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Immunology

Three distinct antigens associated with human T-lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3

(anti-HLA-DR cytolytic T lymphocyte/monoclonal antibody/inhibition of killing)

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ABSTRACT **Monoclonal antibodies were prepared to anti-HLA-DR cytolytic T lymphocytes (CTLs) and screened for inhibition of CTL-mediated killing. Binding of monoclonal antibodies to four types of molecules, LFA-1, LFA-2, LFA-3, and HLA-DR, inhibited killing, suggesting that these molecules participate in the CTL–target cell interaction. The antigens were characterized by immunoprecipitation, crosslinking, $NaDodSO_4$/polyacrylamide gel electrophoresis, and immunofluorescence flow cytometry. The LFA-1 antigen contains α and β polypeptide chains of M_r 177,000 and 95,000 that are noncovalently associated in an $\alpha_1\beta_1$ structure. It is present on both B and T lymphocytes and marks subpopulations that differ in quantitative expression. Human LFA-1 appears to be the homologue of mouse LFA-1. Human LFA-2 is of M_r 49,000 with a minor component of M_r 36,000. It is expressed on CTL lines but not on a B-cell line and in peripheral blood preferentially on T lymphocytes. Human LFA-3 is of M_r 60,000 and is expressed on both B and T lymphocytes.**

Antigen-specific T-lymphocyte-mediated killing is a multistep process involving antigen recognition and adhesion of the cytolytic T lymphocyte (CTL) to the target cell, delivery of the lethal hit, and target cell lysis (1–3). Bystander cells and the CTL itself are unharmed in the killing reaction, and the CTL can detach and engage in further killing encounters. The molecular basis of CTL-mediated killing is of considerable interest, both in itself and for our understanding of T lymphocyte-mediated immunity in general. In previous studies designed to identify the molecules involved in mouse CTL-mediated killing, rat monoclonal antibodies (MAb) were prepared to mouse CTLs and screened for their ability to inhibit killing (4–9). MAb to the Lyt-2,3 and lymphocyte function-associated 1 (LFA-1) antigens, but not MAb to many other types of surface structures, were found to inhibit killing. The LFA-1 antigen appears generally important in mouse T-lymphocyte immune interactions, since MAb to it block not only allogeneic and xenogeneic CTL-mediated killing but also T-helper-cell antigen-specific proliferative responses and T-cell-dependent B-cell responses (4). Anti-LFA-1 MAb exert their effect on killing by binding to the CTL rather than to the target cell and block the formation of CTL-target conjugates and the Mg^{2+}-dependent adhesion step but not the Ca^{2+}-dependent lethal hit stage (4, 7). The mouse LFA-1 antigen contains two noncovalently associated polypeptide chains of M_r 180,000 and 95,000 (10, 11). No human homologue of mouse LFA-1 has been defined, but Leu-2a (OKT8) is the human homologue of Lyt-2,3 (12).

We have probed the molecular mechanisms of human T-lymphocyte-mediated killing by preparing MAb to human anti-HLA-DR CTLs and directly selecting for MAb that block killing. Anti-HLA-DR CTLs express OKT4 but not the OKT8 antigen and thus differ in surface phenotype from anti-HLA-A and -B CTL, which are $OKT4^-$ $OKT8^+$ (13–16). Previous CTL blocking studies done with MAb that were initially selected for labeling of lymphocyte subpopulations have suggested that OKT3 (15, 17–19), Leu-2a (OKT8) (19–21), and OKT4 (14, 15) are associated with CTL-mediated killing; however, none of these antigens has thus far been shown to be the T-cell antigen receptor. Direct screening for MAb blocking killing should be a more efficient method of identifying the antigen receptor and other molecules involved in CTL–target cell interactions and lethal hit delivery. In addition, it was of interest to determine whether antigens associated with $OKT4^+$ killers would be associated with the function of $OKT8^+$ killers or $OKT4^+$ helpers and whether the LFA-1 antigen found in the mouse and its association with T-cell function had been evolutionarily conserved in humans.

