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Refinement of *HLA* Gene Mapping with Induced B-Cell Line Mutants

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Abstract. The lymphoma cell line BJAB.B95.8.6 was gamma-irradiated to induce mutations of major histocompatibility complex (MHC) encoded genes. Cloned "wild-type" cells were phenotyped HLA-A1, A2, B13, B35, Bw4, Bw6, Cw4, DR5, DRw52, DQw1, DQw3, DPw2, DPw4, GLO1*1, PGM3*2-1, and ME1*0 and possessed two apparently normal chromosome 6s prior to mutagenesis. Loss mutants were selected 5 days after 3 Gy gamma-irradiation employing three complement-fixing monoclonal antibodies specific for HLA-A2 (TÜ101) and Bw4 (TÜ48, TÜ109). Fifteen independently arising mutants were isolated and cloned. Typing with monospecific alloantisera and cell-mediated lympholysis revealed the presence of HLA-A1, B35, Bw6, Cw4, DR5, DRw52, DQw3, and DPw4 specificities on all mutant clones. HLA-A2, B13, and Bw4 were absent. Mutants differed in their expression of class II antigens. One group retained DQw1 and DPw2, another was DQw1$^-$, DPw2$^+$, and a third was DQw1$^-$, DPw2$^-$. Karyotyping of the "wild-type" line and selected mutant clones showed that the loss of HLA specificities correlated with deletions which map the *HLA-A* and *-B* loci directly to the distal part of the 6p21.33 region and the class II genes to the region 6p21.33 (proximal) to 6p21.31 (distal) on the short arm of chromosome 6.

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Abbreviations used in this paper: CML, cell-mediated lympholysis; CTX, cytotoxicity; DBBA, direct bacterial binding assay; EBV, Epstein-Barr virus; GLO, glyoxalase; IBBA, indirect bacterial binding assay; LU, lytic units; ME1, cytoplasmic malic enzyme; MHC, major histocompatibility complex; MOAB, monoclonal antibody; NADP, nicotinamide-adenine dinucleotide phosphate; PGM3, phosphoglucomutase isozyme 3.

Introduction

The human histocompatibility complex (*HLA*) comprises a set of linked genes on the short arm of chromosome 6 which code for cell-surface glycoproteins and certain complement components. Lymphoid cell line mutants are useful in the study of gene number, arrangement, localization on the chromosome, and control of expression within the *HLA* complex. After mouse mutant cell lines were established by an analogous approach (Hyman 1973, Rajan 1977), the first stable *HLA* mutants of lymphoid cell lines occurring either spontaneously or after induction with chemical mutagens were described by Pious and co-workers (Pious et al. 1973, 1977, Pious and Soderland 1977). Gamma-irradiation may result in large chromosomal deletions (Abrahamson and Wolff 1976). Mutant cells with a deletion on the short arm of chromosome 6 were used to map the gene encoding the HLA-DR alpha chain by genomic blotting techniques (Erlich et al. 1983). Characterization of other deletion mutants provided evidence that the *DP* and *DR* loci are encoded by distinct genes and that *DP* lies between the *GLO1* and the *DR* loci (Kavathas et al. 1981). Furthermore, DeMars and co-workers (1983a) confirmed by using HLA-DR loss mutants that the DQw1 and DR1 products are under separate genetic control. The results described by Orr and colleagues (1982) showed the usefulness of *HLA* loss mutants in combination with DNA probes for the analysis of the gene organization in the human major histocompatibility complex (MHC), and Orr and DeMars (1983) found class I genes which map telomeric to the *HLA-A2* locus and may therefore correspond to the mouse *Tla* and/or *Qa* genes. In addition, the availability of mutants which have lost all *HLA* genes would be useful for examining the characteristics of products of transfected genes.

This article describes the production of two groups of mutated cell lines. The first has lost the expression of class I antigens from one of the haplotypes, while the other has lost different class II antigens from the same haplotype in addition. Cytogenetic studies revealed deletions of various sizes on the short arm of one of the chromosome 6s, which could be correlated with losses of particular MHC gene products at the cell surface, thereby enabling a precise mapping of certain *HLA* genes. These mutants could also provide tools for examining the regulation of MHC gene expression and function. In addition, such mutants might be valuable reagents for the production of monoclonal antibodies (MOABs) against HLA alloantigens, as well as convenient material for the absorption of complex alloantisera.

Materials and Methods

Cell lines. The human B lymphoma-derived, Epstein-Barr virus-superinfected cell line BJAB.B95.8 (Klein et al. 1976) as well as clones and mutants obtained from it were routinely maintained in Eagles' minimal essential medium supplemented with 10% fetal calf serum (FCS; Medac, Hamburg, Federal Republic of Germany), penicillin (100 units/ml), streptomycin (100 μg/ml), 2 m*M* glutamine, and 4.5 g/l *D*-glucose. The cells were kept at a density of $1–2 \times 10^5$ per ml.

