0022-1767/85/1355-3102$02.00/0
THE JOURNAL OF IMMUNOLOGY
Copyright © 1985 by The American Association of Immunologists
Vol. 135, No. 5, November 1985
Printed in U.S.A.

HERITABLE LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN-1 DEFICIENCY: ABNORMALITIES OF CYTOTOXICITY AND PROLIFERATION ASSOCIATED WITH ABNORMAL EXPRESSION OF LFA-1[1]

ALAN M. KRENSKY,* STEVEN J. MENTZER,† CAROL CLAYBERGER,* DONALD C. ANDERSON,§ FRANK C. SCHMALSTIEG,¶ STEVEN J. BURAKOFF,† AND TIMOTHY A. SPRINGER[2‡]

*From the *Department of Pediatrics, Stanford University, Stanford, CA 94305; the †Division of Pediatric Oncology and the ‡Department of Pathology, Harvard Medical School and the Dana-Farber Cancer Institute, Boston, MA 02115; the §Departments of Pediatrics, Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030; and the ¶Department of Pediatrics, University of Texas Medical Branch, Galveston, TX 77550*

The effect of heritable LFA-1 deficiency on T lymphocyte function was measured. After primary mixed lymphocyte stimulation, all six patients studied showed diminished allospecific T lymphocyte cytolytic and NK activity as compared with kindred and normal controls. MLR and mitogen-induced proliferative responses were consistently depressed. LFA-1-deficient, EBV-transformed B cell lines were poor stimulators of T cell responses. Primary cytolytic responses by lymphocytes from severely LFA-1-deficient patients (<0.2% of normal surface expression) were consistently more profoundly depressed than those by lymphocytes from moderately deficient patients (about 5% of normal surface expression). These results demonstrate the importance of LFA-1 in lymphocyte function. After repeated MLR restimulation, proliferative and cytolytic capacity improved and CTL lines could be established from all patients. Cytolysis by lines from one but not a second severe patient, and by four of four moderate patients, was inhibited by anti-LFA-1 MAb, and at 10-fold lower concentrations than required for inhibition of killing by control CTL lines. The locus of inhibition was on the target cell for the severely deficient CTL line, and on both the target and effector cells for moderately deficient CTL lines. In contrast, the locus of inhibition for normal CTL is on the effector cell. These findings show that LFA-1 can participate bidirectionally in cell interactions. The in vitro results are discussed in terms of the clinical findings in patients.

A number of cell surface molecules important in the interaction between T lymphocytes and other cells have recently been identified with monoclonal antibodies (MAb).[3] A similar set of molecules appears to mediate the response of helper T lymphocytes to antigen-presenting cells and killing by cytolytic T lymphocytes (CTL) of target cells. This suggests that these diverse cell functions are initiated by cell interactions that have a similar molecular basis. Thus far, conclusions regarding the functional importance of these surface molecules are based primarily on the ability of MAb to lymphocyte function-associated antigen-1 (LFA-1) (1–4), T8 (5, 6), and T4 (7–9) to inhibit function, and the ability of MAb to LFA-2/T11 (2, 3, 10–12), T3 (13, 14), and the antigen receptor (15) to stimulate or inhibit function. A complementary and rigorous method of evaluating the importance of these molecules would be to examine the functional capacity of mutant cells deficient in a specific molecule. The recent discovery of a heritable deficiency disease (16–20) has now made this possible for the LFA-1 molecule.

LFA-1 was initially defined by MAb that inhibit CTL-mediated cytolysis in the absence of complement. MAb blocking studies have shown that LFA-1 is involved not only in CTL-mediated cytolysis, but also in a wide variety of leukocyte functions. Anti-LFA-1 MAb inhibit antigen-specific and mitogen-driven T lymphocyte proliferative responses (1, 3, 21), natural killer (NK)-mediated cytolysis (3, 4), and granulocyte antibody-dependent cellular cytolysis (ADCC) (22, 23). Inhibition by anti-LFA-1 MAb of CTL conjugation to target cells suggests that LFA-1 functions in cell-cell adhesion (1, 24). The LFA-1 glycoprotein is expressed on essentially all leukocytes but is absent from nonhematopoietic cells (1). The LFA-1 molecule consists of a M_r = 177,000 α subunit and a M_r = 95,000 β subunit noncovalently associated in a heterodimer (1, 2, 4).

In this report, we have investigated the effect of genetic deficiency of LFA-1 on lymphocyte function. A group of six patients were studied, all of whom appear to have a primary deficiency of the M_r = 95,000 β subunit (16, 25). The LFA-1 α subunit is synthesized but is not expressed on the surface in the absence of the β subunit. Mac-1 and p150,95, two related molecules that utilize distinct α subunits but share the same β subunit as LFA-1, are also deficient on granulocytes and monocytes of the patients. These Mac-1-, LFA-1-deficient patients have no other known biochemical or developmental defects, yet they manifest life-threatening immune dysfunction. The patients' clinical course is characterized by recurrent bacterial infections, poor wound healing, and lack of

Received for publication May 9, 1985.
Accepted for publication August 2, 1985.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by National Institutes of Health Grants CA31798, CA34129, CA09280, AI05877, and AM35008.
[2] Address correspondence to T. A. Springer, Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney St., Boston, MA 02115.
[3] Abbreviations used in this paper: CTL, cytotoxic T lymphocyte; MAb, monoclonal antibody; MLR, mixed lymphocyte response; PBL, peripheral blood lymphocyte; E:T, effector to target cell.

granulocyte infiltration of inflammatory sites, despite marked granulocytosis (reviewed in Reference 25). We find that the lymphocytes from all patients show decreased CTL- and NK-mediated killing and T cell proliferative responses compared with their kindred and normal controls. The role of LFA-1 in lymphocyte function and in the clinical manifestations of the disease is discussed in relation to these findings.

MATERIALS AND METHODS

Peripheral blood lymphocytes (PBL). PBL from LFA-1-deficient individuals, family members, and normal volunteers were purified on Ficoll/Hypaque gradients as described (16). Tissue typing was performed by the Immunology Research Laboratory at the Baylor University School of Medicine and the Histocompatibility Laboratory at the Dana-Farber Cancer Institute. The human leukocyte antigen (HLA) types were as follows: patient 1 (HLA-A2,32; B35,51; Cw4; DR3,4), mother 1 (HLA-A2,2; B27,51; Cw1; DR3,?), father 1 (HLA-A3,32; B7,35; Cw4,7; DR2,4), patient 2 (HLA-A1,2; B14,14; Cw7,w8; DR1,w6), mother 2 (HLA-A1,23; B14,w57; Cw3,w7; DRw6,w9), father 2 (HLA-A2,28; B14,w62; Cw1,w8; DR1,?), patient 4 (A30,32; B14,W53; DR1), patient 6 (HLA-A1,?; B8,14; C?; DR3,w7), and his following three children: patient 7 (daughter) (HLA-A1,29; B8,44; C?; DR3,w6); patient 8 (son) (HLA-A1,32; B8,40(61); Cw2; DR3,w6); heterozygote daughter SFO (HLA-A1,32; B8,40 (61); Cw2; DR3,w6); heterozygote daughter HFO (A1,29; B8,44; DR3).

