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Antigens Involved in Mouse Cytolytic T-Lymphocyte (CTL)-Mediated Killing: Functional Screening and Topographic Relationship[1]

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To investigate the repertoire of molecules which are associated with cytolytic T-lymphocyte (CTL)-mediated killing, function-blocking monoclonal antibodies (MAb) have been selected and characterized. Spleen cells from rats immunized with secondary mouse CTL were fused with mouse myeloma cells. Antibodies secreted by 2400 hybrid cultures were selected solely by their ability to block CTL-mediated killing in a mouse anti-rat xenogeneic system. Fifteen cultures with antibodies which blocked CTL-mediated killing were chosen for cloning and further characterized by immunoprecipitation and immunofluorescence flow cytometry. One group of five monoclonal antibodies recognized the Lyt-2,3 molecule of 35,000 M_r. The second group of six MAb recognized the LFA-1 antigen containing two subunits of 180,000 and 95,000 M_r. One MAb giving only partial inhibition of killing was an IgM anti-Thy-1. It strongly agglutinated CTL. The target antigens defined by three other MAb were not definitively identified. Competition in cell binding between anti-Lyt-2,3 and anti-LFA-1 MAb suggested that their blocking effect in cytolysis is due to binding to distinct and spatially separate molecules on effector cells. The results of direct screening for functional blockade support the important role of Lyt-2,3 and LFA-1 molecules in T-cell-mediated cytolysis.

INTRODUCTION

Recently, a number of monoclonal antibodies to T lymphocytes have been tested for their ability to block cytolytic T-lymphocyte (CTL)[2]-mediated killing. The idea has been to identify CTL-surface antigens which participate in the killing pathway. In a previous report from this laboratory (1), anti-CTL MAb were selected for preferential binding to T cells versus B cells and then tested for inhibition of CTL-mediated killing. MAb to 12 antigens had little or no effect on killing. In contrast, two MAb were able to block CTL-mediated killing. One MAb recognized the Lyt-2,3 antigen, antibodies to which had previously been reported to block killing

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[2] Abbreviations used: BNLΦ, Brown Norway rat lymphoma; BSA, bovine serum albumin; Con A blasts, Concanavalin A-stimulated spleen cells; CTL, cytolytic T lymphocyte; FCS, fetal calf serum; LFA-1, lymphocyte-function-associated antigen 1; MAb, monoclonal antibody(ies); PAGE, polyacrylamide gel electrophoresis; PBS, 0.14 *M* NaCl, 0.01 *M* $NaPO_4$, pH 7.3; SDS, sodium dodecyl sulfate.

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(2–7). The second MAb defined a previously undescribed mouse lymphocyte cell surface antigen designated LFA-1. LFA-1 contains two polypeptide chains of 180,000 and 95,000 M_r and is present on B as well as T lymphocytes (8). The MAb to LFA-1 blocked T helper cells as well as CTL functions (9). In all of these studies, the antibodies were selected primarily for their ability to bind to cell surface antigens, and were then secondarily tested for blockade of function. Furthermore, only a single MAb to LFA-1 was obtained in the original study, and it remained possible that the relationship between immunoprecipitation of 180,000 and 95,000 M_r polypeptides and blockade of CTL-mediated killing was fortuitous.

To define molecules associated with a functional pathway, the most direct approach would be primary selection for hybridomas which secrete blocking antibodies. There are several advantages of using functional screening instead of immunobinding techniques. First, a larger number of different blocking MAb should be obtainable, allowing a better correlation between the type of antigen defined and blocking activity to be established. Second, antigens having an important role in function but expressed only transiently during effector–target interaction or only in low quantities would be selected by functional criteria but not by binding techniques.

In the present study, 2400 rat anti-mouse CTL hybridoma cultures were directly tested for the ability of their supernatants to inhibit T-cell-mediated cytolysis. Fifteen inhibitory cultures were selected, and the MAb specificity determined.

Five of the selected MAb recognized the Lyt-2,3 antigen, while a second group of six recognized the LFA-1 antigen, emphasizing the importance of these antigens in CTL-mediated killing. The topographic relationships of these two antigens on the cell surface has been studied using MAb cross-inhibition experiments.

