0022-1767/87/13810-3130$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 138, 3130–3136, No. 10, May 15, 1987
Printed in U.S.A.

MEMBRANE GLYCOPROTEIN p150,95 OF HUMAN CYTOTOXIC T CELL CLONES IS INVOLVED IN CONJUGATE FORMATION WITH TARGET CELLS[1]

GERRIT D. KEIZER,[2]* JANNIE BORST,* WIETZE VISSER,* ROLAND SCHWARTING,†
JAN E. DE VRIES,† AND CARL G. FIGDOR*

*From the *Division of Immunology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Huis, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands; †Steglitz Klinikum, Free University of Berlin, W. Berlin, Federal Republic of Germany; and ‡Immunology Laboratories, Unicet, Dardilly, France*

The p150,95 heterodimer, one of three members of the leukocyte function associated antigen (LFA) family, is expressed by monocytes, granulocytes, NK cells, and a small percentage of lymphocytes. We now report that the p150,95 glycoprotein is expressed by some cytotoxic T cell clones and that it is involved in cell-mediated cytolysis by these clones. Two CTL clones, clone JS-93 ($CD3^+$ $CD4^+$ $CD8^-$) and clone JS-102 ($CD3^+$ $CD4^-$ $CD8^+$) expressed high levels of p150,95 and were shown to be specifically directed against HLA-DR and HLA-A2, respectively. Immunoprecipitations followed by two-dimensional gel electrophoresis demonstrated no heterogeneity in the p150,95 molecule isolated from both clones. Furthermore, we demonstrated that monoclonal antibodies (moab) directed against p150,95 could inhibit the cytotoxic activity of both clone JS-93 and clone JS-102 (50% and 47%, respectively). Single cell assays revealed the inhibition to occur at the level of conjugate formation rather than at the level of the lethal hit.

Similar results were obtained with moab directed against LFA-1 (p170,95). The capacity of the moab directed against LFA-1 and p150,95 to inhibit CTL activity and conjugate formation were additive, resulting in a similar percentage of inhibition as found with moab directed against the common β-chain of these molecules. It is concluded that at least some CTL clones express the p150,95 antigen at their cell surface, and that this molecule, like LFA-1, acts at the level of conjugate formation between effector and target cells.

Cytolytic T lymphocytes (CTL) are crucial in the defense against intracellular parasites, viruses, and immunogenic neoplasms (1–3). CTL-mediated killing is a multistep process that takes place within 10 to 15 min at 37°C (4). The initial step is conjugate formation followed by the strengthening of the CTL-target cell interaction (4–7). The second step involves the delivery of the lethal hit, followed by lysis of the target cell independently of the presence of the CTL (4).

Two main phenotypes of CTL clones have been described, namely $CD3^+$ $CD4^+$ $CD8^-$ and $CD3^+$ $CD4^-$ $CD8^+$ subsets (8–10). $CD3^+$ $CD4^+$ $CD8^-$ CTL clones were primarily found to be directed against class II major histocompatibility complex (MHC) antigens, whereas $CD3^+$ $CD4^-$ $CD8^+$ CTL clones are mainly reactive with class I MHC antigens (9–12). Monoclonal antibodies (moab)[3] directed against CD4 and CD8 are able to prevent conjugate formation (13–15). However, there is recent evidence that the CD8 antigen may be involved in the triggering of the lethal hit and has a regulatory role (16, 17). Furthermore, several investigators noted that moab directed against the CD3-T cell receptor complex (18) inhibited CTL-mediated lysis (13, 19–21), probably by preventing triggering of the lethal hit (13–15).

Another molecule involved in CTL-mediated lysis is the leukocyte function associated antigen-1 (LFA-1) (22–24). Monoclonal antibodies directed against this antigen have been shown to prevent conjugate formation (13, 25, 26), but are probably not involved in the delivery of the lethal hit (13). The role of LFA-1 is not restricted to CTL-target cell binding, since moab directed against this structure inhibit also various other cell-cell interactions, for instance B-B (27, 28), B-T (29–31), T endothelial cell (32), and T cell-macrophage interactions (29–31).

The LFA-1 antigen consists of an α subunit of 170 kilodaltons (kD) and a β subunit of 95 kD and is related to complement receptor 3 (CR3) (Mo1, Mac-1; 165, 95 kD) and p150,95 (150, 95 kD) antigens because these molecules share the β subunit (30, 33, 34). LFA-1 is expressed by all leukocytes in contrast to CR3 (35) and p150,95 (36–38), which have been shown to be expressed by monocytes, granulocytes, and NK cells (36). Monoclonal antibodies directed against CR3 and p150,95 were unable to inhibit the mixed lymphocyte reaction and NK cell-mediated killing (36).

We now report that the p150,95 antigen can be expressed on both $CD3^+$ $CD4^+$ $CD8^-$ and $CD3^+$ $CD4^-$ $CD8^+$ CTL clones. Furthermore, we provide evidence that moab reactive with this antigen can inhibit CTL-mediated killing at the level of conjugate formation.

MATERIALS AND METHODS

Monoclonal antibodies. The moab SPV-L7 (IgG1), S-HC1 3 (IgG2b), and Bear-1 (IgG1) are directed against the α-chains of human

Received for publication October 3, 1986.
Accepted for publication January 27, 1987.
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 Grant NKI 83-19 from the Koningin Wilhelmina Fonds (The Netherlands Cancer Foundation).
[2] Correspondence should be sent to: Dr. G. D. Keizer, Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

[3] Abbreviations used in this paper: LFA-1, leukocyte function associated antigen 1; moab, monoclonal antibody; TEA, triethanolamine; kD, kilodalton; E:T, effector to target cell.

LFA-1, p150,95, and CR3 (Mol, Mac-1), respectively (30, 36). The moab CLB-54 (IgG1) reacts with the common β-chain of this antigen family and was kindly provided by Dr. F. Miedema (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) (30, 39). SPV-L7 and CLB-54 were obtained after fusion of SP2/0 myeloma cells with spleen cells of mice, immunized with T lymphocytes. The moab were selected for their capacity to inhibit CTL activity. The moab Bear-1 and S-HC1 3 were obtained after fusion of SP2/0 myeloma cells with spleen cells of mice immunized with monocytes and hairy cell leukemia cells, respectively. The following moab were used as controls: SPV-T3A (anti-CD3, IgG2b), CEM (anti-KLH, IgG1), anti-Leu-14 (B cell specific, IgG2b).

