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Lymphocyte adhesion mediated by integrins

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Introduction

In order to effectively defend the body against infectious organisms, cells of the immune system should be able to circulate as non-adherent cells in blood and lymph, and to migrate as adherent cells throughout the tissues. To arrive at sites of infection and to adhere to target cells bearing foreign antigen, cells need to cross endothelial and vessel walls. To control this rapid transition between adherent and non-adherent states, lymphocytes are equipped with different adhesion receptors. Integrins form a major group of adhesion receptors mediating the adhesion events of lymphocytes with antigen-bearing cells as well as between lymphocytes and endothelial cells (fig. 1). Lymphocytes express several integrin molecules at their cell surface which may all participate in the same intercellular interaction. Apart from integrins, lymphoid cells express other adhesion receptors which may regulate cell adhesion or contribute to the integrin-mediated cell-cell interaction. Cloning of the genes encoding these molecules and their counterreceptors revealed that they belong to distinct families of structurally related proteins: the selectins, immunoglobulin superfamily and the CD44 adhesion receptor (fig. 1). Here we focus on integrin-mediated adhesion of lymphocytes; therefore, only integrins will be discussed in detail.

Integrins expressed on lymphocytes and their ligands

All integrins are heterodimers composed of an α-subunit non-covalently associated with a β-subunit (Hynes, 1987). Eight different β-subunits and fourteen different α-subunits have been identified thus far. Many distinct α-subunits can associate with only one β-subunit (Rouslahti, 1991; Hynes, 1992). In addition, certain α-subunits can associate with different β-subunits (Holzmann and Weissman, 1989; Hemler, 1990). Based on shared β-subunits, subfamilies can be distinguished within the integrin family. Only the molecules belonging to the β1, β2 and β7 subfamilies are expressed on lymphocytes (table 1), and will be discussed below.

The β1 integrins are also called VLA (very late activation antigens). Thus far, eight members of this group are defined. They are expressed on a wide variety of cells, including non-haematopoietic cells (Hemler, 1990; Hemler et al., 1990; Hemler, 1988). Most VLA bind to extracellular matrix (ECM) components such as fibronectin (FN), laminin (LM) and collagen (Coll), and thus play a role in lymphocyte adhesion and migration into tissues (Shimizu et al., 1991; Rouslahti and Pierschbacher, 1987). VLA-4 (CD49d/CD29) is the only member of the β1 integrins which recognizes, apart from the ECM component FN, a transmembrane molecule. VCAM-1 (vascular cell adhesion molecule-1) (Osborn et al., 1989; Elices et al., 1990) the ligand of VLA-4, is expressed by activated endothelium (fig. 1). Therefore, VLA-4 is also involved in lymphocyte-endothelial cell interactions. The site on the ligands recognized by many VLA contains the sequence RGD or EILDV (Rouslahti and Pierschbacher, 1986; Wayner et al., 1989).

The β2 integrins are also called the Leu-Cam family (leukocyte cell adhesion molecules), since their expression is restricted to leukocytes (T and B lymphocytes, monocytes, granulocytes), in contrast to all other integrin families. This group consist of three members: LFA-1, CR3 (or Mac-1) and p150,95 (Sanchez-Madrid et al., 1982; Keizer et al., 1985).

LFA-1 (leukocyte-function-associated antigen-1) consists of a 180-kDa α-subunit (CD11a) and a 95-kDa β-subunit (CD18) (Kurzinger et al., 1981), and is expressed on all leukocytes. It is involved in a broad range of leukocyte functions such as T-cell-mediated killing, T helper cell and B-cell responses, natural killer cell activity, monocyte-mediated antibody-dependent cytotoxicity and leukocyte adhesion to endothelial cells (Springer, 1990; Martz, 1987). The involvement of LFA-1 in all these responses has been revealed by monoclonal antibodies directed against LFA-1 that inhibit these processes (Krensky et al., 1983). LFA-1 mediates cell-cell interaction through binding of one of its three counterreceptors, ICAM-1 (intercellular adhesion molecule-1) (Marlin and Springer, 1987), ICAM-2...
Fig. 1. Multiple adhesion receptors can contribute to the interaction of lymphocytes with endothelial cells (A) or of lymphocytes with leukocytes (T, B lymphocytes, monocytes and dendritic cells) (B).

Different families of adhesion molecules can be distinguished: blank = integrins, hatched = immunoglobulins, cross-hatched = selectins, stippled = unclassified molecules.

Table I. Integrins expressed on lymphocytes.