Monoclonal antibodies. Designation, specificity, and references of all MOABs used to generate and to characterize the mutants are listed in Table 1.

Table 1. HLA phenotype of BJAB.B95.8.6 wild-type and MOABs employed for serological analysis of mutants. I. Phenotype of BJAB. B95.8.6: HLA-A1, A2, B13, B35, Bw4, Bw6, DR5, DRw52, DQw1, DQw3, DPw2, DPw4. II. Monoclonal antibodies

Designation	Specificity	Reference
W6/32.HL	HLA-A, B, C heavy chains	Barnstable et al. 1978 Ziegler and Milstein 1979
W6/32.HK	Inactive variant	Ziegler and Milstein 1979
TÜ 25	Epithelial antigen	Ziegler et al. 1981
SFR8-B6	HLA-Bw6	Radka et al. 1982
TÜ109	HLA-Bw4	Fauchet et al. 1984
TÜ 48	HLA-Aw23, Aw24, Aw32, Bw4	Müller et al. 1982
TÜ110	HLA-B13	Müller et al. 1983a
TÜ101	HLA-A2, A28	Müller et al. 1983b
XV-17.1	HLA-A2, A28	Bourel et al. 1984
4B	HLA-A2	Yang et al. 1984
A2, 28M1	HLA-A2, A28	Grumet et al. 1982
FMC-5	HLA-A2, A28	Beckman et al. 1981
TÜ 22	DQ molecules	Shaw et al. 1984
TÜ 34	DR molecules	Shaw et al. 1984
TÜ 35	DR, DP molecules*	Shaw et al. 1984
TÜ 36	DR molecules	Shaw et al. 1984
TÜ 37	DR, DP molecules*	Shaw et al. 1984
TÜ 39	DR, DP molecules*	Shaw et al. 1984
TÜ 43	DR, DP molecules*	Shaw et al. 1984
YD1/63.HLK	DR, DP molecules	Shaw et al. 1984
NDS 15.38	DR molecules*	Fuggle et al. 1983

* The possibility cannot be excluded that with at least some haplotypes, DQ molecules may also be bound by these MOABs.

Complement. Pooled rabbit serum was absorbed at 0 °C with BJAB.B95.8.6 cells (6×10^7 cells/ml of complement) to render it free of cytotoxicity for BJAB.B95.8.6 cells, and was stored in small aliquots at −70 °C for subsequent use.

Cloning. BJAB.B95.8 cells were cloned under limiting dilution conditions (Levy et al. 1979) in the presence of irradiated (20 Gy) mouse macrophages from a BALB/c mouse (1×10^4 cells per well in flatbottom microtiter plates; Tecnomara, Fernwald, Federal Republic of Germany). Clones were picked from wells seeded on average with 0.2 cells/well.

Mutagenesis. BJAB.B95.8.6 cells were suspended in culture medium and irradiated with various doses (1.5, 2.0, 2.5, 3.0, 3.5 Gy) from a 137-cesium source. After 5 days, viable cells were counted with a Leitz microscope equipped with phase contrast optics in order to establish the dose which allows only 10% of the cells to survive.

Selection. Exponentially growing BJAB.B95.8.6 cells were irradiated with 3 Gy. Five days later, 1×10^7 cells were washed twice with Hanks' balanced salt solution (Gibco-Biocult), and 2.5 ml of pooled undiluted supernatants from the myeloma hybrids TÜ48, TÜ101, and TÜ109 was added to the cell pellet. After mixing, cells were incubated for 30 min at 22 °C with continuous gentle shaking. The cells were then centrifuged, the supernatant aspirated and 12.5 ml complement was added. After mixing, cells were incubated at 22 °C with continuous gentle shaking. Seventy minutes later, cells were centrifuged, and the pellet resuspended in culture medium containing 20% FCS and distributed equally into 960 flat-bottom microtiter plate wells. Each well received, in addition, 1×10^4 irradiated (20 Gy) BALB/c macrophages. After 2 weeks of culture at 37 °C in 5% CO_2, growing colonies were transferred to 24-well

Costar plates. Fifteen cultures, designated BM1 through BM15, were subsequently cloned by limiting dilution for extensive study.

Bacterial binding assay. The bacterial binding assay was carried out either as a single-step ("direct") procedure (DBBA; Uchánska-Ziegler et al. 1982) or following the two-step ("indirect") test (IBBA; Ziegler et al. 1982). *Staphylococcus aureus* Cowan I bacteria were either coated with affinity-purified anti-mouse IgG (Medac), anti-mouse μ-chain (Medac or Conco, Wiesbaden, Federal Republic of Germany), or anti-rat IgG (Conco). In the direct assay MOABs bound to these coated bacteria were used for the analysis of cell-surface antigens, whereas in the indirect test the coated bacteria were applied after cells had been treated with the MOABs. The MOABs W6/32.HK and W6/32.HL served as negative and positive controls, respectively, in all assays. TÜ25 (an IgM molecule) was used to control for nonspecific binding of mouse IgM antibodies.