MATERIALS AND METHODS

Cell Lines. JY (HLA-$A2^+$, -$B7^+$, -DR4,6^+) and Daudi (HLA-A^-, -B^-, -$DR6^+$) B-lymphoblastoid cell lines were used as stimulator and target cells and maintained in RPMI 1640 medium/10% fetal calf serum. Generation of long-term $OKT4^+T8^-$ anti-HLA-DR6 CTL lines and $OKT4^-T8^+$ anti-HLA-A2 and -B7 CTL lines has been described (13, 15, 22).

Hybridomas. BALB/c mice were injected with 10^7 cells of the HLA-DR CTL line intraperitoneally on days −49 and −35 and intravenously on day −3. Spleen cells from three mice were fused on day 0 with P3X63Ag8.653 or NSI, divided in selection medium into ten 96-well plates (Costar, Cambridge, MA), and grown as described (23, 24). The 44 cultures showing >30% inhibition of killing were transferred to 2-ml culture wells. Those cultures positive for immunoprecipitation or maintaining blocking activity after further growth were cloned and subcloned in soft agar. Selection of active clones and subclones was by both inhibition of CTL-mediated killing and the ^{125}I-labeled anti-mouse Ig indirect binding assay. Anti-mouse Mac-1 and LFA-1 MAb (7) and anti-Leu-1 and -4 MAb (12, 20), provided by R. Evans, Sloan–Kettering, New York, have been described.

CTL Inhibition Assay. Anti-HLA-DR CTL effectors (2.5–10 × 10^3 in 50 μl) were mixed with 50 μl of hybridoma culture supernatants in V-well microtiter plates and incubated for 15 min at 37°C. ^{51}Cr-Labeled JY or Daudi target cells (10^3 in 50 μl) were added and ^{51}Cr release was measured after 2–4 hr. The assay and calculation of ^{51}Cr release have been described (4, 13). Effector/target ratios were chosen to give 50% specific ^{51}Cr

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Abbreviations: CTL, cytolytic T lymphocyte; LFA, lymphocyte function associated; MAb, monoclonal antibody(ies).

release. Inhibition experiments were carried out in the absence of complement.

Iodination, Immunoprecipitation, and Electrophoresis of Cell Surface Proteins. Hypaque/Ficoll-purified OKT4$^+$ CTL, JY cells, C57BL/6J mouse anti-P815 CTL, and 4-day thioglycollate-elicited mouse peritoneal exudate cells were iodinated using chloroglycoluril (IODO-GEN, Pierce) (5). Triton X-100 cell lysates were mixed with 50 μl of MAb-containing culture supernatants and immunoprecipitation was carried out as described (25). As second antibodies, a mixture of 30 μl of rabbit anti-mouse IgG and 5 μl of sheep anti-mouse IgM or 100 μl (7.4 μg) of the 187.1 anti-mouse κ chain MAb (26) (a gift of M. Scharff) together with 50 μl of a 10% suspension of *Staphylococcus aureus* were used for mouse MAb, and 30 μg of RG7/

