

LFA-1 IMMUNODEFICIENCY DISEASE

Definition of the Genetic Defect and Chromosomal Mapping of α and β Subunits of the Lymphocyte Function-associated Antigen 1 (LFA-1) by Complementation in Hybrid Cells

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Cell adhesion molecules are thought to play an important role in guiding cell migration and localization in the development of the embryo and in organogenesis. In the immune system, cell adhesion molecules mediate leukocyte-endothelial cell interactions, and in many types of cell interactions, they enhance the efficiency of specific receptor-mediated processes. The importance of a family of three leukocyte cell adhesion proteins, LFA-1 (lymphocyte function-associated antigen 1),¹ Mac-1 (macrophage antigen 1), and p150,95, has recently been highlighted by the discovery (1-9) of an immunodeficiency disease in which expression of these molecules on the surface of leukocytes is lacking. Patients with a deficiency of these three molecules (for brevity, designated the LFA-1 deficiency here) present with recurrent life-threatening infections, and display profound defects in adhesion-dependent granulocyte, monocyte, and B and T lymphocyte functions (2, 4-12).

LFA-1 has been defined both in mice and in humans (13, 14). The LFA-1 molecule on normal cells is critically involved in mediating adhesive interactions by T and B lymphocytes, natural killer cells, and granulocytes, and mediates homotypic lymphocyte adhesion stimulated by phorbol esters (12, 13, 15, 16). The LFA-1 molecule contains an α L subunit of M_r 177,000, and a β subunit of M_r 95,000 (14). Its β subunit is identical to that of the related Mac-1 and p150,95 molecules, which have distinct α M and α X subunits (14).

Leukocytes from LFA-1-deficient patients lack surface expression of both the α L and β subunits of LFA-1 (3). To determine whether the α L, β , or both subunits are defective in patients' cells, we have tested for interspecies subunit complementation after fusion of human patient lymphocytes to an LFA-1⁺ mouse

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¹ *Abbreviations used in this paper:* H-ActD, 33258 Hoechst and actinomycin D; HGPRT, hypoxanthine/guanine phosphoribosyl transferase; LFA-1, lymphocyte function-associated antigen 1; QM, quinacrine mustard dihydrochloride.

T cell line. In parallel, we produced hybrids with normal human T cells. We show here that human LFA-1 α L and β subunits from normal cells can associate with mouse LFA-1 subunits to form interspecies hybrid $\alpha\beta$ complexes. After fusion of mouse and LFA-1-deficient patients' cells, surface expression of the patients' α L, but not β subunit could be rescued by interspecies subunit complementation, demonstrating that the defect in patients' cells is in the β subunit. Furthermore, we have mapped the gene encoding the β subunit and hence the putative defect to human chromosome 21. We have mapped the α L subunit gene to human chromosome 16.

Materials and Methods

Cells and Cell Culture. The hypoxanthine/guanine phosphoribosyl transferase (HGPRT)-negative LFA-1⁺ mouse BW5147.G.1.4 thymoma line was maintained in RPMI-1640 medium supplemented with 20 mM L-glutamine, 50 μ M 2-ME, 50 μ g/ml gentamycin, and 20% FCS (complete medium). PHA blasts were prepared (3) from isolated peripheral blood mononuclear cells from LFA-1 deficiency patient 1 (7) or her asymptomatic mother. PHA blasts were used 10 d after the initiation of culture.

Monoclonal Antibodies. The rat mAbs M7/14 and M18/2a have previously been shown (17) to be specific for the mouse LFA-1 α L and β subunits, respectively. The mouse mAbs TS1/22 and TS1/18 have previously been shown to be specific for the human LFA-1 α L and β subunits, respectively (14). The P3X63 myeloma IgG1 was used as control. Hybridoma culture supernatants were used for immunofluorescent staining, and mAb purified from culture supernatants or ascites were used for precipitation.

Fusion and Selection of Mouse \times Human Hybrids. Human patient or control PHA blasts were fused with murine HGPRT-deficient BW5147 cells at a 4:1 ratio with 50% PEG 1600 according to the method of Kontiainen et al. (18). After a 2-wk selection in complete medium plus HAT, hybrid cells were grown and expanded in complete medium plus HT. PHA blasts do not grow in medium lacking IL-2, and HGPRT-deficient BW5147 cells do not grow in HAT medium. After 1 wk of growth in HT medium, hybrids were stained by indirect immunofluorescence (see below) and selected by flow cytometry for surface membrane expression of human LFA-1 subunits. The most brightly fluorescing 1% of the cells were aseptically sorted out using a Coulter Epics V cell sorter. Sorted cells were regrown in complete medium, then reanalyzed by flow cytometry, or sorted again for hybrids positive or negative for human LFA-1 subunit expression.

