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LFA-1, LFA-2, and LFA-3 Antigens Are Involved in CTL-Target Conjugation[1]

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The cytotoxic T lymphocyte-target cell interaction involves antigen recognition, cell adhesion, and delivery of the "lethal hit" (1). Monoclonal antibodies (mAb)[3] specific for a number of cell surface molecules can interrupt cytolytic T lymphocyte (CTL)-mediated cytolysis, demonstrating the complexity of this process at the molecular level (2). MAb that bind to OKT3 (3, 4), OKT4 (5–9)/OKT8 (4, 10, 11), lymphocyte function-associated antigen (LFA)-1 (2, 12, 13), LFA-2 (2, 12, 14), and Ti (15) on the effector cell all block CTL-mediated cytotoxicity. Target cell structures involved in cytotoxicity include LFA-3 (2, 12) and the major histocompatibility complex (MHC) antigens, HLA-A,B,C (16), HLA-DR (5–8), SB (9), and DC (17, 18).

Landegren *et al.* (19) showed that mAb recognizing Leu-2a (OKT8) inhibited cytolysis by interrupting CTL-target conjugation whereas OKT3 monoclonal antibody did not. Tsoukas *et al.* (20) similarly showed that OKT3 blocks cytolysis at a post-recognition stage. We have recently described the function, cell distribution, and structure of three cell surface molecules (LFA-1, LFA-2, and LFA-3) involved in the CTL-target interactions (2, 12). In this report, we show that monoclonal antibodies that recognize these three structures interfere with CTL-target conjugation.

MATERIALS AND METHODS

Monoclonal antibodies. MAb to LFA antigens were derived from subcloned lines TS1/12 (LFA-1), TS1/18 (LFA-1), TS2/18 (LFA-2), TS1/8 (LFA-2), and TS2/9 (LFA-3). Culture supernatants were routinely used, although purified immunoglobulin (Ig) from ascitic fluid gave similar results.

Commercially available mAb used included OKT3, OKT8, OKT4, Leu-1, Leu-2a, Leu-3a, and Leu-4. (Leu-1-4 antibodies were provided by Dr. Robert Evans, Sloan-Kettering Cancer Institute, NY).

Fluorescence-activated cell sorter (FACS) analysis. Immunofluorescence flow cytometry was performed on an FACS II after labeling cells with mAb and affinity-purified fluorescein isothiocyanate anti-mouse IgG (Zymed Laboratories, South San Francisco, CA).

Long-term cytolytic cell lines. OKT8$^+$ HLA-A2, B7-specific CTL lines were generated as previously described (5). Peripheral blood lymphocytes (PBL) from a normal volunteer (M.P.: HLA-A11, Aw32, B27, Bw51, Cw2, DR7) were separated on a Ficoll/Hypaque gradient and were co-cultured with irradiated lymphoblastoid cells (JY or Daudi) in interleukin 2 (IL 2)-containing medium. All cells were propagated in complete media, i.e., RPMI 1640 (M.A. Bioproducts, Bethesda, MD) medium supplemented with 10% fetal calf serum, 2 mM L-glutamine (GIBCO, Grand Island, NY), penicillin 100 U/ml, streptomycin 100 μg/ml (GIBCO), and 25 μM 2-mercaptoethanol (Eastman Organic Chemicals, Rochester, NY). IL 2 was the culture supernatant from phytohemagglutinin (PHA) stimulated PBL, purified by taking the fraction that precipitated between 50 and 75% ammonium sulfate saturation and dialyzing it against phosphate-buffered saline (5).

^{51}Cr-release assay. CTL assays were performed in duplicate or triplicate in V-bottomed microtiter wells, and the percentage specific release was calculated. The human lymphoblastoid cell line JY (HLA-A2,2; B7,7; DR4,6) was used as the target for CTL.

MAb were added at various concentrations directly to the microculture well at the start of the incubation of the ^{51}Cr-release assay, and the percentage of blocking was calculated by the formula: percent blocking = 100 × (SR control) − (SR + MAb)/(SR control) in which SR control is the specific release in the absence of mAb.

Assays were performed at effector to target (E:T) ratios of 10:1 to 12:1 unless otherwise specified, and were harvested at 3 to 4 hr, whenever 50% specific release had occurred in the absence of added antibody.

