The β₂ integrin lymphocyte function-associated antigen 1 (LFA-1) mediates activation-dependent adhesion of lymphocytes. To investigate whether lymphocyte-specific elements are essential for LFA-1 function, we expressed LFA-1 in the erythroleukemic cell line K562, which expresses only the integrin very late antigen 5. We observed that LFA-1-expressing K562 cannot bind to intercellular adhesion molecule 1-coated surfaces when stimulated by phorbol 12-myristate 13-acetate (PMA), whereas the LFA-1-activating antibody KIM185 markedly enhanced adhesion. Because the endogenously expressed β₁ integrin very late antigen 5 is readily activated by PMA, we investigated the role of the cytoplasmic domain of distinct β subunits in regulating LFA-1 function. Transfection of chimeric LFA-1 receptors in K562 cells reveals that replacement of the β₂ cytoplasmic tail with the β₁ but not the β₇ cytoplasmic tail completely restores PMA responsiveness of LFA-1, whereas a β₂ cyttoplasmic deletion mutant of LFA-1 is constitutively active. Both deletion of the β₂ cytoplasmic tail or replacement by the β₁ cytoplasmic tail alters the localization of LFA-1 into clusters, thereby regulating LFA-1-mediated adhesion to intercellular adhesion molecule 1. These data demonstrate that distinct signaling routes activate β₁ and β₂ integrins through the β-chain and hint at the involvement of lymphocyte-specific signal transduction elements in β₂ and β₇ integrin activation that are absent in the nonlymphocytic cell line K562.
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1990. \( \alpha_\beta \) mediates adhesion to VCAM-1, \( \alpha_\delta \) and the extracellular matrix component fibronectin, whereas \( \alpha_\beta \), predominantly binds to fibronectin (Hemler et al., 1987; Takada et al., 1987; Hemler et al., 1988; Al-tevogt et al., 1995).

LFA-1/ICAM-1 adhesion requires activation of LFA-1 through intracellular signals (Martz, 1987; Dustin and Springer, 1989; van Kooyk et al., 1989; Hyenes, 1992). This activation process, termed "inside-out" signaling, is common to all integrins including the \( \beta_1 \) and \( \beta_2 \) integrins (Keizer et al., 1988; Dustin and Springer, 1989; van Kooyk et al., 1989; Altieri, 1991; Dransfield et al., 1992b; Andrew et al., 1993; Landis et al., 1993). Activation of LFA-1 is thought to result in a conformational change in the \( \alpha/\beta \) heterodimer, leading to an enhanced binding affinity LFA-1 for its ligand ICAM-1 (Lollo et al., 1993). Both affinity (active conformation) and avidity (clustering) changes are required to obtain strong binding of LFA-1 to ICAM-1 (Dustin, 1990). Affinity/avidity changes in LFA-1 depend on an intact cytoskeleton, physiological temperature, and binding of divalent cations, in particular Mg\(^{2+}\) (Rothlein and Springer, 1986; Dransfield and Hogg, 1989; Figdor et al., 1990; Dransfield et al., 1992a).

Binding of Ca\(^{2+}\) to LFA-1 supports clustering (high-avidity state) of LFA-1 on the cell surface, resulting in enhanced LFA-1-mediated adhesion (Figdor et al., 1990; van Kooyk et al., 1994).

Alternatively, binding of certain activating anti-integrin antibodies, or the divalent cation Mn\(^{2+}\), to the extracellular part of the integrin, can also induce an active conformation (high-affinity state) of the \( \beta_1 \beta_2 \beta_3 \) integrin, resulting in increased adhesion to the ligand (Keizer et al., 1988; Robinson et al., 1992; Andrew et al., 1993; Landis et al., 1993). It is thought that these activating anti-integrin monoclonal antibodies (mAbs) mimic ligand binding and stimulate postligand binding signaling ("outside-in" signaling). Outside-in signaling generates different intracellular signals, including phosphorylation of distinct tyrosine kinases and other proteins (Hyenes, 1992; Kanner et al., 1993; Arroyo et al., 1994).

