Divalent Cation Regulation of the Function of the Leukocyte Integrin LFA-1

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Abstract. The integrin lymphocyte function-associated antigen-1 (LFA-1) expressed on T cells serves as a useful model for analysis of leukocyte integrin functional activity. We have assessed the role of divalent cations Mg²⁺, Ca²⁺, and Mn²⁺ in LFA-1 binding to ligand intercellular adhesion molecule-1 (ICAM-1) and induction of the divalent cation-dependent epitope recognized by mAb 24. Manganese strongly promoted both expression of the 24 epitope and T cell binding to ICAM-1 via LFA-1, suggesting that Mn²⁺ is able to directly alter the conformation of LFA-1 in a manner that favors ligand binding. Since Mn²⁺ also promotes functional activity of other integrins, parallels in mechanism of ligand binding may span the integrin family. In contrast, induction of 24 epitope expression by Mg²⁺ required removal of Ca²⁺ from T cell LFA-1 with EGTA. Furthermore, binding of mAb 24 to T cell LFA-1 in the presence of either Mn²⁺ or Mg²⁺ was found to be specifically inhibited by Ca²⁺, suggestive of a negative regulatory role for Ca²⁺ in the control of leukocyte integrin function. Analysis of T cell binding to ICAM-1 via LFA-1 in the presence of Mg²⁺ or Mn²⁺, confirmed that Ca²⁺ exerted inhibitory effects upon LFA-1 function. The implication of our findings is that Ca²⁺ bound with relatively high affinity to LFA-1 may serve to maintain an inactive state. Thus induction of function and 24 epitope expression may occur as a result of displacement of Ca²⁺ from leukocyte integrins or alternatively, such activators may be able to impose the required conformational change in the presence of bound Ca²⁺.

The CD11/CD18 subfamily of integrins, otherwise known as the β₂ or leukocyte integrins, are receptors that regulate dynamic adhesion processes of leukocytes (Springer, 1990; Sanchez-Madrid et al., 1983). The sequence of events resulting in induction of leukocyte integrin functional activity has been only partly determined. Thus intracellular processes are able to exert control over leukocyte integrin function, switching these receptors rapidly from an "inactive" to an "active" form, thereby modulating adhesive interactions of immune cells. Altered associations between leukocyte integrins and cytoskeletal elements within the cell occur after treatment with activating agents. Co-capping of lymphocyte function-associated antigen-1 (LFA-1) and talin following phorbol-ester treatment has been observed (Kupfer and Singer, 1989) and integrin function might be controlled in part by association with proteins such as α-actinin and talin (Horwitz et al., 1986; Otey et al., 1990). Altered cell surface distribution of cytoskeletal-associated leukocyte integrins has been suggested to localize them at sites of adhesive interaction, providing a mechanism for avidity regulation (Detmers et al., 1987; Figdor et al., 1990; Dransfield, 1991).

Control of leukocyte integrin functional activity independent from that of avidity regulation may be possible. Conformational changes in the extracellular domains of leukocyte integrins could cause altered affinity of ligand binding. Although the precise alterations that occur following activation have yet to be defined, the presence of putative divalent cation domains on the α subunits of integrins and the critical role of extracellular divalent cations in integrin-ligand interaction, suggests that structural alterations necessary for ligand recognition may occur as a result of divalent cation binding. For many integrins, ligand recognition is augmented by the presence of the divalent cation Mn²⁺. However, the basis for this phenomenon is poorly understood.

We have been investigating the binding characteristics of a unique mAb named 24, that is specific for the leukocyte integrin α subunits (Hogg and Selvendran, 1985; Dougherty et al., 1988; Dransfield and Hogg, 1989). Since the epitope is present on the three related polypeptides, it may define a conserved region of these molecules. More interestingly, and of direct relevance to the control of leukocyte integrin function, antibody recognition of the intact leukocyte integrin

