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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[1]

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I. INTRODUCTION

Killer cells are an important component in the immune response to cells with altered or foreign cell surface antigens. Antigen-specific killing is mediated by a subset of Thy-1$^+$ thymus-derived (T) lymphocytes which have been termed cytolytic T lymphocytes (CTL). CTL can be elicited in response to allogeneic or xenogeneic cells bearing foreign histocompatibility antigens, or in response to syngeneic cells bearing foreign antigenic determinants introduced by chemical haptenation or viral infection (reviewed in Engers & MacDonald 1976, Golstein 1976, Martz 1977, Henney 1977, Berke 1980, Burakoff et al. 1980). Induction of maximal cytolytic activity requires 5 to 10 days for a primary response. Cytolytic activity displays all the hallmarks of specific immunity, i.e. it is highly specific for antigens on the eliciting cells, and it shows accelerated development and reaches

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higher levels in secondary responses. CTL are not only specific for the foreign antigen on the target cells, but also for the type of histocompatibility antigens on the target. The majority of CTL are restricted by the H-2K,D antigens of the major histocompatibility complex (MHC) (Burakoff et al. 1980, Zinkernagel & Doherty 1979). CTL elicited to virally infected cells of $H\text{-}2^a$ histocompatibility type will lyse such cells. However, target cells differing from the eliciting cells either in their H-2 histocompatibility antigens (e.g. of the different $H\text{-}2^b$ type) or in the type of infecting virus, are not lysed. Helper T lymphocytes are also MHC restricted, but by another product of the MHC, Ia antigens (reviewed in Benacerraf & Germain 1978). Antigen recognition by CTL and by helper T cells thus is governed by similar rules, and what is learned about the CTL may be of general importance.

Cytolytic T lymphocyte effector activity has been resolved into two steps by studies on its requirement for divalent cations (Golstein 1976, Martz 1977, Berke 1980). In the first step, which requires Mg^{+2} and specific antigen recognition, CTL tightly adhere to target cells in 0.5 to 5 min at 37°C, forming CTL-target conjugates. In the second step, which is Ca^{+2}-dependent, the 'lethal hit' is delivered to the target cell in 5 to 15 min at 37°C. After this, the target cell is 'programmed for lysis' independently of any further interactions with the CTL, as may be demonstrated by disruption of the conjugates. Target cell lysis requires about 1.5 h at 37°C, and is commonly measured by the release of cytoplasmic labels such as $^{51}CrO_4^{-2}$. Bystander cells and the CTL itself are unharmed in the killing reaction, and the CTL can detach and engage in further killing cycles.

The molecular basis of CTL-mediated killing is of considerable interest, not only in itself but also for our understanding of T lymphocyte-mediated immunity in general. The molecules involved are quite distinct from those of humoral (B lymphocyte-dependent) immunity. Antibodies and complement components are not required for CTL-mediated killing, and anti-immunoglobulin sera or antisera to C2, C3, or C5 do not block CTL-mediated killing (Henney et al. 1972).

One approach for determining the molecular basis of CTL function would be to assess the activity of isolated CTL components in reconstituted, cell-free systems. However, this method has not been appropriate to the CTL, because disrupted CTL or CTL supernatants have been unable to mediate lytic activity (Henney 1977).

Therefore, we have taken a different approach of using monoclonal antibodies (MAb) to probe for the functional moieties of the CTL *in situ*. The idea behind these studies was that binding of MAb to functionally important CTL surface components would result in steric hindrance and inhibit killing, whereas binding of MAb to other CTL surface components would have no effect (Figure 1). Using this approach, we identified a novel CTL surface component,

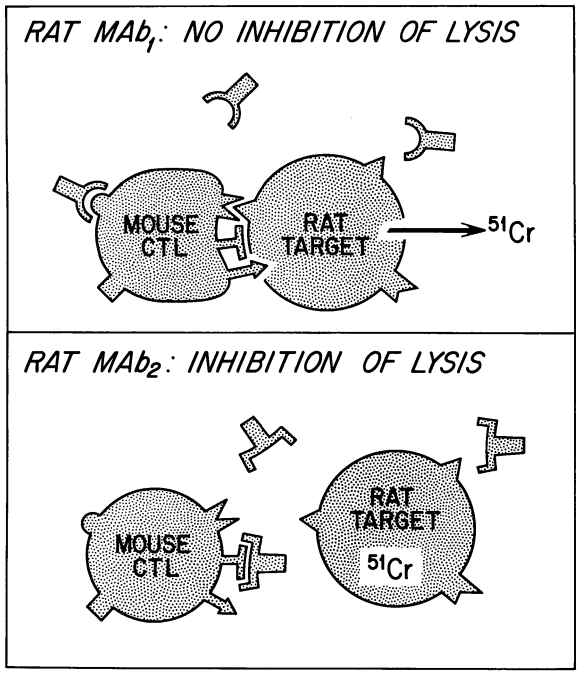

Figure 1. Doubly xenogeneic system for screening monoclonal antibodies blocking CTL effector cell function. Rat MAb are tested for blocking of B6 mouse CTL with specificity for rat BN lymphoma (BNLΦ) target cells. Antibodies which might block by binding to the target are avoided since the target cell is syngeneic with the rat antibody donor.

LFA-1, described its association with killing, and confirmed the association with killing of another antigen, Lyt-2,3 (Davignon et al. 1981a, b, Kürzinger et al. 1981, Martz et al. 1982). This review will summarize published work and highlight more recent work on the structure and role in CTL function of LFA-1 and Lyt-2,3. The homology of LFA-1 to Mac-1, a macrophage differentiation antigen (Kürzinger et al. 1982), and association of Mac-1 with the type three complement receptor (CR_3) function on myeloid cells (Beller et al. 1982) will also be described.

II. IDENTIFICATION AND CHARACTERIZATION OF LFA-1 AND LYT-2,3 AS CELL SURFACE SITES FOR BLOCKADE OF CTL-MEDIATED KILLING

To obtain anti-CTL monoclonal antibodies, rats were immunized with mouse CTL, and their spleen cells fused with NSI myeloma cells. In our initial experiments, we wished to determine how many different structures on the CTL surface could be identified, and which of these structures played an essential role

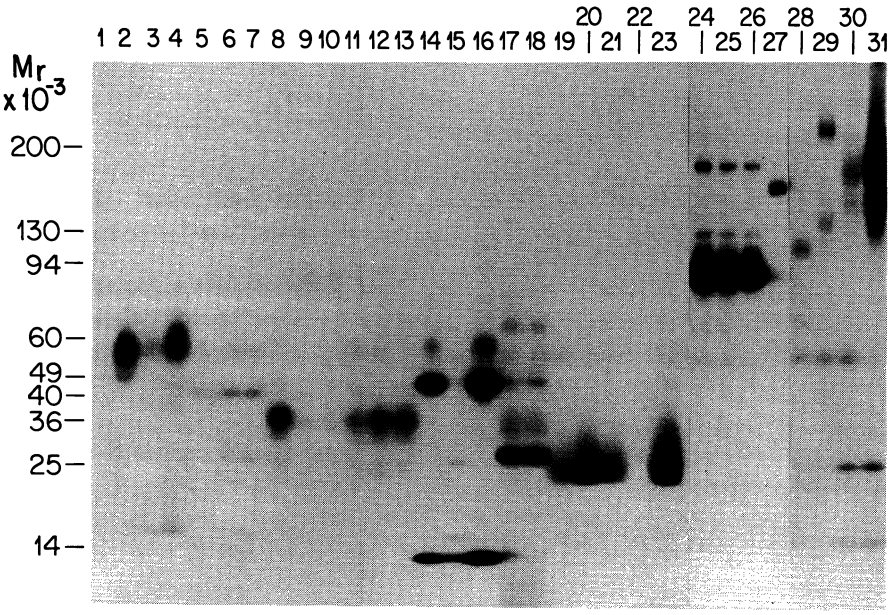

Figure 2. Antigens on mouse T lymphocytes immunoprecipitated by rat anti-mouse MAb. MAb to mouse CTL were selected in the ^{125}I-anti-rat IgG indirect binding assay and tested in immunoprecipitation. MAb-containing culture supernatants (50 μl) were mixed with lysates of surface-iodinated concanavalin A-stimulated spleen cells, immunoprecipitated with rabbit anti-rat IgG serum, and were subjected to reduction and SDS 5-15% gradient PAGE and autoradiography as described (Kürzinger et al. 1981). Lanes contain immunoprecipitates of the following antibodies: 1, NSI supernatant containing 50 μg/ml normal rat IgG as control; 2, M1/84; 3, M7/20; 4, M12/2; 5, M5/111; 6, M5/116; 7, M5/120; 8, M5/24; 9, M5/118; 10, M5/119; 11, M12/4; 12, M12/5; 13, M12/7; 14, M7/21; 15, M5/26; 16, M12/3; 17, M5/114; 18, M7/81; 19, M5/49; 20, M5/54; 21, M5/56; 22, M5/78; 23, M12/6; 24, M5/113; 25, M7/83; 26, M12/1; 27, M7/14; 28, M5/35; 29, M7/7; 30, M7/84; 31, M1/9.3.

