PHENOTYPIC EXPRESSIONS OF THE MAJOR HISTOCOMPATIBILITY LOCUS IN MAN (*HL-A*): LEUKOCYTE ANTIGENS AND MIXED LEUKOCYTE CULTURE REACTIVITY*

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In each species wherein a major histocompatibility locus has been defined (mouse (1), rat (2), chicken (3), and man), each allele determines multiple antigenic specificities. In each species numerous alleles have been defined. Information relating to minor loci is less complete; many of these appear to show considerably less polymorphism, and the alleles are presumably less complex. Incompatibility for a complex locus is quantitatively more pronounced since more individual specificities are involved. It is possible that these quantitative differences account for much of the distinction between a major and a minor locus, but there is also strong evidence for qualitative differences. Most salient in this regard are the comparative studies with mixed leukocyte cultures and differential resistance to immunosuppression as described by Silvers, Wilson, and Palm for the rat (4). Similar distinctions have been made in the mouse.

In man, many of the antigens detected serologically are under the control of a single genetic system (5–8). A single genetic locus also controls reactivity in mixed leukocyte culture (MLC) tests (9). Serologic and MLC test precedures are predictive of skin-graft survival between siblings (10–13). Skin grafts between siblings identical at *HL-A* persist between 14 and 42 days with a mean of approximately 25 days, whereas skin grafts between most pairs dissimilar at *HL-A* are rejected in 7–14 days, with a mean between 11 and 12 days.

A previous brief report from our laboratories suggested that there is a direct correlation between the failure of leukocytes to stimulate in mixed cultures and antigenic similarity (7). Both of these tests probably measure properties of the same locus—the major histocompatibility locus in man, *HL-A*. We now present additional data supporting these conclusions and discuss some interesting exceptions to the correlation between the mixed leukocyte culture test and antigenic reactivity.

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Materials and Methods

Serologic Determinations.—Antisera used were obtained from multiparous women, from male volunteers immunized by skin grafting or by subcutaneous inoculation with lymphocyte suspensions, or from the National Institutes of Health serum bank. Some of the sera containing more than one antibody have been purified by absorption, some are believed to be monospecific as collected, and others have been deliberately used as polyvalent reagents. A description of the antigenic specificities has been given (14). Serologic testing was by the cytotoxicity test. In the semimicromethod used, 25 microliters of antiserum is incubated for 25 min at 37°C with equal volumes of a lymphocyte suspension (adjusted to approximately 8×10^5 cells per milliliter) in barbital buffer with saline and rabbit complement. Excess supernatant is replaced with 0.1% trypan blue in saline, and the proportion of stained cells is determined. In the microtest, 1 μl each of serum, lymphocyte suspension (at a concentration of 1.6×10^5 cells per milliliter), and rabbit complement are incubated in a Terasaki microtiter plate.[1] Evaporation is prevented by adding water to the moat outside the wells and by keeping the lid tightly closed. After incubation, the wells are flooded with trypan blue solution, excess dye is shaken off, and the test is read under direct or phase microscopy. Test suspensions containing more than 20% damaged cells are read as positive, provided that a normal serum control contains less than 10% stained cells.

Mixed Lymphocyte Culture.—Following is a brief description of a method previously published (15), with details of modifications of that method. Heparinized blood is allowed to sediment for approximately 2 hr at room temperature. The plasma is removed and centrifuged for 10 min at 150 *g* to obtain leukocytes; the supernatant is again centrifuged at 1000 *g* to obtain cell-free plasma. Cells to be used as "responding cells" are suspended in Eagle's minimal essential medium modified for suspension culture (MEM-S, Grand Island Biological Co., Grand Island, N. Y.) and supplemented to contain glutamine, 2 mmole/liter; penicillin, 100 units per milliliter; streptomycin, 100 μg/ml; and pooled cell-free plasma, 20 ml/100 ml, (20% MEM).

