbit antiserum produced against purified, denatured β subunit has recently confirmed that the β subunit is defective and has revealed that in some patients the β-chain precursor is of an aberrant size (T. K. Kishimoto, D. C. Anderson, and T. A. Springer, manuscript in preparation).

Chromosomal analysis of human × mouse hybrids shows that the LFA-1 α-chain gene is encoded on human chromosome 16 (11). The β-chain gene, and hence the genetic defect, is on chromosome 21 (11, 60). This agrees with the autosomal recessive inheritance of LAD (6).

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Functional Consequences of LFA-1 Deficiency

Defects in adhesion-dependent lymphocyte functions have been observed in patients with LAD. Moderately to profoundly impaired proliferative responses to mitogens, allogeneic cells, and antigen were found in all (37, 55, 56, 61, 62) but one (63) study. Proliferation was most impaired at suboptimal mitogen doses ($\bar{x} = 12\%$ of normal) (55, 56, 61, 62) and the dose-response curve was shifted (62). Mitogen proliferative responses of lymphocytes of LFA 1–deficient patients were further depressed when LFA-1 MAb was added (37, 55, 56). This agrees with the finding that deficiency in most patients is quantitative rather than absolute and shows that small amounts of LFA-1 present on patient lymphocytes can be functionally important. IFN-α and IFN-γ production by MLR or mitogen stimulated patient lymphocytes was severely deficient (62, 63).

After primary mixed lymphocyte culture, cytolytic T lymphocyte–mediated killing was 8–40% of normal ($\bar{x} = 18\%$ for 4 patients) and was more depressed in the severe than moderate phenotypes (61, 63). Natural killing ranged from strikingly deficient ($\sim 10\%$ of normal) (34, 61–63) to normal (37, 64). Antibody-dependent cytotoxicity by K cells and polymorphonuclear leukocytes was markedly depressed (34) or normal (37). These differences may be related to the extent of LFA-1 deficiency (34, 61). After repeated restimulation with allogeneic cells, CTL lines could be established from patient lymphocytes that showed cytolytic activity somewhat lower (61) or comparable (37, 55) to normal. The improvement in patient T-cell function after secondary stimulation (61) suggests that T lymphocytes can partially adapt to LFA-1 deficiency, perhaps by clonal selection of T lymphocytes with high-affinity antigen receptors.

For 5 of 6 patients, cytolysis by CTL, NK cells, and ADCC effectors was further diminished by LFA-1 MAb (37, 55, 61), and killing by patient cells was inhibited by much lower than normal concentrations of anti–LFA 1 MAb (37, 61). The above studies used LFA-1^+ target cells. Killing by LFA-1–deficient patients' cells was more markedly depressed with LFA-1^- target cells (65). With normal CTL, LFA-1 MAb have been found to exert their inhibitory effect by binding to the CTL (8, 13, 25, 61), although pretreatment with MAb of both target cells and effectors has been shown to be more inhibitory than pretreatment of effector cells only (8). In comparable studies with patient CTL, LFA-1 MAb pretreatment of LFA-1^+ target cells only (severe deficiency) or of both CTL and LFA-1^+ target cells (moderate deficiency) was found to be inhibitory (61). This shows that LFA-1 on both the CTL and target cell can be functionally important.

Antibody production by B lymphocytes in LAD patients was found to

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be abnormal in vitro (37, 43) and abnormal in vivo in some patients (37, 43, 66). Strikingly, repeated immunization with tetanus and diphtheria toxoids, *Bordetella pertussis*, and influenza virus produced no response, but anti-mannose antibodies were produced in response to chronic candida infection and total Ig levels were elevated (43). In other patients, antibody production, T lymphocyte–dependent DTH, and recovery from viral infection occur, but very little quantitative data has been published.

Lymphocytes are present in normal amounts and other lymphocyte surface antigens are normal in LAD. T- and B-lymphocyte defects occur in LAD, but it is not surprising that they are milder than in a disease in which lymphocytes are missing altogether, as in severe combined immunodeficiency disease. Other accessory molecules, levels of LFA-1 still present on patient cells, and antigen-receptor-affinity maturation may contribute to patient lymphocyte responsiveness. The dramatic defects in granulocyte and monocyte mobilization in LAD have clinically overshadowed lymphocyte defects. However, lymphocyte defects in antibody production to bacteria (66) may contribute to the recurring bacterial infections. In addition T lymphocyte defects may be related to characteristic chronic mucous membrane and cutaneous candidal infections, and to the death of one patient due to picorna virus infection [54].

Prevention of Graft Rejection by LFA-1 MAb

The poor prognosis of LAD patients prompted Griscelli, Fischer, and coworkers (67) in France to attempt bone marrow transplants to correct the deficiency in two patients. Although only HLA-mismatched bone marrow was available, both transplants were accepted and the recipients are disease-free. Fischer et al (68) observed that LAD patients, all of whom did not mount allogeneic mixed lymphocyte responses, did not reject grafts. In their previous experience with bone marrow transplantation, this group found that T cell-depleted, HLA-mismatched bone marrow could be accepted by patients with severe combined immunodeficiency but was rapidly rejected by patients with other immune disorders who could mount allogeneic mixed lymphocyte responses.