Cell lines and T cell clones. Two different human cytotoxic T cell clones were mainly used in this study, a $CD3^+$ $CD4^+$ $CD8^-$ clone (JS-93) and a $CD3^+$ $CD4^-$ $CD8^+$ clone (JS-102). These clones were directed against HLA-DR and HLA-A2, respectively (40). The specificities of the CTL clones JS-104, -107, -122, -132, and -141, all directed against JY, have been described (40). The T cell clones were cultured in serum-free medium as described (41). The human Epstein-Barr virus (EBV) transformed B cell line JY and the B cell line Daudi were cultured in RPMI 1640 supplemented with 5% fetal calf serum (FCS).

Immunofluorescence. Cells were incubated (30 min, 0°C) in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) and 0.01% sodium azide with appropriate dilutions of the different moab followed by incubation with fluorescein isothiocyanate (FITC)-labeled goat $F(ab')_2$ anti-mouse IgG antibody (Nordic, Tilburg, The Netherlands) for 30 min at 0°C. The relative fluorescence intensity was measured by FACS IV analysis, which was calculated as

$$\frac{\text{fluorescence intensity of a moab reactive with the cells}}{\text{fluorescence intensity of a moab nonreactive with the cells}}$$

^{51}CR-release assay. Cytotoxicity assays were carried out in microtiter plates with U-shaped wells as described (8). The capacity of moab to block the cytotoxic activity of CTL clones was determined by incubating 1 or 5×10^3 effector cells with or without moab for 15 min at room temperature. After this incubation period, 1×10^3 ^{51}Cr-labeled JY cells were added. Subsequently, the microtiter plates were centrifuged at 50 × G and were incubated for 4 hr at 37°C. The percent specific ^{51}Cr release was calculated as described (10, 42).

Single cell assay. The single cell assay, which was used to determine the capacity of various moab to inhibit binding of effector cells to target cells was performed according to a method developed by Grimm, Bonavida, and Bradley (43, 44). One hundred microliters of carboxy fluorescein-labeled effector cells (2×10^6/ml) in Iscove's modified MEM (Gibco, Glasgow, Scotland), supplemented with 0.25% BSA and 200 μl of target cells (2×10^6/ml), were mixed in 10 x 75-mm glass tubes in the presence or absence of moab and were equilibrated to 30°C in a waterbath for 10 min. After this incubation period the mixture was centrifuged for 5 min at 200 × G, and the supernatant was carefully removed. Subsequently, 50 μl medium were added, followed by 50 μl medium containing 1% agarose, which was kept at 39°C. (The agarose used in this assay was a blend of Sigma type I and VII agarose in a ratio of 1:5, respectively.) After the cell pellet had been resuspended, the agarose-cell suspension was plated on microscopic slides that had been precoated with agarose. After gelation of the agarose, the slides were placed in Iscove's medium with 0.25% BSA and were incubated for 3 hr at 37°C in a humidified 5% CO_2 atmosphere. After this incubation step the cells were stained with 0.4% trypan blue dissolved in PBS for 10 min, followed by fixation with 0.6% formalin. The total number of conjugates was calculated according to the formula

$$\% \text{ Conjugates} = \frac{\text{total number of conjugated effector cells}}{\text{total number of effector cells}} \times 100\%$$

The percentage of kill was enumerated according to the formula

$$\% \text{ Kill} = \frac{\text{dead number of conjugated target cells}}{\text{total number of conjugates}} \times 100\%$$

Radiolabeling and immunoprecipitation. Cell surface radioiodination with Na ^{125}I (Amersham, UK) was catalyzed by 1,3,4,6-tetrachloro-3α,6αdiphenylglycoluril (Iodogen; Pierce Chemical Co., Rockford, IL) (45) as described (46). For immunoprecipitation (46), radiolabeled cells were lysed with 1% Nonidet P-40 in 0.01 M triethanolamine-HCl, pH 7.8, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.02 mg/ml ovomucoid trypsin inhibitor (Sigma; TEA/NaCl buffer). Nuclear debris was removed from the lysates by centrifugation at 13,000 × G for 15 min at 4°C. Material insoluble in the lysis buffer was removed by centrifugation at 100,000 × G for 30 min in an air-driven centrifuge (Beckman Instruments, Palo Alto, CA). Lysates were precleared further by successive incubations with formalin-fixed *Staphylococcus aureus* bacteria (strain Cowan I) and mouse IgG coupled to protein A-Sepharose. Precleared lysates were incubated for 3 to 4 hr with a nonspecific moab coupled to protein A-Sepharose. The immunoprecipitates were removed from the lysates by centrifugation at 13,000 × G. Precipitates were resuspended in 0.2 ml TEA/NaCl buffer with 0.5% sodium deoxycholate and were centrifuged for 15 sec at 13,000 × G. Subsequently immunoprecipitates were washed extensively in 0.01 M TEA/HCl, pH 7.8, 0.2% Nonidet P-40.

Enzyme treatments. Neuraminidase treatment was carried out on immunoprecipitates for which protein A-Sepharose beads were resuspended in 50 μl of 0.05 M sodium acetate, pH 5.5, 0.9% NaCl, 0.1% $CaCl_2$, 1 mM phenyl methyl sulfonyl fluoride (PMSF) and 0.1 U per neuraminidase sample (Sigma N2876), and incubation took place for 3 hr at 37°C while shaking.