Binding assay. Binding of target cells to effectors was investigated by a modified method of Grimm *et al.* (21). Target cells were labeled with fluorescein diacetate to distinguish them from effector cells under the fluorescence microscope (22). Effector cells were preincubated for 20 min with 20 μg/ml of mAb or medium alone. Target cells were mixed with effector cells in the presence of mAb at E:T ratios of 1:1 to 10:1, and were centrifuged at 500 × G. E:T ratios of 1.5:1 and 2:1 yielded binding ratios of approximately 50%. After a 20-min incubation at room temperature, the sediment was resuspended and the cells were scored by two separate observers for binding. All experiments were performed in duplicate or triplicate.

RESULTS

Anti-HLA-A2,-B7 CTL line. CTL lines were generated and tested for target specificity as previously described (5). The target specificity of the line A1 was assessed by using a panel of target cells expressing various HLA-A,B and DR specificities. Table I shows that A1 is specific for

TABLE I
Specificity of CTL line A1[a]

E:T	HLA	Targets					
		JY	DAUDI	MST	PRIESS	MANN	K562
	A	2	—	3	2	29	—
	B	7	—	7	15	12	—
	DR	4, 6	6, -	2	4	7	—
10:1		56	3	42	39	2	0
2:1		21	0	17	12	0	0

[a]Target specificity for the CTL line A1 is shown. Data are expressed as percentage specific release at E:T ratios of 10:1 and 2:1.

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[3] Abbreviations used in this paper: LFA, lymphocyte function-associated antigen; CTL, cytolytic T lymphocyte; mAb, monoclonal antibody; PBL, peripheral blood lymphocytes; FACS, fluorescence-activated cell sorter analysis; IL 2, interleukin 2; E:T, effector-to-target ratio; MHC, major histocompatibility complex.

HLA-A2 and -B7-expressing cells. The ability of anti-MHC mAb to block cytolysis was also assessed (Table II). A1 is inhibited by mAb that recognize HLA-A,B,C (W6/32), but not by mAb that recognize HLA-DR (TS1/16) or DC1 (Genox 3.53).

Conjugate binding assay. A fluorescein-labeled target cell binding assay was performed to evaluate inhibition of CTL-target conjugate formation by the anti-LFA-1, LFA-2, and LFA-3 mAb (Table III). ^{51}Cr-release cytotoxicity assays were performed in parallel to assess mAb inhibition of cytolysis (Table IV). MAb recognizing LFA-1, LFA-2, LFA-3, OKT8, and HLA-A,B,C (W6/32) inhibited CTL-target (A1-JY) conjugate formation (Table III) and cytolysis (Table IV). All inhibitory mAb, but especially anti-OKT8, inhibited conjugate formation better at lower levels of effector-target binding (compare Expts. 1 and 2, Table III). Anti-OKT3 mAb inhibited cytolysis (Table IV) but not conjugate formation (Table III). Anti-OKT4 mAb did not inhibit cytolysis (Table IV) or conjugate formation (Table III). Of note, anti-Leu-3a mAb inhibited conjugate formation and cytolysis of a Leu-3a$^+$, HLA-DR6-specific CTL clone (data not shown). Anti-Leu-1 mAb did not inhibit conjugate formation or cytolysis (Tables III and IV).

TABLE II

Inhibition of cytolysis by anti-MHC mAb[a]

Antibody added	% Specific Release
No antibody	51
W6/32 (anti-HLA-A,B,C)	15
TS1/16 (anti-HLA-DR)	52
Genox 3.53 (anti-DC1)	53

[a]The CTL line A1 was assayed for cytotoxicity on JY target cells by a 4-hr ^{51}Cr-release assay at E:T ratio of 10:1. The mAb were added at the start of the 4-hr incubation period.