The acceptance of HLA-mismatched bone marrow by LFA-1 deficient patients suggested that LFA-1 may be important in graft rejection. Since (*a*) graft rejection can be mediated by both T and non-T cells, (*b*) LFA-1 MAb inhibit both T cell and NK immune functions in vitro and (*c*) LFA-1 is low or absent on hematopoietic stem cells (15, 16), Fischer et al (68) treated graft recipients with 0.1 mg kg^{-1} anti-LFA-1 α subunit MAb from 3 days before to 5 days after transplantation. Recipients had a variety of inherited diseases such as Wiskott-Aldrich syndrome and osteopetrosis, and all received HLA-mismatched transplants. The use of LFA-1 MAb

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resulted in 7/7 successful engraftments, a dramatic improvement over previous experience. Thus, the clinical experience with LAD, and concepts based on the functional effects of LFA-1 MAb in vitro, have led to new treatment modalities in the therapy of other diseases.

LFA-1 in Antigen-Independent Adhesion

Lymphocyte activation is accompanied by increased adhesiveness and motility. Although specific antigen may be used to stimulate increased adhesiveness, stimulated lymphocytes show a generalized increased adherence to cells lacking specific antigen. Cells cultured in the MLR acquire the ability to adhere to a wide variety of tumor cell types. There is no MHC restriction in this adhesion, although species specificity has been shown (69). Adhesion of lymphocytes to one another can also be measured as cluster formation, i.e. aggregation. After autologous MLR or periodate stimulation, 5–35% of the viable lymphocytes are found in clusters (70). Lymphocytes isolated from clusters by vigorous vortexing readily reaggregate. Aggregation is also induced by phorbol esters, perhaps by bypassing specific activation mechanisms through direct stimulation of protein kinase C (71). Within 15 min, human PBL show uropod formation, and within 30 min, exhibit hairy surface projections, ruffled membranes, and the beginnings of aggregation (72). Similar aggregation is seen with monocytes (72) and some leukocyte cell lines, including EBV-transformed B lymphocytes (73).

Antigen-independent aggregation of a single cell type (homotypic adhesion) has recently been found to be an excellent model system for studying the cell biology of LFA 1–dependent adhesion. Phorbol ester–induced homotypic aggregation of T, B, and myeloid lineage cells (22, 30) and peripheral blood lymphocytes (22, 23) is inhibited by anti-LFA-1 MAb (Figure 1A,B). Clustering of MLR, autologous MLR, lectin, periodate, and lipopolysaccharide-activated lymphocytes (70), spontaneous clustering of EBV B-lymphoblastoid cells (74), and clustering of monocytes cultured in IFN-γ [75] are also LFA-1 dependent.

The importance of LFA-1 in homotypic adhesion has been further demonstrated by the finding that phorbol ester–activated lymphocytes from LFA 1–deficient patients fail to aggregate (22). LFA-1^- lymphocytes, however, are able to coaggregate with LFA-1^+ lymphocytes (22). This demonstrates that LFA 1–dependent adhesion is not mediated by homophilic interactions whereby LFA-1 molecules on one cell interact with those on another, and it is consistent with observations of LFA 1–dependent CTL and NK interactions with LFA-1^- targets (8, 48, 65, 76–78).

The characteristics of phorbol ester–stimulated lymphocyte aggregation and the adhesion step in CTL-mediated killing are similar. LFA 1–depen-

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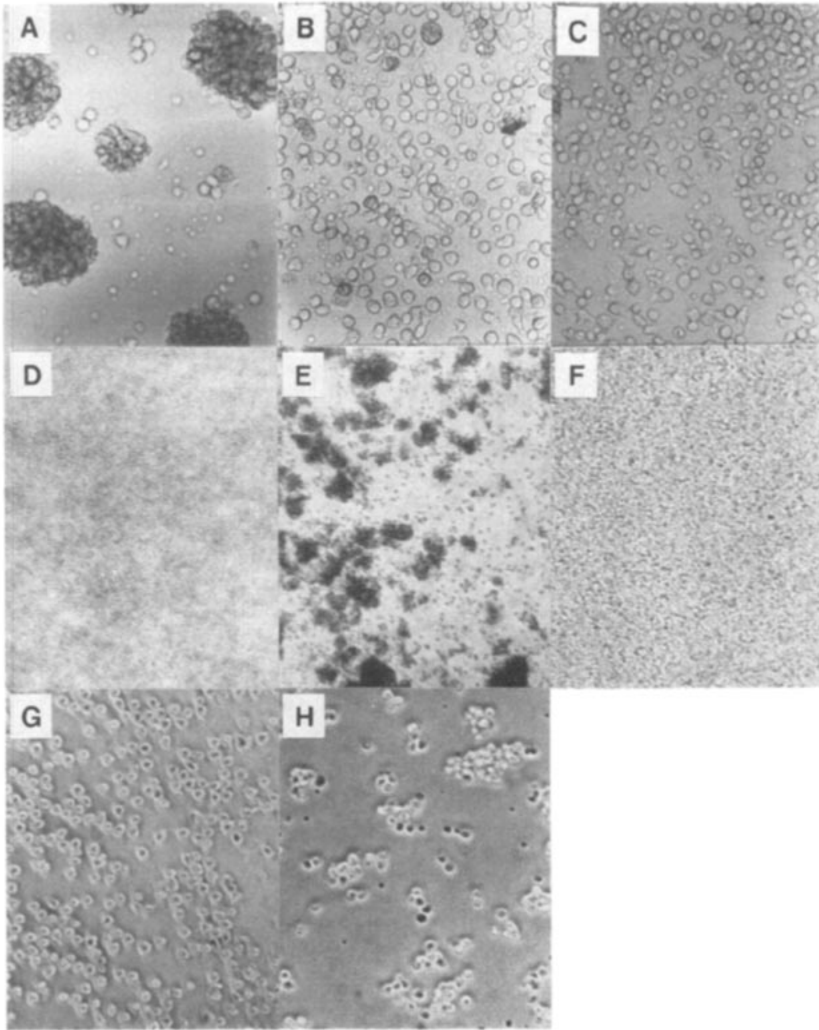