Electrophoresis and autoradiography. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on vertical slab gels according to a modification of the procedure of Laemmli (47). Two-dimensional gel electrophoresis was done according to the method of O'Farrell (48). For isoelectric focusing, ampholytes (LKB, Bromma, Sweden) of pI 3.5 to 10, 4 to 6, and 5 to 8 were used in a ratio of 1:1:4 in sample buffer and gel. Samples were dissolved in 8.8 M urea, 0.4% SDS, 5% 2-mercaptoethanol, and 0.2% ampholytes. After 45 min, an equal volume of 8.8 M urea, 2% Nonidet P-40, 5% 2-mercaptoethanol, and 2% ampholytes was added and electrophoresis was performed (16 hr at 300 V followed by 4 hr at 800 V). The second dimension consisted of a 7.5% SDS-polyacrylamide gel. Kodak XAR-5 film was used in combination with intensifier screens (Cronex Lightning Plus; Dupont Chemical Co., Newton, CT) for autoradiography of ^{125}I-labeled materials.

RESULTS

Expression of p150,95 by CTL clones. The p150,95 antigen has been shown to be expressed on only a minor population of peripheral blood lymphocytes (36). Furthermore, it has been reported that immunoprecipitations carried out with antibodies to the common β-chain of the LFA-1 family from CTL clones sometimes show the presence of the p150,95 antigen (33, 49). This prompted us to screen our CTL clones for reactivity with the S-HC1 3 moab (anti-p150,95α). The expression varied widely between the 16 CTL clones that were tested and was also variable in time within the individual clones (results not shown). Two clones were selected for detailed analysis, clone JS-93 ($CD3^+$ $CD4^+$ $CD8^-$) and clone JS-102 ($CD3^+$ $CD4^-$ $CD8^+$), which expressed high levels of the p150,95 antigen (relative fluorescence intensity >10) as shown by immunofluorescence (Fig. 1). Furthermore, these clones were found to be reactive with moab directed against LFA-1, but not with moab directed against CR3, the third member of the LFA-1 family (Fig. 1). Similar results were obtained with the other CTL clones (not shown).

Inhibition of the cytotoxic activity of the CTL clones. It is well established that moab directed against the LFA-1 α- and β-chain are able to inhibit the lytic activity of CTL clones. Because the α-chains of LFA-1 and p150,95 are associated with a common β-chain, and are therefore structurally related, we tested whether antibodies directed against p150,95α could block CTL activity. As depicted in Table 1, moab directed against the α- and β-chains of LFA-1 and p150,95 were able to inhibit the cytotoxicity of both clone JS-93 and clone JS-102. In addition, the moab SPV-T3b (anti-CD3) blocked the cytotoxicity of both clones. In contrast, the moab Bear-1, which reacts with CR3, and the moab CEM (anti-KLH) were ineffective. Similarly, other moab directed against CR3 (OKM-1) were unable to inhibit cytotoxicity. At low effector to target cell (E:T) ratios, the inhibition was more