MATERIALS AND METHODS

CTL generation and assay. C57BL/6J mice were primed by intraperitoneal injection of DBA/2 P815 tumor cells or rat BN lymphoma cells, and secondary CTL obtained by culturing immune spleen cells for 4 to 6 days with irradiated BALB/c $\times$ DBA/2 F_1 spleen cells or irradiated BN cells to obtain anti-P815 or anti-BN CTL, respectively, as previously described (1, 9).

Immunization and fusion. Wistar/Furth male rats were primed ip on Day -30. Two rats were injected with 10^7 C57BL/6J anti-P815 CTL per rat in 1 ml of L15 medium. The third rat was injected with 10^7 C57BL anti-P815 CTL "activated" by incubation on phytohemagglutinin-coated L cells for 3 hr at 37°C, before injection (10). On Day -3, rats were boosted intravenously with the identical amount and type of cells. On Day 0, NSI or P3X63Ag8.6.5.3 myeloma cells were fused with spleen cells from the immunized rats, using 50% (w/w) polyethylene glycol as described (11, 12). Cells were distributed into ten 96-well plates (Costar) per spleen and fed with 20% fetal calf serum/Dulbecco's modified Eagle's medium/hypoxanthine/aminopterin/thymidine as described (11). After 3 weeks, hybridoma culture supernatants were harvested and screened for inhibition of CTL-mediated killing. Specificity for Lyt-2,3, LFA-1, or other antigens was determined within the following 2 weeks by immunofluorescence and immunoprecipitation. Uncloned lines appeared monospecific by these criteria. Cloning in soft agar was attempted for all blocking cultures except for the M16 and M17 hybrids recognizing Lyt-2,3. Clones active in the CTL blocking assay were successfully isolated from M15/5, M15/15,

M17/4, M17/5, M17/7, M16/1, and M15/32. Results are reported for both cloned and uncloned lines.

Other MAb. The M7/14 anti-LFA-1 MAb was previously described (1). MAb specific for Lyt-2,3 were obtained from the hybridomas M5/24 (1), M12/4, M12/5, and M12/7. The M12 clones were obtained by immunization of rats with C57BL/6J concanavalin A-stimulated spleen cells and selection for binding to the latter cells but not to BALB/c *nu/nu* spleen cells (T. A. Springer, unpublished).

CTL assay and inhibition of cell-mediated cytolysis by culture supernatants. C57 anti-BNLΦ effector cells (50 μl) were mixed with 50 μl of hybridoma culture supernatant in microtiter V-well plates (Linbro) and incubated for 15 min at 37°C. ^{51}Cr-labeled BNLΦ targets (10^4 cells in 50 μl) were then added to each well. Plates were shaken at room temperature, centrifuged 5 min at 500*g*, and incubated at 37°C for 2–4 hr. Plates were then again shaken and centrifuged at 4°C for 10 min at 800*g*. Aliquots of 75 μl were harvested using a 12-channel pipettor (Titertek) and counted for released ^{51}Cr in a gamma spectrometer. Each culture supernatant was tested in duplicate in two separate microtiter plates.

Percentage inhibition of ^{51}Cr release was calculated as $100 \times [1\text{-}[(e - c)/(u - c)]]$, where e represents the amount of ^{51}Cr released in wells containing effector and target cells and inhibitor supernatant, u is the uninhibited ^{51}Cr release using control NSI culture supernatant, and c is the spontaneous ^{51}Cr release in control wells in which medium was substituted for the effector cells.

Iodination, precipitation, and electrophoresis of cell surface proteins. Ficoll-purified C57BL anti-P815 CTL (2×10^7 cells, viability >90%) were washed three times with cold phosphate-buffered Earle's balanced saline, pH 7.2. The cells were vectorially iodinated, using chloroglycoluril (IODO-GEN, Pierce) as described (13).

