Hu-1: Major Histocompatibility Locus in Man

Abstract. *A single locus with 15 or more alleles controls reactivity in mixed leukocyte culture tests. Genes at this locus also control most of the specificities measured by cytotoxic antiserums to leukocytes. Both of these tests can be used to predict survival of skin grafts. It is proposed that this is the major histocompatibility locus in man.*

Homograft rejection follows host reaction against histocompatibility antigens present on donor tissue and absent from the host. Antigenic disparity can be determined by "matching" procedures such as the mixed leukocyte culture (MLC) test or by the detection of individual antigens with suitable isoimmune antiserums.

In one-way MLC tests, peripheral-blood leukocytes of one of the donors are treated with mitomycin C (*1*), which prevents them from synthesizing DNA yet allows them to stimulate the division of untreated cells from the other donor. The response of the untreated cells is judged by the incorporation of radioactive thymidine into acid-precipitable material.

In leukocyte typing, serums from certain multiparous women, or from subjects specifically immunized against leukocytes or skin grafts, are mixed with lymphocytes in the presence of complement. If the cell being tested carries the appropriate antigen, the cell membrane is damaged, and a dye such as trypan blue will readily enter and stain it. If the antigen is not present, the cell remains intact and does not stain (*2*).

Both MLC tests and typing results are predictive of skin-graft survival. Skin grafts between siblings whose cells do not stimulate in MLC culture show a prolonged survival compared to grafts between siblings whose cells do stimulate in culture (*3*). Similarly, grafts between individuals who are antigenically very similar show prolonged survival compared to those between individuals with many differences (*4*).

In our experiments, siblings from seven large families were studied in one-way MLC tests, in all combinations of two within each family. The cells were also typed with 31 selected cytotoxic antiserums. Results of the two methods are compared. All studies were done in Madison, Wisconsin, on cells from freshly drawn blood.

Two or more siblings whose cells are mutually nonstimulatory in reciprocal one-way MLC tests are referred to as "MLC-identical siblings." Table 1 shows MLC results on seven siblings from one of the families studied. Cells treated with mitomycin C are indicated by the subscript m. The numbers in the table represent the number of counts per minute of tritiated thymidine incorporated by untreated cells during a 5-hour period of labeling on the 7th day of culture less the number of counts per minute in control cultures (for example, AA_m) whose values are given in parentheses. Nonstimulation can readily be distinguished from positive stimulation—the maximum ratio between an allogeneic mixture and the control in nonstimulating combinations being 1.35, whereas the comparable ratios for the positively stimulating cultures vary between 8 and 264. Three siblings (A, B, and C) form one group, two others (D and E) form a second, and the remaining two (F and G) are unique.

Typing serums used for genetic study should be monospecific. Serums containing more than one antibody can be used if the components react against antigens of the same genetic system. While such antiserums do not give the maximum resolution possible, observed differences are still valid. Previous tests with the antiserums used in this study have shown that no two of the serums contain the same antibodies, and that where more than one antibody is present the components appear to react against antigens controlled by the same system. Some serums detect previously defined antigenic specificities [such as LA-2 (also called 8a) and 4a], while others react with antigens that have not been classified. The reactions of some of the serums against the same siblings and their parents are summarized in Table 2. Only those serums capable of detecting the polymorphism for any given antigen in this particular family are included. Other serums reacted with all the cells or failed to react with any.

The results of MLC tests correlate well with the results of leukocyte typing. For reasons stated below, we are interested in this study only in this type of correlation. In the family presented, there are two MLC-identical groups—each tested with 13 antiserums. For each group we can compare patterns of reaction to each antiserum. For instance, the reaction patterns of A, B, and C with antiserum Pay is considered a single comparison, with antiserum Cou a second comparison. Similarly the reactions of D and E with these two antiserums represent two further comparisons. Each of these

Table 1. Results of MLC tests on siblings tested in one-way culture in all combinations. Results represent the net number of counts per minute incorporated in allogenic mixed cultures. Control cultures (treated and untreated cells from the same donor) are listed in parantheses. Radioactivity of the controls was subtracted from that incorporated in allogeneic mixtures to obtain the net results listed.

