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Recognition of HLA-A2 and -B7 antigens by cloned cytotoxic T lymphocytes after gene transfer into human and monkey, but not mouse, cells

(major histocompatibility antigen/antigenic determinant/monoclonal antibody/allospecific cytotoxic T lymphocyte/DNA transfection)

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ABSTRACT **The genes that code for the human major histocompatibility class I antigens, HLA-A2 and HLA-B7, were introduced into human, monkey, and mouse cell lines by cotransfection with suitable biochemical markers and the fluorescence-activated cell sorter was used to identify and/or select stable cell populations expressing high surface levels of these antigens. Levels of expression obtained were similar to those observed for endogenous HLA antigens on various human cell lines and were 25–80% of those observed on the human B-lymphoblastoid cell line JY. Serologically defined HLA-A2 and HLA-B7 polymorphic determinants remained intact on all transfected recipient cells analyzed. Cloned human allospecific cytotoxic T lymphocytes (CTL) specific for HLA-A2 or HLA-B7 were capable of lysing appropriate HLA-transfected human cells with comparable efficiency to JY cell lysis. Two of 10 CTL clones lysed appropriate monkey cell transfectants with ≈20% the efficiency of human cell transfectants. No specific lysis of any HLA-transfected mouse cell lines, including a B cell lymphoma, was observed despite comparable levels of surface antigen expression or after induction of higher levels by mouse γ-interferon. Furthermore, L cells expressing human β_2-microglobulin in addition to HLA-A2 or -B7 were not lysed by these CTL. Thus, an additional species-specific component may be involved in lysis by allogeneic CTL—possibly related to the function(s) of other surface proteins on target cells.**

Human class I antigens, HLA-A, -B, -C, encoded in the major histocompatibility complex (MHC) on chromosome 6, play an important role in self/non-self recognition, serving as restricting elements for cell-mediated lysis by virus-immune cytotoxic T-lymphocytes (CTL) or directly as target antigens for allospecific CTL (reviewed in ref. 1). As integral membrane glycoproteins, insertion of these highly polymorphic HLA heavy chains (M_r, 44,000) in the membrane is dependent on a noncovalent association with β_2-microglobulin (β_2m; M_r, 12,000) (reviewed in ref. 2). In somatic cell hybrids, human HLA heavy chains can associate with heterologous β_2m for appropriate surface expression (3, 4).

Multiple genomic sequences that hybridize to class I cDNA probes have been isolated from human, mouse, and porcine gene libraries. Because of the genetic complexity displayed by these multigene families, functional members have been identified using gene-transfer techniques (5–10). The products of mouse *H-2* genes introduced into mouse L cells by DNA-mediated gene transfer (DNA-MGT) can be recognized by appropriate monoclonal antibodies (7–9) and by allospecific or *H-2*-restricted virus-immune CTL (9, 11, 12). Although both monomorphic and polymorphic serological determinants of human class I antigens can be detected following DNA-MGT into mouse L cells (5, 6, 13–15), recognition by human allospecific CTL or mouse xenogeneic CTL raised against human HLA antigen specificities has not been successful (ref. 14; unpublished results). Recently, however, some xenogeneic CTL clones have been shown to recognize HLA antigens expressed in L cells after DNA-MGT (15).

To investigate the cellular recognition of HLA antigens in more detail, we have cotransferred human class I genes coding for an *A* locus and a *B* locus antigen (HLA-A2 and HLA-B7) with suitable selectable markers into human, monkey, and mouse cell lines. Human allospecific CTL clones recognizing HLA-A2 and HLA-B7 antigens have been established, and they were used to show that human CTL can specifically lyse appropriate HLA-transfected human and monkey but not mouse cells. These results suggest that another species-restricted factor may play a role in the cellular but not serological recognition of human MHC class I proteins.

MATERIALS AND METHODS

Cell Culture. The human osteosarcoma cell line, 143b, and the human rhabdomyosarcoma cell line, RD, obtained from C. Croce (Wistar Institute) and M. Zuniga (California Institute of Technology), respectively, were deficient in thymidine kinase (TK) activity. The African green monkey kidney cell line CV-1, the mouse embryonic liver cell line C127, and the mouse B-lymphoma cell line M12.4.1 were gifts of W. Haseltine (Dana-Farber Cancer Institute), D. DiMaio (Yale University), and L. Glimcher (Harvard Medical School), respectively. Mouse L cells deficient in TK and adenine phosphoribosyltransferase have been described (16).