Microcytotoxicity assay. The standard NIH complement-dependent microcytotoxicity assay was used to define HLA-A, B, C, and D region antigens with the Ninth International Histocompatibility Workshop antisera and well-defined local antisera.

DP typing. Typing for alleles of the *HLA*-linked *DP* locus was performed using primed cellular reagents kindly provided by Dr. S. Shaw, NCI, NIH, Bethesda, Maryland (Shaw et al. 1980). Typing was performed by cell-mediated lympholysis (CML) techniques. Effector cells specific for DP antigens were prepared by restimulating primed reagents with pooled peripheral blood mononuclear cells (30 Gy gamma-irradiated) in the presence of a source of interleukin-2 (20% Lymphocult T, Biotest Serum Institute, Frankfurt, Federal Republic of Germany), as described (Pawelec et al. 1982). After a 5-day culture period, these cells were titrated onto ^{51}Cr-labeled target cells from wild-type BJAB.B95.8.6 and selected mutants at a starting effector-to-target ratio of between 50:1 and 100:1; we proceeded by tripling the dilution. Maximal ^{51}Cr release was achieved by incubating target cells with 1% Triton-X 100; spontaneous release was measured after incubation of the target cells with medium alone. For unknown reasons, the spontaneous ^{51}Cr release from the parental cells and most of the mutants was somewhat higher than expected (up to 35%), but results were still interpretable. Specific cytolysis after 4 h coculture was expressed in lytic units (LU) per 10^7 effector cells, one LU being defined as the number of effectors required to achieve 25% specific ^{51}Cr release, calculated from a titration curve of at least three effector cell dilutions, as described (Pawelec et al. 1982).

Determination of GLO1, PGM3, and ME1. Cells (1×10^7) were pelleted by centrifugation and lysed by adding 100 μl of distilled water followed by sonication. Horizontal starch gel electrophoresis and zymogram techniques were performed as described (Kömpf et al. 1975, Siebert et al. 1980). For comparison, phosphogluconate dehydrogenase and glutamic-oxaloacetic transaminase 2 (mitochondrial) were stained on the same gels. *GLO1* activity was determined by densitometrical assay of the zymograms (Eppendorf-Photometer, 546 nm filter, W+W recorder).

Cytogenetic techniques. Colcemid (0.1–0.5 μg/ml medium) was added to a cell suspension containing 5×10^5 cells in 2 ml medium a half an hour or 2 h before harvesting. After the cell suspension was centrifugated at 1000 rpm and the supernatant discarded, the cells were treated with hypotonic KCl solution (0.075 M) at room temperature for 20 min. Fixation, dropping on ice-cold slides, air-drying, and staining procedures (by a modified Giemsa-banding technique) were performed as described elsewhere (Fonatsch et al. 1980). About 100 metaphases per cell line were analyzed.

Results

Production of HLA mutants. To obtain a homogeneous "wild-type" population, we cloned the cell line BJAB.B95.8 under limiting dilution conditions. The cloning efficiency was 70–80%. One of these clones, BJAB.B95.8.6, was used for mutagenesis. Cell-survival experiments with the wild-type cells gave a consistent dose-dependent killing effect by ionizing radiation. A dose of 3 Gy was chosen to

maximize the frequency of mutations in a small surviving population. Within 3 weeks of immunoselection of such irradiated cells with a "cocktail" of the MOABs TÜ48, TÜ101, andTÜ109, 15 independent cell populations (BM1 through BM15) could be propagated further in culture. These cell lines were tested in the IBBA with MOABs used for selection and with TÜ110, an anti-HLA-B13 reagent as well as with W6/32.HL and W6/32.HK as positive and negative controls. The results demonstrated that the MOABs used for selection and MOAB TÜ110 no longer reacted with BM1-BM15 (data not shown). The lack of reactivity with TÜ48, TÜ101, TÜ109, and TÜ110 indicated that the HLA-A2 specificity as well as both the B13 and Bw4 specificities were no longer expressed by BM1 through BM15. Since Bw4 and B13 determinants are known to be carried by the same structure, this finding strongly suggests that the HLA-B molecule was indeed no longer present on the cell surface. However, other class I products were still present on the cell surface, as shown by the retention of reactivity with W6/32.HL.

Characterization of the HLA mutants. The "wild-type" line BJAB.B95.8.6 was shown to express the serologically defined HLA antigens A1, A2, B13, B35, Bw4, Bw6, Cw4, DR5, DRw52, DQw1, and DQw3 and the cellularly defined DPw2 and DPw4 specificities (Fig. 1). A second DR specificity could not be established as a classical allospecificity using pregnancy sera. However, reactivity with the MOAB NDS 15.38 suggested the possible presence of HLA-DR2, although this MOAB seems to react also with DQ products.

