when the problems we are exploring will be ready for a rigorous biochemical approach. It may turn out to be a kind of biochemistry as novel as that of gene function and replication was in its own time. Maybe we will again turn up something meaningful and exciting.

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Transplantation: Pairing of Donor and Recipient

Lymphocyte typing and stimulation of leukocytes in mixed cultures are used to select donors for transplants.

Fritz H. Bach

Homotransplantation of the kidney is now the therapy of choice for selected patients with end-stage kidney disease. Advances that have made possible the rapid increase, from four kidneys transplanted in 1958 to 434 in 1968 (*1*), in utilization of this procedure must be traced in large measure to research (i) in immunosuppression, in which various drugs are used to suppress the body's rejection reaction to the transplanted tissue, and (ii) in histocompatibility testing, in which the attempt is made to pair (*2*) donors and recipients who have antigenically similar tissues. In this article I discuss histocompatibility research including the tests that are used to pair donor and recipient, the methods used to analyze the data obtained, the genetic conclusions which can be drawn, the evidence establishing these tests as practically applicable to donor-recipient pairing, and the problems confronting us at present. Although the results discussed are those concerned mainly with man, I include a brief discussion of histocompatibility in the mouse to help our understanding of histocompatibility problems related to transplantation of organs or tissues in man.

Studies in the mouse (*3*) have been facilitated by the availability of inbred strains which, for all practical purposes, are genetically identical (co-isogenic) except for differing with respect to a single histocompatibility gene. An animal of an inbred strain will accept skin from another animal of the same strain; a member of one strain will reject a skin graft from a member of another coisogenic strain differing by only one histocompatibility gene. Rejection is associated with recognition by host cells of foreign (nonself) histocompatibility antigens. These histocompatibility antigens are associated with the gene by which the two animals differ. The concept that a tissue is rejected because that tissue carries antigens that are foreign to the recipient—that is, not possessed by the recipient—can be illustrated by grafting experiments between two inbred parental strains differing in their histocompatibility genes and their F_1 hybrid offspring. In such a situation the F_1 animal carries all the antigens of both parents. Either parent will reject a graft from the F_1 since that animal's cells will carry the foreign antigens of the other parent, but the F_1 will not reject grafts from either parental strain.

Many histocompatibility (H) systems, named H-1 through H-13, H-Y, and H-X, have been discovered in the mouse. Incompatibility at any one will lead to graft rejection. One of these, the H-2 system, seems to be of much greater importance than any of the other systems and has been termed the "major histocompatibility" system in the mouse. Similarly, in both the rat (*4*) and the chicken (*5*), a single ma-

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jor histocompatibility system has been identified. More than 20 different alleles of the mouse H-2 system (designated H-2^a, H-2^b, and so forth) have been identified; each allele is responsible for the presence on cells of a set of different antigenic specificities. One allele can be associated with ten or more specificities. A unique combination of antigenic specificities constitutes the phenotypic expression of each allele.

The separation of the major from the other minor histocompatibility systems is based on several facts. In experimental species, skin graft rejection is very prompt (8 to 12 days) in the great majority of cases when donor and recipient differ at the major system. However, when only a minor H system mismatch is present, skin grafts survive longer. Whereas differences at multiple minor H systems appear to be cumulative (*6*) in that skin graft rejection is more rapid with two minor H systems incompatibilities than with either one alone, such differences may still not represent as strong a barrier to graft survival, effectiveness of immunosuppression, or ability to induce tolerance. In addition, certain antigenic incompatibilities at H-2 appear "stronger" in that they will lead to more rapid rejection than other "weaker" H-2 antigenic incompatibilities. Thus, in mice or rats, one could pair with respect to either major system identity or minimization of major system antigenic incompatibility. This concept becomes of major importance for pairing in man.

Methods for pairing in man can be divided into two categories. In the "typing" method suitable antiserums are used to identify antigens on donor and recipient tissue. This permits one to pick a donor who has antigens identical, or nearly so, to those of a given recipient. The "matching" method depends on the physiological response which the cells of a recipient show to antigens on the allogeneic tissue of a donor. Although there are several tests for matching (*7*, *8*), I will consider only the mixed leukocyte culture (MLC) test.

Both typing and matching procedures utilize peripheral blood lymphocytes. The lymphocyte carries some histocompatibility antigens on its surface, making it a suitable test cell for typing procedures—although there is no assurance that different tissues will have the same quantitative or even qualitative distribution of transplantation antigens (*9*, *10*). In addition, the lymphocyte has been implicated as one of the important cells involved in homograft rejection, although little is known about the mechanism of graft destruction that follows recognition of foreign histocompatibility antigens by host lymphocytes. In the MLC test, lymphocytes of two subjects are mixed in vitro; the lymphocyte is used both as a responding cell (reacting to foreign tissue antigens by enlargement, DNA synthesis, and division), and as a stimulating cell (carrying the foreign antigens). The extent of the response of the lymphocytes in culture is used to measure the amount of antigenic disparity between the two test subjects.

The development of our knowledge about histocompatibility in man came from studies in which both typing and MLC techniques were used. The suggestion that one genetic system controls most of the now recognized lymphocyte (histocompatibility) antigens in man was first made on the basis of typing results (*11*, *21*). This suggestion was confirmed by MLC tests (*12*), giving evidence that this was the major histocompatibility system in man—HL-A (*13*). It is either by typing for the HL-A antigens, of which more than 25 are now recognized, with the view to minimizing the number of antigenic incompatibilities or by matching with the MLC test with the purpose of minimizing HL-A disparity as measured in MLC that a donor is chosen for a given recipient.

