Signalling and adhesive properties of the integrin leucocyte function-associated antigen 1 (LFA-1)

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Leucocyte function-associated antigen 1 (LFA-1; also known as CD11a/CD18) belongs to the family of integrins which is one of the most important families of cell adhesion molecules and includes receptors that bind to the extracellular matrix as well as receptors involved in cell-cell interactions. Recent findings have demonstrated that integrins are both adhesion receptors and signalling molecules. LFA-1 plays a crucial role in many cellular processes (migration, antigen presentation, cytotoxicity, cell proliferation, haemopoiesis) by mediating cell-cell adhesion by binding to ligands such as intercellular adhesion molecules 1, 2 and 3 (ICAM-1, -2 and -3) [1]. All integrins are $\alpha\beta$ heterodimers, with the $\alpha$-subunit non-covalently associated with a $\beta$-subunit [2]. Integrins can be grouped into subfamilies according to a common $\beta$-subunit [3]. Leucocytes express $\beta_1$ (VLAs), $\beta_2$ (LeuCAMs, CD11a/CD18) and $\beta_3$ (homing) integrins. LFA-1 belongs to the subfamily of $\beta_2$ integrins, which includes Mac-1 ($\alpha_4\beta_2$; CD11b/CD18), p150,95 ($\alpha_5\beta_2$; CD11c/CD18) and $\alpha_6$ ($\alpha_6\beta_1$; CD11d/CD18), being exclusively expressed by leucocytes [4,5].

Integrin structure/ligand-binding sites

Ligand-binding sites in integrin $\alpha\beta$ heterodimers have recently been identified. Three regions of LFA-1 are involved in ligand binding (Figure 1). The first consists of sequences close to or within the EF-hand-like motifs located in the N-terminal half of the $\alpha$-subunit with similarity to motifs in classical $\text{Ca}^{2+}$-binding proteins, such as calmodulin [6,7]. The second is made up of sequences located in a region of approx. 200 residues, designated the A or inserted (I) domain, located in the $\alpha$-subunit. The I-domain is structurally similar to the ligand-binding domain in von Willebrand factor, complement factors B and C2 and cartilage matrix protein [8–10]. Only a few integrins (p3, x1p, x2p) contain an I-domain. Analysis of recent crystal structures of the I-domain of $\alpha_4$ revealed a conserved DXSXS motif which forms a $\text{Mg}^{2+}$ co-ordination site, which is also termed the metal-ion-dependent adhesion site (MIDAS) [11]. Mutation of the MIDAS co-ordination residues abrogates ligand binding [12–14], demonstrating that bivalent cations are critical for interaction of integrins with their ligands [15]. The third region is a 200-amino acid sequence located N-terminal of the cysteine-rich domain that is highly conserved in $\beta$-subunits and shows simi-
larity to the I-domain [11]. This site has also been demonstrated to contain a MIDAS-like motif [16]. Mutations within this sequence also eliminate ligand binding [17,18].

It can be speculated that the MIDAS-containing I-domain, the EF-hands in the α-subunit and the MIDAS-like motif in the β-subunit, function co-operatively in ligand binding. All integrin-ligand interactions are bivalent-cation-dependent, and, consistent with this finding, all three regions are implicated in ligand binding and have been shown to contain sequences that interact with bivalent cations. Asp-137, Thr-206 and Asp-239 located in the I-domain of LFA-1 are also conserved in other I-domains and are involved in co-ordination of the Mg^{2+} cation; they are therefore critical for binding of ligands (e.g. ICAMs, C3bi and collagen) [19]. Furthermore residues located in the proximity of the bivalent-cation-binding pocket, Met-140, Glu-146, Thr-243 and Ser-245, have also been shown to be critical for binding of LFA-1 to ICAM-1 [20].

