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Immunology

Gamma ray-induced loss of expression of HLA and glyoxalase I alleles in lymphoblastoid cells

(HLA typing/haplotype variants/hybridoma screening)

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ABSTRACT Gamma rays from a cesium source were used to generate human lymphoblastoid cell line variants that had lost expression of all major histocompatibility complex antigens coded for by a single haplotype. The cell line was heterozygous at the glyoxalase I locus and had the HLA haplotypes *HLA-A1, B8, DRw3,* and *HLA-A2, B5, DRw1.* We selected with anti-HLA-B8 antiserum in a population of cells that had been irradiated with 300 R. The incidence of B8-loss variants was 4.1×10^{-5} on day 5 after irradiation. Analysis of variants showed that expressions of *HLA* and *GLO* alleles *trans* to B8 were retained. However, expression of additional *cis*-linked *HLA* and *GLO* gene products was lost in 12 of 17 variants. Variants that had lost expression of (*i*) *HLA-B8,* (*ii*) *HLA-B8, A1,* (*iii*) *HLA-B8, A1, DRw3,* or (*iv*) *HLA-B8, A1, DRw3* and the *cis*-linked glyoxalase I allele were obtained. Karyotype analysis was performed on eight variants that had lost expression of two or more *cis*-linked alleles. Three variants had two normal appearing no. 6 chromosomes, four variants had a deletion that included the region coding for *HLA* genes on the short arm of one no. 6 chromosome, and one variant had an inversion or translocation involving the short arm of one no. 6.

One useful approach to studying major histocompatibility complex (MHC) antigens is to isolate antigen variants of somatic cells. Klein and associates (1, 2) first attempted to obtain H-2 antigen variants of tumor cells by *in vivo* selection. More recently, *in vitro* selection for MHC antigen variants of mouse cell lines (3, 4) and of human lymphoblastoid cell lines (5, 6) has been reported. A single exposure of cell lines to H-2 or HLA antiserum and complement sufficed for isolation of resistant clones.

Variants of human lymphoblastoid cell lines with altered expression of a MHC antigen were first reported by Pious *et al.* (5). Variants that originated spontaneously or after treatment with chemical mutagens were obtained (7, 8). The great majority of these variants had lost expression of only the HLA antigen selected against, but simultaneous loss of two or more HLA antigens in three variants that originated after unspecified mutagenesis has been briefly reported in an abstract (9). The mechanism of variant formation was not determined precisely. Evidence against antigenic modulation, chromosome loss, mitotic recombination, and antigenic masking was reported in several variants tested (10).

We have used anti-HLA-B8 antiserum and rabbit complement to select for variants of a human lymphoblastoid line that carried the HLA haplotypes *HLA-A1, B8, DRw3* and *HLA-A2, B5, DRw1* and was heterozygous at the linked glyoxalase I locus (*GLO*). This allowed us to select for loss of *HLA-B8* and then determine expression of *cis* and *trans* HLA and glyoxalase I alleles in the variants. Our aim was to obtain variants of lymphoblastoid cell lines that had lost expression of all HLA antigens coded for by a single haplotype. Ionizing radiations were used as the mutagen because they are known to induce terminal and interstitial deletions ranging in size from intragenic deletions to losses of most or all of a chromosome (11).

Variants were obtained after selecting, with anti-HLA-B8 antiserum and complement, against lymphoblastoid cells that had been irradiated with 300 R (0.08 coulomb/kg) of gamma radiation. Variants never lost expression of *trans* alleles; there were four types that had lost expression of (*i*) *HLA-B8* alone, (*ii*) *HLA-B8* and *A1,* (*iii*) *HLA-B8, A1* and *DRw3,* or (*iv*) *HLA-B8, A1, DRw3* and the *cis*-linked glyoxalase I allele. Deletions, mitotic recombinations, and polar regulatory changes resulting from mutations, inversions, and translocations could account for the spectrum of variants we observed. The frequency with which these variants were obtained is high enough to make this a useful approach for obtaining haplotypic loss variants of human lymphoblastoid lines that can be used in serological, cellular, and biochemical approaches to typing and studying HLA antigens.

