Ins and outs of LFA-1

Marijke Lub, Yvette van Kooyk and Carl G. Figdor

Leukocyte function-associated molecule 1 (LFA-1) is an integrin that plays a major role in the immune system. Recent findings demonstrate that LFA-1 has a two-way signaling function, mediating cell adhesion and stimulating intracellular processes at the same time. Here, Marijke Lub, Yvette van Kooyk and Carl Figdor discuss the 'inside-out' and 'outside-in' signaling properties of LFA-1, as a prototype leukocyte integrin, in normal and malignant T cells. They integrate data into a model that highlights the role of the cytoskeleton in the regulation of LFA-1.

Leukocyte function-associated molecule 1 (LFA-1; CD11a/CD18) is similar to other integrins in that it comprises an α and a β chain that are noncovalently associated. It is a member of the β2 group of integrins, which includes Mac-1 (CD11b/CD18) and p150,95, these being exclusively expressed by leukocytes. Activation of LFA-1 is a prerequisite for ligand binding and, thus far, three ligands of LFA-1 have been identified: intercellular adhesion molecule 1 (ICAM-1), ICAM-2, and ICAM-3 (Ref. 4). Circulating peripheral blood lymphocytes (PBLs) generally express an inactive form of LFA-1; this is crucial to maintain homeostasis, since constitutively active LFA-1 would cause instantaneous aggregation of circulating cells and clogging of the vessels. Intracellular signals are generated only after activation of a lymphocyte, for instance through the T-cell receptor TCR/CD3 complex or by phorbol 12-myristate 13-acetate (PMA), and these signals cause transient activation of LFA-1 (Refs 2,3). This process is referred to as 'inside-out' signaling (Fig. 1). In addition to the TCR/CD3 complex, several other leukocyte surface receptors can activate LFA-1 through G proteins or protein tyrosine kinases (PTKs). These activate protein kinase C (PKC) and increase intracellular calcium levels ([Ca\(^{2+}\)]) as a result of phospholipase C\(_{y}\) (PLC\(_{y}\))-mediated inositol breakdown. Influx of extracellular Ca\(^{2+}\) and lipids have also been demonstrated to activate LFA-1 (Refs 6,7).

Recent findings show that crosslinking of LFA-1 at the cell surface by antibodies can induce intracellular signals\(^{8,9}\), suggesting that ligand binding can affect cellular functions such as apoptosis, cytotoxicity, proliferation, cytokine production and antigen presentation\(^{10-12}\). This is referred to as 'outside-in' signaling (Fig. 1). The relatively short cytoplasmic tails of leukocyte integrins do not contain any known catalytic domains, therefore any ligand-induced stimulation of PTKs or inhibition of protein tyrosine phosphatases (PTPs) must be indirect. Although there is ample precedent for modulation of non-receptor PTKs and PTPs by ligation of the TCR/CD3 complex, it is not known which protein–protein interactions are responsible for integrin-mediated tyrosine phosphorylation\(^{13,14}\). Ligand-induced oligomerization of surface receptors may initiate signaling by bringing together catalytic units of PTKs on the cytoplasmic tails of receptor subunits or on associated proteins\(^{15}\). Alternatively, oligomerization may organize cytoskeletal complexes that serve as frameworks for the association of PTKs. Possible candidate PTKs in leukocytes include p72\(^{56k}\), ZAP-70, p56\(^{\text{fyn}}\), p65\(^{\text{shc}}\) and p125\(^{\text{Fyn}}\) (Ref. 16). Indeed, recent information links integrin-mediated p125\(^{\text{Fyn}}\) phosphorylation to the Ras/mitogen-activated protein kinase (MAPK) signal transduction pathway\(^{17}\). Furthermore, dephosphorylation by CD45 may negatively regulate LFA-1-mediated signaling in lymphocytes\(^{18}\), although, on thymocytes, CD45 is reported to stimulate LFA-1-mediated adhesion\(^{19}\).

The highly conserved GFFKR motif in the cytoplasmic domain of the α chain of integrins seems to play a major role in modulating integrin function. The observation that the nuclear protein calreticulin, a negative regulator of gene expression, can directly bind to the GFFKR region\(^{20}\) suggests that sequestration of calreticulin to the cell surface by binding to integrins\(^{21}\) is involved in the activation process of integrins, and may disclose a new signaling pathway. Furthermore, the observation that deletion of this region results in a constitutively active form of LFA-1 underscores the importance of this motif in LFA-1 function\(^{22}\).