"Typing"—Definition of Antigens

There are several "indicator" systems for cell antigen-antibody reactions (cytotoxicity, agglutination, complement fixation, and others). The most commonly used methods are based on lymphocyte cytotoxicity testing, with antiserums obtained from multiparous females (which could contain antibodies to antigens of the fetus which were inherited from the father and were not possessed by the mother) or from specifically immunized individuals. In these tests, cells are incubated with each of many antiserums in the presence of complement and trypan blue. If the cells carry an antigen for which there is complement-fixing antibody in the particular serum being tested, in the presence of complement the cells will be damaged and will stain with the trypan blue (*14*). Antiserums containing antibodies against more than a single specificity (polyspecific antiserums) are widely used in leukocyte serology to define "antigens." Since the interpretation of the reactions of polyspecific antiserums is a major problem in leukocyte genetics, I will discuss the different approaches used to define antigens before discussing the current state of knowledge.

An antigen is defined either with a group of polyspecific antiserums or with operationally monospecific antiserums. If two polyspecific antiserums react identically with cells of many individuals, the antiserums may be identical. Even if a few of the cell samples react with one antiserum and not the other, the antiserums may still be significantly correlated. In operational terms, the reactions of two polyspecific antiserums with a panel of cells can be entered in a 2 by 2 table as follows.

		Antiserum 1	
		+	−
Antiserum 2	+	a	b
	−	c	d

where *a*, *b*, *c*, and *d* are the number of samples in each classification. Thus when $b = c = 0$ (where *b* and *c* are the number of individuals reacting with one antiserum but not the other) and the number of samples in the panel is large, the two antiserums are tentatively regarded as being identical. When either or both *b* and *c* are not equal to zero, the reactions of the antiserums may still be closely correlated even if each has antibodies which are not present in the other. If a series of polyspecific antiserums all show significant positive correlations with each other they may be supposed to share at least one antibody in common and thus to define an antigen. Any sample of cells that does not react with every antiserum in the series does not possess that antigen, provided that there is one shared antibody and that there are no technical errors. A sample that does react with all of the antiserums still may not have that antigen, but the probability of reacting with only the unshared antibodies should decrease rapidly as the number of antiserums against which the cells are tested increases.

While the general approach to the definition of antigens with groups of positively correlated antiserums is a very reasonable one, it is possible to misinterpret these results when a set of antiserums contains antibodies against antigens that occur together in a population. Such antiserums, although pos-

Table 1. Leukocyte typing on a family used in the 3rd Histocompatibility Workshop (Torino). Adapted from Ceppellini (*22*).

Subject	Antigens											Chromosomes
	2	3	4	5	6	7	8	9	10	11	12	
						Parents						
Father	+	+	−	−	+	−	−	+	+	−	−	a/b
Mother	+	−	+	−	+	−	+	−	−	−	−	c/d
						Siblings						
1	+	−	−	−	+	−	+	−	−	−	−	b/d
2	+	−	+	−	+	−	−	−	+	−	−	b/c
3	+	−	+	−	+	−	−	−	+	−	−	b/c
4	+	−	+	−	+	−	−	−	+	−	−	b/c
5	+	−	+	−	+	−	−	−	+	−	−	b/c
6	+	+	−	−	+	−	+	+	−	−	−	a/d
	+	+						+				a
					+				+			b
	+		+									c
	+				+		+					d

sessing different antibodies, would still have similar reactions with samples from that population. For example, if there are two different antiserums, anti-A and anti-B, and if the antigens A and B are highly associated, such that the frequency of individuals with either A or B alone is low, then the two antiserums will show a positive correlation even though they are detecting different antigens. Evidence that this sort of complexity occurs in leukocyte typing comes from the work of Dausset *et al.* (*15*) who showed that the pattern of reactions of a group of antiserums varied with respect to races, dramatically illustrating that antigens of the HL-A system are logical constructs defined in populations and do not necessarily have a one-to-one correspondence with single chemical substances in a cell membrane.

Since a great many tests of association are performed during this kind of analysis, spurious correlations can confuse the final interpretation of a set of reactions. Most workers agree that the description of antigens by association analysis of reactions should be regarded as only preliminary, with absorption studies being necessary to confirm the classification. In absorption studies, cells of an individual are mixed with an antiserum to remove from the antiserum any antibodies which can react with the given cells, that is, for which there is an antigen on those cells. Suppose an antiserum contains only two antibodies, anti-A and anti-B. If we have two cells, one of which has only antigen A on its surface, the other only antigen B, then both these cells would react positively with the antiserum. If we absorb the antiserum with cells carrying antigen A, only the antibody to B would be left in the antiserum; such a "monospecific" serum reacts only with cells bearing the B antigen.

Walford *et al.* (*16*) attempted to mollify the problem of associated antigens by giving an operational definition of monospecificity for antiserums obtained after specific immunization (it would also apply for the serums of pregnant women if the cells of the fetus or of the father are available). They showed that, when an antiserum contains antibodies against two antigens, if the serum does not react with the cells used to immunize the antiserum donor after absorption with cells from any one of 20 to 30 unrelated individuals reacting positively with the antiserum (and this is true for all 20 to 30 cell samples), then the probability is less than 0.05 that the two antigens are found together less than 90 percent of the time. Operationally these two antigens are considered "an antigen." The more frequently two antigens are found together, the more they behave as a single antigen, since the presence of one almost always specifies the presence of the other. An operationally monospecific antiserum (by the definition of Walford *et al.*), then, is an antiserum that does not react with the cells of the original donor after individual absorptions with cells from any one of 30 positively reacting unrelated people.

There is, of course, nothing magical about the number 30, but the frequency of association and the probability used are convenient. While the use of operationally monospecific antiserums helps to lower the risk of misinterpreting the reactions of polyspecific antiserums against associated antigens, the problem of cross-reacting antibodies remains. Absorption studies cannot provide an unambiguous interpretation of the reactions of two antiserums with a series of cells (*17, 18*).

The distinction must be made between the term "antigen" as defined by a group of antiserums with positively correlated reactions ("antiserum group"), and the definition as a chemical entity such as one of the ABO blood group antigens. It is perhaps unfortunate that the word "antigen" should even be used for the first, for we might easily be lulled into believing that we have more information than there actually is: a high positive correlation between the reactions of two or more antiserums does not guarantee that a single antigenic specificity is being detected. Perhaps a term like "antiserum group" should be substituted for "antigen" when speaking of the pattern of reactions that a given sample of cells possesses: for example, we may say that "an individual typed positive for antiserum group A" rather than "positive for antigen A," and leave the word "antigen" for operationally defined monospecific antiserums. Nevertheless, the difference between an antiserum group and an operationally monospecific antiserum is more of degree than of kind.

