Role of $\alpha_L\beta_2$ Integrin Avidity in Transendothelial Chemotaxis of Mononuclear Cells[1]

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The leukocyte integrin $\alpha_L\beta_2$ (LFA-1) is important in transendothelial migration. Since it is not fully understood how LFA-1 mediates transmigration, we studied the effects of α_L and β_2 cytoplasmic domain mutants that alter LFA-1 adhesiveness for intercellular adhesion molecule-1. Monocyte chemotactic protein-1 (MCP-1) induced LFA-1-dependent transendothelial migration of Jurkat and J-β2.7 transfectants coexpressing the MCP-1 receptor CCR2B and wild-type α_L. No transendothelial chemotaxis was observed with truncation mutants of the α_L cytoplasmic tail, which rendered LFA-1 constitutively active or locked LFA-1 in a low avidity state unresponsive to cellular activation. Moreover, transendothelial chemotaxis of lymphoblastoid SLA transfectants was abolished by truncation of the β_2 cytoplasmic domain, but not by mutation of its TTT motif, which is important in phorbol ester-induced adhesion. These data indicate that transmigration may require both α_L and β_2 cytoplasmic domains. We further show that MCP-1-induced transendothelial chemotaxis of PBMC was inhibited by sustained activation of LFA-1 with Mn^{2+} or a stimulatory mAb to β_2. Dimeric soluble intercellular adhesion molecule-1 also reduced transendothelial chemotaxis of PBMC. Taken together, our data suggest that transendothelial chemotaxis of mononuclear cells may involve dynamic changes in LFA-1 avidity. *The Journal of Immunology,* **1997, 159: 3968–3975.**

Transendothelial emigration of leukocytes and recirculation of lymphocytes are thought to be controlled by the sequential action of traffic signal molecules on endothelium, which mediate rolling, activation-dependent arrest, and diapedesis (1, 2). Chemokines that are released or immobilized within the endothelial vicinity may promote firm adhesion and transendothelial migration of leukocytes (3–6). The importance of β_2 integrins in leukocyte extravasation in vivo is evident from patients with leukocyte adhesion deficiency-1 (LAD)[3] (7) and the profound effects of Abs to β_2 integrin subunits in inflammatory disease models (8). Several β_1 and β_2 integrins and their ligands participate in transendothelial migration of leukocytes. However, studies in vitro with neutrophils, monocytes, and lymphocytes show that the leukocyte β_2 integrin heterodimer LFA-1 ($\alpha_L\beta_2$, CD11a/CD18) is more important than Mac-1 ($\alpha_L\beta_2$, CD11a/CD18) and the β_1 integrin very late antigen-4 ($\alpha_4\beta_1$, CD49d/CD29) (9–12). Furthermore, the inducible LFA-1 ligand intercellular adhesion molecule-1 (ICAM-1) on endothelium is more important than the constitutively expressed ligand ICAM-2 (11). Platelet endothelial cell adhesion molecule-1 is also involved in transendothelial migration of leukocytes (13).

The adhesiveness of LFA-1 for ICAM-1 requires activation. Cellular stimulation by CD3 engagement or by protein kinase C activation with phorbol ester generates intracellular signals that result in transient or sustained increases in LFA-1 avidity, respectively (14, 15). This may involve increased multivalent or monovalent affinity, association with cytoskeletal proteins, or cell spreading (16–18). Truncation of the β_2 cytoplasmic domain or mutation of three contiguous threonine residues (758–760) in its C-terminal region eliminates constitutive binding of transfected COS cells to ICAM-1 and prevents stimulation with phorbol ester (19, 20), indicating that the β_2 cytoplasmic domain is required for regulating LFA-1 adhesiveness. The TTT motif also modulates PMA-induced adhesion of CHO cells by affecting post-ligand binding events, such as cytoskeletal interactions or spreading (21). Mutations that disrupt the conserved GFFKR motif in the cytoplasmic domain of αIIb and α_L result in constitutively active ligand binding of αIIbβ_3, an αIIbβ_3 hybrid containing the α_L cytoplasmic domain, and LFA-1 (22–24). This suggests that this motif maintains a default, low affinity state of these integrins. The divalent cations Mg^{2+} and Mn^{2+}, especially in the absence of Ca^{2+}, can directly induce high affinity forms of LFA-1 by imposing an "active" conformation (18, 25). Activation can also be mimicked by mAbs to α_L or β_2 (26–28).

Cell migration involves extension of the lamellipodium, formation of adhesive complexes to provide traction, and detachment at the rear, allowing the cell to advance over its substrate (29, 30). Little is known about the coordination of this process at the molecular level or whether LFA-1-dependent transendothelial migration induced by chemokines may require changes in LFA-1 avidity to allow optimal traction and detachment. Chemokines have been shown to activate the β_2 integrin Mac-1 in neutrophils (31, 32). However, the CC chemokine monocyte chemotactic protein-1 (MCP-1), which attracts mononuclear cells (33, 34), failed to significantly modulate the avidity of LFA-1 for ICAM-1 in adhesion assays with T cells (35). Hence, the mechanisms by which LFA-1 mediates transendothelial chemotaxis remain to be elucidated.

Here we study the role of LFA-1 avidity regulation in transendothelial chemotaxis. We used α_L- and β_2-deficient lymphoid cell lines (20, 36) to explore the effects of α_L and β_2 cytoplasmic

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[3] Abbreviations used in this paper: LAD, leukocyte adhesion deficiency; ICAM, intercellular adhesion molecule; MCP-1; monocyte chemotactic protein-1; BCECF/AM, 2',7'-bis-2-carboxyethyl-5-(6)-carboxy-fluorescein-acetoxymethyl-ester; sICAM-1, soluble intercellular adhesion molecule-1; HSA, human serum albumin; HHMC, HBSS, 10 mM HEPES, 1 mM Mg^{2+}, and 1 mM Ca^{2+}, pH 7.4.