in CTL-mediated killing (Davignon et al. 1981a). Xenoimmunization and selection for monoclonal antibodies binding to T lymphocytes was found to identify a large number of T lymphocyte cell surface antigens (Figure 2), including antigens of 55,000 to 58,000 M_r (lanes 2-4), antigens of 45,000 M_r (lanes 5-7), the Lyt-2,3 antigen of 35,000 M_r (lanes 8 and 11-13), H-2 antigens containing subunits of 48,000 and 12,000 M_r (lanes 14 and 16), Ia antigens containing polypeptides of 35,000 and 28,000 M_r (lanes 17 and 18), the Thy-1 antigen of 25,000 M_r (lanes 19-21 and 23), a component of 95,000 M_r (lanes 24-26), the LFA-1 antigen containing two subunits of 180,000 and 95,000 M_r (lane 27), a moiety of 115,000 M_r (lane 28), an antigen containing two polypeptides of 250,000 and 140,000 M_r (lane 29), and the Ly-5, common leukocyte, or T200 antigen of 200,000 M_r (lanes 30 and 31).

TABLE I
Monoclonal antibodies inhibiting CTL-mediated killing

Antigen	MAb	Ig	Inhibition of Killing (%)[a]
LFA-1	M7/14	$\gamma 2a\kappa$	88
LFA-1	M15/15	$\gamma 2a\lambda$	59
LFA-1	M17/4	$\gamma 2a\kappa$	83
LFA-1	M17/5	$\gamma 2b\kappa$	95
LFA-1	M17/7	$\gamma 2b\kappa$	92
LFA-1?	M15/32	$\gamma 2b\lambda$	54
Lyt-2,3	M5/24	$\gamma 2b\kappa$	40
Lyt-2,3	M12/4	$\gamma 2b\kappa$	63
Lyt-2,3	M12/5	$\gamma 2a\lambda$	72
Lyt-2,3	M12/7	$\gamma 2b\kappa$	50
Lyt-2,3	M15/5	$\gamma 2b\kappa$	78
Thy-1	M16/1	$\mu\kappa$	38[b]

[a] Inhibition of secondary (1-1) (primed *in vivo*, boosted *in vitro*) xenogeneic B6 anti-BNLΦ killing. CTL were treated with an equal volume of hybridoma culture supernatant for 15 min, the ^{51}Cr-labeled BNLΦ cells were added and ^{51}Cr release was measured after 2 to 4 h. The data are averages of two to 24 experiments. Specific ^{51}Cr release was in the range of 25 to 90% and spontaneous release was 8 to 19%, both in the presence of NSI culture supernatant.

[b] M16/1 strongly agglutinated CTL.

Monoclonal antibodies to these and other antigens were next tested for inhibition of CTL-mediated killing. Most of the MAb had little effect on killing, giving −8% to 26% inhibition (Davignon et al. 1981a). In contrast, the M7/14 MAb, which immunoprecipitated polypeptides of 180,000 and 95,000 M_r, consistently inhibited killing by an average of 90% (Table I). This inhibition suggested the antigen defined by M7/14 was essential for killing, and it was designated lymphocyte function-associated antigen 1, or LFA-1. Two MAb to Lyt-2,3, M5/24 and 53.6, also significantly inhibited killing by 40% and 90%. This confirmed, in the xenogeneic CTL system, previous reports that antibodies to Lyt-2,3 block CTL-mediated killing (Shinohara & Sachs 1979, Nakayama et al. 1979, Fan et al. 1980, Shinohara et al. 1980, Hollander et al. 1980, Sarmiento et al. 1980).

In further hybridization experiments, we have screened directly for function-blocking MAb (Sanchez-Madrid et al. 1982). There were several advantages to using blockade of killing, rather than cell binding assays, as the primary screen. Firstly, a larger number of different blocking MAb should be obtainable, allowing a better correlation to be established between blocking activity and the type of surface structure recognized. Secondly, antigens having an important role in function but expressed in low quantities or only transiently during CTL-

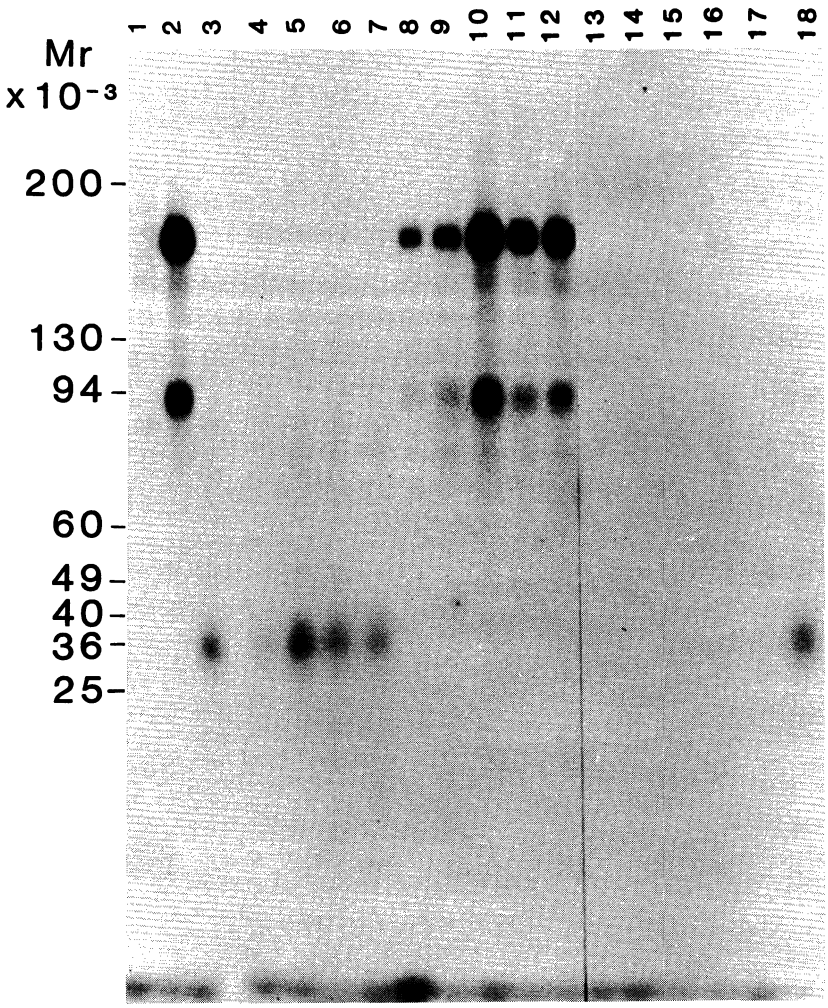

Figure 3. SDS-PAGE of ^{125}I-surface antigens immunoprecipitated by CTL-blocking MAb. Spleen cells from B6 mice primed with P815 and restimulated *in vitro* were surface labeled with ^{125}I using Iodogen. Cell lysates were immunoprecipitated with 100 μl NSI supernatant plus added normal rat IgG as control (lane 1); or culture supernatants containing the following MAb: M7/14 anti-LFA-1 standard, lane 2; M12/7 anti-Lyt-2,3 standard, lane 3; M15/5, lane 4; M17/2, lane 5; M17/8, lane 6; M17/9, lane 7; M15/15, lane 8; M17/1, lane 9; M17/4, lane 10; M17/5, lane 11; M17/7, lane 12; M15/32, lane 13; M16/1, lane 14; M17/3, lane 15; M17/6, (weakly visible in original), lane 16; M17/10, lane 17; M17/11, lane 18. Purified rabbit anti-rat IgG (mouse IgG absorbed) coupled to Sepharose (2.1 mg/ml, 30 μl) was used to precipitate the immune complexes. Reduced samples were subjected to SDS 5-15% PAGE and autoradiography.

target cell interactions would be selected by functional criteria but not by binding techniques. In three different experiments, spleen cells were removed from rats immunized to B6 anti-P815 CTL and fused with NSI or P3Ag8.6.5.3 myeloma cells. Over 2,400 different hybridoma culture supernatants were screened for antibodies which would block B6 mouse anti-BNLΦ rat xenogeneic CTL-mediated killing. Fifteen inhibitory hybridoma cultures were selected for detailed analysis and cloning.

