The Journal of Immunology
RESEARCH ARTICLE | OCTOBER 01 1983

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C Bernabeu; ... et. al

J Immunol (1983) 131 (4): 2032–2037.
https://doi.org/10.4049/jimmunol.131.4.2032

0022-1767/83/1314-2032$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 131, No. 4, October 1983
Printed in U.S.A.

EXPRESSION OF THE MAJOR HISTOCOMPATIBILITY ANTIGENS HLA-A2 AND HLA-B7 BY DNA-MEDIATED GENE TRANSFER[1]

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Genes coding for the heavy chain of the class I antigens HLA-A2 or HLA-B7 of the human major histocompatibility complex have been introduced into mouse LtK$^-$ cells by cotransfection with the herpes simplex virus thymidine kinase gene. HAT-resistant colonies were isolated expressing either HLA-A2 or HLA-B7 as monitored by indirect immunofluorescence. Immunoprecipitation analysis of both antigens by either sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or isoelectric focusing (IEF) showed that they were identical to the HLA-A2 and HLA-B7 expressed in the human lymphoblastoid cell line JY (homozygous HLA-A2, HLA-B7). However, human cytotoxic T lymphocytes (CTL) generated against JY and CTL clones specific for HLA-A2 or HLA-B7 were unable to recognize the transfectants as targets. These results indicate that the human HLA-A2 (or B7) complexed with the murine β_2-microglobulin could be an inappropriate target structure for the CTL. However, because the transfectants are not killed by human CTL even in the presence of lectins, it is suggested that other molecules that are not able to overcome the human-mouse species barrier may be involved in the killing mechanism.

The class I antigens of the major histocompatibility complex (MHC)[4] play an important role in graft rejection as well as in the T cell-mediated cytotoxicity to allogenic or to virus-infected target cells (1, 2). In humans, the HLA-A, HLA-B, and HLA-C antigens, are cell surface glycoproteins composed of a variable heavy chain (m.w. 45,000) encoded on chromosome 6 and a constant light chain β_2-microglobulin (β_2m) (m.w. 12,000) encoded on chromosome 15. The insertion of the HLA in the membrane is dependent on its noncovalent association with β_2m (3). The role of β_2m in HLA maturation can also be accomplished by mouse β_2m as shown in somatic cell hybrids (4, 5). In the mouse, the class I MHC antigens H2-K, H2-D, and H2-L have a similar structure and function as HLA-A, HLA-B and, HLA-C in humans (6). DNA probes that cross-hybridize between human class I genes allow insight into the organization of these genes (7, 8). By Southern blotting experiments, at least 15 to 20 HLA-like genes were revealed (9). So far, the number of proteins identified is much smaller than the predicted number of genes. Much work is left to be done in order to identify the proteins encoded by those HLA-like genes. In this sense, DNA-mediated gene transfer into mouse L cells has proved to be a very useful technique. Gene transfer, followed by screening of the gene products by specific monoclonal antibodies, has been used with mouse (10–12), pig (13), and human (14, 15) class I MHC genes.

In the present report, we have analyzed the protein structure and immunologic function of the HLA-A2 and HLA-B7 antigens expressed into mouse L cells. As determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) the HLA molecules expressed in mouse L cells are similar to the HLA molecules expressed on the parental human cell line. We have also studied these HLA expressing mouse L cells as possible targets for cytotoxic T lymphocytes (CTL) directed to HLA-A2 and HLA-B7.

MATERIALS AND METHODS

Cells. The mouse LtK$^-$ cell line described previously (16) was a generous gift from Dr. Richard Axel (College of Physicians and Surgeons, Columbia University, New York, NY). The human lymphoblastoid cell line JY (homozygous HLA-A2 and HLA-B7) was cultured in either RPMI 1640 containing 5% fetal calf serum (FCS) (GIBCO Laboratories, Grand Island, NY) (17) or in Iscove's modified Dulbecco's minimal essential medium (DMEM) supplemented with insulin (5 μg/ml), human transferrin (35 μg/ml), and bovine serum albumin (Cohn's fraction V, 2.5 mg/ml) (Sigma Chemical Co., St. Louis, MO), 2×10^{-5} M ethanolamine (Merck, Darmstadt, West Germany), and 1% v/v of a dispersion of human plasma lipids made as previously described (18). Isolation and culture of the cytotoxic T cells clones JR-2-16 and HG-31 have been described (18). These clones are specific for HLA-A2 and HLA-B7 antigens as shown by panel studies using HLA-typed peripheral blood lymphocytes and by family studies (19). Peripheral blood lymphocytes (PBL) from healthy donors were purified on Ficoll-Hypaque density gradients (20).