Human tumor cell lines. The Epstein Barr virus (EBV)-transformed B lymphoblastoid cell lines JY and MANN, as well as the erythroleukemia cell line K562, were typed by the Histocompatibility Laboratory at the Dana-Farber Cancer Institute: JY (HLA-A2, B7, DR4, DRw6), Mann (HLA-A29, B12, DRw7), and K562 (HLA-A$^-$, B$^-$, DR$^-$).

Culture conditions. Primary culture was performed as described (7). Briefly, 7×10^6 PBL were stimulated with 1×10^5 JY cells or medium alone in 2-ml wells (Linbro; Flow Laboratories, McLean, VA). The JY stimulator cells were irradiated with 10,000 R. The culture medium was RPMI 1640 supplemented with 10% fetal calf serum (M. A. Bioproducts, Bethesda, MD), 2 mM L-glutamine (Gibco Laboratories, Grand Island, NY), penicillin at 100 μg/ml and streptomycin at 100 μg/ml (Gibco), and 25 μM 2-mercaptoethanol (Eastman Organic Chemicals, Rochester, NY). Long-term CTL lines and NK cells were generated as described (26).

^{51}Cr-release assay. Cells were harvested after 6 days of culture, and cytolytic assays were performed in duplicate or triplicate in V-bottom microtiter wells (Linbro). Target cells (JY, K562, or MANN) were incubated with 0.1 mCi of [^{51}Cr]sodium chromate for 1 to 2 hr at 37°C, and 1×10^3 target cells were incubated with 1×10^5, 2.5×10^4, or 6×10^3 effector cells (corresponding to effector to target [E:T] ratios of 100:1, 25:1, and 6:1) for 3 to 4 hr at 37°C. After incubation the plates were centrifuged, and 100-μl samples of cellfree supernatant were taken and counted in a gamma counter. Percentage specific release (% SR) was calculated according to the formula: $SR = 100 \times [(E - C)/(T - C)]$, where E is cpm released by incubating targets with immune cells, C is cpm released from targets incubated with medium alone, and T is the total cpm releasable from targets with 100 μl of 5% Triton X-100.

MAb blocking experiments. MAb were added at various concentrations directly to the microculture well at the start of the 3- to 4-hr incubation of the ^{51}Cr-release assay, and the percentage of blocking was calculated according to the formula: Percent blocking = 100 × [(SR control) − (SR + MAb)/(SR control)], where SR control is the specific release in the absence of MAb and SR + MAb is specific release in the presence of MAb. To establish the locus of inhibition by anti-LFA-1 MAb, effector or target cells were pretreated with MAb at 50 μg/ml for 30 min at room temperature, washed extensively four times, and used in standard 3- to 4-hr ^{51}Cr-release assays.

MAb to the LFA-1 α subunit, TS1/12, TS1/22, TS2/4, and TS2/14, and TS1/18 to the β subunit have been described (2). These MAb recognize three unique and two overlapping determinants on the LFA-1 molecule (27). MAb were derived from subcloned hybridoma lines. Other antibodies used include TS2/18 (anti-LFA-2), TS2/9 (anti-LFA-3), w6/32 (anti-HLA-A,B,C), TS1/16 (anti-HLA-DR) [2] OKT3, OKT4, OKT8, OKT11 (Ortho, Raritan, NJ), Leu-1, Leu-3a, and Leu-5 (Becton-Dickinson, Mountain View, CA).

Proliferation assays. Mixed lymphocyte response (MLR) cultures were established at 1×10^5 responder cells and 10^4 stimulator cells (2500 R) per 0.2 ml microtiter well. The cultures were pulsed for 18 hr with 1 μCi/well of [^{3}H]thymidine (New England Nuclear, Boston, MA), harvested with a cell harvester (Cambridge Technologies, Cambridge, MA), and counted in a beta counter.

The blastogenic response of lymphocytes to phytohemagglutinin (PHA) was measured with 1×10^5 responder cells/well in 0.2 ml of complete medium and harvested on day 3. Pulsing, harvesting, and counting were performed as in the MLR assay.

Immunofluorescence. Immunofluorescence flow cytometry was performed on an Epics V (Coulter) after labeling cells with MAb and affinity-purified fluorescein isothiocyanate F(ab')$_2$ goat anti-mouse IgG (Tago, Burlingame CA), as described (16).

RESULTS

Two populations of patients have been defined that differ in the severity of deficiency of Mac-1 and LFA-1 on the cell surface. The severity of the clinical syndrome, characterized by recurrent bacterial infections, poor wound healing, and a lack of granulocyte infiltration of inflammatory sites, has been shown to correlate with the degree of the deficiency (16, 25). Severely deficient patients 1 and 2 and moderately deficient patients 4 through 8 express <0.2% and 5%, respectively, of the normal amounts of Mac-1, LFA-1, and p150,95 on their neutrophils and monocytes and of LFA-1 on their lymphocytes. Both types of patients have been examined in these studies.

Defective allospecific CTL-mediated killing and natural killing. PBL from LFA-1-deficient patients, family members, and unrelated individuals were stimulated with EBV-transformed B cell lines (JY or MANN) that were mismatched at both class I and class II HLA loci. After 6 days, cells were harvested and tested for cytolytic activity against ^{51}Cr-labeled JY target cells. All LFA-1-deficient individuals showed low levels of cytolysis of JY cells at all effector to target ratios tested (Fig. 1*A–C*): patient 1 was less than 10% of control levels, patient 2 was less than 15% of control levels, and patient 4 was less than 40% of control levels of cytolysis. Similar results were obtained in other CTL assays after short-term