1. Abbreviations used in this paper: ICAM-1, intercellular adhesion molecule-1; LFA-1, lymphocyte function-associated antigen-1; MFI, mean fluorescence intensity; PBMC, peripheral blood mononuclear cells; PdBu, phorbol-12,13-dibutyrate.
heterodimer requires the presence of Mg$^{2+}$. This antibody has therefore been used to probe the Mg$^{2+}$ occupancy of leukocyte integrins on intact cells and we have previously suggested that alteration of affinity of divalent cation binding represents part of the mechanism for control of leukocyte integrin functional activity (Dransfield and Hogg, 1989; Dransfield et al., 1990). In this paper, LFA-1 expressed on T cells has been used as a model system for analysis of leukocyte integrin functional activity. The role of divalent cations has been examined in the induction of expression of the 24 epitope and control of the interaction between T cell LFA-1 with its ligand, intercellular adhesion molecule-1 (ICAM-1). Both Mn$^{2+}$ and Mg$^{2+}$ promoted interaction of LFA-1 with ICAM-1 and also induced the 24 epitope. Although both divalent cations were effective, 20-fold higher concentrations of Mg$^{2+}$ were necessary and unlike Mn$^{2+}$, required chelation of Ca$^{2+}$. Indeed, Ca$^{2+}$ specifically inhibited induction of LFA-1 ligand binding activity and of the 24 epitope by Mn$^{2+}$/Mg$^{2+}$. The conclusion from these studies is that divalent cation binding, and implicitly, function of leukocyte integrins is finely controlled. Thus high-affinity Ca$^{2+}$ binding may serve to maintain leukocyte integrins in an "inactive" state, with transition to the Mg$^{2+}$-bound "active state" requiring displacement of bound Ca$^{2+}$ either by Mn$^{2+}$ or by the mechanisms of physiological activation.

**Materials and Methods**

**Monoclonal Antibodies**

Production, isolation, and characterization of mAb 24 has been described previously (Hogg and Selvendran, 1985; Dougherty et al., 1988; Dransfield and Hogg, 1989). Control mAbs used were 5.5, recognizing p814 cytoplasmic protein (I5), and 38, (CD11a) recognizing the LFA-1 α subunit (Dransfield and Hogg, 1989).

**ICAM-1**

ICAM-1-expressing L cells transfectedants have been described previously (Altman et al., 1989). In addition, ICAM-1Fc protein was prepared by replacing the CD8 portion of an Fc (hinge, CH2, and CH3 domains of immunoglobulin G) expression plasmid (Aruffo et al., 1990) with the three N$	ext{H}_{2}$-terminal domains of ICAM-1 (Simmons et al., 1988) (up to the asparagine residue at position 287). The recombinant protein was produced by transient expression in COS-1 cells (Seed and Aruffo, 1987) and purified by adherence to protein A-coupled Sepharose.

Both ICAM-1-expressing L cells and ICAM-1Fc protein were used as targets in the T cell binding assay and were prepared in the following manner. The ICAM-1-expressing L cell transfecants were grown in flat bottom tissue culture 96-well plates (Becton Dickinson & Co., Mountainview, CA) in selection medium containing 0.5 mg/ml of Geneticin (Sigma Chemical Co., St. Louis, MO). After reaching confluence, supernatants were discarded and the L cells were fixed with 2% formaldehyde in PBS-A before addition of T cells. Purified ICAM-1Fc protein (40 μl of a concentration of 20 μg/ml in PBS-A) was added to each well of 96-well flat bottom ELISA plates (Dynatech, Cambridge, MA) and incubated at 4°C overnight. The plates were subsequently saturated with 2% BSA and PBS-A (100 μl/well) by incubation for 2 h at room temperature. The wells were then washed five times with 200 μl of PBS-A before addition of T cells.

**T Cells**

Peripheral blood mononuclear cells (PBMC) were prepared from freshly drawn heparinized blood by centrifugation over Ficoll/Hypaque (Pharmacia, Uppsala, Sweden). T cells were prepared by passage of plastic non-adherent PBMC over a nylon wool column (Julius et al., 1973) yielding >90% CD3 positive cells. Activated T cells were expanded from unstimulated PBMC by culture in RPMI 40 containing 10% FCS medium plus treatment with phytohaemagglutinin (10 μg/ml) and phorbol-12,13-dibutyrate (PdBu) (50 nM) for 48 h (Cantrell et al., 1985). Cells were then washed and cultured in medium plus 10% supernatant from the IL-2 producing cell line MLA. After 1-2 wk culture, quiescence was induced by removal of IL-2 from the culture medium for 2-3 d. The resultant quiescent T cells serve as useful models for analysis of T cell activation (Cantrell and Smith, 1984; Cantrell et al., 1985).