MATERIALS AND METHODS

Cell Lines. Lymphoblastoid cultures were established by infecting peripheral blood lymphocytes from members of local families with Epstein–Barr virus by the procedure of Sugden and Mark (12), except that infected lymphocytes were plated directly on gamma-irradiated feeder layers of fibroblasts without an intervening layer of agarose.

Variants were isolated from LCL-721, which had been in culture for 10 months. The HLA haplotypes and their *cis*-linked glyoxalase alleles on the short arm of chromosome 6 starting from the centromere were Glo^{s}, *HLA-DRw3, -B8, -A1* and Glo^{fj}, *HLA-DRw1, -B5, -A2* (Fig. 1). Typing results for the lymphoblastoid culture LCL-721 and the normal lymphocytes from which they originated were identical with regard to specificities we studied.

Lymphoblastoid lines were grown in Falcon flasks (no. 3013 or 3024) in RPMI-1640 medium containing 25 mM Hepes buffer, penicillin (100 units/ml), streptomycin (100 μg/ml), and 15% heat-inactivated fetal bovine serum that had been screened for ability to support cell growth. This medium is referred to as 15% FCS/RPMI. The cell density was maintained at 1×10^5 to 1×10^6 cells per ml. During the 4 days prior to irradiation, portions of cultures were removed daily and replaced with fresh medium in order to maintain exponential growth.

Before selection, LCL-721 growing in exponential phase was suspended at 2×10^5 cells per ml and irradiated with 300 R at

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Abbreviation: MHC, major histocompatibility complex.

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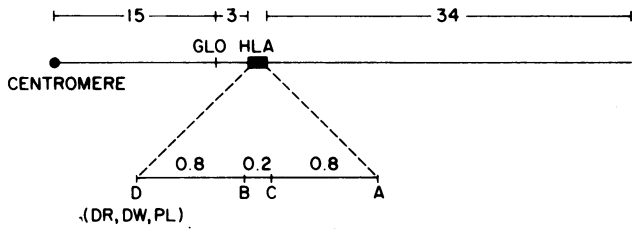

FIG. 1. Locations of relevant loci on the short arm of human chromosome 6. Recombination frequencies (expressed in centimorgans) are for males (13). GLO stands for glyoxalase I. The major histocompatibility gene products HLA-A, HLA-B, HLA-C, and HLA-D are identified by serological methods. Products of the HLA-D region are identified by serological means (DR antigens) and by two cell-mediated methods, the mixed lymphocyte test (DW antigens) and the primed lymphocyte test (PL antigens). It is not known if the molecules detected by serological and cellular methods are coded for by the same genes.

a dose rate of 1.5×10^4 R/hr from a mark I cesium source. Some cells were immediately plated in soft agarose to measure survival. Cells not plated were fed every day and kept at a cell density of $2–6 \times 10^5$/ml.

Antisera and Complement. HLA-A and HLA-B antisera were purchased from the American Red Cross (Madison, WI). The sera, from multiparous donors, were obtained as recalcified plasmas and were defined with a panel of lymphocytes (unseparated) from 50 donors. Sera with desired specificity and strong cytotoxicity (80–100%) were designated Zei. anti-HLA-B8, Til. anti-HLA-A1, Teb. anti-HLA-A2, and Hen. anti-HLA-B5. Variants were tested once with serum Teb. and at least twice with all the other sera. The serum used for selection was Ruc. anti-HLA-B8. The HLA-DRw sera were kindly provided by the University of Minnesota Histocompatibility Laboratory. The sera, from multiparous donors, were absorbed with a pool of platelets from 100 donors. Equal volumes of packed platelets and serum were incubated at 37°C for 1 hr and overnight at 4°C. The anti-DRw3 sera were Craig, Bachman, and Ihrig and the anti-DRw1 sera were Lewondowski and Lee. The Craig, Bachman, and Lewondowski sera were absorbed with lymphoblastoid cells to remove nonspecific cytotoxic effects on the LCL lines. Sera that were used to characterize variants either killed more than 95% of the cells (positive typing response) or gave a negative typing response of less than 25% cytotoxicity. Serological testing of HLA antigens was performed by using the standard National Institutes of Health microcytotoxicity assay with Terasaki plates (14).