Cells to be used as stimulating cells are suspended in 5–10 ml of 20% MEM, incubated for 20 min at 37°C with mitomycin C at 25 μg/ml, twice washed in 20% MEM, and resuspended in 20% MEM. Responding cells are present in all cultures at final concentrations of 0.3×10^6–0.5×10^6 mononuclear cells per milliliter. Stimulating cells are present at a final concentration of 0.6×10^6–0.8×10^6 cells per milliliter. Immediately after being mixed, cultures are distributed in replicate 2.5-ml volumes in glass tubes (16×100 mm) with metal closures and incubated at 37°C in a humidified 4% CO_2 atmosphere for 7 days.

After approximately 160 hr of incubation, 2 μc of tritiated thymidine (specific activity 1.9 c/mmole; Schwartz Bio Research Inc., Orangeburg, N. Y.) are added to each culture. Replicate cultures are harvested 5–5½ hr later by the addition of a 1000-fold excess of nonradioactive thymidine in 0.1 ml normal saline, immersion of the culture tubes in ice, centrifugation for 10 min at 1000 *g*, and freezing of the cell sediment at −20°C. For the determination of the incorporation of thymidine, the cultures are thawed, 5 ml of cold 5% TCA is added to each, and the tube contents are mixed on a Vortex mixer. 10 min later the precipitate is recovered by centrifugation, dissolved in 1 ml of 0.1 N NaOH, and precipitated again with 4.5 ml of 6.7% TCA. The acid precipitation procedure is repeated twice.

The final precipitate is dissolved in 0.1 ml of tetraethylammonium hydroxide, mixed with scintillation fluid, and counted. The results are expressed as counts per minute in each sample. The values plotted are averages of the replicate cultures.

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[1] Falcon Plastics, Los Angeles, Calif.

RESULTS

Results of MLC tests in a family studied are given in Table I. Seven siblings are studied in one-way MLC tests in all combinations of two. The numbers represent counts per minute of tritiated thymidine taken up by the cells in each culture. Results of sibling mixtures which do not stimulate (MLC-identical siblings) are enclosed in double lines, and can be easily separated from stimulating cell combinations. Thus, siblings A, B, C, and D make up one MLC-identical group; siblings E and F make up a second group; and sibling G is unique. Two siblings of that family were not tested in all combinations in this experiment. One of these, I (see Table II) was tested in combination with G, the results of which were as follows: GG_m, 43 cpm; GI_m, 1138 cpm; II_m, 430 cpm; and IG_m, 862 cpm.

TABLE I

Results of MLC Tests on Seven Siblings Tested in One-Way Culture in all Combinations

Sibling	A_m	B_m	C_m	D_m	E_m	F_m	G_m
A	(52)	16	1	29	2415	1507	2974
B	−46	(70)	−55	−35	784	734	451
C	6	5	(13)	12	186	221	598
D	−6	−36	−41	(79)	2055	2033	3184
E	2099	1906	1241	2517	(61)	−13	2100
F	3190	2015	1491	2581	30	(254)	4412
G	812	538	1082	1325	837	1134	(43)

The clear separability of MLC-identical siblings from nonidentical ones is supported by the analysis shown in Fig. 1, in which the highest value of stimulation seen with each responding cell is equated to 100. Thus, for responding cells A (Table I), incorporation in the mixture AG_m (2974) is equated to 100. Proportionally, that for AB_m is 0.5, for AC_m is 0.0, for AE_m is 82, and for AF_m is 51. Similarly, numbers can be plotted for B and other responding combinations. Fig. 1 gives the combined data from Table I and from previously published material (7) plotted in this manner. Very rarely some mixtures have given ambiguous results. Such mixtures on being retested have always permitted a clear classification into one of the two aforementioned categories. An example of this is the mixture EG_m previously reported. In that case the radioactive thymidine incorporated gave the following results with responding cells of E. The control culture EE_m incorporated 52 cpm. Allogeneic mixtures incorporated as follows (cpm in excess of the control value) EA_m, 8663; EB_m, 5763; EC_m, 12815; ED_m, 56; EF_m, 2431; and EG_m, 515. EG_m accounts for only 4% of the maximum stimulation of cells of E in the mixture EC_m, and is represented by the bar at 4% in Fig. 1. That this does represent a case of stimulation is evi-