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Names</th>
<th>CD</th>
<th>Distribution</th>
<th>Counterstructure</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1a1</td>
<td>VLA-1</td>
<td>CD49a/CD29</td>
<td>no</td>
<td>LM, Coll</td>
</tr>
<tr>
<td>a2</td>
<td>VLA-2</td>
<td>CD49b/CD29</td>
<td>no</td>
<td>LM, Coll</td>
</tr>
<tr>
<td>a3</td>
<td>VLA-3</td>
<td>CD49c/CD29</td>
<td>no</td>
<td>LM, Coll, FN</td>
</tr>
<tr>
<td>a4</td>
<td>VLA-4</td>
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<td>no</td>
<td>FN, VCAM-1</td>
</tr>
<tr>
<td>a5</td>
<td>VLA-5</td>
<td>CD49e/CD29</td>
<td>no</td>
<td>FN</td>
</tr>
<tr>
<td>a6</td>
<td>VLA-6</td>
<td>CD49f/CD29</td>
<td>no</td>
<td>LM</td>
</tr>
<tr>
<td>β2aL</td>
<td>LFA-1</td>
<td>CD11a/CD18</td>
<td>yes</td>
<td>ICAM-1, -2, -3</td>
</tr>
<tr>
<td>aM</td>
<td>Mac-1, CR3</td>
<td>CD11b/CD18</td>
<td>yes</td>
<td>ICAM-1, FG, fc X, C3bi</td>
</tr>
<tr>
<td>aX</td>
<td>p150,95</td>
<td>CD11c/CD18</td>
<td>yes</td>
<td>FG, C3bi</td>
</tr>
<tr>
<td>β7a4</td>
<td>—</td>
<td>CD49d/CD29</td>
<td>yes</td>
<td>FN, VCAM-1</td>
</tr>
<tr>
<td>aHML</td>
<td>—</td>
<td>—</td>
<td>yes</td>
<td>?</td>
</tr>
</tbody>
</table>

Abbreviations used: Coll (collagen), fc X (factor X), FG (fibronigen), FN (fibronectin) and LM (laminin).
ADHESION MOLECULES IN LEUKOCYTE-ENDOTHELIUM INTERACTIONS

(Staunton et al., 1989a) or ICAM-3 (de Fougerolles et al., 1991; de Fougerolles and Springer, 1992).

All three ICAM are members of the immunoglobulin superfamily. ICAM-1 has five Ig-like domains, ICAM-2 has two Ig-like domains, that are 35% identical to the two N-terminal domains of ICAM-1 (Staunton et al., 1990). Recent cloning of ICAM-3 revealed that it consists of five Ig-like domains, 48% identical to ICAM-1 and 31% identical to ICAM-2 (Vazeux et al., 1992; Fawcett et al., 1992). β2 integrins which bind immunoglobulin superfamily counterreceptors recognize specific domains within the counterreceptor (Staunton et al., 1990; Diamond et al., 1991). Recently, it has been described that the LFA-1/ICAM-1 interaction can also be inhibited by peptides (Dustin et al., 1986). In contrast to LFA-1, ICAM-1 is not exclusively expressed on leukocytes but on a wide variety of cells, including endothelial cells (Dustin et al., 1986). Inflammatory mediators strongly induce ICAM-1 expression on endothelium (Dustin et al., 1986; Pober et al., 1986). ICAM-2 is found on haematopoietic cells and is highly expressed on endothelial cells. Its expression cannot be upregulated by inflammatory cytokines (Staunton et al., 1989a; de Fougerolles et al., 1991). Because ICAM-2 expression is high on endothelial venules in lymph nodes and vascular tissue, it has been hypothesized that it plays a role in leucocyte trafficking through the lymph. ICAM-3 has thus far only been found on leucocytes. Because of its high expression on resting leucocytes, it is thought to play an important role in the initiation of the immune response (de Fougerolles and Springer, 1992).

CR3 (CD11b/CD18; Mac-1) consists of a 170-kDa α-subunit and a 95-kDa β-subunit. It is expressed at low levels on a subset of T lymphocytes, whereas expression on monocytes and granulocytes is high (Miller et al., 1986; Keizer et al., 1987). In resting monocytes and granulocytes, CR3 is stored in peroxidase-negative granules (Bainton et al., 1987). Activation of these cells by inflammation mediators results in rapid degranulation and increased surface expression of CR3 (Bainton et al., 1987; Miller et al., 1987). CR3 functions as a receptor for the complement component C3bi and mediates binding of unopsonized microorganisms (Wright et al., 1983). Other ligands of CR3 are fibrinogen, Factor X (fX) and ICAM-1 (Loike et al., 1991; Diamond et al., 1990). LFA-1 and CR3 recognize distinct Ig domains on the ICAM-1 molecule (Diamond et al., 1991).