Indirect Immunofluorescence Flow Cytometry. Indirect immunofluorescent staining of cells was done by treating the cells with hybridoma culture supernatants specific for the α L and β subunits of mouse LFA-1, the α L and β subunits of human LFA-1, or control supernatant as previously described (19). Cells were indirectly stained with affinity-purified FITC-labeled anti-rat or mouse IgG (Zymed, Burlingame, CA) and analyzed on an Epics V flow cytometer gated by forward angle light scatter to exclude dead cells. Data from the analysis of 10^4 cells are expressed as fluorescence intensity on an arbitrary logarithmic scale.

Immunoprecipitation and SDS-PAGE. Cells were enzymatically cell-surface radioiodinated by the glucose oxidase/lactoperoxidase method (3). Triton X-100 lysates of ¹²⁵I-labelled cells were precleared with mAb coupled to Sepharose CL-4 B (1 mg/ml) or CNBr-activated, glycine-quenched Sepharose CL-4 B by three sequential rounds of immunoprecipitation (3). The precleared lysates were immunoprecipitated with mAb-Sepharose and subjected to SDS-8% PAGE and autoradiography as previously described (3).

Chromosome Analysis. The human chromosomal content of each hybrid cell line was determined after a routine metaphase harvest (20) and karyotyping using two different banding techniques. $\sim 5 \times 10^6$ cells from each line were harvested for metaphase chromosomes after a 45-min incubation in 0.00625 μ g/ml colcemid at 37°C, a 7-min incubation in 0.075 M KCl at 37°C, and fixation at room temperature in 3:1 methanol/acetic acid. Slides were prepared from each harvest and stained with quinacrine mustard dihydro-

chloride (QM) (21) for identification of chromosomes by quinacrine-fluorescein Q-banding (QFQ-banding) and with 33258 Hoechst and actinomycin D (H-ActD) for discrimination of mouse and human chromosomes (22). After staining with H-Act D, mouse centromeres fluoresced brightly in comparison to human centromeres. One notable exception to this staining pattern is the somewhat brighter fluorescence seen in secondary constrictions of human chromosomes 1 and 16 (22).

Southern Hybridization. DNA was extracted from cells by the method of Bell et al. (23). After digestion with Eco RI, samples were electrophoresed on 1.0% agarose gels and Southern blotted (24) to blotting membranes (Zeta-Probe; Bio-Rad Laboratories, Richmond, CA). The presence of human chromosome 16 was determined by probing with a cloned human α_1 globin gene (25). Human chromosome 21 was identified using DNA sequences from plasmid pPW228C. This plasmid was derived from the WA17 mouse \times human hybrid cell, which contains only human chromosome 21 and contains sequences originating from that chromosome. Cloned insert DNA was isolated from plasmid DNA, labeled with ^{32}P by nick-translation, and hybridized to Southern blots by standard procedures (24).

Results

Selection of Human \times Mouse Hybrids. BW5147, a mouse thymoma line expressing LFA-1 (26), was fused with PHA-stimulated T lymphoblasts derived from either an LFA-1 deficiency patient, or from her asymptomatic, heterozygous mother. After selection in HAT medium, hybrids expressing human αL or β subunits were then separately selected by fluorescence-activated cell sorting with mAb specific for the respective human LFA-1 subunits. The mouse anti-human LFA-1 α and β subunit-specific mAb used here do not crossreact with murine LFA-1 (see below, Fig. 2, *g* and *h*). Similarly, the rat anti-mouse LFA-1 αL and β subunit-specific mAb used here do not crossreact with human LFA-1 (see below; Fig. 2, *a* and *b*). 3 wk after cell fusion, only a minor fraction of the hybrid cell population expressed human LFA-1 subunits as detected by immunofluorescence flow cytometry. Therefore, hybrid cells expressing human LFA-1 α or β subunits were enriched by serial fluorescence-activated cell sorting. Representative results from the enrichment of the heterozygous mother \times BW5147 (M \times BW) hybrids with human αL -specific mAb are shown in Fig. 1. The initial sort of the 1% most brightly fluorescing cells (Fig. 1*a*) produced populations with at least 10% of the cells positive for surface human LFA-1 αL (Fig. 1*b*). Repeated enrichment by serial sorting (Fig. 1*b-d*) resulted in a hybrid population that contained >90% human LFA-1 αL^+ (Fig. 1*f*). Hybrids negative for human LFA-1 subunit expression (Fig. 1*e*) were simultaneously selected during the last sort for comparative karyotyping.