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domain mutations that alter LFA-1 avidity and its cellular regulation. Our results suggest that transendothelial chemotaxis may involve a dynamic modulation of LFA-1 avidity.

Materials and Methods

Reagents and mAbs

Human recombinant MCP-1 was obtained from Genzyme (Cambridge, MA). Accudenz was purchased from Accurate Chemicals (Westbury, NY), and 2′,7′-bis-2-carboxyethyl-5-(6)-carboxy fluorescein-acetoxy methyl ester (BCECF/AM) was obtained from Molecular Probes (Eugene, OR). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). The murine IgG myeloma X63, mAbs TS1/22 (α_L), TS1/18 (β_2) (37), CBR-LFA-1/2 (β_2) (28), and R6.5 (ICAM-1) (9) were purified with protein A. Purified mAb 5A11 (CCR2) was provided by LeukoSite, Inc. (Cambridge, MA) (38).

Blood cell isolation

Leukocyte-rich plasma was prepared from citrate-anticoagulated blood by dextran sedimentation of erythrocytes. PBMC were prepared from leukocyte-rich plasma by Ficoll-Hypaque density gradient centrifugation. Monocytes were isolated by Accudenz 1.068 hyperosmotic gradient centrifugation of leukocyte-rich plasma and separated from platelets by four washes at 300 × *g*, yielding approximately 85% purity (39). PBL were obtained from PBMC by plastic absorption of monocytes.

J-β2.7 transfectants and SLA cell clones

The α_L-deficient Jurkat T cell clone J-β2.7 (36) and J-β2.7 transfectants expressing truncation mutants of α_L (α1095*, α1090*) or wild-type α_L (α_L-wt) have been described previously (24) and were maintained in RPMI 1640 medium with 10% FCS. EBV-transformed B lymphoblastoid cells (SLA) derived from a patient with severe LAD (7) and transfected with wild-type β_2 or with β_2 mutants (β_2-731*, β_2-T758TT/AAA) have been described previously (20). SLA cell clones were screened for comparable β_2 surface expression and chemotaxis across bare polycarbonate Transwell inserts (Costar, Cambridge, MA; 5-μm pore size) to MCP-1 and maintained in RPMI 1640 medium with 200 μg/ml hygromycin.

Jurkat CCR2 transfectants

The cDNA that encodes for MCP-1 receptor B (CCR2B) (38, 40) was provided by LeukoSite. For transfection, cells were washed with PBS and incubated on ice for 5 min (10^7 cells/0.5 ml PBS) with 10 μg/ml pcDNA-3/CCR2B or pcDNA-3 linearized with *ScaI*. After electroporation (260 V, 960 μF) with a Gene-Pulser (Bio-Rad), transfected cells were selected with G-418 (Life Technologies, Grand Island, NY) at 0.8 mg/ml. G-418 resistant cells were subcloned by limiting dilution. Clones expressing functional CCR2B were identified by flow cytometry and chemotaxis across bare polycarbonate Transwell inserts (5-μm pore size) to MCP-1, and maintained in RPMI 1640 medium with G-418 (0.8 mg/ml).

Soluble forms of ICAM-1

Soluble ICAM-1 (sICAM-1) was purified from mutant CHO Lec 3.2.8.1 cells that express high mannose carbohydrates by immunoaffinity chromatography with ICAM-1 mAb R6.5 coupled to Sepharose (41). Bivalent ICAM-1/IgA chimera (IC1-IgA) and two-domain ICAM-1 (IC1-2D, domain 1 and 2) were previously described (42). Monomeric and dimeric forms of two-domain ICAM-1 were prepared by deglycosylation of IC1-2D with endoglycosidase H, followed by separation of monomeric and dimeric forms by gel filtration (J. M. Casasnovas, C. Weber, and T. A. Springer, unpublished data). Before assays, proteins were purified by size exclusion chromatography using a 1- × 50-cm Sepharose 6B-CL column eluted with PBS.

Cell adhesion assays

Cell adhesion to ICAM-1 or BSA adsorbed at 10 μg/ml onto 96-well microtiter plates was performed as previously described (43). Nonspecific adhesion was blocked with 1% HSA treated at 56°C for 2 h. Cells were labeled with the fluorescent dye BCECF-AM (1 μg/ml) and resuspended in HHMC (HBSS, 10 mM HEPES, 1 mM Mg^{2+}, and 1 mM Ca^{2+}, pH 7.4) with 0.5% HSA. Labeled cells (5×10^4 in 50 μl) were added to coated wells with or without (control) stimuli and allowed to settle on ice. Plates were rapidly warmed and incubated at 37°C. The fluorescence of input and adherent cells was quantified with a Fluorescence Concentration Analyzer (Iddex, Westbrook, ME). Nonadherent cells were removed by a standardized washing procedure in a Microplate Autowasher EL-404 (Bio-Tek Instruments, Winooski, VT) with HHMC (39). Specific binding was expressed as a percentage of input after subtracting background binding to BSA-coated wells.

Flow cytometry

Transfectants were reacted with mAbs in HHMC and 0.5% HSA, stained with FITC goat anti-mouse IgG mAb (Zymed, San Francisco, CA), and analyzed by flow cytometry in a FACScan (Becton Dickinson, Mountain View, CA). Staining of HUVEC showed expression of ICAM-1, but not VCAM-1, and concentrations of mAbs for blocking experiments were saturating (data not shown).

