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A Dominant Jurkat T Cell Mutation That Inhibits LFA-1-Mediated Cell Adhesion Is Associated with Increased Cell Growth

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LFA-1 exists in a low avidity state on resting leukocytes and is believed to adopt a high avidity state when the cells are exposed to a stimulus. Current evidence supports both aggregation of LFA-1 on the cell surface and conformational changes in the reversible acquisition of a high avidity state. We studied this regulation by selecting a Jurkat T cell clone, J-lo1.3, that expresses LFA-1 yet fails to bind to purified ICAM-1 despite treatment of the cells with PMA or Mn2+. Several lines of evidence demonstrated the absence of any changes within LFA-1 itself. LFA-1 protein purified from the J-lo1.3 clone and the wild-type Jurkat clone, Jn.9, were found to be functionally equivalent. The cDNA sequences encoding the LFA-1 α- and β-chains from J-lo1.3 were identical with the published sequences except for nine base pairs. However, these differences were also found in a Jurkat mutant with a constitutively avid phenotype, J+hi1.19 or the wild-type Jn.9 genomic or cDNA. Fusion of J-lo1.3 with Jn.9 yielded hybrids that exhibited the J-lo1.3 adhesion phenotype, which indicated a dominant mutation in J-lo1.3. This phenotype was relatively specific for LFA-1 among all integrins expressed by Jurkat. Interestingly, the J-lo1.3 cells had a 1.2-fold faster doubling time than did the Jn.9 cells. Reversion of J-lo1.3 to the wild-type adhesion phenotype by mutagenesis and selection also decreased the growth rate. These data support a connection between cellular growth and cellular adhesion in lymphocytes. The Journal of Immunology, 2001, 167: 6171–6179.

A t sites of tissue inflammation, members of the integrin family serve to arrest leukocyte rolling and they mediate firm cellular attachment to the vascular endothelium before extravasation. The dynamic regulation of integrin adhesiveness is an essential element of this function. LFA-1 is an integrin of the β2 class that is expressed exclusively on leukocytes and mediates cell adhesion to the Ig superfamily members ICAM-1 and -2 on endothelium (1, 2). LFA-1 also supports adhesion of leukocytes to other leukocytes via ICAM-1, -2, and -3 (3) and possibly ICAM-4 and -5 under selected experimental circumstances (4).

LFA-1 on resting lymphocytes binds only weakly to cell surface-expressed ICAM, but binding can be enhanced by intracellular or extracellular stimuli. Various cytokines and chemokines have been reported to trigger LFA-1-mediated adhesion (5, 6). Cross-linking of cell surface receptors CD2, CD3, CD7, CD28, or CD45 among others (7, 8, 9, 10) can alter LFA-1 avidity for ICAM and can induce cell-cell adhesion (7, 8, 11). LFA-1-mediated adhesion may also be induced by activation of protein kinase C with phorbol esters (12, 13), leading to LFA-1 release from the cytoskeleton and clustering on the membrane (14). On the extracellular side of the membrane, divalent cations such as Mn2+ and Mg2+/EGTA have been considered to bind LFA-1 and activate it directly by altering its conformation (15, 16). LFA-1 adhesiveness can be modulated by these cellular cues with no change in the level of LFA-1 on the cell surface (7, 8). Thus, at least two forms of LFA-1 may exist before binding ICAM, an active form and an inactive form, and interconversion between these two forms is thought to occur by changes in conformation and/or aggregation state.

In this report, we describe a Jurkat T cell clone, J-lo1.3, bearing LFA-1 that is locked in a low avidity state and is unable to mediate adhesion to ICAM-1. These cells were also observed to grow at a rate 1.2 times that of a wild-type clone, Jn.9. Multiple lines of evidence showed that the altered adhesion phenotype was not due to changes within the LFA-1 coding sequence. Reversion of the J-lo1.3 adhesion phenotype to that of the wild-type Jurkat phenotype by mutagenesis of J-lo1.3 and selection for phorbol ester-stimulated adhesion to ICAM-1 resulted in a cellular growth rate that was also decreased. These data establish a link between the regulation of LFA-1-mediated lymphocyte adhesion and cell growth.