Cloned lines derived from the 15 *BM* mutant populations were subjected to detailed serological analyses of HLA antigen expression employing microcytotoxicity. In addition, they were tested in the DBBA with a panel of different MOABs against HLA class I and class II antigens. This technique has the advantage of a semi-quantitative demonstration of the presence of an antigen on the surface of individual cells, as judged by the number of bound bacteria. Representative results on "wild-type" cells and two different mutants used in this assay are shown in Table

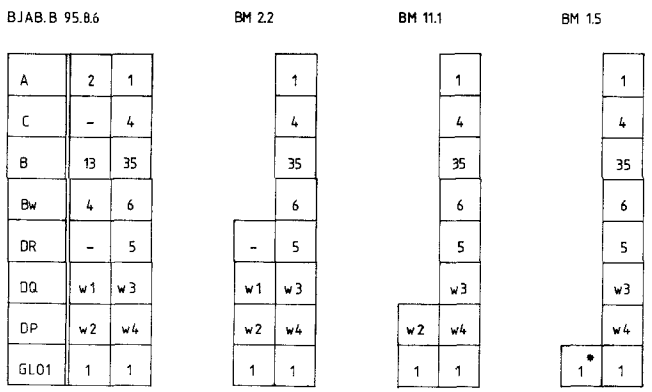

Fig. 1. Reconstruction of the BJAB.B95.8.6 *HLA* haplotypes as inferred by comparative phenotypic studies of the three mutant cell lines *BM2.2*, *BM11.1*, and *BM1.5*. "*" indicates an increase of about 70% in GLO1 activity.

Table 2. HLA class I and class II antigen expression of two mutants and the parental line as defined in the direct bacterial binding assay

Cell lines MOAB*	Specificity	BJAB.B95.8.6			*BM 1.5*			*BM 2.2*		
		%rc	Range	av	%rc	Range	av	%rc	Range	av
W6/32.HL	A, B, C	100	80–200	150	100	80–180	120	100	80–180	110
W6/32.HK	–	0	0	0	0	0	0	0		0
TÜ 25	–	0	0	0	0	0	0	0		0
TÜ101	A2	97	20– 80	40	0	0	0	0		0
XV-17.1	A2	99	10– 80	40	0	0	0	0		0
TÜ 48	Bw4	99	20– 80	40	0	0	0	0		0
TÜ109	Bw4	100	10– 80	30	91	5– 50	15	99	10– 50	15
TÜ110	B13	99	20–100	40	0	0	0	0	0	0
SFR8.B6	Bw6	100	10–100	35	100	20– 80	40	100	20– 80	30
TÜ 22	DQ	100	20–100	50	97	10–100	50	99	20–120	60
TÜ 34	DR	99	10– 60	45	100	10– 60	35	100	10– 70	50
TÜ 35	DR,DP	99	25–100	50	94	10– 80	40	100	10–100	35
TÜ 36	DR	100	10– 80	45	100	10– 80	30	98	20– 80	50
TÜ 37	DR, DP	98	20– 80	50	98	20–100	50	99	20– 80	55
TÜ 39	DR, DP	99	20–100	70	98	20–100	70	100	20–100	65
TÜ 43	DR, DP	100	15–100	70	99	20–100	70	100	20–100	70
YD1/63.HLK	DR, DP	95	6– 50	25	70	4– 30	10	56	4– 50	15

* Hybridoma cell supernatant; relevant specificity of the MOAB supernatant.
%rc, percent reactivity; av, average number of bacteria per cell.

2. The cells from each line retained 100% reactivity with W6/32.HL, but the level of class I molecule expression by the mutants was reduced by approximately 30% in comparison to the wild-type line. The HLA-A2 antigen (TÜ101, XV-17.1), the "private" specificity HLA-B13, and the HLA-B13-associated "supertypic" specificity HLA-Bw4 were no longer present on any mutant. While TÜ109, one of the HLA-Bw4-specific MOABs used for selection, did bind well to the "wild-type" cells, it reacted consistently weaker to the mutants in the DBBA (Table 2) and not at all to the mutants in the IBBA (results not shown). The HLA-Bw6 determinant (SFR8.B6) remained detectable on the mutants. Using class II-specific MOABs, with the exception of YD1/63. HLK, no detectable differences were found between "wild-type" cells and the mutants either in the number of positive cells or in the amount of bacteria bound to the cell surfaces. With YD1/63. HLK, which detects a subpopulation of HLA-DR and DP molecules on certain haplotypes, mutants could be distinguished from the "wild-type" line.