Pooled serum from 6- to 12-week-old rabbits (purchased from Pel-Freez) was used as the complement source. Cytotoxic activity of the complement was reduced by diluting the complement with heat-inactivated human AB serum that lacked antibody reactive with T or B lymphocytes, as suggested by Ferrone *et al.* (15).

Selection. Cells were centrifuged for 5 min at $150 \times g$ and washed once with 4 ml of cold RPMI-1640 containing 0.5% fetal calf serum (designated 0.5% FCS/RPMI). One milliliter of antiserum was added to each tube which contained a pellet of 1×10^6 cells. After mixing, cells and antiserum were incubated for 45 min at 22°C with gentle shaking every 10 min. Then, 4 ml of cold medium was added and the cells were centrifuged as before. The supernatant was decanted and 5 ml of complement diluted 7:3 with AB serum was added per 10^6 cells. Cells and complement were incubated for 90 min at 22°C with gentle shaking every 10 min. Ten milliliters of cold 0.5% FCS/RPMI was added and the cells were centrifuged, resuspended in soft agar plating medium, and distributed into P60 petri dishes (2×10^5 cells per dish) for cloning.

Selection against cells that expressed HLA-B8 was not complete. Therefore, cytotoxicity tests (13) were used to determine if colonies that grew after selection were true variants.

Absorption. Cells (10×10^6) were spun for 5 min at $150 \times g$, washed once with cold 0.5% FCS/RPMI, transferred to a Microfuge tube and spun for 1 min at $8700 \times g$ in a Beckman Microfuge B. After removal of the supernatant, 1 ml of antiserum was added and absorption was carried out for 30 min at 22°C; the tube was shaken every 10 min. To remove absorbing cells from the serum, the suspension was centrifuged twice for 4 min in the Microfuge.

Cloning of Lymphoblastoid Cells. Cloning was performed in semisolid agarose with a fibroblast feeder layer by the method of Sugden and Mark (12) except that dishes were not sealed with adhesive tape. Dishes were fed with 1 ml of 15% FCS/RPMI on day 1 and with 2 ml of medium every 6 days thereafter. The cloning efficiency of LCL-721 was 25%.

Amplifying Clones from Soft Agar. Linbro 24-well plates (no. 76-033-05) were seeded with 1×10^5 fibroblasts per well. When the fibroblasts had reached confluency they were irradiated with 4000 R. After 2 days, 1 ml of 15% FCS/RPMI was added per well. Fresh medium (0.5 ml per well) was added the next day. By using pasteur pipettes, 2-week-old colonies isolated from soft agarose were individually placed in wells and vigorously pipetted against the side of the well to disperse the cells. Wells received additions of 0.5 ml on each of the next 2 days; on every second day after that, 1 ml of medium was removed and replaced with fresh medium. After 1–2 weeks most wells had at least 5×10^5 cells. Cells from a well were then transferred to a 35-mm petri dish (P30). In some wells, however, the cells grew so slowly that 1 month elapsed before the cells were transferred to a P30 dish. Cells were maintained as suspension cultures without further selection, and aliquots were stored in 25% FCS/RPMI with 10% (vol/vol) dimethyl sulfoxide in liquid N_2.

Starch Gel Electrophoresis. Horizontal starch gel electrophoresis of glyoxalase I was performed by the method of Kompf *et al.* (16) as outlined by Harris and Hopkinson (17). Cells (5×10^6) growing exponentially were centrifuged for 5 min at $150 \times g$, washed once with phosphate-buffered saline (Ca^{2+}-, Mg^{2+}-free), transferred to Microfuge tubes, and centrifuged for 10 sec at $8700 \times g$ in a Beckman Microfuge B. After the supernatant was removed, 60 μl of gel buffer was added and the cells were resuspended. The suspension was freeze-thawed three times in acetone/dry ice and then centrifuged for 20 min at $35,000 \times g$ in a Sorvall RC-5 centrifuge. Fifty microliters of lysate was added to slots in the gel and then overlayered with paraffin. Electrophoresis was for 17 hr at 3.6 V/cm at 4°C. The enzyme was located in the gel by staining with iodine according to the procedure of Parr *et al.* (18).

