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

BY ERIC J. BERNHARD,* AI-XUAN T. LE,† JAMES A. BARBOSA,*
ELIZABETH LACY,* AND VICTOR H. ENGELHARD*†

From the *Department of Microbiology and †Department of Surgery, University of Virginia,
Charlottesville, Virginia 22908; ‡Molecular Diagnostics, Inc., West Haven, Connecticut 06516;
and the †DeWitt Wallace Research Laboratory, Program in Molecular Biology,
Memorial Sloan-Kettering Cancer Center, New York, New York 10021

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 antiga-
ten. 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 con-
served among many different human tissues, contribute to the formation of epitopes
recognized by class I MHC-specific CTL.

Cells. Lymphoid cells were cultured in RPMI 1640 medium, and nonlymphoid cells were
cultured in a 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 trans-
fectants, 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 trans-
genic or normal C57BL/6 mice immunized with 2 × 10⁷ JY cells (HLA-A2; B7; DR4,6),

This work was supported by National Institutes of Health grants AI-20963, AI-21393, AI-24815 and
American Cancer Society Grant ACS-IM-437. E. Lacy is the recipient of an American Heart Associa-
tion Established Investigator Award. A.-X. Le is the recipient of Public Health Service Fellowship AI-
07263. E. J. Bernhard is a predoctoral trainee supported by National Institutes of Health grant CA-09109.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/88/09/1157/06 $2.00
Volume 168 September 1988 1157–1162
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).

Cr Release Assays. Assays were carried out for 4 h as described previously (14). The exchange of endogenous class I-associated β₂m 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<sup>β</sup> (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
CTL Clones from HLA-A2.1 Transgenic Mice Recognize HLA-A2.1 Expressed on both Transformed and Non-transformed Human Lymphoid Cells

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; B27; DR1,3), LA2.1, an HLA-A2.1-expressing cell line derived from HMY2.CIR (HLA-A2; B27; DR8) by electroporation, WT49 (HLA-A2; 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 MIA2.1, an HLA-A2.1-expressing cell line derived from HMY2.CIR (HLA-A2; B27; DR8) by electroporation, WT49 (HLA-A2; 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).

Table I

<table>
<thead>
<tr>
<th>Target HLA-A2 antigen</th>
<th>Percent-specific (^{51}{}_{\text{Cr}}) release</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.1 A2.1</td>
<td>80.6</td>
</tr>
<tr>
<td>310 A2.1</td>
<td>90.5</td>
</tr>
<tr>
<td>PBL A2.1</td>
<td>83.7</td>
</tr>
<tr>
<td>LA2.1 A2.1</td>
<td>50.2</td>
</tr>
<tr>
<td>HMY2.CIR Negative</td>
<td>1.3</td>
</tr>
<tr>
<td>WT49 A2.2</td>
<td>0.7</td>
</tr>
<tr>
<td>DK1B A2.3</td>
<td>4.0</td>
</tr>
<tr>
<td>DAUDI Negative</td>
<td>-0.2</td>
</tr>
<tr>
<td>MST Negative</td>
<td>3.1</td>
</tr>
<tr>
<td>JM Negative</td>
<td>3.0</td>
</tr>
</tbody>
</table>

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; B27; DR1,3), LA2.1, an HLA-A2.1-expressing cell line derived from HMY2.CIR (HLA-A2; B27; DR8) by electroporation, WT49 (HLA-A2; 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).

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).
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 II

<table>
<thead>
<tr>
<th>Target</th>
<th>Representative clones</th>
<th>Number of clones reactive with target</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDA2</td>
<td>AT1-3</td>
<td>58</td>
</tr>
<tr>
<td>RD mock</td>
<td>AT1-19</td>
<td>44</td>
</tr>
<tr>
<td>MIA2.1</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>MIA3.1</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>GM126</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>GM2708</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>GM2709</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>143bTK-</td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>CV1P5A2</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td>CV101 mock</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>EL4-A2</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>EL4</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>EL4</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>EL4</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

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; MIA2.1 and MIA3.1, 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.
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.

M1A2.1 and M1A3.1 were generously provided by Dr. William Biddison. We thank John Ridge, Christopher Eichman, and Lia Kwee for excellent technical assistance, and Marie Burton for preparation of the manuscript.

Received for publication 2 May 1988 and in revised form 6 June 1988.

References