Transendothelial chemotaxis assay

Isolation and culture of HUVEC and transendothelial migration assays were previously described (34, 39). HUVEC were grown on collagen-coated 6.5-mm-diameter Transwell inserts (8-μm pore size). MCP-1 in assay medium (RPMI 1640/medium 199 and 0.5% HSA) was added to 24-well tissue culture plates. Transwells were inserted, and cells were added to the top chamber. A dilution of cells served as a measure of input. For inhibition studies, transfectants or mononuclear cells were preincubated with the α_L mAb TS1/22 on ice, and HUVEC were preincubated with ICAM-1 mAb R6.5 or isotype control X63 for 20 min. Monocytes were allowed to transmigrate for 1 h, and PBL or PBMC were allowed to transmigrate for 3 h. Input and transmigrated cells were detached with 5 mM EDTA and counted by flow cytometry with monocytes; lymphocytes were resolved by light scatter gates. Jurkat and SLA transfectants were labeled with BCECF/AM and allowed to migrate for 3 h. Input and migrated cells were counted by fluorescence microscopy.

Results

Effect of α_L cytoplasmic domain mutations on transendothelial chemotaxis

To study the relationship between LFA-1 avidity and transendothelial chemotaxis, we tested the effect of α_L cytoplasmic domain mutations on adhesion to ICAM-1 and transendothelial migration induced by MCP-1. Mutant α_L-deficient Jurkat J-β2.7 cells (36) expressing wild-type α_L (α_L-wt) or α_L truncated before (α1090*) or after (α1095*) the GFFKR motif (24) were transfected with MCP-1 receptor CCR2B cDNA or vector alone. Clones with comparable surface expression of both CCR2 and α_L were selected for further analysis (Fig. 1*A*). Adhesion to ICAM-1 of α_L-deficient J-β2.7 cells expressing CCR2B was undetectable and was not stimulated by MCP-1, PMA, Mn^{2+}, or an activating mAb to the β_2 subunit, CBR-LFA-1/2 (Fig. 1*B*). By contrast, transfectants coexpressing α_L-wt and CCR2B, like wild-type Jurkat cells, showed stimulation of binding to ICAM-1 with PMA, Mn^{2+}, or CBR-LFA-1/2 mAb. However, stimulation with MCP-1 at various concentrations (1–100 ng/ml) for 5, 15, or 30 min had no effect on adhesion (Fig. 1*B* and data not shown), as previously seen with lymphocytes (35). Transfectants coexpressing CCR2B and mutant α1090*, which lacks the GFFKR motif, showed constitutively high binding to ICAM-1 that could not be further stimulated with any agonist. Transfectants coexpressing CCR2B and mutant α1095* showed little binding to ICAM-1, and binding could be activated with Mn^{2+} or CBR-LFA-1/2 mAb but not with the cellular agonists PMA or MCP-1 (Fig. 1*B*). Binding was inhibited by mAbs to α_L and ICAM-1 (not shown). Thus, the truncation mutants α1090* and α1095* appear to lock LFA-1 in a high avidity state or a low avidity state, respectively.

Transmigration of PBL across uncoated filters is not inhibited by mAbs to integrin subunits (11). This suggests that migration in the bare filter system may serve as a measure of cell motility independent of integrins. Jurkat cells, J-β2.7 cells, and α_L-wt or mutant α_L transfectants expressing similar levels of CCR2B revealed comparable spontaneous and MCP-1-induced migration across bare filters (Fig. 1*C*). MCP-1 did not affect the migration of mock CCR2B transfectants (Fig. 1*C*). Migration was chemotactic