denced by two facts. First, stimulation in the mixture EG_m, although representing 4% of the stimulation seen in the mixture EC_m, is still 10 times higher than that in the mixture ED_m, a case of nonstimulation, which represents only 0.44% of the stimulation in EC_m. Second, the mixture EG_m when retested by the method described in the following paper showed unequivocal stimulation.

Using the criterion of zero stimulation as an indication of identity for genes controlling reactivity in MLC tests, we have done a genetic analysis based on the frequency of nonreactivity in MLC tests between individuals of different

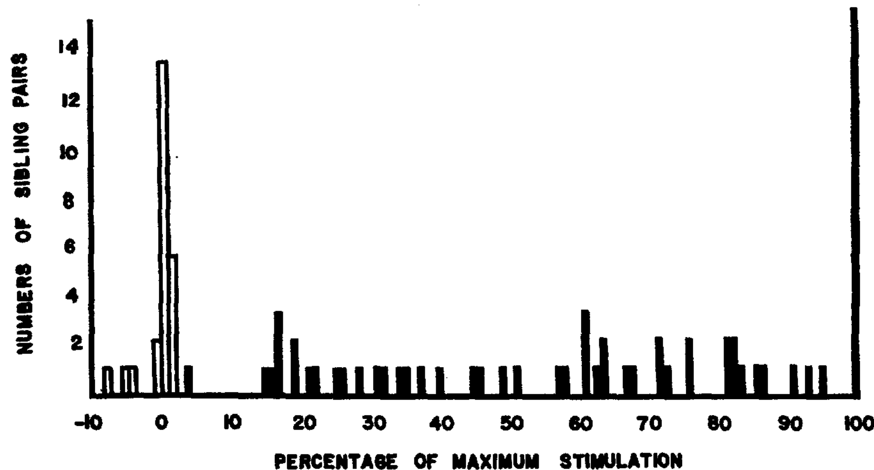

FIG. 1. Values for stimulation in MLC tests plotted to show separability of positive stimulation and zero stimulation. Solid bars represent cultures showing stimulation, open bars those that showed no stimulation. Six allogeneic mixtures with each of 14 sibling responders are plotted. The highest stimulation obtained with each responding cell is equated to 100 and all other values of "stimulation" with that responding cell are expressed as a percentage of 100.

genetic relationships (16). Results of these studies showed that a single genetic locus with 15 or more alleles controls reactivity in MLC tests.

In Table II are given the reactions of sera, known to measure antigens associated with the *HL-A* system, on the same seven siblings presented in Table I and on their parents. This table also gives a genotypic analysis of the distribution of the *HL-A* alleles. The genotypes of the siblings in a family can be determined by transmissional analysis of the leukocyte antigens of the parents. Because *HL-A* is a very polymorphic locus, both parents will usually be heterozygous for different alleles, and four groups of siblings can be identified. Thus, if both parents are heterozygous for different alleles and the father has alleles a and b (ab) and the mother has alleles c and d (cd), then the four groups of

siblings would be respectively characterized by allele pairs ac, ad, bc, and bd. When the father's cells react positively with a given antiserum and the mother's cells do not react with that antiserum, we can assume that the father is heterozygous for the allele determining that antigen, since some of the children do not inherit that allele. If we assign that antigen to one of the father's alleles at the