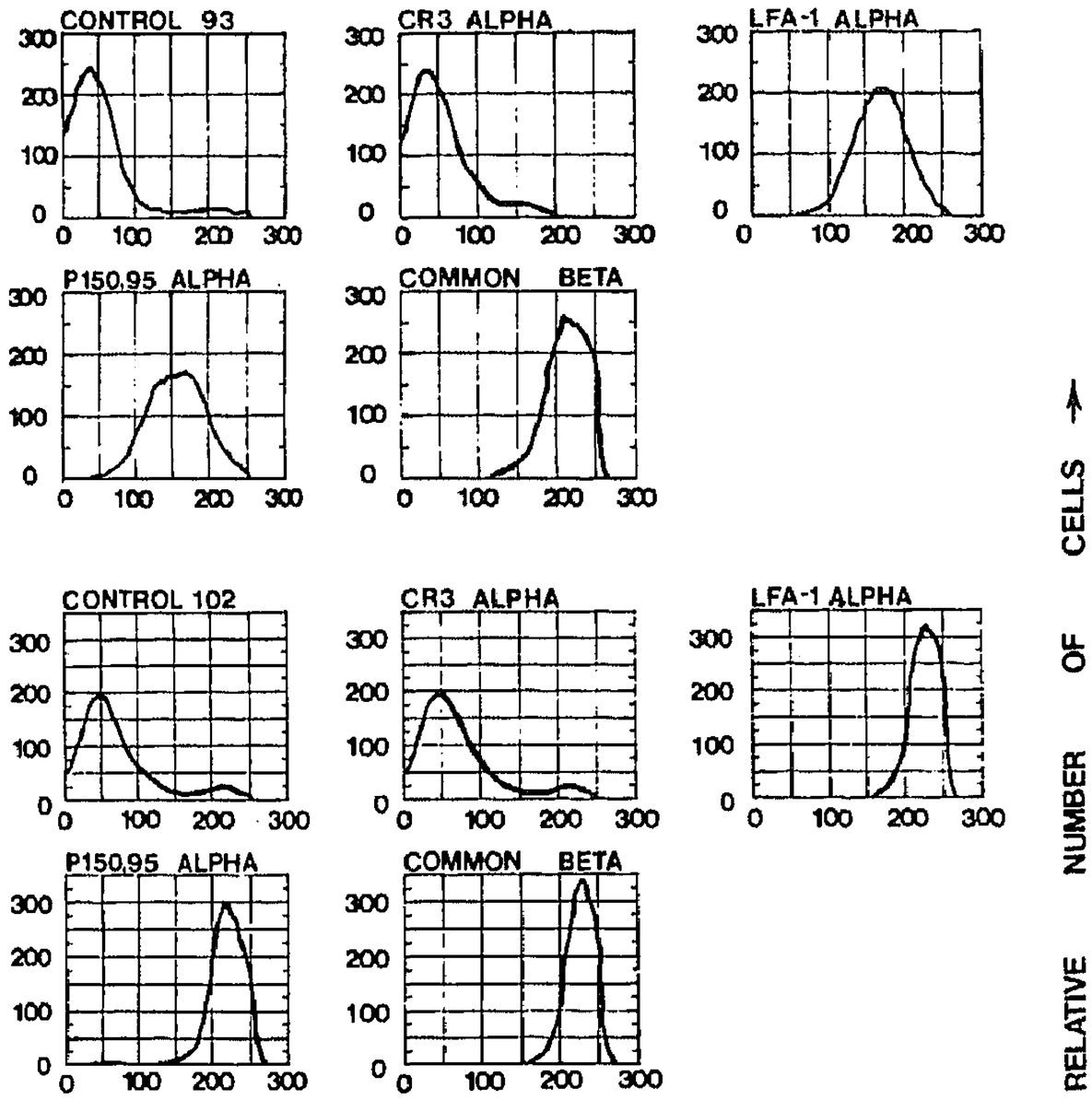

Figure 1. The expression of the LFA-1 family antigens on the CTL clones JS-93 and JS-102 as measured by immunofluorescence. The moab Bear-1 (CR3 α), SPV-L7 (LFA-1α), S-HC1 3 (p150,95α), and CLB-54 (common β) were used ina 1/500 dilution of ascites. The moab CEM (anti-keyhole limpet hemocyanin) and anti-Leu-14 (B cell specific) served as negative controls for the IgG1 and IgG2b moab, respectively. Because no differences were observed between reaction patterns of CEM or anti-Leu-14, only one control is shown.

pronounced than at high E:T ratios.

Combinations of the antibodies directed against the p150,95 and LFA-1 α-chains resulted in additive blocking effects on the cytolytic action of both clones (Table 1). In addition, it is observed that the CTL activity of several other clones with a low expression of p150,95 could not be inhibited significantly by moab directed against p150,95 alone (Table II). Although anti-LFA-1α moab could inhibit CTL activity, a much higher inhibition was observed if a combination of anti-LFA-1α and anti-p150,95α moab were used, indicating that anti-p150,95 under these conditions can synergize with anti-LFA-1. Furthermore, we noted that inhibition of CTL activity by anti-β-chain moab was comparable to the inhibiton found with mixtures of anti-LFA-1α or anti-p150,95α moab (Table II). These results indicate that both LFA-1 and p150,95 are involved in the process of cell-mediated cytolysis.

Inhibition of conjugate formation by moab. Several investigators have shown that moab directed against LFA-1 block cell mediated cytolysis at the level of conjugate formation (13, 25, 26). To determine whether the inhibiton of CTL activity by moab directed against p150,95 was also caused by the inhibiton of conjugate formation, single cell assays were carried out. As shown in Table III, both moab directed against p150,95α and the LFA-1α were able to inhibit conjugate formation of clone JS-93 and clone JS-102 with JY cells. Mixtures of these moab had additive blocking effects and were as effective in inhibiting conjugate formation as moab directed against the common β-chain. In addition, it is shown that SPV-T3b (anti-CD3) did not prevent conjugate formation but was able to inhibit CTL-mediated killing (Table III). Conjugates formed in the presence of moab directed against the LFA-1 family antigens showed the same ratio viable/nonviable target cells as control samples. These results indicate that the LFA-1 family antigens do not play a significant role in the delivery of the lethal hit.

Recently it has been described that CTL clones can form nonspecific conjugates with target cells that do not express the specific antigen to which the CTL is directed (13, 26). Although conjugates are formed, lysis of these target cells does not occur (13). This process was shown to be LFA-1 dependent. To investigate whether p150,95 is also involved in this "antigen nonspecific" conjugate formation, clone JS-93 and clone JS-102 were conjugated to Daudi cells, which cannot be lysed by these clones, since they do not express class I or proper class II molecules (40). As shown in Table IV, formation of these nonspecific conjugates could be inhibited both by moab directed against p150,95 and against LFA-1. From these data it is concluded that the inhibition of cell-mediated cytolysis by moab directed against p150,95 and LFA-1 is primarily caused by inhibition of conjugate formation and not by blocking of the delivery of the lethal hit.

Biochemical analysis of p150,95 on CTL clones. As mentioned above, clone JS-93 has the $CD3^+$ $CD4^+$ $CD8^-$ phenotype, whereas clone JS-102 has the $CD3^+$ $CD4^-$ $CD8^+$ phenotype. To investigate whether the different CTL clones expressed biochemically identical p150,95, two-dimensional gel electrophoresis was performed from iodinated material. The p150,95 α- and β-chains were

TABLE I

Inhibition of CTL activity by moab[a]

moab	Antigen	% Inhibition of CTL Activity			
		Clone JS-93		Clone JS-102	
		E:T ratio		E:T ratio	
		5:1	1:1	5:1	1:1
Bear-1	CR3α	2 ± 3	3 ± 3	3 ± 5	1 ± 3
SPV-L7	LFA-1α	34 ± 5	55 ± 10	30 ± 8	49 ± 8
S-HCl 3	p150,95α	26 ± 6	50 ± 9	34 ± 6	47 ± 7
CLB-54	Common β	38 ± 8	65 ± 4	49 ± 7	60 ± 10
S-HCl 3 + SPV-L7		40 ± 6	72 ± 8	54 ± 11	80 ± 11
SPV-T3b	CD3	78 ± 12	98 ± 4	65 ± 8	85 ± 7
CEM	KLH	1 ± 1	0 ± 2	1 ± 2	0 ± 4

[a] CTL clones were mixed with ^{51}Cr-labeled JY cells and incubated for 4 hr in the presence of moab (10 μg/ml) as indicated. In the absence of moab the cytotoxicity always exceeded 30%. Mean percentages inhibition ± SD of four experiments are shown. The phenotypes of clones 93 and 102 are $CD3^+$ $CD4^+$ $CD8^-$ and $CD3^+$ $C4^-$ $CD8^+$, respectively.

