Brief Definitive Report

CYTOTOXIC T LYMPHOCYTES FROM HLA-A2 TRANSGENIC MICE SPECIFIC FOR HLA-A2 EXPRESSED ON HUMAN CELLS

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Cytotoxic T lymphocytes (CTL) use class I molecules of the MHC in the recognition of virally infected and foreign cells (reviewed in reference 1). It has recently been demonstrated that class I molecules can present specific peptide fragments derived from processed antigens to influenza virus-specific CTL (2), and to H-2-restricted CTL specific for HLA antigens expressed in murine transfectants (3). These results suggested that CTL specific for alloantigens and xenoantigens might also recognize epitopes composed of MHC antigens together with peptides derived from the target cell. It has been shown that CTL clones raised against HLA-A2 on human cells often did not recognize the same antigen expressed on murine transfectants (4–10), and evidence was obtained that suggested this might be due to a difference in the epitopes associated with HLA-A2.1 on human and murine cells (11). This has now been investigated directly using transgenic mice that express the human HLA-A2.1 antigen. Although these mice were tolerant to HLA-A2.1$^+$ murine cells, specific CTL responses were elicited by HLA-A2.1 expressed on human cells. These results strongly suggest that proteins that differ between mouse and human cells, but are highly conserved among many different human tissues, contribute to the formation of epitopes recognized by class I MHC-specific CTL.

Materials and Methods

Cells. Lymphoid cells were cultured in RPMI 1640 medium, and nonlymphoid cells were cultured in α MEM (Hazelton Research Products, Denver, PA), both supplemented with 2 mM glutamine and 10% FCS. Transfected cells were maintained in the presence of 250 μg/ml geneticin (Gibco Laboratories, Grand Island, NY) with the exception of the M1 transfectants, which were maintained in 25 μg/ml mycophenolic acid. The expression of HLA-A2.1 by all appropriate cells was verified by flow cytometry using the HLA-A2-specific mAb MA2.1 (12).

CTL clones were derived by limiting dilution culture of spleen cells from HLA-A2.1 transgenic or normal C57BL/6 mice immunized with 2×10^7 JY cells (HLA-A2; B7; DR4,6),

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as previously described (13). CTL clones were also derived from HLA-A2 transgenic mice by limiting dilution cloning after secondary in vitro restimulation with JY. CTL clones were maintained as described (14).

^{51}Cr Release Assays. Assays were carried out for 4 h as described previously (14). The exchange of endogenous class I-associated β_2m on target cells was accomplished by washing cells three times in serum-free RPMI 1640 with subsequent culture for 14 h in the presence of 10% human serum.

Results

HLA-A2.1-expressing transgenic mice were produced from the C57BL/6 strain. The expression of the HLA-A2.1 molecule on the surface of lymphoid cells from these mice was determined to be approximately equal to that of H-2D^b (Le, A. T., E. J. Bernhard, M. J. Holterman, P. Parham, E. Lacy, and V. H. Engelhard, submitted for publication). HLA-A2.1 transgenic and normal C57BL/6 mice were primed in vivo with the HLA-A2.1 expressing human cell line, JY. 1 mo later, spleen cells from these animals were restimulated in limiting dilution microcultures with JY cells. The lytic activity of these cultures was assessed using the HLA-A2.1-positive human cell line HSB, and EL4-A2, a derivative of the syngeneic murine EL4 cell line transfected with the gene for HLA-A2.1. CTL from normal C57BL/6 animals showed a distribution of reactivities ranging from exclusive recognition of the human cell line to recognition of both mouse and human targets at comparable levels (Fig. 1 *A*). In contrast, no CTL from HLA-A2.1 transgenic animals that lysed the human target showed significant reactivity on EL4-A2 (Fig. 1 *B*).

To characterize the epitopes recognized by CTL from the HLA-A2.1 transgenic animals, 20 independent clones were derived from limiting dilution culture. Three additional clones were obtained from limiting dilution cloning of a secondary mixed lymphocyte culture. All clones lysed the HLA-A2.1-positive EBV-transformed B lymphoblastoid cell lines 23.1, 310.B27#4, and the human HLA-A2.1 transfectant LA2.1 (Table I). However, none of the clones lysed: HMY2.C1R, the HLA-A2-negative cell from which LA2.1 was derived; MST, JM, and Daudi, which share MHC antigens other than HLA-A2.1 with JY; or WT49 and DK1B, which express HLA-A2.2 and -A2.3, respectively. All clones exhibited high levels of lysis on normal human HLA-A2.1-positive PBL (Table I). These clones have been shown to recognize at least four different epitopes (14a). Taken together, these results indicate that these