Cell lines were maintained in α minimal essential medium (α ME medium; GIBCO) or RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Microbiological Associates), penicillin (100 units/ml), streptomycin (100 μg/ml), and 2 mM glutamine. The RD cell line reverted to a TK^+ phenotype at a frequency of 1×10^{-6} and was maintained in α-ME medium containing BrdUrd at 30 μg/ml.

Effector Cytotoxic T Lymphocytes. Human A2- and B7-specific CTL clones were generated from the peripheral blood lymphocytes (PBL) of a single donor (HLA-A11, Aw32, B27, Bw51, Cw2; DR7,7). PBL were separated on a Ficoll/Hypaque gradient and stimulated in culture with irradiated JY lymphoblastoid cells (HLA-A2, B7; DR4,6) as described (17). Each line was stringently subcloned twice by limiting dilution and maintained in complete RPMI medium plus 10% interleukin 2-conditioned supernatants from phyto-

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Abbreviations: MHC, major histocompatibility complex; β_2m, β_2-microglobulin; TK, thymidine kinase; NEO, neomycin; IIF, indirect immunofluorescence; DNA-MGT, DNA-mediated gene transfer; CTL, cytotoxic T lymphocyte(s); E:T, effector/target ratio; mAb, monoclonal antibody; PBL, peripheral blood lymphocytes.

hemagglutinin- and mixed lymphocyte culture-activated PBL.

DNA-MGT. This procedure was carried out using calcium phosphate-precipitated DNA and monolayer cultures as described (5), except that in some cases a 2-min glycerol shock (15% glycerol in Hepes-buffered saline at 37°C) after transfer was used. Cotransfection of 1×10^6 cells was carried out in the presence of culture medium with 1–5 μg of recombinant plasmid containing either the gene encoding HLA-B7 (p158R1) or that encoding HLA-A2 (pB3.2R1) (5) and 100–500 ng of a plasmid containing either the herpes simplex virus *tk* gene (PTKX1) (18) or the dominant selectable Tn*5* neomycin-resistance (*neo*^{r}) gene (pSV2neo) (19). Additional high molecular weight recipient cell DNA was used as carrier. Appropriate hypoxanthine/aminopterin/thymidine (HAT medium) or G418 (geneticin, 1 mg/ml, 40% purity; GIBCO) selection was initiated 60 hr after transfer. Fresh medium was added every 2–3 days. Either individual colonies were isolated or entire flasks of colonies were harvested 10–14 days after selection.

DNA-MGT using calcium phosphate-precipitated DNA and B lymphocytes was carried out in suspension as described (20). Cotransfection of 1×10^7 cells was carried out with pB3.2R1 (20 μg) or p158R1 (20 μg) and pSV2neo (1 μg). Alternately, a plasmid containing both the HLA-A2 and the Tn*5* *neo*^{r} gene, pA2neo (5 μg), was used (a gift of D. Levy, Harvard). Cells were plated at 5×10^5 per 6-well plate (Costar) or at 5×10^4 per 24-well plate (Falcon) and G418 selection was initiated 60 hr after transfer. Semi-adherent colonies were visible 10–14 days later and were expanded at 3 weeks following Ficoll/Hypaque gradient separation of viable cells.

Monoclonal Antibodies (mAbs). mAb W6/32 recognizes a monomorphic determinant on all HLA-A, -B, -C heavy chains that are associated with β_2m (21). mAbs BB7.2, PA2.1, 4B3, and MA2.1 recognize allotypic determinants on HLA-A2, HLA-A2/A28*, HLA-A2/A28, and HLA-A2/B17, respectively (22–24). mAbs BB7.1, MB40.2, MB40.3, and ME1 recognize human allotypic determinants on HLA-B7, B7/B40, B7/B40, and B7/B27/B22, respectively (22, 25, 26).

Indirect Immunofluorescence and Fluorescence-Activated Cell Sorter Analysis. Indirect immunofluorescence (IIF) was carried out with saturating amounts of antibody as described in Table 2. Cells were either fixed in 1% paraformaldehyde for analysis or further treated with propridium iodide (5 μg/ml; Sigma) to circumvent nonviable cell staining. Evaluation of immunofluorescence and cell sorting were carried out with an EPICS V laser system and an MDADS multichannel analyzer (Coulter Electronics).