Karyotyping. Lymphoblastoid cells were grown at 2×10^5 to 8×10^5 cells per ml in spinner cultures in 15% FCS/RPMI containing 0.1% Pluronic F68 (BASF, Wyandotte, MI). An aliquot (2.5×10^6 cells) was incubated for 2 hr at 37°C with vinblastine sulfate (Velban, Eli Lilly) at 0.05 μg/ml. Cells were spun at $350 \times g$ for 1.5 min and slowly resuspended in 5 ml of hypotonic solution (40 × stock: NaCl, 90 g/liter; Na_2HPO_4, 13.65 g/liter; $NaH_2PO_4 \cdot H_2O$, 2.14 g/liter) at 37°C. After a 15-, 20-, or 30-min incubation, cells were spun as before and slowly resuspended in 1.5 ml of ice-cold methanol/acetic acid, 3:1 (vol/vol), as fixative. After a 10-min incubation on ice, the cells were given two more fixative rinses separated by a 10-min interval on ice. Cells were resuspended in a final volume of 0.7 ml of fixative and stored at 4°C overnight. Cells were then usually resuspended in methanol/acetic acid, 1:1 (vol/vol), and, after 4 hr, 3 drops of cell suspension were dropped 3 feet onto

Table 1. Isolation of variants and mutation frequency

Selection		Cells plated,	Cloning efficiency		Colonies isolated,	Colonies tested		True variants,	Variant frequency
Day	Treatment	no. $\times 10^{-6}$	Without treatment	After complement treatment	no.	No.	%	no.	$\times 10^{5}$
5	Irradiated	4	0.154	0.121	247	144	58	20	4.1
7	Irradiated	4	0.182	0.107	71	43	60	5	1.17
	Spontaneous	1	—	0.182	8	6	75	0	<0.55
9	Irradiated	4	0.194	0.158	65	35	53	13	2.06
	Spontaneous	1	—	0.206	29	22	76	1	0.48
13	Irradiated	4	0.158	0.148	49	31	63	5	0.91
	Spontaneous	1	—	0.144	23	13	56	0	<0.7

Variant frequency was calculated as no. of variants/(total cells times cloning efficiency after complement treatment alone).

wet (cold water) slides and dried with warm air from a hair dryer.

After 7 days of storage, cells were subjected to brief trypsinization and stained with Giemsa by a modified Seabright method (19). Slides were immersed in a 0.0125% trypsin solution (20–22°C) for 20–35 sec. The stock trypsin solution, made just before use, was 0.0325 g of Difco 1:250 trypsin in 5 ml of DP rinsing solution (0.1 g of KCl, 4.0 g of NaCl, 0.1 g of KH_2PO_4, and 0.58 g of Na_2HPO_4 made up to 500 ml with distilled water and adjusted to pH 7.0 with 0.2 M Na_2HPO_4 or 5 M HCl); 1 ml of this stock solution was diluted in 49 ml of DP solution. After trypsinization, slides were put through two 45-sec rinses with DP solution. Slides were then stained for 4–6 min in 1.5 ml of Giemsa (Harleco, no. 620) plus 50 ml of pH 6.8 buffer made up with Gurr's pH 6.8 buffer tablets. Slides were rinsed in distilled water for 5–10 sec and air dried. Sometimes, aged slides were incubated overnight in a 56°C oven before staining.

RESULTS

During initial selection experiments with anti-HLA-B8 antiserum, several variants were identified that survived the selection procedure but were sensitive to killing effects of the antiserum, although less so than the parental LCL-721 cells. It seemed possible that the antibody reacting against the survivors was directed against antigens other than B8, such as DRw3. The antiserum was subsequently absorbed with an anti-B8-sensitive variant. This absorbed serum was used in subsequent experiments because it no longer killed variants but was still able to kill essentially all of the parental LCL-721 cells. Any contaminating anti-HLA-A1 or anti-HLA-DRw3 antibody that may have existed in the serum would have been removed by the absorption procedure because the B8 variant used for absorption expressed HLA-A1 and -DRw3.