p150,95 (CD11c/CD18) consists of a 150-kDa α-subunit and a 95-kDa β-subunit. Like CR3, it is expressed on a small subset of lymphocytes, whereas it is present at high levels on monocytes, macrophages, granulocytes and dendritic cells (Miller et al., 1986; Keizer et al., 1987; Lanier et al., 1985). The α-subunit of p150,95 shows 63% homology with the α-subunit of CR3. Like CR3, p150,95 is stored in peroxidase-negative granules (Bainton et al., 1987). p150,95 binds to fibrinogen and C3bi (Loike et al., 1991; Myones et al., 1988). No transmembrane ligand of p150,95 has yet been identified. Both p150,95 and CR3 bind to denatured proteins (Davis, 1992), indicating that they are involved not only in cell-cell adhesion but also in cell-substrate adhesion.

β7 integrins α4/β7 (also called α4βp) and HML/β7 have recently been identified on lymphocytes (Erle et al., 1991; Holzmann et al., 1989). Similar to α4/β1, α4/β7 binds to fibronectin as well as to VCAM-1 (Ruegg et al., 1992).

Structure and function of integrins

Both α- and β-subunits of integrins are type I transmembrane glycoproteins with a single hydrophobic transmembrane segment and a short cytoplasmic tail. The extracellular domains of both subunits associate to form the αβ heterodimers (Hynes, 1992). The characteristics of each subunit will be discussed in detail below (fig. 2).

The extracellular domains of the α- and β-subunits

The β-subunit of integrins contains a large extracellular domain consisting of 750 amino acids (β2 integrins). Characteristic of all β-subunits is a 4-fold repeat of a cysteine-rich segment in the extracellular part of the β-subunit (fig. 2) (Kishimoto et al., 1989b). The N-terminus may be tightly folded due to internal disulphide bonding (fig. 3). The extracellular domain of the β-subunit contains a region of 100-200 amino acids that is highly conserved between different β-subunits. Point mutations in this area revealed that this region is important for α/β association and ligand binding (Kishimoto et al., 1989; Wardlaw et al., 1990; Arnout et al., 1990).

The α-subunits are also transmembrane glycoproteins and have a large extracellular part (LFA-1: 1,150 amino acids). In the N-terminal part of the protein, a 7-fold repeat of a homologous segment is located. Three (β2 integrins) or four (almost all β integrins) of these repeats at the C-terminal part contain a sequence which shows homology with the calcium binding proteins troponin C, calmodulin and parvalbumin (Tuckwell et al., 1992; Kirchhofer et al., 1991). These repeats most likely contribute to the divalent cation-binding properties of the integrins (Kirchhofer et al., 1991; Martz, 1980). This EF-h and motif is made up of 13 residues, with a coordination typically supplied by residues 1, 3, 5, 7, 9 and 12 (Larson et al., 1989; Kretsinger, 1980). The α-subunits of the β2 integrins (CD11a-c) contain the metal-binding consensus nanopptide DXDGDGXXD (D = aspartic acid, G = glycine, X = any amino acid)
Fig. 2. Schematic diagram of the structure of $\beta_2$ integrins.

The extracellular part of the $\alpha$ chain ($\alpha_L$) contains an I domain and 7 tandem repeats, of which three contain cation binding motifs (hatched). The extracellular part of the $\beta$ chain ($\beta_2$) contains four cystein-rich repeats (cross-hatched) and a 100-200 amino acid long region (stippled) which is highly conserved among the $\beta$ subunits of integrins. (*) represents sites of naturally occurring amino acid substitutions or deletions in the $\beta_2$ subunit, which results in impaired $\alpha \beta$ heterodimer formation, or the LAD syndrome. In the cytoplasmic tails of the $\alpha$ and $\beta$ chain, serine (S), tyrosine (Y) and threonines (T) residues are mentioned which can be phosphorylated (p) upon PKC activation and are important for functional activity of LFA-1 (see text).

