Soviet Union for the 1969 Mars opportunity. If any mission is planned for 1969, its development must by now be well along. The impact of an unsterilized bus or of a superficially sterilized capsule could be a catastrophe for the biological exploration of Mars.

Uncertainty about sterilization also has serious consequences. If there is not a detailed exchange of knowledge concerning mission sterilization and procedures, then a wide range of estimates can be made about the probability that the planet is infected. This uncertainty, and pressure of other competition for resources, will lead to an estimate that will serve to minimize allocations for sterilization efforts.

It should be stressed that, to be effective, information exchange among space-faring nations must take place enough years before planetary missions to have a meaningful impact on design and planning. We hope that all our colleagues throughout the world will consider these issues carefully.

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Transplantation: Problems of Histocompatability Testing

Typing and matching tests are useful in pairing donors and recipients for organ transplantation.

Fritz H. Bach

Pairing (*1*) of donors and recipients for organ transplantation has been important in determining the success and survival of kidney allografts. Two methods are available to achieve pairing: "typing," in which specific histocompatibility antigens are detected with suitable isoimmune antiserums, and "matching," in which tests in vivo or in vitro, such as the mixed leukocyte culture test (*2*), are used to measure the degree of incompatibility between two individuals.

It is increasingly evident that results of typing are predictive of skin graft survival (*3*), and that they are correlated, at least retrospectively, with kidney graft survival (*4*). Although these correlations are very strong in sibling pairs, predictions are less reliable in unrelated pairs (*5*). Although less extensively studied, there is evidence that the mixed leukocyte culture test is also predictive of skin graft survival between siblings (*6*), and that it correlates, retrospectively, with kidney graft survival (*7*).

I will discuss the genetic reasons that make predictions more simple in sibling pairs and an approach to the problem of histocompatibility pairing.

The purpose of pairing tests is to achieve "compatibility." Complete compatibility exists when donor and recipient tissues have identical histocompatibility antigens or when donor tissue has no foreign histocompatibility antigens (antigens which the recipient does not also have). Incompatibility exists if the donor has some antigens foreign to the recipient—the degree depending on the number and relative "incompatibility strengths" of such antigens.

Recent evidence (*8*) confirms earlier suggestions (*9*) that most of the human leukocyte antigens measured by antiserums now available are determined by alleles of a single genetic system, *HL-A*. A single polymorphic genetic locus also controls reactivity in mixed leukocyte cultures (*10*). Comparison of results obtained by such tests with those of leukocyte typing suggests that the locus controlling reactivity in mixed leukocyte cultures is the same one which determines the leukocyte antigens. We have proposed that this is the major histocompatibility locus in man (*11*). An undetermined number of "minor" loci undoubtedly exist, each determining histocompatibility antigens which, however, probably represent weaker incompatibility barriers.

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A major histocompatibility locus has also been defined in the mouse (*H-2*), the rat (*Ag-B*), and the chicken (*B*). The concept of a single major locus strongly influencing graft survival has developed because: (i) rejection of skin grafts occurs in 8 to 12 days in the vast majority of cases in which there is a difference at the major histocompatibility locus, whereas survival for much longer periods of time is possible when two animals are identical at this locus even though they may differ at any one or more of the minor loci, and (ii) it is more difficult to achieve either effective immunosuppression or tolerance when two animals differ at the major locus than when they differ at even several minor loci. In mixed cultures of allogeneic peripheral blood leukocytes, the cells react only when the two individuals differ at the major locus, but not when they differ only at minor loci (see *12*).

Based on analogy with other animals and from what is known directly in man, pairing for the *HL-A* system is of the utmost practical importance for graft survival, and I will concern myself only with this system.

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. It is very probable that not all antigens of *HL-A* are yet defined. Second, even in cases where the donor is known to carry some antigens foreign to the recipient, neither the "strength" of such antigens, nor the relationship of strength to the histocompatibility genotype of the recipient is known. [It has been demonstrated in the ABO system, that the "incompatibility strength" of the A antigen varies with the ABO genotype of the recipient (*13*).]

Why can typing procedures predict skin graft survival between siblings but only rarely between unrelated individuals? If there is only a single locus, no matter how many alleles there are in the population, two parents can have at most four different alleles and there is a maximum of four genotypes for the siblings. If we designate the father's alleles *a* and *b* and the mother's alleles *c* and *d*, then the siblings can be *ac, ad, bc*, and *bd*. In the mouse, each *H-2* allele determines several antigens, some of which may be identical with those determined by other alleles. If we assume that this is the case in man, we need not define all the antigens in a sibship to find those siblings who have inherited the same two alleles from their parents. This is because we know that each sibling receives one allele from each parent. If we have a single antiserum that differentiates the two alleles of a single parent, we will be able to say unambiguously (barring crossing-over) which allele that parent contributed to a given offspring, regardless of how many antigens are specified by that allele. Hence, with two antiserums, one differentiating the two alleles of the father and the other those of the mother, we can completely define the genotypes of the offspring with respect to that locus. For instance, if allele *a* codes for antigens 1, 2, 3, and 4; allele *b* codes for antigens 1, 2, 5, and 6; allele *c* codes for antigens 1, 3, 7, and 8; and allele *d* codes for antigens 2, 4, 7, and 9; then antiserums for antigens 5 and 9 (as one possible pair) will permit us to detect the differences between siblings inheriting the *ac, ad, bc,* or *bd* allelic combinations. Thus any two siblings not showing a difference with these two antiserums will be identical at *HL-A*.