Because there was no published account of the mutagenic effects of ionizing radiation on LCL lines, cell survival and the incidence of hypoxanthine phosphoribosyltransferase (HPRT) variants at different doses of irradiation were determined for LCL-721. The HPRT selection system was used as a "range-finder" for mutagenizing *HLA* genes. The D_0 dose (the dose giving a survival of 37%) was 161 R and the back-extrapolate of the exponential portion of the survival curve to 0 dose was 1.2. A dose of 300 R was chosen for irradiating cells before antibody selection because it gave a survival of 10% and the incidence of HPRT mutants increased to 3.9×10^{-5} per survivor (details of this work will be published elsewhere).

At the beginning of a selection experiment, 8×10^6 cells were irradiated with 300 R. The total number of cells began to increase on the second day after irradiation and increased exponentially thereafter. On days 5, 7, 9, and 13, selection with antiserum was imposed on 4×10^6 cells from the irradiated culture and on 1×10^6 unirradiated cells to measure the background of spontaneous variants. After selection the cells were plated in soft agarose medium and colonies were isolated after 14–18 days. Only about 60% of the isolated clones grew well enough to be analyzed. Most of those that died contained less than about 1000 cells. Because the highest proportion of variants occurred in small clones and small clones were most likely to die before being analyzed, the mutation frequency observed may be an underestimate.

A total of 43 induced and 1 spontaneous variant was obtained (Table 1). The highest mutant frequency occurred on day 5 and was 4.1×10^{-5}. The spontaneous frequency was 0.5×10^{-5} or lower. Variants were stable because the B8 phenotype did not reappear in any variant after 6 months in culture without selection.

Analysis of Variants. The 44 variants that had lost expression of the HLA-B8 antigen were analyzed to determine if other *HLA* encoded histocompatibility antigens or allelic products of glyoxalase I had been lost. Of those tested, none had lost histocompatibility antigens or the Glo^1 type coded for by genes in the *trans* position relative to the *B8* allele. However, expressions of *cis*-linked *HLA* loci were simultaneously lost in some of the variants. Some of the variants lost expression of the *cis*-linked Glo^2 allele as well (Fig. 2). The highest incidence of variants with multiple losses occurred on days 5 and 7. Variants that had lost expression of (*i*) HLA-B8 alone, (*ii*) HLA-B8, A1, (*iii*) HLA-B8, A1, DRw3, or (*iv*) HLA-B8, A1, DRw3, and Glo^2 were found (Table 2). Any variant that had lost expression of

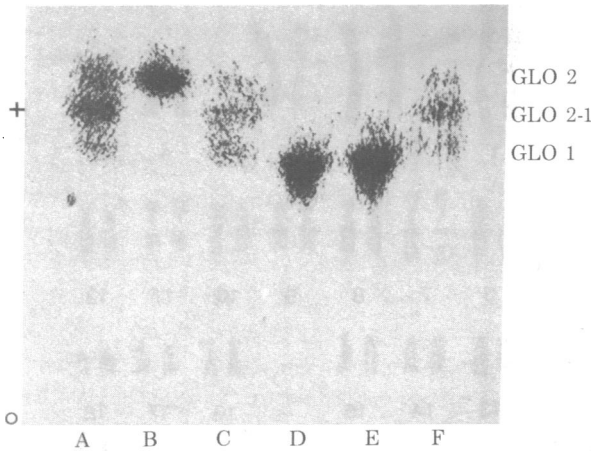

FIG. 2. Starch gel electrophoresis of cells for glyoxalase I. Three common phenotypes (GLO 1, GLO 2-1, and GLO 2) have been identified. GLO 1 (slow type) and the GLO 2 (fast type) are the two homozygous phenotypes of a two-allele system. The intermediate type, GLO 2-1, is the heterozygous phenotype in which subunits contributed by both alleles associated randomly in pairs to form three different isoenzymes: two homodimers and a heterodimer. Variants 1 and 2 originated independently. Lanes: A, LCL-sibling; B, LCL-father; C, LCL-721; D, LCL-mother; E, variant 1; F, variant 2.