Genetic Analyses—HL-A Antigens

If a series of unrelated individuals are typed with two antiserums, anti-A and anti-B, the distribution of positive and negative reactions for the two antiserums will differ if genes determining *A* and *B* are the only alleles of a single genetic sytem rather than genes at unlinked loci. If they belong to a single system, an individual can either be homozygous *AA* or *BB*, in which case he can transmit only an *A* gene or a *B* gene respectively, or heterozygous *AB* in which case he can transmit either the *A* or the *B* gene. The proportions of each class, if we assume random mating, will follow the binomial distributions $p^2 + 2pq + q^2 = 1$, where p and q are the frequencies of the *A* and *B* genes. In this situation, it is impossible to find cells which have neither the A nor the B antigen. If the genes are at different loci, one possibility is that a father and mother will both be heterozygous at both loci. The mating would be $A/-$, $B/- \times A/-$, $B/-$ (with the dashes representing genes which specify neither A nor B antigens, although they may specify other antigens). Offspring of such a mating could be $-/-$ resulting in cells

which carry neither the A nor the B antigen.

Because the same population data can be used for grouping antiserums to define antigens as described above, and testing for association between antigens, 2 by 2 table analysis has played a major role in the development of leukocyte genetics. The above situation could be depicted as follows, with anti-A and anti-B each giving 75 percent positive reactions, that is, the frequency of A is equal to the frequency of B, which is 0.5.

One Locus

	Anti-A +	Anti-A −	
Anti-B +	.50	.25	.75
Anti-B −	.25	0	.25
	.75	.25	

Unlinked loci

	Anti-A +	Anti-A −	
Anti-B +	.56	.19	.75
Anti-B −	.19	.06	.25
	.75	.25	

This simple example illustrates how it may be possible to show that two antigens belong to the same system or to closely linked systems (with gametic disequilibrium) by testing for associations between antigens in population data.

Because of the numerous calculations necessary when many antiserums are used, van Rood and van Leeuwen (*19*) introduced the use of computers to tentatively define antigens by grouping antiserums with positively correlated reactions and then to compare reactions among antiserum groups. Since then, this approach has been extended (*20*).

The most powerful method for demonstrating whether antigens belong to the same genetic system is linkage analysis. An example of this type of analysis is given in Table 1, where the reactions of antiserums against some HL-A antigens for a family are given, along with the genotypic analysis. The genotypes of the siblings are determined by transmissional analysis of the leukocyte antigens of the parents. The HL-A system is a very polymorphic one; both parents will thus usually be heterozygous for different alleles, and four groups of siblings can be identified. If the father has alleles *a* and *b* (*ab*) and the mother has alleles *c* and *d* (*cd*), then the four groups of siblings will be characterized respectively by allelic pairs *ac, ad, bc,* and *bd.* When the father's cells react positively with a given monospecific 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 if some of the children do not react with the antiserum. If we arbitrarily assign that antigen to one of the father's alleles at the 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).

Van Rood *et al.* (*12*) and Dausset *et al.* (*11*) were the first to present data suggesting that most of the detected leukocyte antigens belonged to one system. Studies at the Third Histocompatibility Workshop (1967) showed quite convincingly not only that almost all known leukocyte antigens belong to one linkage group (HL-A) but also that additional independently segregating systems exist (*22*).

In 1967, at the Third Histocompatibility Workshop at which a panel of 41 individuals was used, many laboratories had antiserums which showed similar patterns of reactivity with the panel. Seven of the antiserums gave identical patterns of reaction and other antiserums gave very similar patterns to the first seven. This degree of agreement between different laboratories led to the adoption of a uniform nomenclature for several antigens. Many other antigens that have not been given HL-A numbers are recognized in individual laboratories. In Table 2 are the names of HL-A designated and other antigens and the corresponding names from some of the participating laboratories. Since the writing of this article, four

Table 2. Nomenclature equivalents of HL-A specificities described in different laboratories. All specificities in any single row of the first two divisions of the table are thought to be exactly equivalent. In the third division equivalences are not yet established. The numbers in parentheses give the population frequencies of the various specificities according to the city and nationality of the investigating laboratory and therefore are of anthropological meaning.

Name	Amos	Batchelor	Ceppellini	Dausset	Engelfriet	Kissmeyer	Payne & Bodmer	Rood	Shulman	Terasaki	Walford
Official HL-A nomenclature											
HL-A 1	19	1(40)	8(21)	11(22)	4	LA1	LA1(25)	LA1(34)		1	Lc-1(23)
HL-A 2	1	5(36)	9(44)	1(53)	2	LA2	LA2(51)	8a(61)	Bl(46)	2	Lc-2(55)
(Mac)											
HL-A 3	4		10(26)	12(30)		LA3	LA3(35)	LA3(25)	Hill	8	Lc-3(37)
HL-A 5	12	25(4)	5(24)	5(19)	7	MH				6	Slaughter
HL-A 7	2		20(14)	10(31)	3	7C	4d(36)	7c(37)		11	Lc-8(40)
HL-A 8	56	2(29)	7(14)	8(18)	1	7D	4c(15)	7d(23)		5	Lc-7(17)
HL-A 9	Stewart	3(22)	12(29)	16(19)		LA4	SLA4			4	Lc-11(23)
Undesignated HL-A specificities found to be alike in two or more laboratories											
	3		3(75)	3(51)		4A	4a(60)	4a(66)		3	
			6(78)	7(65)			4b(65)	4b(90)		7	
	15		11(23)	4(39)		T12				9	Merrit
			13(19)							12	Lc-20(31)
				14(40)					F1		
								7a(51)	C1(30)		
				15(12)		Ba*					
Undesignated HL-A specificities, not alike or not yet compared											
	24, 28	4(45), 6(21)	2(90), 4(65)			BB		6a(90)			Lc-9, 10(27), 12
	34, 39	7(11), 8(8)	14(41), 21(8)	2(79)		ILN		6b(55)			13(30), 14(15)
	40, 42	9(7), 10(51)	22(17)	6(34)		HN		7b(55)			15(40), 16(20)
		13(33), 19(9)		17		R					17(23), 18(28)
		21(8), 24(7)									19(20), 21, 22, 23