Parallel serial FACS selections of hybrids derived from the patient's or her mother's cells were done with human LFA-1 αL or β subunit-specific mAb. By selection with the appropriate mAb, cells expressing the human αL subunit or β subunit, respectively, were isolated from hybrids derived from the mother's cells (Fig. 2, *i* and *j*). These hybrids were designated M \times BW, α^+ and M \times BW, β^+ , respectively. The hybrid cells expressed only the selected human LFA-1 subunit. M \times BW, α^+ hybrids expressed the human αL subunit, but not the human β subunit (Fig. 2*i*). M \times BW, β^+ hybrids expressed the human β subunit, but not the human αL subunit (Fig. 2*j*). All hybrids were positive for both the murine αL and β subunits (Fig. 2, *c-f*). Serial selection of patient-derived hybrids also produced cells positive for the human αL subunit (Fig. 2*k*). These hybrids were

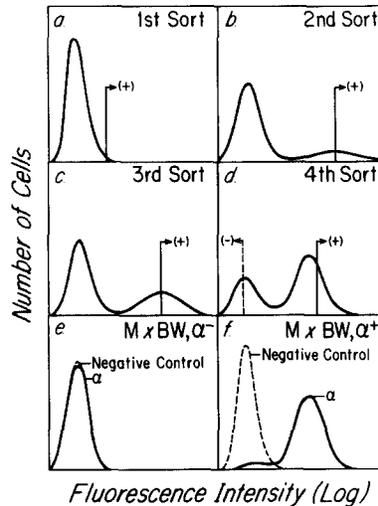


FIGURE 1. Representative selection of mouse \times human hybrids expressing human LFA-1 by serial fluorescence-activated cell sorting. Hybrids derived from the mother's cells were stained by indirect immunofluorescence with mAb specific for the human LFA-1 α L subunit, and the brightest 1% of the cells were sorted aseptically, as indicated by the arrow in *a*. This population was expanded, then serially resorted for cells staining positive for the human α L subunit with sorting gates as indicated in *b*, *c*, and *d*. During the fourth serial selection (*d*), both positive ($M \times BW, \alpha^+$) and negative ($M \times BW, \alpha^-$) populations were isolated, regrown, then indirectly stained with either anti-human α L mAb or negative control antibody (*e* and *f*).

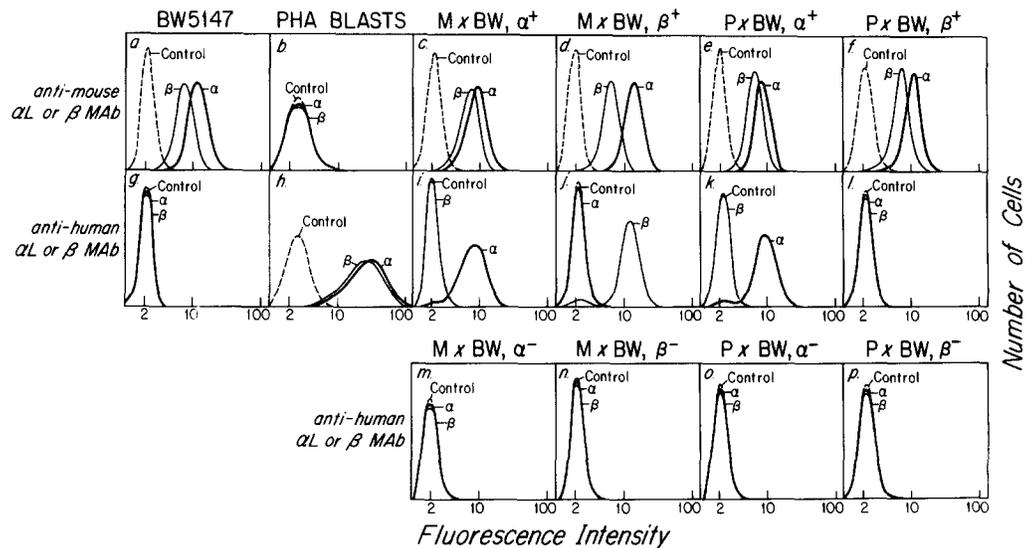


FIGURE 2. Cell surface expression of human and murine LFA-1 α L and β subunits on hybrid cells detected by immunofluorescence flow cytometry. Cells were directly stained with mAb specific for the α L and β subunits of mouse LFA-1, the α L and β subunits of human LFA-1 (solid lines), or with control mAb (dashed line). $M \times BW$ and $P \times BW$ designate hybrids derived from the mother's and patient's cells, respectively. α^+ , α^- , β^+ , β^- denote hybrids selected by sorting for positive or negative expression of human LFA-1 α or β subunits.