DNA. The cloned herpes simplex thymidine kinase gene was a generous gift from Dr. Victor Corces (John Hopkins University, Baltimore, MD). The HLA clones JY150 and JYB3.2 (P. A. Biro, J. Pan, and S. Weissman, manuscript in preparation) in the lambda vector Charon 4A were isolated and identified as described (14, 21). The nucleotide sequence of JY150 and JYB3.2 genomic clones corresponds to the amino acid sequence of HLA-B7 and HLA-A2, respectively. The clone JY150 is identical to the clone JY158 previously described (14, 20), as judged by nucleotide sequence and restriction maps. High m.w. salmon sperm DNA was obtained from Sigma Chemical Co. and purified as described (16).

Antibodies. The monoclonal antibodies W6/32 and MB40.2.2 were obtained from Accurate Chemical and Scientific Corp., Hicksville, NY. W6/32 recognizes a common determinant to HLA-A, HLA-B and HLA-C antigens when associated with β_2m (22). MB40.2.2 recognizes a specific determinant on HLA-B7 and HLA-B40 (23). The monoclonals 4B and 4E recognize antigenic determinants on HLA-A2, A28 and a common epitope on HLA-B, respectively, and they were a generous gift from Dr. Bo Dupont (Memorial Sloan-Kettering Cancer Institute, New York, NY). Fluorescein isothiocyanate (FITC) goat anti-mouse Fab was purchased from Coulter Immunology, Hialeah, FL. The rat monoclonal M1/42.3 anti-mouse H2 (24) and the rabbit anti-rat IgG antisera were kindly provided by Dr. T. Springer (Dana-Farber

Received for publication March 29, 1983.
Accepted for publication June 15, 1983.
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[1] This work was supported by a National Institutes of Health grant AI-15066.
[2] Recipient of a fellowship from the United States-Spanish Joint Committee for Scientific and Technological Cooperation.
[3] Scholar of the Leukemia Society of America.
[4] Abbreviations used in this paper: HAT, hypoxantine-aminopterin-thymidine CTL, cytotoxic T lymphocyte; CML, cell mediated lympholysis; MLC, mixed lymphocyte culture; MLR, mixed lymphocyte reaction; LDCC, lectin dependent cellular cytotoxicity; IEF, isoelectric focusing; SDS, sodium dodecyl sulfate; PHA, phytohemaglytinin; Con A, Concavalin A; β_2m, beta-2-microglobulin; FITC, fluorescein isothiocyanate; TPCK, L-P-tosylamino-2-phenylethyl chloromethyl ketone; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis.

Cancer Institute, Boston, MA). Monoclonal anti-human β_2m was a generous gift from Dr. L. Nadler (Dana-Farber Cancer Institute, Boston, MA). The rabbit anti-HLA antiserum C41 recognizes the denatured HLA heavy chain (25).

Immunofluorescence. Mouse L cells were detached from the petri dishes with 0.05% trypsin and washed with PBS. Cells (2×10^6) were incubated for 30 min with the specific mouse monoclonal antibody in 150 μl of phosphate buffered saline (PBS) containing 0.1% sodium azide. After two washes, the cells were incubated with FITC-goat anti-mouse Fab for 30 min, washed twice and analyzed with a fluorescence microscope or a cytofluorograph (System 30L, Ortho Instruments Co., Westwood, MA).

Transfection. LtK$^-$ cells were transfected with the HLA clones, JY150 and JYB3.2, according to the procedure described by Wigler *et al.* (16). HAT-resistant colonies were screened with W6/32 for their expression of HLA antigens by indirect immunofluorescence.

Cytotoxic T cell lysis (CML). Labeling with ^{51}Cr and lysis of the target cells in the presence of effector clones were carried out as described (18, 26). In the mixed lymphocyte reaction (MLR), PBL (HLA-A2, HLA-A11, HLA-B8, HLA-BW51 and HLA-AW24, HLA-A29, HLA-B17, HLA-BW35) were stimulated three times with the JY cell line (HLA-A2, HLA-A2, HLA-B7, HLA-B7) on days 1, 7, and 14. On day 20, they were assayed for direct cytotoxicity *vs* the transfectants. L cells were trypsinized before ^{51}Cr labeling (26). Allogeneic killing of B6 mice primed against B6.C3H or B6.D2 was carried out as described (27).

Cold target inhibition assay. Varying numbers of unlabeled target cells that served as inhibitor cells were mixed with a fixed number of effector cells; 2×10^3 ^{51}Cr-labeled target cells were then added, and the plates were centrifuged for 3 min at 150 × G and incubated for 4 hr at 37° C in 5% CO_2. After this incubation period, 100 μl of supernatant were collected and counted in a gamma counter.

Radiolabeling. Cells were enzymatically iodinated with Na^{125}I catalyzed by lactoperoxidase (Calbiochem-Behring, La Jolla, CA) and hydrogen peroxide (28). L cells were trypsinized and washed in PBS before iodination.