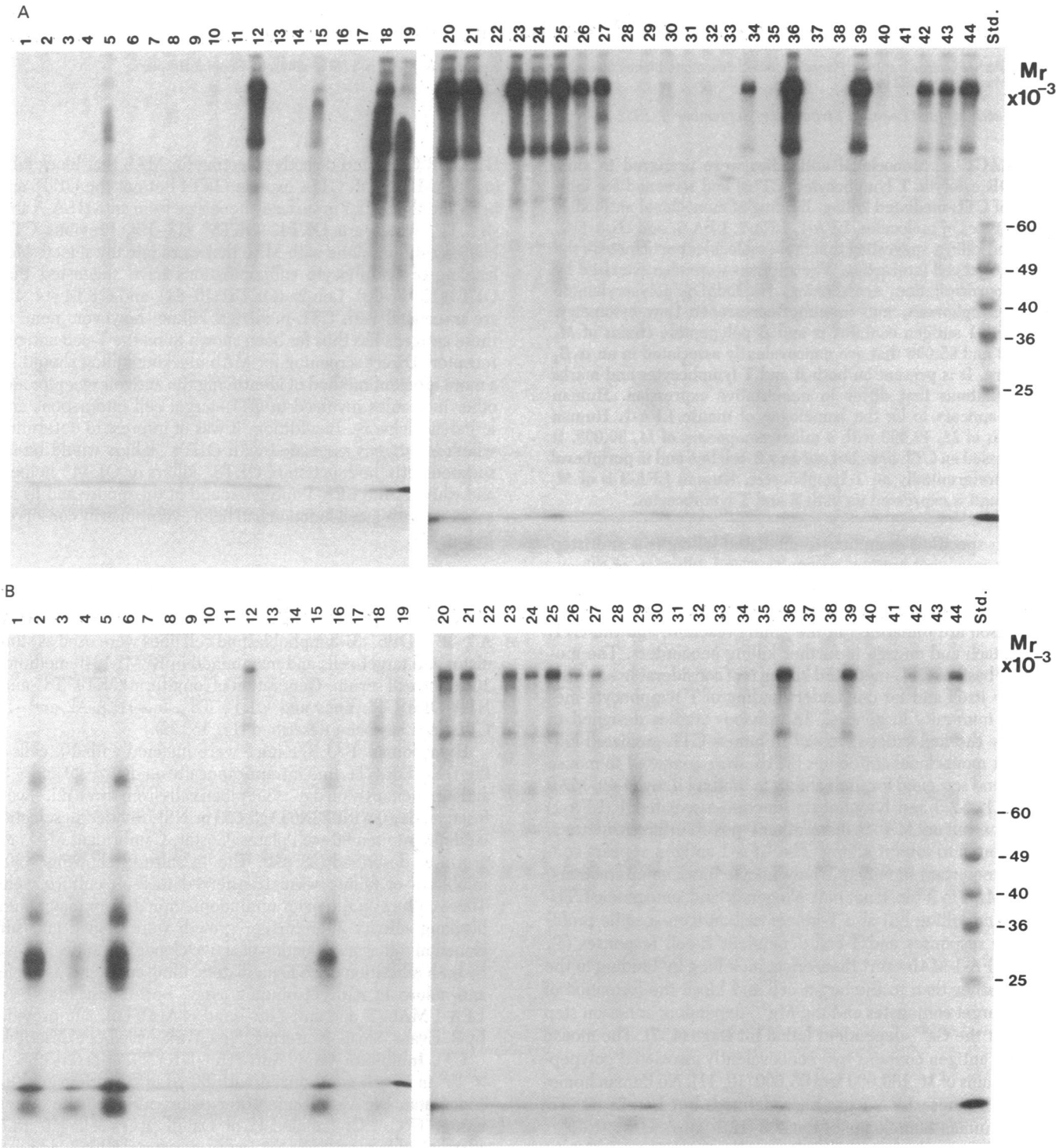

FIG. 1. NaDodSO$_4$/polyacrylamide gel electrophoresis of ^{125}I-labeled antigens immunoprecipitated by CTL-blocking hybridoma culture supernatants. Human anti-HLA-DR CTL (*A*) or JY target (*B*) cell lines were surface labeled with ^{125}I by using IODO-GEN. Cell lysates were immunoprecipitated with 50 μl of hybridoma culture supernatant from 44 selected culture lines: TS1/1–TS1/18, lanes 1–18, respectively; TS1/21 and TS1/22, lanes 19 and 20; TS2/1–TS2/24, lanes 21–44, respectively. Reduced samples were subjected to NaDodSO$_4$/10% polyacrylamide gel electrophoresis and autoradiography.

7.6 anti-rat κ chain MAb (27) coupled to Sepharose CL-4B at 2 mg/ml was used for rat MAb. Samples were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography with enhancing screens (28). Molecular weight standards included filamin, myosin, α_2-macroglobulin, β-galactosidase, phosphorylase a, serum albumin, catalase, fumarase, and creatine kinase.