Responding cells	Stimulating cells (count/min)						
	A_m	B_m	C_m	D_m	E_m	F_m	G_m
A	(43)	35	−9	2426	1737	11340	1685
B	57	(95)	14	2846	1623	4695	1165
C	22	23	(17)	1672	1763	1193	1284
D	2055	2365	1970	(25)	13	392	746
E	8663	5743	12815	56	(52)	2431	515
F	4251	5030	6989	1554	1138	(89)	1325
G	1505	1253	1649	472	432	263	(33)

Table 2. Results of cell typing on siblings and parents. Pluses refer to the percentage of cells killed in the assay. Any rank from + to 4+ is considered positive.

Anti-serums	Siblings							Parents	
	A	B	C	D	E	F	G	Father	Mother
Pay	+++	+++	+++	0	0	0	+++	0	+++
Cou	+++	+++	+++	0	0	0	++++	0	+++
Roy	+++	++	++	0	0	0	++	0	++
Nw	+++	++++	+++	0	0	0	+++	0	+++
Eh	++	++	++	0	0	0	++	++	++
Dic	++	++	+++	0	0	0	+++	0	+++
Sd	++	+	++	0	0	0	++	0	++
Fs	++	++	++	0	0	0	++	0	++
Hf	+++	+++	+++	0	0	++	+	+++	0
Lbu	++	++	++	+++	+++	0	+++	++	+++
Bc	0	0	0	++	+	0	0	0	+
Rb	0	0	0	++	++	0	++	++	0
Ra	0	0	0	++	0	0	0	+	0

four comparisons fits the prediction that MLC-identical siblings are identical in their reactions to the antiserums. Of the 26 comparisons, only one does not agree with the prediction, since cells of individuals D and E react differently with antiserum Ra. In the seven families studied, 11 MLC-identical sibling groups are detectable. Individuals in these groups can be compared for identity in reaction with from 11 to 21 antiserums, depending on the family—a total of 185 comparisons. In 177 cases, all the members of an MLC-identical group type identically; in eight cases the MLC-identical siblings do not type identically (such as siblings D and E with antiserum Ra). Two antiserums are associated with five of the eight cases of noncorrelation.

The random probability of two siblings typing identically depends on the genotypes of the parents. We have included only those parental genotypes which result in both positive and negative offspring. 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. The random probability that two siblings will react identically, whether positive or negative, with respect to a given antiserum will thus vary between 1/2 and 5/8. Thus, if the locus controlling MLC reactivity segregates independently of a locus controlling antigens, the cells of a maximum of five out of eight of MLC-identical siblings will react identically with the antiserums. The probability of obtaining the correlation we have found (177/185) by chance alone is vanishingly small.

Previous analysis of MLC data has suggested that reactivity in culture is controlled by the genotype at a single locus with many alleles (*5*). At such a locus, regardless of the number of alleles present in the population, a maximum of four different alleles are represented in any family and a maximum of four genotypes can be found in the siblings. Thus, a minimum of 25 percent of siblings will be identical at this locus and should not stimulate. In unrelated individuals, however, as the number of alleles in the population becomes very great, the frequency with which cells of two individuals fail to stimulate will approach zero. In Table 3 the expected values for the proportion of nonstimulators (for different genetic relationships) are given for varying numbers of loci and alleles, if there are equal allelic frequencies, full penetrance, and no genetic or gene-product interactions.

Table 3. Expected percentages of nonreacting mixtures when one-way stimulation was used.