Anti-LFA-1α antibodies that inhibit interaction with the ligands ICAM-1, ICAM-2 and ICAM-3 map three distinct epitopes (IdeA, IdeB and IdeC; Figure 2) within the I-domain that are located close to the cation-binding face [21]. In contrast, anti-LFA-1α antibodies that interfere with ICAM-2 and ICAM-3 binding but do not affect ICAM-1 binding recognize a fourth epitope in the I-domain (IdeD; Figure 2) at the opposite side of the cation-binding site [23]. This epitope appears to be in close proximity to Ile-126 and Asn-129 (IdeA; Figure 2), which we identified as being involved in binding of ICAM-3 but not ICAM-1. This epitope is located at the extreme N-terminus of the I-domain [24], which is linked to the rest of the integrin. Synthetic short peptides that contain the sequences of the ICAM-3-binding site block LFA-1-ICAM-3 adhesion and proliferation, but LFA-1-ICAM-1 interaction is unaffected. This indicates that the I-domain contains different binding sites for different ligands, and that antibodies that interfere with LFA-1 function actually affect the ligand-

![Ribbon representation of the CD11a I-domain based on a co-ordination provided by Qu and Leahy [22]](image-url)
binding residues by steric hindrance or by altering the conformation of the residues. ICAMs are known to use an LDV sequence for integrin binding (amino acids IETP in ICAM-1 and LETS in ICAM-2 and ICAM-3). How precisely these motifs interact with the various sites in the I-domain is not yet clear and awaits the production of I-domain-ligand co-crystals.

**Regulation of integrin function**

Leucocytes normally circulate in the bloodstream as non-adherent cells, but during infection, tissue damage and wound healing, they must be recruited to sites of inflammation. A feature of integrins is their ability to rapidly and reversibly modulate the level of adhesiveness. Integrins play an essential role in the two-way communication between the interior of a cell and its extracellular environment. In response to signals from the cytoplasm or from interaction with the extracellular ligand, adhesiveness can be regulated via conformational alterations, such as changes in avidity (multivalency) and affinity of the integrin, with no change in surface density of the adhesion molecules [25,26]. When changes in both avidity and affinity occur, integrins can strongly bind their ligands. Mechanisms that generate $\beta_2$-integrin-mediated adhesion will be discussed below.

**Affinity regulation**

On most cells, integrins are of low affinity and do not bind to their ligands. The affinity of integrins for their ligands is controlled by intracellular signals that activate the integrins, the so-called 'inside-out' signalling [27]. Under physiological conditions, cross-linking of the T-cell receptor–CD3 complex generates intracellular signals which result in a transient LFA-1–ICAM-1 interaction [28,29]. This results in conformational changes in LFA-1 generated through the cytoplasmic tails in response to intracellular signalling. Activation of protein kinase C and elevation of intracellular cAMP, Ca$^{2+}$ and lipid levels have been demonstrated to result in activation of LFA-1 [30,31]. Most of these intracellular events also lead to affinity changes in $\beta_1$ and $\beta_2$ integrins. The affinity alterations within LFA-1 are accompanied by Mg$^{2+}$-dependent conformational changes, which can be detected by expression of the 24 epitope [32].

Although the cytoplasmic tails of the $\alpha$- and $\beta$-subunits of integrins are relatively short (58 and 46 amino acids respectively), several sequences within them have been implicated in the alterations that occur in integrin affinity. Deletion of a stretch of five amino acids containing three adjacent threonines and phenylalanine in the C-terminal part of the $\beta_2$ cytoplasmic domain has shown that this sequence is required for LFA-1–ICAM-1 binding [33,34]. PMA-induced triggering of LFA-1-mediated adhesion, which activates protein kinase C, results in phosphorylation of Ser-756 in the cytoplasmic tail of the $\beta_2$ subunit. However, mutation of this serine residue has demonstrated that phosphorylation is not crucial for the affinity modulation of LFA-1 [34]. Recently, a regulatory protein (cytohesin) has been identified that specifically interacts with the cytoplasmic tail of $\beta_2$ integrins. Expression of this protein enhances $\beta_2$-integrin-mediated adhesion [35]. A conserved membrane-proximal region of the cytoplasmic $\beta$-subunit (LLvXIIIhDR; less conserved amino acids are shown as lower case, and X indicates a non-conserved residue) affects heterodimer affinity and specificity [36,37], and appears to interact with the conserved membrane-proximal site in the $\alpha$-chain (GFFKR) [38]. Deletion of these conserved regions results in increased ligand-binding affinity, suggesting that the conserved membrane-proximal region of the integrin cytoplasmic domains may serve to constrain these receptors into a default low-affinity conformation [39].