Conformational changes and multimerization of LFA-1

Despite the wealth of information gathered, the precise mechanism that controls LFA-1-mediated ligand binding remains unknown. The expression of integrin neo-epitopes upon activation or after ligand binding suggests that alterations in the conformation of integrins are important for ligand binding\(^{11,12,23,24}\). This notion is supported by the observation that certain monoclonal antibodies raised against LFA-1 have the capacity to induce ligand binding, rather than inhibit LFA-1–ICAM interactions (Table 1). Alterations in the conformation of integrins can also be achieved upon divalent cation binding, since integrin-mediated adhesion depends on the presence of Mg\(^{2+}\), Ca\(^{2+}\) and Mn\(^{2+}\) (Refs 25–27). Whereas binding of Mn\(^{2+}\) directly activates LFA-1 by itself, Mg\(^{2+}\) supports LFA-1-mediated adhesion only after additional stimuli (e.g. intracellular signals or activating antibodies). Although the role of Ca\(^{2+}\) has remained obscure, previous observations showing that integrins can aggregate at the cell surface\(^{28}\).
have implicated Ca\(^{2+}\) in the surface distribution of LFA-1. Using an antibody directed against a Ca\(^{2+}\)-dependent activation epitope on LFA-1 (L16), direct evidence has been obtained that binding of Ca\(^{2+}\) by LFA-1 is associated with multimerization of LFA-1 on the surface of activated T cells\(^{27}\). This finding may explain why activated T cells (L16\(^{lo}\)) readily bind ligand upon appropriate stimulation, whereas resting lymphocytes express less-clustered LFA-1 (L16\(^{hi}\)) and bind ligand less avidly.

The importance of multimerization of integrin receptors has also been reported by others\(^{28,29}\), and is essential to trigger ‘outside-in’ signaling. Indeed, it has been convincingly demonstrated that the induction of a high-affinity state by deleting the GFFKR motif is not sufficient for stable LFA-1-mediated cell adhesion; it still requires multimerization of LFA-1 (Ref. 22). Interestingly, divalent cations have been shown to exert distinct effects on integrin function. Multimerization of LFA-1 is exclusively mediated by Ca\(^{2+}\), while Mg\(^{2+}\) and Mn\(^{2+}\) have no such effect (Ref. 27; Y. van Kooyk et al., unpublished). These findings demonstrate that divalent cations control LFA-1-mediated adhesion by two distinct mechanisms (Fig. 2): (1) Mg\(^{2+}\)- or Mn\(^{2+}\)-dependent alterations in the ligand-binding affinity of LFA-1; and (2) Ca\(^{2+}\)-dependent multimerization of LFA-1, which enhances the avidity of LFA-1 ligand interactions. Because of these differences, Mn\(^{2+}\), Mg\(^{2+}\) and Ca\(^{2+}\) probably bind to distinct sites on integrins, and may have a Cooperative effect on ligand binding\(^{22-27}\).

**Active and inactive forms of LFA-1**

As discussed above, LFA-1 is only transiently activated after stimulation by agonists (Fig. 1). This holds true not only for freshly isolated lymphocytes, but also for a number of long-term T-cell cultures (Table 1). This observation, and the fact that \(\beta_3\) integrins are exclusively expressed by leukocytes, raises the question as to how LFA-1 is regulated in non-leukocytic cells, which lack leukocyte-specific elements.

When LFA-1 is expressed on non-leukocytic cells, distinct phenotypes are observed. Adherent mouse L-cell fibroblasts and monkey COS cells abundantly express the L16 epitope, whereas non-adherent K562 cells are L16\(^{lo}\). Furthermore, as summarized in Table 1, L or COS cells express a constitutively active form of LFA-1, whereas K562 cells express a form that is not responsive to PMA, suggesting that at least some regulatory elements are missing; they can only be stimulated by activating antibodies (Ref. 24; M. Lub et al., unpublished). The importance of intracellular signals in the regulation of LFA-1 is demonstrated further by the finding\(^{30,31}\) that some leukemic T-cell lines (Jurkat, CEM) cannot mediate adhesion through LFA-1 after stimulation with physiological agonists or PMA (Table 1). Interestingly, these leukemic cells are unable to form multimers of LFA-1 at the cell surface (L16\(^{hi}\), despite

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**Table 1. Ability of T cells to bind ICAM-1 after stimulation by PMA or activating anti-LFA-1 antibodies**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Stimulation</th>
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<tbody>
<tr>
<td></td>
<td>None</td>
<td>PMA</td>
</tr>
<tr>
<td>Normal T cells</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Resting PBLs</td>
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<td>-</td>
</tr>
<tr>
<td>Activated PBLs</td>
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<td>-</td>
</tr>
<tr>
<td>T-cell clone</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leukemic T cells</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CEM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jurkat</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LFA-1 transfectants</td>
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<td>-</td>
</tr>
<tr>
<td>COS-LFA-1</td>
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<td>-</td>
</tr>
<tr>
<td>L-cell-LFA-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K562-LFA-1</td>
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</tbody>
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Abbreviations: Ab, activating antibody; ICAM-1, intercellular adhesion molecule 1; LFA-1, leukocyte function-associated molecule 1; NT, not tested; PBL, peripheral blood lymphocyte; PMA, phorbol 12-myristate 13-acetate.
significant LFA-1 expression\textsuperscript{31}. Preliminary findings indicate that this phenomenon originates from defective signaling pathways rather than from structural defects in LFA-1 (Ref. 30; Y. van Kooyk et al., unpublished).