The results strongly confirmed the correlation between LFA-1 and Lyt-2,3 structures and CTL-mediated killing. Of the 15 MAb selected by blockade of CTL-mediated killing, five immunoprecipitated Lyt-2,3 (Figure 3, lanes 4-7, and 18) five immunoprecipitated LFA-1 (Figure 3, lanes 8-12) and two weakly immunoprecipitated LFA-1 (data not shown) from B6 anti-P815 CTL. Inhibition by the cloned anti-Lyt-2,3 and anti-LFA-1 antibodies derived from several experiments averaged 61% and 83%, respectively (Table I). Recently, Pierres et al. (1982) and Sarmiento et al. (1982) have also obtained CTL-blocking MAb which appear to recognize the same LFA-1 molecule. The M16/1 anti-Thy-1 IgM MAb which gave weak inhibition of 38%, was also selected by blockade of killing (Table I). However, this inhibition was probably due to the fact that M16/1 strongly agglutinated CTL (Sanchez-Madrid et al. 1982). Attempts to clone the two other inhibitory lines were unsuccessful, hindering identification of the target antigens.

III. ANTI-LFA-1 AND ANTI-LYT-2,3 MAb BLOCK KILLING IN A HIGHLY SPECIFIC MANNER

In these studies, we have used a doubly xenogeneic system in which rat anti-mouse MAb are tested on mouse anti-rat CTL (Figure 1). The MAb cannot bind to the target cell, since both are of rat origin. Inhibition therefore should be due to binding to LFA-1 or Lyt-2,3 determinants on the CTL effector. This was confirmed by immunofluorescence measurements (Davignon et al. 1981a).

A number of trivial explanations for inhibition by anti-Lyt-2,3 and anti-LFA-1 MAb have been ruled out (Davignon et al. 1981b). The anti-LFA-1 MAb is not toxic to CTL as measured by trypan blue exclusion. Furthermore, the antibody can be washed out and CTL activity recovered, perhaps due to *de novo* LFA-1 synthesis during the assay period. Anti-LFA-1 had no significant effect on CTL motility, as measured by the ability of CTL to crawl under monolayers of 3T3 cells. To test for agglutination of CTL, MAb were added and the CTL assay was initiated as usual, then cells were resuspended and examined in a hemocytometer. Anti-LFA-1 (Davignon et al. 1981b) and anti-Lyt-2,3 MAb (unpublished) produced little or no agglutination of CTL. The M5/49 anti-Thy-1 MAb agglutinated CTL-containing populations (CTLP) but had little effect on killing. The M16/1 IgM anti-Thy-1 MAb agglutinated CTLP into large clumps

and inhibited killing by 38%. We believe that the small and variable inhibition of killing seen with anti-Thy-1 MAb is due to agglutination. Indeed, considering that CTL are Thy-1$^+$ and are undoubtedly included in the agglutinated clumps, it is remarkable how little the anti-Thy-1 MAb inhibit.

Inhibition by anti-LFA-1 and anti-Lyt-2,3 also is not related to non-specific 'blanketing' of the CTL surface with antibody. LFA-1 and Lyt-2,3 MAb bind to about 7.2×10^4 and 10.5×10^4 sites per cell, respectively, on secondary MLC cells which have potent killing activity (Kürzinger et al. 1981). Anti-H-2 and anti-Thy-1 MAb bind to a higher number of 18×10^4 and 75×10^4 sites per cell, respectively, but have little effect on killing.

MAb bound to a surface molecule could prevent it from carrying out its function either by steric hindrance, or by causing its removal from the surface by patching followed by capping and endocytosis or shedding. When secondary MLC were incubated with anti-LFA-1 MAb for 1 h at 37°C, then fixed and labeled with FITC anti-IgG, the antigen remained uniformly distributed over the cell (unpublished). Since antibodies can potently inhibit killing within as little as 15 min (see below), it is clear that patching or removal from the cell is not involved. However, it remains possible that crosslinking monomeric antigens with bivalent antibody might be required for inhibition. Inhibition experiments with Fab fragments, which would most definitively test whether inhibition is purely steric or requires crosslinking, have not yet been carried out.

IV. CELLULAR DISTRIBUTION OF LFA-1 AND LYT-2,3 AND EFFECTS ON OTHER T AND B CELL FUNCTIONS

To study the distribution of LFA-1 and Lyt-2,3 (Kürzinger et al. 1981, Sanchez-Madrid et al. 1982), cells were labeled with MAb, then with affinity-purified, mouse IgG-absorbed, FITC-anti-rat IgG and subjected to immunofluorescence flow cytometry. As previously reported, (Ledbetter & Herzenberg 1979), Lyt-2,3 is present on 12 to 17% of spleen cells, representing a subset of about $\frac{1}{3}$ to $\frac{1}{2}$ of splenic T lymphocytes, and on 83% of thymocytes. LFA-1 is on $>97\%$ of spleen cells, and is thus present on both B and T lymphocytes. The number of LFA-1 sites/spleen cell is heterogeneous, and labeling of purified populations has shown T lymphocytes express 3.5-fold more LFA-1 than B lymphocytes. LFA-1 is present on $>97\%$ of thymocytes and on $>95\%$ of blood lymphocytes. A subpopulation of 79% of bone marrow cells bear LFA-1, implying expression on nonlymphoid cells which probably include granulocytic precursors. However, not all leukocytes are LFA-1$^+$, since thioglycollate-elicited peritoneal macrophages are $>94\%$ LFA-1$^-$. LFA-1 does not appear to be expressed outside hematopoietic tissues, since lung, liver, brain, and kidney are all negative. Changes in antigen expression associated with CTL generation have been examined by comparing naive spleen cells with spleen cells after a 5 day

secondary MLC stimulation. The quantity of antigen per positive cell was measured by immunofluorescence flow cytometry. LFA-1 expression was increased 4.8-fold, compared to increases in H-2, Thy-1, and Lyt-2 expression of 1.8-fold, 3.6-fold, and 1.6-fold, respectively. This specific increase in LFA-1 relative to the other antigens was accompanied by an increase in Lyt-2^+ cells from 11 to 84%.

The effects of anti-LFA-1 MAb on a number of lymphocyte functions (Davignon et al. 1981b) have led to the working hypothesis that LFA-1 is crucial in interactions of lymphocytes with other cells. The M7/14 anti-LFA-1 MAb blocks T lymphocyte proliferative responses to antigens under Ir gene control, while an antibody to Thy-1 has no effect. Similarly, the allogeneic mixed lymphocyte proliferative response (MLR) was blocked by anti-LFA-1 but not by anti Thy-1 or anti-H-2. The xenogeneic MLR response of mouse spleen cells to irradiated rat lymphocytes was also blocked, showing blockade is due to binding to LFA-1 molecules on the responder cells. The MLR was blocked if antibody was added to cultures at day 0, but not if added on days 1, 2, or 4 of the 6-day culture period. This showed that the antibody blocked induction of proliferation rather than proliferation itself, which was measured with a [^{3}H]thymidine pulse at day 5. Furthermore, anti-LFA-1 MAb does not inhibit the proliferative response of cloned T cell lines to IL-2 (T. Strom and D. Beller, unpublished). These results suggest that LFA-1 participates in the induction of T helper cell responses to antigen, perhaps at the level of the T helper cell-macrophage interaction, but is not essential for maintenance of T cell proliferation.