more HL-A antigens have been given numbers at the Fourth International Histocompatibility Workshop (*23*). The designations in parentheses refer to the previous names used by the individual laboratories: HL-A 10 (Da 17, To 13 and Te 12); HL-A 11 (ILN*, Te 13, Da 21, To 26); HL-A 12 (Da 4, T 12, Ao 15, To 11, Te 9); and HL-A 13 (HN, To 21, BT 23).

At least 27 antigens of the HL-A system are now recognized. While all these antigens are associated with this single genetic system, they can be further separated into groups on the basis of their behavior in populations and their segregation within families. For instance, antigens HL-A-1, -2, -3, -9, -10, and -11 are mutually exclusive with respect to inheritance and behave in the population and in family studies as though they were determined by a single genetic site. That is, one individual never possesses more than two of these antigens and, in family studies, alleles for the two antigens never segregate together. Two or more antigens showing this relation to each other constitute a segregant series. Five other antigens, HL-A5, -7, -8, -12, and -13 fit into a second segregant series (*24*). The antigens of the first segregant series do not have a segregant relationship with antigens of the second series. Walford has suggested that a third and possibly a fourth segregant series of antigens exists (*25*). Recogntion of these segregant series facilitates pairing by typing since one individual can possess only two antigens for each segregant series and can have no other antigens of that series.

Because there are several segregant series at HL-A, it has often been suggested that the HL-A system consists of two or more distinct regions. This suggestion is consistent with findings of recombination within the HL-A system (*26*). In each case the recombinant chromosome presumably consists of genetic material associated with an antigen of the first segregant series and an antigen associated with the second series. One possible explanation of these findings is that the HL-A system consists of a series of linked loci. In such a scheme, the HL-A allele would actually consist of a series of linked alleles, each belonging to a separate genetic locus. An allele of one locus might determine one antigen of a segregant series; other alleles of that same locus would determine the other antigens of that segregant series. Alleles of a linked locus would determine the antigens of a second segregant series, and so forth. In this scheme, the recombinational events would have occurred between the two loci.

However, this is only one explanation of the findings (*18*). In the absence of detailed information on the chemical nature of the HL-A antigens, terms such as "mutational sites" and "subloci" are inappropriate. I would also caution that the data presented by Davies *et al.* (*27*) cannot be used to subdivide the HL-A region. These workers fractionated the HL-A antigens by column chromatography and obtained several fractions with factors capable of inhibiting different HL-A antiserums separated into different peaks. Although the results of this type of experiment can be suggestive, they cannot in principle determine the existence of multiple cistrons, and in this particular case the initial use of enzymes to disrupt the cells further complicates the interpretation. The only evidence for the nature of the gene is the identification and characterization of the gene product and comparison of the different products formed as the result of genetic recombination. Our suggestion, more extensively discussed previously (*18*), that the term segregant series be used for these groups of antigens is based on a desire not to prejudice our concept of the nature of the genetic material at HL-A. The unproductive controversy about similar problems in the Rh system emphasizes the futility of premature conclusions about the nature of that genetic region.

Walford (*28*) has described an antigen that is included with one of the HL-A segregant series on the basis of population data but the gene for which segregates independently of the HL-A genes in family studies. One explanation for the inclusion of an antigen into a segregant series is that the gene for that antigen interacts with the gene at the genetic site in question. Such an interaction has been demonstrated in immunogenetic systems (*29*). Walford's case could be the first instance of epistasis (gene interaction) at HL-A.

"Matching"—Mixed Leukocyte Cultures

In mixed leukocyte culture (MLC) tests (*30*), the amount of incompatibility between two individuals is measured without defining the antigens responsible for the incompatibility. In one-way MLC tests (*31*, *32*), peripheral blood lymphocytes of one individual (responding cells of a potential recipient) enlarge and divide in response to foreign antigens on the mitomycin C (subscript "m")–treated stimulating cells of a second individual (a potential donor); the response (stimulation) is assayed by measuring the incorporation of radioactive thymidine into the untreated responding cells (*8*, *32*, *33*). Mitomycin C prevents treated cells from incorporating thymidine. In previously described methods, MLC tests have required 6 to 7 days of incubation to obtain results. While our present method can give a result at 2 or 3 days (*34*), it is still only applicable in a prospective fashion to living donor or occasionally to transplants from cadavers.

Results by these methods can be interpreted either (i) as stimulation in contrast to lack of stimulation between pairs of individuals or (ii) as an attempt to assess the amount of stimulation. It is possible to distinguish stimulation from nonstimulation in most cases (*8*, *32*, *33*). For instance, in Table 3, cells of six siblings in the same family are grouped into three MLC identical sibling pairs (AB, CD, and EF). Whereas cells of A and B do not stimulate each other significantly (AB_m = 1211 count/min compared with the control AA_m, 930 count/min, and BA_m = 773 count/min compared with BB_m, 758 count/min), both A and B cells can respond and stimulate (as shown in the mixtures AX_m, BX_m, XA_m, and XB_m). The need for testing more extensively with several doses of stimulating cells to assure MLC identity is discussed below.