For immunoprecipitation, 50 to 100 μl of MAb culture supernatants was incubated with labeled cell lysates in a final volume of approximately 150 μl for 2 hr at 4°C. Then, 30 μl of affinity-purified, mouse IgG-absorbed, rabbit anti-rat IgG coupled to Sepharose CL-4B (10 mg/ml) was added. After shaking for 2 hr, the beads were washed three times with 0.01 *M* Tris–HCl, pH 8.0, 0.1% hemoglobin, 0.1% Triton X-100, 0.14 *M* NaCl; once with 0.01 *M* Tris–HCl, pH 8.0, 0.14 *M* NaCl; and once with 0.05 *M* Tris–HCl, pH 6.8. Samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (14) and autoradiography with enhancing screens (15).

Competition cell binding assay. M17/4.4 and M17/7.1 anti-LFA-1 MAb were purified and iodinated on RG7/9.1–Sepharose CL-4B and eluted with 1% BSA, 0.1 *M* Glycine–HCl, pH 2.5 (16). RG7/9.1, a mouse anti-rat kappa chain MAb (16), was purified with *S. aureus* protein A–Sepharose affinity chromotography (17) and coupled to Sepharose at 3.3 mg/ml. M12/5.2 and M12/7.2 anti-Lyt-2,3 MAb were purified by DE-52 cellulose (Whatman) chromatography (18) and iodinated in solution with chloroglycoluril (19).

In competition assays, 25 μl of hybridoma culture supernatants, appropriately diluted in 10% FCS–PBS, was mixed with 25 μl of 5×10^7 Con A blasts/ml in 10% BSA in PBS and shaken at 4°C in microtiter plates for 1 hr on a Microshaker II (Cooke, Alexandria, Va.). ^{125}I-MAb (25 μl in 10% BSA) was added and shaking continued for ½ hr. Plates were washed three times with 0.25% BSA in PBS, and pellets were removed with two washes of 0.25% BSA in PBS and gamma counted. All assays were in duplicate in separate microtiter plates.

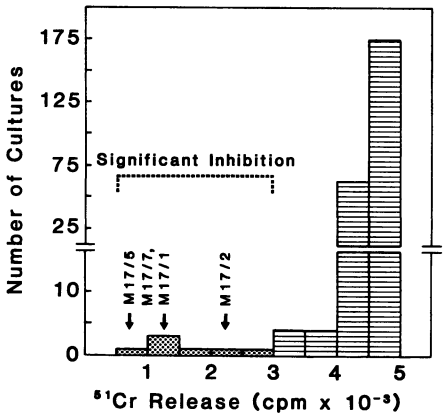

FIG. 1. Identification of hybridoma culture supernatants blocking CTL function in the ^{51}Cr-release assay. Effector cells were pretreated with an equal volume of hybridoma culture supernatant for 15 min at room temperature, then ^{51}Cr-labeled BNLΦ cells were added, and the assay was completed as described in Materials and Methods. Arrows indicate hybridoma lines selected in this assay. Spontaneous ^{51}Cr release in the absence of CTL was 526 cpm, and uninhibited ^{51}Cr release in the presence of NSI culture supernatant and CTL was 4295 cpm.

Other methods. Immunoglobulin subclass was determined by double immunodiffusion with subclass-specific antibodies (Miles Laboratory, Elkhart, Ind.). Glutaraldehyde-fixed cells were prepared as reported (20). Fluorescent labeling and analyses on a Becton–Dickinson FACS II were as previously described (8).

RESULTS

Selection of rat anti-mouse monoclonal antibodies blocking CTL-mediated killing. Results from three independent fusions are reported here. Wistar/Furth rats were immunized with mouse anti-P815 (allogeneic) CTL-rich populations for fusions M15 and M17, and with the same cells but after "activation" by incubation on PHA-coated L cells (10) for fusion M16.

Over 2400 hybridoma culture supernatants were screened for antibodies which could block CTL-mediated killing by binding to determinants on the CTL effector cell. Supernatants were incubated in the absence of complement with mouse CTL specific for rat BN lymphoma cells, ^{51}Cr-labeled BN lymphoma target cells were added, and ^{51}Cr release was measured after 2 to 4 hr. Since both the MAb and target cells are of rat origin, this xenogeneic CTL assay system avoids the selection of hybridomas secreting blocking antibodies that bind to antigens on the target cell (1).