Figure 1 Visualization of LFA molecule–dependent adherence in homotypic aggregation assays [22, 82, 104]. A-C: LFA-1– and ICAM-1-dependent adherence of phorbol ester-stimulated JY lymphoblastoid cells at 37°C. JY cells aggregated in the presence of control MAb (A), but not in the presence of LFA-1 MAb (B), or ICAM-1 MAb (C). D-F: LFA-3-dependent aggregation of human erythrocytes in the presence of added CD2 at 4°C. Human erythrocytes were aggregated at 4°C by 100 μg ml[1] purified, native CD2 protein (E) but not by heat denatured CD2 (D). Aggregation was reversed by addition of 5 μg ml^{-1} LFA-3 MAb (F). G, H: LFA-3-dependent aggregation of JY lymphoblastoid cells in the presence of added CD2 at 4°C. JY cells do not aggregate at 4°C (not shown) except in the presence of purified CD2 (H). Aggregation is reversed by LFA-3 MAb (G).

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dent aggregation requires Mg^{+2}, and Ca^{+2} has a synergistic effect with suboptimal concentrations of Mg^{+2} (5, 22). A cytoskeleton with functional microfilaments is required for aggregation as shown by inhibition with cytochalasin B (22, 79). Aggregation is energy and temperature dependent (5, 80). The similar requirements for Mg^{+2}, a functional cytoskeleton, energy, and temperature in both CTL adhesion (5, 40) and phorbol ester–stimulated aggregation may all be intimately related to the involvement of LFA-1 in these processes.

Lymphocyte activation appears to be required for the LFA 1–dependent, antigen-independent adhesion system to become operative. LFA-1$^+$ T and B lymphocytes and thymocytes do not aggregate significantly in the absence of activation by culture, antigen, mitogen, phorbol ester, or EBV transformation (22, 23, 70, 80). Since phorbol-ester activation does not increase the amount of cell surface LFA-1 and protein synthesis is not required (22, 79, 81, 82), some other mechanism must be responsible for the enhanced LFA 1–dependent adhesion after activation. Phorbol esters are pleiotropic, inducing pseudopod formation, motility, and more rapid capping in blood lymphocytes (72, 83) (but not EBV-transformed cells: 22). Some phosphorylation of the LFA-1 β subunit is induced by phorbol esters (84). However, the molecular mechanism(s) which regulate LFA 1–dependent adherence remain unknown.

Antigen-Independent Interactions with Nonhematopoietic Cells

The interaction of T cells with vascular endothelium is a prerequisite to the migration of lymphocytes into sites of inflammation and is important in the pathophysiology of graft rejection. T-lymphocyte adherence to endothelial cells, augmented by phorbol-ester stimulation of the T cells, is inhibited by LFA-1 MAb (81). T lymphoblast adherence to endothelial cells and fibroblasts is also LFA-1 dependent (85, 86). Binding of lymphocytes to high endothelial venules in vitro and homing of lymphocytes to peripheral lymph nodes in vivo is specifically inhibited 50% by LFA-1 MAb; MAb to a lymphocyte homing receptor inhibits more completely (87).

LFA-1 in Antigen-Dependent Adhesion to Nonhematopoietic Cells

Elegant studies with antigen-specific T-cell hybridomas have shown that LFA-1 MAb block antigen presentation by lymphoid cells but do not block presentation by Ia-transfected fibroblasts or artificial Ia-containing membranes (48, 78, 88–90). Similarly, anti–LFA 1 MAb can inhibit the killing of hematopoietic target cells but not nonhematopoietic targets by

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the same CTL line (77, 90, 91). However, other studies have shown LFA-1 MAb can block killing of transfected fibroblasts even across a species barrier (92) and can moderately inhibit killing of endothelial cells (76, 91).

These discrepancies are not understood, and the idea that a ligand for LFA-1 is absent from nonhematopoietic cells is one of several possible explanations. Some feature of the surface of nonhematopoietic cells, which correlates with their tendency to grow as adherent cells, may facilitate stronger and hence LFA 1–independent adherence by lymphocytes. Both LFA 1–dependent and –independent mechanisms of adherence to endothelial cells have been defined (81). Endothelial cells and fibroblasts secrete extracellular matrix components such as fibronectin; perhaps extracellular matrix receptors (which appear related to LFA-1, see below) can substitute for LFA-1. Variable expression of the putative cell surface ligand for LFA-1, ICAM-1 (see below) might also explain some of the conflicting results.

A Unifying Hypothesis on the Involvement of LFA-1 in Specific Receptor-Dependent and -Independent Adhesion

The T-lymphocyte antigen receptor was held early on to have three functions: to dictate antigen specificity, to stabilize adhesion to the antigen-bearing cell, and to trigger delivery of effector functions. However, the function of the antigen receptor in stabilizing adhesion has received varying experimental support.