TABLE III

Inhibition of conjugate formation by mAb[a]

mAb added	Experiment 1		Experiment 2	
	% Binding	% Inhibition of binding	% Binding	% Inhibition of binding
Medium	65 ± 3	—	36 ± 6	—
Control (Leu-1)	61 ± 3	6	34 ± 3	9
TS1/18 (anti-LFA-1)	46 ± 4	30	16 ± 9	56
TS2/18 (anti-LFA-2)	25 ± 5	62	10 ± 3	63
TS2/9 (anti-LFA-3)	40 ± 7	39	11 ± 3	70
OKT8	56 ± 8	14	14 ± 3	62
OKT4	65 ± 7	0	36 ± 4	0
OKT3	61 ± 2	6	33 ± 2	9
W6/32 (anti-HLA-A,B,C)	46 ± 14	30	9 ± 6	75

[a]Conjugate-binding assay was performed as described in *Materials and Methods*. MAb were added before addition of fluorescenated target cells (JY). Data shown are the percentage of fluorescenated cells conjugated to effector cells and the percentage inhibition of binding compared with incubation with medium alone. E:T ratios were 2:1 (Expt. 1) and 1.5:1 (Expt. 2). Percentage binding is shown ±1 SD.

TABLE IV

Inhibition of cytolysis by mAb[a]

mAb Added to the Assay	Antigen Recognized	% Specific Release	% Inhibition of Cytolysis
None	—	47 ± 4	—
Anti-Leu-1	Leu-1	46 ± 4	3
TS1/18	LFA-1	21 ± 4	56
TS2/18	LFA-2	19 ± 4	60
TS2/9	LFA-3	24 ± 10	49
Anti-OKT8	OKT8	25 ± 4	47
Anti-OKT4	OKT4	50 ± 7	0
Anti-OKT3	OKT3	10 ± 3	79
W6/32	HLA-A,B,C	23 ± 6	52

[a]The CTL line A1 was assayed for cytotoxicity in a 4-hr ^{51}Cr-release assay at E:T ratio of 12:1. The mAb were added at the start of the 4-hr incubation period. The data shown are percentage specific release ± 1 SD.

DISCUSSION

Antigen-specific cytolysis is known to involve antigen recognition, cell adhesion, and delivery of the "lethal hit". MAb that block CTL-mediated killing by binding to the CTL or target cell surface have identified cell surface proteins involved in lymphocyte-target cell interactions, including Lyt-2,3, LFA-1 (23–25), and L3T4 (26) in the mouse, and OKT3 (3, 4), OKT8 (4, 10, 11), OKT4 (5–9), and Ti(15) in the human. We recently identified three new antigens involved in CTL-mediated cytolysis by using mAb derived after immunization with human HLA-DR-specific CTL and screening for the ability of these mAb to block HLA-DR-specific cytolysis. In this report, we have shown that these mAb inhibit cytolysis by blocking CTL-target cell conjugate formation.

In addition to the antigen-specific interaction between the T cell antigen receptor (15) and MHC antigens expressed on the target cell, other T cell and target cell surface molecules appear to mediate accessory (i.e., not antigen specific) interactions. The ability of anti-accessory molecule mAb to inhibit cytolysis lends further support to the hypothesis that an "adhesive strengthening" process accompanies antigen-specific recognition (23, 27). The anti-LFA-1, LFA-2, and LFA-3 mAb apparently act by steric interference of this antigen-nonspecific adhesion process.

Our data do not preclude the possibility that these molecules are also involved in the lethal hit *per se*, although we have no data to support this contention. We have not yet been able to separate antigen recognition and adhesion from the lethal hit.

Thus, it appears that CTL-target conjugation involves two steps: a) specific immunologic recognition and b) adhesion strengthening. Specific immunologic recognition involves an interaction between the CTL receptor and target antigen. We hypothesize that adhesion strengthening depends on the interaction of antigen-nonspecific cell surface structures that form receptor-ligand pairs between effectors and targets. It appears that LFA-1, LFA-2, and LFA-3 are involved in this adhesion strengthening process.

SUMMARY

Three cell surface antigens associated with the CTL-target cell interaction were previously identified by generation of mAb against OKT4$^+$, HLA-DR-specific CTL, and selection for inhibition of cytolysis in a ^{51}Cr-release assay. In this report, we showed that these mAb inhibit cytolysis by blocking CTL-target cell conjugate formation. It appears that LFA-1, LFA-2, and LFA-3 are cell surface structures involved in strengthening effector-target adhesion that accompanies antigen-specific recognition.

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