TABLE II

Leukocyte Typing and Proposed Genotyping in the H Family

Antisera	Father	Mother	Siblings								
			A	B	C	D	E	F	G	H	I
PAY	+++	0	0	0	0	0	+++	+++	+++	+++	+++
RA	+++	0	++	++	+++	++	0	0	0	0	0
BC	++++	0	++++	++++	+++	++++	0	0	0	0	0
LBU	0	+++	++	+++	+++	++	+++	+++	0	++	0
ROY	0	+++	++	+++	+++	++	++	+++	0	++	0
NW	0	++++	+++	++++	++++	+++	+++	+++	0	++++	0
SD	0	+++	++	+++	+++	+++	++	++	0	+	0
KH	0	++	++	++	++	+	+	++	0	+	0
FS	0	+++	++	+++	+++	+++	++	++	0	++	0
HM	0	+++	+++	++++	++++	++++	+++	++++	0	+++	0
DAL	++	++	++	++	++	+	0	0	+	0	0
DK	0	+++	+++	+++	+++	+++	+++	+++	++	+++	++
BH	++++	+++	+++	++++	++++	++++	0	0	++	0	++

Father		Mother		Sibling	Genotype
a	b	c	d	A	b/c
+PAY		+LBU		B	b/c
		+ROY		C	b/c
		+NW		D	b/c
	BH+	+SD		E	a/c
		+KH		F	a/c
	RA+	+DK	DK+	G	a/d
	BC+			H	a/c
	DAL+		BH+	I	a/d
		+FS	DAL?		
		+HM			

locus in question, we can then say that all children with positively reacting cells have received that allele from the father and that all those with negatively reacting cells have received the other allele. This can be checked if we have another antigen, associated with the father's second allele. All children negative for the first antigen should be positive for this second antigen, and those positive for the first should now be negative (contrasting distribution). This is the case in the family listed in Table II. If we assign the antigen measured by antiserum PAY to allele a of the father and the antigens measured by antisera RA and BC to allele b of the father, we can say that siblings E, F, G, H, and I received allele

a from the father; and siblings A, B, C, and D receive allele b. We can do a similar analysis for the two alleles c and d of the mother. Allele c, determining antigens measured by antisera LBU, ROY, SD, KH, FS, HM, and NW is given to children A, B, C, D, E, F, and H; and allele d is presumably given to children G and I. Although we have no antigen that we can clearly associate with allele d of the mother, we can assign allele d by the argument of contrasting distributions. There is one additional antiserum, BH, which is informative. Although both parents are heterozygous, the data does fit if we assume that siblings A, B, C, and D are positive because they have inherited allele b from the father and siblings G and I are positive because they have inherited allele d of the mother.

TABLE III

Comparison between Siblings for Identity of Allelic Inheritance by Genotyping and MLC Identity

	a MLC identity		*b* MLC identity	
	+	−	+	−
HL-A identity by genotyping, +	25	1	8	1
HL-A identity by genotyping, −	0	70	0	21

Genotyping is not practical in certain families, notably some small families, especially if one of the parents is not available.

Two forms of comparison between the MLC data and the typing results are presented. In Table III, those families are compared in which complete genotyping was possible. Siblings inheriting the same *HL-A* alleles (*HL-A* identical) are compared for their identity in MLC tests; those differing in allelic inheritance (*HL-A* dissimilar), for the presence of stimulation in MLC tests. The data can be expressed in two ways. If an *HL-A* identical group contains three siblings and all are MLC identical, then we can either count each possible combination of two siblings separately, thus three separate comparisons (Table III, *a*); or, more conservatively, we may consider any one *HL-A*-identical group of siblings as a single unit (Table III, *b*). Eight families are genotyped. Two of these had two *HL-A*-identical groups each, four had one each, and two had no siblings that were identical at *HL-A*. In the eight *HL-A*-identical groups, 26 different sibling pairs could be compared for their reactivity in MLC tests. In 25 of these cases cells of the siblings did not stimulate; however, in one case, cells from siblings shown to be identical by genotyping did, on two occasions, clearly stimulate each other. 70 pairs of siblings who differed in their allelic inheritance

at *HL-A* were available in the eight families. Cells in all of these combinations stimulated in MLC tests. In Table III, *b*, the more conservative comparison is made in which each *HL-A*-identical group is considered as only a single "unit" in the *HL-A*-identical category, and the *HL-A* group is considered only once in each *HL-A*-dissimilar combination. Nine *HL-A*-identical groups were detected, eight of which did not stimulate in MLC tests, one of which did stimulate. Of the 21 *HL-A*-dissimilar comparisons, all 21 did stimulate.