The antigen specificity of adhesion can be measured by comparing CTL conjugation with target cells bearing or lacking specific antigen. This topic has recently been reviewed in more detail by Martz (5). The ratio of specific: nonspecific adhesion for binding of mouse CTL, generated in vivo, to allogeneic tumor target cells is typically five-fold, and ranges from two- to fifteen-fold in different studies (5). In contrast, studies with cloned human CTL stimulated in vitro have shown equally strong conjugation with specific alloantigen positive and negative tumor target cells (42, 93). Thus, while the antigen receptor can contribute to target cell adhesion, antigen-independent conjugates are always found at significant levels, and sometimes they predominate.

In both mouse and human studies, the specificity of target cell killing is much (~thirty-fold) higher than that of conjugate formation (5). Thus, much or all of the specificity contributed by the antigen receptor appears to be in triggering effector function, while the contribution of the antigen receptor to adhesive specificity is variable. Antibodies to framework determinants of the antigen receptor and to the associated T3 molecule have been reported to inhibit killing but not conjugate formation, while anti–LFA 1 and Lyt-2 (CD8) MAb inhibited adhesion in parallel experiments

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(42, 94). However, these results are from systems in which conjugation was not antigen specific (42) or in which specificity was not tested (94). It has been suggested that accessory molecule–dependent adhesion precedes antigen receptor ligation (42); however, results of experiments in the mouse system demonstrating antigen-specific adhesion argue that the antigen receptor can contribute to adhesion. While antigen-independent adhesion may procede antigen receptor engagement in systems in which conjugation is not antigen specific (42, 93), there is no kinetic evidence bearing on this question. The existence of both antigen-dependent and -independent conjugates suggests variations in the contribution of antigen-dependent and -independent mechanisms.

How is LFA 1–dependent adhesion activated physiologically? Reasoning by analogy to phorbol ester–stimulated homotypic adhesion, we hypothesize that binding of the antigen receptor during initial T cell–antigen presenting cell or effector cell–target cell contact releases diacyl glycerol (the physiologic activator of protein kinase C) and stimulates LFA 1–dependent adherence. Thus, binding of specific receptors to their ligands would provide only a small part of the decrease in free energy required to stabilize cell-cell adhesion; most would be provided by LFA-1. LFA-1 thus is hypothesized to provide a mechanism for amplifying adherence. Triggering of adhesion strengthening mechanisms by antigen receptor ligation would make antigen recognition more sensitive. We believe that the difference between CTL which show antigen-specific conjugate formation and those which show antigen-independent conjugate formation is that in vitro propagation and repeated stimulation with antigen of the latter cells has already activated LFA 1–dependent adherence to such an extent that it cannot be further elevated by antigen recognition.

Relation to Extracellular Matrix Receptors

Like LFA-1, the cell surface receptors for extracellular matrix components such as fibronectin are $\alpha\beta$ heterodimers (95). The N-terminal sequences of the α subunits of the vitronectin receptor and platelet gpIIb IIIa protein (a receptor for fibronectin, fibrinogen, and vitronectin) are homologous to the LFA-1 and Mac-1 α subunits (96a,b). The cDNA sequence of the human LFA-1 β subunit (Kishimoto, O'Connor, Lee, Roberts, Springer, 96c) shows $\sim$45% homology to the chicken integrin (fibronectin receptor) β subunit (97). These results suggest that the LFA-1, Mac-1, and p150,95 family and the extracellular matrix receptor family constitutes a supergene family of adhesion molecules. Position-specific proteins that appear to control cell migration and localization in the developing Drosophila embryo may also belong to this supergene family (95). Although it has been proposed that the α subunits of Mac-1, LFA-1, and gpIIb IIIa are

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derived by differential splicing (98), the Northern blots with a Mac-1 DNA clone (101) and the absence of shared peptides (99, 100) do not support this idea.

Structural similarities between extracellular matrix receptors and the LFA-1, Mac-1, and p150,95 leukocyte adhesion proteins strongly suggest functional similarities. Extracellular matrix receptors recognize a core sequence of *arg-gly-asp* within ligands, and additional ligand sequences can modify specificity (95). Although a synthetic peptide containing the fibronectin recognition sequence failed to inhibit phorbol ester–stimulated lymphocyte aggregation (R. Rothlein, M. Pierschbacher, T. A. Springer, unpublished), it remains possible that LFA-1 recognizes ligand(s) containing similar sequences.

Matrix receptors form a link between the extracellular matrix and the cytoskeleton and are localized at adhesion plaques (97). It is interesting that LFA-1 has been found co-localized with actin at the site of adhesion between NK cells and targets (102). Modulation by lymphocyte activation of the interaction between LFA-1 and the cytoskeleton would be an attractive mechanism for regulating LFA 1–dependent adherence.