designated $P \times BW, \alpha^+$. Thus, fusion with mouse lymphocytes rescued surface expression of the human LFA-1 α L subunit from LFA-1-deficient patient lymphocytes. However, when patient-derived hybrids were selected with anti-human β subunit mAb, hybrids positive for human β subunit expression could not be isolated. Similar results were obtained in a second independent selection experiment: hybrids expressing the human β subunit could be isolated from hybrids derived from the mother's cells (75% β^+ after one sort), but not the patient's cells.

Human and Mouse LFA-1 Subunits Coassociate. The presence of either human α L or β subunits on the surface of the hybrid cells along with murine α L and β subunits might be accounted for by either the presence of nonassociated human subunits, or by the formation of interspecies $\alpha\beta$ complexes. When LFA-1 is solubilized with nonionic detergents from human or murine cells, the α and β subunits remain noncovalently associated and hence are coimmunoprecipitable (Fig. 3, A and B), as previously described (14, 26). Thus, if the human subunits were associated with mouse subunits in the hybrid cells, they should coimmunoprecipitate. Therefore, we solubilized LFA-1 molecules from ^{125}I -labeled hybrid cells and tested for the presence of interspecies $\alpha\beta$ complexes by immunoprecipitation (Fig. 3, C–H). When lysates of $M \times BW, \alpha^+$ and $P \times BW, \alpha^+$ hybrids were immunoprecipitated with an mAb specific for the human LFA-1 α L subunit, a band comigrating with the mouse β subunit was coprecipitated with the human α L subunit (Fig. 3, C and E, lane 3 in each). The human α L subunit was of higher M_r than the mouse α L subunit from the same hybrid cells (compare Fig. 3, C and E, lanes 2 and 3), and resembled human α L from PHA blasts in M_r (Fig. 3A, lane 3). This human α L subunit was removed when lysates of the hybrids were precleared with mAb to the mouse β subunit (compare Fig. 3C, lane 3; and D, lane 3). These results provide immunological and biochemical evidence that these hybrids express authentic human α L subunits, and that these subunits are associated with murine β subunits in $\alpha\beta$ complexes. The results with $P \times BW, \alpha^+$ hybrids further suggest that the rescue of surface expression of the human α L subunit from LFA-1-deficient patient's cells is due to the formation of an interspecies hybrid complex with the murine β subunit.

Similar evidence showed that the $M \times BW, \beta^+$ hybrids expressed the human β subunit noncovalently associated with the mouse α L subunit. The anti-human β mAb coprecipitated the mouse α L subunit and the human β subunit (Fig. 3G, lane 4). The human β subunit was of lower M_r than the mouse β subunit precipitated from the same hybrid cells (Fig. 3G, compare lanes 4 and 2). Furthermore, preclearing with anti-mouse α L subunit mAb removed most of the mouse α L subunit precipitable with the anti-human β mAb (Fig. 3, H and G, compare lane 4 in each).

Chromosome Mapping of Human LFA-1 α L and β Subunit Genes. Mouse-human hybrids generally retain mouse chromosomes, but rapidly lose human chromosomes. As described above, only a small percentage of hybrid cells was positive for human α L or β subunits 3 wk after fusion. Furthermore, cell surface expression of the human α L and β subunits on the serially sorted, enriched hybrid cell populations was not stable, and was lost with repeated passage. We took advantage of this instability to map the chromosomal locations of the human

