Characterization of Lymphocyte Function-Associated Antigen 1 (LFA-1)-Deficient T Cell Lines

The α_L and β_2 Subunits Are Interdependent for Cell Surface Expression[1]

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The leukocyte, or β_2, integrins are heterodimeric cell surface molecules that share a common β subunit, and have unique α subunits. LFA-1 is the predominant β_2 integrin on lymphocytes, and plays an important role in many lymphocyte functions; however, most studies of the cytoplasmic regions of LFA-1 have been performed in transfected epithelial cells, such as COS, in part because no lymphoid cell lines deficient in the LFA-1 α subunit have been described. To address structure-function studies of β_2 integrins in relevant cell types, two T lymphoblastoid cell clones that completely lack cell surface LFA-1, J-β_2.7 and SK-β_2.7, derived from Jurkat and SKW3, respectively, were prepared by chemical mutagenesis and selection. Biosynthetic labeling and immunoprecipitation showed that the J-β_2.7 clone did not translate any LFA-1 α subunit protein, while the SK-β_2.7 cells did not synthesize any β_2 subunit protein. Northern blot analysis of poly(A$^+$) RNA from these cells revealed an absence of the corresponding mRNA in each case. By transfection analysis, only the α subunit reconstituted LFA-1 expression in the J-β_2.7 cells, while only the β subunit restored cell surface LFA-1 expression in the SK-β_2.7 cells. Functional studies with the parental cell lines, the J-β_2.7 and SK-β_2.7 cells, and the transfectants showed that all binding of Jurkat and SKW3 cells to purified ICAM-1 is mediated by LFA-1, and the reconstituted LFA-1 expressed by the J-β_2.7 and SK-β_2.7 transfected cells is regulated normally. *The Journal of Immunology,* **1997, 158: 273–279.**

Lymphocyte function-associated antigen 1 (LFA-1),[3] Mac-1, p150,95, and α_d/β_2 comprise the β_2 integrin subfamily of cell surface adhesion molecules (1–4). The β_2 integrins are heterodimeric glycoproteins defined by a common β-chain (CD18) paired noncovalently with distinct α-chains (α_L or CD11a in LFA-1, α_M or CD11b in Mac-1, α_X or CD11c in P150,95 and α_d). The primary structure of each chain of the β_2 integrins reveals a long NH_2-terminal extracellular region, a hydrophobic transmembrane domain, and a short cytoplasmic sequence (5, 6). The sequence homology of the β_2 integrin-α subunits is higher within this subfamily than when compared with other integrin α subunits (5). This is paralleled by the finding that the genes encoding the α subunits of LFA-1, Mac-1, and p150,95 are clustered together on the short arm of chromosome 16 (7). The gene encoding the β_2 protein is located on chromosome 21 (7).

The β_2 integrins are expressed exclusively on leukocytes and related cells thought to be derived from bone marrow such as dendritic cells and Langerhans cells, and mediate cell adhesion in inflammatory and immune responses (1, 4, 8). The importance of the β_2 integrins is underscored by the disease, leukocyte adhesion deficiency (LAD) type I, a genetic disorder caused by mutations affecting the β_2 chain (9–11) and resulting in deficiency of β_2 integrin cell surface expression. Patients with this syndrome, usually diagnosed in early childhood, suffer from severe and recurrent infections which result from an impairment of a wide range of leukocyte adhesion-dependent functions. The clinical severity correlates with the level of β_2 integrin cell surface expression, however, the genetic defects are heterogeneous as both moderately and severely deficient patients may have normal β_2 mRNA expression and protein precursor synthesis or very little at all (11, 12). In LAD patient cells, the α chains are not expressed on the cell surface alone (10–12). However, transfection of EBV-transformed B cell lines derived from patients with LAD type I with wild-type β_2 cDNA restored the surface expression and function of LFA-1, which indicates no intrinsic defect in the α subunit (10). It has also been observed that precursor proteins of the α_L and β_2 chains may be synthesized but are then rapidly degraded, perhaps due to the instability of the precursor protein or the nonassembled subunits (13, 14).

Association of the α- and β-chains of β_2 integrins is important for further glycosylation and maturation of the heterodimer and its transport to the cell surface (15–17). In COS cells, the α_L subunit required co-transfection of the β_2 cDNA to enable its surface expression, however, the β_2 chain could be detected on the cell surface at lower levels when transfected alone (18, 19). In these studies, association of the β_2 subunit with an endogenous COS cell α subunit was not observed. In some LAD patient cells, it has been found that point mutations of the β_2 chain occur in highly conserved regions of the extracellular domain, which may be important for $\alpha\beta$ dimerization (20–22). In addition, conserved sequences in the membrane proximal cytoplasmic region of the α_L and β_2 chain that stabilized the heterodimer have been identified in COS cell transfectants (19). The role of the α subunit of LFA-1 in

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Received for publication June 25, 1996. Accepted for publication September 30, 1996.

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[1] This work was supported in part by a grant from the Arthritis Foundation (L.B.K.).

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[3] Abbreviations used in this paper: LFA-1, lymphocyte function-associated antigen 1; LAD, leukocyte adhesion deficiency; ICAM, intercellular adhesion molecule; EMS, ethylmethane sulfonate.