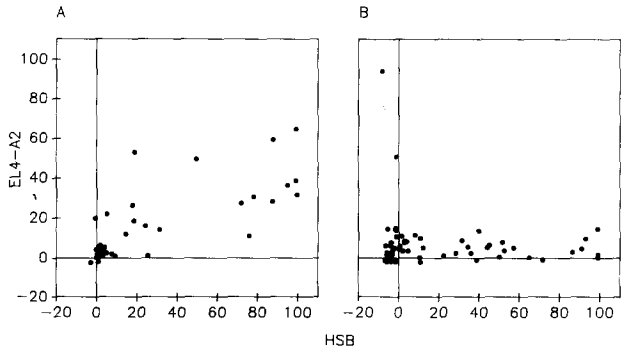

FIGURE 1. Clonal reactivity patterns from a C57BL/6 (*A*) or an HLA-A2 transgenic (*B*) mouse using human (HSB) and murine (EL4-A2) target cells expressing HLA-A2.1. The data shown are derived from plates in which >37% of wells were negative for HLA-A2 reactivity.

TABLE I

CTL Clones from HLA-A2.1 Transgenic Mice Recognize HLA-A2.1 Expressed on both Transformed and Non-transformed Human Lymphoid Cells

Target	HLA-A2 antigen	Percent-specific ^{51}Cr release	
		Mean	Range
23.1	A2.1	80.6	65–96
310	A2.1	90.5	69–102
PBL	A2.1	83.7	59–98
LA2.1	A2.1	50.2	19–82
HMY2.C1R	Negative	1.3	−2–8
WT49	A2.2	0.7	−3–4
DK1B	A2.3	4.0	1–17
DAUDI	Negative	−0.2	−4–4
MST	Negative	3.1	0–14
JM	Negative	3.0	−2–10

23 CTL clones were assayed at an E/T ratio of 10:1. Target cells include the B lymphoblastoid cell lines 23.1 (HLA-A2;B27;DR8), 310.B27#4 (HLA-A2; B^-; DR1,3), LA2.1, an HLA-A2.1-expressing cell line derived from HMY2.CIR (HLA-A^-; B^-; DR8) by electroporation, WT49 (HLA-A2.2; B17; DR3), DK1B (HLA-A2.3,33; B40,44; DR6,-), MST (HLA-A3; B7; DR2), DAUDI (HLA class I^-; DR6); the T lymphoblastoid line JM (HLA-A3,25; B7,37); and PBL, 3-d PHA (Gibco Laboratories) -stimulated normal PBLs from donor TJM (HLA-A2,24; B7,62; C3,7; DR1,2).

CTL are specific for several different epitopes found on both normal and transformed HLA-A2.1-positive human lymphoid cells.

The expression of the epitopes recognized by these CTL was further investigated using HLA-A2.1-positive cells derived from nonlymphoid tissue. All of the CTL clones lysed RDA2, an HLA-A2-expressing transfectant of the human rhabdomyosarcoma cell line RD, and M1A2.1, an HLA-A2.1-expressing transfectant of the xeroderma pigmentosum fibroblast M1 (Table II). All clones also lysed two HLA-A2-expressing primary fibroblast lines, GM126 and GM2708. The HLA-A2-expressing osteosarcoma cell line 143bTK$^-$ was lysed by only 17 of 22 clones tested, although it expressed HLA-A2 at levels comparable with RDA2 and GM2708. Only three of the clones were able to lyse CV1P5A2, an HLA-A2.1-expressing transfectant of the African green monkey cell line CV-1. None of the clones lysed HLA-A2.1-expressing murine transfectants derived from EL4 (Table II), the C127 mammary carcinoma, or the P815 mastocytoma (data not shown).

To rule out the possibility that the recognition of HLA-A2 on the surface of murine cells required the association of human β_2-microglobulin (β_2m) with the HLA-A2 antigen, exchange of human for the endogenous β_2m was carried out on the murine EL4-A2 cell line. EL4-A2 was not lysed by any clone after this manipulation, nor was any effect noted on lysis of similarly treated human cell lines (Table III).

Discussion

The failure of certain human CTL specific for HLA class I molecules to recognize these molecules when expressed on murine cells has been repeatedly observed (6–10).