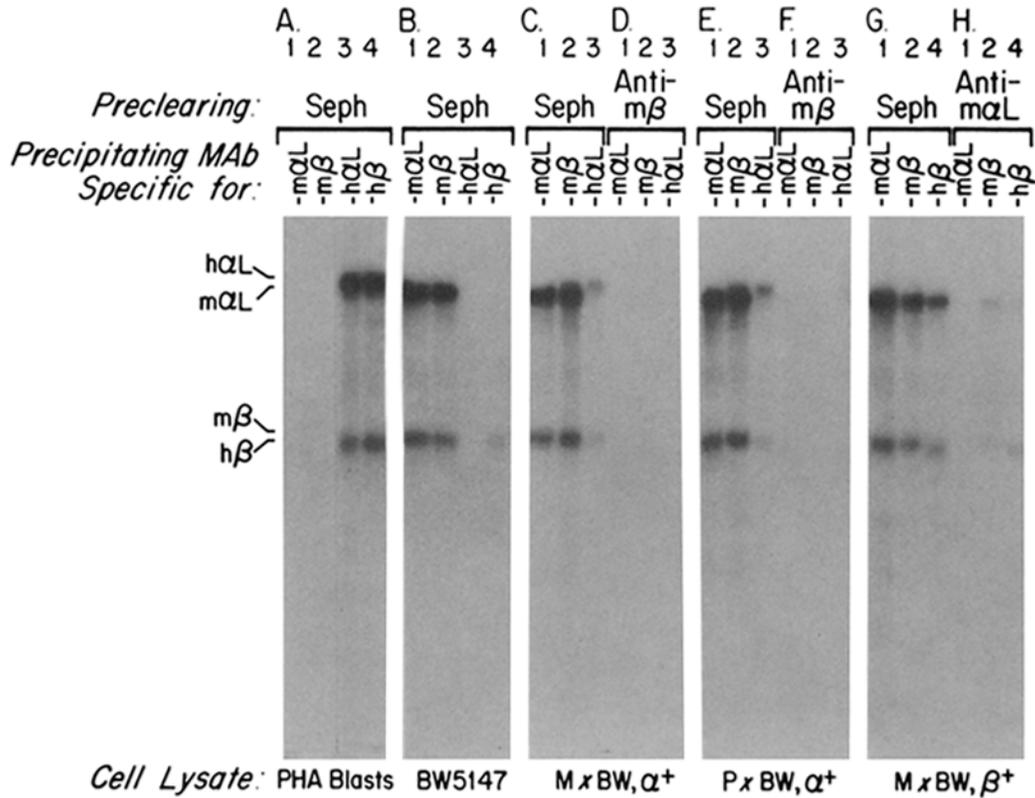


FIGURE 3. Coassociation of human and murine LFA-1 α L and β subunits on the cell surface of hybrid cells. Triton X-100 lysates of ^{125}I -labeled mother's PHA blasts, mouse BW5147 cells, and hybrids were precleared with mAb coupled to Sepharose CL-4 B (1 mg/ml) or CNBr-activated, glycine-quenched Sepharose CL-4 B (*Seph*) as a control. The precleared lysates were immunoprecipitated with mAb specific for the designated LFA-1 subunits, and the immunoprecipitates were subjected to SDS-8% PAGE and autoradiography. The anti-mouse and human α and β subunit mAb immunoprecipitated the expected subunits in a species-specific manner. In addition, a spurious band was immunoprecipitated by the anti-human β subunit mAb from mouse BW5147 cells as well as human β^+ and α^+ (data not shown) hybrids prepared from it. This band migrated distinctly from the mouse β subunit in several other experiments, and was not associated with the mouse α subunit. The identity of this protein is not known.

LFA-1 α L and β subunit genes. During the last serial selection, both positive and negative hybrids were isolated for karyotypic analysis. The negative hybrids were isolated from cells that had been positive during the previous serial selection. Positive and negative hybrids as well as unfused mouse parental BW5147 cells were karyotyped after metaphase preparations were stained with QM or with H-Act D (Fig. 4). At the time of the karyotypic analyses, $\geq 90\%$ of the cells of positive hybrid lines expressed the appropriate cell surface human LFA-1 subunits, as detected by flow cytometry (Table I). Very few human chromosomes were retained in the hybrids, and the genes encoding the human α L and β subunits could be assigned by comparison of positive hybrids with the negative hybrids derived from the same population. Expression of human α L was concordant with the presence of human chromosome 16, and therefore the human α L