0022-1767/97/$02.00

dimer formation, cell surface expression, and function has been analyzed only in non-physiologic cell types, such as COS, because no α subunit-deficient leukocyte has been described. To evaluate the extent to which the α_L and β_2 chains are interdependent for cell surface expression and function in T cell lines, two LFA-1-deficient cell clones, J-β_2.7 and SK-β_2.7, were derived from Jurkat and SKW3 cells, respectively. Characterization of the J-β_2.7 cells revealed absence of α_L mRNA expression, while the SK-β_2.7 cells lacked β_2 mRNA. Since neither clone had detectable LFA-1 protein on the cell surface, the α- and β-chains are mutually interdependent for cell surface expression in a cell type that normally expressed LFA-1. Furthermore, the J-β_2.7 cells are the first lymphoid cells described that lack the α subunit of LFA-1 and will prove valuable in future studies of LFA-1 structure and function.

Materials and Methods

mAbs, cell lines, and reagents

The previously described mAbs TS2/4 (anti-α_L, IgG1) (23), TS1/22 (anti-α_L, IgG1) (23), TS1/18 (anti-β_2, IgG1) (23), TS2/16 (anti-β_1, IgG1) (23), TS2/18 (anti-CD2, IgG1) (23), CBR-IC3/1 (anti-ICAM-3, IgG1) (24), and YZ.1 (anti-CD35, IgG1) (25) were purified with protein A. The mAbs CBR-LFA1/2 (anti-β_2, IgG1) (26) and CBR-LFA 1/7 (anti-β_2, IgG1) (27) were gifts from Dr. L. Petruzzelli (University of Michigan, Ann Arbor, MI). The 187.1 mAb (IgG, rat anti-mouse κ-chain) has been previously described (28). The T lymphoma cell lines Jurkat (originally from the American Type Culture Collection, Bethesda, MD) and SKW3 (originally from Dr. P. Cresswell, Yale University, New Haven, CT) were maintained in RPMI 1640 supplemented with 10% FCS, 25 μg/ml gentamicin, and 2 mM L-glutamine (culture medium). All other reagents were from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Medford, MA) unless otherwise specified.

Mutagenesis of wild-type cells and selection for LFA-1-deficient cells

Jurkat and SKW3 cells (1×10^8 cells in total) in log phase growth were treated with ethylmethane sulfonate (EMS) (200 μg/ml) in sealed flasks for 24 h at room temperature (29). Cells were then washed and cultured in RPMI 1640 medium until growing well. Selection for cells deficient in LFA-1 was performed by placing cells on plates coated with the anti-β_2 mAbs TS1/18 or LFA 1/7, a procedure termed immunopanning (30). Briefly, mAb at 5 to 40 μg/ml in 50 mM Tris, pH 8.0, was coated onto 10-cm petri dishes for 1 h at room temperature. The plates were blocked for 15 min with culture medium to decrease nonspecific binding and then washed three times to remove unbound mAb. Cells suspended in culture medium were layered onto the plates and incubated for 10 min at room temperature. Nonadherent cells were saved and expanded in culture. The process was repeated until a population of cells substantially negative for LFA-1 expression was observed by flow cytometric analysis, a clone from each, J-β_2.7 and SK-β_2.7, was obtained by plating at limiting dilution.

Flow cytometry

Cells were washed and incubated with saturating concentrations of mAb for 30 min on ice, washed, and stained with a 1:20 dilution of FITC-conjugated goat anti-mouse IgG (Zymed, San Francisco, CA), washed, and fixed in 2% paraformaldehyde/PBS. Surface expression was then analyzed by flow cytometry in a FACScan (Becton Dickinson, Mountain View, CA).

Surface iodination

Cells in log phase growth were surface labeled with $Na^{125}I$ (Dupont/NEN, Boston, MA) using iodogen as previously described (31). Cells were washed once and then solubilized with lysis buffer (HBSS, 0.5% Nonidet P-40, 2 μM pepstatin, 2 μM leupeptin, 18 μg/ml aprotinin, 1.5 mM PMSF, and 20 mM HEPES, pH 7.4) for 20 min on ice. Nuclei and cell debris were pelleted and lysates precleared twice with protein A-coupled Sepharose beads. Aliquots of the precleared lysates were then incubated with mAb-coupled Sepharose for 1 h at 4°C. The beads were washed three times in lysis buffer and once in 50 mM Tris, pH 8.0 and subjected to 7.5% SDS-PAGE (32) and autoradiography.

Biosynthetic labeling

For biosynthetic labeling, 5×10^7 cells in log phase growth were preincubated in RPMI 1640 medium without cysteine and methionine for 30 min. Cells (5×10^6/ml) were labeled for 60 min with 0.5 mCi Tran^{35}S-label (ICN, Costa Mesa, CA) for 60 min, then incubated with complete RPMI 1640 for 2 h in a 37°C, 5% CO_2 incubator. Cells were washed once in PBS and then solubilized in the lysis buffer (see above) for 20 min on ice. Nuclei and cell debris were pelleted and the lysates were precleared with protein A-coupled Sepharose beads and rat-anti mouse κ-chain mAb 187.1. Immunoprecipitation was carried out with protein A-coupled Sepharose beads, mAb 187.1, and the mAbs TS1/22 or CBR-LFA1/2 at 4°C for 60 min. Samples were subjected to 7.5% SDS-PAGE and autoradiography.