**T Cell Binding Analysis**

Detection of T cell binding to ICAM-1-L cells or ICAM-1Fc was carried out as follows. T cells were labeled with 200 μCi of $^{35}$Cr/ml of cells (2 × 10$^5$/ml) for 1 h at 37°C, and then washed in 20 mM Hepes, 140 mM NaCl, 2 mg/ml glucose, pH 7.4 (Hepes/NaCl). Labeled cells were added to 96-well tissue culture plates containing ICAM-1Fc or confluent monolayers of ICAM-1-L cells. For analysis of T cell binding in different divalent cations, appropriate dilutions of cations were made in Hepes/NaCl buffer. In one set of experiments, as stated in text, the assay was performed either in 50 mM MnCl$_2$, with or without PdBu added to the cells at a final concentration of 20 mM during the binding assay.

Plates were then centrifuged at 75 g for 1 min before incubation at 37°C for 30 min to allow binding and washed three times before radiometric quantitation of cell binding. Bound cells were lysed in PBS-A containing 1% NP-40 and counted using a Betaplate counter (LKB Instruments Inc., Bromma, Sweden).

**Flow Cytometric Analysis**

Before flow cytometric analysis, cells were washed in Hepes/NaCl buffer or in some experiments, as stated in text, were incubated for 5 min at 37°C either in Hepes/NaCl or Hepes/NaCl containing 5 mM EDTA. Washed cells were then added to flexi-well plates at 2 × 10$^5$/well and 50 μl of antibody added to each well. mAb 24 was used either as ascitic fluid (1:200 dilution) or purified IgG (20 μg/ml) at which concentration, binding was saturating. Dilutions of the divalent cation chlorides were made in Hepes/NaCl buffer as appropriate. Primary antibody incubations were carried out for 20 min at 37°C as previously described (Dransfield and Hogg, 1989). Cells were then washed twice in ice cold Hepes/NaCl and bound mAb was detected using FITC-conjugated goat anti-mouse IgG (used at 1:400, Cappel Laboratories, Cochranville, PA). Secondary antibody incubations were carried out for 20 min at 4°C followed by two further washes in Hepes/NaCl. Analysis of fluorescence was made using a FACScan (a registered trademark of Becton Dickinson & Co.) fitted with a logarithmic amplifier. Mean fluorescence intensity (MFI) of binding of mAb 24 is calculated relative to binding in the absence of divalent cation (or in the presence of 5 mM EDTA). The MFI recorded for mAb 24 in the absence of divalent cation was always lower than control values when compared with relevant non-binding mAbs, indicating that no detectable binding of mAb 24 occurred under these conditions.

**Radiodination and Immune Precipitation**

Cells were labeled with $^{125}$Iodine using lactoperoxidase/glucose oxidase as previously described (Dransfield and Hogg, 1989). Cell lysis was in Hepes/NaCl buffer containing 1% NP-40 and protease inhibitors apronitin (0.4 U/ml), PMSF (2 mM), iodoacetamide (10 mM), and 0.1% NaN$_3$ with the subsequent lysate centrifuged at 11,000 g for 30 min to remove cellular debris. Lysates were pre-cleared with either mAb 5.5-Sepharose or glycine-Sepharose for 30 min on a rotary mixer. Immune precipitation using mAbs 24 and 38 directly coupled at 1 mg/ml to Sepharose CL-B (25 μl of a 10% suspension per 100 μl of lysate) was carried out for 1 h on a rotary mixer. After precipitation, beads were washed twice in Hepes containing 500 mM NaCl and 1% NP-40, twice in Hepes containing 140 mM NaCl and 1% NP-40, and once in Hepes containing 0.5% SDS before SDS-PAGE analysis on 7% acrylamide gels under reducing conditions. Dried gels were exposed to XOMAT-AR photographic film (Eastman Kodak Co., Rochester, NY). Molecular weight markers used were myosin heavy chain (200 kD), phosphorylase B (97 kD), and bovine albumin (66 kD).

**Results**

**Effect of Manganese upon LFA-1 Ligand Binding Activity**

We have previously shown that Mn$^{2+}$ was able to substitute for Mg$^{2+}$ in restoration of LFA-1 binding to ICAM-1 as
Figure 1. LFA1 functional activity is induced by Mn²⁺ alone. °Cr-labeled resting T cells were allowed to bind to ICAM-1 expressing L cells either in E4 medium (o) or in Hepes/NaCl containing 500 µM Mn²⁺ (w). Binding was for 30 min at 37°C either in the presence (PdBu) or absence (none) of 20 nM PdBu as indicated. For each set of conditions the percentage of T cell binding in the presence of anti-LFA-1 mAb 38 is shown (anti-CD11a). Results from triplicate assays are expressed as the mean percentage of cells ± SEM of the total number of T cells added to each assay (total T cell counts = 26, 623 ± 500 cpm).