A representative functional screening of 240 hybridoma cultures from the M17 fusion is shown in Fig. 1. Only those hybridoma supernatants giving more than 30–35% inhibition in ^{51}Cr release were selected. They were retested, transferred to larger 2-ml culture wells, and retested again. Fifteen different hybridoma culture lines which gave consistent inhibition of T-cell-mediated cytolysis were selected for detailed analysis and cloning.

Cell structures recognized by blocking MAb. To characterize the structures defined by the selected MAb, lysates of ^{125}I-surface-labeled B6 anti-P815 CTL preparations were immunoprecipitated and analyzed by SDS–PAGE and autoradiography (Fig.

2). One group of MAb, including M15/5, M17/2, M17/8, M17/9, and M17/11, precipitated one polypeptide of 35,000 M_r (Fig. 2, lanes 4–7 and 18). This polypeptide corresponded to the Lyt-2,3 antigen precipitated by the M12/7.2 MAb (Fig. 2, lane 3). A second group of MAb, namely, M15/15, M17/1, M17/4, M17/5, and M17/7, immunoprecipitated an antigen containing two polypeptide chains of 180,000 and 95,000 M_r (Fig. 2, lanes 8–12). This antigen appeared identical to the LFA-1 antigen (1) defined by the M7/14 MAb (Fig. 2, lane 2). M17/6 and M15/32 also specifically precipitated LFA-1, but more weakly. M15/32 clones and subclones were inactive in indirect immunoprecipitation, but partially purified M15/32.2.9 IgG coupled to Sepharose immunoprecipitated small amounts of LFA-1. No bands

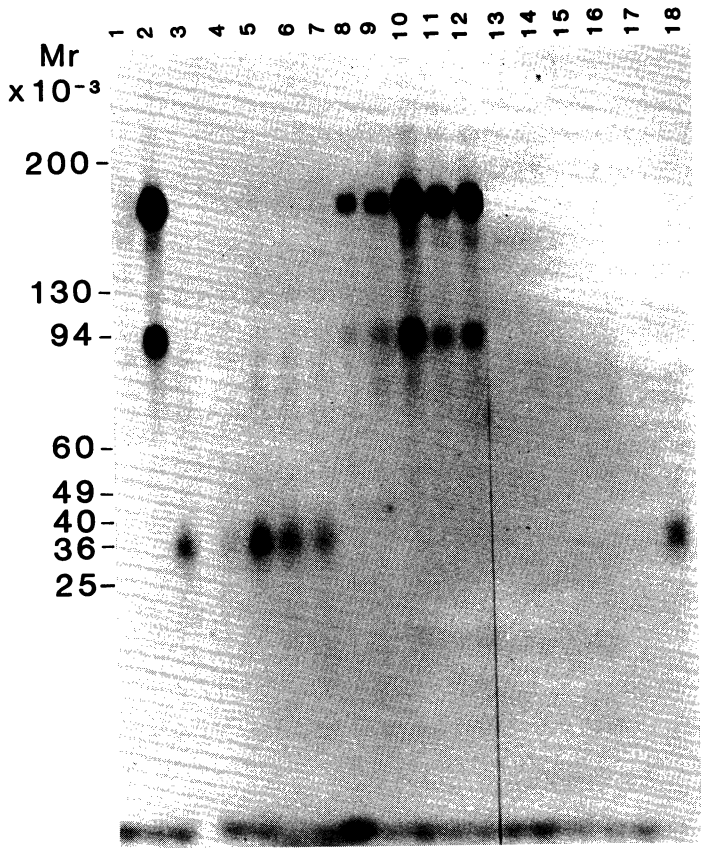

FIG. 2. SDS–PAGE of ^{125}I-surface antigens immunoprecipitated by CTL-blocking MAb. Spleen cells from B6 mice primed with P815 and restimulated *in vitro* were surface-labeled with ^{125}I using Iodogen. Cell lysates were immunoprecipitated with 100 μl NSI supernatant plus added normal rat IgG as control (lane 1); or with 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–PAGE in a 5–15% polyacrylamide gradient gel and autoradiographed for 3 days using an intensifying screen. Proteins run in the same gel as molecular weight standards were as described previously (8).

were immunoprecipitated in this and in two to six other experiments by the M16/1, M17/3, and M17/10 MAb.