Immunoprecipitations. After labeling, cells were lysed with 1% Nonidet P-40 (NP-40) (Particle Data Laboratories, Elmhurst, IL) in 0.01 M Tris-HCl pH 7.8, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.02 mg/ml ovomucoid trypsin inhibitor (Sigma Chemical Co., St. Louis, MO). The lysates were centrifuged for 30 min at 100,000 × G and precleared overnight with heat-inactivated formalin-fixed *Staphylococcus aureus*. Later preclearings also included a preformed complex of mouse IgG and rabbit anti-mouse IgG. Precleared lysates were incubated for 2 to 4 hr with a preformed complex of the monoclonal antibody and rabbit anti-mouse IgG at 4°C (29). In the case of the H2 antigen, the preformed complex was formed by rat anti-H2 IgG and rabbit anti-rat IgG. The precipitate was resuspended in 0.01 M Tris-NaCl buffer and 0.5% deoxycholate sodium salt and washed on a discontinuous gradient consisting of 0.4 ml of 10% sucrose, 0.5% NP-40 in the Tris/NaCl buffer and 0.8 ml of 20% sucrose in the same buffer without detergents. The precipitate was then washed once more in 0.01 M Tris-HCl, pH 7.8, 0.2% NP-40 before electrophoresis. Immunoprecipitations with C41 rabbit antisera were carried out as described (30) on lysates that had been previously denatured in the presence of 0.2% SDS for 5 min at 90°C.

Electrophoresis. SDS-PAGE was carried out on discontinuous vertical slab gels according to a modification of the Laemmli procedure (31). Gels were made with 10 to 15% acrylamide gradients. IEF was performed on vertical slab gels basically as described (32). The gels contained: 5% acrylamide, 0.5% NP-40, 8 M urea, and a mixture of ampholytes pI 3.5 to 10 (LKB Instruments Inc., Rockville, MD), 4 to 6, 6 to 8, and 8 to 10 (Accurate Chemical and Scientific Corp., Hicksville, NY) as 10:2:2:2 (v/v). Samples were run from anode to cathode. To measure the pH gradient, gel slices of 1.5 cm × 0.5 cm were incubated in 2 ml of water.

Peptide mapping. Specific bands were cut out of gels previously fixed, dried, and autoradiographed. The gel slices were then soaked overnight in destaining solution (7.5% acetic acid, 20% methanol) and lyophilized. Then, 1 ml of 0.1 M NH_4HCO_3 containing 25 μg of Trypsin (TPCK, 217 U/g, Worthington Millipore Co., Freehold, NJ) was added per gel slice. The samples were trypsinized twice while incubating for a total of 16 hr at 37°C. The supernatants were pooled and lyophilized. The dry protein was dissolved in 50% pyridine, and electrophoresis was performed on cellulose thin-layer chromatography plates (nr 5502 E. Merck, Darmstadt, Germany) in pyridine, acetic acid, H_2O as 1:10:89, pH 3.5. Chromatography was done in the second dimension with pyridine, acetic acid, butanol, H_2O as 10:3:15:12 (v/v).

Autoradiography. For ^{125}I-labeled proteins, Kodak XAR-5 film was used in combination with intensifier screens (Cronex Lighting Plus, Dupont Chemical Co., Newton, CT).

RESULTS

Study of the HLA-A2 and HLA-B7 gene products in mouse L cells. The transfectant colonies MC.2.2 and CM7.13 expressing HLA-A2 and HLA-B7, respectively, were selected by indirect immunofluorescence with the monoclonal antibody W6/32. These cells were grown for 9 months without any noticeable change in their HLA expression. Figure 1 shows a cytofluorograph analysis of these clones by using the monoclonals 4B, 4E, and MB40.2.2, which recognize specific determinants on HLA-A2, HLA-B, and HLA-B7, respectively. The clone KT.1, which had been transfected only with the thymidine kinase gene, did not react with any of the antibodies. The clone MC2.2 was positive with a monoclonal antibody specific for HLA-A2, whereas the clone CM7.13 showed a strong immunofluorescence with the two antibodies recognizing HLA-B7. In both clones, there was less staining with the monomorphic antibody W6/32 than with the polymorphic antibodies.

According to the above results, the HLA-A2 and HLA-B7 antigenic determinants recognized by the antibodies W6/32, 4B, 4E, and MB40.2.2 were present in the transfectants. In order to examine the protein structure of the HLA antigens, we carried out an immunoprecipitation analysis. Figure 2 shows that in the clones CM71.3 and MC.2.2 the m.w. of HLA-A2 and HLA-B7 heavy chain is the same as in the human cell line JY (45,000). The H2 antigen (lane A), which is the HLA homologue in the mouse, has a higher m.w. (about 48,000). This difference is due to two carbohydrate side chains in the H2, as opposed to one in the HLA antigen (33). It is also interesting to note that the human β_2m (lane B) runs slightly faster than the mouse β_2m associated with either H2 or HLA (lanes A, C, and D).