Alleles (No.)	Genetic relationship	
	Sib-sib	Unrelated
	One locus	
1	100	100
2	78	63
3	61	33
10	35	3.7
15	32	1.7
20	30	0.96
30	28	0.43
∞	25	0.00
	Two loci	
2	61	39
3	37	11
4	27	4
5	21	1.8
6	18	0.94
	Three loci	
2	47	24
3	22	3.6
4	14	0.8
	Four loci	
2	37	15
3	14	1.2

The total Madison series includes 209 sibling pairs in 29 families. Of these, the cells of 61 pairs (29.2 percent) have not stimulated. Of 282 mixtures of cells from unrelated pairs, none have not stimulated. The 95 percent confidence limits for these percentages are 23 to 35 percent for the siblings and 0 to 1.4 percent for the unrelated individuals. These combined data only fit the expectation for one locus with 15 or more alleles. Although the data on siblings also fit the possibility that there are two loci, each with four alleles, the data for unrelated tests do not fit. Results of two-way MLC tests in more than 500 cell mixtures from unrelated subjects—all of which have stimulated—further support the interpretation in terms of multiple alleles at a single locus. This analysis requires no imposition of the extent of compatibility, since nonstimulators are clearly separated from stimulators; nor is it sensitive to the assumption of equal allelic frequencies since, with a large number of alleles, any change from equality will closely approximate a simple decrease in the number of effective alleles (*6*).

Finally, the assumption of a single locus with many alleles gains strength from the serological results. The genetic analysis of the antigen data can be illustrated with the data in Table 2. The mother must be heterozygous for the genes controlling the antigens determined by the top eight antiserums since she has negatively reacting children. Under such circumstances, when several siblings have a group of antigens, we can assume as a first approximation that these antigens are controlled by a single gene or by a group of closely linked genes. This could be the case for the genetic factor or factors controlling the reaction to the first eight antiserums. Thus siblings A, B, C, and G react with these antiserums, and D, E, and F do not. From similar analyses of the seven families it appears that specificities measured by most of the antiserums are controlled by linked genes. (Two errors due to typing appear possible in Table 2: the weak reaction of G and Hf, and the positive reaction of the father with Eh.) Two antiserums accounted for five of the eight cases of noncorrelation between MLC grouping and leukocyte typing.

We have two cases in which stimulation occurs in only one direction. In the first case, the cells of a child do not stimulate the cells of its father but the cells of the father stimulate those of his child. The father's cells type positively for eight antigens not possessed by the child, whereas none of the antiserums used detect antigens on the child's cells, which are not also present on the cells of the father. The second case is exactly analogous except that the cells of a girl, which do not stimulate the cells of her mother, do react positively with one antiserum with which the mother is negative. The mother has 12 antigens that the daughter does not have.

There were two unrelated individuals who, from the typing data, might have been expected to show stimulation in only one direction. One reacted strongly with only three antiserums and very weakly with two others. The second subject reacted with these five antiserums and with ten others. However, there was stimulation in both directions in MLC tests. Thus, there are apparently antigens recognized in MLC tests which are not detected by the antiserums used in our experiments. Furthermore, we recognized three groups in some families by MLC tests and only two by leukocyte typing. Since some of the antigens controlled at this locus are not yet defined and since it is probable that some of the antigens detected are controlled by genes not at this locus it is only possible to group individuals on the basis of MLC tests and then correlate this with the results of antigen typing.

There are some interesting exceptions to the general correlation between

the MLC test and typing. Cells of dizygotic twins did not stimulate, but typed differently with respect to 10 of 29 antiserums. Leukcocyte chimerism in these twins might account for nonstimulation in MLC tests. Bach and Uchida have studied a pair of chimeric human twins whose cells did not stimulate (*6a*). In a study of the donors and recipients of kidney transplants [(done retrospectively) in the Denver program (*7*)] there were several combinations in which cells from the donor failed to stimulate cells from the recipient in one-way cultures although typing of donor and recipient shows clear antigenic differences. In these cases, possible changes in the immunological status of kidney recipients on long-continued immunosuppressive therapy must be considered.