The results of the complement-mediated cytotoxicity (CTX) tests on selected mutants using MOABs against class I antigens are summarized in Table 3. This assay was employed to determine whether the conflicting results of the DBBA and the IBBA with TÜ109 could be ascribed to the particular test system or were due to varying concentrations of MOABs or of the antigen on the cell surface. Furthermore, three additional HLA-A2-specific MOABs (4B; A2,2M1; FMC-5) were introduced in an attempt to determine whether the loss of HLA-A2 expression was due to structural variation of the molecule or to the molecule's disappearance. Unlike the "wild-type" cells, the mutant cells were not lysed in the presence of

Table 3. Reactivity of *BM* mutants in the CTX employing class I-specific MOABs

MOABs	TÜ48		TÜ109		TÜ110		TÜ101	4B	A28, 2M1	FMC-5	SFR8-B6*
HLA specificity	Bw4		Bw4		B13		A2	A2	A2	A2	Bw6
Cells	S	A	S	A	S	A	S	S	S	S	S
BJAB.											
B95.8.6	+	+	+	+	+	+	+	+	+	+	+
BM 1.5	−	−	−	+	−	−	−	−	−	−	+
BM 2.2	−	−	−	+	−	−	−	−	−	−	+
BM 4.2	−	−	−	+	−	−	−	−	−	−	+
BM 5.2	−	−	−	+	−	−	−	−	−	−	+
BM 8.5	−	−	−	+	−	−	−	−	−	−	+
BM 9.1	−	−	−	+	−	−	−	−	−	−	+
BM10.1	−	−	−	+	−	−	−	−	−	−	+
BM14.1	−	−	−	+	−	−	−	−	−	−	+
BM15.3	−	−	−	+	−	−	−	−	−	−	+

S, hybridoma supernatant; A, ascites fluid; *, positive control.
"+" indicates reactivity, "−", lack of reactivity.

MOABs TÜ48 and TÜ110, thus indicating that the mutants had indeed lost HLA-Bw4 and B13 antigens, respectively. This phenomenon occurred regardless of MOAB concentration. In contrast, in the presence of increasing monoclonal antibody concentration, TÜ109 appeared to recognize additional structures on the cells, most likely Bw6 antigens (C. Müller et al., unpublished results), thereby confirming the results of the DBBA. Furthermore, the mutant cells did not react with MOABs recognizing different epitopes on the HLA-A2, A28 molecules (compare Table 2 and 3).

To investigate the expression of DP molecules, we performed cell-mediated lympholysis (CML) studies on selected mutants, using two independent DPw2-, and two independent DPw4-specific reagents as effector cells. The effector cells failed to lyse targets not expressing the relevant DPw2 or DPw4 target antigens (data not shown). Initial typing studies indicated that lysis of wild-type cells by anti-DPw2 and anti-DPw4 reagents was as significant as that measured on the control DPw2, w4+ lymphoblastoid cell line KR3598 (Table 4). In contrast, *BM1.5* was not lysed by the two DPw2-specific reagents, but retained full susceptibility to lysis by DPw4-specific reagents, whereas *BM2.2* was killed by both DPw2 and DPw4-specific effectors. DP typing studies from all remaining mutants revealed that the cells retained the DPw4 specificity. Mutants *BM7.4*, *BM10.1*, and *BM15.3*, in addition to *BM1.5*, were found to have lost expression of DPw2 (Table 5, Fig. 1).

"Wild-type" cells as well as mutants were tested in CTX assays using alloantisera from the Ninth International Histocompatibility Workshop in order to define their remaining HLA antigen expression. The presence of HLA-A2, B13, and Bw4 antigens could not be demonstrated among the mutants, whereas HLA-A1, B35, Bw6, and Cw4 antigens were still present on all cell lines. In addition, all mutants retained expression of class II specificities DR5, DRw52, and DQw3. However, a clear-cut distinction was found between the different mutant lines in the expression of the allosera-defined DQw1 specificity. Thus *BM2.2*, *BM3.1*, *BM4.1*, *BM12.1*,

Table 4. DP typing of BJAB.B95.8.6 and two mutant cell lines

Effectors for DP[†]	Target cells			
	KR3598*	BJAB.B95.8.6	BM 1.5	BM 2.2
2A	128[‡]	190	0.1	136
2B	203	598	0.1	116
4A	106	178	275	202
4B	248	136	123	738

* Preestablished DP type of the control target cell KR3598 was DPw2, DPw4.
[†] Effector cells specific for designated DP antigens.
[‡] Cytotoxicity presented in lytic units (25%) per 10^7 effectors.