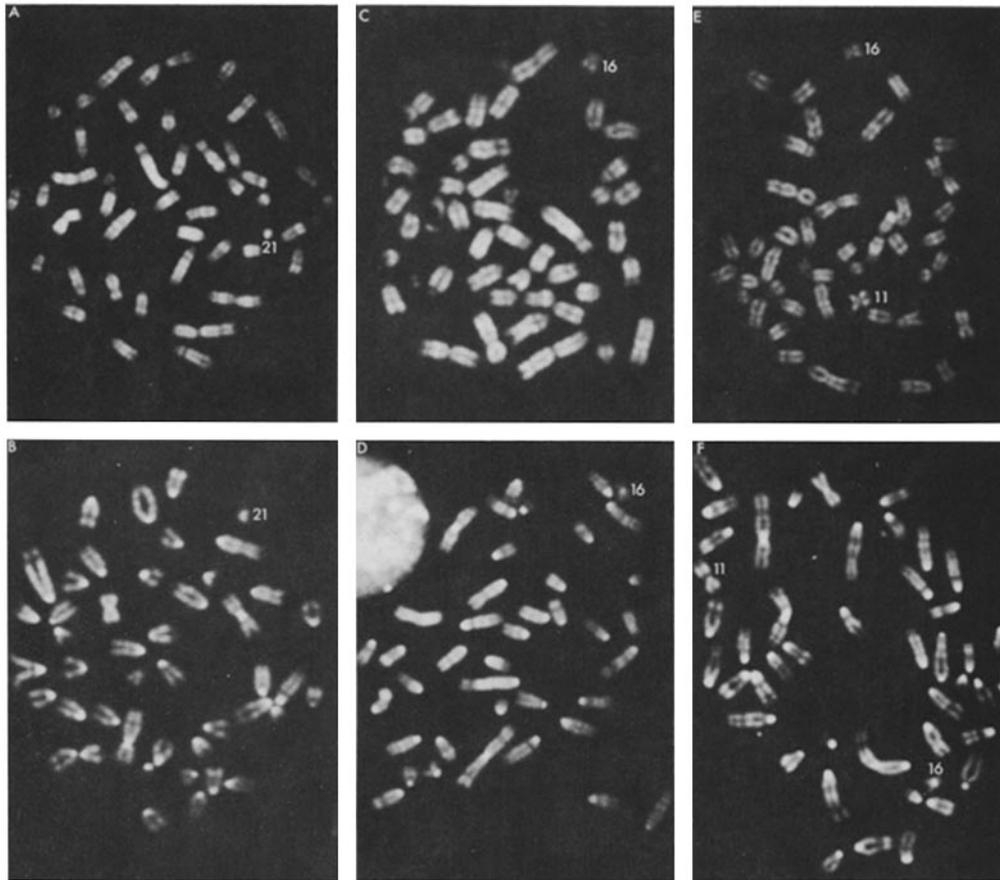


FIGURE 4. Metaphase chromosome spreads of mouse \times human hybrids stained with QM or H-ActD. *A*, *C*, and *E* are representative metaphase spreads from the positive hybrids $M \times BW, \beta^+$, $M \times BW, \alpha^+$ and $P \times BW, \alpha^+$, respectively, stained with QM. *B*, *D*, and *F* are typical metaphase spreads from $M \times BW, \beta^+$, $M \times BW, \alpha^+$, and $P \times BW, \alpha^+$, respectively, stained with H-ActD. Human chromosomes 21 and 16 are indicated. In addition, human chromosomes X and 11 are noted in several of the photographs.

gene can be assigned to this chromosome. $\sim 90\%$ of $M \times BW, \alpha^+$ hybrids and 30% of $P \times BW, \alpha^+$ hybrids contained human chromosome 16. These differences may reflect a faster rate of loss of chromosome 16 from $P \times BW, \alpha^+$ than $M \times BW, \alpha^+$ hybrids and the fact that mRNA and protein on the hybrid cell surface could remain for some time after chromosome loss. Similarly, the human β gene could be assigned to human chromosome 21 by comparing human β subunit-positive hybrids with hybrids that had lost human β subunit expression. The presence of a human X chromosome in all hybrids was not unexpected, since this chromosome codes for HGPRT, the enzyme necessary for growth in selective HAT medium. No other human chromosome was detected in these hybrids except for the nonconcordant presence of human chromosome 11 in both $P \times BW, \alpha^+$ and $P \times BW, \alpha^-$ cell populations (Table I).

The presence of human chromosomes 16 and 21 in human αL^+ and β^+ hybrids,

TABLE I
Chromosome Analysis of Hybrids

Hybrid	Positive cells (%) [*]	Number of metaphases examined		Metaphases (%) with human chromosome: [‡]							
				16		21		X		11	
		QM	H-ActD	QM	H-ActD	QM	H-ActD	QM	H-ActD	QM	H-ActD
M × BW, α^+	98	33	40	91	82			24	25		
M × BW, α^-	0	25	25	4	20			28	40		
P × BW, α^+	90	21	52	33	26			14	55	90	94
P × BW, α^-	0	23	40	9	0			30	65	87	97
M × BW, β^+	91	28	17			89	82	43	53		
M × BW, β^-	0	22	15			4	7	27	27		

^{*} Cells expressing surface human α L or β , as detected by indirect immunofluorescence flow cytometry as described in Fig. 2.

[‡] Determined by karyotyping after staining of metaphase preparations with quinacrine mustard dihydrochloride (QM) or 33258 Hoechst-actinomycin D (H-ActD) as described in Fig. 4. For human chromosomes where no data are shown, they were not detected.