Although the cytoplasmic tail of the \( \alpha \) and \( \beta \)-chains of \( \beta_1 \beta_2 \beta_3 \) integrins are relatively short (45, 45, and 51 amino acids for the \( \beta_1 \beta_2 \beta_3 \) cytoplasmic tail, respectively) and do not contain any intrinsic kinase activity, the cytoplasmic tails seem to be involved in transmitting inside-out signals as well as outside-in signals to and from the integrin molecule. It has been demonstrated that the adhesiveness of LFA-1 is controlled by the cytoplasmic domain of the \( \beta_2 \) subunit, because truncation of the cytoplasmic \( \beta_2 \) tail, but not the \( \alpha_\delta \) tail, eliminates LFA-1 binding to ICAM-1 (Hibbs et al., 1991b). In particular, mutations of a triplet of threonines (positions 758–760) and the phenylalanine residue at position 766, in the \( \beta_2 \) cytoplasmic tail profoundly reduced the adhesiveness of LFA-1 (Hibbs et al., 1991a; Peter and O’Toole, 1995). It has been suggested that the altered adhesiveness due to mutation of the threonine triplet is caused by an altered cytoskeletal association/organization and not to an affinity change in LFA-1 (Peter and O’Toole, 1995). Because deletion of the cytoplasmic domain of the \( \alpha_\delta \) subunit does not affect binding to ICAM-1, it is hypothesized that the cytoplasmic tail of \( \alpha_\delta \) is predominantly involved in "postligand binding" events of this integrin (Hibbs et al., 1991b).

Similarly, truncation of the cytoplasmic domain of the \( \beta_2 \) integrin subunit impairs adhesion to both fibronectin and laminin and has been shown to be important for cell spreading and localization to focal contacts (Solowska et al., 1989; Hayashi et al., 1990; Marcantonio et al., 1990; Reszka et al., 1992). Moreover, also partial removal of the \( \beta_2 \) cytoplasmic domain displayed no ligand binding activity of \( \alpha_\beta \) (Crowe et al., 1994).

In this study, we investigated whether a lymphocytic environment is required to mediate adhesion through the \( \beta_2 \) integrin LFA-1 and whether this depends on the cytoplasmic tail of the \( \beta_2 \)-chain. Therefore, we used the erythroleukemic K562 cells to express either wild-type LFA-1, LFA-1 in which the entire cytoplasmic tail of the \( \beta_2 \) was deleted (ΔT24), or LFA-1 in which the cytoplasmic domain of the \( \beta_2 \) was exchanged for the cytoplasmic domains of \( \beta_1 \) or \( \beta_7 \).

We demonstrate that the cytoplasmic tail of the \( \beta_2 \)-chain of integrins plays a pivotal role in regulating ligand binding affinity (active conformation) and avidity (clustering) and suggest that K562 and lymphocytes use different signaling elements to activate integrins.

**MATERIALS AND METHODS**

**mAbs**

mAbs SFV-L7 (IgG1), NKI-L15 (IgG2a), and NKI-L16 (IgG2a) reactive with the \( \alpha \)-chain of LFA-1 were raised as described previously (Keizer et al., 1985, 1988). The nonblocking mAb TS2/4 (IgG1) reactive with \( \alpha_\delta \) was provided by Dr. E Martz (Sanchez Madrid et al., 1982); mAb 60.3 (lgG1), directed against \( \beta_2 \) was obtained from Dr. J.M. Harlan (Beaty et al., 1983). The anti-\( \beta_2 \) mAb KIM185 (IgG1) was used to activate \( \beta_2 \) integrins (Andrew et al., 1993) and the anti-\( \beta_2 \) mAb TS2/16 to activate \( \beta_2 \) integrins (Hemler et al., 1984; van de Wiel-van Kemenade et al., 1992). The anti-\( \alpha_\delta \) mAb SAM-1 (IgG1) was used to block very late antigen 5-dependent adhesion (Keizer et al., 1987).