Effects on B cell antibody responses were assessed by including MAb in *in vitro* cultures and determining the number of plaque-forming cells. M7/14 inhibited the T-dependent B cell response to sheep red blood cells. Since both T and B cells are required for this response and both express LFA-1, it was not possible to determine the locus of inhibition. The B cell response to NP-Ficoll, which is not dependent on T cells, was unaffected. The B cell proliferative response to lipopolysaccharide was also unaffected. Thus, B cell responses which do not require interactions with other cells are not inhibited by anti-LFA-1.

V. MECHANISM OF BLOCKADE OF CTL-MEDIATED KILLING

The MAb to LFA-1 has been shown to block killing in a number of CTL systems (Davignon et al. 1981b). Blocking of xenogeneic mouse anti-rat BN-lymphoma killing averaged 90%. Inhibition was not diminished even when highly active CTL or high effector/target ratios were employed. CTL directed to TNP-modified self determinants were blocked by 100%. Inhibition of allogeneic killing was somewhat less (50-80%).

TABLE II

Comparative inhibition of xenogeneic and primary and secondary allogeneic killers by anti-Lyt-2,3 and anti-LFA-1 MAb

MAb	Dilution	2°α-BNLΦ	1°α-P815	2°α-P815
αLyt-2,3 M12/4.2	1/6	68	72	5
αLyt-2,3 M12/4.2	1/60	58	61	5
α-Lyt-2,3 M12/4.2+M12/5.2	1/6	76	79	18
α-Lyt-2,3 M12/4.2+M12/5.2	1/60	65	72	13
α-LFA-1 M15/15	1/6	88	96	65
α-LFA-1 M15/15	1/60	93	86	46
α-LFA-1 M17/5	1/6	100	85	32
α-LFA-1 M17/5	1/60	91	74	22
α-LFA-1 M15/15+M17/5	1/6	83	91	56
α-LFA-1 M15/15+M17/5	1/60	96	86	49
α-LFA-1+α-Lyt-2,3 M17/5+M12/4	1/6	103	94	60
α-LFA-1+α-Lyt-2,3 M17/5+M12/4	1/60	97	86	49

Secondary (1-1) xenogeneic B6 anti-BNLΦ CTL (E:T=5), primary (0-1) B6 anti-P815 CTL (E:T=5) or secondary (1-1) B6 anti-P815 CTL (E:T=0.5) were incubated with MAb, each at the appropriate concentration, and the appropriate ^{51}Cr-labeled target cells were added and the assay completed as described (Davignon et al. 1981b). Inhibition was determined relative to specific ^{51}Cr release in the presence of NSI supernatant, which was 53, 49, and 76%, respectively. Secondary anti-P815 CTL were consistently less well inhibited than anti-BNLΦ and primary anti-P815 CTL by the anti-Lyt-2,3 and anti-LFA-1 MAb in other experiments, whether secondary CTL gave higher or lower specific release.

In experiments with secondary allogeneic killers, the inhibition by anti-Lyt-2,3 and anti-LFA-1 MAb has been consistently lower than with secondary xenogeneic killers (Table II). MacDonald et al. (1981) noted that killing by peritoneal exudate lymphocytes was much less susceptible than that by MLC cells to inhibition by anti-Ly-2. CTL with high avidity for target cells might be correspondingly more difficult to inhibit. CTL avidity would be expected to increase after priming. Therefore, we compared inhibition of killing by primary (0:1) and secondary (1:1) MLC cells (Table II). Both anti-Lyt-2,3 and anti-LFA-1 MAb gave stronger inhibition of the primary than secondary killers. The anti-LFA-1 MAb gave comparatively better inhibition of the allogeneic secondary killers.

Inhibition by anti-Lyt-2,3 and anti-LFA-1 MAb was at least partially additive, suggesting that these molecules have different roles in the killing process. Furthermore, anti-LFA-1 and anti-Lyt-2,3 MAb do not cross-inhibit one another, suggesting these antigens are topographically distinct on the cell surface (Sanchez-Madrid et al. 1982).

In the presence of the lectins PHA and Con A, CTL are capable of killing in an antigen-nonspecific fashion. CTL or mitogen-activated T cells but not normal T

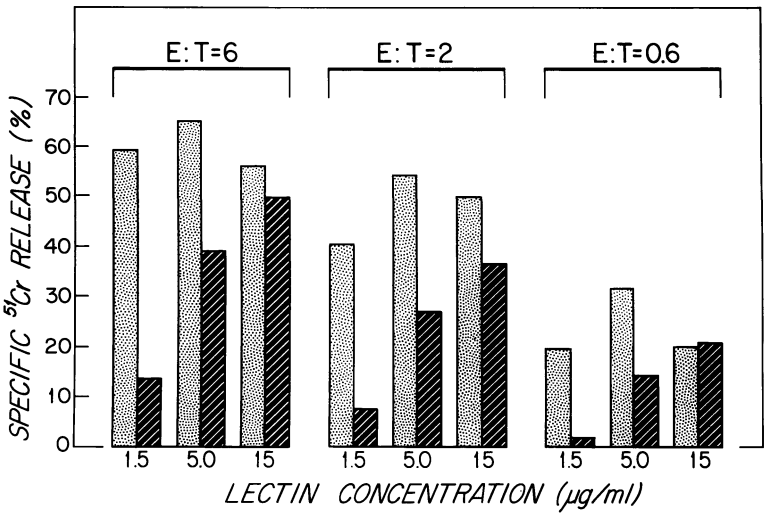

Figure 4. Anti-LFA-1 MAb inhibits lectin-dependent killing more strongly at low than high lectin concentrations. B6 anti-P815 CTL were assayed on ^{51}Cr-labeled BNLI target cells at the final concanavalin A concentrations and effector: target ratios indicated. CTL were pretreated with M7/14 anti-LFA-1 (filled bars) or M1/69HK control (open bars) supernatants, containing the same IgG and FCS concentrations, target cells and lectin were added, and specific ^{51}Cr release was determined after 4 h.

lymphocytes are active in lectin-dependent killing (Asherson et al. 1973, Bevan & Cohn 1975, Gately & Martz 1977, Green et al. 1978). Lectins have been hypothesized to form a bridge between the CTL and the target cells, replacing the function of the CTL antigen receptor, or to provide an activating function, but the exact mechanism of action is not clear. To test the effect of anti-Lyt-2,3 and anti-LFA-1 MAb on lectin-dependent killing, B6 anti-H-2^d allogeneic CTL were used with rat BNLΦ target cells. Interestingly, the inhibitory effect of anti-LFA-1 MAb was highly dependent on the lectin concentration (Figure 4). Concanavalin A concentrations of 1.5, 5.0 and 15 μg/ml gave similar amounts of lectin-dependent killing. However, anti-LFA-1 MAb gave strong inhibition of killing (77 to 91%) at 1.5 μg/ml but little or no inhibition (0 to 26%) at 15 μg/ml (compare with Berke et al. 1981). Higher lectin concentrations should result in a higher number of cross-bridges between the CTL and target cell, i.e. a higher affinity interaction. Anti-Lyt-2 MAb were similarly effective in inhibiting killing at low lectin concentrations giving inhibition of 48 to 96%, $\bar{x}=72\%$.