^{51}Cr Release Assay. Target cells were labeled overnight at 37°C with 0.2 ml of ^{51}Cr (0.2 μCi of Na_2CrO_4; 1 Ci = 37 GBq; New England Nuclear). Monolayer culture cells were lifted with 0.03% EDTA in phosphate-buffered saline. Cytotoxic T-cell-mediated lysis assay was carried out and percentage specific ^{51}Cr release was calculated as described by Reiss *et al.* (12).

RESULTS

DNA-MGT and Serological Analysis of HLA on Transfectants. Various human, monkey, and mouse recipient cell lines were used to establish a panel of HLA-A2$^+$ and HLA-B7$^+$ cells by cotransfecting these cloned HLA gene sequences with plasmids containing either the herpes simplex virus *tk* gene or the Tn*5* *neo*^{r} gene (Table 1). Transfection frequencies varied among the different cell lines used. Individual HAT^R or G418^R colonies were picked, expanded, and analyzed for appropriate surface expression by IIF with the HLA-B7-specific mAb BB7.1 or the HLA-A2-specific mAb BB7.2. Alternately, entire flasks of colonies were harvested and the fluorescence-activated cell sorter was used directly to sort out homogeneous HLA-A2$^+$ or HLA-B7$^+$ subpopulations displaying different levels of expression. HLA-A2- and HLA-B7-expressing cell lines representing the highest levels of surface expression obtained on each recipient line and mock transfectants produced by using only the selectable *tk* or *neo* gene are shown in Table 2. Because the recipient cell lines differ in size, the relative levels of expression of HLA-A2 and HLA-B7 on human, monkey, and mouse transfectants are presented as functions of both total cellular fluorescence and surface antigen density.

Table 1. Recipient cell lines used for DNA-MGT

Cell line	Description	Selection used/ resistance	Transfection frequency, no. $\times 10^6$
143b	Human osteosarcoma	HAT/TK$^+$	5
RD	Human rhabdomyosarcoma	HAT/TK$^+$	100
CV-1	African green monkey kidney	G418/NEO$^+$	50
LTKA	Mouse fibroblast	HAT/TK$^+$	100–1000
C127	Mouse embryonic liver	G418/NEO$^+$	5–50
M12.4.1	Mouse B lymphoma	G418/NEO$^+$	50

The human osteosarcoma line 143b was shown by IIF with a variety of mAbs specific for HLA-A2 or HLA-B7 to express endogenous HLA-A2 but not HLA-B7 determinants, while the human rhabdomyosarcoma line RD lacked determinants for both HLA-A2 and HLA-B7. These lines have been tentatively typed as (HLA-A2; BW21,BW42; CW2) and (HLA-A1; BW51,B14), respectively (M. Pollack, Baylor College of Medicine; personal communication). Neither the monkey nor mouse recipient cell lines cross-reacted with any of the polymorphic HLA-A2 or HLA-B7 mAbs used.

Expression levels ranged from 10 to 80% of those found on JY cells. Both the human cell transfectant RDWHS and the mouse cell transfectant C1272B expressed levels of HLA-A2 ≈25% that of the high-expressing JY cell line (28) but equivalent to that of the endogenous *HLA-A2* gene present in the human osteosarcoma line 143b. The HLA-A2$^+$ monkey cell line CV1P5 expressed nearly 80% of JY cell total fluorescence but, because of relative cell size differences, the antigen density was only 25% that of JY cells. The mouse L-cell transfectant LTKA2S and the mouse B-cell transfectant M5C expressed ≈10%. As expected, mock transfectants did not express HLA-A2 determinants. Similar results were observed for HLA-B7 transfectants (Table 2, Exp. B).

A panel of HLA-A2-specific mAbs that recognize at least three separate epitopes on the HLA-A2 glycoprotein (29) displayed similar levels of binding to each HLA-A2-transfected cell population (Table 3). When any of the different polymorphic mAbs were used, the comparative levels of expression among cell lines remained relatively constant. A small increase in MA2.1 antibody binding was reproducibly observed on the monkey and mouse cell transfectants but not on the human cell lines assayed. The significance of these small differences is not clear, but they may indicate minor conformational alterations or processing differences of the HLA-A2 glycoprotein in the different cell backgrounds.