Another analysis of these HLA gene products was done by IEF. Figure 3 shows that the mobility on IEF of the transfected HLA-A2 and HLA-B7 antigens (lanes B and C) corresponds to the HLA-A2, B7 antigens in the human cell line JY (lane A). Although all the bands present in the human HLA antigens were found in the transfectants, their intensity seem to differ slightly. These two proteins are different from the H2 antigens (lane D). The charge heterogeneity displayed in this system shows that the oligosaccharide side chain maturation process of the HLA has occurred in the murine L cells. The H2 molecules present in the L cells (lane D) show a large heterogeneity because the monoclonal M1/42.3 reacts with H2K, H2D, and H2L (24) and, in addition, the sialic acids in the H2 antigens can be linked to two different carbohydrate side chains instead of just one on the HLA (33).

Mouse L cells expressing HLA-A2 or HLA-B7 as target cells for human anti-HLA-A2 or anti-HLA-B7 CTL. Because the gene products of the HLA-A2 and B7 genes appeared to be expressed correctly, as judged by the monoclonal antibodies, recognition by cytotoxic T lymphocytes was studied. Previously, we generated the CTL clones HG-31 JR-2-16 against the HLA-B7 and HLA-A2 antigens, respectively (18). This specificity has been corroborated by panel studies using peripheral blood lymphocytes from HLA-typed individuals and by family studies (19). In addition, the cytotoxicity of the clones can be inhibited by monoclonal antibodies directed against the HLA-A2 or HLA-B7 antigens (19, 26). When these clones were used as effector cells with ^{51}Cr labeled mouse MC2.2 and CM7.13 cells as targets, no lysis could be observed (Table I). The transfectants were killed, however, by B6 anti-C3H mouse CTL (Table II). In order to exclude the possibility that the CTL clones HG-31 and JR-2-16 could be specific for a unique determinant not accessible in the L cells, we decided to use, instead, a mixture of CTL from a tertiary MLC. Under these conditions, the pool of CTL will recognize multiple determinants. However, negative results were also obtained when a MLC stimulated with JY was assayed for CTL activity against the transfectants (Table III). It remains possible that JY contains multiple nonallelic products and, therefore, the antigens encoded by JY150 and JYB3.2 are not recognized by the CTL clones and there is a minor population of