We have based several studies on this clear separability of MLC identical siblings from nonidentical ones, which have confirmed the impressions of previous authors (*7*, *8*). (i) There is a correlation between MLC test results and prolonged skin survival (*35*). Further we have found that there is a highly significant correlation between MLC nonstimulation and identity for the HL-A leukocyte antigens measured by Amos' antiserums. That is, siblings whose cells do not stimulate in MLC tests show virtually identical reactions with the antiserums (*13*, *36*). Also, a genetic analysis of the MLC data to ascertain how many loci and alleles are involved in determining reactivity in MLC tests suggests that a single genetic locus with a minimum of 20 alleles is involved (*12*, *13*). This analysis is based on a 28.2 percent frequency of nonstimulation in 291 sibling pairs,

with no cases of nonstimulation in more than 80 parent-child and more than 600 unrelated combinations. The foregoing studies suggested that the locus controlling MLC reactivity is the same as that determining the majority of the leukocyte antigens and is the major histocompatibility locus in man (*13*).

We have some direct evidence that leukocyte antigens associated with genetic systems other than HL-A—such as 5a, 5b, and 9a—do not result in stimulation. Two sibling pairs, in which the members of each pair typed identically for HL-A antigens and did not stimulate in MLC tests (in Dr. T. E. Starzl's transplantation program in Denver, Colorado), differed at the 5a antigenic system in one case and at 5a and 9a in the other. In neither case was there stimulation (*37*).

The genetic variability of the HL-A system is of such a high degree that the likelihood of finding two individuals other than sibs who are phenotypically identical is very small. For transplantation programs, as well as for reasons discussed below, it would be useful to measure degrees of antigenic disparity at HL-A in a meaningful manner. Our approach to demonstrating that the MLC test does this was to work within families in which it was possible to assign the parental HL-A alleles on the basis of leukocyte typing results (Table 3). With any sib group as reference (for example, sibs with genotype ac) two sib groups (ab and bc) differ by one allele, and one group (bd) has no common allelic inheritance and thus differs by two alleles. Since allele frequencies are very low, the parents will differ from any child by one allele. Within the family, one might predict that sibs differing from the responder by two alleles should have a greater degree of immunogenetic disparity from that responder than sibs differing by only one allele. This hypothesis was substantiated in two separate studies.

In one study (*33*), five families, each tested from two to four times, were examined with the use of a modified MLC method testing each mixture (for example, AB_m) at four concentrations of stimulating cells. Parental HL-A alleles were assigned on the basis of lymphocyte typing. In those experiments we predicted that the cells of the sib differing by two alleles would "stimulate the most" of all family members tested in every experiment. In one experiment, cells of one of the parents

Table 3. MLC test results in six siblings of one family. Three MLC identical sibling pairs are present. Cells of a member of one pair stimulate and respond to all of a member of a different pair (not shown). X is an unrelated individual.

Responding cells	Stimulating cells						
	A_m	B_m	C_m	D_m	E_m	F_m	X_m
A	930	1211					8040
B	773	758					27926
C			529	480			24433
D			621	604			30272
E					777	477	21322
F					766	681	38178
X	2716	8366	6755	9958	5235	11822	431

stimulated more than those of sib differing by two alleles—representing a clear exception to our prediction. Despite this one exception the probability of obtaining the predicted correlation by chance alone was, by the most conservative estimate, 1/570. In the second study (*38*) seven families at the Third Histocompatibility Workshop were studied by blind experimental protocol. In Fig. 1 are given the results of one of those experiments. Three mixtures failed to show stimulation (AB_m, AC_m, and AD_m). Shown

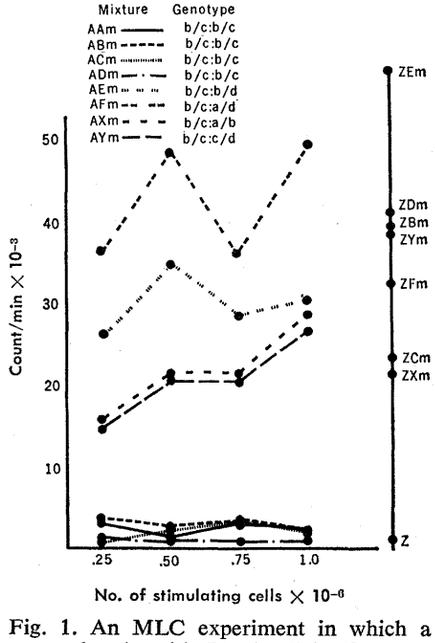

Fig. 1. An MLC experiment in which a responder A, with HL-A alleles *b* and *c* from his father and mother, respectively, is tested with mitomycin C–treated stimulating cells from siblings B-F, and parents X and Y. Each combination, such as AB_m, is tested at four concentrations of stimulating cells. Siblings B-D, who have inherited the same HL-A alleles, do not stimulate cells of A although they are capable of stimulation in the mixture with responding cells of an unrelated individual, Z (right vertical axis). Sibling E and the two parents each differ from A by one allele and stimulate A less than sibling F who differs by two HL-A alleles.

on the right vertical axis are the control cultures in which every stimulating cell type used in the experiment is tested with the responding cells of an unrelated individual, Z. Unless there is stimulation in the control cultures (for example, ZD_m), a failure to stimulate in the test mixture (such as AD_m) cannot be believed. In this case all the instances of nonstimulation involved treated cells that were capable of eliciting stimulation when mixed with unrelated responding cells. The cells from those sibs inheriting the same HL-A alleles show no stimulation. The cells from the sib differing from the responder by two alleles stimulate the most, whereas the cells of the parents and one sib differing by one allele show intermediate stimulation. The correlation in this second study was also convincing—seven parent-offspring pairs and three sib pairs differing by one allele were tested in contrast to sibs differing in two alleles. Each of these ten mixtures resulted in less stimulation than the relevant sib pair combination which differed by two alleles. It must be stressed that these correlations indicate only that what are probably fairly major relative differences in antigenic disparity in the majority of cases can be detected with good reproducibility, and in such instances the amount of stimulation observed is immunogenetically meaningful. Similar results have been obtained by others (*39*).