TABLE IV
Siblings in MLC-Identical Groups compared for Identity of Reaction with Leukocyte Antisera

Antisera	Families															
	Sc		Hi		Jo	Th	Sa	Ha	Se		Ny	De	OB	Gr	Ths	
	4*	2	4	3	2	3	3	2	2	2	2	2	2	2	2	2
RH	−	+			+	−		+	+	+		+			−	−
KP	+	+			+			+	+	+		+	+			
PAY	+	+	+	+	+			+	+	+		+	+	+		
AJ					+		+	+				+	−	+		
COV	+	+			+		+	+				+	+			
ENN	+	+			+			+	+	+		+	+			
RIL			+	+			+		+	+	+	+		+		
CAM								+	+	+					+	+
PBU											−			+		
DAL			+	+		+	+		+	+	+	+		+		
LBU	+	+	+	+				+				+			+	+
ROY	+	+	+	+	−				+	+	+		+	+		
NW	+	+	+	+	+	+	+					+	+	+	+	+
EH	+	+	+	+	−	−	−					+	+	−	+	+
DK	+	+			+	+	−	+				+	+	+	+	+
PIG			+	+	+	+	+					+			+	+
SD	+	+	+	+					+	+		+	+	+		
KH			+	+	+	+								+		
HF	+	+	+	−	+				+	+			+		+	+
RA	+	−	+	+	+		+		+	+	+	+		+	+	+
BH			+	+	+						+	+		+		
BC	+	+	+	+	+			+			+	+		+		
FS	+	+	+	+				−			+	+	−	+	+	+
McM			+	+		+	+							−		
CAR			+	+	+		+		+	+		+		+	+	+
SHO						+						+				
HM			+	+								+				
RB	+	+				+	+					+	+		+	+

+, indicates that all siblings in MLC-identical group typed identically.
−, indicates that there was some difference in typing results.
* Refers to number of siblings in that particular MLC-identical group.

A more extensive comparison is possible if all siblings showing nonstimulation in MLC tests are tested for their similarity in typing. This allows inclusion of families for whom genotyping is not possible. 12 families can be included in this comparison, with a total of 16 MLC-identical groups (Table IV). Members of

TABLE V

Studies of Two Nonidentical Twin Sisters

Typing results		
Sera	A	B
Complem. cont.	2/100*	8/100*
Serum control	−−−	−−−
COU	+++	++++
AJ	++++	+++
KP	+++	++
PAY	+++	+
WB	+++	+
GRIF	+++	+++
ENN	++++	+++
WITH	−−−	−−−
JOH	++	−−−
CAM	−−−	−−−
GW	++++	+
RIL	+++	−−−
P.BU	+++	++
L.MO	++	++
L.BU	+	+++
ROY	++	+++
NW	+++	++++
EH	−−−	++
DK	++	+++
WAS	−−−	+
SA	−−−	++
BH	−−−	+++
HM	+++	+++
JT	+	++
JD	−−−	++
BC	−−−	+++
BL	−−−	−−−
CAR	++++	++
McM	−−−	+
SHO	−−−	−−−
SD	−−−	++
MR	+	+
HF	−−−	−−−
PIG	−−−	−−−

MLC test results
cpm
AA_m—31
AB_m—55
BB_m—98
BA_m—91
AX_m—10122
BX_m—8698
XA_m—15608
XB_m—13214

* Percentage of cells "killed" equivalent to −. Pluses (+) refer to different percentage of cells killed in the assay.