TABLE II

Synergistic effects of moab on the cytotoxic activity of weakly p150,95-positive CTL clones[a]

moab	Antigen	% Inhibition of Cytotoxicity				
		CTL clone				
		JS-104	JS-107	JS-122	JS-132	JS-141
Bear-1	CR3α	4	0	2	3	0
SPV-L7	LFA-1α	25	25	48	27	30
S-HCl 3	p150,95α	14	10	3	2	0
CLB-54	Common β	52	41	62	54	38
S-HCl 3 + SPV-L7		48	37	64	44	42
SPV-T3b	CD3	72	66	83	68	92
CEM	KLH	0	3	1	4	2
% p150,95-positive cells		55	78	25	33	18

[a] CTL clones were mixed with ^{51}Cr-labeled JY cells (ratio 2:1) and incubated for 4 hr in the presence of moab (10 μg/ml), as indicated. In the absence of moab the cytotoxicity always exceeded 25%. The percentage of inhibition of cytotoxicity of representative experiments of five CTL clones are shown. The clones 104, 107, 122, and 141 expressed CD4 but not CD8, whereas clone 132 was shown to be CD4$^-$ CD8$^+$. The percentage of p150,95-positive cells was determined by FACS IV analysis. The relative fluorescence intensity did not exceed 3 (see *Materials and Methods*).

TABLE III

Inhibition of different stages of the cytotoxic reaction of CTL clones against JY cells by moab[a]

moab	Antigen	% Inhibition			
		Clone JS-93[b]		Clone JS-102[b]	
		Conjugate formation	Lysis	Conjugate formation	Lysis
Bear-1	CR3α	0 ± 4	1 ± 3	0 ± 3	3 ± 2
SPV-L7	LFA-1α	38 ± 8	3 ± 4	42 ± 12	1 ± 3
S-HCl 3	p150,95α	27 ± 3	4 ± 4	24 ± 6	0 ± 4
CLB-54	Common β	65 ± 12	3 ± 3	64 ± 5	4 ± 3
S-HCl 3 + SPV-L7		55 ± 10	2 ± 4	68 ± 7	4 ± 4
SPV-T3b	CD3	2 ± 4	46 ± 10	0 ± 2	56 ± 11
CEM	KLH	2 ± 4	0 ± 2	1 ± 3	2 ± 1

[a] Single cell assays were carried out as described in *Materials and Methods*. In the absence of moab the percentage of conjugate formation and target cell lysis was 50 ± 10% and 48 ± 15%, respectively, for both clones. Mean percentages of inhibition ± SD of four experiments are shown. The moab were added at a concentration of 10 μg/ml. The phenotypes of clones 93 and 102 are CD3$^+$ CD4$^+$ CD8$^-$ and CD3$^+$ CD4$^-$ CD8$^+$, respectively.

[b] E:T ratio 1:1.

TABLE IV

Inhibition of non specific conjugate formation between CTL and Daudi cells by moab[a]

moab	Antigen	% Inhibition of Conjugate Formation	
		Clone JS-93[b]	Clone JS-102[b]
Bear-1	CR3α	2 ± 4	1 ± 1
SPV-L7	LFA-1α	63 ± 7	65 ± 4
S-HCl 3	p150,95α	30 ± 8	35 ± 7
CLB-54	Common β	85 ± 5	75 ± 11
S-HCl 3 + SPV-L7		85 ± 9	86 ± 8
SPV-T3b	CD3	3 ± 3	1 ± 1
CEM	KLH	0 ± 2	1 ± 2

[a] Single cell assays were carried out as described in *Materials and Methods*. Both clone 93 and clone 102 were not able to lyse Daudi cells as measured by the chromium-release assay (40). In the absence of moab the percentage of conjugate formation was 65% ± 15 for both clones. Values represent mean ± SD of four experiments. The moab were added at a concentration of 10 μg/ml. The phenotypes of clone 93 and 102 are CD3$^+$ CD4$^+$ CD8$^-$ and CD3$^+$ CD4$^-$ CD8$^+$, respectively.

[b] E:T ratio 1:1.

found to be slightly heterogeneous in charge, but no significant differences were observed between the clones (Fig. 2*A*). To reduce the charge heterogeneity, sialic acids were removed by neuraminidase treatment. The PI of the p150,95 α-chain shifted for both clones from 5.7–6.1 to a PI of 6.0–6.2 (Fig. 2*B*). Interestingly, the β-chain of p150,95 separated into four distinct spots, probably due to incomplete digestion or other posttranslational modifications. No significant differences were observed between the clones. Taken together, these results indicate that the p150,95 antigen is nonpolymorphic expressed by CTL clones with different phenotypes.

DISCUSSION

Evidence is provided that the p150,95 antigen can be expressed by both class I and class II HLA-specific CTL clones. Monoclonal antibodies directed against p150,95 are demonstrated to inhibit the cytotoxic reaction at the level of conjugate formation. Furthermore, the mode of action of p150,95 is similar to that of LFA-1, and additive inhibitory effects were observed if mixtures of moab directed against both antigens were used. In addition, synergistic effects were observed in those situations where anti-p150,95 moab had no significant blocking effects. Finally, the p150,95 antigen was not found to be heterogeneous, since biochemical analysis of the p150,95 antigen derived from different CTL clones showed identical results.

Sanchez-Madrid et al. (33) described a variable presence of the p150,95 antigen in lysates of CTL clones, since they frequently observed the presence of a 150 kD band in immunoprecipitations from CTL clones with moab directed against the β-chain of the LFA-1 family. These researchers suggested that the variability of expression might be attributed to variations in the presence of feeder cells. Immunofluorescence experiments with moab directed against p150,95 revealed the expression of this antigen on CTL clones (Fig. 1). During the preparation of this paper, similar findings were reported by Miller et al. (50). The possibility that our results are due to the expression of the p150,95 antigen on monocytes present in the feeder cell suspensions, instead of on CTL clones, could be excluded for several reasons.

