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CELL SURFACE STRUCTURES INVOLVED IN THE HUMAN CYTOLYTIC T LYMPHOCYTE RESPONSE

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ABSTRACT Human cytolytic T lymphocyte (CTL) lines specific for a variety of MHC antigens (HLA-A2, HLA-DR6, and DC1) were used to define cell surface structures involved in lymphocyte-target cell interactions. Monoclonal antibodies (mAbs) recognizing LFA-1 (180K, 95K), LFA-2 (Leu 5/OKT11-49K), LFA-3 (55-65K), and Leu 4 (OKT3-19K) antigens blocked cytolysis by all three CTL lines. Monoclonal antibodies recognizing Leu 2a (OKT8) inhibited cytolysis by the Leu 2a+, HLA-A2-specific CTL line and mAbs recognizing Leu 3a (OKT4A, B, and E) inhibited cytolysis by the Leu 3a+, HLA-DR6-specific CTL line, supporting the hypothesis that Leu 2a/Leu 3a mAbs define T cell surface structures involved in the recognition of MHC antigens. No mAb recognizing either Leu 2a or Leu 3a blocked cytolysis by the Leu 2a+, DC1-specific CTL, suggesting that an as yet undefined cell surface structure may be involved in DC recognition.

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Monoclonal antibodies which recognize LFA-1, LFA-2, Leu 2a, Leu 3a, and Leu 4 blocked cytolysis by binding to the effector cell while anti-LFA-3 and anti-MHC (HLA-A,B, DR, and DC) mAbs inhibited by binding to target cells. Furthermore, using a fluorescein-labelled target cell binding assay, anti-LFA-1, LFA-2, LFA-3, and Leu 2a/Leu 3a mAbs were shown to block conjugate formation while anti-Leu 4 mAb did not.

Thus, at least six cell surface molecules (Leu4, Leu 2a, Leu 3a, LFA-1, LFA-2, and LFA-3), in addition to the antigen receptor and the specific target antigen, are involved in the CTL-target cell interaction.

INTRODUCTION

Cytolytic T lymphocytes (CTL) are important effectors in the cell mediated response to viruses (1), allografts (2), and some tumors (3). The definition of cell surface molecules important in the CTL response may elucidate the general mechanisms of cellular recognition, cell interactions, and the "lethal hit" of cytotoxicity. We have generated CTL lines and clones and monoclonal antibodies to further define the cell-surface molecules involved in the CTL-target interaction.

RESULTS

Human Allogeneic CTL Recognize Class II MHC Antigens In Addition to Class I MHC Antigens.

We have generated long-term CTL lines that recognize HLA-A2 and -B7 antigens by stimulating normal human peripheral blood lymphocytes with JY, an Epstein-Barr virus (EBV)-transformed lymphoblastoid cell line (HLA-A2,2; B7,7; DR4,6) in the presence of interleukin-2 (IL-2) containing conditioned media (4).

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HLA-DR6 specific CTL were similarly generated by long-term stimulation with the cell line Daudi, which expresses no HLA-A or B antigens but is HLA-DR6+ (5). We recently generated CTL specific for a sub-group of target cells expressing the DCl antigen (6). The specificity of these CTL lines was determined by 1) cytotoxicity against panels of target cells expressing various HLA-A,B,DR and DC specificities (data not shown-see 4,5,6) and 2) inhibition of cytolysis by anti-major histocompatibility complex (MHC) monoclonal antibodies (Table 1).

TABLE 1

Inhibition of Cytolysis by Anti-MHC Monoclonal Antibodies

Line:	A1	F1	A9
Specificity: / Treatment	HLA-A2	HLA-DR6	DCl
No antibody	49	53	49
Leu 1 (pan-T cell)	54	55	51
w6/32 (HLA-A,B,C)	21	47	51
PA2.1 (HLA-A2)	16	51	50
TS1/I6 (HLA-DR)	53	8	52
L243 (HLA-DR)	50	16	48
LB3.1 (HLA-DR)	47	11	47
Genox 3.53 (DCl)	50	52	4
Leu 10 (DC subgroup)	53	53	0

The data shown are percentage specific release in a 4 hour ^{51}Cr-release assay. Monoclonal antibodies were added at the initiation of the four hour incubation period.

Anti-Leu 2a Monoclonal Antibodies Inhibit Cytolysis of HLA-A,B Specific CTL While Anti-Leu 3a Monoclonal Antibodies Block HLA-DR Specific Cytolysis.

All of the CTL lines generated express the Leu 4 (OKT3) antigen. The HLA-A2 specific CTL express Leu 2a (OKT8) and the HLA-DR6 specific CTL express Leu 3a (OKT4). Surprisingly, the DC specific CTL express Leu 2a, although Leu 2a monoclonal antibodies do not inhibit cytolysis by these CTL (Table 2).

TABLE 2
Inhibition of Cytolysis by Anti-T Cell MAb

Line:	A1	F1	A9
Phenotype:	Leu2a+	Leu3a+	Leu2a+
Specificity:	HLA-A2	HLA-DR6	DC1
Treatment			
No antibody	49	53	49
Leu 1	54	55	51
Leu 4 (OKT3)	20	16	21
Leu 3a (OKT4)	50	21	51
Leu 2a (OKT8)	26	53	53

Data shown are percentage specific release in a 4 hour ^{51}Cr-release assay.