within these repeats, but lack the glutamic acid at position 12, as is found in other typical EF-hand motifs (Tuckwell et al., 1992). It has been proposed that the glutamic acid residue, critical for cation binding, is provided by: 1) a segment in the $\alpha$-subunit; 2) an oxygenated residue or segment within the $\beta$-subunit, or; 3) an aspartate residue present in the ligands recognized by these receptors (Dransfield, 1991; Ginsberg et al., 1991). Binding of the divalent cations Ca$^{2+}$ and Mg$^{2+}$ is necessary for receptor function (Martz, 1980; Staatz et al., 1989; Elices et al., 1991; Rothlein and Springer, 1986). They can modulate $\alpha/\beta$ associations as well as ligand binding affinity (Kirchhofer et al., 1991). Recently, a novel site within the I domain of CR3 (CD11b/CD18) has been shown to function as a divalent cation binding site, which seems essential for ligand binding (Michishita et al., 1993). The $\alpha$-subunit of the $\beta_2$ family contains an inserted region (I domain), which shows homology to cartilage matrix protein, von Willebrand factor and complement factor B and C$_2$. The I domain is thought to be a flexible recognition element within the $\alpha$-chain, involved in ligand binding (Diamond et al., 1993). Electron microscopic studies of the $\beta_3$ integrins revealed that the $\alpha/\beta$ complex has a globular head with two tails. Similarities in structure are expected for the leukocyte-specific $\beta_2$ integrins (fig. 3) (Carrell et al., 1985; Dana et al., 1991). Thus, the ligand binding site of the integrins seems to be formed by the cation domains and the I-domain within the $\alpha$-subunit and the conserved region within the $\beta$-subunit (fig. 3).

The intracellular domains of the $\alpha$- and $\beta$-subunits

The cytoplasmic domains of the integrins are thought to be important for receptor function. Both phosphorylation of the cytoplasmic domain and its induced association with the cytoskeleton may direct receptor activation through induction of a conformational change. Interaction with cytoskeletal proteins, to contribute to focal adhesion, is important for integrin function, since disruption of the cytoskeleton abolishes receptor-mediated adhesion (Reszka et al., 1992; Pardi et al., 1992b).

The cytoplasmic domain of the $\beta$-subunits is short (47, 46 up to 52 amino acids, $\beta_1$, $\beta_2$ and $\beta_7$, respectively) and can interact with cytoskeletal proteins such as talin and $\alpha$-actinin ($\beta_1$ and $\beta_2$ integrins) (Pardi et al., 1992b; Otey et al., 1990; Burn et al., 1988; Pavalko et al., 1991; Pavalko and Burridge, 1991). Moreover, the cytoplasmic domains may also interact with other cytoplasmic components (Brown et al., 1990). Deletion of the cytoplasmic domain of the $\beta_1$-$\beta_2$-subunit can inactivate receptor function (Hibbs et al., 1991a,b; Buyon et al., 1990). Three threonine residues, which are highly conserved within the $\beta_1$, $\beta_2$ and $\beta_7$ integrin subfamilies, are most important for the $\beta_2$ integrin receptor function (fig. 2) (Hibbs et al., 1991b). Upon stimulation with PMA or fMLP, serine$^{736}$ present in the cytoplasmic domain of the $\beta_2$ subunit is phosphorylated (Pardi et al., 1992; Chatila et al., 1989; Buyon et al., 1990). Whether this phosphorylation affects receptor function is not known.
the regulation of distinct post-receptor binding events (Shimizu and Shaw, 1991).

Regulation of lymphocyte adhesion

Lymphocytes are equipped with several mechanisms to direct and regulate adhesion and migration to sites of inflammation (table II). First, upregulation or downregulation of surface expression, or the state of glycosylation of particular adhesion receptors is employed to control adhesive processes. Moreover, clustering of integrin receptors may increase the avidity of cell-cell interactions. Enhanced receptor expression and receptor clustering on the cell surface do not necessarily correlate with enhanced adhesiveness of cells. This requires activation of the adhesion receptor itself, resulting in a higher affinity for its ligand. Activation of integrins can be achieved through "inside out" signalling, whereby signals are generated from within the cell (Hynes, 1992) (fig. 4). Activation of integrins involves conformational changes within the receptor, which lead to a higher affinity for its ligand, establishing cell-cell interaction. Binding of the ligand by the integrin can result in "outside in" signalling, which may affect migration or proliferation and differentiation of the cell (Shimizu and Shaw, 1991; Pardi et al., 1992a). The following sections will describe each of these processes in more detail.