Materials and Methods

mAbs and cell lines

The previously described mAbs TS1/22 and TS2/4 (anti-αL, IgG1; Ref. 17), TS1/18 (anti-β2 and IgG1; Ref. 17), and P3×63 (IgG1) were obtained from the American Type Culture Collection (Manassas, VA). CBR1 LFA1/2 (anti-β2 and IgG1; Ref. 18) was a gift from Dr. L. Petruzzelli (University of Michigan, Ann Arbor, MI) and Dr. T. Springer (Center for Blood Research, Boston, MA), and CBR M1/29 was a kind gift from Dr. R. Rothlein (Boehringer-Ingleheim, Ridgefield, CT). These mAb and 2/1A4.1 (anti-CD16 and IgG1; Ref. 19) were purified from hybridoma...
supernatants by protein A affinity chromatography. HP1/7 (anti-α1 and IgG1), mAb616 (rat anti-α1 and IgG2a), and OKM1 (anti-αM and IgG1) were obtained via the Fifth Leukocyte Typing Workshop (20). The T lymphocyte cell line Jurkat (originally from American Type Culture Collection) was maintained in RPMI 1640 supplemented with 10% FCS, 25 μg/ml gentamicin, and 2 mM l-glutamine in a 5% humidified CO2 atmosphere. Jurkat clones were isolated by limiting dilution cloning under the same conditions. SKW3 cells (originally from Dr. P. Creswell, Yale University, New Haven, CT) were cultured similarly.

CHO cells producing the ICAM-1-Fc fusion protein

The sequence encoding the entire extracellular portion of human ICAM-1 was amplified by PCR using Vent polymerase (New England Biolabs, Beverly, MA). Additional 5′-BamHI and 3′-NheI restriction sites were included in the PCR primers, and the amplified product was digested with BamHI and Nhel and was ligated to the corresponding sites of the expression vector pCDNIg1 (kindly provided by Dr. B. Seed, Massachusetts General Hospital, Boston, MA). This vector contains genomic sequence encoding the hinge, CH2, and CH3 regions of human IgG1 and sequence encoding the signal peptide from the cell surface molecule CD5. A clone, selected with the appropriate restriction map and the ICAM-1 cDNA was confirmed by DNA sequence analysis. A DNA fragment encoding the entire fusion protein construct was removed by digestion with XhoI and NotI, and was transferred to the expression vector pαNeo, digested with the same enzymes, to yield the plasmid pαNIC-1. The expression plasmid, pαNIC, was prepared by ligation of available linkers to a 5′-3′ fragment of plMT.neo.1 (provided by Dr. K. Peden, Johns Hopkins University, Baltimore, MD) encoding the G418 resistance and then ligating this fragment into the BamHI site of AprM8 (21) such that the direction of transcription was the same as that of the CMV promoter. The plasmid, pαNIC-1, was introduced into Chinese hamster ovary (CHO) cells (a kind gift from Dr. A. Dana-Farber Cancer Institute, Boston, MA) by CaPO4 precipitation, and a stable CHO cell clone, CHO-pαNIC1.4, was selected that expressed 10 μg/ml ICAM-1-Fc recombinant protein in the culture media.

Purified proteins

Twenty-five milligrams of recombinant ICAM-1-Fc protein were purified by protein A affinity chromatography from 3 liters of conditioned media from culture of CHO-pαNIC1.4. The protein was >95% pure by SDS-PAGE and was aliquoted and stored at -80°C. ICAM-1 was purified from human plasma by R6.5 mAb affinity chromatography, as described (22). LFA-1 protein from Jn.9 and Jn.9 and Jn.9 cells was purified by TS2/4 monoclonal affinity chromatography (23). Purified LFA-1 protein was immortalized in 96-well plates at site densities varying from 150 to 450 molecules/μm2. Site densities were determined by radioimmunomassay with TS1/2, a mAb that recognizes the ligand binding site of LFA-1 and should thus represent the density of immobilized molecules of available for binding (17, 24). Chymotryptic fragments of fibronectin, FN 120 and FN 40, were purified by PRO-TRAN-MXR (Bio-Rad), and the fragments were used as described (25). The sequence encoding the entire extracellular portion of human ICAM-1 was amplified by PCR using Vent polymerase (New England Biolabs, Beverly, MA). Additional 5′-BamHI and 3′-NheI restriction sites were included in the PCR primers, and the amplified product was digested with BamHI and Nhel and was ligated to the corresponding sites of the expression vector CD5-IgG1 (kindly provided by Dr. B. Seed, Massachusetts General Hospital, Boston, MA). This vector contains genomic sequence encoding the hinge, CH2, and CH3 regions of human IgG1 and sequence encoding the signal peptide from the cell surface molecule CD5. A clone, selected with the appropriate restriction map and the ICAM-1 cDNA was confirmed by DNA sequence analysis. A DNA fragment encoding the entire fusion protein construct was removed by digestion with XhoI and NotI, and was transferred to the expression vector pαNeo, digested with the same enzymes, to yield the plasmid pαNIC-1. The expression plasmid, pαNIC, was prepared by ligation of available linkers to a 5′-3′ fragment of plMT.neo.1 (provided by Dr. K. Peden, Johns Hopkins University, Baltimore, MD) encoding the G418 resistance and then ligating this fragment into the BamHI site of AprM8 (21) such that the direction of transcription was the same as that of the CMV promoter. The plasmid, pαNIC-1, was introduced into Chinese hamster ovary (CHO) cells (a kind gift from Dr. A. Dana-Farber Cancer Institute, Boston, MA) by CaPO4 precipitation, and a stable CHO cell clone, CHO-pαNIC1.4, was selected that expressed 10 μg/ml ICAM-1-Fc recombinant protein in the culture media.