ICAM-1: A Putative LFA-1 Ligand

Coaggregation of LFA-1$^+$ cells with LFA-1$^-$ cells and LFA 1–dependent interactions of CTL and NK cells with LFA-1$^-$ targets suggest that at least one other molecule, perhaps a ligand for LFA-1, is involved in LFA 1–dependent leukocyte adhesion. To detect such molecules, MAb were elicited to lymphocytes from LFA-1 deficiency patients and screened for inhibition of phorbol ester–induced aggregation of LFA-1$^+$ cells (82). One MAb inhibited aggregation by reacting with a novel antigen distinct from LFA-1. This antigen, intercellular adhesion molecule-1 (ICAM-1), is widely distributed on cells of both hematopoietic and nonhematopoietic origin (86). ICAM-1 is expressed in low levels on peripheral blood cells and in higher levels on mitogen-activated T lymphoblasts, EBV-transformed B cells, and some cell lines of T cell and myeloid lineage (82, 86). Immunohistochemical staining of thin sections has shown that ICAM-1 is expressed on most vascular endothelial cells, tissue macrophages, germinal center dendritic cells, and thymic and mucosal epithelial cells (86). Expression on vascular endothelium is greatest in inflammation. ICAM-1 is a heavily glycosylated protein with a heterogeneous weight ranging from 90 kd to 114 kd. The deglycosylated precursor is 55 kd (86).

The ICAM-1 MAb inhibits the LFA 1–dependent phorbol ester–induced aggregation of T-lymphoblasts, B-lymphoblastoid, and myeloid cell lines, and also the binding of T lymphocytes to fibroblasts (Figure 1A, C) (82, 86). The binding of T cells to fibroblasts can be inhibited by either

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anti–ICAM 1 treatment of fibroblasts or by anti–LFA 1 treatment of the T cells. The inhibitory effects of anti–LFA 1 and anti–ICAM 1 are not additive. These results suggest a possible receptor-ligand interaction. ICAM-1 expression on dermal fibroblasts is increased several fold over a 4–10 hour period after treatment with interleukin-1 (IL-1) or interferon-γ (IFN-γ), and increased expression directly correlates with increased ICAM 1–dependent T-cell binding (86). Similarly, IL-1 and IFN-γ, as well as tumor necrosis factor, induce a rapid rise in ICAM-1 expression on endothelial cells (103).

The expression of ICAM-1 on cells at inflammatory sites and its induction on fibroblasts and endothelial cells by cytokines suggest that ICAM-1 may regulate adherence during inflammatory and immune responses. Up-modulation of ICAM-1 may facilitate margination and subsequent movement of lymphocytes into inflammatory regions and may potentiate the immune response. ICAM-1 expression may also regulate adhesion of monocytes. When monoblastoid U937 cells are induced to differentiate along the monocytic pathway, ICAM-1 expression increases almost twenty fold and correlates with the induction of LFA-1 dependent aggregation (82, 86).

The distribution of ICAM-1 and its role in many LFA 1–dependent adhesion systems make it an attractive candidate for the ligand of LFA-1. However, ICAM-1 MAb fails to inhibit the LFA 1–dependent aggregation of one T-cell line (82), and this argues against a hypothesis that ICAM-1 is the sole ligand for LFA-1. ICAM-1 may be a member of a family of related LFA-1 ligands, only some of which bear the determinant defined by the ICAM-1 MAb.

CD2 AND LFA-3

CD2 Is a Receptor for the Cell Surface Ligand LFA-3

The widely distributed surface molecule LFA-3 has recently been shown to be the ligand for the T-lymphocyte surface molecule CD2 (93, 104, 105). Inhibition of CTL-mediated killing and helper-T-lymphocyte functions by anti-CD2 and anti-LFA-3 appears to be due to the ability of these MAb to inhibit binding of CD2 to its ligand, LFA-3. The emphasis of the following section is to discuss the data supporting this hypothesis. Another mechanism involving negative signaling via CD2 or LFA-3 is also considered. Finally, the concept of stimulation of T-lymphocyte function through CD2 and the potential role of LFA-3 in this signaling is examined.

CD2 is a glycoprotein of 45–50 kd found on all T lymphocytes, large

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granular lymphocytes, and thymocytes (9, 13, 106–109). CD2 (cluster of differentiation 2) is the internationally accepted nomenclature (110) for the antigen variously referred to in earlier work as OKT11, T11, LFA-2, Leu5, Tp50, and the sheep erythrocyte receptor (9, 13, 106–109, 111). MAb to CD2 inhibit a variety of T-lymphocyte functions including antigen-specific T lymphocyte–mediated cytolysis and T lymphocyte–proliferative responses to lectins, alloantigens, and soluble antigens (9, 13, 109, 112, 113). CD2 MAb inhibit CTL-mediated killing by binding to the CTL rather than the target cell (which is often $CD2^-$) (13). CD2 MAb inhibit conjugation of CTL to target cells (29). Some (109, 114, 115) but not other (13, 109) CD2 MAb partially inhibit NK activity. CD2 MAb also inhibit rosetting of sheep erythrocytes with human T lymphocytes (106–108), as described in more detail below, and inhibit antigen-independent conjugation of thymocytes, T lymphoblasts, and CTL to B lymphoblast and K562 cells (93, 116, 117).

Inhibition of proliferation of peripheral blood lymphocytes and T-cell lines by anti-CD2 MAb is accompanied by a failure to induce IL-2 mRNA accumulation and IL-2 secretion, and a failure to express IL-2 receptor mRNA and protein (113, 118–122). The effects of anti-CD2 MAb are overcome in some systems by addition of exogenous IL-2, suggesting that the failure to express IL-2 receptor is secondary to the failure to secrete IL-2 (119, 121).