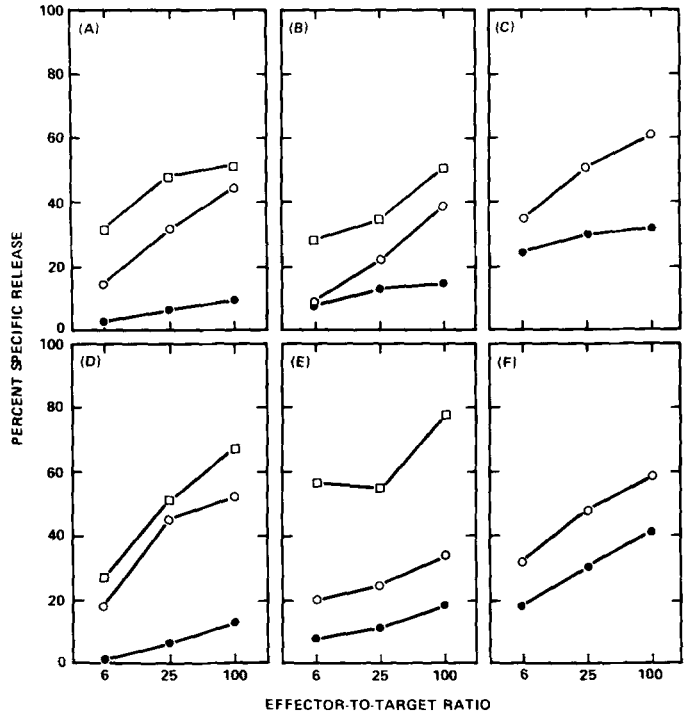

Figure 1. CTL (*A–C*) and cultured NK cells (*D–F*) from patients (●) 1 (*A, D*), 2 (*B, E*), and 4 (*C, F*), their mothers (○) and fathers (□), 6 days after primary MLC stimulation with JY, were assayed for cytotoxicity in a 3- to 4-hr ^{51}Cr-release assay at E:T ratios of 100:1, 25:1, and 6:1. Target cells were JY for CTL and K562 for NK cells.

(primary or secondary) MLR stimulation. Representative results with patients 1, 6, 7, and 8 are shown in Table I. The defect in short-term CTL-mediated killing was consistently more pronounced in severely deficient patients 1 and 2 than in moderately deficient patients 4, 6, 7, and 8 (Fig. 1 and Table I).

Because primary human mixed lymphocyte cultures contain both specific CTL and nonspecific NK cells, we used MAb blocking to estimate the relative contribution of each type of killer cell to anti-JY cytolysis. Inclusion of anti-HLA-A,B,C (W6/32) and OKT3 monoclonal antibodies inhibited greater than 50% of the cytolysis by both control and patient CTL, demonstrating that the majority of the observed lysis was allospecific and mediated by $OKT3^+$ CTL (data not shown). LFA-1-deficient CTL were also defective in their ability to lyse PBL cultured for 1 wk (data not shown), indicating that the defect in cytolysis was not limited to EBV-transformed target cells.

Long-term CTL from LFA-1-deficient individuals show improved cytolytic activity. The long-term in vitro culture of LFA-1-deficient CTL provided an opportunity to study the evolution of cytolytic function and the stability of the LFA-1-deficient phenotype. CTL were maintained in culture in medium supplemented with interleukin 2 (IL 2) (supernatant from PHA and MLR-stimulated PBL) and stimulated at 1- to 2-wk intervals with irradiated JY or Mann cells. After 7 wk in culture, the efficiency of cytolysis by LFA-1-deficient lymphocytes increased significantly. Examples of the improvement in lytic function of CTL lines are shown in Table I. CTL cultures of patients 1, 6, 7 and 8 and of related and unrelated controls were tested after both short- and long-term culture. Separate cultures from patient 1, established from blood donated at different times and stimulated with either JY or Mann, demonstrated comparable levels of cytolysis, showing that the improvement in lytic activity was not unique to a particular stimulator or target cell line (Table I). The lytic activity of cultures from patients 1, 6, 7, and 8 demonstrated considerable improvement (Table I), but did not reach control levels even after 20 wk in culture (not shown). Comparisons in experiment 3 are particularly relevant, because patients 6, 7, 8 and the heterozygote control are from the same family. Moreover, patient 8 and the heterozygote control are HLA identical.

TABLE I

CTL response in short-term and long-term culture of LFA-1-deficient patients[a]

Effector Cells (Donor)	Target Cells	^{51}Cr Release			
		Short-Term E:T		Long-Term E:T	
Experiment 1		15:1	45:1	11:1	33:1
Patient 1	Mann	5	8	52	68
Mother	"	31	51	61	87
Control	"	45	69	ND	ND
Experiment 2		25:1		25:1	
Patient 1	JY	12		50	
Mother	"	32		60	
Father	"	65		78	
Experiment 3		15:1	45:1	5:1	15:1
Patient 6	JY	30	39	52	65
Patient 7	"	40	45	48	63
Patient 8	"	33	42	59	63
Heterozygote daughter	"	68	75	61	85
Control	"	59	79	53	77

[a] Lymphocytes from each donor were co-cultured with irradiated JY or Mann cells in the presence of IL 2 (see *Materials and Methods*). Effector cells were assayed for cytolytic activity against the appropriate target cells. Data shown are percent specific release in 3- to 4-hr ^{51}Cr-release assays. In experiments 1 and 3 the cultures were tested after 2 and 8 wk of culture; experiment 2 was performed after 1 and 7 wk of culture.

The improvement in CTL function was not accompanied by an increase in LFA-1 expression. The CTL line from severe patient 2 showed <0.1% of normal expression of the LFA-1 α and β subunits (Fig. 2*d*, *e*). LFA-1 was detectable on the CTL line from moderate patient 4 at 0.7% and 0.6% of the amounts on a control CTL line for the LFA-1 α and β subunits, respectively (Fig. 2*j*, *k*). LFA-1 expression as a percentage of that on the relevant healthy control cell was consistently lower on CTL and EBV-transformed cell lines (16) than on normal peripheral leukocytes (25) of moderately deficient patients. CTL lines from unaffected family members expressed normal levels of LFA-1 (Fig. 2*g*, *h*, *m*, and *n*). Cell surface expression of HLA (Fig. 2*f* and *l*), T3, T4, T8, LFA-2, and LFA-3 (not shown) by LFA-1-deficient CTL did not differ from that of control cells.

Natural killing. In a parallel series of experiments, we tested whether LFA-1 deficiency affected NK activity. PBL from LFA-1-deficient individuals, their families, and unrelated controls were cultured alone or with JY cells for 6 days (similar NK activity was noted whether stimulator cells were present or absent). NK cell-mediated cytolysis was assessed on the K562 erythroleukemia cell line (HLA negative.) All LFA-1-deficient individuals showed low levels of NK-mediated cytolysis compared with that of family members and many unrelated individuals (Fig. 1*D–F*), although some unrelated individuals also showed low levels of NK cell-mediated cytolysis (data not shown).