Flow cytometry

Cells were washed and incubated in microtiter plates with saturating amounts of mAbs (typically 10–20 μg/ml) for 30 min on ice, washed three times, and stained with a 1/100 dilution of FITC-conjugated goat anti-mouse IgG1 (Zymed Laboratories, San Francisco, CA), washed three times, and resuspended in PBS (5 mM phosphate and 150 mM NaCl, pH 7.4, at 4°C). Surface expression of proteins was analyzed immediately by flow cytometry in a FACSscan (BD Biosciences, Mountain View, CA).

Adhesion assay

Adhesion assays were performed as previously described (22, 27). Briefly, purified ICAM-1-Fc in 50 mM Tris, pH 9.0, was added to wells of a 96-well Linbro Titertek plate (Fisher Scientific, Medford, MA) at a concentration of 2.4 μg/ml and was incubated overnight at 4°C. For other proteins, input concentrations in 50 mM Tris, pH 9.0, are indicated in the relevant figure legend. Wells were blocked with HBBS 1% BSA (assay buffer) for 30 min. The wells were washed and 1 × 105 cells labeled with 2′,7′-bis-(2-carboxyethyl)-5′-(and-6′)-carboxyfluorescein, acetoxy-methyl ester (Molecular Probes, Eugene, OR) were added to each well and incubated at 37°C for 1 h. Cells were assayed for binding to ICAM-1-Fc in the absence and presence of PMA (50 ng/ml), Mn2+ (1 mM), or CBR LFA1/2 (20 μg/ml). Nonadherent cells in the plate were gently removed by filling the wells with assay buffer, inverting the plate in a 5-liter container of wash buffer (5 mM HEPES, pH 7.3, 150 mM NaCl, 0.1% glucose, and 2 mM MgCl2) at 37°C for 30 min, and allowing the nonadherent cells to fall out of the wells. Bound cells were detected by fluorescence measurements of the cells taken before and after washing, and as expressed as the percentage of input cells bound.

LFA-1 cDNA sequence analysis

Primers for amplification of fragments of the LFA-1 α and β subunit cDNAs were designed based on the published sequences (28, 29). Briefly, cDNA was prepared using the Marathon cDNA Kit (Clontech Laboratories, Palo Alto, CA). RT-PCR was performed with the proofreading Advantage cDNA polymerase (Clontech Laboratories) under calculated optimal temperature conditions for each primer set. To minimize the likelihood of encountering PCR errors, amplified fragments of DNA were purified from 0.5–1% low-melt agarose gels and sequenced directly, without subcloning, and both strands were sequenced.

Cell fusion experiments

Jn.9 cells and Jn.9.I cells were each transfected separately with a retroviral vector, pBabePuro (30), containing a puromycin acetyltransferase gene or with the pBSneo plasmid, which confers resistance to the G418 antibiotic, and stable transfectants of each were selected. Pairwise combinations of the cells (Jn.9 with Jn.9, Jn.9 with Jn.10.1, or Jn.9.I with Jn.10.1) were fused with PEG 4500 (American Type Culture Collection) (17), and selected in puromycin (0.6 μg/ml) plus G418 (700 μg/ml) simultaneously. Pooled heterokaryons that grew in tissue culture and survived this double selection were assayed to determine their avidity toward ICAM-1.

