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Regulation of Leukocyte Integrin Function: Affinity vs. Avidity

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Abstract Leukocytes circulate freely in the bloodstream until receiving signals which activate adhesive mechanisms essential for immune responsiveness. Key mediators of these adhesion events are heterodimeric cell surface *receptors* called integrins. It is now apparent that several components may contribute to successful integrin-mediated adhesion: alterations in individual receptors lead to enhanced affinity for ligand; integrin clustering causes an increase in avidity; by spreading, the adhering cell is less susceptible to shear force. Model systems have allowed us to examine the contribution of each of these factors in generating adhesion. In more physiologically relevant situations, it can now be questioned whether integrin-mediated adhesion is regulated via alterations in receptor affinity or avidity, or whether both these mechanisms are involved. © 1996 Wiley-Liss, Inc.

Key words: adhesion, integrin, LFA-1, ICAM-1, leukocyte

Cells do not prosper in isolation. For efficient functioning they require adhesive contacts with either neighboring cells or the tissue matrix elements within their immediate surroundings. This adhesion is frequently mediated by the integrins which are a large family of αβ heterodimers expressed by the majority of cells in the body [Hynes, 1992]. The indispensible role of integrins is illustrated by their participation in the many activities of white blood cells termed leukocytes. These cells circulate in the blood in nonadherent form but make transient bonds when contacting other leukocytes, moving across inflamed endothelium and trafficking through tissues. How the regulation of this adhesive state is accomplished has been the subject of much research and even more speculation. The exact mechanics of stimulation are not well understood but possible end effects include increases in integrin affinity and avidity changes brought about by receptor clustering plus other accessory features such as cell spreading. In addition, integrin function is dynamically controlled through the action of the divalent cations Mn^{2+}, Mg^{2+}, and Ca^{2+}. In this review we discuss current information about the elements which contribute to the regulation of leukocyte adhesion. This topic has been addressed in other recent reviews [Pardi, 1994; Diamond and Springer, 1994; Faull and Ginsberg, 1995; Stuiver and O'Toole, 1995; Lub et al., 1995].

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INTEGRIN STRUCTURE: METAL ION AND LIGAND BINDING SITES

Integrins are metalloproteins which have the potential to bind 4–5 divalent cations. It is not yet clear whether all these sites are active in the native receptor or equivalent in terms of cation specificity. A representation of the α and β subunits of a typical integrin is depicted in Figure 1. All α subunits have seven short homologous domains of ~60 residues, of which the last four (domains IV–VII) or three (domains V–VII), in I domain–containing integrins, contain a sequence resembling the Ca^{2+} binding EF-hand motif. Isolated peptides which include these motifs from the platelet αIIbβ3 integrin will bind Ca^{2+} as measured by a terbium luminescence technique [D'Souza et al., 1994]. Binding studies with the α subunits of LFA-1 (αLβ2) and αIIbβ3 show a site for ligand in this region [reviewed in Hogg et al., 1994]. These are the first recognized examples in integrins of a close association between ligand and divalent cation binding.

The "I" domain which is "inserted" within the short α subunit domains is found in only