**Avidity regulation**

Integrin–ligand interaction can also be regulated by changes in cell-surface distribution of the integrin (avidity). Clustering of LFA-1 on activated T-cells facilitates ICAM-1 binding, and it has been demonstrated that Ca$^{2+}$ is involved [40]. With the use of the anti-LFA-1$\alpha$ antibody NKI-L16, which recognizes a Ca$^{2+}$-dependent epitope on CD11a, evidence has been obtained that binding of Ca$^{2+}$ to LFA-1 is associated with multimerization of LFA-1 on the cell surface of activated T-cells [25,26]. Since there is evidence that ICAM-1 is naturally found as a dimer, ligand binding may also induce LFA-1 dimerization [41]. Similarly, clustering of Mac-1 has been reported to correlate with its ability to bind ligand [42]. Evidence is now emerging that the interaction of integrins with the cytoskeleton may regulate their distribution on the cell surface,
and may thus affect the avidity of integrin–ligand interactions. Accumulation of cytoskeletal proteins, such as α-actinin, F-actin, vinculin, paxillin, talin and filamin, into focal adhesion complexes (FACs) requires the combination of both aggregation of the integrin receptor by antibodies and ligand binding [43]. Integrins associate with different cytoskeletal components (α-actinin, talin) and focal adhesion kinase (pp125<sup>Fak</sup>), in particular through the cytoplasmic tail of the β<sub>3</sub>-subunit [44]. A conserved region (EYRRFEKE), N-terminal of the β<sub>3</sub> cytoplasmic tail, appears to be critical not only for interaction with the cytoskeleton but also for endoplasmic reticulum retention, assembly and transport to the cell membrane of LFA-1 [37]. Mutation of three threonines results in impaired spreading and decreased ability to form FACs and to organize the cytoskeleton into stress fibres [45]). A conserved NPXY motif in the β-chain has been implicated in the localization in FACs [36].

Coupling and uncoupling integrins to and from the cytoskeletal elements provides leukocytes with a mechanism of locomotion. 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 the cytoskeletal elements (derepression). We have indeed observed that uncoupling of LFA-1 from the cytoskeletal network, by cytochalasin D, facilitates lateral movement of LFA-1 (cluster formation), which results in high avidity and sequentially high-affinity ligand interaction on resting leukocytes [46]. Dynamic interaction of integrins with the cytoskeleton has recently also been demonstrated by others, showing that uncoupling of β<sub>i</sub> integrins from the cytoskeleton by cytochalasin D enhances the motility of ligand-coated gold particles on stimulation with PMA [47]. This indicates that the cytoskeleton actively maintains LFA-1 in its non-adhesive state and that release from the cytoskeletal constraints is indeed an important early step in activation of adhesion.

**Post-ligand signalling through integrins upon ligand binding**

Besides regulation of the adhesion receptor by signals generated inside the cell, increasing evidence is emerging that adhesion receptors can also transmit signals into the cell. This so-called ‘outside-in’ signalling by integrins has been shown by the use of monoclonal antibodies or immobilized ligands to cross-link integrin receptors. Cross-linking of integrins by their ligands results in cellular functions such as programmed cell death (apoptosis), cytotoxicity, proliferation, differentiation, antigen presentation and specific transcriptional regulation of target genes and cytokines [30,48].

Cross-linking of LFA-1 by antibodies triggers phosphorylation of phospholipase C-γ1, ZAP-70 and an 80 kDa protein [49]. We recently observed that binding of LFA-1 to only ICAM-1 and not to ICAM-3 generates tyrosine phosphorylation of distinct proteins (Y. van Kooyk, unpublished work). Interaction of β<sub>i</sub> integrins with extracellular matrix has been shown to induce rapid tyrosine phosphorylation of several proteins including pp125<sup>Fak</sup> (focal adhesion kinase of 125 kDa), paxillin, tensin and the Src family of kinases such as p130<sup>Cas</sup> [50]. Furthermore LFA-1 cross-linking results in a rise in intracellular Ca<sup>2+</sup> levels and pH [31]. pp125<sup>Fak</sup> has been shown to bind to the cytoplasmic tail of β<sub>i</sub> integrins and to associate with two non-receptor protein tyrosine kinases, pp60<sup>Src</sup> and pp59<sup>Shc</sup>, via their SH2 domains [51]. Other signalling pathways, stimulated through clustering of certain β<sub>i</sub> integrins, are: ERK and JNK signalling pathways [43], activation of Rho and p190-B, a new member of the Rho GAP family of the signalling molecules, and integrin-linked kinase, a new 59 kDa threonine-serine kinase that associates with the cytoplasmic tail of the β<sub>i</sub> subunit. Binding to fibronectin results in down-regulation of kinase activity of this integrin-linked kinase (p59<sup>ILK</sup>). It is now thought that it is not pp125<sup>Fak</sup> but p59<sup>ILK</sup> that is the most receptor-proximal protein kinase in the regulation of integrin-mediated signal transduction.