Transfection of Jurkat cell clones with α1β2γ

Jn.9 and Jn.10.1 cells in log phase growth were washed once and 105 cells were resuspended in 1 ml of PBS. A cDNA expression plasmid, pNaN-Mac-1, containing the full-length human α1β2γ (Mac-1) α cDNA in the expression vector pαNeo was added, and electroporation was performed at 250 V and 960 mT. The transfected cells were cultured in complete RPMI1640 media for 2 days and were selected in complete media supplemented with G418 at 750 μg/ml. The transfected cells were immunopanned on purified anti-α1β2γ mAb OKM1 or CBR-M1/29 for several cycles to yield a uniformly positive pool of cells by assay.

Reversion of the Jn.10.1 phenotype

Jn.10.1 nonadherent cells in mid-log phase growth were subjected to insertional mutagenesis by coculture with adherent GP+envAM12/PUS3.1 cells (Ref. 31 and L. K. Cherry and L. K. Klickstein, unpublished observations) at 80–90% confluency. These cells produce the PUS3.1 retrovirus at a titer of 5 × 105/ml. Eight micrograms per milliliter Polybrene were added to reduce electrostatic interactions between cells. Following coculture for 48 h, the mutagenized Jn.10.1 cells were separated from adherent cells and cultured for an additional 24 h. The pool of mutagenized Jn.10.1 cells in RPMI 1640 was added to petri dishes coated with ICAM-1-Fc, and the cells were incubated for 30 min at 37°C with phorbol dibutyrate (10 ng/ml). Nonadherent cells were removed by gentle washing, and adherent cells were saved and expanded in culture. An enrichment of cells that bound to ICAM-1 was seen following three cycles of selection, and cells fitted best by limiting.

Cell proliferation assays

Cells in log phase growth were subcultured in quadruplicate at an initial concentration of 1 × 105 cells/ml. Cells were then counted daily using a hemacytometer after staining with trypan blue. Differences in the proliferation rate during mid-log phase growth at 145 h were analyzed for statistical significance by the Wilcoxon rank sum test. In addition, cells (2.5 × 105/ml) were labeled with CFSE at a final concentration of 0.1 μM. CFSE may be used to track cell division due to the progressive halving of fluorescence intensity of the dye after each cellular division (32). Fluorescence intensity was measured at 12- to 24-h intervals by flow cytometry. Differences in the cell doubling time were analyzed for statistical significance by Student’s t test. Propidium iodide staining of the CFSE-labeled cells was also performed to determine the extent of cell death (33).
Results

Initial characterization of the J-lo1.3 clone

Jurkat T cells have been reported to exhibit variable LFA-1-dependent adhesion to ICAM-1 (34, 35), and we also noticed this variability in preliminary studies. In an attempt to understand this phenomenon, we cloned cells from our laboratory Jurkat line and screened for cell clones that exhibited different avidity for ICAM-1 when stimulated by PMA. Several clones were identified that did not adhere to immobilized ICAM-1 and one of the clones, J-lo1.3, was selected for further studies. Similarly, the clone Jn.9, with a low basal adhesiveness and relatively high PMA-inducible adhesion, was selected as a wild-type control. The phenotypes of these two clones remain stable over at least 2 mo in continuous culture.

Flow cytometric analysis of the wild-type Jn.9 and nonadhesive J-lo1.3 Jurkat clones (Fig. 1A) revealed that J-lo1.3 expressed both LFA-1 α6 and β2 subunits on the cell surface, albeit at reduced levels compared with the wild-type Jn.9 clone. Levels of other proteins, including the β1 integrin subunit (Fig. 1A, last column), were unchanged. A previously described clone, J-β2.7 (22), which lacks cell surface LFA-1, is shown for comparison. In our standard adhesion assay to immobilized, purified ICAM-1-Fc, the wild-type clone Jn.9 exhibited inducible adhesion when treated with PMA, 1 mM Mn2+, or the activating mAb CBR LFA1/2 (Fig. 1B). In contrast, J-lo1.3 binding to purified ICAM-1-Fc could not be induced by either phorbol esters or Mn2+. Similar results were found for binding to ICAM-2 and ICAM-3 (data not shown). Indeed, the binding of J-lo1.3 was comparable with the LFA-1-deficient Jurkat clone, J-β2.7. However, the anti-LFA-1 mAb that activates adhesion, CBR LFA1/2, was able to induce J-lo1.3 binding to ICAM-1. These data suggest that the LFA-1 protein in the J-lo1.3 cells is functional when a direct means of stimulation is used rather than the indirect stimulation provided intracellularly by phorbol esters or extracellularly by Mn2+. Furthermore, although there are decreased levels of LFA-1 on the surface of the cells, the amount of LFA-1 is adequate to support adhesion under these conditions.