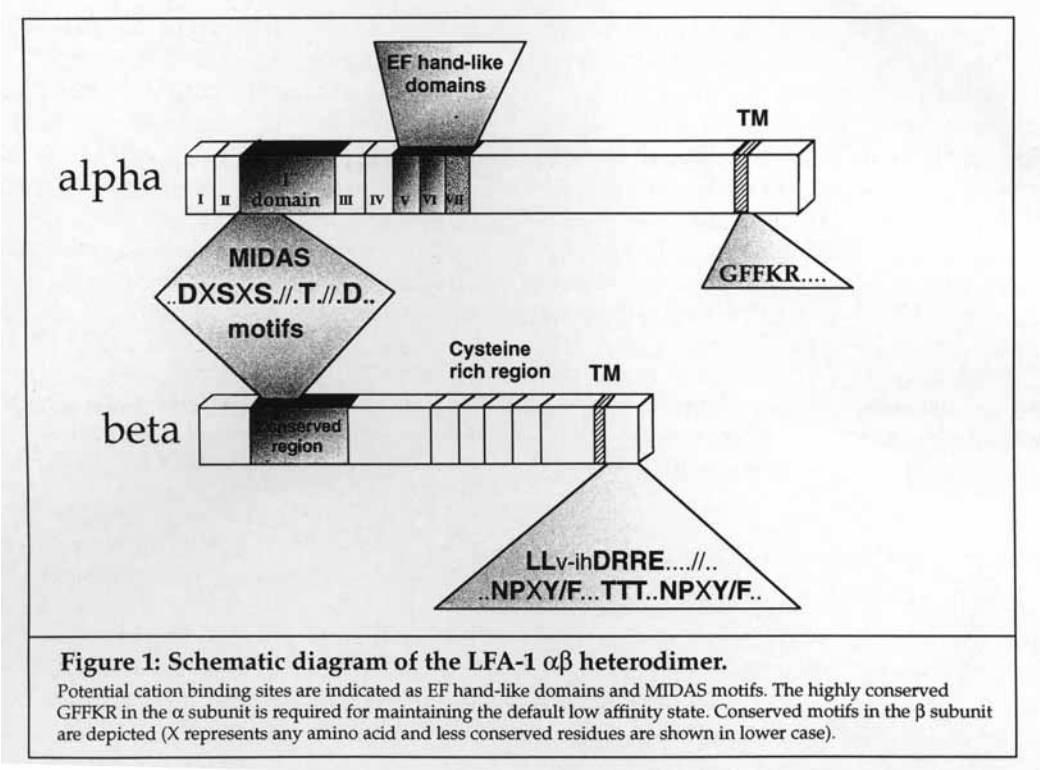

Figure 1: Schematic diagram of the LFA-1 αβ heterodimer.
Potential cation binding sites are indicated as EF hand-like domains and MIDAS motifs. The highly conserved GFFKR in the α subunit is required for maintaining the default low affinity state. Conserved motifs in the β subunit are depicted (X represents any amino acid and less conserved residues are shown in lower case).

Fig. 1. Schematic diagram of the LFA-1 αβ heterodimer. Potential cation binding sites are indicated as EF-hand-like domains and MIDAS motifs. The highly conserved GFFKR in the α subunit is required for maintaining the default low-affinity state. Conserved motifs in the β subunit are depicted (X represents any amino acid, and less conserved residues are shown in lower case).

seven integrins, all of which are expressed on leukocytes: the β2 family members, LFA-1 (αLβ2), Mac-1 (αMβ2), p150,95 (αXβ2), and the newly discovered integrin αDβ2; α1β1 (VLA-1), α2β1 (VLA-2), and the intraepithelial T cell integrin, αEβ7. The interest in I domains has been intense not only because they bind ligand but also because they have a metal binding site, originally recognized in the I domain of Mac-1, which differs from the EF-hand-like cation binding domains [Michishita et al., 1993]. This I domain has now been crystallized [Lee et al., 1995] and is revealed to be a homolog of the classical Rossmann fold of the dinucleotide binding type displaying a row of six β strands surrounded by seven α helices [reviewed in Bergelson and Hemler, 1995]. In the crystal structure, an Mg^{2+} coordination site is located at the "top" of the fold and composed of a D×S×S motif plus two noncontiguous "T" and "D" residues, which has led to the term MIDAS motif (metal ion–dependent adhesion site). It is now certain that the I domains can recognize all the major ligands of the I domain–containing integrins as well as the pathogens Echovirus (α2β1) and the NIF protein of canine hookworm (Mac-1) [reviewed in Hogg et al., 1994]. A close connection between ligand and metal binding for the I domain is also evident from studies showing that the iC3b and NIF protein binding sites impinge on the MIDAS motif [Ueda et al., 1994] and mutation of this motif prevents ligand binding [Kamata et al., 1995]. It is now fairly certain that a similar domain expressing a MIDAS motif is present within the conserved region in all β subunits, although the homology is evident only at the level of secondary structure prediction [Lee et al., 1995] (see Fig. 1).