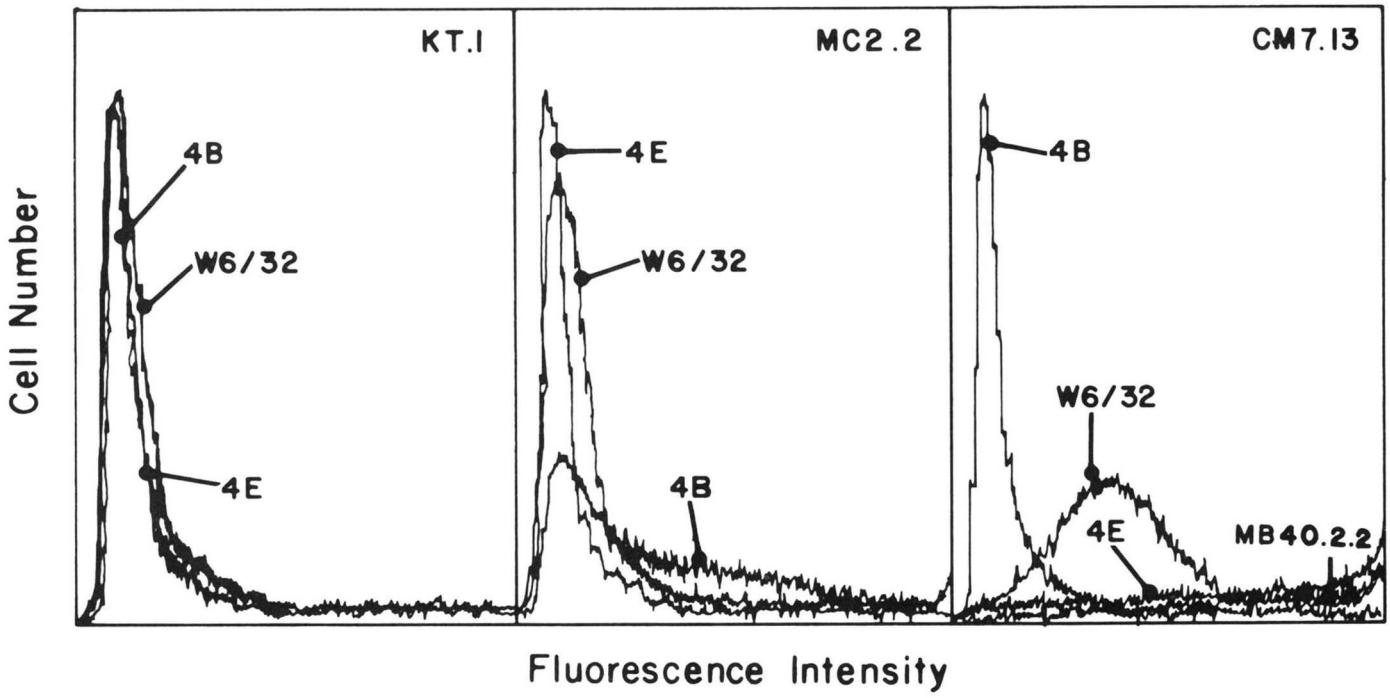

Figure 1. Expression of HLA-A2 and HLA-B7 antigenic determinants on the transfectants. The cells were labeled with the monoclonal antibodies W6/32 (monomorphic for HLA heavy chain), 4E (specific for HLA-B polymorphic epitopes) 4B (specific for HLA-A2 and HLA-A28), and MB40.2 (specific for HLA-B7 and HLA-B40). After washing, the cells were stained with FITC goat anti-mouse Fab and analyzed on a cytofluorograph as described in *Materials and Methods*. The fluorescence intensity is displayed on a linear scale.

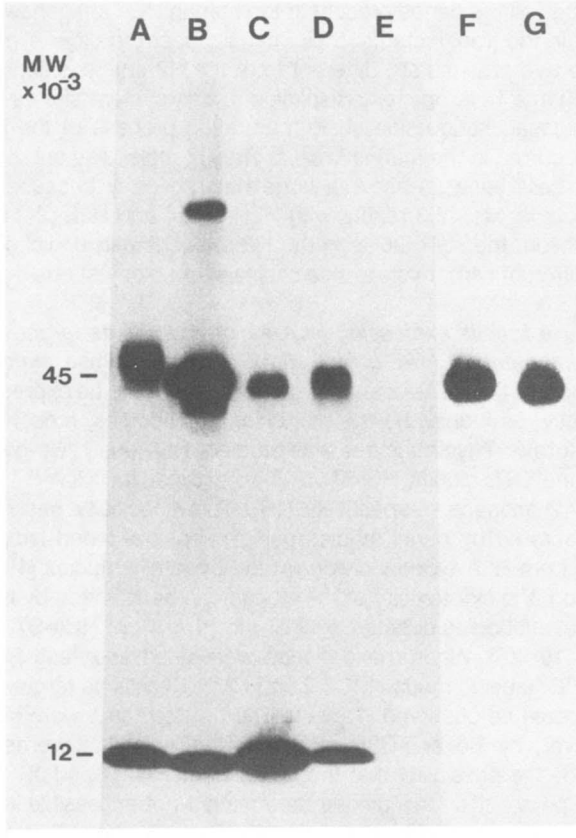

Figure 2. Immunoprecipitation analysis by HLA-A2 and HLA-B7 antigens on SDS-PAGE. Cells were ^{125}I labeled with lactoperoxidase, and the lysates were immunoprecipitated as described in *Materials and Methods*. A: KT.1 clone, anti-H2 monoclonal antibody (M1/42.3); B: JY cells, anti-human β_2m monoclonal antibody; C: CM7.13 clone, anti-HLA-B monoclonal antibody (4E); D: MC.2.2 clone, anti-HLA-A2 monoclonal antibody (4B); E: KT.1 clone, 43 plus 4B monoclonal antibodies; F: MC2.2 clone, rabbit anti-HLA heavy chain antiserum (C41); G: CM7.13 clone, rabbit anti-HLA heavy chain antiserum (C41).

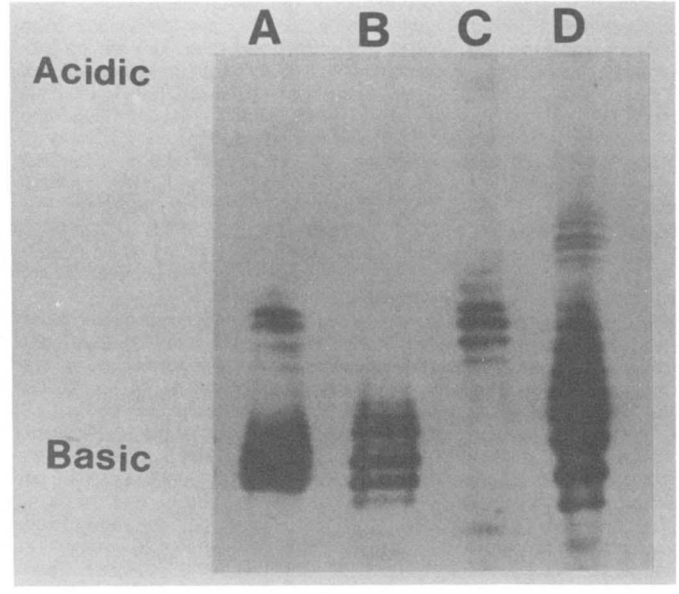

Figure 3. Immunoprecipitation analysis of HLA-A2 and HLA-B7 antigens by IEF. Cells were ^{125}I-labeled with lactoperoxidase, and the lysates were immunoprecipitated. The immunoprecipitates were run on SDS-PAGE, and the bands of heavy chains were cut out and subjected to IEF as described in *Materials and Methods*. A: JY cells, anti-human β_2m monoclonal antibody; B: MC.2.2 clone, anti-HLA-A2 monoclonal antibody; C: CM7.13 clone, anti-HLA-B monoclonal antibody; D: KT.1 clone, anti-H2 monoclonal antibody (M1/42.3)