Table 5. Expression of HLA antigens on wild-type and mutant cells as determined by CTX and CML assays

Cell lines	HLA products												
	CTX assay											CML assay	
	A1	A2	B13	B35	Bw4	Bw6	Cw4	DR5	DRw52	DQw1	DQw3	DPw2	DPw4
BJAB. B95.8.6	+	+	+	+	+	+	+	+	+	+	+	+	+
BM 1.5	+	−	−	+	−	+	+	+	+	−	+	−	+
BM 2.2	+	−	−	+	−	+	+	+	+	+	+	+	+
BM 3.1	+	−	−	+	−	+	+	+	+	+	+	+	+
BM 4.1	+	−	−	+	−	+	+	+	+	+	+	ND	ND
BM 5.2	+	−	−	+	−	+	+	+	+	−	+	+	+
BM 6.3	+	−	−	+	−	+	+	+	+	−	+	+	+
BM 7.4	+	−	−	+	−	+	+	+	+	−	+	−	+*
BM 8.5	+	−	−	+	−	+	+	+	+	−	+	ND	ND
BM 9.1	+	−	−	+	−	+	+	+	+	−	+	ND	ND
BM10.1	+	−	−	+	−	+	+	+	+	−	+	−	+
BM11.1	+	−	−	+	−	+	+	+	+	−	+	+	+
BM12.1	+	−	−	+	−	+	+	+	+	+*	+	+	+*
BM13.1	+	−	−	+	−	+	+	+	+	+	+	+	+
BM14.1	+	−	−	+	−	+	+	+	+	+	+	ND	ND
BM15.3	+	−	−	+	−	+	+	+	+	−	+	−	+*

* Only one test was done; reagents for typing were from the Ninth Workshop, and at least four sera per specificity were employed.

BM13.1, and *BM14.1* had retained expression of the DQw1 specificity, whereas this had been lost from the remaining mutants (Table 5).

In lysates from the original cell line BJAB.B95.8.6, as well as from the mutant cell lines, no activity of the cytoplasmic NADP-dependent malate dehydrogenase (ME1) could be detected. Each of these lines exhibited the heterozygous phenotype PGM3*2-1. The GLO1 phenotype of each of the lines investigated was GLO1*1.

From densitometrical assays it was determined that the mutant *BM1.5* contained 170%, whereas *BM2.2* and *BM11.1* had 94% of the activity of the wild-type BJAB.B95.8.6 line, respectively (mean values from three assays).

The exposure of the BJAB.B95.8.6 line to gamma-irradiation, resulting in dramatic changes of HLA phenotypes in the mutants suggested that changes in the genotype could be expected. Cytogenetic studies were therefore undertaken. The karyotype of "wild-type" BJAB.B95.8.6 already contained a number of structural chromosomal anomalies, but two normal unchanged chromosome 6s were present. Aberrations of the banding pattern of chromosome 6 were analyzed in three mutants, *BM1.5*, *BM2.2*, and *BM11.1*. The banding pattern of the short arm of chromosome 6 of BJAB.B95.8.6 and the changes in *BM1.5*, *BM2.2*, and *BM11.1* are depicted in Figure 2. In one of the chromosome 6s of *BM1.5*, breaks had occurred, leading to a deletion most likely from the distal part of 6p21.31 to the distal part of 6p21.33. In addition, the band regions 6p21.1 and 6p21.2 appeared to be duplicated, resulting in an elongated short arm of chromosome 6. In *BM2.2*, loss of material from 6p21.33 (distal) to 6p23.05 could be observed. The *BM11.1* had the largest deletion with the break in 6p21.31 (distal) and 6p23.05. A synthesis of phenotyping and karyotyping results obtained with the present mutant lines is given in Table 6.

With regard to the location of the MHC genes in the mutants *BM1.5*, *BM2.2*, and *BM11.1*, the conclusion can be drawn that class I genes are located distally in 6p21.33, the *DQ* gene proximally in 6p21.33, 21.32 or distally in 6p21.31, and the *DP* genes of class II distally in 6p21.31. Furthermore, it can be stated that the *GLO1* gene is not found in the area between 6p21.31 (distal) and 6p23.05, but rather in the area 6p21.1-6p21.2. An overall synopsis of this situation as it pertains to the results obtained with the mutants *BM1.5*, *BM2.2*, and *BM11.1* is shown in Figure 2.

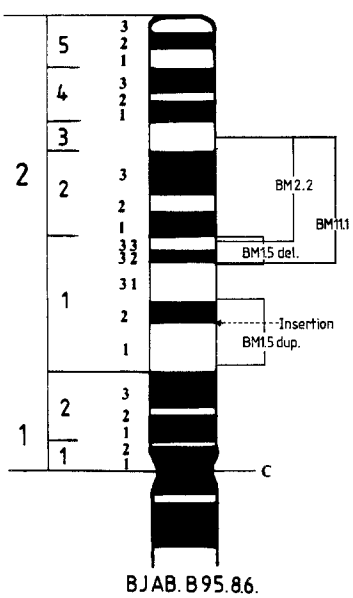

Fig. 2. Schematic representation of the short arm of a normal chromosome 6 (BJAB.B95.8.6) and its mutated counterpart from mutants *BM1.5*, *BM2.2*, and *BM11.1*. c, the centromere; del, deletion; dup, duplication.

Table 6. Conclusions from phenotypic and karyotypic data.