Cell distribution. The cellular distributions of the antigens recognized by the blocking MAb were studied by labeling spleen and thymus cells with MAb, followed by FITC-rabbit anti-rat IgG and immunofluorescence flow cytometry. The M15/5 and M17/2 MAb (Fig. 3) and M17/8, M17/9, and M17/11 MAb (data not shown) gave the same pattern of labeling of spleen and thymus cells as a previously described anti-Lyt-2,3 MAb, M5/24 (Fig. 3). They labeled 17% of spleen cells and 83% of thymocytes. The immunofluorescence data, together with the immunoprecipitation results, strongly suggest these MAb recognize Lyt-2,3. The intensity of labeling by anti-Lyt-2,3 MAb was twofold greater for γ2a MAb, e.g., M17/2, than for γ2b MAb, e.g., M15/5. This was due to the presence of subclass-specific antibodies in the mouse IgG-absorbed, FITC-rabbit anti-rat IgG reagent (21). The data are consistent with all anti-Lyt-2,3 MAb binding to the same number of sites/cell. Indirect binding

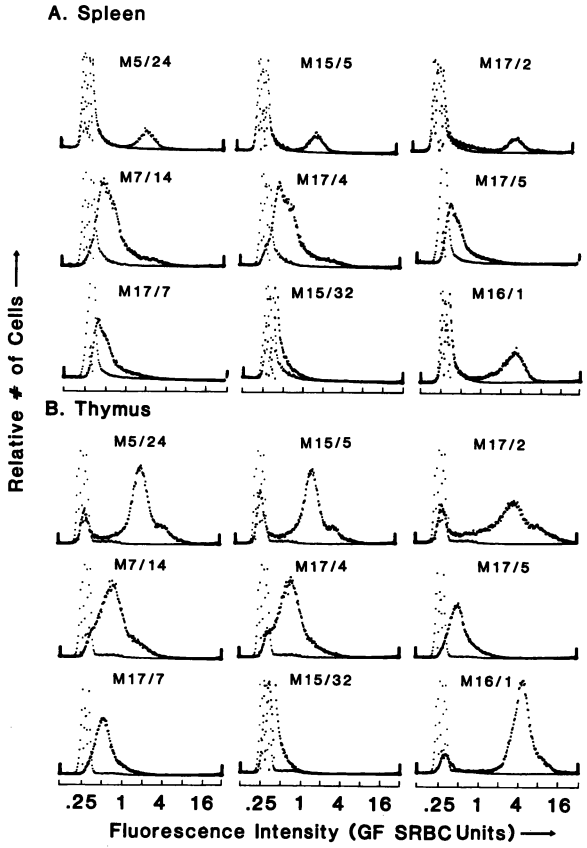

FIG. 3. Immunofluorescence flow cytometry of spleen and thymus cells stained by blocking MAb. Cells were labeled with blocking hybridoma culture supernatants (dark dots), or with the M1/69.16 HK non-binding MAb as control (dim dots), then with FITC-rabbit $F(ab')_2$ anti-rat IgG absorbed with mouse IgG. Fluorescence analyses of 40,000 cells were carried out on a Becton–Dickinson FACS II with a log amplifier as previously described (8). Fluorescence intensity was calibrated relative to glutaraldehyde-fixed sheep red blood cells (GF SRBC).

experiments (not shown) using these MAb showed they bound to T but not B lymphocytes.

The second group of MAb, namely, M17/4, M17/5, and M17/7 (Fig. 3), and M15/5, M17/1, and M17/6 (not shown), gave a staining identical to that of the M7/14 MAb defining LFA-1 (Fig. 3). As previously described for the M7/14 MAb, >87% of spleen cells were labeled with heterogeneity in the intensity of staining. Thymocytes were >86% positive. The γ2a MAb stained twice as brightly as γ2b MAb.