Similar results were observed with a panel of HLA-B7-reactive mAbs (Table 4). mAbs BB7.1, MB40.2, MB40.3, and ME1 bound to all human, monkey, and mouse cell transfectants and displayed similar relative levels of expression among the transfectants. Thus, comparative quantitative binding of the HLA-A2- and HLA-B7-specific mAbs that recognize different epitopes on the appropriate molecules established that these proteins are expressed in association with either human, monkey, or mouse β_2m, without significant conformational alteration of these epitopes.

Table 2. Relative HLA-A2 and HLA-B7 expression levels

Cell line	HLA gene transferred*	Relative linear fluorescence	Relative antigen density	% JY total fluorescence	% JY antigen density
Exp. A: Relative HLA-A2 expression using mAb BB7.2					
JY	—	(1.2)†			
JY	—	105.0	105.0	100.0	100.0
143b1D	None	(6.2)			
143b1D	None	26.0	9.3	24.8	8.8
RDV	None	3.1			
RDWHS‡	HLA-A2	23.5	9.8	22.4	9.3
CV101	None	5.2			
CV1P5	HLA-A2	82.0	27.3	78.1	26.0
C127M	None	4.6			
C1272B	HLA-A2	23.5	10.7	22.4	10.2
LTKM	None	2.8			
LTKA2S‡	HLA-A2	11.4	6.7	10.8	6.4
M12412E	None	2.8			
M12415C	HLA-A2	11.8	9.1	11.2	8.7
Exp. B: Relative HLA-B7 expression using mAb BB7.1					
JY	—	(1.2)			
JY	—	29.5	29.5	100.0	100.0
143b1D	None	5.6			
143b10B	HLA-B7	45.0	15.5	152.5	52.5
143b7E	HLA-B7	8.8	3.8	29.8	12.9
RDV	None	3.1			
RDXHS‡	HLA-B7	16.0	6.4	54.2	21.7
CV101	None	5.2			
CV1Q2	HLA-B7	29.5	10.2	100.0	34.6
C127M	None	4.8			
C127B7S‡	HLA-B7	16.0	7.3	54.2	24.7
LTKM	None	2.8			
LTK24A	HLA-B7	5.2	2.9	17.6	9.8
M12412E	None	2.8			
M1241E5	HLA-B7	5.9	4.5	20.0	15.3

Cells (1×10^6) were incubated in medium containing 2% heat-inactivated fetal calf serum and 0.02% sodium azide with saturating levels of mAbs BB7.2 (HLA-A2) or BB7.1 (HLA-B7) in 50 μl at 4°C for 1 hr with shaking. Cells were washed three times and incubated for another hour on ice in 50 μl of fluorescein-conjugated goat anti-mouse Ig [$F(ab')_2$, 5 μg total]. Cells were fixed in 1% paraformaldehyde and analyzed by flow cytometry. Channel numbers, representing the median peak value of the relative logarithmic fluorescence distribution obtained by the MDADS multichannel analyzer, were converted to relative linear fluorescence values as described by Muirhead *et al.* (27), using beads of various fluorescence intensities as standards. All values were derived for a single experiment and represent similar relative levels of expression seen on three separate occasions. Cell volume analysis relative to JY cells was carried out with a Coulter Counter channelizer after calibration with different-sized microspheres. Relative antigen density equals relative linear fluorescence/relative surface area.

*None, mock-infected cells.

†Control staining with the myeloma supernatant P3 is shown for JY and 143b in parentheses and for each mock transfectant was equivalent to BB7.1 or BB7.2 staining.

‡Population of transfectants derived from fluorescence-activated cell sorting.