Characterization of LFA-1 on J-lo1.3

To determine whether the functional abnormality of J-lo1.3 cells was a consequence of a mutation in LFA-1 itself, the LFA-1 protein from Jn.9 and J-lo1.3 cells was purified and immobilized in 96-well plates. The LFA-1 protein purified from J-lo1.3 supported adhesion of ICAM-1 bearing SKW3 T lymphoma cells identically to the LFA-1 purified from wild-type cells (Fig. 2A). These results demonstrated that when the LFA-1 protein from J-lo1.3 was assessed outside of the cellular environment, it functioned equivalently to that from Jn.9.

Sequence analysis of LFA-1 cDNA from J-lo1.3

PCR fragments encoding LFA-1 were amplified from genomic DNA or cDNA synthesized from poly(A)+ RNA purified from Jn.9, J-lo1.3, or J+hi1.19, a clone bearing constitutively active LFA-1 (K. S. C. Weber, L. K. Cherry, C. Weber, and L. B. Klickstein, unpublished results), using LFA-1-specific primers. The PCR fragments were sequenced directly without subcloning, and both strands were sequenced. In the α6 cDNA, the presence of a 3-bp deletion upon comparison with the published sequence allowed us to clearly recognize the presence of two α6 alleles co-expressed in the Jurkat cells, one with the deletion and one without. On the basis of peak height on the electropherograms, we have assigned one allele as the major allele and one as the minor; the 3-bp deleted allele is thus the major allele expressed in Jurkat. The other changes are as shown in Fig. 2B; there were 10-bp differences in total, including the 3-bp deletion, when the α6 cDNA sequence was compared with the published sequence from myeloid cells (28). However, all these differences between the published sequence and the J-lo1.3 sequence were also found in the Jurkat mutant with a constitutively avid phenotype, J+hi1.19, or the wild-type Jn.9 genomic or cDNA (data not shown). Hence, the differences that we observed were either allelic differences between Jurkat and the published sequences or they represented one or more sequencing errors. To resolve this issue, we resequenced the regions in question from the original LFA-1 α subunit cDNA plasmid (28) and confirmed the published sequence in every case except for bases 2072 and 2073, where we obtained our major sequence (Fig. 2B) instead of the published sequence. In either case, these few sequence changes are not causally related to the mutant phenotype of J-lo1.3.

Four sequence differences were found in the cDNA encoding the β2 subunit (Fig. 2B) and none of the changes altered the derived...
The L-10l.3 cells bear a dominant inhibitor of LFA-1-mediated adhesion

Stable heterokaryons were prepared as described in Materials and Methods to determine whether wild-type Jn.9 Jurkat cells could complement the mutation in L-10l.3. Pooled heterokaryons were assayed, rather than cloned cells, to ensure the outcome was representative. As controls, each cell was fused with itself to determine any effect on the LFA-1 adhesion phenotype of the process of cell fusion and drug selection. Flow cytometry confirmed that LFA-1 expression on all the heterokaryons was similar to that on Jn.9, and all heterokaryons treated with the activating mAb CBR bound well to immobilized ICAM-1 (data not shown), which indicated the presence of intact LFA-1 on the hybrid cells. LFA1/2 bound well to immobilized ICAM-1 (data not shown), indicating the presence of a dominant mutation in the L-10l.3 that inhibits LFA-1-mediated adhesion to ICAM-1.

The effect of the L-10l.3 mutation on other integrins

Jurkat cells express the α1 integrins αβ1 (VLA-4) and α3β1 (VLA-5), which are present at wild-type levels on the L-10l.3 cells (Figs. 1A and 4A). The chymotryptic fragments of fibronectin, Fn40 and Fn120, contain the CS1 peptide and RGD sequence that are the ligands for VLA-4 and VLA-5, respectively. In experiments of PMA-stimulated cells binding to a range of input concentrations of fibronectin peptides, L-10l.3 bound less well to the Fn40 peptide via the α2 (Mac-1). Expression of α3β1 on the surface of Jn.9 and L-10l.3 cells was similar after transfection and immunopanning (Fig. 5A), but was lost after 2 wk in culture in the absence of selection (data not shown). Adhesion of Jurkat cells bearing αMβ2 to the specific ligand iC3b (Fig. 5B) revealed that these cells, Jn.9/αM and L-10l.3/αM, were both responsive to stimulation with a phorbol ester; however, the L-10l.3/αM cells bound less well than did the Jn.9/αM. Consequently, the mutation in the L-10l.3 cells is predominantly LFA-1 specific with smaller effects on the β1 integrin VLA-4 (αβ1) and the β2 integrin αMβ2.