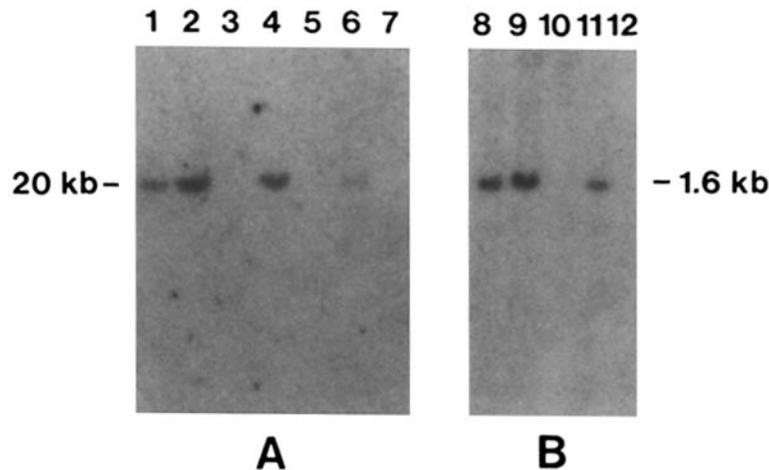


FIGURE 5. Identification of human chromosomes in hybrid cells by Southern hybridization. 5 μ g of high molecular weight DNA from normal human control 1 (lanes 1 and 8); normal human control 2 (lanes 2 and 9); BW5147 (lanes 3 and 10); M × BW, α^+ (lane 4); M × BW, α^- (lane 5); P × BW, α^+ (lane 6); P × BW, α^- (lane 7); M × BW, β^+ (lane 11); and M × BW, β^- (lane 12) was digested with Eco RI, electrophoresed on a 1% agarose gel, and transferred to nylon membranes. Filters were probed with nick-translated ³²P-labeled probes specific for human chromosomes 16 (A) and 21 (B), and autoradiographed.

respectively, was further tested by Southern hybridization of hybrid cell DNA with cloned human DNA segments specific for these two human chromosomes. A cloned human α_1 globin gene from chromosome 16 hybridized to an appropriate 20 kb band in control human DNA (Fig. 5A, lanes 1 and 2), but not to mouse BW5147 DNA (Fig. 5A, lane 3). This probe detected similar-sized sequences in human α L subunit-positive hybrids derived from the mother's (M × BW, α^+) or patient's (P × BW, α^+) cells (Fig. 5A, lanes 4 and 6), but did not detect human chromosome 16 in hybrids that had lost expression of the human α L subunit (Fig. 5A, lanes 5 and 7). Note that the lower intensity of hybridization with P × BW, α^+ DNA relative to the M × BW, α^+ DNA is consistent with the relative numbers of cells containing human chromosome 16, as detected by

karyotyping (Table I). A human chromosome 21-specific probe hybridized to control human DNA and $M \times BW, \beta^+$ DNA (Fig. 5B, lanes 8, 9, and 11), but not to DNA from either mouse BW5147 cells or $M \times BW, \beta^-$, a hybrid that had lost human β subunit expression (Fig. 5B, lanes 10 and 12). The specific presence of human chromosomes 16 and 21 in hybrids expressing the human αL and β subunits, respectively, and their absence in hybrids that had lost expression confirm our assignment of the human LFA-1 subunit genes to those chromosomes.

Discussion

Hybrid cells expressing interspecies hybrid LFA-1 molecules containing a human αL subunit complexed with a mouse β subunit or a human β subunit complexed with a mouse αL subunit were isolated here from fusions of normal human lymphocytes with a mouse T cell line. Based on similarities in subunit structure, cell distribution, and function, it has previously been proposed (27) that human and murine LFA-1 are homologs. The observation here that human and murine LFA-1 subunits can coassociate to form interspecies hybrid molecules that are transported to and expressed on the cell surface demonstrates that the mouse and human LFA-1 complexes are truly homologs of each other. In particular, the sites of intersubunit interaction must be closely conserved between the two species. A similar type of interspecies subunit complex formation between human HLA and murine β_2 -microglobulin has previously been reported (28).