**DNA Constructs**

The 4.2-kb \( \alpha \)-chain of LFA-1 was cloned in the XbaI site of the pCDM8 vector that directs expression of \( \alpha_\delta \) from the cytomegalovirus (CMV) AD169 immediate early promoter (pCDM8). The 3' end of \( \beta_2 \) was cloned as an EcoRI-BglII fragment in the pCMV CMV vector (containing a neomycin resistance gene; Invitrogen, San Diego, CA). Within this sequence is a unique Apal site at position 1980. The C-terminal end was rebuilt from this site with 10 overlapping oligonucleotides and amplification by the polymerase chain reaction to obtain the appropriate hybrids. For the \( \beta_2/\beta_1 \) chimeric protein,
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aL (cytoplasmic domain) was joined to amino acid 723 of β2, and for the β2/β1 chimeric protein, amino acid 747 of β1 (cytoplasmic domain) was joined to amino acid 723 of β2. The deletion mutant of LFA-1 was made by truncation of the β2 cytoplasmic tail from amino acid 724 (see Figure 2).

Cell Culture and Transfection
Stable LFA-1-expressing K562 transfectants were established by electroporation of 10^7 cells in 0.8 ml of phosphate-buffered saline at 280 V and 960 μF with the aL (pCDMA) and the wild-type β2 subunit (pIA/CMV), the aL and β2 cytoplasmic truncated subunit (Δ724), or the aL and the chimeric β2/β1- or β2/β2-chain of LFA-1. K562-LFA-1 transfectants were cultured in RPMI 1640 medium (Life Technologies, Paisley, Scotland), supplemented with 10% fetal calf serum (BioWhittaker, Verviers, Belgium), and 1% antibiotics/antimycotics (Life Technologies). After 48 h the neomycin analogue, G418 (2 mg/ml, Life Technologies) was added to the culture medium. The different transfectants were sorted three times to obtain a homogenous population of cells expressing high levels of LFA-1. Positive cells were stained directly with fluorescein isothiocyanate (FITC)-labeled TS2/4 mAb. Cells were sorted with the Coulter Epics Elite (Coulter, Hialeah, FL).

Immunofluorescence Analysis
Expression of LFA-1 on the transfectants was determined by immunofluorescence. Cells (2 x 10^5 cells) were incubated (30 min, 4°C) in phosphate-buffered saline, containing 0.5% (wt/vol) bovine serum albumin (BSA, Boehringer Mannheim, Mannheim, Germany) and 0.01% sodium azide (10 mM, Merck, Hohenbrunn, Germany), with appropriate dilutions of either an anti-integrin mAb or an isotype-matched control antibody, followed by incubation with FITC-labeled goat F(ab')2 anti-mouse IgG (Zymed Laboratories, San Francisco, CA) for 30 min at 4°C. The relative fluorescence intensity was measured by FACScan analysis (Becton Dickinson, San Jose, CA).

Adhesion Assay
Binding of LFA-1-positive cells to ICAM-1 was performed with ICAM-1 fusion proteins consisting of the five Ig-like domains of ICAM-1 fused to a human IgG1 Fc fragment (ICAM-1Fc). ICAM-1Fc was generated by transfecting mouse L cells with the vector pCI-C1-IgG1 by calcium phosphate precipitation (calcium phosphate transfection system, Life Technologies) using a standard protocol (Fawcett et al., 1992). Culture supernatant was purified by protein A chromatography and eluted with 3.5 M MgCl2 and 0.5% glycerol.