There have been several speculations as to why it might be beneficial for ligand and metal ion binding to be associated. One possibility has been that a stable ternary structure is formed in which ligand directly interacts with the metal ion site via one of its coordinating residues, or alternatively that metal ion binding may have a long-range role in altering conformation, perhaps by making a binding site accessible. Evidence is now available which suggests a slightly different option. D'Souza and colleagues have demonstrated that a three-way complex be-

tween integrin, cation, and ligand may be an unstable intermediate. Their idea is that bound cation holds the conformation of the binding site in a form recognized by ligand, but when ligand binds the cation is displaced, perhaps through another shape change [D'Souza et al., 1994]. These thoughts are also consistent with the finding that ligand ICAM-1 binds to LFA-1 domain V/VI in the absence of divalent cation [Stanley et al., 1994]. It will be interesting to examine structures of the I domain and the domain V/VI region of the α subunit, in the presence and absence of bound metal ion, to determine how these distinct cation binding motifs in the different subunits assist ligand binding.

INTEGRIN ACTIVITY IS A REFLECTION OF CELL BACKGROUND

The activity of integrins is dependent on the cell type on which they are expressed. One early example of this feature was the collagen and laminin specificity of α2β1 expressed by endothelial cells compared to the strict collagen binding activity of the same integrin on leukocytes [Kirchhofer et al., 1990]. This cell type–specific behavior has been confirmed in transfection experiments using the integrin α2β1 [Chan and Hemler, 1993], and by analysis of α4β1 on various cell lines [Masumoto and Hemler, 1993]. In addition to cell type–specific differences, the differentiation state of the cell can also define integrin activity. Differentiation of a monocyte cell line with phorbol ester for several days converts α5β1 into a state capable of binding fibronectin without altering integrin expression levels [Faull et al., 1994]. The ability of the β2 integrin, Mac-1, on monocytes to bind fibrinogen is also related to the state of cellular maturation and differentiation [Altieri et al., 1988]. These examples suggest that the correct intracellular apparatus must be present in order to transmit activation signals to integrins. This may depend on the lipid composition of cell membranes [Conforti et al., 1990] or on intracellular events such as altered connection with the cytoskeleton [reviewed in Lub et al., 1995].

As previously mentioned, leukocytes differ from other cells in being able to transiently regulate the activity of their integrins. The integrins are normally expressed in an inactive form as befits a circulating cell, but when the leukocyte is triggered by a receptor such as the antigen-specific T cell receptor (TCR)/CD3 complex on T lymphocytes, then "inside out" signaling promotes a transient adhesive state mediated by LFA-1 [Dustin and Springer, 1989] and the β1 receptors α4β1, α5β1, and α6β1 [Shimizu et al., 1990]. The adhesiveness of integrins can also be influenced by stimulation through other cell surface receptors as well as pharmacological agents such as phorbol ester [Dustin et al., 1989], calcium ionophore [van Kooyk et al., 1993], and lipid [Hermanowski-Vosatka et al., 1992]. How these stimulants achieve increased adhesion is an area of intense research interest.

AFFINITY REGULATION OF INTEGRINS

One direct way of increasing the adhesiveness of a receptor for its ligand is by the induction of a high affinity state. This method of regulation consists of an alteration within the receptor itself which is conveniently described by many researchers as a conformational change, although structural evidence for this is lacking. The divalent cations Mg^{2+}, Mn^{2+}, and Ca^{2+}, special activating monoclonal antibodies (mAbs), and ligand mimetic peptides are all agents which can alter integrin affinity [reviewed in Faull et al., 1995; Stuiver et al., 1995].