Immunofluorescence. Immunofluorescence flow cytometry was done on a FACS II after labeling cells with MAb-containing culture supernatants and affinity-purified fluorescein isothiocyanate-conjugated anti-mouse IgG (Zymed, S. San Francisco, CA) as previously described (5).

RESULTS

The anti-HLA-DR CTL lines used in these studies were obtained after repeated stimulation of human blood lymphocytes with Daudi, an HLA-DR6$^+$ mutant cell line that lacks HLA-A and -B antigens. Spleen cells from mice immunized to anti-HLA-DR CTL were fused with P3X63Ag8.653 or NSI myeloma cells, for fusions TS1 or TS2, respectively. After 2 wk, supernatants from hybridomas showing vigorous growth were screened for their ability to inhibit killing of ^{51}Cr-labeled JY targets by anti-HLA-DR CTLs. Of 864 supernatants tested in duplicate, 44 gave reproducible inhibition of 30–86%. The nature of the molecules recognized by the blocking antibodies was investigated by immunoprecipitation. Because blockade could be due to binding to molecules on either the CTL effectors or the JY targets, immunoprecipitation was carried out from both types of cells (Fig. 1).

A strikingly high proportion of the blocking supernatants, 20 of 44, immunoprecipitated polypeptide chains of M_r 177,000 and 95,000 from the CTL line and, in lesser amounts, from JY (Fig. 1, lanes 5, 12, 15, 18–21, 23–27, 30, 32, 34, 36, 39, and 42–44). Seven subcloned independent hybridomas have been isolated that produce MAb that immunoprecipitate this antigen (Fig. 2A, lanes 1–7; Table 1), which has been designated human lymphocyte function-associated 1 (LFA-1) antigen. Six of these MAb gave inhibition of killing ranging from 32% to 60%; one gave only 9% (Table 1). The strongest anti-LFA-1 blocker, TS1/18, gave potent dose-dependent inhibition of killing at concentrations as low as 0.2 μg/ml (Fig. 3).

Human LFA-1 is very similar to mouse LFA-1 (Fig. 2A, lanes 8–12). $NaDodSO_4$/polyacrylamide gel electrophoresis of human LFA-1 and mouse LFA-1 showed that the β polypeptides are of identical M_r (95,000; Fig. 2A, lane 7, and Fig. 2B, lane 2). Both α chains are of M_r close to 180,000 but that of the mouse is slightly higher, by about 3,000. The α chain of human LFA-1 is closer in M_r to the α chain of mouse LFA-1 than to the α chain of the related mouse Mac-1 macrophage antigen (Fig. 2A, lanes 13–15). The human LFA-1 α and β polypeptides were also present after isolation under nonreducing conditions (Fig. 2C, lane 1), showing they are not disulfide-linked. The higher mobility of the chains in the absence of reduction (apparent M_r, 155,000 and 90,000; Fig. 2C, compare lanes 1 and 5) indicates the presence of intrachain disulfide linkages.

Precipitation by the anti-human LFA-1 MAb of two different polypeptides could be because both bore the antigenic determinant(s) or because the chains were noncovalently associated. To examine this, lysates were treated with the cleavable crosslinking reagent dithiobis(succinimidyl)propionate, immunoprecipitated, and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis. With higher concentrations of crosslinker the α and β polypeptides disappeared, and crosslinked products of M_r 225,000–270,000 appeared (Fig. 2C, lanes 2–4). The M_r of the crosslinked products is very close to the combined apparent M_r of the nonreduced subunits—i.e., 245,000. Reductive cleavage of the crosslinks showed that the crosslinked product contains the α and β polypeptides (Fig. 2C, lane 6). These findings strongly suggest that the α and β subunits are present in an $\alpha_1\beta_1$ complex in human LFA-1.

