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The leukocyte function-associated molecule 1 (LFA-1) plays a key role in cell adhesion among leukocytes and between leukocytes and other cell types. Although two ligands of LFA-1 have been identified, namely the intercellular adhesion molecules 1 (ICAM-1) and 2 (ICAM-2), the exact mechanism by which LFA-1 binds to and detaches from its ligand(s) has remained obscure. Based on novel findings, Carl Figdor, Yvette van Kooyk and Gerrit Keizer now present a model of LFA-1-mediated lymphocyte adhesion, showing that LFA-1-dependent cell adhesion is a dynamic process in which ligand-binding affinity is regulated by intramolecular changes in LFA-1. This process is controlled by other lymphocyte surface structures, notably CD2 and CD3.

The LFA-1 family of adhesion receptors consists of three structurally related glycoproteins, LFA-1, CR3 and p150,95, which comprise the CD11-CD18 group of adhesion molecules. They have similar architecture and are composed of two non-covalently linked polypeptides: a unique \( \alpha \) subunit (CD11a-c) and a common \( \beta \) subunit (CD18). Cloning of the CD11 genes revealed the presence of three potential cation-binding sites on each of the individual \( \alpha \) chains.

Based on structural homology, the LFA-1 family of adhesion molecules belongs to the integrin family of adhesion receptors. However, in contrast to other integrins, the LFA-1 family of antigens is exclusively expressed on leukocytes. LFA-1-mediated adhesion is accomplished by binding to ICAM-1, ICAM-2 or to other, as yet unidentified, ligands. In contrast to the restricted tissue distribution of LFA-1, ICAM-1 is widely distributed, and its expression can be induced by a variety of inflammatory cytokines.

LFA-1-ligand interaction

Much has been learned about the conditions required for LFA-1-ligand interaction. LFA-1-mediated cell adhesion is a temperature- and energy-dependent process that requires an intact cytoskeleton and the presence of divalent cations, notably Mg\(^{2+}\) (Refs 6, 13, 14).

Apart from these conditions that must be fulfilled to enable LFA-1-mediated cell–cell interactions, several observations indicate that LFA-1-dependent adhesion is controlled by other cellular processes. Resting leukocytes do not adhere spontaneously, but several stimuli can induce LFA-1-mediated cell–cell interactions. Phorbol myristate acetate (PMA) strongly induces LFA-1-dependent cell adhesion. In addition, monoclonal antibodies directed against the cell surface molecules CD2 and CD3 have now been shown to stimulate cell aggregation.

One possible conclusion from these observations is that surface molecules, like CD2 and CD3, can activate LFA-1 through intracellular signalling pathways, and that activation of LFA-1 leads to enhanced ligand binding. This hypothesis implies the existence of at least two different forms of LFA-1 – an inactive and an active form. A strong argument in favour of the existence of these two states of LFA-1 is that if only one form of LFA-1 existed, spontaneous aggregation of peripheral blood leukocytes would cause clogging of blood vessels. This notion is supported by the fact that freshly isolated leukocytes do not tend to aggregate to each other, although they express significant levels of LFA-1 and ICAM-1. Similarly, cloned cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, which express extremely high levels of LFA-1 and ICAM-1, do not aggregate spontaneously. However, rapid aggregation of CTL can be observed upon stimulation with antigen, PMA, or through CD2 or CD3. This cell clustering is LFA-1-dependent, since it is abrogated by anti-LFA-1 antibodies. Furthermore, the induced cell adhesion cannot be attributed to a short-term augmentation of surface expression of LFA-1 and ICAM-1 (Refs 5, 6, 13). These observations, therefore, provide further evidence for the existence of an inactive and an active form of LFA-1.

Activation of LFA-1

What causes LFA-1 to transform from an inactive into an active state? An anti-LFA-1 antibody, termed NKL-L16, was recently described that, in contrast to other anti-LFA-1 antibodies, stimulates cell adhesion rather than inhibiting LFA-1-dependent cell–cell interactions. NKL-L16-induced cell aggregation does not involve intracellular signalling, but appears to alter the conformation of the LFA-1 molecule such that its affinity for ligand binding is...
Fig. 1. Model for regulation of LFA-1-mediated cell adhesion. Binding of antigen to TCR–CD3 complex stimulates phospholipase C (PLC) that catalyses the hydrolysis of phosphatidyl inositol bis-phosphate (IP$_2$) producing inositol triphosphate (IP$_3$) which mobilizes intracellular calcium and diacylglycerol (DAG). Subsequent phosphorylation of the $\beta$ chain of LFA-1 causes a conformational change leading to high-affinity ligand binding. LFA-1 dependent cell adhesion is downregulated by phosphorylation of CD2 resulting in modulation of the TCR–CD3 complex. As a consequence, PKC levels fall and phosphatases dephosphorylate LFA-1$, reversing it into its inactive state, thus facilitating de-adhesion. PMA and NKI-L16 activate LFA-1 by direct activation of PKC or by binding to LFA-1, respectively.