Two steps in CTL-mediated killing have been distinguished by their divalent cation-dependence. The first step, formation of a tight adhesion between the CTL and the target cell, is Mg^{+2}-dependent. The second step, delivery of the lethal hit, is Ca^{+2}-dependent. Anti-LFA-1 MAb can block formation of CTL-

TABLE III

Effect of adding anti-Lyt-2,3 or anti-LFA-1 MAb at different time points relative to a calcium pulse

		Time of MAb Addition (min)			
		−50	−5	0	+5
		^{51}Cr Specific Release (%)			
Expt. 1	Control	65	59	48	49
	α-LFA-1	0	9	19	45
	α-Lyt-2,3	33	8	4	44
Expt. 2	Control	57	59	64	63
	α-LFA-1	−2	25	22	60
	α-Lyt-2,3	19	15	8	46
Expt. 3	Control	57	62	66	64
	α-LFA-1	−2	21	18	61
	α-Lyt-2,3	12	11	6	55

B6 anti-BNLΦ CTL and ^{51}Cr-labeled BNLΦ cells in L15 medium, 5% FCS, 3m EGTA, were centrifuged and incubation at 37°C was begun at time −50 min. Ca^{+2} (5 mM final) was added at 0 min, EDTA (10 mM final) was added at 10 min, and specific ^{51}Cr release was measured at 2 h. MAb were added at −50 min (before prespin), at −5 min, at 0 min (mixed with the Ca^{+2} in one aliquot), or at 5 min.

target conjugates, enumerated by microscopy, and thus appear to block the first step (Davignon et al. 1981b). Anti-Lyt-2,3 MAb have been reported to block conjugate formation (Fan et al. 1980). To investigate which divalent cation-dependent step was blocked, MAb were added at various times in the following protocol. CTL and target cells were mixed together at −50 min in the presence of Mg^{+2} and EGTA, which allows conjugate formation but not the lethal hit; Ca^{+2} in excess of EGTA was added at 0 min to initiate the lethal hit; EDTA was added at 10 min to chelate Ca^{+2} and terminate the lethal hit; and ^{51}Cr release was measured after 2 h to allow completion of killer cell-independent lysis. The anti-Lyt-2,3 and anti-LFA-1 MAb were inhibitory when added at −50, −5, or 0 min relative to the Ca^{+2} pulse (Table III). Since conjugates are formed within 5 min at 37°C, i.e. by time −45 min, the data suggest that the MAb either inactivate or dissociate preformed conjugates. Counting of conjugates showed that anti-LFA-1 MAb, added simultaneously with Ca^{+2} at time 0, could reverse conjugate formation (Table IV). Thus, the inhibition of ^{51}Cr release when the MAb were added with the Ca^{+2} at time 0 appears to be due to rapid reversal of adhesions, rather than to inhibition of lethal hit delivery. MAb added 5 min after Ca^{+2} had little effect on ^{51}Cr release (Table III), while EDTA added at 5 min was partially inhibitory (data not shown). This lends further support to the idea that anti-Lyt-2,3 and anti-LFA-1 do not inhibit the lethal hit. CTL can readily exchange one target cell for another during the Mg^{+2}-dependent period (Balk & Mescher 1981),

TABLE IV
Anti-LFA-1 MAb added simultaneously with Ca^{+2} can reverse conjugate formation

	Expt. 1		Expt. 2	
	Targets in Conjugates (%)	Inhibition (%)	Targets in Conjugates (%)	Inhibition (%)
Treatment				
0°C, Control MAb 45 min.	10		12	
20°C, Control MAb 45 min.	41		50	
45 min 20°C, Control MAb+Ca^{+2} 10 min 37°C	39		ND	
45 min 20°C, α-LFA-1+Ca^{+2} 10 min 37°C	18	73	26	63

B6 anti-BNLΦ CTL and BNLΦ cells were mixed together in L15 medium, 5% FCS, 3 mM EGTA with or without control (M1/69HK) supernatant, centrifuged, and incubated at specified temperatures. Control M1/69HK supernatant or M17/5.2 anti-LFA-1 MAb-containing supernatant at 37°C, mixed together with Ca^{+2} (5 mM final), was added after 45 min to some samples and incubation continued another 10 min at 37°C. Samples were vortexed, placed on ice, coded for blind analysis, and the % of large cells (targets) with adherent small cells (CTL) was determined by counting in a hemocytometer as previously described (Davignon et al. 1981b). Inhibition of specific conjugate formation was calculated after subtraction of the % of conjugates at 0°C.

implying that their receptors can rapidly engage and reverse (breathe). This apparently allows reversal of conjugates by MAb. For anti-Lyt-2,3 MAb, Shinohara et al. (1981) reached the same general conclusion that the Mg^{+2}-dependent stage was inhibited, although our results differed in that we consistently found inhibition at time 0. The most important point to emerge from these studies is that both anti-Lyt-2,3 and anti-LFA-1 MAb can inhibit very late in the Mg^{+2}-dependent period. This rules out promotion of an irreversible step within the Mg^{+2}-dependent phase by either the LFA-1 or the Lyt-2,3 molecule.

Two models for the function of LFA-1 and Lyt-2,3 based on the above evidence are shown in Figure 5. Model A proposes that LFA-1, Lyt-2,3 and the antigen receptor all have affinity for ligands on the target cell. Thus all contribute to the overall avidity of the CTL for the target. Changes in avidity affect CTL activity by changing the proportion of conjugates formed (Balk et al. 1981, Thorn & Henney 1977). Model B proposes that LFA-1 and Lyt-2,3 reversibly promote different steps in the Mg^{+2}-dependent recognition-adhesion pathway. The order of the steps shown is arbitrary. In this model, LFA-1 and Lyt-2,3 might enhance or regulate the rate of steps, rather than serve as obligatory catalysts. The net effect of these steps could be to increase the avidity of the CTL for the target cell. Thus, Models A and B are both compatible with present data. Features of the models and justifications for them follow.

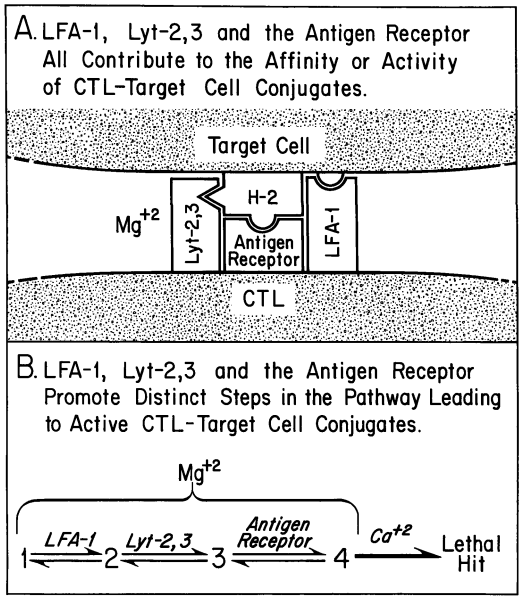

Figure 5. Models for mechanism of action of LFA-1 and Lyt-2,3 in CTL-mediated killing.

1) The antigen receptor is hypothesized to be distinct from LFA-1 and Lyt-2,3. LFA-1 is expressed not only on T lymphocytes but also on B lymphocytes and 79% of bone marrow cells (Kürzinger et al. 1981). This suggests it is not the T lymphocyte antigen receptor. It appears unlikely that Lyt-2,3 is an antigen receptor, since certain CTL hybridoma lines directed to H-2 allogeneic determinants kill in an antigen-specific manner but are Lyt-2,3$^-$ (Kaufman 1982). Further, mature T cells in F_1 animals which are heterozygous for alleles at the *Lyt-2* locus show codominant expression of Lyt-2.1 and 2.2 alloantigens (Ledbetter et al. 1981). These markers are not allelically excluded, as would be expected for antigen receptors in analogy to immunoglobulin allotypes on B lymphocytes. However, the caveat should be added that a small contribution to specificity by LFA-1 or Lyt-2,3 is possible.

2) Cytolytic T lymphocytes are hypothesized to differ in avidity for target cells, due to differences in receptor affinity. CTL have been shown to vary in avidity depending on the form and concentrations of antigen used in elicitation (Thorn & Henney 1977, Balk et al. 1981). At equilibrium, even in target excess, not all CTL are engaged to targets (Balk et al. 1981). Thus, an increase in avidity should lead to increased ^{51}Cr release in a CTL assay.