TABLE VI

Studies on Two Unrelated Donor (D)-Recipient (R) Kidney Transplantation Pairs

Antisera	Typing results			
	H(R)	I(D)	K(D)	L(R)
MR	+	–	+	–
JOH	+	–	+	–
PAY	+	–	+	–
KP	+	–	+	–
WB	+	–	+	–
ENN	+	+	+	+
COU	+	+	+	–
AJ	+	+	+	+
RH	+	–	–	–
CAM	+	–	–	–
PBU	+	+	+	+
LMO	–	+	–	–
GW	+	–	+	+
LBU	–	+	–	–
GRIF	–	+	+	–
ROY	+	+	+	–
EH	+	+	+	–
NW	+	+	+	–
SD	–	+	–	–
PIG	+	+	+	–
DK	+	+	+	+
JT	–	–	+	+
HM	–	–	+	–
SHO	+	+	+	–
McM	+	+	+	+
BC	–	+	+	+
BH	+	+	+	+
VER	+	+	+	+
JD	–	+	+	–
SA	+	+	+	–
BL	–	–	+	–
CAR	+	–	+	+
HF	+	+		
DAL	–	–	+	–
RA	+	–	+	+
BST	–	–	+	–
KH			+	–
FS	+	+	+	–
RB	+	+	+	–
THA	+	+	–	–
BM	–	–	+	+
BL_2	–	–	+	–
MH	–	–	+	+
RH	+	–	+	+

MLC test results
cpm
HH_m— 231
HI_m— 547
II_m— 258
IH_m— 3577
HX_m— 4921
IX_m— 10681
KK_m— 90
KL_m— 181
LL_m— 125
LK_m— 2525
KX_m— 1471
LX_m— 9482

an MLC-identical group can only be usefully compared for their reactions with an antiserum when there is a polymorphism for this antigen within that family. Thus, each MLC-identical group can be compared for its reactions with from 8 to 22 antisera. A total of 226 comparisons are possible. Any MLC-identical group within a family containing two or more siblings is listed. All the members of that group are compared for their reactions with each of the antisera listed. A "comparison" is made by correlating typing results obtained with one antiserum on members of one MLC-identical group, regardless of the size of that group. 209 comparisons fit the prediction that all members of an MLC-identical group type identically, 17 do not.

In Table V are given the typing results and results of mixed leukocyte culture tests for a pair of nonidentical twin sisters. There is a marked discrepancy, in both directions, in the antigen profile of these two individuals; however, their cells do not stimulate each other in mixed leukocyte cultures. Results of control cultures to show that nonstimulation is not a technical artifact are also included.

In Table VI are given the typing results on two pairs of unrelated donors and recipients of kidneys for transplantation[2] and results of mixed leukoctye culture tests on these individuals. In each case there is a marked antigenic discrepancy between the individuals; results of the mixed leukocyte culture tests indicate that the recipient does not respond to the donor, whereas the donor does respond to the recipient in both cases.

DISCUSSION

We have previously published results suggesting that both leukocyte typing and MLC tests define the major histocompatibility locus in man, *HL-A* (previously known as *Hu*-1). These conclusions are strengthened by the additional data presented here and by results obtained at the Third Histocompatibility Workshop in Turin, Italy in 1967. In Turin, it became clear that most of the antigens detected by available leukocyte antisera are determined by a single genetic system (8). Most of the antisera used in the experiments presented above were used in Turin and were found to detect antigens of the *HL-A* series. Some of the antigens detected were similar to, or identical with, previously designated antigens such as LA2, 7c, and 4a. Others measured new specificities, also belonging to the *HL-A* system.