Cellular Recognition of HLA-A2 and HLA-B7 in Transfected Cells. Human CTL clones were isolated that are allospecific for HLA-A2 or -B7 (unpublished data). The specificity of these clones was shown by using a panel of human lymphoblastoid cell lines of different HLA types as targets. No evidence of natural killer activity (assessed by K562 lysis) or MHC class II-directed lysis was observed. Furthermore, target cell lysis could be blocked with anti-class I but not anti-class II mAbs. Two representative clones, CTL-A2.1 and CTL B7.1, phenotypically $T3^+$, $T8^+$, $T4^-$ and allospecific for HLA-A2 and -B7, respectively, were used to assess the ability of human, monkey, and mouse cells to function as CTL targets after the introduction of human *HLA-A2* or *-B7* genes (Table 5). Clone CTL-B7.1 specifically lysed the HLA-B7-transfected human RD cell line but not the HLA-A2- or mock-transfected RD cells (Table 5, Exp. B). The percentage lysis of RDXHS cells was ≈80% that of JY cells at similar effector/target (E:T) ratios. Further, the high HLA-B7 expressing human 143b transfectant, 143b10B, was lysed by CTL-B7.1 with efficiency comparable with that of RD cells while the lower expressing population, 143b7E, was lysed 30% as efficiently (data not shown). The HLA-B7-expressing monkey cells, CV1Q2, were also specifically lysed by CTL-B7.1 but only one-third as well as the human RDXHS transfectant. Four additional HLA-B7-specific CTL clones, CTL-B7.2–B7.5, lysed human but not monkey HLA-B7 transfectants (data not shown). CTL-B7.1 (or CTL-B7.2–B7.5) did not lyse HLA-B7-expressing mouse cell transfectants. Identical results were found for both mouse C127- and LTK-transfected cells, even at E:T ratios as high as 50:1. These cells could be lysed by complement and mAb W6/32. In addition, mouse L-cell transfectants could function as appropriate targets for anti-$H\text{-}2^k$-allospecific CTL lysis.

The HLA-A2-specific CTL clone, CTL-A2.1, also demonstrated specific lysis of HLA-A2-transfected human (RD) and monkey (CV-1), but not mouse (LTK^- or C127), cell transfectants (Table 5, Exp. A). Nonspecific background lysis was found with all RD populations and is thought to represent minor crossreactivity of CTL-A2.1 with a determinant expressed on an endogenous HLA antigen. RDWHS cells were lysed by CTL-A2.1 approximately one-third as efficiently as JY cells (45% at E:T = 15:1, vs. 5:1). At these E:T ratios, 143b cells expressing an endogenous *HLA-A2* allele are lysed to a similar degree as RDWHS cells. Four addition-

Table 3. Allotypic expression of HLA-A2 on a panel of transfectants

Cell line	Relative linear fluorescence: P3 control	BB7.2 (A2)	PA2.1 (A2/A28*)	MA2.1 (A2/B17)	4B3 (A2/A28)
JY	0.7	365	350	365	790
143b1D	1.2	25	25	26	50
RDW	1.0	36	36	36	68
CV1P5	2.5	158	158	204	335
C1272B	1.2	82	100	126	228
LTKA2S	1.6	25	25	29	50
M12415C	1.0	28	28	30	48

IIF and fluorescence-activated cell sorter analyses were carried out as in Table 2. HLA specificities are shown in parentheses.

Table 4. Allotypic expression of HLA-B7 on a panel of transfectants

Cell line	Relative linear fluorescence: P3 control	BB7.1 (B7)	MB40.2 (B7/B40)	MB40.3 (B7/B40)	ME1* (B7/B27)
JY	0.5	25	2.3	37	42
143b10B	1.6	34	5.2	126	130
RDX	1.1	12.8	4.5	29	30
CV1Q2	2.0	23	9.4	51	52
C127B7S	1.8	23	6.4	60	64
LTK24A	0.7	3.2	1.8	7.4	9
M1241E5	1.0	3.8	2.0	8.9	11.0

IIF and fluorescence-activated cell sorter analyses were carried out as in Table 2. HLA specificities are shown in parentheses.

*Assayed in a separate experiment.