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Calcium Specifically Inhibits Manganese-induced mAb 24 Recognition

Binding of mAb 24 to T cells was maximal at concentrations of Mn²⁺ >50 µM (Fig. 3 a), 20-fold lower than the concentrations of Mg²⁺ required as previously determined (Dransfield and Hogg, 1989). In the presence of Ca²⁺ alone, no reactivity with mAb 24 was induced (Dransfield et al., 1990; Dransfield and Hogg, 1989; and data not shown). However, when the effect of Ca²⁺ upon Mn²⁺-induced reactivity was assessed by performing Mn²⁺ titrations in the presence of 1 mM Ca²⁺, binding of mAb 24 was inhibited (Fig. 3 a). To determine whether the observed inhibition was specific, the ability of other non-inductive divalent cations (Dransfield et al., 1990) to inhibit binding of mAb 24 to T cells in the presence of 100 µM Mn²⁺ was assessed. Results shown in Fig. 3 b demonstrate that Ca²⁺, and not Sr²⁺, Ba²⁺, or Mg²⁺, specifically inhibited Mn²⁺-induced recognition.

Induction of the 24 Epitope on T Cell LFA-1 by Magnesium Requires Removal of Calcium

In these present experiments, we had been surprised to find that Mg²⁺ was not able to induce the 24 epitope as in previous studies (Dransfield and Hogg, 1989). One possible explanation for the lack of induction of mAb 24 reactivity by Mg²⁺ was suggested by the ability of Ca²⁺ to inhibit Mn²⁺ induction of the 24 epitope. The speculation was that Ca²⁺ might already be bound to LFA-1 present on the T cell surface. In our previous studies, cells were pretreated with the divalent cation chelator EDTA before incubation with selected cation (Dransfield and Hogg, 1989). However, in...
Manganese is a potent inducer of mAb24 recognition of resting T cell LFA-1. Recognition of T cell surface LFA-1, or solubilized LFA-1 from 125I-labeled cells by mAbs 24 and 38 (CD11a, LFA-1 α) in the presence or absence of 500 μM Mn2+ was determined by flow cytometric analysis (a and b) or immunoprecipitation analysis (c). Indirect immunofluorescence FACS profiles of antibody binding are shown for mAbs 24 (a) and 38 (b). Binding of antibody in the presence of 500 μM Mn2+ (------) or absence of added divalent cation (-----) are shown compared to irrelevant nonbinding control mAbs 5.5 (IgGl) and 4 U (IgG2a) ( ). In c SDS PAGE analysis of polypeptides immunoprecipitated from 125I-labeled T cells by mAb 24 (lane a and b) and mAb 38 (lane c and d) in the presence (lane a and c) or absence (lane b and d) of 500 μM Mn2+.

these present studies T cells had been treated only by washing in Ca2+/Mg2+-free Hepes-buffered saline before incubation in the presence of divalent cation. The implication was that Ca2+ must be bound to leukocyte integrins with sufficient affinity that it was not removed simply by washing the cells in Ca2+/Mg2+-free medium.

To test the possibility that Ca2+ already bound to leukocyte integrins was inhibiting expression of the 24 epitope in the presence of Mg2+, the following experiments were performed. T cells were first washed either in buffer containing the Ca2+-chelating agent EGTA or in buffer lacking Ca2+/Mg2+, before an assessment of binding of mAb 24 to these treated cells in different conditions. Using cells that were washed only in Ca2+/Mg2+-free buffer, there was observed to be only low levels of binding of mAb 24 in the presence of Mg2+ (or Mg2+/Ca2+), compared to cells incubated with Mn2+ (Fig. 4 a). However, when these T cells were pre-treated with EGTA, binding of mAb 24 in the presence of Mg2+ was increased nearly 10-fold. Control samples in which Ca2+ was removed from T cells with EGTA or EDTA, and then incubated with Ca2+ alone or no divalent cation did not permit detection of the 24 epitope. The same experiments were repeated using immunoprecipitation analysis to assess whether these observations reflected divalent cation binding directly to solubilized LFA-1. Polypeptides immunoprecipitated by mAb 24 from 125I-labeled T cells under conditions equivalent to those used in the flow cytometric analysis are shown in Fig. 4 b. These results confirm that for T cells, Mg2+-dependent recognition of LFA-1 by mAb 24 requires removal of Ca2+ from the molecule by chelating agents.