The M16/1 MAb labeled 35% of spleen cells and 92% of thymocytes (Fig. 3). The percentage and intensity of labeling of spleen and thymus cells, and the observation of strong binding to brain and to T but not B lymphocytes (not shown) suggest that M16/1 MAb recognizes the Thy-1 antigen (22).

The M15/32 MAb gave weak but significant labeling of spleen and thymus cells (Fig. 3). This suggests either that M15/32 MAb recognizes an antigen expressed in low quantity or that M15/32 MAb has a low avidity for its target antigen.

Inhibition of function. The characteristics and the inhibitory effect of the 15 selected MAb are summarized in Fig. 4. The anti-Lyt-2,3 MAb gave inhibition of cytotoxicity ranging between 60 and 80%, with the exception of M17/11 MAb, which only gave a partial inhibition of 35%.

The group of six anti-LFA-1 MAb gave the strongest inhibition of 60 to 96%.

The M16/1 anti-Thy-1 MAb gave weak inhibition of 38%, which was variable from one experiment to another. A different IgM anti-Thy-1 MAb has also been reported to significantly inhibit CTL-mediated killing (cited in Ref. (23)). Microscopic examination of CTL treated with M16/1 showed they were agglutinated into large clumps. It thus appears that blocking by M16/1 is due to CTL agglutination, rather than to binding to a CTL structure essential for killing.

The partial inhibition of CTL activity by M15/32 MAb seemed to be consistent in more than 20 different experiments.

The M17/10 and M17/3 MAb gave about 50% inhibition of killing. Attempts to clone these lines were unsuccessful, hindering identification of the target antigen.

Inhibition by anti-Lyt-2,3 and LFA-1 MAb is exerted by binding to different sites on the effector cells. Inhibition of CTL-mediated killing by both anti-Lyt-2,3 and anti-LFA-1 MAb could be due either to a close topographic situation of these antigens on the cell surface membrane or to a specific separate effect by MAb against the two antigens. In order to distinguish between these two possibilities, competition experiments between unlabeled and labeled MAb were carried out. Competition was tested on both fresh and glutaraldehyde-fixed cells, and at both 4 and 37°C. Results were similar in all cases, and a representative experiment on glutaraldehyde-fixed cells is shown in Table 1. Binding of the ^{125}I-labeled M17/4 and M17/7 anti-LFA-1 MAb was inhibited by all four anti-LFA-1 MAb tested, suggesting that these MAb define identical or topographically related epitopes on the LFA-1 molecule. Cross-blocking with the M12/5 and M12/7 anti-Lyt-2,3 MAb was also tested. These MAb were chosen because they recognize topographically distinct determinants on Lyt-2,3 molecule (Table 1, and T. A. Springer, unpublished), increasing the probability of finding a contact between Lyt-2,3 and LFA-1. Neither M12/5 nor M12/7 gave significant inhibition of ^{125}I-anti-LFA-1 MAb binding. The reciprocal experiment showed that the anti-LFA-1 MAb also did not inhibit binding of the anti-Lyt-2,3 MAb to Con A blasts. These experiments show LFA-1 and Lyt-2,3 are spatially distinct on the cell surface.