Ninety-six-well flat-bottomed plates (Maxisorp, Nunc, Roskilde, Denmark) coated with 50 μg/ml goat anti-human Fc-specific Fab'2 (4 μg/ml; Jackson ImmunoResearch Laboratories, Westgrov, PA) for 1 h at 37°C and blocked with 1% BSA in TSM (20 mM Tris-HCl, pH 8, 150 mM NaCl, 1 mM CaCl2, 2 mM MgCl2) for 30 min at 37°C were coated with 0.2 μg/ml ICAM-1Fc protein overnight at 4°C. Stably transfected CD11a/CD18 K562 cells were labeled with Na251CrO4 (Amersham International, Buckinghamshire, England) for 45 min at 37°C. Radiolabeled cells were washed and preincubated for 15 min at room temperature with different stimuli (200 mM phorbol 12-myristate 13-acetate (PMA, Calbiochem, La Jolla, CA), 5 μg/ml KIM185, or 5 μg/ml TS2/161 and/or blocking mAbs (10 μg/ml). Cells were allowed to adhere for 45 to 60 min at 37°C. Unbound cells were removed by washing with TSM supplemented with 0.5% (wt/vol) BSA. The adherent cells were lysed with 100 μl of 2% Triton X-100 and radioactivity was quantified in a gamma counter. Results are expressed as the mean percentage of cells binding to triplicate wells. Values are depicted as integrin-specific adhesion: percentage of cells binding = percentage of cells binding in the presence of an integrin-blocking mAb (NKI-L15 or SAM-1).

Radiolabeling and Immunoprecipitation
Transfectants were surface labeled with Na251CrO4 (Amersham International). For immunoprecipitation, 10^7 cells were solubilized for 1 h at 4°C in immunoprecipitation buffer (IPB), which contained 1% Nonidet P-40 (Sigma, St. Louis, MO), 50 mM triethanolamine (pH 7.8, Sigma), 150 mM NaCl, 1 mM CaCl2, 2 mM MgCl2, and as protease inhibitors (Sigma) 1 mM phenylmethylsulfonyl fluoride, 0.02 mg/ml ovomucoid trypsin inhibitor, 0.02 mg/ml leupeptin, and 1 mM Nα-tosyl-L-lysine chloromethyl ketone were added. Nuclear debris was removed from the lysates by centrifugation at 13,000 x g for 15 min at 4°C. Lysates were preclarified by successive incubation with mouse IgG covalently coupled to protein A-Sepharose CL-4B beads (Pharmacia, Piscataway, NJ). Preclarified cell lysates were immunoprecipitated with specific mAb, directed against either LFA-1 or VLA-5, coupled to protein A-Sepharose CL-4B for 1 h at 4°C. The immunoprecipitates were removed from the lysates by centrifugation at 13,000 x g. Subsequently, immunoprecipitates were washed extensively in IPB and analyzed under reducing conditions with 5% β-mercaptoethanol in SDS sample buffer. SDS-PAGE was visualized on a vertical slab gel (5-15%) according to a modification of the Laemmli procedure (Laemmli, 1970). Kodak XAR film was used in combination with intensifier screens (Cronex Lightning Plus; DuPont, Newton, CT) for autoradiography of Na251CrO4-labeled materials.

Confocal Microscopy
Cells were fixed with 0.5% paraformaldehyde. Fixed cells were stained with TS2/4 mAb (10 μg/ml) for 30 min at 37°C followed by incubation with FITC-labeled goat (Fab')2 anti-mouse IgG mAb (Zymed Laboratories, San Francisco, CA) for 30 min at 4°C. The relative fluorescence intensity was measured by FACScan analysis (Becton Dickinson, San Jose, CA).

RESULTS
Expression and Function of Wild-Type LFA-1 in the Erythroleukemic K562 Cells
LFA-1 (α7β1) is an adhesion receptor that is exclusively expressed on lymphocytes. To investigate the importance of a lymphocytic environment for the adhesion function of LFA-1, we transfected both wild-type α7- and β1-chain cDNA in erythroleukemic K562 cells that do not express the lymphocyte-specific β2 and β1 integrins endogenously. The transfectants express high levels of the heterodimer LFA-1 on their cell surface as detected by staining of the cells with mAb directed against either α7 (SPV-L7) or β1 (60.3; see Figure 3A).