T cell proliferation. PBL from LFA-1-deficient patients,

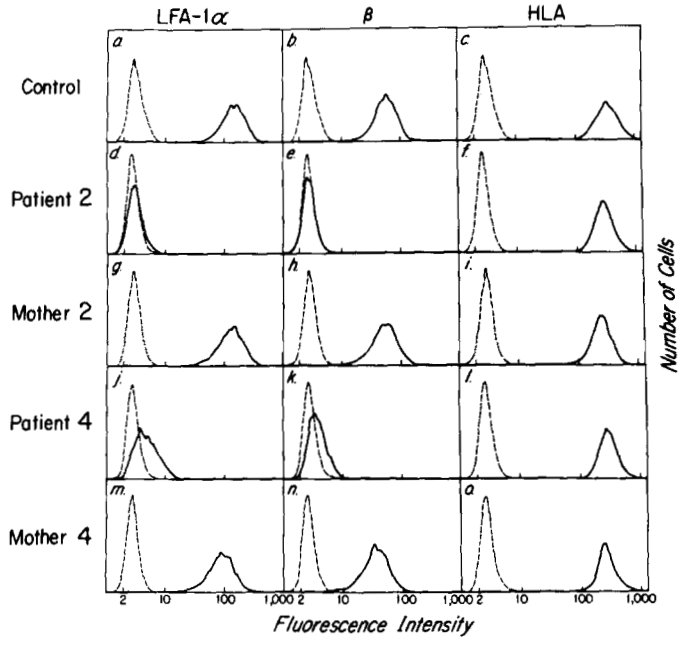

Figure 2. Immunofluorescence flow cytometry of CTL from Mac-1-, LFA-1-deficient patients. Patient, kindred, and control anti-JY CTL lines, 12 wk after establishment and 5 days after the last JY stimulation, were purified by Ficoll-Hypaque sedimentation. CTL were labeled with a mixture of anti-LFA-1 α MAb (TS1/22, TS2/4, TS2/6, TS2/14, and TS1/12), anti-LFA-1 β MAb (TS1/18), and anti-HLA MAb (W6/32) as indicated (*solid curves*) or with X63 myeloma IgG as control (*dashed curves*), a second step of fluoresceinated anti-mouse IgG, and scatter-gated immunofluorescence was determined.

family members, and unrelated controls were tested for proliferation after a) allostimulation (JY cells or unrelated normal PBL) or b) lectin stimulation (PHA). After 3 days in culture with irradiated JY cells, PBL from LFA-1-deficient individuals incorporated less than 30% as much [^{3}H]thymidine as did cells from control individuals (Fig. 3). Similarly poor proliferative responses occurred after stimulation with normal donor PBL (not shown). LFA-1-deficient individuals also showed an impaired proliferative response to PHA, at both 2.5 and 0.25 μg/ml PHA (Table II). The response could not be improved by addition of IL 2. Of interest, the long-term CTL lines demonstrated an improvement in proliferative response to allostimulation that paralleled the improvement in lytic activity. After 20 wk in culture, CTL lines from deficient patients proliferated as well as controls when stimulated by the appropriate allogeneic target cell (JY or Mann; data not shown).

Defects in the ability of LFA-1-deficient B cells to stimulate T cell responses. These results show that T cells from LFA-1-deficient individuals responded poorly in a number of in vitro functional assays. Because LFA-1 is present on virtually all leukocytes, it was of interest to determine whether B cells from these patients could normally stimulate in the MLR. EBV-transformed B cell lines were generated from LFA-1-deficient patients, family members, and control individuals. These cells were used as stimulators for MLR and the generation of allogeneic CTL, with normal PBL used as responders. In all cases, LFA-1-deficient B cells were poor stimulators for primary allogeneic MLR (representative results are shown in Table III). The LFA-1-deficient EBV lines gave near-normal secondary stimulation after primary stimulation with LFA-1$^+$ EBV lines (data not shown). Additionally, LFA-1-deficient B cells were poor stimulators when used in primary culture to generate CTL. We were unable to generate any long-term CTL lines against these LFA-1-deficient B cells.

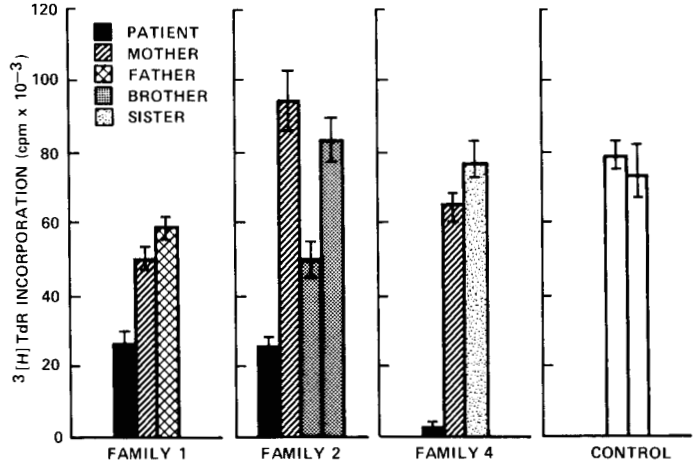

Figure 3. Primary allogeneic proliferative responses. Lymphocytes (10^5) from patients, family members, and two unrelated controls were stimulated with 10^3 irradiated JY cells and assayed for thymidine incorporation on day 5. Data shown are the mean [^{3}H]thymidine incorporation of three to six replicates × 10^{-3} ± 1 SD.

Effect of anti-LFA-1 MAb on cytotoxic responses. To investigate the utilization of LFA-1, we examined the ability of anti-LFA-1 MAb to inhibit long-term CTL lines. As noted previously, all LFA-1-deficient patients demonstrated lower levels of cytolysis than related or unrelated controls (Fig. 4*B*; in Fig. 4*A*, higher E:T ratios and longer incubations were used for patient CTL to obtain levels of killing similar to normal CTL). Interestingly, anti-LFA-1 MAb significantly blocked cytolysis by all but one LFA-1-deficient CTL line (Fig. 4*A*, *B*). Furthermore, the concentration of anti-LFA-1 MAb required for 30% inhibition of cytolysis was 10- to 30-fold less than the concentration required for control CTL lines (Fig. 4*A*, *B*). The exception was patient 2, whose CTL were only slightly inhibited by concentrations of anti-LFA-1 MAb as high as 500 μg/ml. Figure 4 is representative of many similar experiments. The differences between the CTL lines from different patients, and the greater sensitivity of CTL lines from patients 1, 4, 6, 7, and 8 to inhibition by anti-LFA-1 than of control CTL, were consistently observed over many weeks in culture.