LFA-1, LFA-2 and LFA-3 Cell Surface Molecules are Involved in CTL-mediated Cytolysis

We have generated monoclonal antibodies by immunizing mice with a Leu 3a+ CTL line specific for HLA-DR6 antigens; hybridomas were screened for mAb blocking of HLA-DR6 specific cytolysis. Binding of mAb to three types of molecules, designated lymphocyte function associated antigens (LFA)-1, 2, and 3, inhibited cytolysis suggesting that these molecules participate in the CTL-target cell interaction (7,8).

LFA-1 is a broadly distributed leukocyte antigen involved in CTL and NK cell mediated cytolysis (7,8). It is the homologue of murine LFA-1 and appears to be involved in cell adhesion (9).

LFA-2 is the sheep erythrocyte receptor, also recognized by Leu 5, OKT11, and mAb 9.6 (8). It is involved in T cell functions, including cytotoxicity and proliferation to alloantigens, lectins, and soluble antigens (8).

LFA-3 is a novel, broadly distributed antigen associated with lymphocyte function (7,8). Anti-LFA-3 mAb blocks lymphocyte function by binding to the target rather than effector cell (Table 3).

TABLE 3
Site of Inhibition by Anti-LFA Monoclonal Antibodies

Treatment	LFA-1	LFA-2	LFA-3
No antibody	54	54	54
Control mAb	55	52	54
LFA mAb	22	10	20
Effectors pre-treated	27	18	53
Targets pre-treated	57	68	17

Data shown are percentage specific release in a 4 hour ^{51}Cr-release assay. Antibody was added at the start of the 4 hour incubation period or, alternatively, CTL or target cells were pre-incubated with antibody for 30 minutes and washed extensively prior to the assay. No additional antibody was added to these wells.

Landegren et al showed that mAb recognizing Leu 2a (OKT8) inhibited cytolysis by interrupting CTL-target conjugation while OKT3 mAb did not (10). Tsoukas et al similarly showed that OKT3 blocks cytolysis at a post-recognition stage (11). We have used a fluorescein-labelled target cell binding assay to evaluate inhibition of CTL-target conjugate formation by anti-LFA-1, LFA-2, and LFA-3 mAb (Table 4). Monoclonal antibodies recognizing LFA-1, LFA-2, LFA-3, OKT8 and HLA-A,B,C (w6/32) inhibited CTL-target (anti-HLA-A2 specific CTL) conjugate formation. All inhibitory mAb, but especially anti-OKT8, inhibited conjugate formation better at lower levels of effector-target binding (compare experiments 1 and 2, Table 4). Anti-OKT3 mAb inhibited cytolysis (data not shown) but not conjugate formation (Table 4). Anti-OKT4 did not inhibit either cytolysis (data not shown) or conjugate formation (Table 4). Of note, anti-Leu 3a mAb inhibited both 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 (Table 4) or cytolysis (data not shown). These results show that mAb which bind to functional structures on either the effector or target cell can block conjugate formation.

TABLE 4
Inhibition of Conjugate Formation by Anti-LFA mAb

Monoclonal Antibody Added	Experiment 1 % Binding (% Inhibition)	Experiment 2 % Binding (% Inhibition)
Medium	65 (--)	36 (--)
Control (Leu 1)	61 (6)	34 (9)
Anti-LFA-1 (TS1/18)	46 (30)	16 (56)
Anti-LFA-2 (TS2/18)	25 (62)	10 (63)
Anti-LFA-3 (TS2/9)	40 (39)	11 (70)
Leu 2a	56 (14)	14 (62)
Leu 3a	65 (0)	36 (0)
Leu 4	61 (6)	33 (9)
w6/32 (anti-HLA-A,B,C)	46 (30)	9 (75)

Conjugate binding assay was performed using fluorescein-labelled targets. Data shown are percentage fluoresceinated cells conjugated to effector cells and the percentage inhibition of binding in the presence of mAb compared to incubation with medium alone. E:T ratios were 2:1 (Expt.1) and 1.5:1 (Expt.2). All standard deviations were less than 15%.

Some Immunodeficient Patients Lack LFA-1.

LFA-1 has been implicated in the pathophysiology of a recently recognized immunodeficiency state characterized by recurrent bacterial infections, impaired phagocytosis, and deficiency of high molecular weight cell surface protein (11-15). It has recently been shown that at least a subset of these patients lack the high molecular weight protein identified by monoclonal antibodies recognizing the β chain of Mol and LFA-1 molecules (Figure 1).

We have studied three of these patients, their families, and unrelated controls. Cells from these LFA-1 deficient patients show decreased CTL-mediated cytotoxicity, NK cell mediated cytotoxicity, and proliferation to alloantigens and PHA.