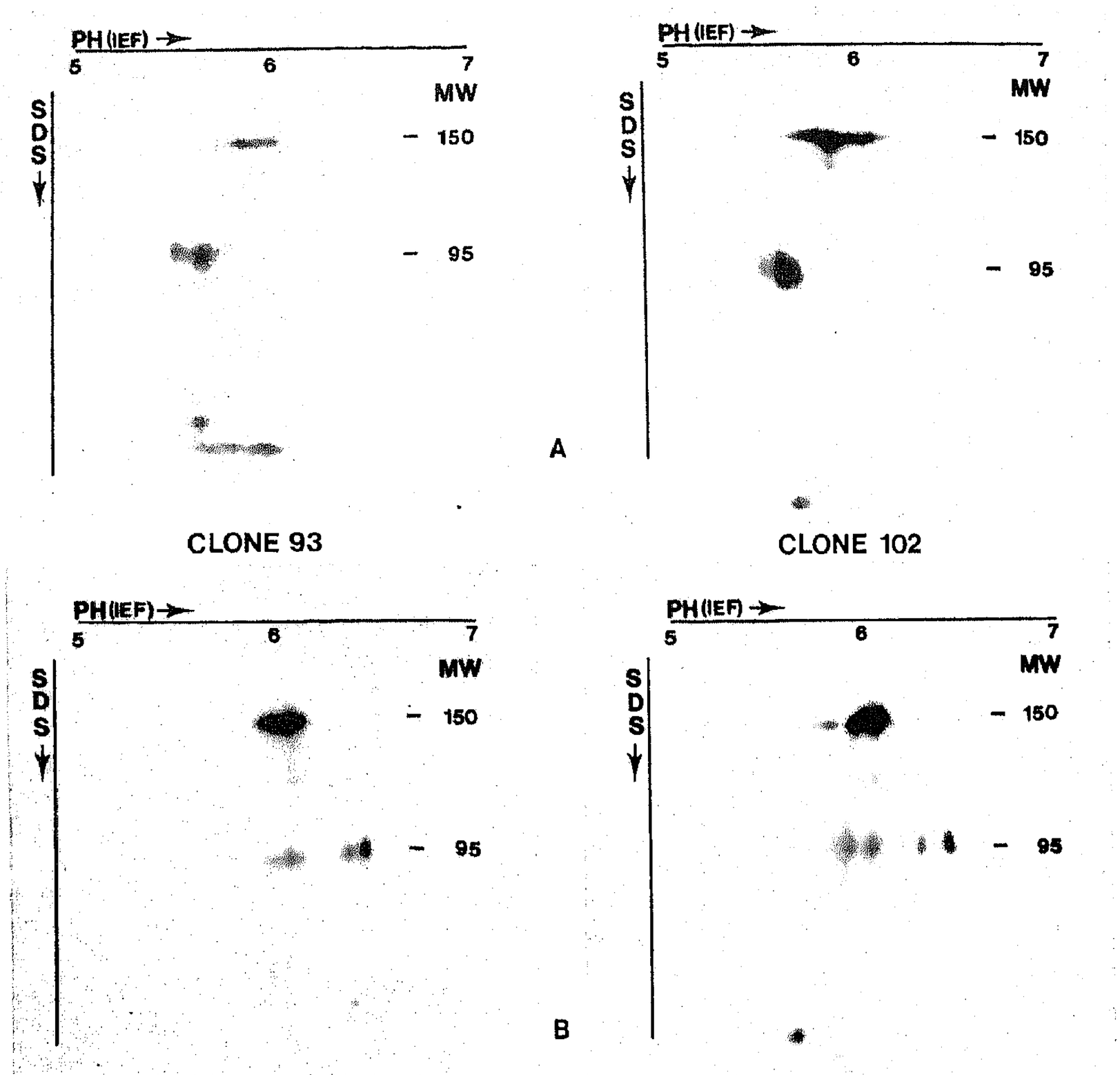

Figure 2. Two-dimensional characterization of the p150,95 antigen precipitated from a $CD4^+$ (clone JS-93) and a $CD8^+$ (clone JS-102) CTL clone. The first dimension involved an isoelectrofocusing (IEF) followed by SDS-PAGE (SDS) in the second dimension. *A*, Untreated immunoprecipitates; *B*, neuraminidase-treated immunoprecipitates.

First, some CTL clones (clone JS-93 and JS-102) expressed the p150,95 molecule, with a high intensity on 100% of the cells (Fig. 1) in the absence of feeder cells. Second, some CTL clones were extensively washed and subsequently cultured for 3 wk or longer in the presence of IL 2 but without the addition of feeder cells and still expressed the p150,95 antigen (not shown). Third, the CTL clones 93 and 102 are shown to have a functionally active p150,95 antigen.

It is well documented that LFA-1, CR3, and p150,95 share a common β subunit (30, 33–38). Monoclonal antibodies directed against the LFA-1 α- or β-chain have been described to inhibit CTL function (13, 22–25, 35). In addition to the structural resemblance between LFA-1 and p150,95, our present data also show functional similarities, since moab directed against p150,95α were also able to inhibit CTL function (Table I). Monoclonal antibodies directed against CR3 were ineffective due to the absence of this antigen on CTL clones.

Furthermore, we (Table I) and other investigators observed that moab directed against the common β-chain of the human LFA-1 family antigens are generally more potent inhibitors of CTL function than moab to LFA-1 alone (34, 49–52). Because the presence of p150,95 on CTL clones has not been described in this respect, these results may be explained by the assumption that the β-chain plays a major role in CTL-target cell interactions (52). On the basis of our results, we do not reject such a possibility, but advocate that the potent inhibitory effects of anti-common β-chain antibodies can be attributed to the blocking of both p150,95 and LFA-1. This notion is supported by the finding that mixtures of moab directed against the α-chains of LFA-1 and p150,95 are at least as effective in inhibiting CTL function as moab directed against the common β-chain (Table I). In addition, results of preliminary experiments indicate that p150,95-negative CTL clones can be equally well blocked by anti-LFA-1α moab as by anti-β-chain moab.

The cytotoxic activity of CTL clones with a low expression of p150,95 could not be inhibited significantly by moab directed against p150,95 alone. These clones were blocked more effectively by anti-common β-chain antibodies than by moab directed against the α-chain of LFA-1. Interestingly, combinations of moab directed to p150,95 and LFA-1 α-chains are as potent in inhibiting the function of these CTL clones as anti-β-chain moab alone (Table II). The experiments suggest that under certain conditions LFA-1α and p150,95 can act synergistically.