BM 1.5 : ABC^-		Class I genes	:$DQw1^-$		This gene is
BM 2.2 : ABC^-	→	are distal in	:$DQw1^+$	→	proximal in
BM11.1 : ABC^-		6p21.33	:$DQw1^-$		6p21.33, 21.32 or distal in 21.31
BM 1.5 : $DPw2^-$		This gene is	:GLO 170%		The *GLO* gene is not
BM 2.2 : $DPw2^+$	→	distal in	:GLO 94%	→	in 6p21.31 (distal)-6p23.05, most likely
BM11.1 : $DPw2^+$		6p21.31	:GLO 94%		in 6p21.1–21.2

Discussion

HLA loss deletion mutant cell lines have been generated from a cloned lymphoma cell line designated BJAB.B95.8.6. This line possessed several advantages for the establishment of mutants: (1) high cloning efficiency, (2) heterozygosity for all known HLA antigens, (3) recognition of the cells by local allospecific MOABs which could be used for immunoselection, and (4) expression of IgM but not IgG on the surface of cells, making them suitable for use in bacterial binding assays, employed as a convenient and accurate screening method. For the production of mutants, a modification of the basic protocol described by Kavathas and co-workers (1980) was used.

After irradiation, a "cocktail" of three MOABs directed against the products of two different *HLA* class I loci was chosen for immunoselection, to increase the probability of obtaining mutants with large deletions. Screening of uncloned mutated lines enabled the rapid selection of 15 mutants for propagation and further study. All had lost the expression of the HLA-A2 and Bw4 antigens detected by MOABs TÜ101, TÜ48, and TÜ109. In addition, the HLA-B13 "private" specificity was also lacking, although selection had only been carried out indirectly with MOABs against the HLA-B13-associated "supertypic" specificity Bw4. A total of 15 mutants were cloned to ensure uniform populations for all further studies. Unexpectedly, in spite of the application of TÜ109 for their selection, all mutant cells reacted with this MOAB in the DBBA but not in the IBBA, albeit to a reduced extent. This is exemplified by the mutants *BM1.5* and *BM2.2*. The most likely explanation for this phenomenon may be a higher avidity of TÜ109 when coupled directly to the bacteria. A comparison of TÜ109 culture supernatant and TÜ109 ascites fluid in the CTX assay demonstrated also that at very high concentrations, TÜ109 may bind to cells which do not carry HLA-Bw4 but rather to Bw6 determinants (C. Müller et al., unpublished data). Such "cross-reactivity" was not observed with ascites fluid of TÜ48 or TÜ110. Experiments are presently being carried out to elucidate the biochemical nature of the TÜ109-reactive molecule on the surface of the mutants.

The results presented in Table 2 show that the total amount of HLA-A, B, C heavy chains as detected by W6/32.HL was reduced in all mutants analyzed. However, the reduction does not fulfill the theoretical expectations, since maximally 30% less antigen as compared with "wild-type" cells was found whereas an antigen loss of up to 50% could be anticipated. These findings are consistent with the results

reported by Gladstone and co-workers (1982), who used radioactive binding assays with two MOABs against HLA-A, B, C heavy chains and obtained a reduction of only 20–25%, although a complete haplotype was deleted in three variant cell lines. In contrast to Gladstone and co-workers (1982), who also found a similarly diminished expression of HLA class II antigens, no such reduction was observed with the present *BM* mutants. One possible exception was the reduced reactivity of *BM1.5* and *BM2.2* with the rat MOAB YD1/63.HLK. These findings were confirmed by a radioactive binding assay using ^{3}H-labeled YD1/63.HLK. Here, a significant reduction of the binding capacity was seen with this MOAB on the two mutants in comparison to wild-type cells (Spring 1984). However, such findings are as yet difficult to interpret since the nature of the YD1/63.HLK-reactive class II molecules of the haplotype lost in *BM1.5* is unknown, although they probably constitute subpopulations of DR and DP antigens (Shaw et al. 1984; A. Ziegler et al., unpublished results). Thus, despite the clearly established loss of class II antigens from one mutated haplotype, the total amount of class II products expressed at the cell surface appears not to have been substantially reduced.