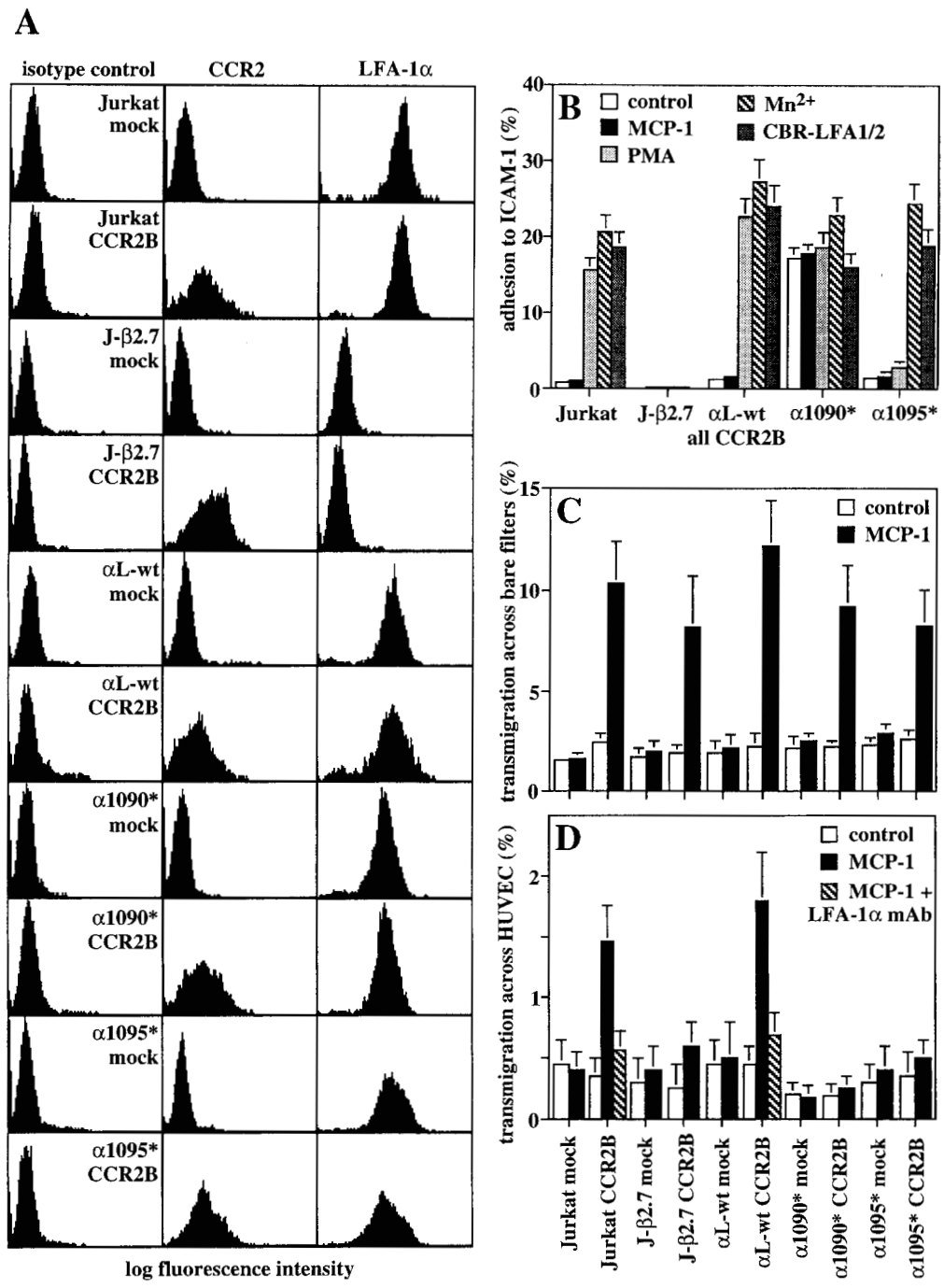

FIGURE 1. Effect of α_L mutations on adhesion to ICAM-1 and transendothelial chemotaxis. *A*, Surface expression of CCR2 and α_L in Jurkat and J-β2.7 cells after transfection with CCR2B and wild-type α_L (α_L-wt) or α_L cytoplasmic domain mutants truncated before (α_L-1090*) or after (α_L-1095*) the GFFKR motif. Cells were reacted with mAb to CCR2 (5A11), α_L (TS1/22), or isotype control and subjected to immunofluorescence flow cytometry. *B*, Adhesion to ICAM-1 of Jurkat cells and J-β2.7 transfectants expressing CCR2B. Cells were subjected to adhesion assays on ICAM-1 in the presence of MCP-1 (50 ng/ml), PMA (100 nM), Mn^{2+} (1 mM), or CBR-LFA-1/2 mAb (1 μg/ml) for 30 min. *C* and *D*, Spontaneous migration and chemotaxis to MCP-1 (50 ng/ml) of mock- and CCR2B-transfected Jurkat cells and J-β2.7 transfectants across bare polycarbonate filters with 5-μm pore size (*C*) or filters coated with endothelial monolayers (*D*). Some transfectants were preincubated with a mAb (TS1/22; 10 μg/ml) to α_L (*D*). The data in *B* through *D* are the mean ± SD of three independent experiments performed in duplicate.

rather than chemokinetic and was not altered by LFA-1 mAbs (not shown). This confirmed that chemotaxis in the bare filter assay did not involve LFA-1 and indicated that the transfectants had equivalent intrinsic motility and CCR2B function. This enabled us to compare their chemotaxis across filters covered with endothelial monolayers.

MCP-1 induced transendothelial chemotaxis of wild-type Jurkat cells or α_L-wt transfectants, but not of α_L-deficient J-β2.7 cells (Fig. 1*D*). Chemotaxis was dependent on transfection with CCR2B. Inhibition with the α_L mAb TS1/22 confirmed that migration was LFA-1 dependent (Fig. 1*D*). The α1090* transfectants