An MAb to a target cell structure (LFA-3) involved in CTL activity was described concurrently with anti-human LFA-1 and LFA-2 (CD2) MAb (9, 13). Since the LFA-3 MAb inhibited CTL-mediated killing by binding to the target cell, it was speculated that LFA-3 might be a ligand for either LFA-1 or CD2; it now appears that the latter is correct. This is discussed below. LFA-3 has a weight of 55–70 kd and has a broad tissue distribution including expression on endothelial, epithelial, and connective tissue cells in most organs studied and on most blood cells including erythrocytes (13, 104). LFA-3 has been mapped to chromosome 1 (123). LFA-3 MAb, like CD2 MAb, also inhibits a number of T helper lymphocyte–dependent functions (13) and inhibits conjugate formation between CTL and target cells (29, 93, 117).

Studies on antigen-independent conjugation of CTL to B-lymphoblastoid target cells have clarified the relationships of the LFA-1, CD2, and LFA-3 avidity-enhancing mechanisms (93). MAb to each antigen partially ($\sim 50\%$) inhibit conjugate formation. Combinations of saturating concentrations of LFA-1 MAb and CD2 MAb or LFA-1 MAb and LFA-3 Mab inhibit conjugate formation totally and thus are additive, while the combination of CD2 MAb and LFA-3 MAb is not any more effective than either MAb alone (13, 93). The LFA-1 dependent and the CD2/LFA-3

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dependent pathways were further resolved by the dependence of the LFA-1 pathway but not the CD2/LFA-3 pathway on Mg^{+2} and temperature (93). Studies with purified CD2 and autologous E-rosetting have recently directly demonstrated an interaction between CD2 and LFA-3 (104).

Binding of human T lymphocytes to sheep (E)rythrocytes is an antigen-independent adhesion termed "E rosetting." E-rosetting has long been used as a technique for the purification of human T cells. Since some MAb against CD2 inhibit E-rosetting, CD2 has been inferred to be the "sheep E rosette receptor" (106–108, 112, 124). CD2 has recently been purified to homogeneity from the tumor cell line Jurkat (125). Purified CD2 inhibits T-lymphocyte E-rosetting and absorbs specifically to sheep E (125), confirming that CD2 interacts directly with a ligand on sheep E.

Human E, like sheep E, rosette with thymocytes, activated T lymphocytes, and some T-cell tumors; however, human E do not rosette with resting T cells (126). Thus, human E may express a ligand for CD2 analogous to that on sheep E. Since CD2 and LFA-3 are involved in the same functional adhesion pathway in CTL-target conjugation, the role of LFA-3 in rosetting of human T lymphocytes with human E was examined (104). Rosetting could be abolished by pretreating T lymphocytes with CD2 MAb or by pretreating E with LFA-3 MAb, suggesting a parallel between the CD2/LFA-3 functional pathway in CTL adhesion and E-rosetting. Experiments with purified CD2 suggest that it binds to LFA-3 (104a,b). Saturable binding of iodinated CD2 to human erythrocytes and to the B-lymphoblastoid cell line JT, a good target in CTL-mediated killing, is inhibited by LFA-3 MAb (104a,b). Reciprocally, preincubation of cells with purified CD2 inhibits LFA-3 MAb binding. High concentrations of purified CD2 aggregate sheep and human erythrocytes and the B lymphoblastoid cell line JY. Aggregation of human E and JY cells by purified CD2 protein is inhibited by anti-LFA-3 MAb (Figure 1D-H). This is the first demonstration that a purified lymphocyte protein can mediate adhesion and suggests that purified CD2 and its membrane bound counterpart bind directly to LFA-3 on human cells. Reciprocal results have recently been obtained with purified LFA-3 (104c), and cell surface CD2 shown to mediate binding of T lymphocytes to purified LFA-3 reconstituted into planar membranes.

An MAb to a sheep erythrocyte cell surface determinant that inhibits sheep E-rosetting with human T-lymphocytes has recently been reported (127). This MAb recognizes a 42-kd glycoprotein which may be the sheep erythrocyte equivalent of LFA-3; it does not cross-react with human erythrocytes. The purified antigen blocks sheep E-rosetting and reduces the staining of human T-lymphocytes by anti-CD2 MAb in immunofluorescence flow cytometry, suggesting that the antigen binds to CD2

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(127). Hence this antigen was called the T11 target structure (T11TS). T11TS MAb inhibits the sheep mixed lymphocyte reaction, and the pattern of expression of T11TS on sheep peripheral blood lymphocytes and activated T-lymphocytes (128) is very similar to that of LFA-3 on human cells (13).