Serological data and MLC test results support the concept that the *HL-A* system is very polymorphic. In Turin, 22 unrelated individuals were extensively studied. All 44 *HL-A* alleles of these 22 individuals had different combinations of antigens associated with them. Similar findings have been obtained by Ward and Amos at Duke University.[3] Estimates of the polymorphism at *HL-A* based on results of MLC tests suggest that a minimum of 15 different alleles are present in the population (16).

[2] Patients from the series of Dr. T. Starzl of Denver, Colo.

[3] Ward, S., and D. B. Amos. Unpublished observations.

Dausset et al. (5) and Ivanyi et al. (17) have suggested that the extent of reactivity in MLC tests correlates with incompatibility for one or more of four leukocyte antigens. We have here presented further data to support our previous suggestion (7) that the loci determining the leukocyte antigens and controlling reactivity in MLC tests are one and the same. In those families for which genotyping is possible, the different *HL-A*-identical siblings should be unambiguously defined and cells of such siblings should not stimulate in MLC tests. In Table III results of such a comparison are presented in two ways. The correlation by either method of comparison is extremely significant. The one exception is discussed below.

For those families in which genotyping was not possible, a different correlation is attempted. Cells of siblings which did not stimulate in MLC tests are compared for their reactions with a series of antisera measuring *HL-A* antigens. 12 families, with a total of 16 MLC-identical groups were included in this study. As pointed out, only those antisera which demonstrate a polymorphism in the family in question are considered. The random probability of two siblings typing identically depends on the genotypes of the parents for the alleles determining the antigen(s) in question. We have included only those situations in which both positive and negative offspring result. Such offspring occur either when both parents are heterozygous for the genes controlling a given antigen (that is carry only one allele for that antigen), or when one parent is heterozygous and the other does not possess the gene for that antigen. If one parent is positive and the other parent and some children are negative, then we know that the parent having the allele determining the antigen in question must be heterozygous for that allele. The probability that two siblings will both receive that allele from the one parent is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$. Thus, the probability that cells of both will react positively is $\frac{1}{4}$. Similarly the probability that they will both be negative is $\frac{1}{4}$. The combined probability that they will react identically with the antiserum in question is, therefore, $\frac{1}{4} + \frac{1}{4} = \frac{1}{2}$. If both parents are heterozygous for a gene determining a given antigen, then the probability that any sibling will inherit the allele is $\frac{3}{4}$, or the probability that two siblings will inherit the allele is $\frac{3}{4} \times \frac{3}{4} = \frac{9}{16}$. The probability that a given sibling will inherit only alleles which do not determine the antigen is $\frac{1}{4}$. The probability that two siblings will both fail to inherit the gene for that antigen is $\frac{1}{4} \times \frac{1}{4} = \frac{1}{16}$. The combined probability that the cells of two siblings will react similarly with the antiserum in question is therefore $\frac{9}{16} + \frac{1}{16} = \frac{5}{8}$. Thus, if the locus controlling MLC reactivity segregates independently of the locus determining the leukocyte antigens, the cells of a maximum of five out of eight MLC-identical siblings will react identically with the antisera.

We were able to make a total of 226 comparisons in which the members of MLC-identical groups could be compared for their reactions with a given antiserum. In 209 of the 226 cases we were correct in our prediction that the members of the MLC-identical group would also type identically. In 17 cases there

was a discrepancy in the typing. Eight of the 17 errors are attributed to only two antisera, EH and RH. This correlation is strengthened further by our including all members of an MLC-identical group in each comparison, even when such a group contained four individuals, and by our counting such a comparison as an error if only one of the four typed differently from the other three. The probability of obtaining the correlation of $^{209}/_{226}$ by chance alone is extremely small.

Both of these correlations then support the concept that the locus controlling reactivity in MLC tests is the same one determining the leukocyte antigens.