The capacity of LFA-1 on K562 cells to bind its ligand ICAM-1 was determined after activation of LFA-1 with PMA or the activating anti-β2 mAb (KIM185) and was compared with the adhesion capacity of VLA-5, the only endogenously expressed β1 integrin. Figure 1A demonstrates that the LFA-1-trans-

β2, Cytoplasmic Tail in LFA-1 Function
Expression of β2-Chimeric LFA-1 Molecules in Erythroleukemic K562 Cells

To determine whether the observed differences between PMA responsiveness of β1 and β2 integrins are due to differences in the cytoplasmic tail, we generated LFA-1 molecules in which the cytoplasmic tail of the β2-chain was truncated close to the transmembrane region at amino acid position 724 (K562-αβ2/Δ724) or replaced with that of the β1-chain or the βγ-chain (K562-αβ1/β1 and K562-αβ2/βγ, respectively). Figure 2 shows the amino acid sequence of the different LFA-1 chimeras. Asterisks mark the amino acid sequence homology of the cytoplasmic domain of the three distinct β-chains. Both β2 and βγ integrins are predominantly expressed by lymphocytes, whereas β1 integrins are widely distributed.

The chimeric β2-chains: β2/β1, β2/βγ and β2/Δ724 were transfected along with the wild-type α-chain (αL) in K562. All transfectants expressed equally high levels of LFA-1 (Figure 3A). Our unpublished observations demonstrated that LFA-1 was expressed as a heterodimer on the cell surface, since all transfectants expressed equally high levels of the MHM23 epitope that has been reported to detect an αβ-association-dependent epitope on LFA-1 (Hildreth and August, 1985). These data demonstrate that deletion of the β2 cytoplasmic tail or replacement of the β2 cytoplasmic tail for the β1 or β2 cytoplasmic tail does not alter the overall conformation of the LFA-1/αβ heterodimer. All transfectants show similar levels of the endogenously expressed β1 integrin, VLA-5 (Figure 3A).

To verify that the chimeric LFA-1 molecules αLβ2/β1 and αLβ2/βγ did not associate with endogenous α5-chain in K562, LFA-1 and VLA-5 were immunoprecipitated from all transfectants. β2 wild-type, chimeric β2-chains, and αLβ2/Δ724 associated with α5 and not with α6 (Figure 3B, lanes C, D, F, G, I, J, L, and M, observed as the thick bands of 185 and 95 kDa). Similarly, immunoprecipitation with an anti-α5 mAb showed that VLA-5 does not associate with α5 or β2 (Figure 3B, lanes A, E, H, and K, observed as one thick band of 130–135 kDa, because, under reduced conditions, the α5- and β1-chains have approximately the same molecular weight).

Cytoplasmic β2 Domain Restores PMA Responsiveness of LFA-1 in K562 Cells

Next, we investigated the capacity of the cytoplasmic tail of the β2 or βγ integrin to restore activation of LFA-1 by PMA. Wild-type LFA-1 (Figure 4) and the chimeric LFA-1 transfectants αLβ2/β1 and αLβ2/βγ showed increased binding when activated by the LFA-1-activating antibody KIM185. The cytoplasmic tail of β7 in the αLβ2/β7 transfectant did not restore PMA-
induced binding to ICAM-1. In contrast, the cytoplasmic tail of \( \beta_1 \) in the chimeric \( \alpha_2\beta_2/\beta_1 \) transfectant restored PMA responsiveness, which is not dependent on expression levels of chimeric \( \alpha_2\beta_2/\beta_1 \), since our unpublished results have shown that transfectants expressing low levels of \( \beta_2/\beta_1 \) also respond to PMA. In addition, increasing the concentration of coated ICAM-1Fc did not alter the PMA nonresponsiveness of wild-type LFA-1 in K562 (Figure 5A). Whereas PMA stimulated binding of chimeric \( \alpha_2\beta_2/\beta_1 \) to ICAM-1 to an extent similar to the KIM185-stimulated adhesion of these cells, both independent of the concentration of coated ICAM-1Fc (Figure 5B). Similarly, also titration using higher doses of PMA did not alter the PMA nonresponsiveness of wild-type LFA-1 in K562 (Figure 5C). In contrast to both the chimeric and wild-type LFA-1 transfectants, the \( \alpha_2\beta_2/\Delta724 \) transfectant binds ICAM-1 equally well in the absence or presence of the activating mAb KIM185 or PMA (Figure 4). Adhesion was LFA-1 mediated since antibodies directed against LFA-1 (Figure 4) or ICAM-1 inhibited the cell binding completely and mock transfectants always showed less than 2% adhesion to ICAM-1 as demonstrated by our unpublished results. These data indicate that the \( \beta_1 \), but not the \( \beta_2 \), cytoplasmic domain is capable of restoring PMA responsiveness of LFA-1 in K562 and that the cytoplasmic tail of \( \beta_2 \) is important in regulating LFA-1 activation. The observation that PMA could still enhance \( \beta_2 \) integrin (VLA-5)-mediated adhesion to fibronectin of all transfectants (Figures 1 and 4) again suggests that distinct intracellular routes are involved in \( \beta_2 \) and \( \beta_7 \) compared with \( \beta_1 \) integrin activation.