Regulation of LFA-1 ligand binding by the cytoskeleton
Integrins can associate with cytoskeletal components (α actinin, talin), particularly through the β chain\textsuperscript{32,33}. Hibbs et al.\textsuperscript{34} identified a TTT motif in the β chain that is important for LFA-1-mediated ICAM-1 binding. However, despite this information, molecular mechanisms involved in the association of LFA-1 with the cytoskeleton remain largely elusive. According to the model proposed in Fig. 3, transient release of LFA-1 from the cytoskeleton, or cytoskeleton-associated molecules, modulates the affinity of LFA-1 for its ligand. This model has two major advantages. First, the capacity to couple and uncouple integrins to and from cytoskeletal elements provides leukocytes with a mechanism to support cell locomotion. Second, the cytoskeleton or associated molecules may act as a repressor, and prevent alteration of the affinity state of LFA-1; thus, activation of LFA-1 would require uncoupling from cytoskeletal elements (de-repression).

In leukocytes, actin polymers assemble into short filaments attached to the cytoplasmic face of the mem-

![Fig. 2. Binding of leukocyte function-associated molecule 1 (LFA-1) to intercellular adhesion molecule 1 (ICAM-1) is regulated by two distinct mechanisms: Mg\textsuperscript{2+}-dependent alteration in the affinity of LFA-1; and Ca\textsuperscript{2+}-dependent multimerization of LFA-1. Maximal adhesion is observed on cells that express LFA-1 in a high-affinity state, clustered on the cell surface.](image)

![Fig. 3. Model describing the interaction between leukocyte function-associated molecule 1 (LFA-1) and the cytoskeleton. (a) The cell cortex (comprising short actin filaments, other cytoskeletal proteins and associated molecules; depicted in yellow) stabilizes LFA-1 at the surface of quiescent leukocytes and prevents it from becoming active (repression). (b) Intracellular signals released upon ligation of the T-cell receptor (TCR)/CD3 complex (not shown), and agonist, temporarily relieve constraints on the cytoplasmic domains of LFA-1, permitting the conformational dynamics required for recognition of intercellular adhesion molecule 1 (ICAM-1), and exposing the conserved GFFKR motif on the α chain. This results in binding of an unknown factor (X) (see text for details). (c) Binding of ICAM-1 initiates a series of post-receptor signaling events - multimerization of LFA-1, actin polymerization (depicted in red), and protein tyrosine kinase activity - which may result in initiation of transcription. The TTT motif on the β chain of LFA-1 plays an important role in post-receptor events.](image)
also directly control integrin function. According to the model, intracellular signals from physiological agonists (i.e. TCR engagement), or stimulation with PMA, cause a temporary dislodgement of LFA-1 from these short actin filaments, allowing LFA-1 to adapt to an active conformation (Fig. 3b). Evidence in favor of this hypothesis comes from several recent findings. First, enhanced motility of ligand-coated gold particles bound by integrins is observed upon stimulation with PMA (D. Kuck and E. Brown, pers. commun.). Furthermore, cytochalasins, which inhibit actin polymerization, not only increase integrin motility\textsuperscript{34} but also clearly enhance the binding of ligand by Mac-1 on monocytes\textsuperscript{35}. Cytochalasin D was also demonstrated to enhance LFA-1-mediated adhesion to ICAM-1 of resting lymphocytes (M. Lub et al., unpublished). Similarly, on U937 cells cultured with PMA, p150,95-mediated rosetting of sheep erythrocytes opsonized with complement factor C3bi (EC3bi) could only be observed when these cells were treated with cytochalasin D (Ref. 37). Together, these findings suggest that enhanced ligand-binding affinity of integrins is preceded by a temporary release from the cytoskeleton.

The release of LFA-1 from the cytoskeleton or associated molecules may expose the GFFKR motif, resulting in binding of an unknown factor (X) (Fig. 3b) that is similar to calreticulin binding to the GFFKR sequence in β integrins\textsuperscript{30}. Subsequent ligand binding (Fig. 3c) results in receptor multimerization, induction of PTK activity and actin polymerization, as well as other post-receptor binding events. Multimers of LFA-1 would then allow firm adhesion to adjacent cells and support functional activity by intracellular signals. Affinity modulation of LFA-1 has been shown to be an independent process\textsuperscript{32} and precedes focal contact formation (multimers of LFA-1). Although the high affinity of LFA-1 is sufficient to bind ligand, strong cell–cell adhesion requires post-receptor reorganization of the cytoskeleton (i.e. actin polymerization, receptor capping).