A) Regulation of lymphocyte adhesion by expression of receptors and ligands

Cell activation by cytokines or chemotactic factors can up- or down regulate cell surface expression. Expression of integrins on lymphocytes seems poorly sensitive to cytokine treatment. Expression of the β1 integrins is not affected by cytokine treatment, while LFA-1 expression (β2 integrin) is enhanced only 2-fold by IL2 or IFNγ (Shimizu et al., 1990). On the other hand, expression of cellular ligands of integrin receptors is most sensitive to cytokines. Inflammatory mediators, such as IFNγ, IL1 and TNFs strongly induce ICAM-1 (Dustin et al., 1986; Pober et al., 1986) or VCAM-1 expression (Osborn et al., 1989) in a wide variety of tissue, such as endothelium, resulting in enhanced binding of lymphocytes (Dustin and Springer, 1988). In contrast, other ligands of LFA-1 (ICAM-2 and ICAM-3) seem less sensitive to inflammatory cytokines (Staunton et al., 1989; de Fougerolles et al., 1991; de Fougerolles and Springer, 1992). Differences in the state of glycosylation may also contribute to the capacity of adhesion receptors to bind to their ligand (Larson et al., 1989; Dahms and Hart, 1986; Shimizu et al., 1990a).
Table II. Mechanisms that regulate β2-mediated cell adhesion.

<table>
<thead>
<tr>
<th>Regulation at the level of</th>
<th>Influenced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Expression of receptors and their ligands</td>
<td>Cytokines, infectious agents, glycosylation</td>
</tr>
<tr>
<td>B. Affinity/avidity modulation of adhesion receptors</td>
<td>(De-)phosphorylation, second messengers</td>
</tr>
<tr>
<td>— &quot;inside out&quot; signalling</td>
<td>Divalent cations, integrin mAb</td>
</tr>
<tr>
<td>— conformational changes</td>
<td>Divalent cations</td>
</tr>
<tr>
<td>— clustering of receptors</td>
<td>?</td>
</tr>
<tr>
<td>— adhesion cascades</td>
<td>Second messengers, kinase activity</td>
</tr>
<tr>
<td>C. Signalling through adhesion receptors</td>
<td></td>
</tr>
<tr>
<td>— &quot;outside in&quot; signalling</td>
<td></td>
</tr>
</tbody>
</table>

![Fig. 4. Activation and inactivation of integrins.](image)

When the receptor is in an inactive state (closed), “inside-out” signalling (1) can activate the receptor by inducing a conformational change (2) whereby the open receptor can bind its ligand. Ligand occupation of the receptor can trigger intracellular events by “outside-in” signalling (3). When signalling has stopped, the activated receptor can convert back into the inactive state.

B) Affinity avidity modulation of lymphocyte adhesion receptors

“Inside-out” signalling

A second mechanism to control cell adhesion is modulation of the affinity of the receptor for its ligand. Resting lymphocytes do not adhere spontaneously, but a variety of stimuli can induce β1-(VLA-4, -5, -6) or β2(LFA-1, CR3)-mediated cell-cell interactions. Exposure of lymphocytes to phorbol ester (PMA) strongly induces β1- and β2-dependent cell adhesion, suggesting that activation of protein kinase C (PKC) changes the affinity of these receptors for their ligands (Martz, 1980; Rothlein and Springer, 1986; Shimizu et al., 1990). More recently, also, elevation of intracellular Ca²⁺ or cyclic AMP (cAMP) levels have been shown to activate the LFA-1 molecule (Haverstick and Gray, 1992a,b; van Kooyk et al., 1993).

In addition, monoclonal antibodies directed against different cell surface structures expressed by T or B lymphocytes can stimulate LFA-1-mediated adhesion (table III). Triggering of these molecules activates intracellular signalling pathways leading to LFA-1 activation, and subsequent enhanced ligand (ICAM-1) binding. The finding that activation of the CD2 and CD3 molecules results in LFA-1-mediated adhesion (Dustin and Springer, 1989; van Kooyk et al., 1989) provides a link between TCR-mediated recognition of antigen and regulation of cell adhesion (Fgdor et al., 1990). The observation that an increase in LFA-1 affinity induced by CD3 triggering is transient provides the cell with a mechanism to facilitate de-adhesion. Triggering of CD2 or CD3 has been shown to activate β1 (VLA-4, -5, -6) (Shimizu et al., 1990b). LFA-1 activation induced through triggering of CD2 or CD3 depends on PKC activation, PTK (protein tyrosine kinase) activity and cAMP levels (Dustin and Springer, 1989; van Kooyk et al., 1989). Also, increased intracellular Ca²⁺ levels induced by CD2 or CD3 triggering directly correlate with the activated state of the LFA-1 receptor (van Kooyk et al., 1989). Recently, other reports have shown that antibodies directed against MHC class I or II (Spertini et al., 1992), CD43 (Axelson et al., 1988), CD44 (Koopman et al., 1990), CD28 (Shimizu et al., 1990b), CD7 (Shimizu et al., 1990b) and CD31 (Tanaka et al., 1992) expressed on T cells, and antibodies directed against CD19 (Smith et al., 1991), CD20, CD40 (Kansas and Tedder, 1991; Barrett et al., 1991) and MHC class II (Mourad et al., 1990) expressed on B cells all induce LFA-1-mediated adhesion (table III). Depending on the surface receptor triggered, different signal transduction pathways seem to be involved. These findings demonstrate that
Table III. Inside-out signalling of β2 integrins.