HL-A Antigens and Transplantation

In attempts to correlate HL-A incompatibility with graft survival, donor-recipient pairs have frequently been pooled without regard to genetic relationship, a circumstance which leads to difficulty in interpreting the results. In a system with many alleles it may be expected that close relatives will have, on the average, fewer incompatibilities than unrelated pairs. Since related pairs should have fewer incom-

patibilities than nonrelated pairs at other loci as well, this genetic stratification can lead to a spurious correlation because of loci other than HL-A that may affect graft survival. Although in many studies related pairs have been separated from nonrelated pairs, the various kinds of familial relationships (like uncle-nephew, sib-sib, and parent-child) are still sometimes mixed.

Several investigators have shown that the antigens measured by their antiserums are transplantation antigens. One method, first tried by van Rood *et al.* (*21*), is to immunize individuals with cells presumed to carry only one or two antigens which the recipients themselves do not possess, and then a short time afterward to transplant skin from individuals—other than the original immunizing donor—who carry and do not carry the relevant antigen. Skin from individuals carrying the antigen or antigens was rejected more rapidly (since the recipient was sensitized to that antigen) than skin not carrying the antigen. This method was used to demonstrate that certain of the leukocyte antigens were indeed transplantation antigens (*15, 21*). While several laboratories have found a significant correlation between typing and skin graft survival in sibling pairs (*15, 40*), they have been unable to find a similar correlation in parent-child grafts, or in unrelated individuals. Since there is one major system that exerts strong control of skin graft survival in man, if one antiserum differentiates the two alleles of the father while reacting negatively with the cells of the mother and another antiserum differentiates the two alleles of the mother while not reacting with cells of the father, these two antiserums will unambiguously define (barring crossover within HL-A) the HL-A alleles of the siblings. In all other genetic combinations all antigens must be defined to pair effectively (*41*). This may well explain the lack of success in nonsib pairs—with only rare exceptions (*42*). Correlations of MLC tests with skin graft survival are mentioned above.

Information on the effect of HL-A incompatibility on kidney graft survival is still rather limited. An important observation of Terasaki *et al.* (*43*) is that kidney grafts can survive for several years even when incompatible for more than one HL-A antigen. Furthermore, no HL-A antigen that they recognized was of critical importance in the sense of certain rejection if an incompatibility existed. Kidney biopsies from 35 patients who had survived about 2 years were examined, and the condition of the graft as determined from the biopsy was significantly correlated with the number of antiserum group mismatches. While different familial relationships were mixed in this analysis, examination of the data they presented reveals no large difference between sib-sib and parent-child grafts.

Terasaki, Mickey, and McClelland (*44*) examined the correlation of clinical rank of 209 surviving transplant patients with mismatches. The clinical ranks were not closely defined and were determined by physicians at different transplant centers. A very highly significant worsening of clinical condition was found when one or more incompatibilities for five antiserum groups was present. This type of data can be very misleading, since the subjective differences in judgment of clinical rank can easily lead to spurious associations due to stratification. Perhaps of more importance is the fact that it would be difficult to make such judgments on a blind basis. Nevertheless, this result tends to confirm the data from kidney biopsies.

A significant improvement has been found in the estimated average survival of compatible transplants compared with incompatible transplants once the patients had survived for at least 3 months, a period of high mortality (*45*). An independent set of data in which survival was considered from the time of transplant did not show a significant difference; the authors suggested that this was because HL-A incompatibility was not important during the early period after transplantation, later differential viability presumably being hidden by random variation. One might expect a statistical correlation between typing results and graft survival even if some important antigens of HL-A cannot be measured, since such a correlation can be based on the exclusion of incompatibility for those transplantation antigens we can recognize. The failures in individual cases can be explained on the basis that we do not recognize all the HL-A antigens yet and do not know their strengths. Patel, Mickey, and Terasaki (*46*), using data from unrelated donors, found highly significant correlations between mismatches and the number of clinically defined rejection episodes, creatinine clearance, and clinical rank but no significant effect of mismatches on transplant survival. The authors suggested that the effect of mismatches becomes important for renal function after a period of time (perhaps a year) has elapsed following transplantation. Mor-

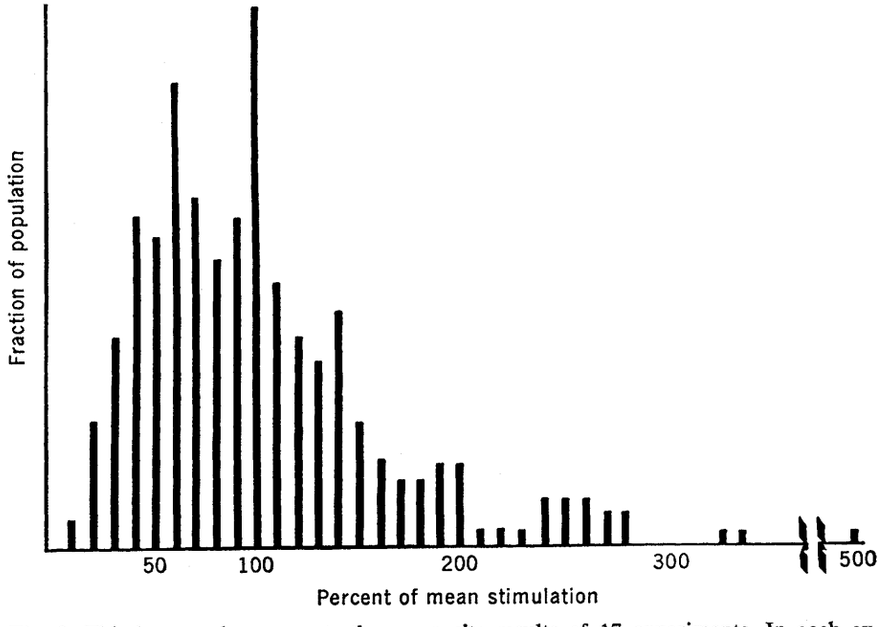

Fig. 2. This bar graph represents the composite results of 17 experiments. In each experiment, cells of one responder are tested with stimulating cells of 10 to 15 unrelated individuals. The average stimulation of the 10 or 15 allogeneic mixtures in each experiment is equated to 100, and all individual values of stimulation are expressed as a percentage of the mean.

ris *et al.* (*47*), however, have reported that HL-A incompatibilities affect the clinical course during the first year. Despite the discrepancy between these reports, an attempt to keep HL-A incompatibility as low as possible for unrelated donors would seem useful.