The Yin and Yang of Divalent Cations Mg^{2+} and Ca^{2+}

Certain divalent cations have been extensively used as a method for increasing the affinity state of integrins. For example, Mn^{2+} increases the affinity of VLA-4 for soluble (s)VCAM-1 with a dissociation constant (K_d) of 33 nM [Jakubowski et al., 1995a]. Mg^{2+} treatment can induce a high affinity form of LFA-1 for sICAM-1, although this is a much lower affinity interaction than that described above, as saturated binding is achieved only at 1.8 μM [Stewart et al., 1996]. For many integrins Ca^{2+} appears to interfere with the activity of Mg^{2+}. For example, Mg^{2+} activates α2β1 anchored in liposomes to bind to collagen, and this is inhibited in the presence of Ca^{2+} ions [Staatz et al., 1989]. This same cation crosstalk is evident in LFA-1, where Mg^{2+} and Mn^{2+} have opposing effects to Ca^{2+} on adhesion of T cells to ICAM-1 and expression of the activation epitope recognized by mAb 24 [Dransfield et al., 1992]. Although Ca^{2+} inhibits generation of high affinity LFA-1, it plays a role in integrin clustering [van Kooyk et al., 1994]. It is not known which of the

cation sites are occupied in any of these situations.

Activating Monoclonal Antibodies

As illustrated in the following two examples, direct evidence for structural alteration in integrins comes from the use of special mAbs which either induce or report affinity increases on integrins in solution or in purified form. The mAb TS2/16 induces a high-affinity state of β1 integrins in solution [Arroyo et al., 1993] and the β2 mAb KIM 127 converts purified Mac-1 from inactive to active form [Cai and Wright, 1995].

The LIBS Step

In 1988, Ginsberg and colleagues described the appearance of ligand-induced binding site (LIBS) epitopes on integrin αIIbβ3 following occupancy by ligand or ligand mimetics such as RGD peptide [Frelinger et al., 1988; Du et al., 1991]. On T cells, the 24 epitope behaves as a LIBS as it is expressed at points of cell contact with ligand ICAM-1 [Cabañas and Hogg, 1993]. These LIBS are considered to mark alterations in integrins reflecting high affinity status. There are two explanations for how the LIBS step might occur. The "induced fit" model suggests that an initial weak interaction between receptor and ligand causes an alteration in the receptor leading to high-affinity binding. A second explanation centers on the idea that integrin on the cell membrane may exist in an equilibrium of active and inactive forms. By binding to a transiently active integrin, ligand may prolong and stabilize this form. So far there is no firm evidence allowing a choice between these two models. There is not always an absolute requirement for the LIBS effect, as high concentrations of Mg^{2+} ions will also produce 24 epitope–positive LFA-1 in the absence of ligand [Stewart et al., submitted]. This observation suggests that the function of ligand in the LIBS step may be to lower the threshold for binding of Mg^{2+}, which in turn facilitates the change to a high affinity form of integrin; this same objective can otherwise be achieved through "swamping" the critical cation site(s) with greatly increased concentrations of Mg^{2+} (Fig. 2).

An Up-to-Date Hypothesis for Activation

Progress is being made in defining the nature of the alteration occurring during integrin activation. In a key experiment Cai and Wright show that the activating mAb KIM 127 can bind