When we deal with a parent and child, or with unrelated pairs, this is no longer true. Although parent and child of necessity share one allele, the second allele is "unrelated." Even if the antigens determined by these unrelated alleles include some that are the same, those which are different will not be detected in the absence of suitable antiserums. The same is true, a fortiori, for unrelated pairs, for in such cases both alleles are unrelated. Thus a failure to demonstrate antigenic differences between donor and recipient does not guarantee compatibility.

In mixed leukocyte cultures, peripheral blood leukocytes of the prospective recipient are mixed in vitro with donor leukocytes treated with mitomycin C, and the response of leukocytes of the recipient to the donor's treated stimulating cells is measured as a function of the incorporation of radioactive thymidine into the cells of the recipient (*14*). Since the assay for stimulation is incorporation of radioactive thymidine, the treated leukocytes (which cannot synthesize DNA) cannot contribute to the assay. Treated cells can, however, stimulate allogeneic cells to respond (incorporate radioactive label). Some test combinations do not result in stimulation. Whereas nonstimulation occurs in approximately 30 percent of sibling mixtures, it has not been found in more than 300 unrelated mixtures. With alleles of equal frequency the proportion of matches is $2/n^2$ (n is the number of alleles), the rare possibility of a homozygous donor who shares an allele with the recipient being ignored. The probability of no matches in 300 trials is then, $P = (1 - 2/n^2)^{300}$. If we solve for P equal to .05, we obtain the minimum estimate of n equals 15 with 95 percent "confidence." This is also minimum for another reason; any inequality in gene frequency will cause the number to be underestimated (*15*). On this basis we have suggested that a single locus with 15 or more different alleles controls reactivity in mixed leukocyte cultures (*11*).

Since stimulation is associated with incompatibility for antigens of the *HL-A* system, it seems unlikely that there are many alleles in the population which determine antigens not detectable in mixed leukocyte cultures—otherwise we would find some unrelated matches which do not stimulate. We, therefore, consider reciprocal nonstimulation between two individuals as a manifestation of "effective identity" at *HL-A*.

Different degrees of stimulation apparently reflect "degrees of incompatibility" at the *HL-A* system (*16*). Siblings and parent-child combinations in which it was known from leukocyte typing whether responder and stimulator differed by one allele or by two alleles at *HL-A* have been studied (*16*). Within such a family any person differing from the responding sibling by both alleles should exhibit greater immunogenetic incompatibility than if he differed from that responder by only one allele. This prediction has been confirmed in 16 tests in five different families with only one exception in one experiment. In that case, the leukocytes of a parent, whose cells always stimulated the responder to a great extent, stimulated the cells of that responder slightly more than did the leukocytes of another child differing from the responder by two alleles. Although it cannot simply be assumed that stimulation between unrelated individuals will meaningfully reflect degrees of incompatibility at *HL-A*, the general finding that the majority of unrelated individuals stimulate more than parent-offspring combinations and generally in the range of stimulation seen between siblings differing by two alleles suggests

the validity of testing unrelated persons in this way.

What then are the questions which confront us? First, we must know whether complete compatibility at *HL-A* is necessary for long-term survival of transplanted organs, and second, if complete compatibility is not necessary, what degree of incompatibility can be tolerated. To answer these questions we must obtain antiserums to measure all the antigens determined by *HL-A* is necessary for long-term sursought in multiparous individuals as well as in subjects who have been intentionally immunized. Even when this is done there will still be the problem of answering the second question posed above.

The pairing procedure used in the kidney transplantation program at the University of Wisconsin illustrates an approach to these questions and emphasizes the importance of performing both matching and typing tests in any such program.

Cells of potential donors in the family of the recipient are typed and tested in mixed leukocyte culture. Only those subjects judged medically and psychologically fit are considered as potential donors. When a donor is found who has identical alleles, as determined in mixed leukocyte cultures, that individual is used. When such a donor does not exist, the donor whose cells stimulate those of the recipient the least is chosen. The amount of incompatibility manifested in that donor-recipient combination tells little, however, about the degree of incompatibility which this represents with respect to some general standard of incompatibility. Therefore, after the related donor is chosen, his cells are tested as stimulating cells against the cells of the recipient along with 15 sets of unrelated stimulating cells.

If all *HL-A* alleles in the population determine antigens which represent strong incompatibilities for any recipient and most unrelated individuals differ by two alleles, such individuals will be very poor donors and represent close to 100 percent incompatibility (the worst incompatibility that could be found in the population). A frequency distribution curve of degrees of incompatibility in such a population would be skewed toward great incompatibility (arbitrarily the right). If there are "weak alleles" determining only antigens which are weak incompatibility barriers, then the curve may be symmetrical or even skewed to the left. Whatever the shape of the curve (which can be empirically determined), if 15 sets of unrelated cells are tested with cells of the potential donor, the strongest stimulator of the 15 will give an estimate of the "effective" maximum incompatibility which will be found in the population. The unrelated individual that stimulates the most can be considered as the "standard of incompatibility," and the related donor-recipient match can be expressed as a percentage of the standard.

If complete compatibility at *HL-A* is not necessary for successful organ transplantation, results with the above approach should 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.

With very rare exceptions, it has been impossible to pair unrelated individuals by typing tests, probably for the reasons stated above. It is, therefore, difficult at the present time to speak of having "good" and "poor" pairing between unrelated individuals, since one can never be certain that important but undetected antigens are not present. Clearly progress is being made in effecting pairing between unrelated individuals, and pairing based on typing and matching tests should be continued. However, all this should be done with the limitations of present methods being recognized and with efforts being made to obtain the maximum amount of information possible from each transplant. In the case of transplants from cadavers, this probably means that all available histocompatibility studies should be done at the time of transplantation, and that leukocytes from the donor should be stored in liquid nitrogen so that they can be retyped as more antigens are defined.

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