3) LFA-1 is hypothesized to contribute to the avidity of the CTL for the target cell. MAb to LFA-1 inhibit CTL-target conjugate formation. If added after conjugates have formed, anti-LFA-1 reverses them. The greater inhibition

by anti-LFA-1 of primary than secondary CTL may be because secondary CTL have such a high avidity for target cells, due to antigen receptor affinity maturation, that the avidity contribution by LFA-1 is less essential. Inhibition by anti-LFA-1 at low but not at high lectin concentrations suggests that it can contribute to the affinity of the CTL for a nonspecific target, and that this affinity contribution by LFA-1 can be mimicked by lectin. It remains possible, however, that LFA-1 is obligatory for CTL activity. Even with MAb bound to all LFA-1 sites on the surface, some residual LFA-1 activity could remain. LFA-1^- mutant CTL clones obtained by immunoselection could resolve this question.

4) Lyt-2,3 is hypothesized to contribute to the avidity of the CTL for the target cell. Anti-Lyt-2,3 MAb inhibit killing by blocking or reversing conjugate formation. Primary MLC CTL are more inhibited than secondary MLC CTL. As in the case of LFA-1, this could be because the affinity contribution by Lyt-2,3 is less important when the antigen receptor has higher affinity. MacDonald et al. (1981) have described heterogeneity among CTL clones in their inhibitability by anti-Lyt-2. Some were completely inhibited, others were not inhibited at all, but all were Lyt-2^+. MacDonald et al. raised the possibility that such differences could be attributable to CTL avidity. Kaufman et al. (1981) obtained hybrid CTL lines from secondary CTL and have shown they are Lyt-2^- and are not inhibitable by anti-Lyt-2 (Kaufman 1982). We have confirmed that these lines lack three different topographically distinct epitopes on the Lyt-2,3 molecule defined by the M12/4, M12/5, and M12/7 MAb (Springer, Kaufman & Eshhar, unpublished). The lines kill in an antigen-specific manner, suggesting Lyt-2,3 is not an antigen receptor and is not obligatory for killing activity. However, the lines appear weaker than secondary CTL, since neuraminidase treatment of targets is required for optimum killing, LPS or Con A blasts are not killed (Kaufman et al. 1981), and anti-LFA-1 MAb is potently inhibitory (Kaufman and Springer, unpublished). It is possible that loss of Lyt-2,3 expression has lowered the avidity of the hybrid CTL lines. Dialynas et al. (1980) mutagenized Lyt-2^+ CTL clones, immunoselected Lyt-2^- variants, and found that they had lost killing activity. This was an important confirmation of MAb-blocking experiments suggesting that Lyt-2,3 was important in killing. The Lyt-$2,3^-$ line was active in lectin-dependent killing, which was interpreted as suggesting that Lyt-2,3 is either an antigen receptor or closely associated with the receptor. However, we have found that anti-Lyt-2,3 MAb block lectin-dependent killing $>90\%$ at low (1.5 μg/ml) lectin concentrations, confirming Fan et al. (1980). Further, Dialynas et al. found that the Lyt-$2,3^+$ line was much more active than the Lyt-$2,3^-$ variant in lectin-dependent killing. Thus, the Lyt-2,3 variant results are consistent with the interpretation that Lyt-2,3 contributes to the affinity of the CTL for the target, but is not the antigen receptor itself.

CTL are usually Lyt-$2,3^+$ and H-2K,D restricted, while helper cells are usually Lyt-$2,3^-$ and Ia restricted. However, Lyt-23^+, H-2,K,D restricted helper cells and

Lyt-2,3$^-$ Ia restricted CTL have been found, which led Swain (1981) to propose that Lyt-2,3 is associated with H-2K,D restriction. The simplest interpretation of this would be that Lyt-2,3 is a receptor for H-2K,D as shown in Figure 5A, but there is no direct evidence for this. In view of the suggestions that Lyt-2,3 is a receptor, the close linkage of immunoglobulin kappa chain and Lyt-2,3 genes on chromosome 6 (Gibson & MacLean 1979) is extremely tantalizing.

VI. BIOCHEMICAL STRUCTURE OF LFA-1 AND ITS HOMOLOGY TO MAC-1

The above studies suggest that LFA-1 and Lyt-2,3 contribute to the avidity of CTL for targets, but a detailed understanding of their mechanism of action is dependent on biochemical characterization. Lyt-2,3 is a dimer of disulfide-linked 30,000 and 35,000 M_r polypeptide chains, but whether these are homodimers (Reilly et al. 1980) or heterodimers (Ledbetter et al. 1981), and which chains bear Lyt-2 or Lyt-3 alloantigenic determinants (Jay et al. 1982), remain controversial. This section will describe the biochemical characterization of LFA-1 and the structurally related Mac-1 antigen (Kürzinger et al. 1982). The relationship between Mac-1 and LFA-1 is particularly intriguing because Mac-1 is associated with macrophage/granulocyte complement receptor function (Beller et al. 1982).

All MAb thus far obtained to LFA-1 immunoprecipitate two polypeptide chains from Triton X-100 lysates of T or B lymphocytes, an α chain of 180,000 M_r and a β chain of 95,000 M_r (Figure 6). MAb to Mac-1 also precipitate two chains, α of 170,000 M_r and β of 95,000 M_r (Figure 6). The α and β chains are not linked by disulfide bonds. Crosslinking with a cleavable bifunctional reagent has shown that, in each antigen, α and β are subunits which are tightly noncovalently associated in $\alpha_1\beta_1$ structures (Kürzinger et al. 1982). The α and β subunits are labeled by ^{125}I, [^{35}S]methionine, and [^{3}H]glucosamine in intact cells, showing both chains are glycoproteins with surface exposure and are synthesized by the cells on which they are expressed (Kürzinger & Springer 1982).

The M7/14 and M1/70 MAb defining LFA-1 and Mac-1, respectively, do not crossreact. The Mac-1 antigen defined by M1/70 has a distribution quite different from LFA-1. Mac-1 is present on macrophages, granulocytes, and natural killer cells, but not on lymphocytes (Springer et al. 1979, Ho & Springer 1982, Holmberg et al. 1981, Flotte et al. 1982). LFA-1 is on lymphocytes but not on thioglycollate-elicited macrophages (Kürzinger et al. 1981). These MAb are also noncrossreactive as shown by immunoprecipitation. M1/70 immunoprecipitates Mac-1 from macrophages but no material from Con A blasts, while M7/14 immunoprecipitates LFA-1 from Con A blasts but nothing from PEC. In contrast, conventional antisera prepared against homogenous Mac-1 antigen crossreactively immunoprecipitate LFA-1 and *vice versa* (Figure 6c, d). Most

MAb to LFA-1 fail to immunoprecipitate Mac-1, and a number of different anti-Mac-1 MAb do not immunoprecipitate LFA-1 (Figure 6a, b). Recently, we obtained a MAb which cross-reactively immunoprecipitates both Mac-1 and LFA-1 (Figures 6a, b, lane 6), and a similar MAb was reported by Trowbridge & Omary (1981). Mac-1 and LFA-1 thus contain unique as well as shared structural features.