The Negative Signal Hypothesis

Negative signal transduction has been suggested as an alternative mechanism for inhibition of function by anti-LFA-3 and anti-CD2 MAb. In this hypothesis, the CD2 or LFA-3 molecules would be critical membrane transport proteins, channels, etc of general importance but not specifically involved in immune interactions; MAb bound to these molecules would inhibit many functions including adherence. It was suggested that anti-CD2 MAb may elicit a negative signal since removal of the CD2 epitope with trypsin eliminated the ability of anti-CD2 to inhibit the response to mitogenic anti-CD3 MAb (113). Anti-CD2 MAb also inhibited proliferation induced by the Ca^{+2} ionophore A23187 suggesting that the 'negative signal' occurred subsequent to the rise in intracellular Ca^{+2} (113). In a study on CTL-mediated killing, removal of the LFA-3 determinant by trypsin treatment of target cells did not affect their susceptibility to lysis, suggesting that LFA-3 did not participate in an adhesion strengthening interaction (129). Similar conclusions were reached in studies with human × mouse hybrids. The presence or absence of human LFA-3 on hybrid cells failed to correlate with their susceptibility to lysis by human CTL, while anti-LFA-3 could inhibit CTL-mediated killing of LFA-3$^+$ but not LFA-3$^-$ hybrid cells (123). The studies with trypsinization and somatic cell hybrids (113, 123, 129), are difficult to interpret, however, since the effects of CD2 and LFA-3 MAb were never examined in the same experiment. It would be interesting to extend the above studies by examining the effects of CD2 MAb and LFA-3 MAb in parallel. The possibility has not been examined that some of the inhibitory effects of anti-CD2 MAb on helper-T-lymphocyte functions (13, 112, 113, 118–122) may be mediated by abrogation of cell-cell contact and adhesion. Therefore, some data that have been interpreted as indicating negative signaling via CD2 may be due to inhibition of critical cell-cell interactions. Indeed, one study noted the failure of mitogen-stimulated lymphocytes to cluster in the presence of anti-CD2 (113). Because our conclusion that CD2 interacts with LFA-3 is supported by direct observations with purified molecules, we believe that it is more convincing than the negative signal hypothesis, which is based on complicated functional experiments.

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Stimulation of Function by CD2 MAb

Activation-related epitopes have been defined on CD2 which are strongly expressed on thymocytes and activated mature lymphocytes but are absent or weakly expressed on resting peripheral blood lymphocytes (111, 130–132). Specific combinations of MAb to certain of these CD2 epitopes can result in proliferation which is IL-2 dependent (111, 133). This has been termed an 'alternative pathway' of T-cell activation, in contrast to the antigen receptor-CD3 complex "classical pathway." In one case an activation epitope (called $T11_3$) was induced on peripheral blood T lymphocytes by MAb to another non-E-rosette blocking epitope ($T11_2$); a combination of anti-$T11_2$ and anti-$T11_3$ led to proliferation in the absence of accessory cells (111). The $T11_2$/$T11_3$ MAb combination was also capable of inducing T-helper activity for antibody responses (111), antigen-independent killing by CTL clones, and killing of inappropriate targets by NK clones (134). Expression of the CD2 activation epitope D66 was increased after incubation with D66 MAb and a Fab fragment of rabbit anti-mouse IgG at 4°C (130). It was subsequently demonstrated that combinations of E-rosette blocking anti-CD2 MAb and D66 MAb could also stimulate proliferation, but only in the presence of accessory cells (133). The accessory cells (monocytes) in this system were contributing an Fc receptor–dependent interaction (133). With thymocytes, the combination of anti-CD2 MAb to the $T11_2$ and $T11_3$ epitopes failed to induce proliferation but did induce expression of IL-2 receptors and addition of exogenous IL-2 resulted in proliferation (135).

It has been suggested that the ability of MAb binding to specific epitopes on CD2 to induce or augment expression of the $T11_3$- and D66-type epitopes may be due to a conformational change in the CD2 molecule, because these effects occur rapidly and at 0°C (111, 130). However, it is also possible that the MAb to the activation epitopes bind with low avidity and that clustering of CD2 by a specific second anti-CD2 (resulting in a specific cluster geometry) enhances binding to the activation epitope by allowing bivalent interaction.

Do components of the 'classical pathway' (the antigen receptor–CD3 complex) regulate the 'alternative pathway' of T-cell activation? The modulation of CD3 (and the antigen receptor) with anti-CD3 prior to exposure to mitogenic combinations of anti-CD2 MAb abrogates subsequent proliferation in response to otherwise mitogenic combinations of anti-CD2 MAb (111, 133). However, CD3 modulation results in a generalized refractory state of T lymphocytes to signals evoking $[Ca^{+2}]^i$ increases (136). Some work suggests that while CD3 may regulate signaling through CD2, CD3 is not required. IL-2 receptor expression is induced by

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combinations of CD2 MAb on both $CD3^+$ and $CD3^-$ thymocytes (135). Binding of CD3 MAb to T cells at 4°C has been shown to induce the 9–1 CD2 activation epitope. However, in this case the CD3 MAb synergizes with the 9-1 CD2 MAb in inducing proliferation in the absence of accessory cells (137).

Stimulation via mitogenic combinations of anti-CD2 MAb results in rapid increases in $[Ca^{+2}]_i$ in T-lymphocyte clones and peripheral blood T lymphocytes (138–140). Furthermore, stimulation of a population of $Fc\gamma R^+$, $CD3^-$ lymphocytes with a single MAb to a D66-like epitope induces a small but significant increase in $[Ca^{+2}]_i$, again suggesting that surface CD3 expression is not required for signaling via CD2. It has also been shown that increases in $[Ca^{+2}]_i$ in response to PHA-P are specifically blocked by anti-CD2 MAb, suggesting that the PHA-P may activate a Ca^{+2} flux in T lymphocytes via CD2 (136).