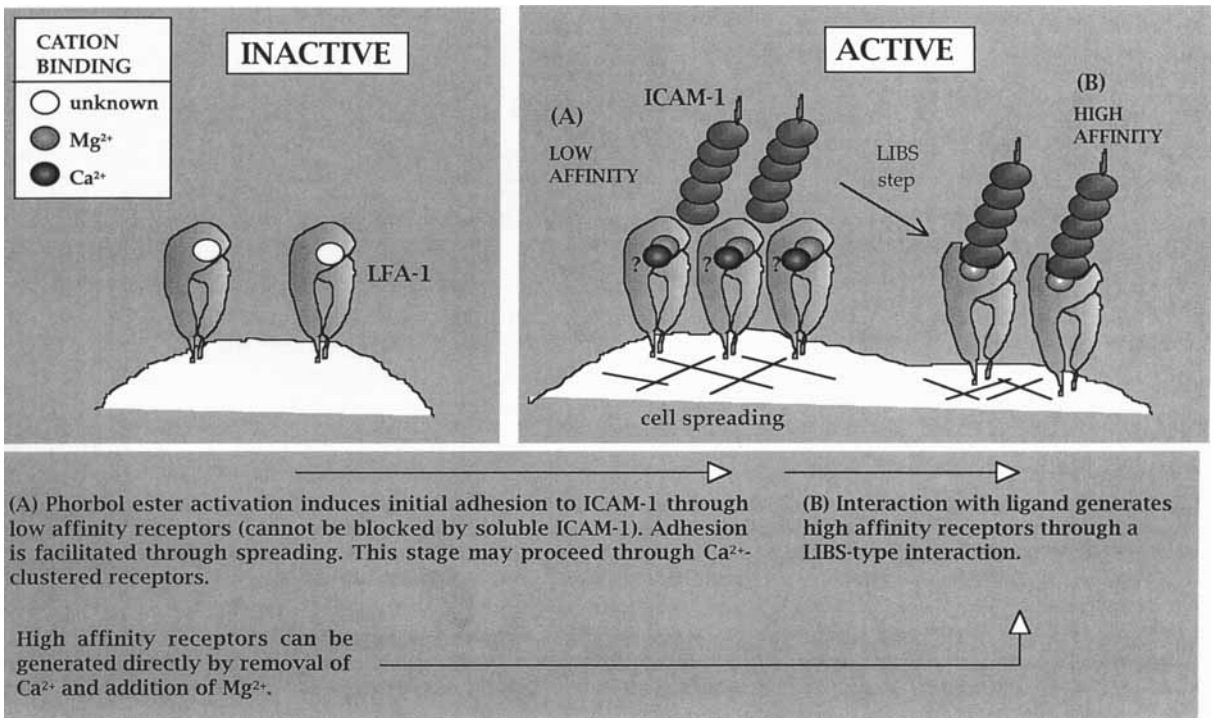

Fig. 2. Hypothetical model of LFA-1 activation states leading to adhesion.

with similar affinity to Mac-1 whether it is in an inactive or active form. This strongly suggests that no conformational change takes place during this process [Cai et al., 1995] and is consistent with a model where the mAb induces exposure of the ligand binding site, possibly through an altered arrangement between α and β subunits. This interpretation is consistent with the fact that the mAb 24 "activation" epitope on LFA-1 is not a neoepitope but preexists on the α subunit [Dransfield et al., 1990], and that it has proved possible to identify ligand binding sequences in the LFA-1 α subunit using isolated fragments of this cation binding domain [reviewed in Hogg et al., 1994].

Inside the Cell

Although the affinity status can be altered from outside the cell, there is evidence that some constraints on integrins are imposed by "active metabolism." This could be explained simply by requirement for membrane fluidity or for intracellular factors. It is now obvious that integrin cytoplasmic domains can control affinity changes [reviewed by Williams et al., 1994]. For example, the GFFKR sequence, which is adjacent to the transmembrane region on the α subunit, behaves as if it determines the default low affinity status of integrins as its elimination confers a constitutive high affinity state on the receptor [O'Toole et al., 1994] (see Fig. 1). An homologous sequence (LLv-ihDRRE) at the cytoplasmic face of the β subunit behaves similarly [Hughes et al., 1995]. Further down the β subunit cytoplasmic domain, mutation within the first of two highly conserved NPXY/F motifs can also alter the affinity status of integrin [O'Toole et al., 1995]. There is so far no explanation as to why a motif associated with the internalization of other receptors affects integrin affinity! Speculation that intracellular factors could bind to and influence these regions has been strengthened by data of this past year identifying several cytoplasmic tail binding factors. For example, the intracellular Ca^{2+} binding protein calreticulin binds to the GFFKR motif [Rojiani et al., 1991], and more recently the focal adhesion kinase $pp125^{FAK}$ and paxillin, both cental players in focal contact formation and signaling, have been shown to bind to peptides containing the LLv-ihDRRE motif [Schaller et al., 1995], to which filamin can also bind [Sharma et al., 1995]. Whether binding of these factors to cytoplasmic tails can regulate affinity has yet to be determined.