Of interest are the findings that conjugate formation between monocytes and target cells can also be inhibited by moab directed against LFA-1 and p150,95 (53). In this combination, target cell lysis was not observed. These results are comparable to our data obtained with Daudi cells that were conjugated to CTL (Table IV) without being killed due to the absence of the antigens needed for specific recognition. Because alloantigen recognition is probably preceded by nonspecific adhesion of cytotoxic T cells and target cells (13, 26), we concluded that both LFA-1 and p150,95 play an important role in the initial

(aspecific) conjugation formation of effector cells and target cells.

Until now, the mechanism that regulates the variable expression of p150,95 on T cells is unknown and requires further study. Preliminary experiments demonstrated that IFN-γ, IL 1, and IL 2 cannot modulate the expression of p150,95. The effects of different feeder mixtures are currently under investigation.

Acknowledgments. We thank Dr. Hergen Spits for his advice and interest in this study, Evert de Vries for technical assistance, and Marie Anne van Halem for her excellent secretarial help.

REFERENCES

1. **Burakoff, S. J., C. S. Reiss, O. F. Finberg, and M. F. Mescher.** 1980. Cell-mediated immunity to viral glycoproteins. *Rev. Infect. Dis. 2:62.*
2. **Cerottini, J.-C., and K. T. Brunner.** 1974. Cell-mediated cytotoxicity, allograft rejection, and immunity. *Adv. Immunol. 18:67.*
3. **Kast, W. M., A. M. Bronkhorst, L. P. de Waal, and C. J. M. Melief.** 1986. Cooperation between cytotoxic and helper T lymphocytes in protection against lethal Sendai virus infection. Protection by T cells is MHC-restricted and MHC-regulated: a model for MHC-disease associations. *J. Exp. Med. 164:723.*
4. **Berke, G.** 1980. Interaction of cytotoxic T lymphocytes and target cells. *Prog. Allergy 27:69.*
5. **Martz, E.** 1977. Mechanism of specific tumor-cell lysis by alloimmune T lymphocytes: resolution and characterization of discrete steps in the cellular interaction. *Contemp. Top. Immunobiol. 7:301.*
6. **Golstein, P.** 1976. Divalent cation requirements as a tool for the study of cell-mediated cytotoxicity systems. *Adv. Exp. Med. Biol. 66:465.*
7. **Henney, C. S.** 1977. T cell-mediated cytolysis: an overview of some current issues. *Comtemp. Top. Immunobiol. 7:245.*
8. **Spits, H., H. Yssel, A. Thompson, and J. E. de Vries.** 1983. Human T4+ and T8+ cytotoxic T lymphocyte clones directed at products of different class II major histocompatibility complex loci. *J. Immunol. 131:678.*
9. **Biddison, W. E., P. E. Rao, M. A. Talle, G. Goldstein, and S. Shaw.** 1982. Possible involvement of the OKT4 molecule in T cell recognition of class II HLA antigens. Evidence from studies of cytotoxic T lymphocytes specific for SB antigens. *J. Exp. Med. 156:1065.*
10. **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.*
11. **Spits, H., H. Yssel, C. Terhorst, and J. E. de Vries.** 1982. Establishment of human T lymphocyte clones highly cytotoxic for an EBV-transformed B cell line in serum-free medium: isolation of clones that differ in phenotype and specificity. *J. Immunol. 128:95.*
12. **Spits, H., J. Borst, M. Giphart, J. Coligan, C. Terhorst, and J. E. de Vries.** 1984. HLA-DC antigens can serve as recognition elements for human cytotoxic T lymphocytes. *Eur. J. Immunol. 14:299.*
13. **Spits, H., W. van Schooten, H. Keizer, G. van Seventer, M. van de Rijn, C. Terhorst, and J. E. de Vries.** 1986. Alloantigen recognition is preceded by nonspecific adhesion of cytotoxic T cells and target cells. *Science 232:403.*
14. **Landegren, U., U. Ramstedt, I. Axberg, M. Ullberg, M. Jondal, and H. Wigzell.** 1982. Selective inhibition of human T cell cytotoxicity at levels of target recognition or initiation of lysis by monoclonal OKT3 and Leu-2a antibodies. *J. Exp. Med. 155:1579.*
15. **Platsoucas, C. D.** 1984. Human T cell antigens involved in cytotoxicity against allogeneic or autologous chemically modified targets: association of the Leu-2a/T8 antigen with effector-target cell binding and of the Leu-4/T3 antigen with triggering. *Eur. J. Immunol. 14:566.*
16. **Fleischer, B., H. Schrezenmeier, and H. Wagner.** 1986. Function of the CD4 and CD8 molecules on human cytotoxic T lymphocytes: regulation of T cell triggering. *J. Immunol. 136:1625.*
17. **Van Seventer, G. A., R. A. W. van Lier, H. Spits, P. Ivanyi, and C. J. M. Melief.** 1986. Evidence for a regulatory role of the T8 (CD8) antigen in antigen-specific and anti-T3-(CD3)-induced lytic activity of allospecific cytotoxic T-lymphocyte clones. *Eur. J. Immunol. 16:1363.*
18. **Borst, J., J. E. Coligan, H. Oettgen, S. Pessano, R. Malin, and C. Terhorst.** 1984. The δ- and ϵ-chains of the human T3/T-cell receptor complex are distinct polypeptides. *Nature 312:455.*
19. **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.*
20. **Platsoucas, C. D., and R. A. Good.** 1981. Inhibition of specific cell-mediated cytotoxicity by monoclonal antibodies to human T cell antigens. *Proc. Natl. Acad. Sci. USA 78:4500.*
21. **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 suppresor function of human T lymphocytes. *Nature 294:168.*
22. **Davignon, D., E. Martz, T. Reynolds, K. Kurzinger, 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.*
23. **Spits, H., G. Keizer, J. Borst, C. Terhorst, A. Hekman, and J. E. de Vries.** 1983. Characterization of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. *Hybridoma 2:423.*
24. **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.*
25. **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.*
26. **Shaw, S., G. E. Ginther Luce, R. Quinones, R. E. Gress, T. A. Springer, and M. E. Sanders.** 1986. Two antigen-independent adhesion pathways used by human cytotoxic T-cell clones. *Nature 323:262.*
27. **Mentzer, S. J., S. H. Gromkowski, A. M. Krensky, S. J. Burakoff, and E. Martz.** 1985. LFA-1 membrane molecule in the regulation of homotypic adhesions of human B lymphocytes. *J. Immunol. 135:9.*
28. **Rothlein, R., and T. A. Springer.** 1986. The requirement for lymphocyte function-associated antigen 1 in homotypic leukocyte adhesion stimulated by phorbol ester. *J. Exp. Med. 163:1132.*
29. **Howard, D. R., A. C. Eaves, and F. Takei.** 1986. Lymphocyte function-associated antigen (LFA-1) is involved in B cell activation. *J. Immunol. 136:4013.*
30. **Keizer, G. D., J. Borst, C. G. Figdor, H. Spits, F. Miedema, C. Terhorst, and J. E. de Vries.** 1985. Biochemical and functional characteristics of the human leukocyte membrane antigen family LFA-1, Mo-1 and p150,95. *Eur. J. Immunol. 15:1142.*
31. **Sanders, V. M., J. M. Snyder, J. W. Uhr, and E. S. Vitetta.** 1986. Characterization of the physical interaction between antigen-specific B and T cells. *J. Immunol. 137:2395.*
32. **Haskard, D., D. Cavender, P. Beatty, T. Springer, and M. Ziff.** 1986. T lymphocyte adhesion to endothelial cells: mechanisms demonstrated by anti-LFA-1 monoclonal antibodies. *J. Immunol. 137:2901.*
33. **Sanchez-Madrid, F., J. A. Nagy, E. Robbins, P. Simon, and T. A. Springer.** 1983. A human leukocyte differentiation antigen family with distinct alpha-subunits and a common beta-subunit: The lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. Exp. Med. 158:1785.*
34. **Hildreth, J. E. K., and J. T. August.** 1985. The human lymphocyte function-associated (HLFA) antigen and a related macrophage differentiation antigen (HMac-1): functional effects of subunit-specific monoclonal antibodies. *J. Immunol. 134:3272.*
35. **Springer, T. A., D. Davignon, M.-K. Ho, K. Kurzinger, 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. 68:171.*
36. **Lanier, L. L., M. A. Arnaout, R. Schwarting, N. L. Warner, and G. D. Ross.** 1985. p150/95, Third member of the LFA-1/CR3 polypeptide family identified by anti-Leu M5 monoclonal antibody. *Eur. J. Immunol. 15:713.*
37. **Hogg, N., L. Takacs, D. G. Palmer, Y. Selvendran, and C. Allen.** 1986. The p150/95 molecule is a marker of human mononuclear phagocytes: comparison with expression of class II molecules. *Eur. J. Immunol. 16:240.*
38. **Springer, T. A., L. J. Miller, and D. C. Anderson.** 1986. p150,95, The third member of the Mac-1, LFA-1 human leukocyte adhesion glycoprotein family. *J. Immunol. 136:240.*
39. **Miedema, F., P. A. T. Tetteroo, W. G. Hesselink, G. Werner, H. Spits, and C. J. M. Melief.** 1984. Both Fc receptors and lymphocyte-function-associated antigen 1 on human T lymphocytes are required for antibody-dependent cellular cytotoxicity (killer cell activity). *Eur. J. Immunol. 14:518.*
40. **Borst, J., A. Voordouw, E. de Vries, A. Boylston, J. E. de Vries, and H. Spits.** 1986. A family of T cell receptor molecules expressed on T cell clones with different specificities for allo-major histocompatibility antigens. *Human Immunol. 17:426.*
41. **Yssel, H., J. E. de Vries, M. Koken, W. van Blitterswijk, and H. Spits.** 1984. Serum-free medium for generation and propagation of functional human cytotoxic and helper T cell clones. *J. Immunol. Methods 72:219.*
42. **Spits, H., M. H. Breuning, P. Ivanyi, C. Russo, and J. E. de Vries.** 1982. In vitro isolated human cytotoxic T-lymphocyte clones detect variations in serologically defined HLA antigens. *Immunogenetics 16:503.*
43. **Grimm, E., and B. Bonavida.** 1979. Mechanism of cell-mediated cytotoxicity at the single cell level. *J. Immunol. 123:2861.*