Table 2. Multiple-loss variants obtained on different days of selection*

Day of selection	No. lost/no. tested			
	B8 only	B8, A1	B8, A1, DRw3	B8, A1, DRw3, GLO^2
5	5/17	2/17	7/17	3/17
7	1/5	1/5	2/5	1/5
9	7/12	2/12	2/12	1/12
13	2/4	0/4	2/4	0/4
9†	0/1	1/1	0/1	0/1

* Five variants that grew poorly could not be completely characterized because of cell death.
† Spontaneous.

DRw3 and Glo^2, in addition to B8, had also lost expression of A1. The spontaneous variant from day 9 had lost HLA-B8 and HLA-A1.

Karyotype Analysis. Karyotypes of the parental line LCL-721 and eight of the variants were determined. The parental line had 46 chromosomes. A small pericentric inversion resulted in transposition of some heterochromatin to the short arm of one no. 16 chromosome. Four of the variants had lost expression of *cis*-linked alleles *A1, B8, DRw3,* and *Glo^2* and had the following characteristics: (*i*) two variants had normal-appearing no. 6 chromosomes in every metaphase examined; (*ii*) one variant had an interstitial deletion of the region of 6p that included the *HLA* genes (20), but multiple rearrangements that were present elsewhere in the cells made it difficult to determine if the genes were present but unexpressed; (*iii*) the fourth variant had a rearrangement, apparently an inversion or translocation, in 6p.

Three of the variants that were analyzed had lost expression of *cis*-linked alleles *A1, B8,* and *DRw3*. Two of these variants had a deletion in one 6p and no other major rearrangements. A complete karyotype of one of these variants is illustrated in Fig. 3. The third triple-loss variant also had an interstitial deletion in 6p, but other major rearrangements again made it difficult to determine if the *HLA* genes had been lost or translocated to another chromosome. The eighth variant had lost expression of *A1* and *B8* and had two normal-appearing no. 6 chromosomes. If the variants analyzed so far are representative of all variants, a large proportion of them should have detectable abnormalities in the short arm of chromosome no. 6.

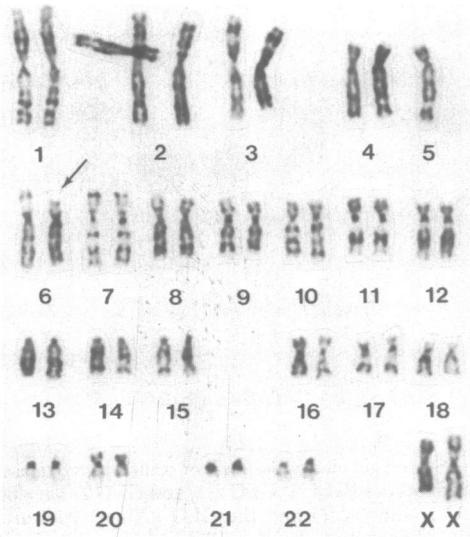

FIG. 3. Karyotype of a variant that had lost expression of *cis*-linked *HLA-A1, B8,* and *DRw3* alleles after irradiation with γ rays. There is an interstitial deletion in 6p of one no. 6 chromosome that includes the region to which the *HLA* genes have been mapped (20).

DISCUSSION

We have shown that loss of HLA antigen expression can be induced in human lymphoblastoid cell lines after treatment with 300 R of γ radiation and selection with HLA antiserum. The maximum frequency of variants, 4.1×10^{-5}, was reached 5 days after irradiation. Because only 58% of the day 5 variants were analyzed, the true mutant frequency may have been about 7.0×10^{-5} per cell surviving irradiation (the survival rate was 10%) if one assumes that variants were as fit as wild-type cells. This assumption is probably false, because we noted that the average variant grew more slowly than normal cells. Therefore, the true mutant frequency may be even higher.

Twelve of 17 variants isolated by selection on day 5 were multiple-loss variants. This is strikingly different from the earlier results (7–9) in which multiple-loss variants were rare among variants that originated spontaneously or after treatment of cells with base substitution- and frameshift-inducing mutagens. Thus, if a particular antigen loss should be desired but the appropriate antiserum is not available or else not powerful enough for use as a selective agent, then selection against *cis*-linked gene products would often suffice if ionizing radiation was used as the mutagenic agent. Even though we did not use antiserum directed against an *HLA-D* region encoded antigen, we readily achieved our objective of obtaining variants that no longer expressed a specific HLA-D antigen.