A major goal of these studies was to examine whether complementation with murine subunits could rescue surface expression of human LFA-1 αL or β subunits in mouse \times human hybrids formed with LFA-1-deficient patient cells. T lymphocytes from LFA-1⁻ patients express neither the LFA-1 αL or β subunit on the cell surface (3). In mouse \times human patient cell hybrids, the formation of interspecies hybrid LFA-1 molecules containing the human αL subunit noncovalently associated with the murine β subunit allowed surface expression of the human LFA-1 αL subunit to be rescued. We were unable to isolate mouse \times human patient cell hybrids that expressed the human β subunit. In contrast, we were able to isolate cells expressing the human β subunit associated with the mouse αL subunit from mouse cells hybridized to cells from the patient's mother. Intracellular LFA-1 αL subunit precursors have previously been detected (3) in lymphocytes from three different LFA-1-deficient patients. However, since the αL precursor was not expressed on the cell surface, it was unclear whether such expression was possible. The results reported here show that the intracellular αL precursor in patients' cells is normal and is competent for surface expression in the presence of an appropriate β subunit. Furthermore, the inability to isolate hybrids expressing the human β subunit from patients' cells argues that the β subunit is defective. These data support the hypothesis that the primary defect in LFA-1 deficiency is in the β subunit, and is consistent with the failure of patients' monocytes and granulocytes to express on their membranes the Mac-1 and p150,95 glycoprotein complexes, which use the same β subunit (3). Formation of an $\alpha\beta$ complex appears to be required for processing and transport to the cell surface.

Although the presence of a murine β subunit was sufficient to rescue cell

surface expression of the patient's LFA-1 α L subunit, we do not know at this time whether surface expression of the related Mac-1 α M subunit and p150,95 α X subunits, which associate with the same β subunit, can be similarly rescued. The hybrids described here did not express human α M or α X subunits (data not shown); however, these proteins are not normally expressed on cells of T lymphocyte lineage, such as the mouse BW5147 cell line. The α subunits of LFA-1, Mac-1, and p150,95 share N-terminal amino acid sequence homology (29 and our unpublished observations). These proteins may thus have evolved as a gene family by gene duplication and divergence. It will be interesting to determine whether the Mac-1 and p150,95 α subunit genes are closely linked to the LFA-1 α subunit gene, and whether α M and α X subunit cytoplasmic precursors are present in the inherited deficiency of Mac-1, LFA-1, and p150,95 (referred to herein as LFA-1-deficiency). Fusion of patients' cells with murine cells of myeloid lineage should serve to answer these questions.

We have mapped the genes encoding the human LFA-1 α L and β subunits to chromosomes 16 and 21, respectively. The α L and β subunit genes were thus assigned to different chromosomes, consistent with our observation that selection with mAb specific for only one human subunit did not isolate hybrids expressing both human subunits. Since our results suggest that the β subunit gene is defective in LFA-1 deficiency, we infer that the inherited defect itself is on chromosome 21. It has been suggested (4, 30) that one patient with LFA-1 deficiency has an X-linked defect. However, studies on the patient whose cells were hybridized here, and on about 20 other patients worldwide show that LFA-1 deficiency is inherited as an autosomal recessive trait (1, 3, 4, 6, 7, 9), consistent with inheritance on chromosome 21. Rescue of human α L subunit expression by the introduction of a functional murine β subunit as reported here suggests that gene therapy for this disease could be accomplished by the introduction of a normal, cloned β subunit gene.

Summary

Lymphocyte function associated antigen 1 (LFA-1) is a leukocyte cell adhesion protein. We have studied a novel human immunodeficiency disease in which LFA-1 and two other proteins which share the same β subunit are lacking from the surface of leukocytes. The basis of the inherited defect in cell surface expression of both the α and β subunits of LFA-1 was determined by somatic cell fusion of patient or normal human cells with an LFA-1⁺ mouse T cell line. Human LFA-1 α and β subunits from normal cells could associate with mouse LFA-1 subunits to form interspecies hybrid $\alpha\beta$ complexes. Surface expression of the α but not the β subunit of patient cells was rescued by the formation of interspecies complexes. These findings show that the LFA-1 α subunit in genetically deficient cells is competent for surface expression in the presence of an appropriate β subunit, and suggest that the genetic lesion affects the β subunit. The human LFA-1 α and β subunits were mapped to chromosomes 16 and 21, respectively. The genetic defect is inferred to be on chromosome 21.

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Note added in proof: Suomalainen et al. (31) have also recently mapped a glycoprotein with characteristics similar to the β subunit to human chromosome 21 by karyotypic analysis of interspecies hybrids. Using similar methods, Tetteroo et al. (32) have reported in abstract form that the human β subunit can associate with murine Mac-1 and LFA-1 α subunits in interspecies hybrids, and have also tentatively assigned the human β subunit to chromosome 21.

Lymphoid hybrids were obtained from the patient's cells, which contained chromosome 21, but did not express the human subunit.

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