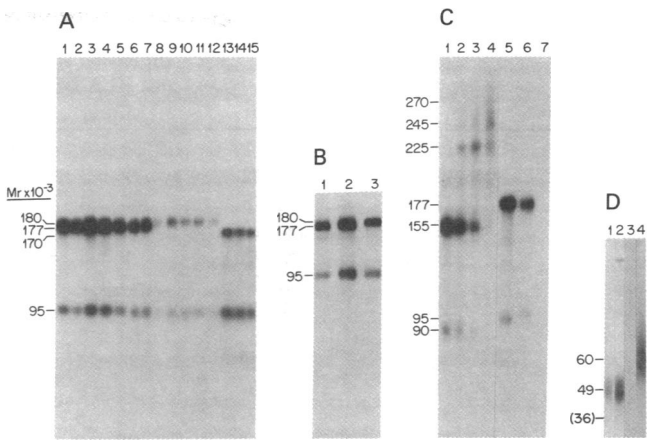

FIG. 2. Biochemical characterization of human LFA-1, -2, and -3 antigens. (*A*) Comparison of human LFA-1 and mouse LFA-1 and Mac-1 antigens. Lysates of ^{125}I-labeled human CTL lines were immunoprecipitated with anti-human LFA-1 MAb TS1/12.1.1 (lane 1), TS2/14.1.1 (lane 2), TS1/22.1.1 (lane 3), TS2/4.1.1 (lane 4), TS2/6.1.1 (lane 5), and TS1/18.1.1 (lanes 6 and 7). Mouse CTL lysates were immunoprecipitated with anti-mouse LFA-1 MAb M17/4.4 (lanes 7 and 8), M17/5.1 (lane 9), M17/7.2 (lane 10), M18/2.9 (lane 11), and M7/14.3.7 (lane 12). Lysates of mouse thioglycollate-elicited peritoneal exudate cells were immunoprecipitated with anti-mouse Mac-1 MAb M19/23.2 (lane 13), M19/24.1 (lane 14), and the anti-LFA-1 and Mac-1 crossreactive MAb M18/2.9 (lane 15). Reduced samples were subjected to $NaDodSO_4$/5–10% gradient polyacrylamide gel electrophoresis. (*B*) Coelectrophoresis of human and mouse LFA-1. Human and mouse LFA-1 antigens were immunoprecipitated as in *A* with TS1/18.1.1 and M17/4.4 MAb, respectively. Lanes: 1, human LFA-1; 2, 1:1 mixture of human and mouse LFA-1; 3, mouse LFA-1. Reduced samples were subjected to 9% $NaDodSO_4$/polyacrylamide gel electrophoresis. (*C*) Crosslinking of human LFA-1 α and β subunits. Lanes: 1–4, respectively, ^{125}I-labeled human CTL lysates (800 μl) were not treated or were crosslinked with 1, 4, or 16 μl of dithiobis(succinimidyl)propionate at 5 mg/ml (10), immunoprecipitated with a mixture of TS1/18.1.1, TS1/11.2.1, TS1/22.1.1, and TS2/6.1.1 MAb, and nonreduced samples were analyzed; 5 and 6, samples that had not been treated or had been treated with 16 μl of crosslinker were analyzed after reduction with 5% 2-mercaptoethanol; 7, a sample immunoprecipitated with P3X63 supernatant as control. Samples were subjected to $NaDodSO_4$/6% polyacrylamide gel electrophoresis. (*D*) LFA-2 and LFA-3 antigens. ^{125}I-labeled human CTL extracts were immunoprecipitated with TS1/8.1 (lane 1), TS2/18.2 (lane 2), NSI supernatant as control (lane 3), and TS2/9.1 (lane 4). Samples were subjected to $NaDodSO_4$/10% polyacrylamide gel electrophoresis and autoradiographed. Exposure times were 1 day (lanes 1 and 2) or 5 days (lanes 3 and 4).