PTP-mediated dephosphorylation of cytoskeletal- or cytoskeleton-associated proteins would ultimately revert LFA-1 into its inactive state\textsuperscript{8}. This inactivation does not necessarily lead to an immediate disruption of the multimers, which may remain at the cell surface, thus favoring rapid binding of (previously) activated T cells as compared with naive resting T cells (Table 1). Interestingly, cytochalasin treatment of activated T cells that express clustered LFA-1 (Fig. 3c) inhibits ligand binding (M. Lub et al., unpublished), indicating that association of LFA-1 with the cytoskeleton of resting (Fig. 3a) and activated lymphocytes (Fig. 3c) is distinct. These differences between cell types may also explain why inhibition of adhesion by cytochalasins has been reported\textsuperscript{38-40}.

Concluding remarks

This article has attempted to integrate data on the regulation of LFA-1-mediated adhesion as a prototype leukocyte integrin. What can be learned from the above discussion of recent progress in the integrin field? First, measurement of expression levels of integrin molecules is only of limited value, since it provides no information on the functional status of these molecules. Second, it is clear that integrins have a two-way signaling function, mediating information from within the cell to outside and vice versa. Third, we begin to gain some insight as to the machinery employed by a leukocyte to control integrin-mediated function. At least two distinct mechanisms can be distinguished: alteration of ligand-binding affinity mediated by structural changes, and the multimerization of LFA-1 molecules at the cell surface that is required for stable cell adhesion. Highly dynamic interactions of the cytoskeleton with LFA-1, and tyrosine phosphorylation of its components, probably controls functional activity. The challenge now is to unravel the precise role of each element in the transmission of signals to the cytoskeleton and the nucleus.

Finally, some leukemic T cells exhibit defective forms of LFA-1, probably due to inappropriate signaling. It will be worthwhile to investigate these cells in more detail, since identification of such defects may be instrumental for an improved understanding of the regulation of LFA-1-mediated adhesion, and might provide insight into whether these defects are related to the progression of leukemia.

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Marijke Lub, Yvette van Kooyk and Carl Figdor are at Tumor Immunology, University Hospital Nijmegen, Philips van Leydenlaan 25, 6525 EX Nijmegen, The Netherlands.

References

Serum gangliosides as endogenous immunomodulators

Lev D. Bergelson

Gangliosides suppress various immune activities in vitro and in vivo. Their level is significantly elevated in tumors and atherosclerotic aorta tissue, as well as in the sera of patients with tumors or atherosclerosis. Here, Lev Bergelson suggests that ganglioside-induced immunomodulation might be involved in atherogenesis and carcinogenesis, and describes a hypothesis that cites gangliosides as a factor interfering with the clearance of low-density lipoproteins (LDLs) and promoting the formation of atherosclerotic plaques.

Gangliosides are a family of acidic glycosphingolipids present in all eukaryotic cells. Their molecular structure comprises a hydrophobic tail (ceramide) and a hydrophilic (oligosaccharide) moiety containing one or several residues of sialic acid (Fig. 1). Gangliosides exhibit receptor or co-receptor functions for many bioactive agents, including cytokines, hormones, toxins and viruses, and are considered to be involved in cell differentiation and morphogenesis, as well as in cellular recognition, cell-cell interaction and growth regulation. Many gangliosides show antigenic properties (e.g. blood group, embryonic and tumor antigens) (reviewed in Refs 1,2).

The majority of gangliosides are situated in the outer leaflet of the cell membrane, such that the sugar moiety protrudes into the extracellular space. However, gangliosides also occur in non-cell-associated forms in blood plasma and other body fluids, and there is a constant exchange between cell-associated and non-cell-associated gangliosides. Moreover, some fast-growing cells actively release gangliosides into the circulation. Indeed, the shedding of gangliosides from some tumor cells may occur at strikingly high rates, and appears to correlate with tumorigenicity and cell density. Since exogenous gangliosides are able to associate with cells via different mechanisms, changes in the ganglioside profile of one cell type ('ganglioside donors') may result in modulation of the surface properties, enzymatic activities and function of another cell type ('ganglioside acceptors'). In this sense, serum gangliosides may be considered as a form of soluble cytokine. This article will discuss the origin, composition and status of circulating gangliosides, the interaction of gangliosides with cells of the immune system and the immunological consequences of this phenomenon.

The origin and status of circulating gangliosides

The presence of gangliosides in serum was discovered as early as 1963 (Ref. 7). Although the concentrations of gangliosides reported in human serum vary, it is now established that elevated levels occur in some pathological conditions such as cancer and atherosclerosis (reviewed in Refs 8,9). The potential significance of these elevations will be discussed below. 

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