Blot hybridization

Total cellular RNA was isolated using guanidine.HCl and differential ethanol precipitation (33). Poly(A^+) mRNA was isolated by oligo(dT) cellulose chromatography. Aliquots of 5 μg of poly(A^+) mRNA were separated by 1.5% formaldehyde gel electrophoresis and transferred to a nylon membrane (34). Southern blots were performed as described (35). The membranes were prehybridized and hybridized with ^{32}P-labeled cDNA fragments of α_L (nucleotides 1–1818) (5), β_2 (nucleotides 1–964) (6) and ICAM-3 (nucleotides 1–1826) (36). The probe to mouse β-actin, which also recognizes human actin sequences, was a *Pst*I fragment from plasmid pAct-1, provided by Dr. Bruce M. Spiegelman, (Dana-Farber Cancer Institute, Boston, MA).

Transfection of LFA-1-deficient cell lines

J-β_2.7 and SK-β_2.7 cells in log phase growth were washed once and 10^7 cells were resuspended in 1 ml PBS. The J-β_2.7 cell suspension was mixed with 25 μg of *Sfi*I linearized α_L or β_2 cDNA (5, 6) in the expression vector AprM8 (36) and 1 μg of the plasmid, paNeo, which confers resistance to G418. The SK-β_2.7 cells were transfected with 25 μg of *Sfi*I-digested α_L cDNA in AprM8 or 25 μg of *Xmn*I-linearized β_2 cDNA in paNeo. The plasmid, paNeo, was constructed by ligation of a 1.9-kb *Sal*I fragment of pMT.neo.1 (provided by Dr. K. Peden) (see Ref. 37) encoding a neomycin phosphotransferase, to *Bam*HI-linearized AprM8 to which *Sal*I linkers had been applied. Electroporation was carried out at 240 V and 960 μF. The transfected cells were cultured in complete RPMI 1640 medium for 2 days and transferred to RPMI 1640 supplemented with 20% FCS and 0.75 mg/ml G418 to select for stable transfectants. Selection of a uniformly positive population of transfected cells was accomplished by immunopanning on TS1/22 for α_L transfectants or TS1/18 for β_2 transfectants, prior to flow cytometry and functional analysis. Similar panning of the control transfectants was carried out in parallel.

Cell-binding assay

Adhesion assays were performed as previously described (38). Briefly, purified ICAM-1 was diluted 1:20 in 50 mM Tris, pH 9.0, and spotted on a 35-mm round petri dish for 1 h. Plates were washed with L-15 medium supplemented with 1% BSA and 10 mM HEPES, pH 7.4 (assay medium), to remove unbound ICAM-1 and incubated with assay medium for 15 min to decrease nonspecific binding. Cells (5×10^6) with or without 100 ng/ml PMA were layered onto plates and incubated at 37°C for 15 min. Plates were washed three times and adherent cells per square millimeter counted in triplicate. For Ab inhibition assays, cells or protein-coated plates were preincubated with mAb for 30 min on ice.

Results

Generation of LFA-1-deficient cell lines

To develop mutant cell lines deficient in LFA-1, the T lymphoma cells, Jurkat and SKW3, which normally express LFA-1 but not Mac-1 or p150,95, were treated with EMS. Cells were incubated on plates coated with β_2 mAb TS1/18 or CBR-LFA1/7, and the nonadherent populations collected and expanded in culture, a procedure termed panning (30). After four and nine cycles of panning for Jurkat and SKW3 cells, respectively, a substantial nonadherent population was evident. The nonadherent clones, J-β_2.7 and SK-β_2.7, derived from the indicated parental cells, were obtained after limited dilution cloning. The surface expression of LFA-1 α- and β-chains was analyzed by flow cytometry and compared with the parental cell lines (Fig. 1*A*). Cells were stained with the mAb TS1/22 (anti-α_L) or CBR-LFA1/2 (anti-β_2). The clones, J-β_2.7 and SK-β_2.7, revealed no surface expression of either α_L or β_2, compared with the wild-type parental cells, demonstrating their deficiency in LFA-1 (Fig. 1*A*). In contrast, surface expression of ICAM-3 (Fig. 1*A*), the T cell marker CD2, and the β_1 integrin