**Clustering of LFA-1 on the Cell Surface Is Regulated by the \( \beta \)-Chain**

We determined whether truncation of the \( \beta \) cytoplasmic domain or replacement of the \( \beta_2 \) cytoplasmic domain by the corresponding \( \beta_1 \) or \( \beta_7 \) cytoplasmic domains affected the distribution of \( \alpha_2\beta_2 \) integrins at the cell surface. Altered distribution of integrins may affect the avidity state of the receptors, facilitating ligand binding (Figdor et al., 1990; van Kooyk et al., 1994; Lub et al., 1995). Therefore, wild-type (\( \alpha_2\beta_2 \)), the deletion mutant (\( \alpha_2\beta_2/\Delta724 \)), and the \( \beta_2 \)-chimeric LFA-1 transfectants (\( \alpha_2\beta_2/\beta_1 \) and \( \alpha_2\beta_7/\beta_1 \)) were stained with the anti-LFA-1 antibody NKI-L16, which detects Ca\(^{2+} \)-dependent clustering of LFA-1 on the cell surface (Keizer et al., 1988; van Kooyk et al., 1994). Figure 6 demonstrates that wild-type LFA-1 transfectants express low levels of the L16 epitope compared with...
Figure 4. Cytoplasmic β domain restores PMA responsiveness of LFA-1 in K562 cells. K562-α1β2/β1 and K562-α1β2/β2 transfecants and K562-α1β2/Δ724 were preincubated in medium (control), PMA (50 nM), or with the activating anti-β2 mAb KIM185 (5 μg/ml) or activating anti-β1 mAb TS2/16 (5 μg/ml) and allowed to adhere for 45–60 min at 37°C. Adhesion was performed in the absence or presence of the LFA-1 blocking mAb (NKI-L15) or VLA-5 blocking mAb (Sam-1) to ICAM-1 or fibronectin, respectively. Depicted is the mean percentage of LFA-1-specific binding to ICAM-1 and VLA-5-specific adhesion to fibronectin of three independent wells. Integrin-specific adhesion: Percentage of cells binding — percentage of cells binding in the presence of an integrin blocking mAb. Data are representative of three experiments.

DISCUSSION

Our results demonstrate that 1) PMA cannot activate LFA-1 when expressed in erythroleukemic K562 cells, despite the fact that PMA readily activates endogenously expressed β1 integrins. 2) Expression of β2 chimeric receptors in K562 reveals that the β1 cytoplasmic tail but not the β2 cytoplasmic tail restores PMA responsiveness of LFA-1. 3) Lymphocyte-specific signal transduction elements may be involved in β2 and β7 integrin activation that are absent in K562 cells. 4) Inside-out signaling (by PMA) is mediated by the β-chain of integrins. 5) Replacement of the β2 cytoplasmic tail with that of β1 alters the surface distribution of LFA-1 into clusters and facilitates ICAM-1 binding. 6) Deletion of the entire β2 cytoplasmic domain localizes LFA-1 molecules into clusters and results in constitutively active receptors.