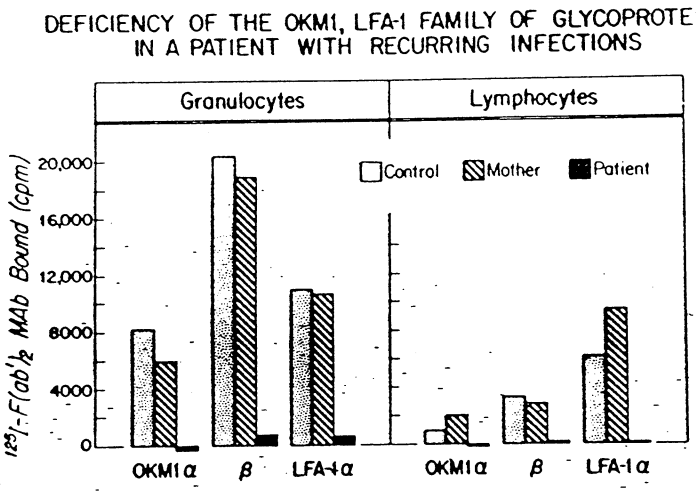

Figure 1. Granulocytes and lymphocytes from an LFA-1 deficient individual, his mother, and an unrelated control are compared with regard to expression of OKM1, LFA-1, and the common chain using a ^{125}I-labelled F(ab')$_2$ direct binding assay.

The Same Cell Surface Molecules are Involved in Recognition and Cytolysis of Endothelial and Fibroblast Targets.

The mechanism of CTL-mediated lysis has been largely studied using lymphoid and other marrow derived target cells. Although the use of lymphoid target cells is experimentally convenient and has provided great insight into the CTL-target cell interaction, the pathophysiologically important targets of allogeneic CTL in transplantation are non-lymphoid cells, especially vascular endothelium and stromal cells. It is possible that CTL recognition and lysis of these cell types proceeds through different mechanisms, perhaps involving different accessory molecules. We have recently shown that an HLA-DR6 specific CTL clone, generated against lymphoblastoid targets, can specifically recognize and lyse cultured human endothelial cells and fibroblasts which have been induced by immune interferon to express class II MHC antigens (16). We have used this system to show that the CTL-vascular endothelium and CTL-fibroblast interactions also involve at least Leu 4, Leu 3a, LFA-1, LFA-2, LFA-3 and HLA-DR (Table 5)(17).

TABLE 5
MAb Inhibition of Fibroblast Cell Cytolysis

Treatment	% Specific Release	% Blocking of Cytolysis
No antibody	35	-
Anti-HLA-DR (TS1/16)	5	87
Anti-HLA-A,B,C (w6/32)	32	9
OKT3 (Leu 4)	12	67
Leu 3a (OKT4)	4	88
Anti-LFA-1 (TS1/18)	18	50
Anti-LFA-2 (TS2/18)	24	32
Anti-LFA-3 (TS2/9)	26	30

Results shown are F1 (HLA-DR6 specific CTL clone) mediated cytolysis of ^{111}indium labeled IFNγ pretreated fibroblasts at an E:T ratio of 20:1. In this experiment detergent (2% NP-40) release was 134,604 cpm; spontaneous release was 6,813 cpm. Data are calculated from means of triplicate determinations where the standard deviation was less than 15%. Concentrations of mAb were chosen which gave maximal inhibition of HDF cytolysis. A mAb reactive with HLA-A,B (w6/32) was used at a concentration of 1/100 ascitic fluid by volume.

DISCUSSION

We have used CTL lines and clones to define target and effector cell surface molecules involved in the human allogeneic response. Target cell specific molecules include class I MHC molecules (HLA-A,B,C), class II MHC molecules (HLA-DR,DC, and SB), and LFA-3. LFA-3 is a novel target cell antigen which is involved in cytolysis of all targets examined thus far, including lymphoblasts, fibroblasts, and endothelial cells.

Effector-cell specific molecules involved in the CTL-target cell interaction include Leu 4 (OKT3), Leu 2a (OKT8), Leu 3a (OKT4), LFA-1, and LFA-3 (Leu 5, OKT 11). Leu 4 blocks cytolysis of both class I and class II specific CTL. It has been suggested that the Leu 4 (OKT3) molecule is a part of the T cell antigen receptor complex (23). Although there is a strong correlation between Leu 2a and class I MHC recognition and Leu 3a with class II recognition, there are exceptions to this paradigm (6,24). Our DC1 specific

CTL are not inhibited by either Leu 2a or Leu 3a monoclonal antibodies, suggesting that perhaps another cell surface molecule is involved in DC recognition.

We have also identified another "family" of molecules, the LFA-1, LFA-2, and LFA-3 antigens, which are more generally distributed than Leu 2a and Leu 3a, but are also involved in the CTL-target cell interaction. These "accessory cell" molecules are antigen-non-specific cell surface structures which appear to be important in conjugate formation. Thus, in addition to the specific interaction between the T cell antigen receptor and MHC antigens expressed on the target cell, other T cell and target cell surface molecules appear important in strengthening adhesion between T cells and the cells with which they interact. The identification and characterization of at least five "accessory" cell surface molecules (Leu 2a, Leu 3a, LFA-1, LFA-2 and LFA-3) involved in the CTL-target cell interactions emphasizes the complexity of this process at the molecular level.

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