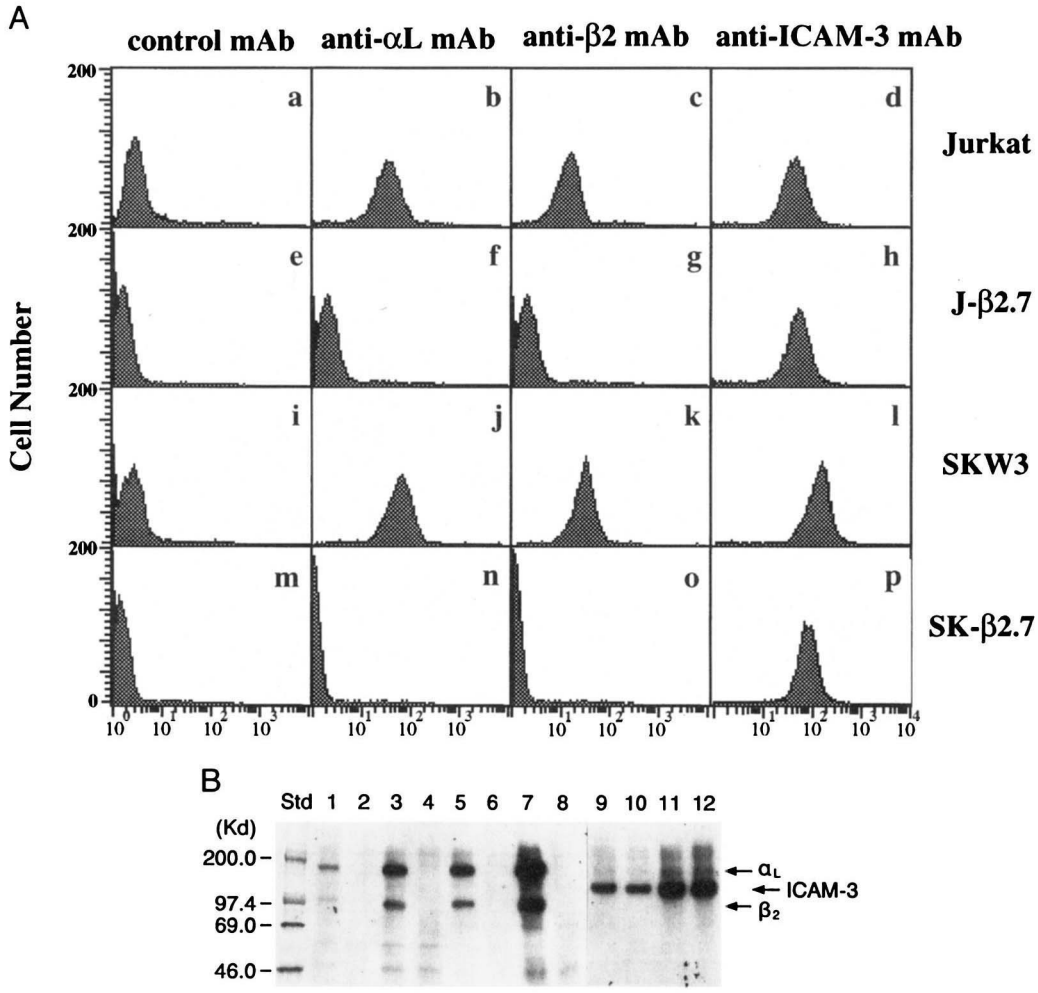

FIGURE 1. LFA-1-deficient cell clones lack cell surface LFA-1. *A*, Immunofluorescence flow cytometry of wild-type Jurkat (*a*, *b*, *c*, and *d*), SKW3 cells (*i*, *j*, *k*, and *l*) and LFA-1-deficient cell clones, J-β_2.7 (*e*, *f*, *g*, and *h*) and SK-β_2.7 cells (*m*, *n*, *o*, and *p*). All cell lines were stained with anti-α_L mAb (TS1/22) (*b*, *f*, *j*, and *n*), anti-β_2 mAb (CBR-LFA1/2) (*c*, *g*, *k*, and *o*), anti-ICAM-3 mAb (CBR-IC3/1) (*d*, *h*, *l*, and *p*) or a nonbinding control mAb YZ.1 (*a*, *e*, *i*, and *m*). *B*, Immunoprecipitation of ^{125}I-labeled surface proteins from Jurkat (*lanes 1*, *5*, and *9*), J-β_2.7 (*lanes 2*, *6*, and *10*), SKW3 (*lanes 3*, *7*, and *11*) and SK-β_2.7 (*lanes 4*, *8*, and *12*). Surface-labeled proteins were solubilized and lysates immunoprecipitated with anti-α_L mAb (TS2/4)-Sepharose (*lanes 1* to *4*) or anti-β_2 mAb (TS1/18)-Sepharose (*lanes 5* to *8*). Anti-ICAM-3 mAb (CBR-IC3/1)-Sepharose was used as a positive control to demonstrate that all cell lines comparably expressed ICAM-3 (*lanes 9* to *12*).

CD29 (data not shown) were comparable in wild-type and LFA-1-deficient cells. To confirm these results, cells were surface labeled and lysates were immunoprecipitated with mAbs TS2/4 (anti-α_L) and TS1/18 (anti-β_2), which recognize distinct epitopes compared with the mAb used for flow cytometric analysis. SDS-PAGE under reducing conditions revealed bands that migrated at the expected m.w. for α_L and β_2, 180 kDa and 95 kda, respectively, from both wild-type cell lines (Fig. 1*B*, *lanes 1*, *3*, *5*, and *7*). In contrast, no bands corresponding to the α_L or β_2 chains were observed upon immunoprecipitation of lysates from the J-β_2.7 or SK-β_2.7 cells (Fig. 1*B*, *lanes 2*, *4*, *6*, and *8*). Immunoprecipitation with the ICAM-3 mAb, CBR-IC3/1, demonstrated similar surface labeling of the wild-type and β_2 integrin-deficient cell lines (Fig. 1*B*, *lanes 9* to *12*). The β_2 integrin-deficient phenotype was stable in culture for more than 2 mo (data not shown).

Characterization of LFA-1-deficient cell lines

To determine whether the α_L and β_2 chains were synthesized but not expressed on the cell surface, cells were metabolically labeled with [^{35}S]methionine and cysteine for 60 min and then incubated with complete RPMI 1640 medium for 2 h. As expected, bands corresponding to both the α_L and β_2 chains were observed from lysates of the parental Jurkat and SKW3 cells (Fig. 2*A*, *lanes 1*, *3*, *5*, and *7*). In contrast, although the J-β_2.7 cells synthesized the β_2 subunit which migrated at the same m.w. as that from the wild-type cells (Fig. 2*A*, *lane 6*), no band corresponding to the α_L chain was observed (Fig. 2*A*, *lane 2*). Furthermore, the mAb LFA1/2 (anti-β_2) clearly co-immunoprecipitated the $\alpha\beta$ heterodimer in the wild-type cells, but only the β_2 chain from the J-β_2.7 cells (Fig. 2*A*, compare *lanes 5* and *7* with *lane 6*). Thus, the failure to express LFA-1 on the cell surface of the J-β_2.7 cells is due to an absence of α_L protein. These experiments also demonstrated that SK-β_2.7 cells were found to synthesize the α_L chain (Fig. 2*A*, *lane 4*), but not the β_2 subunit (Fig. 2*A*, *lane 8*). Thus, the failure of the SK-β_2.7 cells to express surface LFA-1 is due to a lack of β_2 protein synthesis.