To examine the distribution of LFA-1 on normal cells, peripheral blood lymphocytes were labeled with MAb and fluorescein-conjugated anti-mouse IgG and analyzed by immunofluorescence flow cytometry (Fig. 4A). The cells, which were 82–90% T lymphocytes, as shown by staining with Leu-4 and Leu-1 MAb, and 13% B lymphocytes, as shown with anti-IgM, were >98% human LFA-1$^+$, showing that LFA-1 is present on normal T and B lymphocytes. This was also confirmed by double labeling with rhodamine-conjugated anti-human Ig. Two subpopulations were found that differed in quantitative expression of LFA-1 (Fig. 4A). A subpopulation of about 1/3 of the cells expressed 3-fold more LFA-1 than the other 2/3 of the cells. All seven anti-human LFA-1 gave exactly the same pattern and intensity of labeling as shown in Fig. 4A, which represents superimposed curves of two different anti-LFA-1 MAb. A mix-

Table 1. Effect of MAb on anti-HLA-DR CTL-mediated killing

Antigen	$M_r \times 10^{-3}$	MAb	Inhibition of anti-HLA-DR killing
LFA-1	177, 95	TS1/11	34 ± 5
	177, 95	TS1/12	32 ± 5
	177, 95	TS1/18	60 ± 3
	177, 95	TS1/22	41 ± 3
	177, 95	TS2/4	9 ± 3
	177, 95	TS2/6	43 ± 2
	177, 95	TS2/14	41 ± 3
LFA-2	49, (36)	TS1/8	64 ± 5
	49, (36)	TS2/18	65 ± 5
LFA-3	60	TS2/9	73 ± 8
HLA-DR	34, 29	TS1/2	39 ± 8
	34, 29	TS1/16	38 ± 6

All MAb were IgG1 κ as determined by double immunodiffusion with subclass-specific antibodies and by the indirect binding assay using labeled anti-mouse κ chain MAb (26). Inhibition was determined relative to an NSI supernatant of specific ^{51}Cr release from JY or Daudi targets. Results are means ± SEM of 7–19 independent experiments using supernatants from both cloned and subcloned lines.

ture of several of the different anti-LFA-1 MAb gave increased fluoresence intensity, showing binding to distinct sites (not shown).

Four of the 44 inhibitory hybridoma cultures defined the LFA-2 antigen. Active clones and subclones were obtained from two lines, TS1/8 and TS2/18. They immunoprecipitate a diffuse band of M_r 49,000 that is sometimes resolved into a doublet and smaller quantities of a band of M_r 36,000 from CTL lines (Fig. 2*D*, lanes 1 and 2). LFA-2 is present on CTL lines but not on JY B lymphoblastoid target cells, as shown by immunoprecipitation and the indirect binding assay. MAb to LFA-2 block anti-HLA-DR killing by an average of 64% to 65% (Table 1). Specific killing of 50% is inhibited to plateau levels of about 15% by antibody concentrations higher than 0.5 μg/ml (Fig. 3). Immunofluorescence flow cytometry showed that the LFA-2 antigen is on a lymphocyte subpopulation. The two different anti-LFA-2 MAb give superimposable labeling of 89% to 90% of peripheral blood lymphocytes (Fig. 4*B*).

The TS2/9 MAb immunoprecipitates a diffuse band of M_r

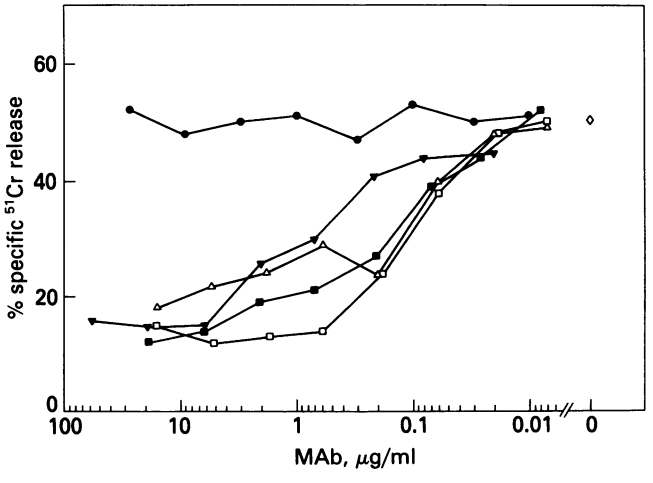

FIG. 3. Inhibition of cytolytic activity by monoclonal antibodies to LFA-1, LFA-2, and LFA-3. Anti-HLA-DR CTLs were assayed using ^{51}Cr-labeled JY target cells. MAb to LFA-1 (TS1/18.1.1; △), LFA-2 [TS1/8.1.1 (□) and TS2/18.1.2 (■)], and LFA-3 (TS2/9.1.2; ▼) and a hamster anti-mouse MAb (M21/3.2.2; ●) as control were added at the initiation of the assay at the indicated final dilutions. ◇, No antibody.