Amino acid sequence when compared with the amino acid sequence derived from β2 cDNA isolated from a tonsillar cDNA library (Ref 29; Fig. 2B). These same changes were also found in PCR fragments amplified from cDNA of the constitutively active clone, J+hi1.19, or the wild-type Jn.9. At three of these four positions, we observed a minor sequence, which likely represents the sequence that was less abundant. B, Amino acids are represented by their one-letter code. Bc, Numbering is based upon the published sequence described by Kishimoto et al. (29).
A correlation between adhesion phenotypes and cellular proliferation

While culturing the Jn.9 and J-lo1.3 cells, it was evident that J-lo1.3 grew faster than Jn.9. This suggested that the LFA-1 adhesive phenotype was somehow related to cellular growth. To address this question, we mutagenized the J-lo1.3 cells and selected for wild-type revertants by panning for cells that regained phorbol ester-stimulated binding to immobilized ICAM-1. After three cycles of selection, an enrichment of cells binding to immobilized ICAM-1-Fc was evident and a clone, J-lo1.3/rev1, was investigated to determine whether these cells, selected by reversion of the adhesive phenotype, also reverted their LFA-1 adhesion to purified ICAM-1, and presented as in A. Adhesion of the Jn.9/J-lo1.9 control hybrid to ICAM-1 was not stimulated by PMA, thus phorbol ester stimulation could not be assessed in the heterokaryons.

A correlation between adhesion phenotypes and cellular proliferation

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FIGURE 5. αβ₂ function of the J-lo1.3 cells. A, Flow cytometric analysis of the wild-type and the J-lo1.3 clones transfected with αβ₂. Negative control mAb, X63, and anti-αM mAb, OKM1, were used. Histograms of cell number vs relative fluorescence intensity are shown. The Jn.9 and J-lo1.3 cells express similar amounts of the αβ₂ integrin. B, An adhesion assay to iC3b, an αβ₂ ligand, was performed with Jn.9 and J-lo1.3 cells transfected with the α subunit of αβ₂. Bars indicate the percentage of cells that remained adherent after washing. αβ₂ is responsive to phorbol ester treatment in both cell types, although to a slightly lesser degree in the J-lo1.3 cells. Data shown are for a representative experiment performed in quadruplicate with error bars of 1 SD.

Discussion
The mechanisms underlying the dynamic regulation of leukocyte integrin avidity are poorly understood. We have created T cell clones with LFA-1 locked in different adhesive states to study this problem. In this report, we describe a Jurkat T cell clone, J-lo1.3, bearing LFA-1 that is locked in a low avidity state and is therefore defective in mediating cell adhesion to purified ICAM-1. Multiple lines of evidence showed that the altered adhesion phenotype was not due to changes within the LFA-1 coding sequence. Surprisingly, these cells grew at a rate 1.2 times that of a wild-type clone, Jn.9. Reversion of the J-lo1.3 adhesion phenotype to that of the wild-type Jurkat phenotype by mutagenesis and selection for phorbol ester-stimulated adhesion to ICAM-1 resulted in a decreased cellular growth rate. These data support a link between the regulation of LFA-1-mediated lymphocyte adhesion and cell growth.

A remarkable difference between the Jn.9 and J-lo1.3 clones is that Mn²⁺ failed to activate LFA-1-mediated adhesion of J-lo1.3 to ICAM-1 (Fig. 1B). Mn²⁺ or Mg²⁺ binding to the metal ion-independent adhesion site (MIDAS) in the I domain of β₂ integrins has been proposed to be critical for binding to ICAM-1 (36). These cations also have been implicated in the regulation of cell adhesion via the LFA-1/ICAM-1 interaction (37). If Mn²⁺ activates all integrin-ligand binding in the same manner, it seems unlikely that Mn²⁺ binding alone to the I domain would be sufficient to trigger cell adhesion because integrins that lack an I domain are also stimulated by Mn²⁺ (37, 38). Crystal structures of isolated I domains of β₂ integrins with and without bound Mn²⁺ were nearly identical (39, 40). Nuclear magnetic resonance analysis of the I domain in solution confirmed this crystal structure and also that a conformational change of the I domain due to binding of Mn²⁺ was unlikely (41). Furthermore, the isolated I domain, expressed as a transmembrane protein on K562 cells, was not stimulated by Mn²⁺ for binding to ICAM-1, which indicated that Mn²⁺ treatment of the I domain alone is inadequate to trigger binding to ICAM-1 (42). However, recent work with site-directed mutagenesis to engineer variants of the I domain of LFA-1 to lock it into either an adhesive or nonadhesive state illustrated that a conformational isomerism model is likely accurate (43), even if it is not directly mediated by metal ion binding to the I domain. The J-lo1.3 cells are unresponsive to Mn²⁺ in the ICAM-1 adhesion assays; however, they bear structurally wild-type LFA-1. Thus, these cells demonstrate that there exist at least two distinct effects of Mn²⁺ in supporting LFA-1-mediated adhesion to ICAM-1, one as a possible component of the ICAM-1 binding site in the I domain and the second as a trigger for cellular adhesion to ICAM-1. Furthermore, both of these effects are probably required for cation-triggered adhesion to ICAM-1 via β₂ integrins.