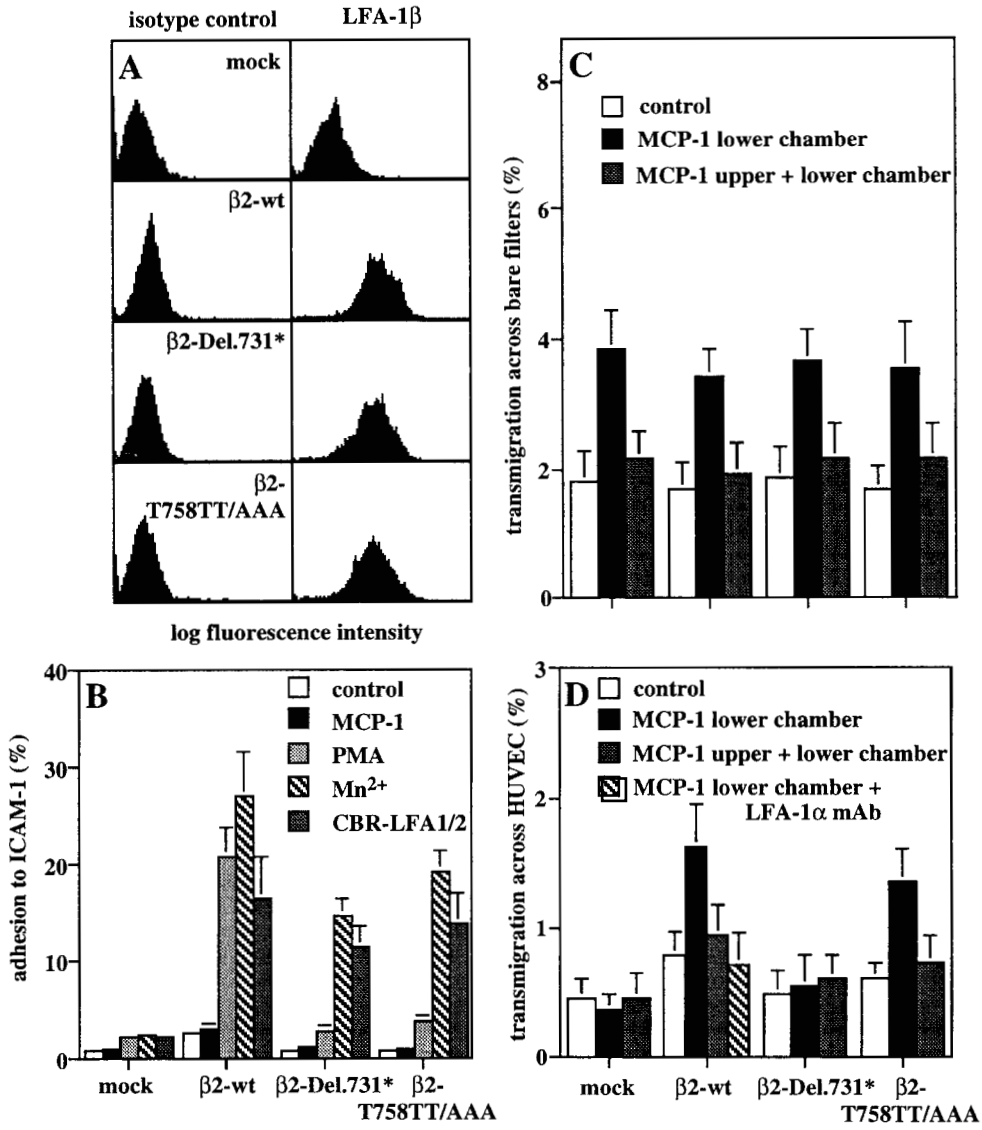

FIGURE 2. Effect of β_2 mutations on adhesion and transendothelial chemotaxis. *A*, Surface expression of β_2 in SLA cells transfected with wild-type β_2 (β2-wt) or the mutants β_2-731* or β_2-T758TT/AAA. Cells were reacted with mAb to β_2 (TS1/18) or isotype control and subjected to immunofluorescence flow cytometry. *B*, Adhesion of SLA transfectants to ICAM-1. Cells were subjected to adhesion assays on ICAM-1 in the presence of MCP-1 (50 ng/ml), PMA (100 nM), Mn^{2+} (1 mM), or CBR-LFA-1/2 mAb (1 μg/ml) for 30 min. *C* and *D*, Spontaneous migration and chemotaxis to MCP-1 (50 ng/ml) of SLA transfectants across bare polycarbonate filters with 5-μm pore size (*C*) or filters coated with endothelial monolayers (*D*). Some transfectants were preincubated with a mAb (TS1/22; 10 μg/ml) to α_L (*D*). The data in *B* through *D* are the mean ± SD of three independent experiments performed in duplicate. Statistical analysis of variance with multiple comparison shows that the MCP-1-induced migration of wt-β_2 transfectants is significantly increased compared with that of controls ($p < 0.05$).

that expressed constitutively active LFA-1 showed minimal spontaneous or MCP-1-induced transendothelial migration (Fig. 1*D*). Similar results were obtained with transfectants expressing an α_L mutant that contains an internal deletion of the GFFKR motif and also renders LFA-1 constitutively active (not shown). The α1095* transfectants expressing LFA-1 that was inactive and was unresponsive to stimulation with PMA also failed to undergo transendothelial chemotaxis in response to MCP-1 (Fig. 1*D*). Thus, transendothelial migration may require the α_L cytoplasmic tail. Our data suggest that high or low avidity states of LFA-1 that cannot be modulated by cellular activation do not support transendothelial chemotaxis.

Effect of β_2 cytoplasmic domain mutations on transendothelial chemotaxis

To study the role of the β_2 cytoplasmic tail in transendothelial chemotaxis, we used an EBV-transformed β_2-deficient lymphoblastoid B cell line (SLA) transfected with wild-type β_2 (β_2-wt), β_2-731* (cytoplasmic tail truncation), or β_2-T758TT/AAA (exchange of TTT 758–760 to AAA) (20). Mock-transfected SLA cells did not express β_2 (Fig. 2*A*) and did not bind to ICAM-1 after stimulation (Fig. 2*B*). We selected β_2-transfected SLA clones with comparable β_2 surface expression (Fig. 2*A*). Wild-type β_2 restored binding to ICAM-1 upon stimulation with PMA, Mn^{2+}, or CBR-LFA-1/2 mAb (Fig. 2*B*). Binding was inhibited by mAbs to LFA-1

(not shown). Truncation of the β_2 cytoplasmic tail (β_2-731*) or the mutation T758TT/AAA eliminated binding in response to PMA but not to Mn^{2+} or CBR-LFA-1/2 mAb (Fig. 2*B*). MCP-1 did not stimulate adhesiveness.