specific CTL not detected in the bulk MLC. To test whether the recognition of the HLA-A2 (or HLA-B7) by CTL was affected, cold target inhibition studies were conducted. As shown in Table IV, no inhibition of the CTL-dependent killing of lymphocytes positive for HLA-A2 or HLA-B7 by the transfectants could be observed even at the highest ratio (50:1). This means that the recognition step in the CTL killing of the transfectants has been affected.

In many systems, it has been shown that lectins can overcome

TABLE I

Cytotoxic activity of clone JR-2-16 and HG-31 vs. transfected cell lines MC 2.2 and CM 7.13 with and without lectin

Target Cell	CTL Clone	Added to Assay	% ^{51}Cr Release at an Effector/Target Ratio of	
			1:1	5:1
MC2.2	JR-2-16	Nothing	1.5	−2.2
		0.1 μg PHA	9.9	7.2
		0.5 μg PHA	9.3	18.6
		0.5 μg ConA	−1.4	−2.0
KT.1	JR-2-16	Nothing	1.9	4.2
		0.1 μg PHA	5.2	9.3
		0.5 μg PHA	6.8	8.9
		0.5 μg ConA	2.6	3.3
CM7.13	HG-31	Nothing	4.1	7.9
		0.1 μg PHA	3.7	11.6
		0.5 μg PHA	3.4	9.1
		0.5 μg PHA	2.9	9.3
KT.1	HG-31	Nothing	1.6	11.8
		0.1 μg PHA	4.6	19.4
		0.5 μg PHA	6.3	14.9
		0.5 μg ConA	4.9	20.2
JY	JR-2-16	Nothing	62.2	80.5
	HG-31	Nothing	41.2	53.6

TABLE II

Cytotoxic activity of mouse CTL against transfected L cells[a]

Target Cell	B6 Cells Primed Against	% ^{51}Cr Release At An Effector:Target Ratio Of		
		3:1	15:1	75:1
MC2.2	B6.C3H	6.6	22	48.8
	B6.D2	0.4	0	6.0
CM7.13	B6.C3H	3.9	20.7	42.8
	B6.D2	0	0.7	5.8
KT.1	B6.C3H	10.0	30.7	47.0
	B6.D2	0.9	1.4	6.8
RDM4	B6.C3H	15.3	34.1	51.7
	B6.D2	1.7	2.5	10.1

[a] Effector cells (5×10^6) were incubated with 5×10^6 stimulator cells that were previously treated with Tris-HCl ammonium chloride and irradiated (1,200 rad). Cultures were incubated for 5 days at 37°C in a 5% CO_2 incubator after which the cytotoxicity assay was performed. The H2 haplotypes are: B6 ($H2^b$), C3H ($H2^k$), D2 ($H2^d$), L cells ($H2^k$), and RDM4 is an $H2^k$ tumor cell line.

TABLE III

Cytotoxic activity of a tertiary human MLC against transfected L cells[a]

Target Cell	Added to Assay	% ^{51}Cr Release	
		MLC-1	MLC-2
MC2.2	None	0.0	0.1
	PHA	0.1	0.2
CM7.13	None	0.0	0.0
	PHA	0.1	0.1
KT.1	None	0.0	0.2
	PHA	0.1	0.2
JY	None	73.1	43.0
	PHA	74.2	69.4

[a] Human PBL were stimulated three times with JY (irradiated with 6000 rad) on days 1, 7, and 14. The assay was performed on day 20. The HLA type of the PBL was: MLC-1 (HLA-A2, A11, B8BW51), MLC-2 (HLA-AW24, A29, B17, BW35). During the assay, the effector/target ratio was 100:1. When present, PHA was 1 μg/ml.

TABLE IV

Cold target inhibition by HLA-A2 and B7 positive cells of CTL killing[a]

Inhibitor/ Target	CTL Clone	Inhibitor Cell Line and % ^{51}Cr Release				
		MC2.2	CM7.13	KT.1	JY	None
50:1	HG-31	—	40.2	38.0	1.3	56.2
	JR-2-16	17.8	—	15.2	0.6	31.4
10:1	HG-31	—	49.8	53.6	2.6	56.2
	JR-2-16	18.8	—	20.4	2.5	31.4
2:1	HG-31	—	55.3	52.6	0.5	56.2
	JR-2-16	32.7	—	36.1	0.2	31.4

[a] The effector clones JR-2-16 and HG-31 were incubated with ^{51}Cr-labeled lymphocytes positive for HLA-A2 and HLA-B7, respectively, as targets. Inhibition by cold targets was carried out as described in *Material and Methods*. The target/effector ratio was 1:1.