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Type of cell</th>
<th>2nd Messenger</th>
<th>Activating</th>
<th>Inactivating</th>
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<tbody>
<tr>
<td>PMA (*)</td>
<td>T, B</td>
<td>PKC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forskolin</td>
<td>B</td>
<td>cAMP, PKA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionomycin</td>
<td>T</td>
<td>Ca^{2+}_i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>B</td>
<td>PKC, PKA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD2 (**)</td>
<td>T</td>
<td>PKC, PTK, Ca^{2+}_i</td>
<td></td>
<td>cAMP</td>
</tr>
<tr>
<td>CD3</td>
<td>T</td>
<td>PKC, PTK, Ca^{2+}_i</td>
<td></td>
<td>cAMP</td>
</tr>
<tr>
<td>CD7</td>
<td>T</td>
<td>PKC</td>
<td></td>
<td>cAMP</td>
</tr>
<tr>
<td>CD19</td>
<td>B</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD28</td>
<td>T</td>
<td>PKC</td>
<td></td>
<td>cAMP</td>
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<tr>
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<tr>
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<td>?</td>
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<td></td>
</tr>
<tr>
<td>CD40</td>
<td>B</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD43</td>
<td>T, B</td>
<td>PKC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD44</td>
<td>T</td>
<td>PTK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC cl I</td>
<td>T, B</td>
<td>PKC, PTK, Ca^{2+}_i</td>
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<td>cAMP</td>
</tr>
<tr>
<td>MHC cl II</td>
<td>T</td>
<td>PKC, PTK, Ca^{2+}_i</td>
<td></td>
<td>cAMP</td>
</tr>
</tbody>
</table>

(*) The addition of PMA, forskolin, ionomycin or LPS to B or T cells generates distinct 2nd messengers activating or inactivating LFA-1-mediated adhesion. (**) Cross-linking of distinct cell surface molecules, by mAb generates different messengers which regulate LFA-1 activation. PKC (protein kinase C), PTK (protein tyrosine kinase), Ca^{2+}_i (intracellular Ca^{2+} mobilization), cAMP (cyclic adenosine mono-phosphate).

Activation of LFA-1 can be induced through triggering of different surface receptors, and illustrate that the LFA-1/ICAM-1 pathway is a common adhesion pathway that may be utilized by lymphocytes under quite different physiological conditions.

Intramolecular alterations (conformational changes)

An increasing amount of evidence indicates that activation of β1 β2 is accompanied by a conformational change within the integrin molecule.

1) Unique monoclonal antibodies have been described that recognize integrins only in their activated state (active conformation). This is determined by the expression of neoepitopes on the integrin or the expression of a ligand-induced binding site (LIBS) on the integrin, which appears upon binding of their ligand (Dransfield et al., 1992a, b; van Kooyk et al., 1991).

2) Binding of M_{g}^{2+} to LFA-1 possibly induces a conformational change, as detected by enhanced reactivity with a certain mAb 24 (Dransfield and Hogg, 1989). Also binding of Ca^{2+} to LFA-1 can alter the configuration of LFA-1, as detected by enhanced reactivity with mAb NKI-L16 (van Kooyk et al., 1991; and submitted). Extracellular binding of Mn^{2+} (Dransfield et al., 1992a; Altieri, 1991) and/or Co^{2+} (Dransfield et al., 1990) can induce a conformational change in both β1 and β2 integrins, which leads to an increased affinity of the receptor for its ligand.

3) Phosphorylation or dephosphorylation of the cytoplasmic tails of the β2 or β1 integrins (α- or β-subunit) or cytoskeletal components may induce a conformational change in the integrin (Pardi et al., 1992).