To analyze whether the absence of the α_L and β_2 protein in the J-β_2.7 and SK-β_2.7 cells, respectively, was due to a lack of specific mRNA or the presence of abnormal mRNA, Northern blot analysis was performed with probes to α_L, β_2 and ICAM-3. As expected, the wild-type cells expressed both α_L and β_2 mRNA (Fig. 2*B*, *lanes 1*, *3*, *5*, and *7*). However, the J-β_2.7 cells lacked α_L mRNA

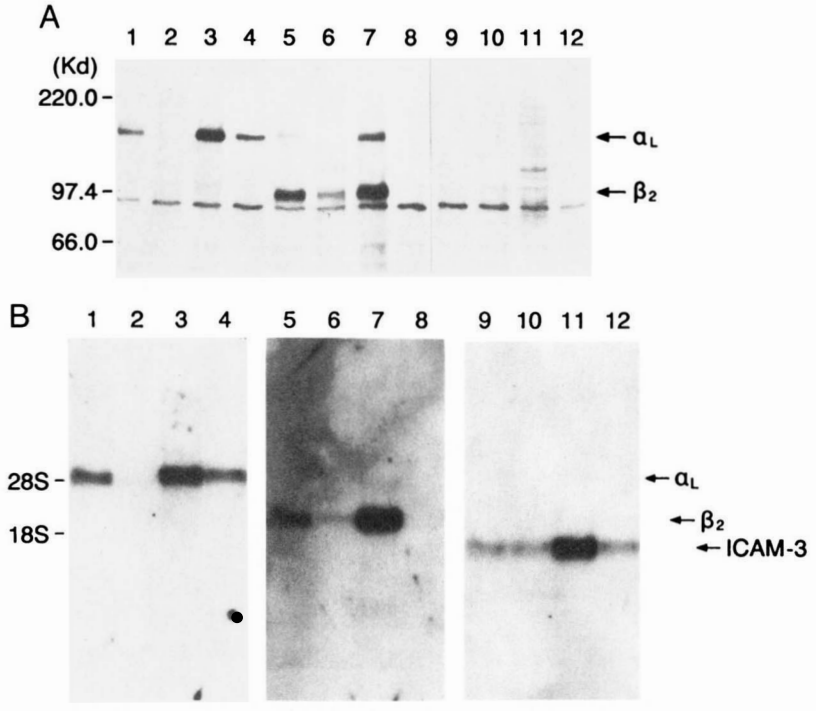

FIGURE 2. A, The LFA-1-deficient cell clones J-β_2.7 and SK-β_2.7 lack the α_L and β_2 subunits, respectively. Wild-type Jurkat (*lanes 1, 5,* and *9*), SKW3 (*lanes 3, 7,* and *11*) and the LFA-1-deficient cell lines J-β_2.7 (*lanes 2, 6,* and *10*) and SK-β_2.7 (*lanes 4, 8,* and *12*) were radiolabeled with [^{35}S]methionine, and cysteine and cell lysates were immunoprecipitated with anti-α_L mAb (TS 1/22) (*lanes 1* to *4*) or anti-β_2 mAb (CBR-LFA1/2) (*lanes 5* to *8*). The anti-murine κ-chain IgG was used as a negative control (*lanes 9* to *12*), which indicated that the 90-kDa band seen in all lanes is nonspecific. *B*, The absence of the α_L and β_2 proteins in the J-β_2.7 and SK-β_2.7 cells, respectively, is caused by the absence of the corresponding mRNA. Northern blot analysis of poly(A$^+$) mRNA from Jurkat (*lanes 1, 5,* and *9*), J-β_2.7 (*lanes 2, 6,* and *10*), SKW3 (*lanes 3, 7,* and *11*) and SK-β_2.7 cells (*lanes 4, 8,* and *12*). Blots were hybridized with probes to α_L (*lanes 1* to *4*), β_2 (*lanes 5* to *8*) and, as a positive control, ICAM-3 (*lanes 9* to *12*). Migration of 28S and 18S RNA is indicated.

(Fig. 2*B*, *lane 2*) but expressed β_2 mRNA (Fig. 2*B*, *lane 6*), whereas the SK-β_2.7 cells were deficient in β_2 mRNA (Fig. 2*B*, *lane 8*) and expressed α_L mRNA (Fig. 2*B*, *lane 4*). Hybridization of the blot with a probe to ICAM-3, performed to assess loading of mRNA, detected bands of equivalent intensity from the Jurkat and J-β_2.7 cells (Fig. 2*B*, *lanes 9* and *10*), while that from the SKW3 cells was more intense than that from the SK-β_2.7 cells (Fig. 2*B*, compare *lanes 11* and *12*). The hybridization pattern seen with a probe to β-actin was similar to that seen with ICAM-3 (data not shown). Hybridization with the probe to β_2 detected less mRNA from the J-β_2.7 cells than from its parental cell line (Fig. 2*B*, compare *lanes 5* and *6*). Nevertheless, it is clear that the lack of the respective protein in the LFA-1 deficient cell clones is caused by the absence of the corresponding mRNA. Southern blot analysis revealed that this was not due to a readily detectable alteration in the gene as no difference in band pattern or intensity was observed upon digestion of genomic DNA from all cell lines with *Bam*HI, *Hind*III, *Pst*I, and *Xba*I (data not shown).