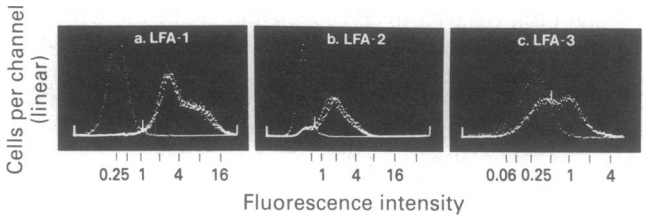

FIG. 4. Immunofluorescence flow cytometry of peripheral blood lymphocytes labeled with LFA MAb. Dim curves represent staining with control P3X63 supernatant. Bright curves represent two different anti-LFA-1 MAb, TS1/12.1.1 and TS1/18.1.1 (*A*, curves superimposed); two different anti-LFA-2 MAb, TS1/8.1.1 and TS2/18.1.1 (*B*, curves superimposed); and a single anti-LFA-3 MAb, TS2/9.1.1 (*C*). Fluorescence intensity is in glutaraldehyde-fixed sheep erythrocyte units (5).

60,000 from CTL lines (Fig. 1*A*, lane 29; Fig. 2*D*, lane 4) and of higher M_r from JY (Fig. 1*B*, lane 29). This MAb, defining the LFA-3 antigen, inhibits killing by an average of 73% in a concentration-dependent fashion (Table 1 and Fig. 3). It weakly labels 51% of peripheral blood lymphocytes and may label the remainder even more weakly (Fig. 4*C*, note the differing fluorescence scales).

Four of the inhibitory supernatants precipitated the HLA-DR antigen, containing chains of M_r 34,000 and 29,000 and present in greater quantities on JY targets than on the CTL line (Fig. 1, lanes 2, 4, 6, and 16). Two of these MAb were successfully cloned and inhibit anti-HLA-DR killing by 39% and 38% (Table 1) but do not inhibit anti-HLA-A and -B CTL-mediated killing. The two MAb label a subpopulation of 18% of peripheral blood lymphocytes. These MAb appear to inhibit killing by binding to the HLA-DR antigen on target cells and confirm the specificity of the CTLs for HLA-DR.

DISCUSSION

To investigate the molecular basis of human CTL-mediated killing, function-blocking MAb probes have been prepared. Four different types of antigens were identified. Three of these have been designated lymphocyte function-associated (LFA) antigens; the fourth is HLA-DR. MAb to these antigens gave consistent significant inhibition of anti-HLA-DR killing that plateaued at MAb concentrations >0.5 μg/ml. LFA-1, LFA-2, and HLA-DR were recognized by multiple independent MAb, demonstrating a good correlation between these structures and inhibition of function. Inhibition was specific in that similar hybridoma culture supernatants containing irrelevant MAb or the W6/32 anti-HLA-A and -B MAb, which binds to a higher number of antigen sites per cell, failed to inhibit killing (data not shown). The identification of LFA-1, LFA-2, and LFA-3 as sites for blockade of killing suggests that these molecules are important in the CTL–target cell interaction.

The human LFA-1 antigen contains polypeptide chains of M_r 177,000 and 95,000. Five anti-LFA-1 MAb inhibited killing by an average of 34–43%; TS2/4 and TS1/18 anti-LFA-1 MAb inhibited by 9% and 60%, respectively. As shown by competitive inhibition of ^3H-labeled MAb binding, the seven MAb define at least four different topographic regions on human LFA-1 (unpublished data). Hindrance by antibody of the function of a molecule would be expected to be governed by the spatial relationship between the antibody binding site and the active site of the molecule. The degree of inhibition given by the different anti-LFA-1 MAb may thus be related to the epitopes to which they bind.