If the I domain MIDAS region is not sufficient for Mn²⁺-triggered binding, could Mn²⁺ bind elsewhere on LFA-1 and trigger adhesion? Lu et al. (42) speculated that Mn²⁺-binding to the MIDAS-like site of the β₂-I-like domain might be a candidate for a regulatory Mn²⁺ binding site. However, if the β₂-I-like domain were a Mn²⁺ binding site and if Mn²⁺ binding to that site alone were sufficient to trigger adhesion, then the J-lo1.3 clone, bearing wild-type LFA-1 protein, should have exhibited Mn²⁺-inducible binding to ICAM-1. Although our observations do not rule out a role for Mn²⁺ binding to the β₂-I-like domain, we can conclude that it is not sufficient to trigger LFA-1-mediated cell adhesion. It seems most likely that the mutation in the J-lo1.3 clone may affect a Mn²⁺-dependent regulatory protein or complex of proteins that...
associates with LFA-1 and governs whether the I domain adopts a conformation that supports ligand binding. Bleijs et al. (44) demonstrated that PMA treatment of K562 cells transfected to express LFA-1 failed to trigger adhesion to ICAM-1, similar to what was found for J-lo1.3. This raises the question of whether the mutation in J-lo1.3 recapitulated the K562 adhesion phenotype. However, we have found that Mn$^{2+}$ is able to trigger LFA-1-dependent adhesion of K562 to ICAM-1 (data not shown), which suggested that the mechanism for the lack of phorbol ester-mediated activation of LFA-1 in J-lo1.3 cells is distinct from that in K562. Kuipers et al. (45) reported a thorough study of a patient who presented with the leukocyte adhesion deficiency (LAD) type 1 phenotype, but had structurally normal CD18 cDNA. This patient apparently has a defect in $\beta_2$ and $\beta_3$ integrin activation. Unlike the phenotype of the J-lo1.3 cells, this patient’s leukocytes exhibited no Mac-1-mediated adhesion but normal $\beta_1$ integrin-mediated adhesion (45). Thus, there are at least three different cellular mutations that may lead to the common phenotype of impaired LFA-1-mediated adhesion despite structurally normal integrins.

Purified LFA-1, when immobilized on plastic, is able to support adhesion of ICAM-1-bearing cells in the absence of any activating stimulus (Fig. 2; Refs. 37 and 46). LFA-1 expressed on nonphysiologic cell types such as COS cells is constitutively active (47), whereas LFA-1 on physiologic cell types requires stimulation to induce ICAM-1 binding (7). These points suggest that LFA-1 adhesiveness on physiologic cells may be regulated by a leukocyte-specific protein or complex of proteins that actively inhibit LFA-1-mediated adhesion. Possible candidates for such proteins present in leukocytes that have been reported to affect LFA-1 function have been described (e.g., Refs. 48–51). One of these or an as yet unidentified protein may be overexpressed or functionally overactive in the J-lo1.3 cells, which could yield the observed dominant phenotype of J-lo1.3 in the experiments with heterokaryons (Fig. 3).

LFA-1 on the stable hybrid Jn.9/J-lo1.3 did not bind to immobilized ICAM-1 in the presence of Mn$^{2+}$. The fusion process itself did not significantly interfere with the activation process triggered by Mn$^{2+}$ because this cation was able to activate the Jn.9/Jn.9 hybrid. In contrast, the control Jn.9/Jn.9 hybrid was not responsive to PMA. This contrasts with the previously reported PMA-inducible adhesion to fibronectin of transiently produced Jurkat hybrids (52). Mobley et al. (52) prepared Jurkat cell clones bearing $\beta_1$ integrins, which failed to bind $\beta_1$ integrin ligands. However, they did not distinguish clearly between true hybrids and cell aggregates in their assay, so either the previous study examined primarily cell aggregates rather than cell hybrids, or there is a difference between the PMA-dependent activation of $\beta_1$ vs $\beta_2$ integrins, or the additional cell culture with the accompanying chromosomal changes required to obtain our stable hybrids was incompatible with maintenance of PMA responsiveness.