TABLE II

Recognition by HLA-A2.1-specific CTL Clones Derived from HLA-A2 Transgenic Mice of Nonlymphoid Human and Simian Target Cells

Target	Representative clones		Number of clones reactive with target
	AT1-3	AT1-19	
RDA2	58	44	23/23
RD mock	- 1	- 5	0/23
M1A2.1	67	69	14/14
M1A3.1	4	3	0/14
GM126	29	24	11/11
GM2708	90	70	11/11
GM2709	62	43	11/11
143bTK$^-$	19	17	17/22
CV1P5A2	- 1	52	3/23
CV101 mock	- 2	- 2	0/23
EL4-A2	0	- 2	0/23
EL4	2	3	0/23

Specificity was determined at an E/T ratio of 10:1. Reactivity was defined as >10% specific lysis. Target cells included RDA2, a human rhabdomyosarcoma cell line (HLA-A1, BW51,B14) transfected with the HLA-A2.1 gene and RD mock, transfected with the neomycin resistance gene alone; M1A2.1 and M1A3.1, HLA-A2.1 and HLA-A3.1-expressing transfectants of the M1 human xeroderma pigmentosum fibroblast line; 143btk$^-$, an HLA-A2-expressing human osteosarcoma line; GM126, a human HLA-A2-expressing primary fibroblast; GM2709, an HLA-A2-expressing lymphocyte, and GM2708, a primary fibroblast from the same donor; CV1P5A2 , an African green monkey kidney cell line transfected with the HLA-A2.1 gene, and CV101 mock, transfected with the neomycin-resistance gene alone; EL4-A2, an HLA-A2.1-expressing transfectant of the murine thymoma cell line EL4, and the untransfected EL4 cell.

It has been suggested that this is due to an inability of accessory molecules on human CTL to bind to their ligands on murine target cells. However, it has also been observed that certain murine CTL raised against HLA antigens expressed on human cells fail to recognize these antigens on murine transfectants (4). This result cannot readily be explained by a lack of participation of accessory molecules, since the CTL and the target are of the same species. It was instead suggested based on fine specificity patterns that this phenomenon is due to differences in the HLA class I molecule expressed on human and murine cells (5, 11). The results of this study provide definitive evidence for the existence of one or more CTL-defined epitopes on the HLA-A2.1 molecule expressed on human cells, which are entirely absent from this molecule when expressed on murine cells.

It is unlikely that these epitopes result from differences in post-translational modification of the HLA-A2.1 molecule in human and murine cells, since no such differences have been detected (6, 7). In addition, the carbohydrate side chain has been shown not to influence CTL recognition of class I molecules (15–17). The recognition of human HLA-A2.1-expressing cells from different individuals and tissues also suggests that these epitopes do not arise from the association of the HLA-A2.1 molecule with human minor histocompatibility or tissue-specific antigens. However, the variable lysis of the 143bTK$^-$ osteosarcoma suggests that some of these epitopes may be lost due to tissue-specific or tumor-specific alteration. It appears that these CTL clones recognize epitopes formed by one or more endogenous, highly conserved but species specific molecules in the context of HLA-A2.1. Given the demonstration

TABLE III

The Epitopes Recognized by HLA-A2.1-specific CTL Clones from HLA-A2 Transgenic Mice Are not Dependent upon Human β2-Microglobulin

	Percent-specific ^{51}Cr release from:			
CTL clone	LA2.1 FBS	LA2.1 HuS	EL4-A2 FBS	EL4-A2 HuS
AT1-1	43	54	−1	2
AT1-11	57	52	2	3
AT1-19	33	41	1	1
AT1-21	45	50	0	6
AT2-1	45	38	−1	3

Target cells include the HLA-A2.1-transfected cell lines LA2.1 (human) and EL4-A2 (murine). Serum mediated β_2m exchange was carried out with either FCS or human serum (HuS) as described in Materials and Methods. The E/T ratio was 25:1.

that peptides may associate with class I molecules to form epitopes recognized by CTL (2, 3, 18, 19), it is tempting to suggest that these epitopes have a similar origin. The data presented here raise the possibility that epitopes recognized by alloreactive T cells may also result from the presentation of endogenously derived molecules in association with a class I MHC molecule on the surface of the stimulator cell.

Summary

CTL clones were derived from HLA-A2.1 transgenic mice by immunization with a human cell expressing HLA-A2.1. None of these clones lysed murine transfectants, and only 3 of 23 lysed monkey transfectants expressing HLA-A2. In contrast, all of these clones lysed a wide variety of human cells expressing HLA-A2.1. These results demonstrate the existence of species-specific epitopes on the HLA-A2.1 molecule, and suggest that these epitopes are formed by the association of class I MHC products with one or more endogenous species-specific molecules. These results provide an explanation for the frequently observed failure of HLA class I-specific CTL to recognize these antigens on murine transfectants. These results also suggest that such endogenous proteins may also contribute to the formation of epitopes recognized by allospecific CTL.

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