4) Specific antibodies directed against the β1 and β2 integrins that enhance instead of inhibit adhesion have been described. Also, Fab fragments of these mAb enhance integrin-mediated adhesion which demonstrates that cross-linking of receptors is not required. Binding of these mAb most likely results in a conformational change in the adhesion receptor, thus increasing the affinity for its ligand. For example, mAb directed against a Ca^{2+}-dependent epitope located on the LFA-1 α-subunit (van Kooyk et al., 1991; Keizer et al., 1988; van Kooyk, unpublished) and mAb directed against an epitope on the common β2-subunit (Robinson et al., 1992) enhance binding of LFA-1 to ICAM-1. Similarly, mAb directed against the VLA-4 α-chain can enhance
VLA-4-dependent cell aggregation (Campanero et al., 1990). However, unique anti-β1 mAb can also stimulate binding of VLA-4 to VCAM-1 as well as binding of leukocyte to ECM components (Kovach et al., 1992; van de Wiel van Kemenade et al., 1992). This indicates that antibodies directed against the α- or β-subunit can activate integrins by inducing a conformational change.

**Clustering of receptors**

Clustering of adhesion receptors has been shown to be important in facilitating integrin-ligand interaction. Clustering of CR3 receptors has been reported to be temporally correlated with its ability to bind their ligand (Detmers et al., 1987). Moreover, binding of Ca²⁺ to LFA-1 is associated with clustering of receptors on the cell membrane, which facilitates LFA-1 activation by increasing the avidity of LFA-1-ligand interaction (van Kooyk, unpublished).

**Adhesion cascades**

Several experiments indicate that adhesion molecules may operate successively in time. For example, leukocyte adhesion to endothelium at sites of inflammation is a multistep process initially involving unstable adhesion, followed by stable adhesion by β2 integrins and finally strong adhesion of β2 integrins to their counterreceptors. Different examples can be given of adhesion cascades leading to stable cell-cell binding.

Recognition of antigen by lymphocytes is mediated by the T-cell receptor-antigen/MHC interaction, which precedes stable LFA-1/ICAM-1 interaction. Similarly, CD2/LFA-3 interaction may activate the LFA-1/ICAM-1 interaction (van de Wiel van Kemenade et al., 1992). Also CD44, which may play a role in lymphocyte homing, can stimulate the LFA-1 adhesion pathway (Koopman et al., 1990).

Selectins or “rolling receptors” act very early in the adhesion cascades (Lawrence and Springer, 1991; Lawrence et al., 1990; Lo et al., 1991). They enable rolling of neutrophils along the endothelial cells, thereby reducing their speed. By bringing the neutrophil into close proximity of the endothelial cell, β2-mediated binding (CR3) to endothelium is facilitated (Lo et al., 1991). More recently, it has been described that cytokines can be immobilized by proteoglycans present on endothelial cells which trigger VLA-4-mediated adhesion of specific lymphocyte subsets and thus regulate adhesion and migration of lymphocytes through endothelium (Tanaka et al., 1993).

**C) Signalling through adhesion receptors**

"Outside-in" signalling

In addition to regulation of the receptor affinity by signals generated inside the cell, an increasing amount of evidence indicates that adhesion receptors themselves can transmit signals into the cell ("outside-in" signalling). The use of monoclonal antibodies or purified immobilized ligands to cross-link specific integrin receptors, has identified cell adhesion molecules as signal transducer molecules.

Clustering of β2 integrins by antibodies directed against the LFA-1 or CR3 α-subunit results in release of intracellular Ca²⁺ as well as an increase in intracellular pH (Ng Sikorski et al., 1991; Schwartz et al., 1991b; Pardi et al., 1989; Richter et al., 1990). Clustering of β1 receptors by mAb directed against the β1-subunit can cause tyrosine phosphorylation of a 115-kDa molecular complex (Kornberg et al., 1991). Moreover, ligation of VLA-4 on T cells stimulates tyrosine phosphorylation of a 105-kDa protein, indicating that engagement of VLA-4 on T cells activates protein-tyrosine kinase (PTK) activity (Nojima et al., 1992). Indeed-specific protein-tyrosine kinases have been identified, which are concentrated in focal adhesions (FAK, or focal adhesion kinases), and which are phosphorylated in response to cell attachment to ECM components (Hanks et al., 1992).