We examined the adhesiveness of the other integrins on J-lo1.3 by examining the binding of $\alpha_4\beta_1$ (VLA-4) and $\alpha_5\beta_1$ (VLA-5) toward their ligands Fn40 and Fn120, respectively. We also transfected $\alpha_2\beta_2$ (Mac-1, CR3) into J-lo1.3 and Jn.9 and studied adhesion to IC3b. In contrast to the complete lack of LFA-1-mediated binding to ICAM-1, we found that these receptor-ligand systems were functional, although there was a slight reduction in the adhesion of J-lo1.3 bearing VLA-4 and $\alpha_5\beta_2$ when compared with Jn.9 that expressed equivalent numbers of receptors (Figs. 4 and 5). The selective regulation of $\beta_1$ and $\beta_2$ integrins coexpressed on the same cell has been reported. For example, $\alpha_2\beta_2$ expressed on eosinophils is activated in a sustained fashion, whereas VLA-4 is transiently adhesive in response to chemokines (53). The sequential regulation of VLA-4 and VLA-5 integrins on monocytes by chemokines demonstrated that integrins can be selectively and differentially regulated even if they share a common subunit, $\beta_1$ (54). Similarly, the selective regulation of $\beta_2$ integrins has been discussed (55). Our observation that the mutation in the J-lo1.3 cells largely affects the LFA-1/ICAM-1 interaction but only marginally affects VLA-4/Fn and $\alpha_5\beta_2$/IC3b interactions demonstrated that the mutation present in J-lo1.3 is largely specific to LFA-1.

In the course of culturing the J-lo1.3 cells, it was clear that they grew more rapidly than the wild-type Jn.9 cells, and this was confirmed in quantitative studies of cell proliferation (Fig. 7). This differential growth rate would alter the proportion of J-lo1.3 and Jn.9 phenotype cells within a Jurkat population over time and this may explain why Jurkat pools in different laboratories are variably adhesive for ICAM-1 (35, 52). Upon reversion of the J-lo1.3 adhesion phenotype to that of the wild-type cells by mutagenesis and selection on purified ICAM-1, the growth rate of the revertant clone, J-lo1.3/rev1, decreased toward that of the wild-type clone Jn.9, but it was not fully reverted. This finding suggested the possibility that these cells may have acquired a compensatory mutation with respect to LFA-1-mediated adhesion, rather than a full reversion (Fig. 7). This correlation between the Jurkat LFA-1 adhesion phenotype and cell growth rate might suggest the presence of contact-dependent inhibition of growth analogous to that seen in nontransformed adherent cells (56, 57). This seems less likely because EBV-transformed lymphocytes from patients with LAD (58) grow more slowly than those from normal individuals (59). Similarly, T cells from patients with LAD grow poorly in response to most stimuli (58, 60). There is precedent for the involvement of LFA-1 in the growth rate of lymphocytes. Van Noesel et al. (61) have shown that growth of purified T cells (1% contaminating monocytes) may be altered by treatment of the cells with blocking anti-CD11a or anti-CD18 Abs. The anti-CD11a Abs
stirred proliferation by increasing IL-2 production, whereas anti-CD18 Abs inhibited proliferation by decreasing IL-2. In both cases, proliferation was triggered by immobilized anti-CD3. The increase in the rate of cell growth triggered by the anti-CD11a Ab was similar to the 1.2-fold increase in growth of our J-lol.1 clone. It has been suggested previously that engagement of LFA/ICAM-1 switches the IL-2 receptor from low to high affinity (62). However, our cell line is IL-2 independent, which suggests there may be another connection between cell adhesion and proliferation. In a more physiologic Ag-dependent model, mAb 24, which promoted $\beta_2$ integrin-dependent adhesion that had been triggered by influenza virus, decreased proliferation of PBMC that had been cultured in PHA and IL-2, compared with control Abs (46). Thus, studies of nontransformed cells are consistent with our findings and they support the existence of a link between cell growth and LFA-1-mediated cell adhesion in lymphocytes.

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References


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