Divalent Cation Requirements for LFA-1 Function Parallel Those for mAb 24 Recognition

The divalent cation requirements for binding of T cell LFA-1 to ICAM-1Fc were compared with those described above for mAb 24 recognition of its epitope. Quantitative assessment of binding of 51Cr-labeled T cells to ICAM-1Fc was performed in a range of concentrations of Mg2+ (Fig. 5 a) or Mn2+ (Fig. 5 b) either in the presence of chelating agent EGTA (1 mM) or Ca2+ (1 mM). In the presence of increasing amounts of Mg2+ and Mn2+, there is a direct correlation between the amount of T cell binding to ICAM-1 and the de-
Extracellular divalent cations have a critical role in permitting interaction of LFA-1 and other integrins with their ligands. For many receptors Mn\(^{2+}\) can replace the requirement for Mg\(^{2+}\) or Ca\(^{2+}\) and provide increased adhesiveness. Thus VLA-5 (Gailit and Ruoslahti, 1988), IbIIa (Kirchhofer et al., 1990), vitronectin receptor (Conforti et al., 1990), VLA-3 (Elices et al., 1991), and VLA-6 (Sonnenberg et al., 1988) all exhibit enhanced ligand binding in the presence of Mn\(^{2+}\). In this present study, LFA-1-mediated binding of T cells to ICAM-1 can be accomplished in the presence of Mn\(^{2+}\) without additional stimuli, suggesting that normal regulatory mechanisms are bypassed. Concentrations of \(~50 \mu M\) Mn\(^{2+}\) restored 50% binding to T cell LFA-1 of mAb 24 which detects a leukocyte integrin activation epitope. The same result was obtained with solubilized receptors indicating that Mn\(^{2+}\) acts directly upon LFA-1 to cause a conformational change. The components required for integrin activation are not yet fully understood but the fact that activation can be induced via an extracellular route using a mAb specific for LFA-1 (van Kooyk et al., 1991) or even after cell fixation (O'Toole et al., 1990), suggests that the required changes are an intrinsic feature of the receptors. The speculation would be that binding of Mn\(^{2+}\) to LFA-1 may be able to directly impose structural alteration, whereas activation in the presence of Mg\(^{2+}\) depends upon a preliminary event leading to a favorable LFA-1 conformation resulting in Mg\(^{2+}\) binding.

Although some integrins such as the fibronectin receptor VLA-5 are functional in the presence of Ca\(^{2+}\) (Gailit and Ruoslahti, 1988), there are now examples of Ca\(^{2+}\) having an inhibitory effect. For example, Ca\(^{2+}\) noncompetitively inhibits Mg\(^{2+}\) activation of VLA-2 (Santoro, 1986; Staatz et al., 1989) and inhibits Mg\(^{2+}\)-dependent αβ receptor function (Kirchhofer et al., 1991). In this study LFA-1/ICAM-1 binding induced by Mn\(^{2+}\) was specifically inhibited by Ca\(^{2+}\) and for LFA-1/ICAM-1 binding in the presence of Mg\(^{2+}\), pre-treatment with Ca\(^{2+}\)-chelating agents was necessary. These results are suggestive of Ca\(^{2+}\) being bound with relatively high affinity when compared to Mg\(^{2+}\). Observed effects of divalent cations on function were paralleled by 24 epitope expression on both intact T cells and solubilized LFA-1. It was therefore concluded that bound Ca\(^{2+}\) imposes a conformation of LFA-1 that is not recognized by mAb 24. These observations suggest that Ca\(^{2+}\) binding to leukocyte integrins (LFA-1) acts as a negative regulator of the functional activity of these molecules. Maintenance of integrin in such an inactive state would prevent leukocytes from randomly adhering to one another in the circulation until an appropriate encounter caused the stimulation necessary for the release of Ca\(^{2+}\) and acquisition of "active" conformation.