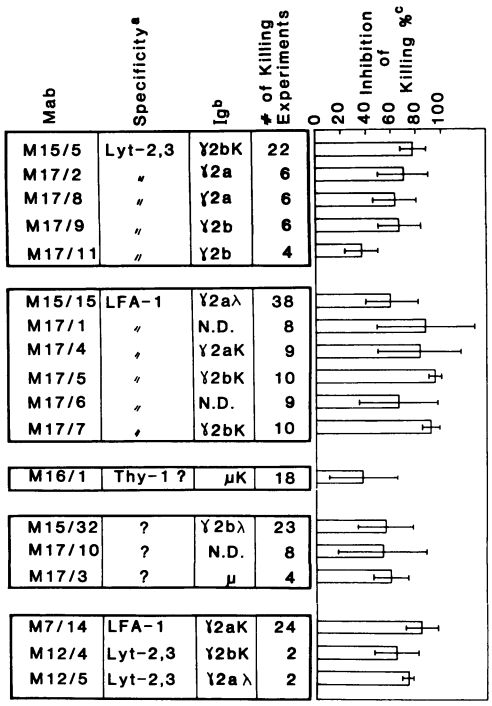

FIG. 4. Inhibition by monoclonal antibodies of B6 anti-BNLΦ CTL-mediated killing. Effector cells were pretreated with an equal volume of hybridoma culture supernatant for 15 min at room temperature; then ^{51}Cr-labeled BNLΦ cells were added, and the assay was completed. Experiments utilized effector-to-target cell ratios ranging between 1:1 and 60:1. Each observation represents the mean of a duplicate test. Data were accumulated from separate experiments performed on different days. Specific ^{51}Cr release in the absence of antibody (NSI or M1/69 HK controls) was in the range 25–90%. The spontaneous release of ^{51}Cr in wells containing the appropriate culture supernatant but with effector cells omitted was 7.7–19%. Percentage inhibition of specific ^{51}Cr release is expressed relative to cultures treated with NSI culture supernatant. [a]Determined by the molecular weight of polypeptides immunoprecipitated from ^{125}I-labeled C57B6 anti-P815 CTL and by cell distribution. [b]Immunoglobulin heavy chain subclass was determined as described under Materials and Methods. Light chain type was determined in a competition assay using mouse monoclonal anti-rat kappa chain (16). [c]Inhibition of specific ^{51}Cr release from BNLΦ target cells. Error bars show the standard deviation. ND, not determined.

The M15/32 MAb inhibited the binding of the ^{125}I-M17/4 anti-LFA-1 MAb to Con A blasts, but not binding by the ^{125}I-anti-Lyt-2,3 MAb. Direct cell binding studies using purified ^{125}I-labeled M15/32 MAb were negative. These findings and the immunoprecipitation results suggest that M15/32 may be a low affinity anti-LFA-1. However, since M15/32 differed from other anti-LFA-1's in its immunofluorescence pattern (Fig. 3) and its inability to block conjugate formation (Davignon *et al.*, unpublished), this assignment must be considered tentative, as indicated in Fig. 4.

DISCUSSION

In this report, a direct functional approach has been used to select xenogeneic rat anti-mouse monoclonal antibodies which inhibit T-cell-mediated cytolysis. MAb that inhibited function by binding to target cell antigens were avoided by the doubly

TABLE 1

Competition between Anti-Lyt-2,3 and Anti-LFA-1 MAb for Binding to Concanavalin A-Stimulated Mouse Spleen Cells

^{125}I-Labeled MAb	Unlabeled MAb						
	M7/14	M17/4	M17/5	M17/7	M12/5	M12/7	M15/32
	% Inhibition						
Anti-LFA-1							
M7/14	(100)	99	89	95	26	8	N.D.
M17/4	93	(100)	95	100	0	0	72
M17/7	97	100	100	(100)	1	0	N.D.
Anti-Lyt-2,3							
M12/5	1	3	0	2	(100)	0	0
M12/7	7	4	0	5	5	(100)	5

Note. Binding of ^{125}I-labeled Lyt-2,3 and LFA-1 MAb to glutaraldehyde-fixed Con A blasts was inhibited by preincubation with spent culture supernatants from hybridoma lines in the competition assay (Materials and Methods). No inhibition and 100% inhibition were determined with the nonactive M1/69HK culture supernatant (1.5–20% of input), and the homologous unlabeled MAb, respectively.

xenogeneic assay system in which MAb and target cells were both of rat origin. This screening method permitted selection of only those MAb that blocked cytolytic activity by binding to antigens on the effector cell. Hybridoma cultures that gave more than 30–35% consistent inhibition of activity were selected for cloning and their specificity was investigated.