The α_L chain requires the β_2 chain for cell surface expression and function

In order to exclude additional mutations in the LFA-1-deficient cells that might affect LFA-1 expression and function, full-length cDNAs encoding the α_L or β_2 subunits were transfected individually into the J-β_2.7 and SK-β_2.7 cells. A uniformly positive population of transfected cells was obtained for each transfectant by immunopanning on TS1/22 for α_L transfectants and on TS1/18 for β_2 transfectants. The control transfectants were treated similarly. Flow cytometric analysis of the J-β_2.7 cells demonstrated that transfection of α_L cDNA restored surface expression of both the α_L and β_2 subunits (Fig. 3, *panels f* and *g*). However, transfection of either the β_2 cDNA or the expression vector alone did not result in expression of the α_L or β_2 chain (Fig. 3, *panels b, c, j,* and *k*). In parallel, the cell surface expression of LFA-1 was restored in the SK-β_2.7 cells by transfection of the β_2 cDNA (Fig. 3, *panels v* and *w*) but not the α_L or paNeo cDNA alone (Fig. 3, *panels n, o, r,* and *s*). The J-β_2.7/α_L and SK-β_2.7/β_2 transfectants bound to purified ICAM-1 on plastic at a level comparable with that observed using wild-type Jurkat cells (Fig. 4, *A* and *B*). Inhibition with the blocking anti-α_L mAb TS 1/22 confirmed that this binding was LFA-1/ICAM-1 dependent. As expected, the LFA-1-deficient cell line J-β_2.7 and SK-β_2.7 cells did not adhere to purified ICAM-1. Moreover, transfection of the J-β_2.7 cells with the β_2 or AprM8 cDNA or of the SK-β_2.7 cells with the α_L or paNeo cDNAs did not restore the LFA-1/ICAM-1-dependent adhesion. (Fig. 4, *A* and *B*). Thus, the mutagenesis resulted in no other functionally significant mutations as the transfectants were regulated normally. These data demonstrate that the J-β_2.7 cells are α_L deficient, the SK-β_2.7 cells lack the β_2 subunit, and the α_L and β_2 subunits are interdependent for cell surface expression and function.

Discussion

In this study, two stable LFA-1-deficient cell lines derived from Jurkat and SKW3 cells have been developed by EMS mutagenesis and selection. Two clones, J-β_2.7 and SK-β_2.7, were chosen for further analysis. Although the negative selection was performed using β_2 mAbs, the cells simultaneously lost surface expression of both the α_L and β_2 subunits, which suggested that the α_L and β_2 subunits are interdependent for cell surface expression. Biosynthetic studies revealed that the J-β_2.7 cells did not synthesize the α_L subunit while the SK-β_2.7 cells lacked the β_2 subunit. However, each cell line did synthesize the partner subunit, which migrated with a relative mobility equivalent to that expressed by the parental cells.

The lack of the α_L subunit in the J-β_2.7 cell, and the β_2 protein in the SK-β_2.7 cell resulted from the absence of the corresponding mRNA (Fig. 2*B*), as judged by Northern blot analysis, but without a gross rearrangement or deletion of DNA. Alkylating agents, such as EMS, are more likely to introduce point mutations in the DNA sequence (39, 40). In previous descriptions of other molecules, mutant cells generated by treatment with an alkylating agent or by gamma irradiation have been found to have grossly normal mRNA