There are several broad categories of possible mechanisms for the origin of single-allele expression in HLA variants of heterozygous cells (21): (*i*) mutation (narrow sense), a change in the sequence of complementary H-bonding groups in DNA bases that is responsible for specificity of replication and transcription; (*ii*) mutation (broad sense), this includes in addition to *i*, partial or complete changes in ploidy, translocations, and inversions that can result in position-effect gene inactivation and mitotic recombination; (*iii*) epigenetic mechanisms, hereditary changes in phenotype that are not included in *i* or *ii* (see ref. 21 for a discussion).

Hypothetical explanations must account for the pattern we observed: there was either loss in expression of just one allele of one locus or, when loss in expression of alleles of more than one locus occurred simultaneously, the alleles were *cis*-linked. This pattern makes it unlikely that cell-wide epigenetic modulation of the expression of *HLA* loci was responsible for the loss of expression of specific alleles, especially when the expression of a *cis*-linked *Glo^2* allele was also lost. Furthermore, none of the variants we isolated displayed the revertibility observed in a multiple-loss variant described earlier (9).

γ-Ray-induced chromosome rearrangements, especially deletions, apparently are responsible for a large proportion of the antigen losses we observed. A total of 44 independent variants were isolated and 5 of the 8 variants karyotypically analyzed so far had microscopically visible aberrations involving the short arm of a no. 6 chromosome. These observations resemble those by Cox and Masson (22), who reported that up to 40% of HPRT-deficient mutants of human fibroblasts that resulted from ionizing radiation had microscopically visible aberrations in the region of the X chromosome containing the *HPRT* locus.

Two independent quadruple-loss variants and a double-loss variant had two normal-appearing no. 6 chromosomes. These variants could have resulted from mitotic recombination, relatively small deletions, or regulatory mutations that had polar effects. Quadruple-loss variants could also have resulted from

loss of an entire no. 6 chromosome followed by rediploidization of the remaining chromosome. *cis*-Acting regulatory mutations have been described in eukaryotes (23), but there is scant evidence that they affected the expression of groups of contiguous loci. There is little reason to think that control over *GLO* and *HLA* genes would be coordinate.

If a deletion were responsible for formation of the variants, then there would be one copy of the allele that was expressed and, perhaps, a commensurate reduction in the amount of gene product. However, if mitotic recombination or chromosome loss and rediploidization had occurred following irradiation, there would be two copies of the expressed alleles and, perhaps, twice as much gene product as in cells with a single active gene copy.

The number of copies of the remaining *HLA* alleles should also strikingly affect the incidence with which expression of the alleles are lost. If a variant has two copies of the remaining *HLA-B5* allele, the incidence of loss of its expression should be approximately the square of the incidence of loss of *B5* expression in the original heterozygous cell line. However, if a variant has only one copy of the *B5* allele, then the incidence of *B5* loss should be comparable to the incidence in the original cell line. Multiple-loss variants that retained both *GLO* alleles could not have resulted from chromosome loss and rediploidization. Therefore, quantification of the amount of HLA-B5 and A1 antigen and studies on the incidence of *HLA-B5* or *A1* loss in such variants provide an opportunity for determining if mitotic recombination has occurred.

Variants that simultaneously lost expression of only *cis*-linked HLA-D(DR) and HLA-B were not observed; whenever HLA-D expression was lost, expression of the *cis*-linked HLA-A and -B antigens were simultaneously lost. This could be fortuitous or could have occurred if most variants that had only lost expression of A1 and B8 resulted from mitotic recombination. This possibility can be examined with procedures stated in the preceding paragraph.

One objective of this work was to produce haplotypic variants that can be used for tissue typing. The haplotypic loss variants we obtained can be used much as one would use MHC homozygous lymphoblastoid cells—e.g., for the preparation of primed lymphocyte test reagents (24), as standards for use as a panel source for screening HLA antiserum (25), for absorption and elution of HLA antiserum, and for purification of specific HLA antigens.