Van Rood and Eernisse (*9*) discussed results of a small retrospective study in which several recipients had died before being typed. These authors attempted to recreate the original data —the frequency of compatible and incompatible donor-recipient pairs of different genetic relationships—by estimating the relevant frequencies from a random population. They then compared these "original" numbers with the frequencies of their transplant survivors. Their results confirmed the impression that HL-A antigens do have an effect on kidney graft survival when related donors are used. They also suggested that, because ten matched patients (sib pairs) were alive and they had estimated that 10.4 were transplanted, perhaps no deaths had occurred in the "identical" group. The agreement between their expected and actual numbers, however, while consistent with their contention, is by no means conclusive.

Some studies suggest a correlation between MLC test results and kidney graft survival (*37*, *48*). However, for the most part these studies have either tested a correlation between MLC identity (as defined above) or low stimulation and kidney graft survival. As such, the correlations shown are dependent to some measure on the presence of HL-A identical sibling pairs in the sample.

In evaluating transplant data, it is important to realize that not only do protocols vary from clinic to clinic, but they are changed with increasing experience. This is illustrated by the sixth report of the Human Kidney Transplant Registry (*49*) which has shown significant increases in 2-year survival of kidneys transplanted after January 1966, compared with previous transplants. The 2-year survival for sib donors increased from approximately 55 to 75 percent, parent donors from 50 to 55 percent, and unrelated donors from 20 to 40 percent. Obviously, pooling of data without the possibility for proper adjustment for extraneous sources of variation, which at present are largely unknown, makes interpretation more difficult.

Present Goals and Approaches

The ultimate goal in histocompatibility testing should be the definition of all important antigens. Important antigens, in this regard, are those for which incompatibility will lead to graft rejection even when immunosuppression or other methods are used to obtain graft survival. Typing procedures can be used to find a donor who has no antigens foreign to the prospective recipient or, less ideally, who has a minimum number of foreign antigens. Two problems arise in this regard. First, two individuals who are judged compatible by typing tests may nonetheless be incompatible for donor antigens not detectable with available antiserums. Second, when incompatibilities exist, neither the "strength" of such antigens nor the relation of strength to the HL-A phenotype of the recipient is known. In the ABO system the "incompatibility strength" of the A antigen varies with the ABO phenotype of the recipient (*50*). A similar situation may obtain in the HL-A system (*41*).

We have presented evidence that cells of unrelated individuals who type identically still stimulate in MLC tests, suggesting that not all antigens of HL-A are yet defined. Whereas stimulation in MLC tests may in some instances reflect minor loci differences, this seems unlikely—at least with any appreciable frequency. Sorensen (*51*) has tested 13 unrelated pairs who were phenotypically identical for the first two segregant series. In 11 of these he found significant stimulation; in the other two he found stimulation in one direction but insignificant stimulation above the control in the other direction. In these last two cases, while the stimulation was not significantly above the control, it was greater than Sorensen has seen in his MLC identical sibling pairs; further, all these mixtures were tested at only one concentration of stimulating cells. In collaboration with Terasaki and his colleagues, we have done similar studies in unrelated pairs (*52*). In some pairs all four antigens of the first and second segregant series were detected; in others, only one antigen of each series. The majority of pairs typing identically did show stimulation, although in some cases there appeared to be zero stimulation (*23*). When the four antigens were defined, there was a correlation between identity of typing and low stimulation. This was not true when only one antigen of each series was defined. Thus, while typing may leave undefined antigens strong enough to cause stimulation in MLC, characterization of a sufficient number of antigens does correlate with MLC compatibility.

Considering only the first two segregant series, the average probability of finding two unrelated individuals identical for those series is less than one per hundred. Considering the possible third and fourth segregant series this frequency will diminish still further. For the logistics of getting an organ to a given recipient, it would be desirable to keep to a minimum the necessary number of recipients in a pool so that any organ which becomes available can go to an acceptable recipient. Kidney transplants can survive for several years with some antigenic mismatches: it thus becomes essential to learn which antigenic mismatches in which combinations are weak enough to permit prolonged organ survival. This information can be obtained by analyzing organ graft survival in donor-recipient combinations with known antigenic phenotypes or by studying the strength of antigens in the MLC test which appears to give a measure of the amount of antigenic disparity at HL-A.

I have previously proposed that one way to approach these problems is the following. If all HL-A alleles in the population determine antigens which represent strong incompatibilities for any recipient, and most unrelated individuals will differ by two alleles, all such individuals will be very poor donors and represent close to 100 percent incompatibility (the worst incompatibility that could be found in the population). We can use 15 unrelated individuals as a sample of the population. If 15 unrelated, stimulating cell types are individually tested with the responding cells of a potential recipient, the average stimulation of the 15 may give an estimate of the average incompatibility that will be found in the population. This average can be considered as a "standard of incompatibility." A given donor-recipient match can be expressed as a percentage of the standard.

If complete compatibility at HL-A is not necessary for sucessful organ transplantation, and there is a simple relation between varying degrees of incompatibility and organ graft survival, results obtained when the above ap-

proach is used may tell us the degree of incompatibility which can be tolerated, and how frequently we will find an unrelated individual who falls into the acceptable incompatibility range. We have now done 17 such experiments. In Fig. 2 is given a composite curve for all 17 experiments done in this manner in which the mean stimulation in each experiment is equated to 100 and all values of stimulation obtained with cells in that experiment are plotted as a percentage of the mean. Obviously, in terms of stimulation in MLC tests, HL-A alleles vary greatly in terms of their antigenic strength and some unrelated pairs stimulate very little compared to our standard (*53*).