LFA-1 is expressed on lymphoid target cells as well as on CTL. Using CTL lines from normal donors, we have previously shown that the dominant site of anti-LFA-1 MAb inhibition was the effector cell and not the target cell. Because anti-LFA-1 inhibited killing by LFA-1-deficient CTL that expressed less LFA-1 than the target cells which they killed, similar studies on the site of inhibition were undertaken for LFA-1-deficient CTL. Effector and target cells were pretreated with anti-LFA-1 MAb (TS1/18-anti-β-chain or TS1-22-anti-α-chain) and were washed extensively before use in a standard ^{51}Cr-release assay (Fig. 5). As noted previously, anti-LFA-1 MAb only weakly inhibited CTL from patient 2; therefore, pretreatment experiments include data only from patients 1, 4, 5, and 6 (Fig. 5*A*). The pattern of anti-LFA-1 MAb inhibition differs in the normal and LFA-1-deficient CTL (Fig. 5*A* and *B*). As previously found, cytolysis by control CTL was inhibited by MAb treatment of the effector cells but not target cells (Fig. 5*B*). In contrast, cytolysis by LFA-1-

TABLE II

Proliferative responses to PHA[a]

	[^{3}H]Thymidine incorporation × 10^{-3} ± SD			
	2.5 μg/ml	2.5 μg/ml + IL 2	0.25 μg/ml	No PHA
Patient 2	28 ± 3	36 ± 2	1.5 ± 0.7	0.3 ± 0.05
Mother	101 ± 8	142 ± 20	9.1 ± 0.4	0.5 ± 0.01
Brother 1	102 ± 2	123 ± 3	4.9 ± 2.1	0.5 ± 0.2
Brother 2	138 ± 9	162 ± 15	10.0 ± 3.0	0.4 ± 0.1
Patient 4	23 ± 5	22 ± 4	0.6 ± 3.2	1.0 ± 1.0
Mother	75 ± 25	90 ± 13	13.9 ± 2.5	3.0 ± 2.4
Sister	108 ± 15	111 ± 16	9.2 ± 1.0	9.0 ± 13
Unrelated control 1	71 ± 14	107 ± 33	4.7 ± 4.9	1.1 ± 1.0

[a] PBL from patients, family members, and an unrelated individual were stimulated with PHA at 2.5 μg/ml or 0.25 μg/ml as designated, pulsed for 18 hr with 1 μCi/well of [^{3}H]thymidine, and harvested on day 3. IL 2-conditioned medium was added on day 0 as indicated.

TABLE III

Proliferative responses to patient and control EBV-transformed B cell lines[a]

Normal Responder	Stimulated with[b]					
	Medium control	LFA-1⁻ lines			Control LFA-1⁺ lines	
		Patient 1	Patient 2	Patient 4	Co1	Co4
AK	386	4,993	83	2,297	2,086[c]	18,631
	±251	±1007	±57	±1456	±560	±3497
MC	543	1,064	2,777	7,746	12,897	27,499
	±217	±428	±572	±869	±1248	±3415

[a] Patient and control lymphocytes were transformed with EBV and passaged in parallel. Proliferative responses to irradiated EBV-transformed stimulator cells are expressed as [^{3}H]thymidine incorporation (cpm). HLA-types: AK HLA-A3, B7, 38, DR6, 6; MC HLA-A3, 30, B7, 13, DR4; line Co1 HLA-A24, B44, w60, DR6, 6; line Co4 HLA-A1, 9, B8, 40, 41, DR3, 5; patient 1 HLA-A2, 32, B35, 51, DR3, 4; patient 2 HLA-A1, 2, B14, DR1, 6; patient 4 HLA-A30, 32, B14, w53, DR1. There were DR locus mismatches in all combinations except for AK with Co1.

[b] With the HLA-DR identical combination omitted (footnote c), the groups differed, $p = 0.012$, by Wilcoxon's exact test.

[c] AK responder and Co1 stimulator were both HLA-DR6, accounting for the low proliferative response.

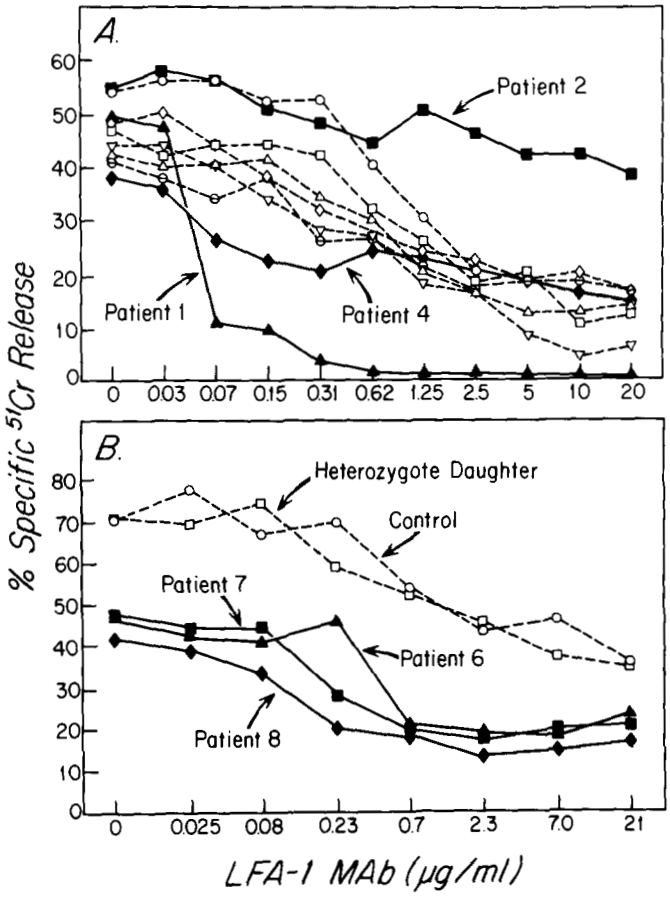

Figure 4. Inhibition of cytolysis by anti-LFA-1 MAb (TS1/18). Long-term CTL lines from patients, family members, and controls (unrelated individuals) were assayed for cytotoxicity in 3- to 4-hr ^{51}Cr-release assays. LFA-1 MAb (TS1/18) was added at the initiation of the assay at the indicated final dilutions. (*A*) Patient 1 (▲), patient 2 (■), and patient 4 (◆) at E:T ratios of 20:1 were compared with father 1 (○), mother 1 (△), mother 2 (□), mother 4 (▽), control 3 (◇), or control 4 (⊖) at E:T ratios of 10:1. (*B*) Patient 6 (▲), patient 7 (■), and patient 8 (◆) were compared with controls at E:T ratios of 45:1 (or at 15:1 with identical results, data not shown).