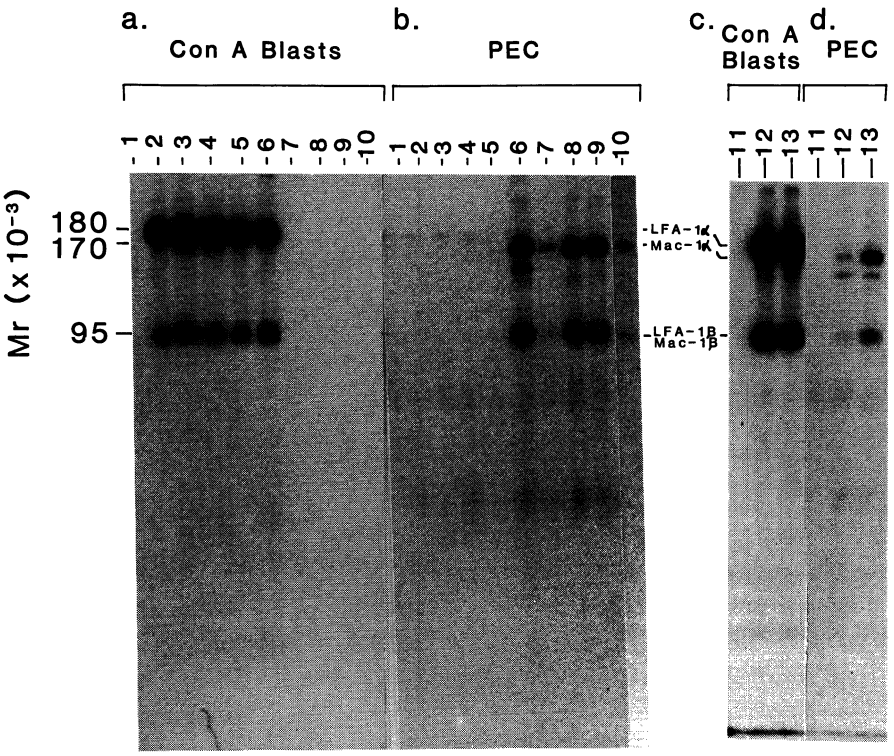

Figure 6. LFA-1 and Mac-1 bear unique and shared antigenic determinants. Day-4 concanavalin A-stimulated spleen cells (Con A blasts) (a,c) and 4-day thioglycollate-elicited PEC (b,d), were prepared and surface labeled with ^{125}I using Iodogen. Cells lysates were immunoprecipitated with 100 μl of supernatants of NSI plus normal rat IgG as control, lane 1; M7/14 anti-LFA-1, lane 2; M17/4 anti-LFA-1, lane 3; M17/5 anti-LFA-1, lane 4; M17/7 anti-LFA-1, lane 5; M18/2 anti-LFA-1, crossreactive with Mac-1, lane 6; M1/70 anti-Mac-1, lane 7; M19/23 anti-Mac-1, lane 8; M19/24 anti-Mac-1, lane 9; M19/1, anti-Mac-1, lane 10; or with 3 μl normal rat serum, lane 11; or antisera to purified (Kürzinger & Springer 1982) Mac-1, lane 12; or LFA-1, lane 13. Immune complexes were precipitated with RG7/7 or RG7/9 anti-rat kappa chain MAb (Springer et al. 1982) coupled to Sepharose. Reduced samples were subjected to SDS 10% PAGE in two separate gels (a-b, c-d) and autoradiography as previously described (Kürzinger et al. 1981). The band appearing just below the Mac-1 α chain in some immunoprecipitates appears to be an α chain degradation product.

Both Mac-1 and LFA-1 have been purified 1,200 to 1,500-fold to homogeneity in 200 to 400 μg quantities by MAb immunoadsorbent affinity chromatography (Kürzinger & Springer 1982). The starting materials were 13×10^9 EL-4 cells, which express 10^5 LFA-1 molecules/cell, or 8×10^9 P388D$_1$ cells, which express 1.6×10^5 Mac-1 molecules/cell. The purification of LFA-1 was the first for an antigen associated with CTL-mediated killing, and lays the groundwork for understanding mechanisms of T lymphocyte-mediated immunity at the molecular level.

The structural basis of the relationship between LFA-1 and Mac-1 was investigated by peptide mapping (Kürzinger et al. 1982, Trowbridge & Omary 1981). The purified antigens were iodinated, the α and β subunits separated by SDS-PAGE (Figure 7A), excised from gels, and subjected to peptide mapping. The LFA-1 and Mac-1 β subunits share at least 10 tyrosyl tryptic peptides (Figure 7E-G) and are thus highly homologous or identical. In contrast, the

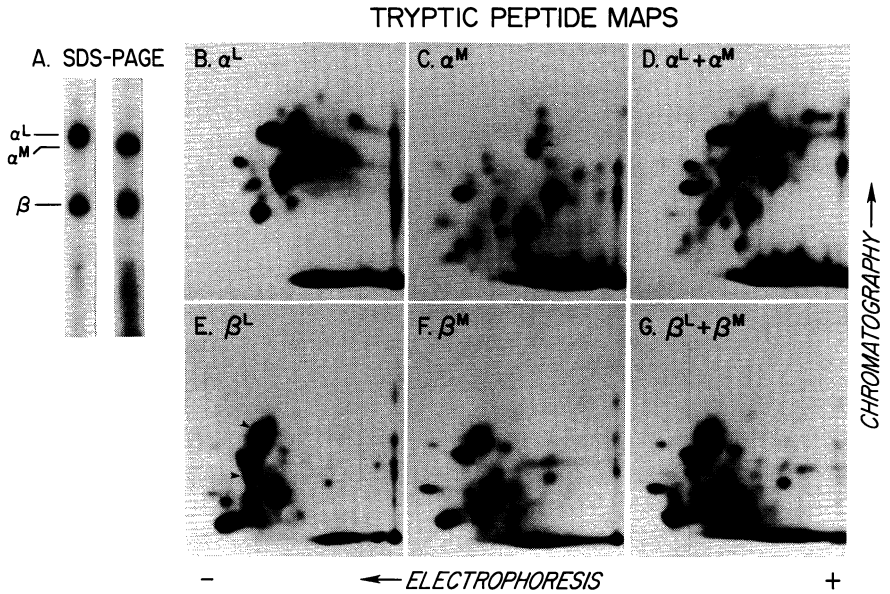

Figure 7. Tryptic peptide maps of SDS-PAGE purified LFA-1 and Mac-1 α and β subunits. LFA-1 and Mac-1 were purified from EL-4 and P388D$_1$ cells and labeled with ^{125}I (Kürzinger et al. 1981). The α and β polypeptides were separated by SDS-7% PAGE, and the wet gel was autoradiographed for 10 min (panel A). A, lane 1, LFA-1; lane 2, Mac-1. Bands were excised and digested with TPCK-trypsin. Peptide aliquots were spotted on cellulose TLC plates and subjected to electrophoresis in 15% acetic acid, 5% formic acid, chromatography in n-butanol: pyridine; acetic acid; water, 65:50:10:40, and autoradiography. B, LFA-1 α chain; C, Mac-1 α chain; D, mixture of α chains; E, LFA-1 β chain; F, Mac-1 β chain; G, mixture of β chains. The arrows in B, C, and D point to a comigrating peptide, those in E point to peptides unique or increased in intensity.

Mac-1 and LFA-1 α subunits (Figure 7B-D) have highly different maps which show at least 17 unique tryptic peptides each. The extensive differences strongly suggest that the Mac-1 and LFA-1 α chains are products of distinct genes. However, since only one amino acid substitution per tryptic peptide would generate different maps, it cannot be ruled out that the α subunits are homologous. Indeed, the α subunits bind to a common or homologous β subunit, and by analogy to other protein families such as the hemoglobins, immunoglobulins, and H-2 histocompatibility antigens which have common or homologous subunits, it would be predicted that the Mac-1 and LFA-1 α subunits have sequence homology and are closely linked genetically.

Mac-1 and LFA-1 comprise a novel family of leukocyte differentiation antigens in which alternative forms of an α subunit of 170,000 to 180,000 M_r are noncovalently associated with a common or highly homologous β subunit of 95,000 M_r in $\alpha_1\beta_1$ structures. The large structural differences in the α subunits suggest they bear the unique epitopes recognized by the M1/70 and M7/14 MAb. The β subunits appear to be immunoprecipitated by virtue of their noncovalent association with the α subunits. It seems likely that the common determinants revealed by polyclonal anti-Mac-1 antibodies and by cross-reacting MAb are principally on the β subunit. The selective expression of the Mac-1 and LFA-1 α chains in the monocytic and lymphoid lineages is a particularly interesting feature of this differentiation antigen family. Studies on how α gene expression is controlled should provide insights into how leukocyte differentiation is regulated at the DNA level.