AVIDITY REGULATION OF INTEGRINS

The activity of integrins may also be regulated through avidity alterations. In theory this allows efficient cell adhesion in the absence of sufficient high affinity receptors. This can most easily be envisaged as a coordinated effort between several low affinity receptors clustered together to increase the overall binding strength in a particular area or through morphological changes in the cell, such as cell spreading, which would either increase the cell surface area available for interaction with ligand and/or reduce shear on the cell by providing a flattened or more streamline shape. Evidence for both integrin clustering and activation-induced spreading exists for the leukocyte integrins and involves connection with the cytoskeleton.

Cell Spreading

Treatment with phorbol esters is a widely used model for inducing integrin adhesion. Several studies have now found that phorbol ester–triggered adhesion occurs without a change in the affinity state of integrins. The spreading of T cells onto fibronectin through α5β1 [Faull et al., 1994] and onto ICAM-1 through LFA-1 [Stewart et al., 1996] occurs with no coincident increase in the affinity of these integrins for their ligands but is dependent on cytoskeletal alterations. Spreading is very much a physiological phenomenon as it occurs following T cell interaction with antigen-pulsed presenting cells [Donnadieu et al., 1994] and with monocytes and neutrophils as part of the transmigration process [Beekhuizen et al., 1992]. Benefits of cell spreading are obvious in venules where cell flattening would provide a means of streamlining leukocytes onto endothelial cells, thus reducing the shear imposed on them by vascular flow. By forcing a larger area of the cell into closer apposition with ligand, spreading could also allow greater opportunity for integrins to bind ligand by means of a zipper-like mechanism [Singer, 1992].

Through mutational analysis it is now clear that specific motifs in integrin β cytoplasmic tails are responsible for the cytoskeletal alterations which allow spreading to take place. Peter and O'Toole show that cell adhesion, spreading, and focal contact formation are dependent on three threonines (T758–760) in the β2 sub-

unit cytoplasmic domain [Peter and O'Toole, 1995] (see Fig. 1). This motif was highlighted in an earlier study [Hibbs et al., 1991] and corresponds to an S752 in β3 which has a role in cell spreading and cytoskeletal interactions [Ylänne et al., 1995].

Clustering

Some of the earliest work on integrins documents their ability to undergo capping, which can be thought of as macroclustering. Phorbol esters also cause cytoskeletal-dependent capping of LFA-1 [Haverstick et al., 1992], concomitant colocalization with the cytoskeletal protein talin [Burn et al., 1988], and aggregation of Mac-1 receptors which is correlated with their adhesion [Detmers et al., 1987]. Ca^{2+} is instrumental for clustering of LFA-1 on a T cell line and is thought to be prerequisite for ligand binding [van Kooyk et al., 1994]. However, LFA-1 on primary leukocytes apparently cannot be clustered in this way and requires prior release from the cytoskeleton with cytochalasin D [Lub et al., 1995]. Cytoskeletal participation is also evident following ligand binding when signals are directed back into the cell. It is an attractive idea that integrins might interact with the cytoskeleton in different ways according to their state of activation and have their functions regulated in this manner.

The Phorbol Ester Model of Adhesion

There is good evidence that phorbol esters orchestrate adhesion through cell flattening and possibly through receptor clustering, although an alteration in receptor distribution could result from cell differentiation. In a number of model systems it seems certain that spreading proceeds via a low-affinity form of integrin. This is further confirmed by the inability of soluble ICAM-1 to block adhesion stimulated by phorbol ester [Stewart et al., 1996] (see Fig. 2). The LIBS step is also involved here, as phorbol ester–treated T cells display the 24 activation epitope once they have adhered to immobilized ICAM-1 [Stewart et al., 1996]. This has the practical benefit of positioning stable LFA-1/ICAM-1 complexes at precisely the locations where they are required, that is, at points of cell–cell contact.