44. **Bonavida, B., T. P. Bradley, and E. A. Grimm.** 1983. Frequency determination of killer cells by a single-cell cytotoxic assay. *Methods Enzymol. 93:270.*
45. **Markwell, M. A. K., and C. F. Fox.** 1978. Surface specific iodination of membrane proteins of viruses and eukaryotic cells using 1,3,4,6-tetrachloro-3α,6αdiphenylglycoluril. *Biochemistry 17:4807.*
46. **Borst, J., M. A. Prendiville, and C. Terhorst.** 1982. Complexity of the human T lymphocyte-specific cell surface antigen T3. *J. Immunol. 128:1560.*
47. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:680.*
48. **O'Farrell, P., II.** 1975. High resolution two dimensional electrophoresis of proteins. *J. Biol. Chem. 250:4007.*
49. **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.*
50. **Miller, L. J., R. Schwarting, and T. A. Springer.** 1986. Regulated expression of the Mac-1, LFA-1, p150,95 glycoprotein family during leukocyte differentiation. *J. Immunol. 137:2891.*
51. **Dongworth, D. W., F. M. Gotch, J. E. K. Hildreth, A. Morris, and A. J. McMichael.** 1985. Effects of monoclonal antibodies to the alpha and beta chains of the human lymphocyte function-associated (HLFA-1) antigen on T lymphocyte functions. *Eur. J. Immunol. 15:666.*
52. **Mentzer, S. J., A. M. Krensky, and S. J. Burakoff.** 1986. Mapping functional epitopes of the human LFA-1 glycoprotein: monoclonal antibody inhibition of NK and CTL effectors. *Hum. Immunol. 17:288.*
53. **teVelde, A. A., G. D. Keizer, and C. G. Figdor.** 1987. Differential function of LFA-1 family molecules (CD11 and CD18) in adhesion of human monocytes to melanoma and endothelial cells. *Immunology. In press.*