The various mutants all shared the loss of HLA-A2 and B13/Bw4 specificities, but differed informatively with regard to class II antigen expression. It was possible to assign the mutants to three groups: (1) six appeared to have retained most or all genes of the *HLA-D* region (e. g., *BM2.2*), (2) at least three mutants had lost DQw1 but retained DPw2 (eg., *BM11.1*), (3) a minimum of four mutants had lost expression of all HLA antigens belonging to the *A2* haplotype, including DPw2 (eg., *BM1.5*). Since the second HLA-DR specificity could not be typed reliably on the "wild-type" cells, it was difficult to evaluate the presence of these HLA-DR antigens in the mutants, although studies with the MOAB NDS 15.38, previously thought to be specific for HLA-DR2 (Fuggle et al. 1983), suggested the expression of this specificity on wild-type cells and on all mutants which had retained DQw1 (A. Ziegler, manuscript in preparation). However, recent sequencing data suggest that of the two β chains precipitated by NDS 15.38, one is DR-like and the other DQ-like (S. V. Fuggle, personal communication). Thus, the reactivity of NDS 15.38 on this DQw1-positive cell line may be caused solely by the presence of DQ molecules. The loss of the HLA-A2 and -B13/Bw4 antigens from all mutants implies that the genes encoding these molecules belong to one haplotype of BJAB.B95.8.6 cells. Further reconstruction of BJAB.B95.8.6 haplotypes is possible from the phenotyping of *BM1.5*, *BM2.2*, and *BM11.1*. The "wild-type" haplotypes would then be *HLA-A1*, *B35*, *Bw6*, *Cw4*, *DR5*, *DRw52*, *DQw3*, *DPw4*, and *A2*, *B13*, *Bw4*, *DQw1*, *DPw2* (Fig. 1).

Deficiency of cytoplasmic malic enzyme (ME1) in each of the cell lines was investigated and confirmed previously reported results (Povey et al. 1981). Furthermore, it was shown that each line expressed the heterozygous phenotype PGM3*2-1. Mutants *BM2.2* and *BM11.1* had the same level of GLO1 activity as BJAB.B95.8.6. This finding indicates that the mutants seem to be genotypically homozygous *GLO1*1/GLO1*1*.

The most important result of the present study is that the region on the short arm of chromosome 6 which carries the different loci comprising the *HLA* complex and closely linked genes was more precisely defined. Somatic cell genetic studies as well as clinical investigations have been used to localize the *HLA* genes between 6p21 and 6p22, probably in the 6p21.3 region (reviewed by Robson and Lamm

1984). The mutants *BM1.5*, *BM2.2*, and *BM11.1* were found to have more informative deletions (see Fig. 2 and Table 6) and to confirm the localization of the *HLA-A, B* and, by inference, also *C* genes to the 6p21.3 region, most likely its distal part. All mutants lost the distal part of 6p21.33, confirming the localization of the class I genes to that region in chromosome 6. The differences in expression of the class II antigen DQ by these mutants may be due to the deletion of genetic material from 6p21.31 (distal) to 6p21.33 (proximal) in mutants *BM1.5* and *BM11.1* in contrast to *BM2.2*, which preserved this chromosomal region. The fact that the cell line BM1.5 did not express the DPw2 antigen, in contrast to BM11.1, could be explained by a break proximally to that in BM11.1. From this data it could be assumed, that the *DQ* gene is located in 6p21.33 (proximal) or 6p21.32 and the *DP* gene lies distally in 6p21.31. The mutant *BM1.5*, presumably bearing a duplication of bands 6p21.1 and 6p21.2, exhibited distinctly elevated levels of GLO1 activity, which can be interpreted as a result of a duplication at the *GLO1* locus. Therefore, cytogenetic findings together with enzyme activity data allow the positioning of the *GLO1* gene in this segment (6p21.1/21.2). The location of the *GLO1* gene in the segment 6p21.31 (distal part)-6p23.05 seems to be excluded by our data. Detailed cytogenetic analysis of *BM1.5*, *BM2.2*, *BM11.1*, and several other mutants will be the subject of a separate communication (C. Fonatsch et al., manuscript in preparation).

A recent report (Morton et al. 1984) presents somewhat discrepant data on *HLA* gene localization. In that report, the HLA-A, B, and C heavy chain genes seemed to be located in 6p at band 6p21.3 (in full agreement with our data), while the *HLA-DR* alpha chain gene was placed in band 6p21.1. However, cytogenetic interpretation of an insertion of chromosomal material from one chromosome into another may be problematic. For class I and II genes, the present results would be in agreement with those of Morton and co-workers if the inserted segment was in fact 6p21.33-6p24 instead of 6p21.1-6p22.

Although the karyotypic data are consistent with the loss of structural genes, it remains theoretically possible that irradiation had instead affected genes with a regulatory function in *HLA* gene expression. Hybridization studies with DNA probes for different class II genes will distinguish regulatory defects from structural gene deletions. The situation with the present mutants may be further complicated by the expression of products of mutated, not deleted structural genes, which are no longer serologically detectable, but could, for example, still be detected by T cells (Gaston et al. 1984). In the present work, this can be excluded only for the cellularly typed DP antigens, but for these, no serology is available.

In conclusion, we have shown that by using a defined set of monoclonal antibodies, it is possible to establish a series of *HLA* mutants originating from a lymphoma-derived cell line. The utilization of different characterized mutant cell lines possessing deletions or point mutations in different haplotypes (Kavathas et al. 1980, Krangel et al. 1982, DeMars et al. 1983b) may contribute to a more precise definition of the regional assignment of genes of the *HLA* complex.

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