The Physiological Setting

Model systems have been an essential means for dissecting the details of integrin activation, but it is obviously of interest to know how integrin regulation occurs in a physiological context. On T cells, crosslinking the antigen-specific TCR/CD3 complex with antibodies has been considered to emulate natural signaling, and this causes integrin-mediated adhesion. However, this procedure does not induce high-affinity integrin as measured by the inability of VLA-4 to bind to sVCAM-1 [Jakubowski et al., 1995a] and LFA-1 to bind to sICAM-1 [Stewart et al., 1996]. Such signaling does result in cell flattening [Donnadieu et al., 1994], actin polymerization [Valitutti et al., 1995], and association of LFA-1 with cytoskeletal proteins [Pardi et al., 1992]. Therefore, signaling through the TCR/CD3 complex does not induce high-affinity LFA-1 and VLA-4 receptors in the first instance, and resembles the model of phorbol ester–induced adhesion. Stimulation of T cells with the chemokine MIP-1α and cell surface receptor CD31 also promotes VLA-4-mediated adhesion to VCAM-1 without altering the integrin affinity state [Jakubowski et al., 1995]. This would seem to be strong evidence that avidity regulation is widespread in the physiological context.

On the other hand, TCR triggering is able to induce high-affinity α5β1 receptors for fibronectin in a T lymphoid cell line [Faull et al., 1994], suggesting that it is too early for generalizations. Other natural agonists increase the affinity of both αIIbβ3 and Mac-1 for their ligands. Thrombin and adenosine diphosphate (ADP) cause αIIbβ3 to bind soluble ligands fibrinogen and fibronectin [reviewed in Faull et al., 1995]. ADP and the chemoattractant fMLP, but not PMA, induce Mac-1 to bind factor X [Altieri et al., 1988] and fibrinogen [Altieri and Edgington, 1988]. Therefore it also seems plausible that affinity regulation can occur through agonist stimulation, and the fact that soluble VCAM-1 can block adoptively transferred autoimmune diabetes in mice gives credence to an in vivo role for affinity changes [Jakubowski et al., 1995b].

The majority of other reports documenting integrin affinity changes make use of activating antibodies and high levels of divalent cation, which are considered of questionable physiological relevance. The divalent cation Mg^{2+} is able to induce high-affinity LFA-1 receptors only when extracellular Ca^{2+} is removed [Dransfield et al., 1992]. Such an exchange ought to be precluded by the plasma levels of Ca^{2+} (2.5–5 mM) compared to 1–2 mM Mg^{2+}. However, the Mg^{2+}/

Ca^{2+} ratio can increase significantly during tissue damage, which may be due to release of some of the 30 mM Mg^{2+} available intracellularly. This altered cation ratio is associated with the ability of wound fluid to induce movement of cells into affected areas [Grzesiak and Pierschbacher, 1995]. Thus direct affinity regulation by divalent cation may be possible in vivo.

In summary, there has now been more detail documenting the essential components which mediate integrin adhesion such as affinity changes, integrin clustering, and a critical role for cell spreading. Discovering which events are *important in vivo* is the next challenge. Whether avidity regulation predominates over affinity regulation or whether a balance between these two mechanisms is the key to successful adhesion to ligands such as ICAM-1 is a major topic for the future.

NOTE ADDED IN PRESS

Two further I domain structures have now been published, one being an LFA-1 I domain (A. Qu and D.J. Leahy, Proc Nat Acad Sci USA 92:19277–10281, 1995) and the other an additional Mac-1 I domain (J.O. Lee et al., Current Biol 3:1333–1340, 1995). These two structures resemble each other in the manner in which metal ion is bound by the MIDAS motif and differ from the first Mac-1 structure which is ligated to another I domain in the crystal form and postulated to resemble the active form of this domain.

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