The SLA clones showed equivalent transmigration to MCP-1 across bare filters that was inhibited by MCP-1 in the upper chamber, confirming that migration was chemotactic and not chemokinetic (Fig. 2*C*). This again suggested that chemotaxis across bare filters is LFA-1 independent and excluded differences in intrinsic motility between the clones. Transfection of β_2-wt conferred chemotaxis to MCP-1 across endothelium, which was inhibited by the α_L mAb TS1/22 (Fig. 2*D*). Truncation of the β_2 cytoplasmic tail completely abolished transendothelial chemotaxis, whereas transfectants expressing β_2 with a mutation of the TTT motif showed normal chemotaxis to MCP-1 (Fig. 2*D*). These data suggest that LFA-1-mediated transendothelial chemotaxis requires the β_2 cytoplasmic tail but not the TTT site, which is involved in PMA-stimulated adhesion.

Effects of sustained LFA-1 activation and dimeric sICAM-1 on transendothelial chemotaxis of PBMC

Using different approaches, we studied the role of LFA-1 avidity in transendothelial migration of PBMC. We used CBR-LFA-1/2 mAb and Mn^{2+} to induce a prolonged activation of β_2 integrin avidity (25, 28). Inhibition with mAbs demonstrated that transendothelial chemotaxis of monocytes stimulated by MCP-1 was primarily mediated by LFA-1 and ICAM-1 (Fig. 3*A*). Stimulation with CBR-LFA-1/2 mAb and Mn^{2+} dose-dependently inhibited transendothelial chemotaxis of monocytes stimulated by MCP-1 to MCP-1 (Fig. 3*A*). In PBL, which express LFA-1 but not Mac-1, activation of LFA-1 with CBR-LFA-1/2 mAb attenuated spontaneous transmigration and markedly inhibited LFA-1-dependent transendothelial chemotaxis to MCP-1 (Fig. 3*B*). Hence, transendothelial chemotaxis of PBMC appears to be impaired by sustained activation of LFA-1.

An increase in LFA-1 avidity would enable binding of bivalent ICAM-1 and, hence, allow inhibition of LFA-1 functions by bivalent ICAM-1, as previously demonstrated in cell adhesion assays (18). To study whether low avidity of LFA-1 is sufficient for transendothelial migration of PBMC or whether it requires increases in LFA-1 avidity, we used monomeric five-domain soluble ICAM-1, (sICAM-1), bivalent five-domain ICAM-1/IgA Fc chimera (IC1-IgA), monomeric (mIC1-2D) and dimeric two-domain ICAM-1 (dIC1-2D) as soluble inhibitors of high avidity LFA-1 interactions. We detected LFA-1-dependent binding of IC1-IgA to PBL in the presence of Mg^{2+}/EGTA, but not with PMA or MCP-1 (not shown). IC1-IgA and dIC1-2D, but not sICAM-1, mIC1-2D, or IgA, markedly inhibited MCP-1-induced transendothelial chemotaxis of monocytes (Fig. 4*A*) and lymphocytes (Fig. 4*B*). IC1-IgA (IC_{50} = 50 nM) was more effective than dIC1-2D (IC_{50} = 150 nM). The affinity of monomeric sICAM-1 for LFA-1 may be too low to inhibit transmigration within the range of concentrations used. Inhibition was due to interactions with LFA-1, since transmigration of lymphocytes that express LFA-1 and not Mac-1 was reduced, and dIC1-2D, which contains the binding site for LFA-1 but not Mac-1 (44), inhibited transmigration of monocytes that express LFA-1 and Mac-1. Chemotaxis through bare filters was not affected by dimeric sICAM-1 (not shown). Thus, transendothelial chemotaxis of PBMC may involve the induction of high avidity LFA-1.