CD2 and LFA-3 in Thymic Ontogeny

The regulation of proliferation and differentiation of immature T lymphocytes in the thymus is very likely a property of the thymic microenvironment which includes a number of cell types in addition to thymocytes (141). A major role has been proposed for the thymic epithelium in this regulation based on observations in pathological and normal states. Immunohistochemical staining of thymus has demonstrated a close association between thymocytes and thymic epithelial cells, particularly in the cortex where the most immature thymocytes are localized and where expression of T-cell antigen receptor first occurs. Recent advances in culture techniques for thymic epithelium have allowed their interactions with thymocytes to be studied in vitro with enriched epithelial cell populations obtained after serial passage (142). The mechanism by which thymocytes adhere to thymic epithelial cells depends largely on CD2 and LFA-3, based on the ability of CD2 MAb and LFA-3 MAb to block rosetting of thymic epithelial cells with thymocytes (105). Furthermore, CD2 MAb and LFA-3 MAb inhibit the accessory cell function of thymic epithelial cells for PHA stimulation of macrophage-depleted thymocytes (143, 144). Thymocyte IL-2 receptor expression is inhibited by CD2 and LFA-3 MAb. Mitogen responses of cells in their native microenvironment (4-mm thymus chunks) are also inhibited.

How immature thymocyte proliferation is triggered and regulated is of key importance in understanding thymocyte ontogeny. Purified thymic epithelial cells have been shown to provide accessory cell support for mitogen-induced proliferation of mature or $CD3^+$, and $CD2^+$ thymocytes and this proliferation is inhibited by CD2 and LFA-3 MAb (143). CD2 antibodies inhibit by binding to thymocytes and LFA-3 antibody inhibits

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by binding to TE cells. Moreover, purified thymic epithelial cells have been shown as well to induce spontaneous proliferation of the most immature or $CD3^-$, $T4^-$, $T8^-$, $CD2^+$, $CD7^+$ thymocytes. These results suggest that LFA-3 is an endogenous ligand for binding and activating thymocytes through CD2.

CD2 and LFA-3 in Mature T-Lymphocyte Function

Since CD2 has a cell surface ligand on human and sheep erythrocytes (LFA-3 and T11TS, respectively) and has the ability to transduce activation signals, it would be plausible for E-rosetting to affect the activation state of T lymphocytes. It has long been known that E-rosetted T lymphocytes are functionally altered. Sheep E-rosetting has been reported to result in acquisition of responsiveness to crude activated lymphocyte supernatants by resting human T lymphocytes (145). We have recently reproduced these results and found that CD2 MAb and LFA-3 MAb inhibit E-stimulated proliferation (M. Plunkett, T. A. Springer, unpublished). It will be interesting to determine whether isolated LFA-3 can duplicate this effect and to determine the effects of LFA-3 in other thymocyte and lymphocyte functional assays.

CD2- and LFA 3–dependent, antigen-independent, CTL-target conujugation does not result in increased $[Ca^{+2}]_i$, while the antigen-dependent interaction does (117). Thus, CD2 and LFA-3 appear to act strictly as an avidity enhancing mechanism in this CTL system. It will be of interest to determine if ligation of CD2 by LFA-3 in other systems can modulate $[Ca^{+2}]_i$ or T-cell function. Localization of T cells in the skin, which is often associated with T-cell activation, is of particular interest since epithelium is rich in LFA-3 (13).

CONCLUDING PERSPECTIVES

Studies on LFA-1, CD2, and LFA-3 have established the functional importance of these molecules in a wide variety of cell-cell interactions of the immune system. They may also be important in vivo in controlling lymphocyte migration and localization in specialized microenvironments. The expression of LFA-3 on thymic epithelial cells, of ICAM-1 on follicular dendritic cells, and the regulated expression of ICAM-1 on endothelial and epithelial cells may be particularly relevant to localization in vivo.

The importance of LFA-1 in the increased adhesiveness accompanying lymphocyte activation has been established, and a model was proposed in which regulation of LFA 1–dependent adhesiveness by specific receptors amplifies adherence. CD2 and LFA 3–dependent adherence may also be

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regulated by lymphocyte activation, as is suggested by the finding that thymocytes and T lymphoblasts, but not resting lymphocytes, show CD2 and LFA 3–dependent adherence to human erythrocytes. Considerable variation in SDS-PAGE mobility of CD2 and LFA-3 on different cell types suggests heterogenous glycosylation; this, increased expression of CD2 on activated lymphocytes, and CD2 activation epitopes are among many possible mechanisms for regulating CD2 and LFA 3–dependent adherence.

Demonstration that CD2 is a receptor for LFA-3 and the identification of ICAM-1 as a putative ligand for LFA-1 have advanced our understanding of how these molecules function. Much remains to be learned at the molecular level about possible additional ligands, receptor and ligand binding sites, regulation of receptor activity, interaction with the cytoskeleton, and signal transduction. The homologies discovered between the LFA-1 family of leukocyte adhesion proteins and extracellular matrix receptors suggest many new concepts concerning functional mechanisms which can now be tested. The complete structure of the LFA-1, ICAM-1, CD2, and LFA-3 proteins will soon be known from cloned genes and these should provide rich insights for future studies on the molecular basis of lymphocyte adhesion and signal transduction.

Acknowledgments

We thank E. Martz, B. Haynes, A. Fischer, W. Golde, A. Hamann, G. Janossy, F. Takei, E. Vitetta, M. Pierschbacher, and J. Hansen for sharing prepublication manuscripts. Work from this lab was supported by NIH grant CA31798 and an American Cancer Society Faculty Award to T. A. Springer.

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