It is suggested that the locus which controls MLC reactivity and in which are included most of the genes controlling the antigens we have measured is the major histocompatibility locus in man. Dausset, Ivanyi, and Ivanyi (*8*) have grouped a series of leukocyte antigens into a single system which they called Hu-1. They also found (*8*) that MLC reactivity was correlated to some extent with incompatibility for at least one of four antigenic factors in the Hu-1 system. Since, in all likelihood, we are measuring some of the same factors which Dausset *et al.* have included in the Hu-1 system, it seems appropriate that the locus be called Hu-1. This locus includes antigens of the Du-1 system (*2*).

This locus may be the major histocompatibility locus for the following reason: (i) In all other species studied there has been one major histocompatibility locus. (ii) Silvers (*9*) has shown that in rats, two strains differing at the major locus will stimulate in MLC. Members of two strains that are compatible at the major locus but that differ by multiple minor loci incompatibilities—with the time of skin-graft rejection being comparable to those of the major incompatibilities—do not stimulate in mixed cultures. (iii) The 8- to 14-day survival of skin grafts between individuals incompatible at Hu-1 (*3, 4*) is comparable to major locus incompatibilities in other species, whereas the time of survival of skin grafts for Hu-1 compatible siblings (15 to 42 days) is not consistent with an H-2 locus difference. In species which have been best characterized, incompatibility at the major locus usually leads to graft rejection within 8 to 13 days, whereas differences at only minor loci can lead to graft rejection in more prolonged periods of time (*10*). (iv) Survival of kidney homografts in individuals who are compatible at Hu-1 seems better (*11*) than that in Hu-1 incompatible siblings. The former group has maintained good function without any steroid therapy, whereas individuals in the latter group have all needed supplemental steroid therapy to maintain good function.

It is unlikely that all the antigens determined by Hu-1 have been identified. In MLC tests cells may respond to antigens that the available antiserums would miss. Antibodies of new specificity could be prepared by reciprocal immunization of siblings whose leukocytes type alike but who mutually stimulate.

The importance of obtaining a complete representation of the Hu-1 locus is considerable both for genetic studies and for matching donors and recipients in a transplantation program.

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References and Notes

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Intrauterine Devices: Effects on Ultrastructure of Human Endometrium

Abstract. *The human endometrium, studied with the electron microscope, undergoes asynchronous and premature cyclic development in response to intrauterine contraceptive devices. Typical nucleolar channel systems and other cellular characteristics of the normal secretory phase appear before ovulation, and decidualization occurs several days prematurely. Disturbance of the synchrony of ovular and endometrial development may be a mechanism of contraceptive action of these devices.*

Reactions of the endometrium to intrauterine foreign bodies vary widely even in closely related species (*1*). For the morphologic response in women to be ascertained, direct studies of the human endometrium are required. The commonly used plastic intrauterine contraceptive devices (IUD's) cause neither interference with transport of spermatozoa (*2*) nor significant chronic infection (*3*), but histological examination of the endometrium in contact with the IUD suggests that there is an alteration of cyclic pattern (*4*). My report of the ultrastructural changes in the human endometrium discloses premature maturation and asynchronous development of epithelium and stroma. The appearance, in the proliferative phase, of ultrastructural features previously described only in association with ovulation or exogenously administered hormones suggests an alteration in endometrial maturation. This effect may represent one mechanism of contraceptive action of the devices.

Endometrial biopsies obtained 6 weeks after insertion of the Lippes loop were repeated at semiannual intervals for periods of up to 3 years; 168 specimens obtained from 51 women were dated according to established histological criteria (*5*) before electron microscopy was performed. Specimens from the immediate vicinity of the loop showed transient inflammation, telangiectasia, and slight fibrosis. Other parts of the endometrium appeared normal histologically. The ultrastructure of each specimen was compared with that of its control (*6*), which was obtained by endometrial biopsy of the same patient before insertion of the device.

Specimens of proliferative endometrium resembled the controls in that an