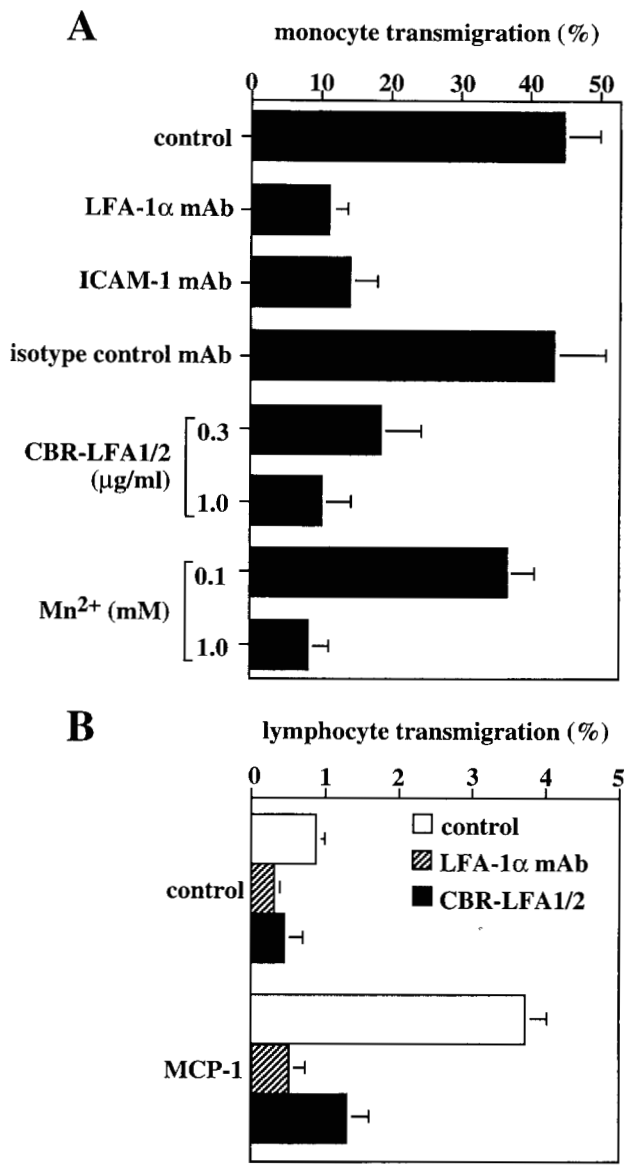

FIGURE 3. Effect of sustained LFA-1 activation on transendothelial chemotaxis of PBMC. *A*, Chemotaxis of monocytes to MCP-1 (100 ng/ml) across endothelial monolayers. Cells were pretreated with CBR-LFA-1/2 mAb or Mn^{2+} at the indicated concentration. Spontaneous migration was 1.8 ± 0.4% of input. Monocytes were preincubated with the α_L mAb TS1/22 (10 μg/ml), and HUVEC were preincubated with ICAM-1 mAb R6.5 or isotype control X63 (both 20 μg/ml). *B*, Spontaneous migration and chemotaxis to MCP-1 (50 ng/ml) of PBL across endothelial monolayers. Cells were pretreated with blocking α_L mAb (TS1/22; 10 μg/ml), stimulatory mAb CBR-LFA-1/2 (1 μg/ml), or isotype control. Data are the mean ± SD of three independent experiments performed in duplicate.

Discussion

We have presented several lines of evidence demonstrating that the transendothelial chemotaxis of mononuclear cells is modulated by the avidity of LFA-1. 1) MCP-1 failed to induce transendothelial chemotaxis of J-β2.7 transfectants expressing α_L that was truncated before the cytoplasmic GFFKR motif and locks LFA-1 in a high avidity state. 2) Transfectants expressing α_L truncated after the GFFKR motif, which are defective in binding to ICAM-1, failed to undergo transendothelial chemotaxis to MCP-1. 3) Similarly, SLA transfectants that expressed a truncated β_2 subunit and

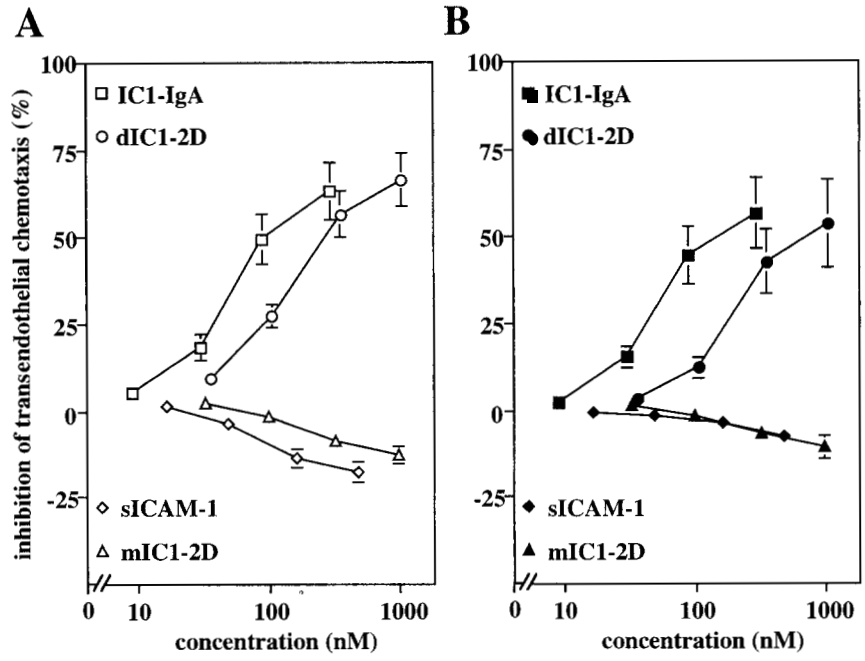

FIGURE 4. Effect of dimeric sICAM-1 on transendothelial chemotaxis of PBMC. Dose response of inhibition of monocyte (*A*) or lymphocyte (*B*) transendothelial chemotaxis. Chemotaxis to MCP-1 (100 ng/ml) of PBMC across endothelial monolayers in the presence of purified IC1-IgA, five-domain ICAM-1 (sICAM-1), and dimeric (dIC1-2D) or monomeric (mIC1-2D) two-domain ICAM-1 at the indicated concentrations. Input and transmigrated cells were counted by flow cytometry using light scatter gates for monocytes (*A*) and lymphocytes (*B*). Molar concentrations are for ICAM-1 monomeric units. Data are the mean ± SD of three independent experiments performed in duplicate.

are defective in binding to ICAM-1 failed to transmigrate. 4) Sustained activation of LFA-1 with CBR-LFA-1/2 mAb or Mn^{2+} inhibited transendothelial chemotaxis of PBMC. 5) PBMC transmigration was inhibited with dimeric forms of ICAM-1, suggesting that transendothelial chemotaxis may involve the induction of high avidity LFA-1 receptors. Taken together, our data suggest that transendothelial chemotaxis may require changes in LFA-1 avidity, rather than a sustained increase in avidity or low avidity that is unresponsive to stimulation by cellular signaling pathways.

MCP-1 induced transendothelial chemotaxis in transfectants that expressed LFA-1, but not in cells that were deficient in either α_L or β_2, indicating that it was largely dependent on LFA-1 (20, 36). In contrast, chemotaxis across bare filters was LFA-1 independent and was equivalent in different transfectants, indicating that intrinsic motility was not affected. This was consistent with findings that T cell clones from LAD patients have an impaired ability to migrate through endothelial monolayers, but similar intrinsic motility, compared with control clones (10).