We have found that all 18 variants that had lost expression of HLA-DRw3 were also negative for the *HLA-D* region-associated PL specificity (26) as defined by the primed lymphocyte test. We have made primed lymphocyte test reagents by using hemizygous variants as stimulators for priming lymphocytes and find that the lymphocytes are primed to HLA-D determinants encoded for by one haplotype only. Thus, these variants can be used as an alternative to homozygous lymphoblastoid cells.

Finally, *HLA* variants will be useful in identifying hybridomas (27) that make antibody against unique HLA antigenic specificities that are no longer expressed on variants. The supernatant fluids from such hybridomas will react with parental cells but not with variants.

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1. Klein, G. & Klein, E. (1958) *J. Cell. Comp. Physiol.* **52,** Suppl. 1, 125–168.
2. Bjaring, B. & Klein, G. (1968) *J. Natl. Cancer Inst.* **41,** 1411–1429.
3. Rajan, T. V. & Flores, C. (1977) *Immunogenetics* **5,** 585–596.
4. Papermaster, B. & Herzenberg, L. (1966) *J. Cell. Physiol.* **67,** 407–420.
5. Pious, D., Hawley, P. & Forrest, G. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1397–1400.
6. Lampson, L., Levy, R., Grumet, F. C., Ness, D. & Pious, D. (1978) *Nature (London)* **271,** 459–462.
7. Pious, D. & Soderland, C. (1977) *Science* **197,** 769–771.
8. Pious, D., Soderland, C. & Gladstone, P. (1977) *Immunogenetics* **4,** 437–448.
9. Gladstone, P., Soderland, C. & Pious, D. (1979) *J. Cell Biol.* **83,** 451 (abstr.).
10. Pious, D., Bodmer, J. & Bodmer, W. (1974) *Tissue Antigens* **4,** 247–256.
11. Abrahamson, S. & Wolff, S. (1976) *Nature (London)* **264,** 715–719.
12. Sugden, B. & Mark, W. (1977) *J. Virol.* **23,** 503–508.
13. Weitkamp, L. & Francke, U. (1978) *Cell. Genet.* **22,** 92–105.
14. Terasaki, P. I., Bernoco, D., Park, M. S., Ozturk, G. & Iwaki, Y. (1978) *Am. J. Clin. Pathol.* **69,** 103–120.
15. Ferrone, S., Pellegrino, M. A. & Reisfeld, R. A. (1971) *J. Immunol.* **107,** 613–615.
16. Kompf, J., Bissbort, S., Gussmann, S. & Ritter, H. (1975) *Humangenetik* **27,** 141–143.
17. Harris, H. & Hopkinson, D. A. (1976) *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam), p. 4.4.15.
18. Parr, C. W., Bagster, I. A. & Welch, S. G. (1977) *Biochem. Genet.* **15,** 109–113.
19. Seabright, M. (1971) *Lancet* **ii,** 971–972.
20. Berger, R., Bernheim, A., Sasportes, M., Hauptmann, G., Hors, J., Legrand, L. & Fellous, M. (1979) *Clin. Genet.* **15,** 245–251.
21. DeMars, R. & Jackson, J. L. (1977) *J. Environ. Pathol. Toxicol.* **1,** 55–77.
22. Cox, R. & Masson, W. K. (1978) *Nature (London)* **276,** 629–630.
23. Dickinson, W. J. & Carson, H. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4559–4562.
24. Reinsmoen, N., Yunis, E. J., Bach, F. H. & Bach, M. L. (1977) *Tissue Antigens* **9,** 11–16.
25. Hansen, J. A., Fu, S. M., Antonelli, P., Kamoun, M., Hurley, J. N., Winchester, R. J., Dupont, B. & Kunkel, H. G. (1979) *Immunogenetics* **8,** 51–64.
26. Kavathas, P., Bach, F. H. & DeMars, R. (1980) *Hum. Immunol.*, in press.
27. Kohler, G. & Milstein, C. (1975) *Nature (London)* **256,** 495–497.