The PMA nonresponsiveness of LFA-1 in K562 cells is not restricted to this integrin only but holds also for the other two β integrins Mac-1 and p150,95 (Ortlepp et al., 1995) and is not dependent on the concentration of the ligand presented or on the concentration of the stimulus (Figure 5). Our finding that the cytoplasmic tail of the β7 integrin cannot revert the PMA nonresponsiveness of LFA-1 in these cells predicts that transfection of β7 integrins into K562 (these cells do

with expression of a regular anti-LFA-1 (NKI-L16: SPV-L7 peak channel ratio is approximately 0.3). Similarly, α1β2/β1 and α1β2/Δ724 transfectants show high expression of the L16 epitope, comparable to expression of the SPV-L7 epitope, indicating that all LFA-1 molecules express the L16 epitope (NKI-L16:SPV-L7 peak channel ratio is roughly 1).

To determine whether deletion or replacement of the β2 cytoplasmic domain for the β1 cytoplasmic domain directly affects the distribution of LFA-1 on the cell surface, CLSM studies were performed with all LFA-1 transfecants. Figure 7 demonstrates that LFA-1 is homogenously distributed on the cell surface of both wild-type (Figure 7A, only two of seven cells show some clustering of LFA-1) and chimeric α1β2/β1 transfecants (Figure 7C), whereas on the cell surface of all chimeric α1β2/β1 (Figure 7B) and α1β2/Δ724 transfected cells (Figure 7D), LFA-1 is distributed in clusters. Clustering was not attributed to a higher expression level of LFA-1 (Figure 3A). These data indicate that either deletion or replacement of the β2 cytoplasmic domain for the β1 cytoplasmic domain dramatically affects the distribution of LFA-1, as well as results in an increment in the L16 epitope expression. Moreover, our unpublished results demonstrated that VLA-5 is similarly distributed into small clusters on all K562 transfecants. It should be noted that despite the strong clustering of α1β2/β1 receptors on the cell surface stable binding to ICAM-1 still depends on activation of the receptor with PMA, in contrast to the α1β2/Δ724 transfecants that express constitutively active LFA-1 molecules.
Figure 5. PMA nonresponsiveness of LFA-1 expressed in K562 is independent on the concentration ICAM-1 coated (A and B) or the amount of stimulus PMA to induce adhesion (C and D). K562-αβ2 (A) and K562-αβ2/β2 (B) were preincubated in medium (0), PMA (50 nM, A), or with the activating anti-β2 mAb KIM185 (5 μg/ml, 0) and allowed to adhere to different concentrations of ICAM-1Fc (500–25 ng/ml) for 45 to 60 min at 37°C in the absence or presence of the LFA-1-blocking mAb (NKI-L15). Similarly, K562-αβ2 (C) and K562-αβ2/β2 (D) were stimulated with different concentrations of PMA (5–300 ng/ml, A). High amounts of PMA were still incapable of restoring the PMA nonresponsiveness of LFA-1, whereas the activating mAb KIM185 enhanced adhesion (C). Depicted is the mean percentage of LFA-1-specific binding to ICAM-1 of three independent wells. LFA-1-specific adhesion: percentage of cells binding – percentage of cells binding in the presence of a LFA-1-blocking mAb (NKI-L15). Data are representative of three experiments.

not endogenously express β2 integrins, such as α4β7, will not allow the cells to respond to PMA. This suggests that K562 cells lack intracellular signaling elements required to regulate adhesion through the lymphocyte-specific β2 and β1 integrins. The finding that the cytoplasmic tail of the β1 integrin completely restores PMA responsiveness of LFA-1 in K562 cells cannot be attributed to a higher expression level of the β2/β1 chimeric receptors, since our unpublished results demonstrate that transfectants expressing low levels of β2/β1 also respond to PMA. Our finding that the β1 integrin VLA-5, which is endogenously expressed by the K562 cells, responds to PMA by allowing the cells to bind its ligand fibronectin demonstrates that the intracellular signaling elements activated by PMA are different for β1 integrins compared with β2 and β3 integrins.