Binding of β1 integrins to FN induces clustering of the integrins and activates the Na⁺/H⁺ antiporter (Schwartz et al., 1991a). In addition, ligation of LFA-1 on B cells improves the ability to present antigen to the T cells (Moy and Brian, 1992). Adherence of neutrophils via β2 integrins together with cytokines induces a respiratory burst and cell motility (Jaconi et al., 1991). Furthermore, monocyte adhesion seems to be associated with PLC activation, whereas intracellular Ca²⁺ mobilization plays a role in cell spreading (Leftkowith et al., 1992). Several reports have indicated that anti-LFA-1-α antibodies (van Severen et al., 1991; van Noesel et al., 1988), anti-ICAM-1 (van Severen et al., 1991a; Cerdan et al., 1989) or anti-ICAM-2 antibodies (Damle et al., 1992) enhance lymphocyte proliferation, when immobilized together with anti-CD3 antibodies (costimulation) (van Severen et al., 1990). Anti-β2-subunit antibodies were ineffective, suggesting that the α- and β-subunits of LFA-1 may have distinct signalling properties. VLA-4 as well as its cellular ligand VCAM-1, can also provide costimulatory signals (van Severen et al., 1991a; Burky et al., 1991; Damle and Aruffo, 1991).

Cross-linking of integrins by antibodies mimics the engagement of receptor with its ligand and affects cytoskeletal organization. "Outside in" signalling through adhesion receptors is mediated by different intracellular messengers and/or cytoskele-
Role of lymphocyte adhesion receptors in vivo

The importance of adhesion receptors in vivo is best illustrated in patients suffering from the leukocyte adhesion deficiency (LAD) syndrome (Staatz et al., 1989; Springer et al., 1984; Anderson and Springer, 1987; Kishimoto et al., 1987; Todd and Freyer, 1988; Fischer et al., 1985), a genetic deficiency of the β2 integrin receptor caused by mutations in the common β2 subunit (fig. 2) (Kishimoto et al., 1989; Wardlaw et al., 1990; Arnaout et al., 1990). The LAD syndrome results in the absence of LFA-1, CR3 and p150,95 on the cell surface of leukocytes. Patients have recurring, life-threatening bacterial infections, and severe defects in adhesion-dependent leukocyte functions, which are often fatal in childhood, unless they can be corrected by bone marrow transplantation (Fischer et al., 1988). The potential of mAb directed against integrins or their counter-receptors to reduce vascular and tissue damage in a variety of clinical disorders, has been examined (acute and chronic allograft rejection, rheumatoid arthritis, inflammatory skin disease, asthma and ischaemia-reperfusion syndromes). Inhibition of leukocyte adhesion processes by "anti-adhesion" therapy represents a novel approach to treat disorders in which leukocytes contribute significantly to vascular and tissue damage (Carlos and Harlan, 1990).

In vivo treatment with anti-VLA-4 antibodies inhibits migration of lymphocytes to cutaneous inflammatory sites (Issakutz, 1991). Anti-ICAM-1 antibodies have been shown to reduce airway inflammation, hyperresponsiveness and asthma symptoms (Wegner et al., 1990). Moreover, a soluble form of ICAM-1, as well as anti-ICAM-1 antibodies inhibit rhinovirus infections, which cause 50% of common colds (Marlin et al., 1990). ICAM-1 has been identified as the cellular receptor for a subgroup of rhinoviruses (Staunton et al., 1989b; Greve et al., 1989) and is one of the receptors for Plasmodium falciparum (malaria)-infected erythrocytes (Ockenhouse et al., 1992; Berendt et al., 1992).

It has been demonstrated that anti-CD18 antibodies prevent ischaemia-reperfusion injury (Vedder et al., 1988), whereas anti-CD11b antibodies reduce infarct size in a canine model (Simpson et al., 1988). Administration of anti-LFA-1 antibodies enhances different tissue transplant survival (Heaggy and Waltenbaugh, 1983; van Dijken et al., 1990; Harning et al., 1991), whereas anti-ICAM-1 antibodies have been demonstrated to extend kidney and heart transplant survival in monkeys in the absence of tolerance induction (Cosimi et al., 1990; Flavin et al., 1991).

A combination of anti-LFA-1 and anti-ICAM-1 mAb leads to tolerance in heart transplantation in mice (Isobe et al., 1992); however tolerance is not observed in skin transplants (van Kooyk et al., unpublished). Pilot studies in humans revealed that anti-LFA-1 mAb prevented acute rejection of kidney transplants without induction of tolerance (Le Mauff, unpublished).

Conclusions

These findings demonstrate that adhesion receptors play an important role in different immunologic reactions. Leukocyte-specific adhesion receptors such as LFA-1 show a dynamic nature by the presence of distinct conformational changes, which can be regulated extracellularly by divalent cations or ligand binding, and intracellularly by the generation of distinct signals. The usage of mAb directed against these receptors or their ligands in vivo by an anti-adhesion therapy demonstrates a promising reduction in tissue destruction, caused by disease or by inflammatory responses. Future experiments will determine if combinations of antibodies directed against distinct adhesion receptors will give optimal acceptance of tissue transplants.

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