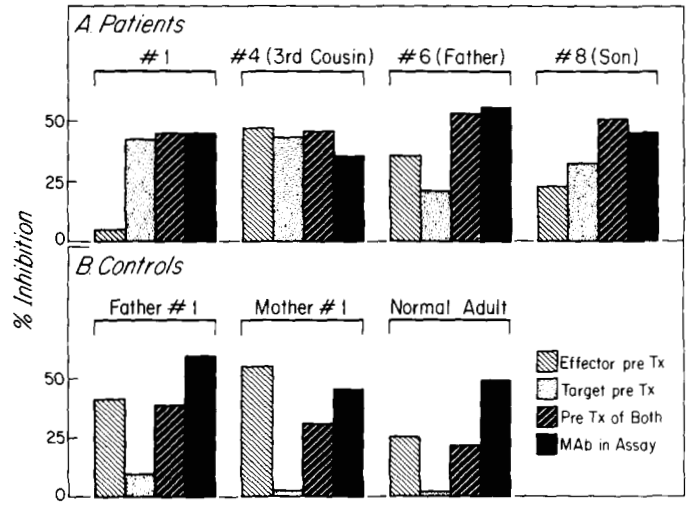

Figure 5. Inhibition of cytolysis after pretreatment of effector and/or target cells with anti-LFA-1 MAb. CTL from *A*) patients 1, 4, 6, and 8 and *B*) related and unrelated controls were tested against the target cell line JY. CTL and JY cells were preincubated with anti-LFA-1 MAb and washed. Pretreatment of the CTL alone, the target cells alone, and both the CTL and the target cells was compared with the inhibition of cytolysis observed when anti-LFA-1 MAb was added at the beginning of the assay. Specific lysis ranged from 40 to 64%.

deficient CTL could be inhibited by MAb treatment of the target cells (Fig. 5*A*). In the case of CTL from the severely deficient patient 1, the target cell was the dominant site of inhibition. For moderately deficient patients 4, 6, and 8, pretreatment of the target cells or pretreatment of the effector cells resulted in comparable degrees of inhibition. These differences between moderately deficient CTL, severely deficient patient 1 CTL, and control CTL were consistently found in over six different experiments.

DISCUSSION

The ability of anti-LFA-1 MAb to inhibit CTL-mediated cytolysis, NK cell-mediated cytolysis, and T cell proliferative responses previously suggested that this cell surface molecule is important in lymphocyte function (1, 2). In this study, we have examined the immune function of lymphocytes from patients with heritable LFA-1 deficiency. This experiment of nature offered a unique opportunity to study the importance and mechanism of action of the LFA-1 molecule. After primary MLR stimulation, all patients studied (two severely and four moderately LFA-1 deficient) showed diminished allospecific cytolysis and NK activity as compared with their kindred and normal controls. MLR and mitogen-induced proliferative responses were consistently depressed. In addition, LFA-1-deficient EBV lines were poor stimulators of T cell responses. These results with genetically deficient cells confirm the importance of the LFA-1 cell surface molecule in lymphocyte function.

The patient cells studied here are deficient in LFA-1, as well as the Mac-1 and p150,95 molecules, which share a common β subunit. The primary defect appears to be in the β subunit; LFA-1 α subunit precursor is made in normal amounts in all patients tested (patients 1, 2, and

4), but is not expressed on the cell surface in the absence of the β subunit (16). Lymphocytes express LFA-1 but not Mac-1 or p150,95. All three molecules are expressed on monocytes, but Mac-1 does not appear to contribute to antigen-presenting function (unpublished observations), and there is no evidence for a contribution by p150,95. No deficiencies in over 20 other surface markers have been found on patient cells. The functions studied in this paper thus appear to be related to the deficiency in LFA-1 only.

Cytolytic activity and proliferative responses of LFA-1-deficient cells improved after repeated restimulation. There was no concurrent increase in LFA-1 expression. It appeared that CTL were selected that had compensated for the LFA-1 deficiency. The mechanism of this compensation is unknown, but it could involve changes in the number or affinity of antigen-specific receptors, or of other accessory molecules. Anti-LFA-1 MAb had previously been shown to block primary killing more potently than secondary CTL killing, and to block lectin-dependent killing more potently at low lectin concentrations than high. It was therefore proposed that LFA-1 makes a more important contribution to low avidity than to high avidity cell interactions (1). The involvement of other T cell accessory molecules in antigen responsiveness has been shown to be inversely related to the affinity of the antigen receptor (28). The existence of such compensatory mechanisms suggests that LFA-1 may not be absolutely required for all lymphoid cell interactions.

One of the most interesting findings to emerge from these studies is that LFA-1 on the target cell can contribute to the CTL-target cell interaction. Previous studies with normal CTL in both mouse and human had demonstrated the functional importance of LFA-1 on the CTL. Mouse anti-rat CTL were inhibited 90% by rat anti-mouse LFA-1 MAb, which bound only to the mouse CTL effector (29). Killing by human CTL was inhibited when effector cells, but not when target cells, were pretreated with anti-LFA-1 MAb (3). In contrast, killing by severely deficient patient 1 CTL was inhibited by anti-LFA-1 pretreatment of target cells but not effectors. Killing by CTL of moderately deficient patients 4, 6, and 8 was inhibited by pretreatment of either effectors or targets. These differences may be a reflection of the relative density of LFA-1 on the target cell and CTL. Normal CTL have more LFA-1 than the B lymphoblastoid target cell line JY. The density of LFA-1 on moderately deficient CTL, however, is less than that on the JY target cell. LFA-1 on severely deficient CTL is undetectable. Consistent with the lower amount of LFA-1 present on patient CTL or involved in patient CTL-target cell interactions, killing was inhibited by 10- to 30-fold lower concentrations of LFA-1 MAb than required for comparable inhibition of control CTL-mediated killing. It is proposed that LFA-1 on both the effector and the target contributes to the interaction between these cells in proportion to its cell surface concentration. Thus, differences in the site of inhibition may be a result of the relative concentration of LFA-1 on the target and CTL. These findings suggest that LFA-1 can function bidirectionally. In agreement with this, JY cells stimulated with phorbol esters can aggregate with themselves, and this homotypic adhesion reaction is inhibited by anti-LFA-1 MAb (30). Furthermore, EBV lines established from normal, but not LFA-1-deficient, lymphocytes undergo self-aggregation (Rothlein and Springer, manuscript submitted).