TABLE V

Labeling of β_2m in different class I antigens[a]

Cell Type	Monoclonal	Class I Antigen	β_2m/Heavy Chain
CM.2.2	M1/42.3	H2	0.16
MC.7.13	M1/42.3	H2	0.15
CM.2.2	4B	HLA-A2	4
MC.7.13	4E	HLA-B7	25
JY	4B	HLA-A2	0.60
JY	4E	HLA-B7	0.53
JY	anti-β_2m	HLA-A2,-B7	0.36

[a] Cells were ^{125}I labeled with lactoperoxidase and immunoprecipitated with the monoclonals described in the Table. After SDS-PAGE and autoradiography, β_2m and heavy chain bands were cut out and counted in a gamma counter.

antigen specificity in CML (34, 35). As shown in Tables I and III, no killing was observed in the presence of PHA or Con A. In control experiments, in a mouse system lectin-dependent cytolysis of L cells could be demonstrated (data not shown). Taken together, these experiments could indicate that the human HLA-murine β_2m complex does not have the appropriate conformation for the human CTL killing. However, the PHA and Con A experiments show that, in addition to recognition of the HLA antigens, other cell surface components involved in the adhesion and or lytic phases could be missing.

Study of the complex between human HLA-B7 or HLA-A2 and mouse β_2m. Although the heterologous association between mouse β_2m and human HLA permitted the expression of the HLA heavy chain, the conformation could be inappropriate for the recognition by CTL. In support of some conformational abnormality, was the observation that in the immunoprecipitates a very high ^{125}I uptake in the β_2m was found (Fig. 2, lanes C and D). Table V shows the ^{125}I labeling ratio of β_2m/heavy chain in different class I antigens. This labeling ratio is between one or two orders of magnitude higher in the case of HLA-A2 and HLA-B7 from the transfectants than the H2 (L cells) or HLA (JY) antigens. The radioactivity associated with the β_2m was absent when the C41 antiserum raised against denatured HLA heavy chain was used to immunoprecipitate HLA-A2 or HLA-B7 from the transfectants (Fig. 2, F and G). This result excludes the possibility of an HLA degradation product comigrating with β_2m. Also, upon labeling with [^{35}S]methionine the expected ratio of HLA-A2/β_2m was found (data not shown). The difference in the ^{125}I-β_2m label can be explained by a conformation in the mouse β_2m-HLA complex that facilitates the access to new tyrosine residues. This possibility was tested by peptide map analysis of the mouse β_2m (Fig. 4). As expected from the different labeling extent, more peptides were labeled in β_2m (HLA-B7) than in the β_2m (H2). These results indicate that the conformation of the HLA-β_2m complex in the transfectants could be, in part, responsible for the absence of killing by the human CTL.

DISCUSSION

The expression of HLA-A2 and HLA-B7 antigens on the transfectant clones could be recognized by immunofluorescence staining with the monomorphic (W6/32) and polymorphic (4E, 4B, and MB.40.2.2) monoclonal antibodies (Fig. 1). The antibody W6/32 recognizes the HLA heavy chain when associated with