Table 5. Lysis of HLA transfectants by CTL clones A2.1 and B7.1

Target cell line	Gene transferred*	% specific ^{51}Cr release 15:1	5:1	1.8:1	0.6:1
Exp. A: HLA-A2-specific CTL clone, A2.1 lysis					
Human					
RDX	A2	45	32	20	11
RDW	B7	28	15	4	1
RDV	None	24	13	5	3
Monkey					
CV1Q2	A2	30	23	9	5
CV1P5	B7	0	0	0	1
CV101	None	0	2	1	0
Mouse					
C1272B	A2	0	1	1	1
C127B7S	B7	0	0	0	3
C1272D	None	1	1	0	—
Exp. B: HLA-B7-specific CTL clone, B7.1 lysis					
Human					
RDX	B7	68	32	19	
RDW	A2	7	2	0	
RDV	None	14	1	0	
Monkey					
CV1Q2	B7	27	16	8	
CV1P5	A2	4	4	2	
CV101	None	1	1	2	
Mouse					
C127B7S	B7	0	0	1	
C1272B	A2	0	0	0	
C1272D	None	1	3	2	

^{51}Cr-labeled target cells (1×10^3) were incubated at various E:T ratios with the indicated CTL effector cells in 96-well round-bottomed microtiter plates at 37°C for 4 hr. Supernatants were harvested after centrifugation and % specific ^{51}Cr release was calculated as $100 \times$ (^{51}Cr released by immune lymphocytes − spontaneous ^{51}Cr release)/(maximal ^{51}Cr released with Triton X-100 − spontaneous ^{51}Cr release). Spontaneous release was <20% of maximal release and SD values among triplicate samples were <5%.
*None, mock-infected cells.

al HLA-A2-specific CTL clones (CTL-A2.2–A2.5) demonstrated a similar ability to lyse HLA-A2-transfected human RD cells. However, these clones were unable to lyse HLA-A2-expressing monkey cells. Thus, isolated HLA-A2- and -B7-specific CTL exhibit at least two patterns of reactivity—recognition of both human and monkey transfectants and recognition of human transfectants only.

Because the level of expression of HLA-A2 on L-cell transfectants was only 10% of that on JY cells, mouse γ-interferon (γ-IFN) was used to induce higher levels of expression (30). Even after an 8- to 10-fold induction of HLA expression by mouse γ-IFN, no lysis by human allospecific

Table 6. γ-interferon (γ-IFN) treated mouse transfectants are not lysed by the HLA-A2-specific CTL clone A2.2

Target cell	γ-IFN	Relative linear fluorescence	% specific ^{51}Cr release 18:1	6:1	2:1
LTKA2S	−	9.0	2	1	1
LTKA2S	+	77.0	0	0	0
LTKM	−	1.8	2	0	0
LTKM	+	1.9	0	0	0
143b	−	23.0	78	50	24

Cells were plated at 5×10^5 cells/25-cm flask with recombinant mouse γ-IFN (Biogen) at 50 units/ml for 48 hr at 37°C, and % specific ^{51}Cr release was determined at various E:T ratios.

Table 7. Mouse B-cell transfectants are not lysed by human CTL clones

Target cell	Gene transferred*	% specific ^{51}Cr release CTLA2.5 (HLA-A2) 24:1	8:1	CTLB7.3 (HLA-B7) 12:1	4:1
JY	—	84	67	51	23
M12415C	HLA-A2	2	1	2	1
M1241E5	HLA-B7	5	6	4	3
M1242E	None	0	0	−4	2

% specific lysis was determined at various E:T ratios.
*None, mock-infected cells.

CTL was seen (Table 6). Furthermore, the mouse B lymphoma M12.4.1, transfected with *HLA-A2* or *HLA-B7* genes and expressing moderately high levels of these surface proteins, was not lysed specifically by human CTL clones (Table 7).

To address whether this lack of killing may be due to conformational changes of the HLA-A2 or HLA-B7 heavy chains due to association with mouse β_2m, the *HLA-A2* gene was introduced into L cells already expressing high levels of human β_2m (31). Surface levels of expression of HLA were typically higher on these transfected cells than on L-cell transfectants (both by mass population or clonal analysis). No lysis of these transfectants expressing good levels of HLA-A2 and human β_2m by CTL clone CTL-A2.2 was observed (Table 8). Because recent experiments suggest that HLA-bound surface β_2m in L cells may exchange to various degrees with bovine β_2m in culture medium (32), these transfectants were also grown in human serum. Again, no lysis by CTL clones was observed. Thus, the absence of killing is not explained solely by association of HLA heavy chains with mouse or bovine β_2m.