Both leukocyte typing and MLC tests, for purposes of this study, are used in an attempt to define identity at the *HL-A* locus. In some families, allelic assignments are easily made, and informative patterns can be discovered even when few sera are used for typing (18, 19). With sera used in this study, although they are limited in number, we can recognize a wide variety of specificities. When there are few children, or families without parents, not infrequently the four alleles cannot be identified. It may then be impossible to distinguish between genotype and phenotype; MLC testing may be useful in resolving these difficulties. When genotyping is not possible, serotyping may give erroneous results, especially when impure sera are used for testing. Serologic tests therefore can give a good definition of *HL-A* alleles in certain families but a poor approximation in some small or incomplete families and in unrelated subjects. Results given in Fig. 1 strongly support the concept that in the majority of cases, results of MLC tests can be assigned unambiguously to one of two classes—those showing stimulation or those not showing stimulation. The latter group distribute around zero as shown in that figure.

Three exceptions to the correlation between typing and MLC results are noted. First, the exception noted in Table III was from the family reported in Tables I and II, and it involves two sisters, G and I. These siblings were alike in their serological reactions except with serum DAL, with which one cell reacted weakly (+) and the other failed to react. A second exception was the case of two nonidentical twin sisters whose antigen profiles were grossly different, but whose cells did not stimulate in MLC tests (Table V). A third was two pairs of unrelated donors and recipients of kidneys for transplantation whose cells typed differently but showed unidirectional nonstimulation in MLC tests (Table VI).

It is difficult to explain the case in which leukocytes of the two sisters with identical *HL-A* genotypes were not mutually nonstimulatory in MLC tests. Results of typing and genotyping are given in Table II, and results of MLC tests in the text. As can be seen, both parental alleles are well defined, and one of the maternal alleles has several antigens associated with it. The other maternal allele, although having no unique antigen associated with it, can be identified with two antisera which were associated with both one allele of the father and the second allele of the mother. There is thus little chance that

the genotyping is incorrect. Further, in two other *HL-A*-identical sibling groups in that family, composed of four and two siblings respectively, the typing-MLC correlation was positive. A discrepancy of this kind might be expected if crossing-over had occurred and the part of the *HL-A* chromosome associated with the cross-over had no detectable antigens associated with it. Save for a rather weak reaction with one serum, DAL, there is no evidence for this.

The overwhelming correlation between MLC identity and identity of typing makes the nonstimulation in the case of the nonidentical twin sisters a clear exception. This could be accounted for by undetected leukocyte chimerism and resultant tolerance. We have previously reported a case of two known human chimeric twins whose cells did not stimulate in MLC tests (9), and it has been shown in rats (20) that cells of animals made tolerant to each other will not stimulate in MLC tests.

Each of the recipients of kidney transplants had received a kidney from an unrelated donor two or more years earlier and had since that time been on immunosuppressive therapy. In each case the donor and recipient typed differently. Although one of us (DBA) did not detect any antigens of the cells of the donor which the recipient did not also possess in one of these cases (Table VI), other antisera used by Van Rood did detect such antigens.[4] Thus both these pairs are antigenically incompatible. In both cases, however, cells of the donor did not stimulate the cells of the recipient, whereas cells of the recipient did stimulate cells of the donor. Also in both cases cells of the recipient were able to respond to allogeneic unrelated cells. Such specific nonreactivity in one direction, which we have not found in more than 324 cases of one-way MLC tests between unrelated individuals, is remarkable (even though the mixtures were tested at only one concentration of stimulating cells, which in some combinations of siblings or of parent and child might not result in stimulation despite MLC nonidentity) (21). One possibility is that the recipient has become "tolerant" to donor antigens.

SUMMARY

The evidence is reviewed that a single genetic system, the major histocompatibility locus in man, *HL-A*, determines most of the antigens measured by presently available leukocyte isoantisera, and also controls reactivity in one-way mixed leukocyte culture tests. Studies in 12 families are presented to support this conclusion. Some interesting exceptions to the general typing—MLC tests correlation are presented and discussed.

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[4] Van Rood, J. J. Personal communication.

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