Quantitative measurement of cell surface expression and immunoprecipitation studies have allowed the definition of the severe and moderate phenotypes of Mac-1, LFA-1 deficiency (25). Mac-1, LFA-1, and p150,95 are expressed in 5% of normal amounts, and α-β complexes are detectable by immunoprecipitation in moderate deficiency and in <0.2% of normal amounts and undetectable by immunoprecipitation in severe deficiency. These differences are related to the severity of clinical expression and abnormalities in adhesion-dependent granulocyte defects.

Similar heterogeneity was apparent among our patients in the severity of T lymphocyte functional defects. Severely deficient patient CTL responses were consistently more depressed than those of moderately deficient patients. Differences were also apparent in long-term patient CTL lines. Killing by CTL lines from all moderately deficient patients was inhibitable at both the target and the effector cell levels. The moderately deficient patients 4, 6, 7, and 8 are all related and are likely to have the same mutation. Killing by severely deficient CTL was either inhibited by MAb at the target cell level only (patient 1) or only slightly inhibitable (patient 2). Findings were similar with patient 1 CTL lines that recognized different HLA disparities; analysis of additional patient 2 CTL lines has been prevented by her death at the age of 18 mo. Whether the differences between patients 1 and 2 relate to the HLA disparities or to differences in the genetic lesion are unknown. Patients 1 and 2 are unrelated. Patients from different kindreds may have different genetic lesions in or affecting the LFA-1 β subunit gene. Further definition of this lesion at the DNA level may result in recognition of subtypes within the moderate and severe deficiency groups.

Recent studies by other investigators on individual Mac-1-, LFA-1-deficient patients (19, 20, 31, 32) obtained quite varied results when lymphoid function was measured. CTL-mediated and natural killing ranged from normal and inhibitable by anti-LFA-1 MAb (19, 31) to profoundly deficient (32). In none of these studies were both primary and long-term CTL-mediated killing responses observed. The contribution to functional responses by target cell LFA-1 was not measured. Furthermore, the patients were not classified as to moderate or severe deficiency phenotype. Our findings have emphasized the importance of considering all of these factors in functional assessments.

The clinical findings of recurrent, life-threatening bacterial infections and severe periodontal disease in Mac-1-, LFA-1-deficient patients appear to reflect a dysfunction in granulocyte and monocyte adherence and migration into inflammatory sites. Predominantly, serum immunoglobulin levels are normal or elevated, delayed hypersensitivity reactions are within the normal range, and viral infections resolve normally (25). Granulocyte function may be more profoundly affected than lymphocyte function becase 1) three as opposed to one cell surface molecules are affected, 2) lymphocytes but not granulocytes can migrate into sites of infection (25), and 3) other accessory molecules on lymphocytes or selection of cells with high-affinity antigen receptors may help compensate for LFA-1 deficiency. It should be pointed out, how-

ever, that some clinical findings have been reported that suggest lymphocyte dysfunction. This is in a minority of cases, all apparently of the severe phenotype. A patient in France described by Fischer et al. (32) developed cutaneous candidiasis, and failed to produce antibody to polio virus, diptheria, and tetanus toxoids after vaccination. An Iranian patient, patient 3 of the Texas series described by Anderson et al. (25), died of an overwhelming pico RNA virus infection involving the oro-hypopharynx, glottis, trachea, and lung. It thus appears possible that lymphocyte defects contribute to the clinical findings in severely deficient patients. Further clinical observations will be required to determine the significance in vivo of the clearly documented lymphocyte dysfunction in vitro in this disorder.

Acknowledgments. We thank J. Casaubon for expert manuscript preparation, and David Harrington, Division of Biostatistics, Dana-Farber Cancer Institute, for statistical consultation.