DISCUSSION

Most, if not all, monomorphic and polymorphic serological determinants of human class I antigens remain intact after transfection of their genes into mouse L cells by DNA-MGT (refs. 5, 6, 13–15, and data presented here). However, the same mouse L cells expressing transfected HLA antigens serve as poor targets for human allospecific CTL (14). Here, we report that human allospecific CTL can recognize and lyse human and monkey, but not mouse, cells transfected with HLA class I genes. Furthermore, the lack of CTL lysis of mouse cells expressing HLA antigens is not limited to mouse L cells, because neither mouse B-cell nor mouse L-cell transfectants could be lysed. Therefore, a more general species-related phenomenon may be involved.

One possible explanation for the lack of killing of mouse transfectants is the low level of HLA antigen expression seen on most L-cell transfectants. These cells expressed only 10–30% of JY cell levels, which may not be sufficient

Table 8. HLA-A2 and human β_2m cotransfected cells are not lysed by CTL clone A2.2

Target cell	Gene transferred*	Relative linear fluorescence	% specific ^{51}Cr release 18:1	6:1	2:1
JY	—	358.0	89	80	46
LE1	HLA-A2	78.0	−1	3	1
LJE1	HLA-A2 + human β_2m	126.0	−1	−1	0
LJCM	human β_2m	2.2	−1	0	0

% specific lysis was determined at various E:T ratios.
*pB3.2R1 (HLA-A2) was cotransferred with pSV2neo into L cells or a human cellular DNA-transfected L-cell line expressing high levels of human β_2m (31).

for successful CTL-mediated lysis. However, human cell transfectants expressing similarly low levels have been specifically lysed, albeit at a lower efficiency (e.g., 143b7E). Furthermore, CTL did not lyse the mouse C127 transfectants even though their levels of HLA expression were identical to those of human RD transfectants. Finally, even after an 8- to 10-fold induction of HLA expression by mouse γ-interferon, no lysis by human allospecific CTL was observed. Thus, although surface expression levels or antigen density may affect the overall efficiency of lysis of human cell transfectants, it does not explain the total absence of lysis observed on mouse cell transfectants. Furthermore, interferon treatment does not induce the expression of other factors that may be necessary to mediate this lysis.

Lack of lysis of mouse cell transfectants by human allospecific CTL could be due to conformational changes of heavy chain determinants as a result of association with mouse, rather than human, β_2m. These conformational changes may affect CTL-defined HLA-A2 and -B7 antigenic determinants to a greater degree than serologically defined determinants. However, association with mouse β_2m is not likely to explain the species-restricted recognition described here, as transfected mouse L cells expressing both human β_2m (31) and HLA-A2 or HLA-B7 were not lysed by our human allospecific CTL. Such experiments are complicated by the observation that surface β_2m bound to HLA or H-2 may exchange to various degrees with bovine β_2m in culture medium (32). However, even after growth of our mouse cell transfectants in human serum, lysis did not occur. Similar results have been obtained by others (33). Furthermore, in murine systems where β_2m exchange is also possible, no effects were observed on the successful lysis of these *H-2*-transfected mouse L cells by murine CTL (9, 11, 12).

Alternative explanations for why human allospecific CTL recognize human cells and not mouse cells expressing transfected HLA antigens seem more likely. For example, (*i*) although gross structural alterations have not been seen (14), slight differences in processing of the human class I molecule in mouse and human cells (e.g., glycosylation) may occur that mask or alter CTL antigenic determinants; (*ii*) since antigen-specific CTL mediated killing is a multistep process involving antigen recognition and adhesion, delivery of the lethal hit, and target cell lysis (34), these human CTL clones may recognize appropriate transfected mouse cell targets but not effectively transmit a lytic signal; (*iii*) other species-specific cell interaction molecules, such as LFA-1, -2, -3 (35) or their ligands, that would increase the ability of the T cell to bind its target may be required; and (*iv*) the possibility that allospecific CTL may in fact recognize self-HLA plus X, where X may be structurally different on mouse and human cells. It is of interest that some mouse xenogeneic CTL clones have been shown to recognize HLA antigens expressed on mouse L cells after DNA-MGT (15). Whether these clones are of high affinity, bypassing the need of other cell surface molecules, or are less dependent on slight conformational or processing changes is unclear. Regardless of which explanation provides the key to lysis of HLA-transfected mouse cells, the successful recognition of human cell transfectants described here provides a model system to study further the cellular recognition of human MHC class I molecules through molecular alteration of the transfected gene.

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