The negative effects of Ca\(^{2+}\) on LFA-1 function are seemingly at variance with reports from several groups suggesting that Ca\(^{2+}\) has synergistic effects in restoration of LFA-1 functional activity at suboptimal concentrations of Mg\(^{2+}\) (Martz, 1980; Rothlein and Springer, 1986; Marlin and Springer, 1987; Makgoba et al., 1988). This apparent discrepancy with results presented here may be explained by differences in protocol. As shown here, Ca\(^{2+}\) already bound to LFA-1 exerts a negative regulatory role upon LFA-1 function. Thus, pre-treatment with EDTA to remove bound divalent cation increases the functional activity of LFA-1 which can be induced with Mg\(^{2+}\). Moreover, both activated cells...
orphorbol ester-treated cells were used in the several studies in which Ca\(^{2+}\) was found to synergize with Mg\(^{2+}\) indicating that under these conditions there are different requirements for divalent cations (Martz, 1980; Rothlein and Springer, 1986; Marlin and Springer, 1987; Makgoba et al., 1988; C. Cabañas and N. Hogg, manuscript in preparation). Interestingly, binding of mAb NKI-L16 to LFA-1 is a Ca\(^{2+}\)-dependent process which causes leukocyte aggregation and has been suggested to be a reflection of LFA-1 membrane distribution (Figdor et al., 1990; van Kooyk et al., 1991). Such alterations in membrane localization might account for synergistic effects of Ca\(^{2+}\) upon Mg\(^{2+}\)-dependent cell binding discussed above.

It would seem likely that similar mechanisms of ligand and cation binding are used by different integrins. Data presented here demonstrating that detection of the 24 epitope upon

Figure 4. Recognition of T cell LFA-1 by 24 in the presence of Mg\(^{2+}\) requires the removal of Ca\(^{2+}\). (a) Indirect immunofluorescence analysis of mAb 24 binding to T cells and (b) immunoprecipitation analysis of polypeptides immunoprecipitated by mAb 24 from \(^{125}\text{I}\)-labeled T cells in the presence of 500 \(\mu\text{M}\) Mn\(^{2+}\) (lane 1), 2 mM Mg\(^{2+}\)/1 mM Ca\(^{2+}\) (lane 2), 2 mM Mg\(^{2+}\)/5 mM EGTA (lane 3), 2 mM Mg\(^{2+}\) alone (lane 4), 1 mM Ca\(^{2+}\) (lane 5), no added divalent cation (lane 6), or 5 mM EDTA (lane 7). In b, lanes 8 and 9 show polypeptides immunoprecipitated by mAb 38 (LFA-1\(\alpha\)) in the presence of 2 mM Mg\(^{2+}\) alone and presence of 2 mM Mg\(^{2+}\)/5 mM EGTA. In a results are shown as the MFI expressed relative to that recorded in the absence of divalent cation (EDTA).
LFA-1 requires removal of Ca\(^{2+}\) parallels that of Ginsberg and co-workers who have shown that expression of the PMI-1 epitope is induced by EDTA treatment of platelets (Ginsberg et al., 1986). Interestingly, ligation of IIb/IIIa with fibronogen \(\gamma\) chain peptide also induces the PMI-1 epitope indicating that ligand binding and cation binding are closely related events (Frelinger et al., 1988). The speculation would be that under conditions of physiological activation, displacement of bound Ca\(^{2+}\) from LFA-1 may allow it to adopt the confor-

mation permitting Mg\(^{2+}\)-dependent recognition of ligand and expression of the 24 epitope. The use of Mn\(^{2+}\) or Mg\(^{2+}\), in the presence of Ca\(^{2+}\) chelators, to induce an active conformation of LFA-1, may mimic ligation of LFA-1 to ICAM-1 induced by physiological activators. Alternatively, ligand binding may not actually displace Ca\(^{2+}\) from LFA-1, but it may impose a conformation similar to that seen in the presence of Mg\(^{2+}\) when Ca\(^{2+}\) is removed. However, since epitope expression parallels functional activity of LFA-1, we have further evidence that conformational changes accompany integrin activation, possibly resulting from divalent cation binding.

In summary, evidence has been presented that divalent cations can induce conformational alterations in the LFA-1 molecule that are detected by mAb 24, paralleling functional activity of LFA-1. In particular, Mn\(^{2+}\), a strong promoter of integrin function, induced both LFA-1/ICAM-1 binding and mAb 24 epitope expression. For Mg\(^{2+}\), both function and mAb 24 epitope expression upon LFA-1 required removal of Ca\(^{2+}\), suggesting that Ca\(^{2+}\) exerts a negative regulatory effect upon leukocyte integrin function. Moreover, these findings suggest that modulation of LFA-1 function may be possible as a result of altered interaction of LFA-1 with divalent cations.

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