Five MAb recognizing the Lyt-2,3 antigen were found to consistently block T-cell-mediated cytolysis. Earlier studies have shown that Lyt-2,3 is a marker for T cells with cytolytic or suppressor functions (24, 25). A number of groups have also reported inhibition of T-cell-mediated cytolysis by alloantisera or monoclonal antibodies against the Lyt-2,3 antigen (1–7). The extent of inhibition by the anti-Lyt-2,3 MAb ranged between 35 and 80%. The heterogeneity in the inhibition might be due to differences in the affinity of the MAb or the topographic site recognized. MacDonald *et al.* (26) found that cytolytic T-lymphocyte clones differed widely in their susceptibility to inhibition by anti-Lyt-2,3 MAb. MAb to Lyt-2,3 block killing during the Mg^{+2}-dependent but not the Ca^{2+}-dependent stage (27) and also block CTL-target conjugate formation (7).

Six different anti-LFA-1 MAb were obtained in these experiments that blocked killing by 60 to 90%. A seventh MAb, M15/32, also appears to be a low-avidity anti-LFA-1 MAb on the basis of immunoprecipitation and cross-blocking experiments. LFA-1 was originally defined by the CTL-blocking M7/14 MAb (1, 8, 9). It contains noncovalently associated (28) α and β polypeptide chains of 180,000 and 95,000 M_r, respectively. The LFA-1 antigen is expressed in both B and T lymphocytes (8). The anti-LFA-1 MAb obtained here recognize exactly the same antigen as defined by the molecular weights of its subunits and its tissue distribution. Three of the anti-LFA-1 MAb selected here were compared to the M7/14 MAb in cell-binding competition assays. All four MAb recognized a very close or identical topographic region on LFA-1. Anti-LFA-1 MAb blocks T-cell-mediated cytolysis by inhibiting the CTL from forming an adhesion to the target cell (9). LFA-1 antigen thus appears to

participate in the Mg^{2+}-dependent antigen-recognition step of CTL-mediated killing (9). However, LFA-1 appears distinct from the T-cell-antigen receptor because it is found on B cells and 75% of bone marrow cells (8). In addition to its effects on CTL-mediated killing, the M7/14 anti-LFA-1 MAb also blocks the induction of antigen-specific T-helper-cell proliferation (9). LFA-1 antigen thus appears crucially important for several lymphocyte functions requiring cell–cell contact. In the original studies, only a single anti-LFA-1 MAb was obtained. Since even MAb might have multiple specificities (29), it remained possible that the relationship between blocking activity and precipitation of an antigen containing 180,000 and 95,000 M_r polypeptides was fortuitous. However, the selection of six MAb by the criterion of inhibition of CTL-mediated killing, all of which recognize the LFA-1 antigen, strongly confirms the correlation between this structure and killing function. Pierres *et al.* (30) have also recently described a MAb which blocks CTL-mediated killing, immunoprecipitates two polypeptides of 180,000 and 94,000 M_r, binds to T and B lymphocytes, and thus appears identical to LFA-1. Sarmiento *et al.* (31) have also described a similar MAb.

Structural homologies between LFA-1 antigen and Mac-1, a macrophage/monocyte lineage marker, have been reported (28, 32). Both molecules have identical or highly homologous β subunits, and different α subunits. All anti-LFA-1 MAb selected by functional criteria described in this study failed to cross-react with the Mac-1 antigen (unpublished results), but cross-reactive antisera and MAb have been reported (28, 32).

Lyt-2,3 and LFA-1 are the only two effector cell antigens thus far suggested by blocking experiments to participate in CTL-mediated killing. It is interesting that both antibodies block the Mg^{2+}-dependent antigen recognition–adhesion step, when the CTL–target conjugate is formed. One possible explanation for this would be a close topographic relationship between Lyt-2,3 and LFA-1. If a MAb bound to one antigen provided sufficient steric hindrance of a neighboring molecule to inhibit its participation in CTL-mediated killing, it would also be expected to inhibit binding of a MAb to that molecule. However, MAb cross-competition experiments could reveal no interaction between Lyt-2,3 and LFA-1, even when two different topographic sites on Lyt-2,3 were examined. This suggests that Lyt-2,3 and LFA-1 play independent roles in CTL-mediated killing.

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