Human LFA-1 appears to be the homologue of the previously described mouse LFA-1 antigen (4–7). The mouse and human antigens are extremely close in molecular weight with β chains

of M_r 95,000 and α chains of M_r 180,000 and 177,000, respectively. As in mouse LFA-1 (10, 11), the α- and β-polypeptide chains of human LFA-1 are subunits that are noncovalently associated in a $\alpha_1\beta_1$ complex. Human LFA-1, like mouse LFA-1 (5), is present on both B and T lymphocytes. The presence of lymphocyte subpopulations that differ 3- to 4-fold in quantitative expression is another similarity between mouse (5) and human. The significance of the relationship between the LFA-1 molecule and CTL-mediated killing is emphasized by its conservation during the evolutionary divergence of the mouse and the human.

In the mouse, the LFA-1 antigen has been found to be structurally related to the Mac-1 differentiation antigen (7, 10, 11), which is also function associated. Mac-1 appears to mediate complement receptor type 3 interactions in both mice and humans (29). In mice, LFA-1 and Mac-1 share a common β subunit; the same relationship may exist in humans.

The LFA-2 antigen has a major component of M_r 49,000 that is sometimes resolved into a doublet and a minor component of M_r 36,000. Titration experiments showed that of all the antibodies tested here, the two anti-LFA-2 MAb gave the most potent inhibition of killing. The MAb also strongly inhibit anti-HLA-A and -B CTL-mediated killing and T-helper-cell proliferative responses (unpublished data). The anti-LFA-2 MAb label a subpopulation of 90% of peripheral blood lymphocytes. Double labeling with rhodamine-conjugated anti-human IgG suggests that T lymphocytes are the LFA-2^+ subpopulation (data not shown). The LFA-2 antigen is present on CTL lines but not on JY B-lymphoblastoid target cells, as shown by immunoprecipitation and the indirect binding assay. The involvement of LFA-2 in T-cell functions and its distribution on T lymphocytes suggest it as a candidate for the T-lymphocyte antigen receptor.

The LFA-3 antigen migrates as a diffuse band with a mean M_r of 60,000 in CTL lines. It can be isolated in greater quantity from B-lymphoblastoid than from CTL lines and is present on >51% of peripheral lymphocytes.

A previous attempt to directly select for MAb inhibiting CTL-mediated killing used anti-HLA-A,B CTLs (30). Blocking MAb were obtained to two different types of antigens, the HLA-A and -B antigen and a molecule apparently identical to the OKT8 (Leu-2a) antigen. By using anti-HLA-DR CTLs, which are not inhibitable by anti-OKT8 MAb (13–15), we avoided the identification of these molecules in the present study.

Other studies have used MAb that were initially selected for labeling of T lymphocytes or their subpopulations and then subsequently tested for inhibition of T-cell functions. Antibodies directed to three types of molecules have been reported to block killing, the OKT3 antigen of M_r 19,000 (17–19), Leu-2a and OKT8 antigens (19–21), which are apparently identical molecules containing disulfide-linked subunits of M_r 32,000 and 43,000 (12, 21), and the M_r 55,000 OKT4 antigen (14, 15). It is unclear why MAb to OKT3 and OKT4 were not obtained in the present study. It is possible that these antigens are less immunogenic than the ones identified here or that antibodies to them gave somewhat weaker inhibition in the initial screening for inhibition of killing.

The identification of three different LFA molecules in this study supports the idea that a number of molecules are involved in the killing process. This is not surprising, because killing is a multistep functional pathway, involving antigen-specific recognition and adhesion of the CTL to the target, delivery of the lethal hit, and CTL-independent target cell lysis (1–3). The particular stage in the killing process in which LFA-1, -2, and -3 participate remains to be elucidated.

Further characterization of the LFA-1, -2, and -3 antigens should provide important insights into the molecular basis of T-lymphocyte immune interactions. The antibodies may be useful clinically, both diagnostically, should function-associated antigens prove to have variable expression in disease states, and therapeutically, to block undesired immune reactions such as graft rejection.

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