greatly enhanced (Y. van Kooyk, P. Weder, F. Hogervorst, A.J. Verhoeven, A.A. le Velde, J. Borst, G.D. Keizer and C.G. Figdor, submitted). In time course studies measuring cell aggregation induced by NKI-L16 or by PMA, strikingly similar kinetics were observed16, suggesting that aggregation induced by PMA may also result from a conformational change leading to high-affinity ligand binding. LFA-1 dependent cell adhesion is downregulated by phosphorylation of CD2 resulting in modulation of the TCR–CD3 complex. As a consequence, PKC levels fall and phosphatases dephosphorylate LFA-1$, reversing it into its inactive state, thus facilitating de-adhesion. PMA and NKI-L16 activate LFA-1 by direct activation of PKC or by binding to LFA-1, respectively.

Role of LFA-1 in target-cell lysis by cytotoxic T lymphocytes

CTL–target-cell interactions have been extensively studied and provide, arguably, the best model to demonstrate the mode of action of LFA-1. Figure 2 shows the various phases through which a cytolytic reaction progresses. It is suggested that, when CTL encounter a target cell, initial cell–cell contact is established via LFA-1–ICAM-1 interaction25–28. At this stage cell binding is non-specific and of low affinity since it does not involve antigen recognition. Nevertheless, target cell binding is strong enough to facilitate antigen recognition by the T-cell receptor (TCCR–CD3 complex. Without antigen recognition, the CTL detach from the target cell. If, however, antigen is recognized, CD3 transmits a signal which ultimately leads to activation of LFA-1 via phosphorylation of the $\beta$ chain. Activated LFA-1 then mediates high-affinity binding between CTL and target cell, thereby strengthening the adhesion between them29. This facilitates the formation of intercellular clefts30, enabling efficient delivery of cytotoxic molecules to the target cell. Furthermore, activated LFA-1 may interact with cytoskeletal elements, thereby directing the migration of cytotoxic granules. Modulation of the TCR–CD3 complex from the cell surface31 is thought to abrogate PKC-mediated fueling of protein phosphorylation, thus acting as a negative feedback signal reverting LFA-1 to its inactive state (Fig. 1) and providing CTL with a mechanism which may lead to detachment from target cells (Fig. 2).

Three different forms of LFA-1 on the cell surface

Fig. 2. Four different forms of LFA-1 on the cell surface: an active and an inactive form. We now propose a third form of LFA-1 which lacks the epitope recognized by the mAb NKI-L16 (Fig. 3). Resting peripheral blood lymphocytes (PBL) express LFA-1, but express extremely low levels of the NKI-L16 epitope in comparison with CTL. It has been observed that the speed with which cells aggregate upon stimulation directly correlates with expression of the NKI-L16 epitope (Y. van Kooyk, P. Weder, F. Hogervorst, A.J. Verhoeven, A.A. le Velde, J. Borst, G.D. Keizer and C.G. Figdor, submitted), suggesting that exposure of the NKI-L16 epitope is a prerequisite for LFA-1-dependent adhesion. This is supported by the finding that in vitro culture (3–6 h) of resting PBL with interleukin 2 (IL-2) increases NKI-L16 expression and correlates with their capacity to aggregate. This is not due to an increased number of LFA-1 molecules expressed, since it is not accompanied by a concomitant rise in expression of LFA-1 epitopes other than NKI-L16. These observations show that the majority of LFA-1 molecules expressed by resting PBL lack the NKI-L16 epitope or that all LFA-1 molecules only partially expose the epitope (inactive LFA-1; Fig. 3(I)). At the moment it is not possible to distinguish between these possibilities.

Further evidence in favour of an NKI-L16-negative form of LFA-1 comes from the observation that the NKI-L16 epitope completely disappears when CTL, expressing high levels of NKI-L16, are treated with ethylenediaminetetraacetic acid (EDTA) or ethylene glycolbis(aminoethyl)tetraacetic acid (EGTA) whereas this does not affect other epitopes of LFA-1 (Y. van Kooyk, P. Weder, F. Hogervorst, A.J. Verhoeven, A.A. le Velde, J. Borst, G.D. Keizer and

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C.G. Fdgord, submitted). Ca²⁺ readily restores NKI-L16 expression. Maturation/activation of PBL by in vitro culture induces the NKI-L16 epitope (Fig. 3(2)).