Two questions we must ask ourselves are whether there is a relatively simple relation between the degree of HL-A incompatibility and satisfactory graft survival and, if there is, how much incompatibility can be tolerated. With the frequency distribution shown in Fig. 2 one might be optimistic since some degree of HL-A disparity in certain cases can be tolerated, so that, even if the amount of permissible disparity is rather small, suitable donors could be found in some appreciable frequency compared to the situation if the frequency distribution curve had been skewed in the other direction, that is, with most alleles being relatively strong. This curve also suggests that the concept of a "one allele different" donor is not of itself quantitatively meaningful except within a family. In terms of HL-A disparity, a two-allele different unrelated donor could be a better donor than a one-allele different related donor.

Both typing results and MLC tests suggest that a very large number of HL-A alleles exist in the population. One of the results of the Third International Histocompatibility Workshop was that, of 44 alleles (22 parents) investigated, no two were identical. The total number of alleles in the system is thus quite large and the frequency of occurrence of each must be extremely low. The results of MLC testing give additional information: in our experience out of more than 600 unrelated pairs of individuals, every pair showed stimulation and therefore, presumably, antigenic disparity at the HL-A system. Since cells of all unrelated individuals examined so far stimulate in MLC tests, this suggests that if there are HL-A antigens which cannot cause stimulation in MLC tests, then the frequency of alleles determining only such antigens is low. These findings are in disagreement with the statement of van Rood and Eernisse (*9*) that some alleles are found in high enough frequency (up to 13 percent) to allow identity at the HL-A system in parent-child combinations and even unrelated pairs. Terasaki's (*54*) estimate of several nonrelated identical pairs also seems unlikely in view of the above findings. Apparent identity under such circumstances could be regarded as an artifact caused by the limited number of antiserums.

It may be that all the important HL-A antigens, as defined above, could be measured if all the available antiserums were used. We must, however, be careful to distinguish between genetic identity at HL-A as exists in sibs inheriting the same parental chromosomes, effective identity as defined by MLC tests, serotypic identity as defined by a limited number of antiserums, and identity for the important antigens—a concept of purely theoretical nature at the present time.

Summary

In man, as in the mouse, rat, and chicken, there is a single genetic system, HL-A, which appears to control the strong transplantation antigens and compatibility that influence graft survival. In addition, compatibility for the ABO blood group system seems essential. The HL-A system is very polymorphic, with more than 20 alleles in the population. Donor-recipient pairing is accomplished either by typing tests in which antiserums are used to define specific HL-A antigens, and in which attempts at pairing are made to minimize antigenic incompatibility, or by mixed leukocyte culture tests in which peripheral blood leukocytes of donor and recipient are mixed in vitro to measure the amount of antigenic disparity at HL-A without specifying the specific antigens responsible for the incompatibility.

Not all antigens of HL-A are defined with the antiserums available in any one laboratory, thus phenotypic identity for the antigens does not insure genotypic identity; very little is known about the incompatibility strength of different antigens, which makes it difficult to evaluate the degree of incompatibility when there are one or more antigen mismatches. Consonant with these considerations, while typing is useful when applied to siblings in whom a simplified genetic situation obtains, the evidence establishing typing as useful for parent-child or unrelated combinations is much weaker. Kidneys from some donors who type very similarly to the recipients are rejected—a circumstance that could be due to mismatches for antigens not measured with the available antiserums; some kidneys that present several antigenic incompatibilities to the recipient do well—a circumstance that could be explained if those antigens are weak in that particular combination.

Results of mixed leukocyte culture tests give a definition of HL-A identity (nonstimulation) as well as meaningful quantitative data on the amount of HL-A disparity. These tests however take 3 days to perform and thus are useful prospectively in only living donors or rare cadaveric donors.

Some kidneys function well despite an HL-A mismatch; however, we do not know how much HL-A incompatibility is consistent with graft survival. It is by a combination of the above methods that we can hope to define which antigens are strong in which combinations, how much HL-A incompatibility can be tolerated without compromising graft function, and how frequently we will find an unrelated donor with an acceptable amount of HL-A incompatibility.

References and Notes

1. I thank Dr. D. Kayhoe for this information.
2. I use the term "pairing" to describe the general problem of picking a donor for a given recipient. "Typing" and "matching" refer to the individual procedures described. "Allogeneic" refers to individuals of the same species who are genetically different; "syngeneic" to members of the same inbred strains; "autologous" to tissue from the same individual. "System" is defined as the unit of closely linked genetic information that cannot be separated into subunits at the level of resolution allowed by formal human genetics [*World Health Organ. Bull.* **39** (1968), p. 483]. "Allele" refers to alternative forms of the genetic material of a system associated with one chromosome and is thus used simply in a transmissional sense.
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Growth versus the Quality of Life

Our widespread acceptance of unlimited growth is not suited to survival on a finite planet.

J. Alan Wagar

In economics, as in most other matters, past experience provides a major basis for current decisions, even though changing circumstances may have diminished the appropriateness of such experience. Such use of "conventional wisdom" may explain our continuing emphasis on economic and other types of growth despite the many problems created by such growth.

When the United States was sparsely populated, emphasis on growth made good sense. Growth of many kinds permitted exploitation of the rich environment at an accelerating rate and provided a phenomenal increase in wealth.

Growth still increases material wealth but has a growing number of unfortunate side effects, as each of us tries to increase his own benefits within an increasingly crowded environment. These spillover effects, which were of minor importance when settlement was sparse and neighbors farther apart, are now of major consequence. For example, a firm may make the most money from a downtown tract of land by erecting a tall office building there. Construction of the building will add to the gross national product, and the builders will be hailed for their contribution to "progress." However, the building will add to traffic congestion, exhaust fumes, competition for parking, the need for new freeways, and social disorder. These problems, which must be handled by someone else, become part of the "environmental mess" or "urban crisis."

When this article was written, the author was leader of the Cooperative Recreation Research Unit maintained by the Intermountain Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, in cooperation with Utah State University, Logan. Since then, he has become leader of a similar unit maintained by the Pacific Northwest Forest and Range Experiment Station in cooperation with the University of Washington, Seattle.