In conclusion, integrin function is not limited to adhesion but they simultaneously also act as signal transducers. The adhesive properties of integrins are controlled by altering both their avidity and affinity either from the outside of the cell or through intracellular pathways. In these processes the short cytoplasmic domains associate with different cytoskeletal proteins, protein tyrosine kinases and many other signalling molecules, resulting in a cascade of intracellular signals. Possible functions of the cytoplasmic tail of integrins are: (1) attachment to the cytoskeleton (e.g. tensin, talin, paxillin, vinculin and α-actinin); (2) interaction with signalling components such as protein kinases and phosphatases (e.g. p59<sup>ILK</sup> and p125<sup>Fak</sup> and CD45); (3) control of cell adhesion, by altering the avidity of the integ-
rin by changing its extracellular conformation (clustering); (4) modulation of the ligand-binding affinity of the integrin.

Introduction

Wounding is defined as a physical disruption of the normal architecture of a tissue and may be caused by trauma, burns, inflammatory processes or metabolic insufficiency. The host initiates a co-ordinated repair response to wounding that serves to prevent infection and ultimately re-establish normal tissue integrity. This response to injury involves a complex interplay of cellular, humoral and connective tissue elements [1,2]. Platelets play an early role by releasing factors that are required for both clotting and the recruitment of peripheral blood leucocytes to the injured site. Peripheral blood leucocytes then produce mediators that combat infection and co-ordinate successive steps of the tissue repair response [1–3]. Connective-tissue cells play a critical role in the reparative phase of wound healing by secreting extracellular matrix proteins. The usual outcome of this cascading series of cellular events is elimination of the invasive stimulus followed by connective tissue scar formation and, over time, remodelling of the injured site.

Connective-tissue fibroblasts are a quiescent cell population that under normal circumstances remain sparsely distributed throughout the extracellular matrix [4]. As a consequence of injury, fibroblasts enter and proliferate within the injured site [5]. The precise origin of the fibroblast-like cells within wounds has been controversial since the original microscopic studies of developing connective tissue performed by Paget in 1863 [6,7]. That wound fibroblasts appeared by migration from adjacent tissue was supported by experiments showing the apparent ingrowth of fibroblasts from local areas, and by the observation that India Ink-tagged monocytes failed to develop into tissue fibroblasts in vivo [7,8]. Other studies, however, reported evidence for the differentiation of leucocytes into fibroblasts within subcutaneous diffusion chambers and the apparent in vivo transformation of peripheral blood mononuclear cells into collagen-producing cells [9,10].

Discovery of peripheral blood fibrocytes

Several years ago, investigations into the cell population present in experimentally implanted subcutaneous wound chambers led to the discovery of an adherent proliferating cell type that displayed fibroblast properties yet expressed distinct haemopoietic/leucocyte cell-surface markers [11]. Wound chambers consist of short lengths of sponge-filled silastic tubing and are a frequently employed model for the study of tissue-reparative responses in vivo. Implantation of these chambers into the subcutaneous space of mice results in a rapid infiltration of peripheral blood inflammatory cells, including neutrophils, monocytes and lymphocytes [12,13]. Large numbers of adherent spindle-shaped cells that resemble fibroblasts were unexpectedly observed to infiltrate wound chambers soon after implantation and coincidentally with the appearance of circulating inflammatory cells. Double-immunofluorescence studies showed that, within 24 h of implantation, as many as 10–15% of the cells present in wound-chamber fluid stain posi-