REFERENCES

1. **Springer, T. A., D. Davignon, M. K. Ho, K. Kürzinger, E. Martz, and F. Sanchez-Madrid.** 1982. LFA-1 and Lyt-2,3, molecules associated with T lymphocyte-mediated killing; and Mac-1, an LFA-1 homologue associated with complement receptor function. *Immunol. Rev. 68:111.*
2. **Sanchez-Madrid, F., A. M. Krensky, C. F. Ware, E. Robbins, J. L. Strominger, S. J. Burakoff, and T. A. Springer.** 1982. Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. *Proc. Natl. Acad. Sci. USA 79:7489.*
3. **Krensky, A. M., F. Sanchez-Madrid, E. Robbins, J. Nagy, T. A. Springer, and S. J. Burakoff.** 1983. The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. *J. Immunol.* 131:611.
4. **Hildreth, J. E. K., F. M. Gotch, P. D. K. Hildreth, and A. J. McMichael.** 1983. A human lymphocyte-associated antigen involved in cell-mediated lympholysis. *Eur. J. Immunol. 13:202.*
5. **Evans, R. L., D. W. Wall, C. D. Platsoucas, F. P. Siegal, S. M. Fikrig, C. M. Testa, and R. A. Good.** 1981. Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to TH2 antigen. *Proc. Natl. Acad. Sci. USA 78:544.*
6. **Reinherz, E. L., R. E. Hussey, K. Fitzgerald, P. Snow, C. Terhorst, and S. F. Schlossman.** 1981. Antibody directed at a surface structure inhibits cytolytic but not suppressor function of human T lymphocytes. *Nature 294:168.*
7. **Krensky, A. M., C. S. Reiss, J. W. Mier, J. L. Strominger, and S. J. Burakoff.** 1982. Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4+. *Proc. Natl. Acad. Sci. USA 79:2365.*
8. **Meuer, S. C., S. F. Schlossman, and E. L. Reinherz.** 1982. Clonal analysis of human cytotoxic T lymphocytes: T4+ and T8+ effector T cells recognize products of different major histocompatibility complex regions. *Proc. Natl. Acad. Sci. USA 79:4395.*
9. **Flomenberg, N., E. Duffy, K. Naito, and B. Dupont.** 1983. Two distinct phenotypes of HLA-DR-specific cytotoxic T-cell lines. *Immunogenetics 17:317.*
10. **Palacios, R., and O. Martinez-Maza.** 1982. Is the E receptor on human T lymphocytes a "negative signal receptor"? *J. Immunol. 129:2479.*
11. **Martin, P. J., G. Longton, J. A. Ledbetter, W. Newman, M. P. Braun, P. G. Beatty, and J. A. Hansen.** 1983. Identification and functional characterization of two distinct epitopes on the human T cell surface protein Tp50. *J. Immunol. 131:180.*
12. **Meuer, S. C., R. E. Hussey, M. Fabbi, D. Fox, O. Acuto, K. A. Fitzgerald, J. C. Hodgdon, J. P. Protentis, S. F. Schlossman, and E. L. Reinherz.** 1984. An alternative pathway of T-cell activation: a functional role for the 50 kd T11 sheep erythrocyte receptor protein. *Cell 36:897.*
13. **Reinherz, E. L., R. E Hussey, and S. F. Schlossman.** 1980. A monoclonal antibody blocking human T cell function. *Eur. J. Immunol. 10:758.*
14. **Chang, T. W., P. C. Kung, S. P. Gingras, and G. Goldstein.** 1981. Does OKT3 monoclonal antibody react with an antigen-recognition structure on human T cells? *Proc. Natl. Acad. Sci. USA 78:1805.*
15. **Möller, G. ed.** 1984. T cell receptors and genes. *Immunol. Rev. 81.*
16. **Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalstieg, and D. C. Anderson.** 1984. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J. Exp. Med. 160:1901.*
17. **Anderson, D. C., F. C. Schmalstieg, M. A. Arnaout, S. Kohl, M. F. Tosi, N. Dana, G. J. Buffone, B. J. Hughes, B. R. Brinkley, W. D. Dickey, J. S. Abramson, T. Springer, L. A. Boxer, J. M. Hollers, and C. W. Smith.** 1984. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of high molecular weight surface glycoproteins (GP138): common relationship to diminished cell adherence. *J. Clin. Invest. 74:536.*
18. **Dana, N., R. F. Todd, J. Pitt, T. A. Springer, and M. A. Arnaout.** 1984. Deficiency of a surface membrane glycoprotein (Mo1) in man. *J. Clin. Invest. 73:153.*
19. **Arnaout, M. A., H. Spits, C. Terhorst, J. Pitt, and R. F. I. Todd.** 1984. Deficiency of a leukocyte surface glycoprotein (LFA-1) in two patients with Mo1 deficiency. *J. Clin. Invest. 74:1291.*
20. **Beatty, P. G., J. M. Harlan, H. Rosen, J. A. Hansen, H. D. Ochs, T. D. Price, R. F. Taylor, and S. J. Klebanoff.** 1984. Absence of monoclonal-antibody-defined protein complex in boy with abnormal leucocyte function. *Lancet 1:535.*
21. **Beatty, P. G., J. A. Ledbetter, P. J. Martin, T. H. Price, and J. A. Hansen.** 1983. Definition of a common leukocyte cell-surface antigen (Lp95-150) associated with diverse cell-mediated immune functions. *J. Immunol. 131:2913.*
22. **Kohl, S., T. A. Springer, F. C. Schmalstieg, L. S. Loo, and D. C. Anderson.** 1984. Defective natural killer cytotoxicity and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity in patients with LFA-1/OKM-1 deficiency. *J. Immunol. 133:2972.*
23. **Miedema, F., P. A. T. Tetteroo, W. G. Hesselink, G. Werner, H. Spits, and C. J. M. Melief.** 1984. Both Fc receptors and LFA-1 on human T gamma lymphocytes are required for antibody-dependent cellular cytotoxicity (K-cell activity). *Eur. J. Immunol. 14:518.*
24. **Krensky, A. M., E. Robbins, T. A. Springer, and S. J. Burakoff.** 1984. LFA-1, LFA-2 and LFA-3 antigens are involved in CTL-target conjugation. *J. Immunol. 132:2180.*
25. **Anderson, D. C., F. C. Schmalstieg, M. J. Finegold, B. J. Hughes, R. Rothlein, L. J. Miller, S. Kohl, M. F. Tosi, R. L. Jacobs, A. Goldman, W. T. Shearer, and T. A. Springer.** 1985. The severe and moderate phenotypes of heritable Mac-1, LFA-1 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features. *J. Inf. Dis. In press.*
26. **Krensky, A. M., S. J. Mentzer, J. L. Greenstein, M. Crimmins, C. Clayberger, T. A. Springer, and S. J. Burakoff.** 1985. Human cytolytic T lymphocyte clones and their function-associated cell surface molecules. In *Hybridoma Technology in the Biosciences and Medicine.* Edited by T. A. Springer. Plenum Press, New York. Pp. 559–574.
27. **Ware, C. F., F. Sanchez-Madrid, A. M. Krensky, S. J. Burakoff, J. L. Strominger, and T. A. Springer.** 1983. Human lymphocyte function associated antigen-1 (LFA-1): identification of multiple antigenic epitopes and their relationship to CTL-mediated cytotoxicity. *J. Immunol. 131:1182.*
28. **Marrack, P., R. Endres, R. Shimonkevitz, A. Zlotnik, D. Dialynas, F. Fitch, and J. Kappler.** 1983. The major histocompatibility complex-restricted antigen receptor on T cells. II. Role of the L3T4 product. *J. Exp. Med. 158:1077.*
29. **Davignon, D., E. Martz, T. Reynolds, K. Kürzinger, and T. A. Springer.** 1981. Lymphocyte function-associated antigen 1 (LFA-1): a surface antigen distinct from Lyt-2,3 that participates in T lymphocyte-mediated killing. *Proc. Natl. Acad. Sci. USA 78:4535.*
30. **Springer, T. A., R. Rothlein, D. C. Anderson, S. J. Burakoff, and A. M. Krensky.** 1985. The function of LFA-1 in cell-mediated killing and adhesion: studies on heritable LFA-1, Mac-1 deficiency and on lymphoid cell self-aggregation. In *Mechanisms of Cell-Mediated Cytotoxicity II.* Plenum Press, New York. Pp. 311–320.
31. **Miedema, F., P. A. T. Tetteroo, F. G. Terpstra, G. Keizer, M. Roos, R. S. Weening, C. M. R. Weemaes, D. Roos, and C. J. M. Melief.** 1985. Immunologic studies with LFA-1- and Mo1-deficient lymphocytes from a patient with recurrent bacterial infections. *J. Immunol. 134:3075.*
32. **Fischer, A., R. Seger, A. Durandy, B. Grospierre, J. L. Virelizier, C. Griscelli, E. Fischer, M. Kazatchkine, M. C. Bohler, B. Descamps-Latscha, P. H. Trung, T. A. Springer, D. Olive, and C. Mawas.** 1985. Deficiency of the adhesive protein complex LFA-1, C3bi complement receptor, p150,95 in a girl with recurrent bacterial infections. Blood. *In press.*