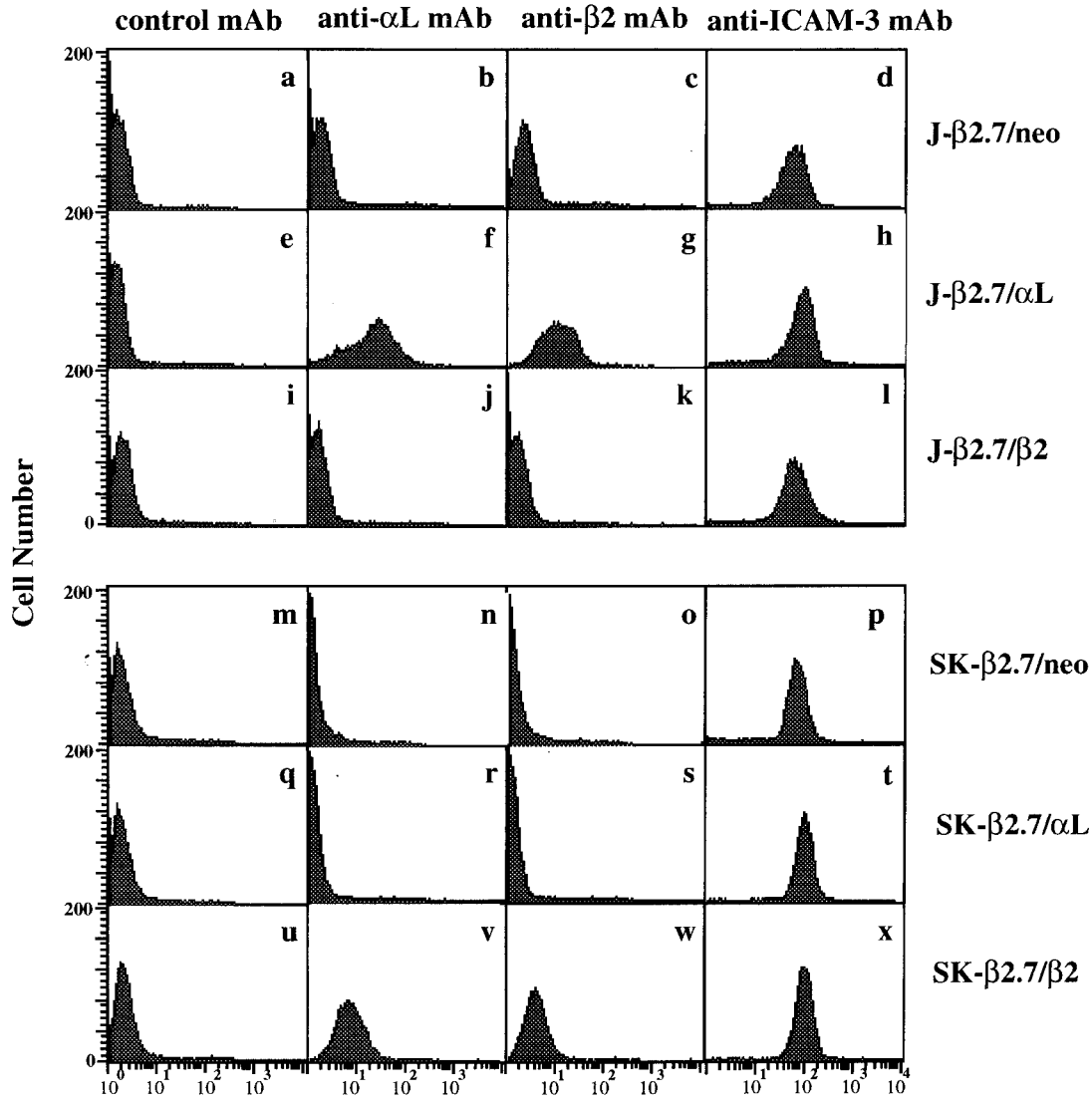

FIGURE 3. Transfection of J-β_2.7 and SK-β_2.7 cells with α_L cDNA and β_2 cDNA, respectively, restores surface expression and function of LFA-1. Immunofluorescence flow cytometry of J-β_2.7 cells transfected with a control plasmid, neo, (*a, b, c,* and *d*), α_L (*e, f, g,* and *h*) or β_2 cDNA (*i, j, k,* and *l*) and SK-β_2.7 cells transfected with a control plasmid (*m, n, o,* and *p*), α_L (*q, r, s,* and *t*) or β_2 cDNA (*u, v, w,* and *x*). Cells were stained with anti-α_L mAb (TS 1/22) (*b, f, j, n, r,* and *v*), anti-β_2 mAb (CBR-LFA1/2) (*c, g, k, o, s,* and *w*), anti-ICAM-3 mAb (CBR-IC3/5) (*d, h, l, p, t,* and *x*) or the control nonbinding mAb YZ.1 (*a, e, i, m, q,* and *u*).

for the protein of interest (41–43). However, more detailed analysis of one mutant cell, the *lck* tyrosine kinase-deficient JCaM1, revealed a splicing error resulting in an exon deletion (43) and in the glycosylphosphatidylinositol-anchor-deficient JY-5 cells, barely detectable levels of phosphatidylinositol glycan class A mRNA were demonstrated by Northern blot analysis (44). In the present study, the absence of the α_L or β_2 subunit mRNA may have been caused by a point mutation in the promoter region, thus affecting the binding of nuclear factors and subsequently impairing transcriptional activation. Alternatively, some stable, local change in chromatin structure may have occurred. Another possibility is that while generating the LFA-1-deficient Jurkat cells by selecting those nonadherent to the β_2 mAb, a pre-existing negative population with a spontaneous mutation may have been isolated. Selection of a pre-existing mutation has been described previously for CD45-deficient Jurkat cells (45, 46), and such a population is suggested by the flow cytometric data (Fig. 1, *B* and *C*). This may explain why only four cycles of immunopanning were required to obtain the LFA-1-deficient Jurkat cells while nine cycles were required for SKW3 cells. Also noteworthy is the lesser amount of β_2 mRNA expressed in the J-β_2.7 cells compared with wild-type Jurkat cells. This suggests that the mutagenesis may have, to a lesser extent, also interfered with the transcription of β_2 mRNA or that α_L and β_2 transcription are in part coordinately regulated.