To elucidate how LFA-1 avidity is correlated with transendothelial chemotaxis, we tested truncation mutants of the α_L and β_2 cytoplasmic domain. Mutations in the αIIb or α_L cytoplasmic domain that disrupt the conserved GFFKR motif increase ligand binding affinity, suggesting that an intact GFFKR motif maintains a default low affinity state (22, 23). We confirmed findings that deletion of the α_L cytoplasmic tail including the GFFKR motif (α1090*) resulted in a constitutively active form of LFA-1 in lymphoblastoid cells (24), whereas deletion of the α_L cytoplasmic domain after the GFFKR motif (α1095*) conferred a low avidity state of LFA-1. Chemotaxis across bare filters, which reflects LFA-1-independent intrinsic motility, was not affected. However, MCP-1 failed to induce transendothelial chemotaxis in the constitutively active α1090* and inactive α1095* truncation mutants of the α_L cytoplasmic domain.

Truncation of the α_L cytoplasmic domain before the GFFKR motif (α1090*) may impair transmigration by increasing LFA-1 avidity rather than by modulating post-ligand binding events or cytoskeletal rearrangement, since its constitutive binding to ICAM-1 was not inhibited by cytochalasin D (24). Despite its high avidity, α1090* failed to promote cell aggregation, which requires cell motility (24). The defect in transendothelial chemotaxis of transfectants expressing the low avidity truncation mutant, α1095*, further emphasized the association between the capacity for avidity regulation and migration as well as the involvement of the α_L cytoplasmic tail.

The β_2 cytoplasmic domain, but not its TTT motif, were also found to be required in transendothelial chemotaxis. The β_2 cytoplasmic domain and the TTT site control constitutive binding of COS transfectants to ICAM-1 and stimulation with PMA (19, 20). Deletion of the β_2, but not the α_L, cytoplasmic tail has been shown to abolish colocalization of LFA-1 with actin and its coprecipitation with vinculin and α-actinin in COS cells, indicating its role in cytoskeletal associations of LFA-1 (45). The TTT site also modulates PMA-induced CHO cell adhesion mediated by the LFA-1 cytoplasmic domain by affecting its cytoskeletal interactions without changes in affinity (21). Hence, transendothelial chemotaxis appears to involve yet to be defined regions in the β_2 cytoplasmic domain distinct from the TTT motif.

Recently, a study in CHO cells expressing $\alpha IIb\beta_3$ with mutations in the αIIb or β_3 cytoplasmic domains associated high affinity for ligand with reduced migration rates on fibrinogen, independent of effects on cytoskeletal organization (46). Truncation of αIIb after the GFFKR motif resulted in low affinity for ligand and increased cytoskeletal organization and was also associated with reduced migration rates. Moreover, truncation of the β_3 cytoplasmic domain results in a loss of focal adhesions or stress fibers and decreased haptotaxis, but increased random migration. These results implicated integrin affinity and cytoskeletal organization as important regulators of cell migration and, consistent with our data, showed that high affinity is dominant in inhibiting migration. We cannot exclude that mutations affecting LFA-1 avidity modulate post-ligand binding events or associations with the cytoskeleton. Our results differ from previous work in the study of directed rather than random migration in the use of a physiologic integrin/cell type combination and in the use of a cell type that lacks focal adhesions and stress fibers.

We further analyzed the role of LFA-1 avidity in transendothelial chemotaxis of PBMC. Prolonged activation of β_2 integrin affinity with CBR-LFA-1/2 mAb or Mn^{2+} inhibited transendothelial chemotaxis of monocytes and lymphocytes. Lymphocytes express LFA-1 but not Mac-1. Inhibition with mAbs confirmed that monocytes use primarily LFA-1 to migrate across unstimulated endothelium in vitro (12). LFA-1 may also play a more important role in monocyte migration to inflammatory sites in vivo, as Mac-1 mAb inhibits only in combination with LFA-1 mAb (47). Thus, transendothelial diapedesis of both monocytes and lymphocytes appears to be impaired by sustained activation of LFA-1. Similarly, activation of β_1 integrin with a stimulatory mAb has been shown to block transendothelial migration of eosinophils (48).

Dimeric forms of ICAM-1, including dIC1-2D, which contains the binding site for LFA-1 but not Mac-1 (44), markedly inhibited MCP-1-induced transendothelial chemotaxis of monocytes and lymphocytes. Similar to our findings with IC1-IgA, it has been reported that dimeric ICAM-1Fc proteins bind to T cells and inhibit T cell adhesion to ICAM-1 when high affinity forms of LFA-1 are induced by Mg^{2+}/EGTA, but not after stimulation with phorbol ester (18). This is consistent with our finding that transmigration did not require the TTT motif, which is important in stimulation with phorbol ester. The inhibition with dimeric ICAM-1 suggests that LFA-1 is present in a high avidity state during transendothelial migration, at least transiently, and that this high avidity state is required for transmigration. The specific targeting of transendothelial chemotaxis with soluble ICAM-1 dimers may allow selective inhibition of mononuclear cell extravasation in vivo. To our knowledge, this is the first demonstration that soluble forms of ICAM-1 inhibit the functional response to a physiologic agonist.

Consistent with previous findings (35), no significant regulation of LFA-1 avidity by MCP-1 was detectable in adhesion or ligand binding assays. However, our data suggest that dynamic changes in LFA-1 avidity are important in transendothelial chemotaxis. Hence, alterations in LFA-1 avidity induced by chemokines may be very transient, polarized to relevant plasma membrane areas (i.e., the leading edge), or restricted to subsets of LFA-1 molecules. Cells expressing LFA-1 cytoplasmic domain mutants that are either in a high or a low avidity state are defective in transendothelial chemotaxis. A dynamic regulation of LFA-1 avidity with transient or cyclic activation and deactivation would enable the temporal coordination of traction and detachment to promote migration of mononuclear cells through endothelial junctions.

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