Expression of the NKI-L16 epitope is, however, not sufficient to induce cell adhesion since CTL do not aggregate spontaneously. Induction of stable cell adhesion requires a strong stimulatory signal (for example antigen, PMA and anti-CD3), which then leads to a third form of LFA-1 -- activated LFA-1 (Fig. 3(3)).

Dispersed and locally concentrated LFA-1

It has been suggested that stable intercellular interactions require the presence of local areas of highly concentrated adhesion receptors on the cell surface. Multiple receptor–ligand interactions formed in such areas would facilitate stable cell adhesion. Along these lines, we hypothesize that resting NKI-L16-negative lymphocytes lack areas of concentrated LFA-1 and therefore cannot adhere efficiently. It is possible that expression of the NKI-L16 epitope is Ca²⁺-dependent suggests that Ca²⁺ is involved in the formation and stabilization of clustered LFA-1. Preliminary light- and electron-microscope studies indicate different distribution patterns of LFA-1 on resting PBL compared to cloned CTL or NK cells.

Regulation of LFA-1-mediated cell adhesion at different levels

What is the benefit of expressing three distinct forms of LFA-1? As discussed above, clear advantages can be envisaged if LFA-1–ligand interactions can be rapidly switched on and off, thereby creating a mechanism to regulate lymphocyte adhesion and de-adhesion. But this does not require the existence of three forms of LFA-1. In our opinion, the answer to this question may be found in the maturation/activation state of a lymphocyte. This is best illustrated by the following example. One of the phases of an immune/inflammatory response is characterized by the recruitment of leukocytes from the peripheral blood pool. Lymphocytes adhere to endothelial cells and migrate into the underlying tissues; this process is regulated by various adhesion pathways, including LFA-1–ICAM-1 interactions. Cytokine production (IL-1, gamma-interferon) will locally raise the expression of ICAM-1 (Ref. 12) on the endothelial cells, thereby facilitating leukocyte adhesion in general. However, instead of random cell binding, it is preferable to recruit only those cells that can make a positive contribution to the immune response. We suppose, therefore, that resting (NKI-L16-negative) lymphocytes are not capable of stable cell adhesion. In contrast, activated or previously activated lymphocytes exhibiting high levels of LFA-1 and exposing high levels of NKI-L16 epitope can immediately bind when LFA-1 is activated by, for instance, antigen presented by endothelial cells. In addition, the release of cytokines during the immune/inflammatory response may invoke activation/maturation of resting cells, which thereby gain the NKI-L16 epitope associated with the capacity to rapidly adhere upon stimulation through CD2 and CD3.

Thus we propose that LFA-1–ligand interactions can be regulated at three levels: (1) the absolute number of molecules expressed at the cell surface, (2) maturation/activation processes which favour cell binding by previously activated NKI-L16-positive cells compared to resting NKI-L16-negative cells and (3) specific activation of LFA-1 into a high affinity state.

Synopsis and discussion

The model presented here formally explains the regulation of leukocyte adhesion by LFA-1. Cell–cell interactions mediated by other members of the integrin
superfamily may well use similar mechanisms. PMA enhances fibronectin receptor (VLA-5)-mediated cell adhesion, and fibronectin synergizes with anti-CD3 antibody to promote lymphocyte proliferation, indicating that the T-cell receptor may regulate fibronectin-receptor-mediated adhesion. Novel findings support and extend this notion, showing that PMA, anti-CD2 and anti-CD3 antibodies can induce VLA4/VLA5- or VLA6-mediated lymphocyte binding to fibronectin or laminin, respectively. Furthermore, two other integrins, CR3 (CD11b–CD18) and GPIIb–IIIa, express neo-epitopes upon activation, indicating that activation can induce a conformational change in these molecules. Chemotactic compounds induce transient neutrophil aggregation and adhesion to endothelium which may be mediated through CR3 (Ref. 40).

Finally, an increasing body of evidence indicates that cell adhesion depends on dynamic membrane (integrin)-cytoskeletal interactions. Taim, an integral membrane protein, may play a major role in this process by linking integrin receptors to the cytoskeleton, since it co-localizes with several integrin molecules at cell contact areas upon antigen- or phorbol-ester-induced stimulation.

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References


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