In an expression system such as the COS cell, the ability of the cell to retain unassembled β_2 but not α_L subunits may be overwhelmed (19). In two studies performed in COS cells, the β_2 chain could be expressed upon transfection of β_2 cDNA alone, albeit at lower levels (18, 19) while the α_L subunit is not observed if transfected alone. This suggests that distinct chaperones may regulate the retention of these two subunits in COS cells or that the β_2 chain is expressed better than the α_L chain in COS cells using vectors with SV40 origins of replication. In the J-β_2.7 and SK-β_2.7 cells, the mechanisms for retaining improperly associated integrin subunits are intact, because neither chain was detected on the cell surface (Fig. 1) if present alone in the cytoplasm (Fig. 2*A*). Recent reports on the function of the endoplasmic reticulum chaperone, calnexin, have shown that it associates transiently with newly synthesized protein

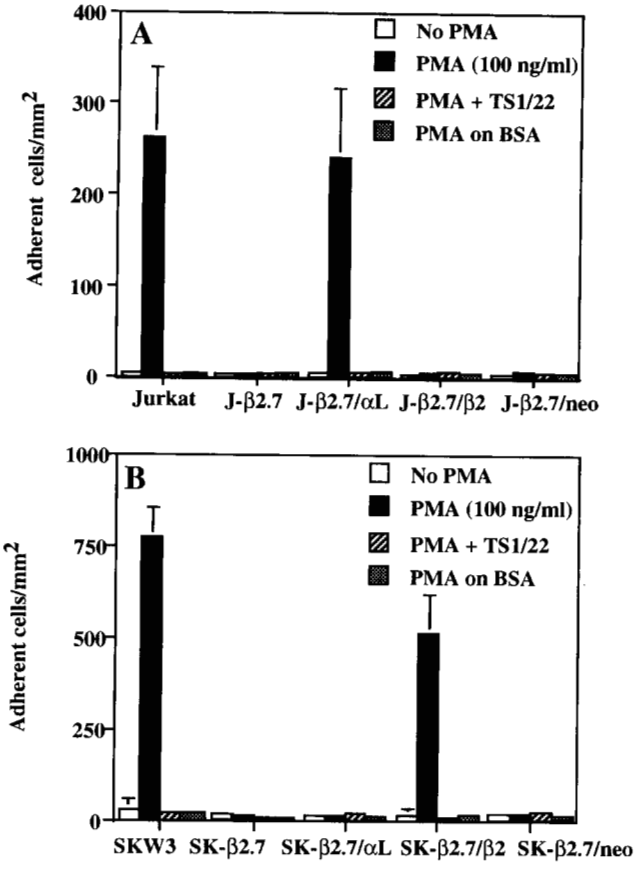

FIGURE 4. Cell binding to purified ICAM-1. Purified ICAM-1 (diluted 1:20) was spotted onto 35-mm petri dishes and nonspecific binding blocked with assay medium. Cells (5×10^6/plate) with or without stimulation with PMA (100 ng/ml) or preincubation with anti-α_L mAb, TS1/22, were layered onto each plate and incubated for 15 min at 37°C. After plates were washed three times with assay medium, the number of adherent cells/mm^2 was scored by microscopic examination. Data are presented as the mean ± SD of three separate experiments.

subunits until they associate to form complete complexes and then progress to the cell surface (46, 47). A related protein, calreticulin, has been reported to bind to integrin α subunit cytoplasmic regions (48, 49). However, in a study using COS cells, the endoplasmic reticulum retention signal of the improperly paired α_L subunits was localized to the extracellular region, not to the cytoplasmic domain (19). Whether calnexin, calreticulin, or a related protein prevents the cell surface expression of incompletely assembled α_L or β_2 chains in the J-β_2.7 and SK-β_2.7 cells is unknown.

There are few previous studies of the effect of α-chain deficiency on β-chain expression in integrins because most β-chains pair with more than one α-chain (2). However in some specialized cell types, such as lymphocytes and platelets, a limited repertoire of integrin expression has permitted similar studies to that reported here. A mouse strain deficient in the α_L subunit was recently prepared using knockout technology (50). In the LFA-1-deficient mice, but not heterozygous or normal mice, two-color immunofluorescence with anti-CD3 and anti-CD18 revealed a near-complete absence of double-positive cells. Glanzmann's thrombasthenia is an inherited bleeding disorder characterized by a deficiency in the integrin α_{IIb}/β_3 of platelets. The molecular defect responsible for this disease may occur in either the α or the β subunit, which then impairs the cell surface expression of the dimer (51, 52). In the present study, the J-β_2.7 cells, which lack the α_L subunit, did not express the β_2 subunit on the cell surface and subsequently, upon transfection of the α_L cDNA, surface expression of the β_2 chain was restored. Moreover, the transfection of α_L reconstituted adhesive functions of LFA-1 to ICAM-1 upon stimulation with phorbol ester, which demonstrated that the chemical mutagenesis did not affect the cellular signal transduction. This is the first report of a lymphoid cell line deficient in the α_L subunit.

Previous studies in EBV-transformed LAD cell lines have demonstrated that the α subunit of LFA-1 requires the β_2 subunit for cell surface expression (10). Similarly, in COS cells, the α_L subunit required co-transfection of the β_2 cDNA to enable its cell surface expression (18, 19). Transfection of the SK-β_2.7 cells with the β_2 cDNA but not the cDNA encoding the α_L chain or the resistance gene alone, restored surface expression and function of LFA-1, as previously reported for EBV-transformed cell lines derived from patients with LAD type I (10, 53). Of interest, the EBV-transformed cell lines derived from patients with LAD type I grow slowly in culture and are difficult to maintain and transfect, for reasons that are not understood. The J-β_2.7 and SK-β_2.7 cells grow well and are readily cloned and transfected, which indicates that the difficulties with the cell lines from LAD patients outlined above are not consequences of the LFA-1 deficiency. These two stable cell lines, deficient in either α_L or β_2, may provide useful models in the study of β_2 integrin function.

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