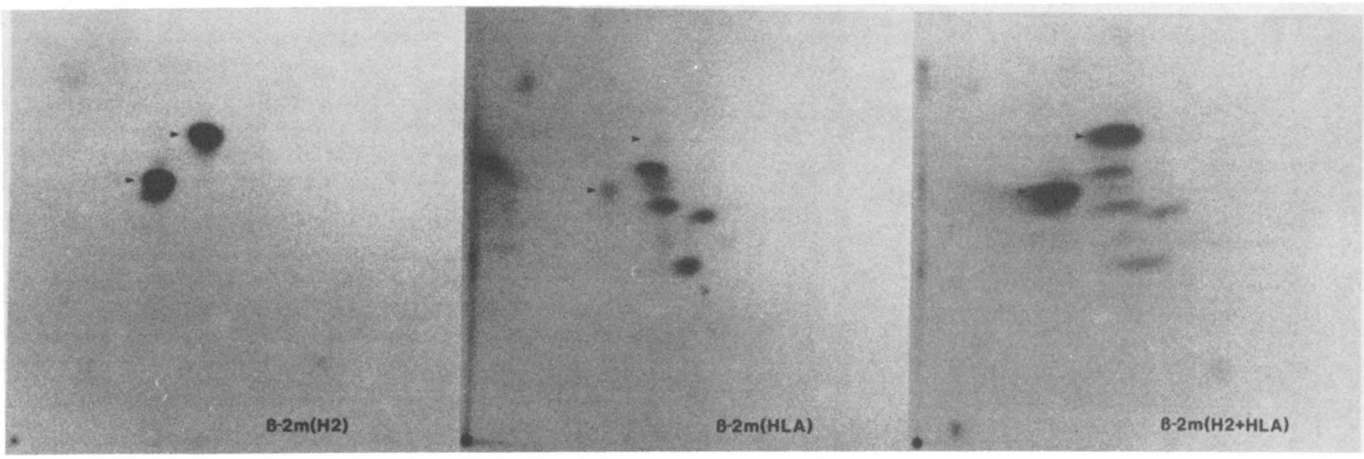

Figure 4. Peptide mapping analysis of mouse β_2m associated with either HLA-B7 or H2 antigens. CM7.13 cells were iodinated with lactoperoxidase and lysed with IPB buffer. The lysate was immunoprecipitated with 4E and M1/42.3 monoclonal antibodies and the immunoprecipitations were run on SDS-PAGE. β_2m bands were detected by autoradiography on SDS-slab gels cut out and trypsinized as described in *Materials and Methods*. The arrows point at the two common spots present in the β_2m peptide maps.

the β_2m (21). Therefore, as in the case of somatic cell hybrids, the HLA heavy chain expressed on the mouse L cells is associated with the murine β_2m (4, 5, 36). The reactivity of the transfectants with the polymorphic antibodies was stronger than with the antibody W6/32. Because β_2m interacts with a monomorphic part of the HLA heavy chain, perhaps the reactivity of the W6/32 antibody is affected by the presence of the murine β_2m. The expression of the H2 antigens was found to be unaltered by the presence of the HLA genes (data not shown), as reported by Lemonnier *et al.* (15). The level of expression of HLA per transfectant cell is lower than the endogenous H2 from the L cells or the HLA from the human lymphoblastoid cell line JY, as reported previously (14, 15). Within this range, HLA-B7 was more expressed than HLA-A2. The nature of this difference is not yet understood.

As indicated by SDS-PAGE and IEF, the primary structure of both heavy chains is basically the same as the HLA of the parental cell line JY (Figs. 2 and 3). These HLA expressing mouse L cells were used to study the role of HLA in T cell-mediated cytotoxicity. In an allogeneic MLR, the presence of cells with a foreign HLA type generates CTL that will lyse cells from individuals with the HLA type used for priming. Although the basic requirement for the CTL killing within one species is only the different HLA, it is not known if this HLA molecule is recognized by itself or in association with another structure(s). Using the transfectants as a model target against human CTL directed to HLA-A2 or HLA-B7 bearing cells, no killing was observed. It has been shown, however, that human T cells primed against murine spleen cells could kill specifically for mouse MHC antigens (37, 38). As a control for lysability, L cells or transfectants were shown to be killed by specific mouse CTL (Table II and References 11, 12, 39, and 40). The recognition step could thus be dependent on the presence of the specific β-2m. Inasmuch as the CTL clones are directed at the allodeterminants, the HLA-mouse β-2m complex is not recognized, because it represents an example of allo plus x. Our result, together with the inability of the transfectants to act as cold targets, might be explained "a priori" by the conformational change detected by our iodination studies on the HLA-β_2m complex (Fig. 4). An abnormal conformation of the murine β_2m associated with human HLA has also been reported by Lemonnier *et al.* (41). In order to elucidate the importance of the β2m in the HLA complex, we are presently trying to transfect the HLA genes into human fibroblasts.

The fact that lectins are not able to overcome the antigen specificity suggests that other molecules, in addition to HLA, are also involved in the lytic mechanism. This is especially true, since in control experiments the transfectants could be lysed by murine cytotoxic T lymphocytes. Whatever extra molecules are necessary for the HLA recognition and killing, they are either absent in the mouse L cells, or, if present, they are not able to overcome the species barrier. Recent reports have described several new antigens that seem to be involved in the CML (34, 42–46). Monoclonal antibodies against these antigens seem to differently affect the cell-mediated cytotoxicity. It has been found, for example, that the adhesion step can be blocked by antibodies against LFA-1 and T8 (44, 45), whereas the antigen T3 seems to be involved in the postadhesion step (44, 46). It is interesting to note that LFA-1 has also been shown to inhibit LDCC (45). Assuming that the LFA-1 interspecies interaction in our xenogeneic system is insufficient, this would explain the absence of killing in the presence of lectins. Further studies will be required in order to elucidate the participation of these antigens in CTL killing.

Acknowledgments. We thank Dr. Victor Corces for his valuable advice on DNA transfection, Dr. Sherman Weissman for his generous gift of the DNA-clones, Drs. Steven Burakoff and Francisco Sanchez-Madrid for their stimulating discussions, and Susan K. Lupul and Donna Moschella for their assistance in preparing the manuscript.

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