VII. ANTI-MAC-1 MAb BLOCKS THE MACROPHAGE AND GRANULOCYTE TYPE THREE COMPLEMENT RECEPTOR (CR_3)

The structural homology between LFA-1 and Mac-1 suggests that their functions would be mediated by similar molecular mechanisms. It was therefore of interest to evaluate the possible relationship of Mac-1 to structures on the macrophage surface of known physiologic function. The M1/70 anti-Mac-1 MAb was tested for inhibition of macrophage Fc and complement receptors (Table V; Beller et al. 1982). The anti-Mac-1 MAb strongly inhibited complement-receptor-mediated rosetting of EAC (erythrocyte-IgM antibody-complement complexes), but a number of other antibodies reactive with macrophage surface antigens had no effect. None of the antibodies significantly inhibited Fc receptor-mediated rosetting of EA (erythrocyte-IgG complexes). Furthermore, M1/70 $F(ab')_2$ preparations strongly inhibited rosetting of EAC but had no effect on rosetting of EA (Table V). Macrophages express two types of complement receptor, CR_1 specific for C3b, and CR_3 specific for C3b inactivator-cleaved C3b (C3bi and further degradation products) (Ross 1980).

TABLE V
Anti-Mac-1 selectively blocks EAC rosetting[a]

	Rosetting Macrophages (%)	
Blocking MAb	EA	EAC
–	93	96
anti-Mac-1 (M1/70)	72	12
anti-H-2 (M1/42)	91	95
anti-H-2 (M7/21)	71	97
anti-Ly5 (M1/89)	82	94
anti-Ly5 (M1/9.3)	89	98
anti-Lgp100 (M7/83)	92	96
anti-pan-leukocyte (M1/84)	93	94
anti-LFA-1 (M17/4)	95	87
normal rat IgG 3μg/ml	88	83
M1/70 IgG 3μg/ml	74	10
M1/70 IgG 1μg/ml	85	19
M1/70 IgG 0.3μg/ml	91	71
M1/70 F(ab')$_2$ 3μg/ml	90	11
M1/70 F(ab')$_2$ 1μg/ml	93	10
M1/70 F(ab')$_2$ 0.3μg/ml	90	32

[a] Peptone-elicited macrophages in 16 mm culture wells were incubated with the indicated MAb (10μg/ml), or purified M1/70 IgG or F(ab')$_2$ at the indicated concentration for 10 min at 20°. Sheep erythrocytes opsonized with IgG antibody (EA) or with IgM antibody and 1:10 C5-deficient A/St serum as source of complement (EAC) were added and centrifuged onto the macrophages. After 30 min at 20°C, unbound erythrocytes were removed by washing, the monolayer was fixed with glutaraldehyde, and the percentage of macrophages rosetting more than five erythrocytes was determined. Groups with strong inhibition are underlined. M1/70 F(ab')$_2$ was prepared and characterized as described in Ault & Springer (1981).

CR_2 which is specific for C3d, is found on B lymphocytes but not on myeloid cells. The M1/70 anti-Mac-1 MAb crossreacts with human granulocytes and monocytes (Ault & Springer 1981). Using erythrocytes coated with purified C3b or C3bi, it was found that anti-Mac-1 blocked CR_3 on both human granulocytes and mouse macrophages, but CR_1 on neither cell (Beller et al. 1982). The most likely interpretation of these results is that Mac-1 antigen is the CR_3. However, the possibility remains that Mac-1 is a distinct moiety which is closely associated with CR_3 or promotes its function.

VIII. FUNCTIONAL SIMILARITIES BETWEEN LFA-1 AND MAC-1

Are there any parallels in the functions of LFA-1 and Mac-1? Both appear involved in adhesion to other cells. Although neither has been directly

demonstrated to be a receptor, this is the simplest interpretation of the results. Mac-1 appears to be the CR_3 receptor. MAb to LFA-1 can block or reverse adhesion to target cells. The simplest interpretation of results with blocking of lectin-dependent killing and of primary and secondary allogeneic killers is that LFA-1 contributes to the avidity of the CTL for the target. LFA-1 thus may be a receptor for a moiety normally present on target cells or deposited on their surface during killer-target interaction. Since LFA-1 and Mac-1 are homologous, it is tempting to speculate that the ligands would also be homologous, i.e. the ligand of LFA-1 would be a homologue of C3. The H-2 and Ia antigens encoded in the MHC are known to be important immune recognition sites for T lymphocytes (Benacerraf 1981), and the possible recognition of H-2 by Lyt-2,3 has already been mentioned. In this regard, it is interesting that C4, C2, and factor B are all tightly MHC-linked (Raum et al. 1980). C4 is a homologue of C3[1], and C2 and factor B help to form C3 convertases in the classical and alternative complement pathways, respectively. The common feature of MHC-linked products may be that they all serve as target molecules in different types of immune recognition.

In addition to the homology between LFA-1 and Mac-1, there appear to be further parallels between CTL-mediated killing and CR_3-mediated adhesion. In 1968, before the three different types of complement receptors had been delineated, Lay and Nussenzweig studied a receptor on macrophages, monocytes, and polymorphonuclear leukocytes, but not on lymphocytes, apparently identical to CR_3, and a second receptor on lymphocytes but not the other cells, apparently identical to CR_2. They found that the adherence of EAC to macrophages (apparently through CR_3) was Mg^{+2}-dependent, whereas adherence to the CR_2 on lymphocytes or the Fc receptor on macrophages was not. As described above, CTL adherence to target cells is also Mg^{+2}-dependent. Furthermore, EDTA reversed the adhesions (rosetting) between EAC and macrophages, and adhesion occurred at 25°C or 36°C but not at 5°C. Thus, the steps in which Mac-1 and LFA-1 participate in CR_3 adherence and CTL-mediated killing, respectively, appear both to be Mg^{+2}-dependent, reversible, and temperature-dependent.

An exciting prospect concerning LFA-1 and Mac-1 is that, since the antigens are structurally related, and both are function-associated, detailed structure-function comparisons are possible. Furthermore, what is learned about the molecular basis of function for one antigen should have heuristic value for the other.

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[1] It seems unlikely that LFA-1 is a receptor for C4, but other C3 homologues might also be MHC-linked.

SUMMARY

The ability of MAb to the Lyt-2,3 and LFA-1 antigens to block CTL-mediated killing suggests that these molecules are important in the killing process. Lyt-2,3 and LFA-1 are distinct in molecular properties and cell distributions. Lyt-2,3 contains disulfide-linked subunits of 30,000 and 35,000 M_r and is expressed on 83% of thymocytes and a subpopulation of 30 to 50% of peripheral T lymphocytes. LFA-1 contains two noncovalently associated subunits, α of 180,000 M_r and β of 95,000 M_r. LFA-1 is expressed on T and B lymphocytes and on 79% of myeloid cells. T cells express 3.5-fold more LFA-1 than B cells and LFA-1 expression increases relative to other surface markers during CTL generation *in vitro*. In addition to inhibiting CTL-mediated killing, MAb to LFA-1 block the induction of T helper cell proliferative responses to antigen, but do not block several lymphocyte responses which are independent of cell interactions.

Anti-LFA-1 MAb inhibited CTL regardless of their specificity. Inhibition is due to binding to molecules on the effector cell. Anti-LFA-1 and anti-Lyt-2,3 MAb inhibit the first resolvable step in killing, the formation of a Mg^{+2}-dependent, strong adhesion between the CTL and the target cell. If added during or at the end of the Mg^{+2}-dependent adhesion stage, both anti-Lyt-2,3 and anti-LFA-1 MAb could reverse adhesions and inhibit killing, but were without effect if added 5 min after initiation of the Ca^{+2}-dependent lethal hit stage. It is proposed that LFA-1, Lyt-2, and the antigen receptor all contribute to the avidity of the CTL for the target cell.

LFA-1 is similar in structure to Mac-1, which contains noncovalently associated α and β subunits of 170,000 and 95,000 M_r. The LFA-1 and Mac-1 95,000 M_r β subunits are identical or highly homologous. The LFA-1 and Mac-1 α subunits have different tryptic peptide maps, showing that they are products of distinct genes. Mac-1 and LFA-1 comprise a novel family of related leukocyte differentiation antigens. Mac-1 is expressed on macrophages, granulocytes, and natural killer cells, but not on lymphocytes. MAb to Mac-1 inhibit the macrophage/granulocyte type three complement receptor for C3bi (CR_3). The homology between LFA-1 and Mac-1 suggests that the steps mediated by these antigens in CTL-mediated killing and CR_3-mediated adherence, respectively, have similar molecular mechanisms.

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