Our results demonstrate that the cytoplasmic domain of the β-chain of integrins plays an important role in the inside-out signaling initiated by PMA. Whether PMA directly or indirectly activates β2 integrins via its β-chain requires further study. Previ-
These distinct findings can be attributed to the sites of the β chain containing deletion greater than 5-15 amino acids at the C-terminal end of the β cytoplasmic domain (Δ759, β cytoplasmic tail is 46 amino acids long) impairs the binding capacity to both laminin and fibronectin. Likewise, partial removal of the C-terminal part of the β₂ cytoplasmic domain (Δ773) of the α₂β₇ integrin displayed no ligand binding activity to VCAM-1 (Hayashi et al., 1990; Crowe et al., 1994). These distinct findings can be attributed to the sites where the cytoplasmic tails of the β-chains were deleted. The different β cytoplasmic tails share high homology in their membrane-proximal region, especially the DRRE sequence is conserved between the different β cytoplasmic tails (Figure 3, D_{759}^{RRE762} of β₂). Comparison of the positions at which the distinct β cytoplasmic tail were truncated suggests that deletion of the conserved aspartic acid residue corresponding to position 731 in the β₂ tail results in a constitutively active molecule, indicating that this residue is most important in regulating integrin activation. In contrast, it seems that in deletion mutants in which this conserved aspartic acid residue is not removed, the integrin activity can still be regulated. Moreover, amino acid sequences more C-terminal of the β cytoplasmic tail are more likely important in ligand binding capacity rather than in regulation of integrin activation (Solowska et al., 1989; Hayashi et al., 1990; Marcantonio et al., 1990; Hibbs et al., 1991a,b; Crowe et al., 1994; Hughes et al., 1995). The hypothesis that the conserved aspartic acid residue (Figure 3, D_{759} of β₂) is pivotal in the regulation of integrin activation, is also supported by our finding that the LFA-1 deletion mutant K562-α₃β₇/Δ724, which lacks the entire cytoplasmic β₂ tail including the conserved DRRE sequence, has shown to be constitutively active.

The observation that β₂/β₇ chimeric LFA-1 receptors localize in clusters on the cell membrane but the β₂/β₇ chimeric LFA-1 and wild-type LFA-1 do not demonstrates that the β₂ cytoplasmic domain plays an important role in mobilizing LFA-1 into clusters. Others have demonstrated that the NPIY motif within the β₂ cytoplasmic domain is important for localization of the integrin into focal contacts (Reszka et al., 1992; Mauro and Dixon, 1994; O'Toole et al., 1995). Interestingly, this motif is absent in the β₂ and β₇ cytoplasmic domain (Figure 2), which may explain the absence of a clustered LFA-1 distribution on the wild-type α₂β₇ and the chimeric α₂β₇/β₇ transfectants. This finding again demonstrates that clustering of LFA-1 in itself is essential but not sufficient to stimulate stable LFA-1/ICAM-1 adhesion. Both high-avidity (clustering) and high-affinity (active conformation induced by PMA) states of LFA-1 cooperated for strong adhesion.

It has been demonstrated that integrins can associate with cytoskeletal components (α-actinin and talin), particularly through the β₇ chain, and thereby regulate the cell surface distribution of the integrin (Burn et al., 1988; Pavalko and LaRoche, 1993). Deletion of the cytoplasmic tail may disconnect the integrin from the cytoskeleton and allow lateral movement of the integrin at the cell membrane, explaining the clustered distribution of LFA-1 on the α₂β₇/Δ724 transfectants. Furthermore, it may well be that clustering of integrins on the cell surface also regulates integrins essential for proper signal transduction (Miyamoto et al., 1995). Not only is the intracellular conformation or association with regulatory proteins affected by clustering of integrins on the cell surface but also the extracellular conformation is altered, as evidenced by enhanced L16 epitope expression when the β₂ cyto-
plasmic domain was deleted or replaced for the βι

We have demonstrated that the cytoplasmic domain of the β-chain of integrins is responsible for the cell

interactions with cytoplasmic proteins that affect the

Furthermore, PMCA can activate βι integrins on

role, either directly or indirectly, in PMCA-induced sig­

PMA activates βι and βι integrins in K562, suggesting that

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REFERENCES


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