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Ranks of Donor-Recipient Histocompatibility for Human Transplantation

Abstract. *Correlation of leukocyte groups with skin and renal allograft survival indicates that ranks of histocompatibility based upon current genetic concepts of the HL-A system may provide an approach to the selection of optimally compatible subjects for clinical organ transplantation. Such ranks may be expressed as a net histocompatibility ratio (NHR) between prospective donors and recipients. The best clinical results have been when this ratio is of 0.5 to 1. Donor-recipient compatibility situations where the ratio was 0.25 or less have been associated with a high incidence of transplant failure, regardless of whether the organ source was a living, related donor or a cadaver donor.*

As a result of genetic studies of the HL-A system of histocompatibility two subloci have been isolated in the HL-A region of the human chromosome; the antigens HL-A1, HL-A2, HL-A3, HL-A9, Da 15, and Da 17 being determined at the first sublocus, and antigens Da 4, HL-A5, HL-A7, and HL-A8 at the second sublocus (*1*). Four additional HL-A antigens [Da 6, Da 9 (*2*), Da 18, HN] may be included on the second sublocus (*3*). The antigens determined at each of the subloci occur as alternative alleles. It is also probable that a large majority of the HL-A antigens capable of conditioning the survival of human organ transplants is included in these two subloci (*4*).

Calculations based on donor-recipient compatibility for the ABO erythrocyte group antigens and the HL-A antigens listed above indicate that a minimum of 500 prospective recipients may be required in order to give a cadaver organ donor a 95 percent chance of being transplanted to a serologically compatible host. Terasaki has indicated that such a waiting list might actually be as high as 1000 subjects (*5*). As progress is made in the isolation of additional HL-A alleles, the size of such a waiting list, if based upon this type of calculation, will inevitably grow in direct proportion to increases in the number of known alleles. Concurrent advances in our understanding of the genetic determinants of the HL-A system may, however, provide for an alternative approach to the selection of donors. Such an approach is based upon the notion that donor-recipient antigenic identities at the two known HL-A subloci are of the greatest importance in conditioning allograft survival. The results of experimental (*6*) and clinical transplantation studies (*7*) support this concept.

During these studies an attempt was made to correlate the survival time of skin allografts from sibling to father with the HL-A genotypes of donor and recipient. The actual ratio of antigenic identities (ID) to incompatibilities (IN) at each of the known HL-A subloci may provide a particularly reliable statistical index of compatibility between donor and recipient (*3*). In an extension of this series to 135 skin allografts from sibling to father, 33 transplants survived for 13.5 days or more (group A), and 27 grafts were rejected on or before day 10 after operation day (group B). In 30 of the 33 grafts (90 percent) in group A, the number of antigenic identities between donor and recipient was as great or greater than the number of incompatibilities at each of the two known HL-A subloci. In contrast, 17 of 27 (63 percent) of the grafts in group B were applied under circumstances where the number of incompatibilities exceeded the number of identities. Genotyping of the subjects in group A has indicated subsequently that 42 percent of the genes at the HL-A subloci in these subjects governed antigens which constituted incompatibilities between donor and recipient, and 36 percent of such genes provided for donor-recipient identities. In contrast, similar genotype studies in group B indicated that 77 percent of the genes at the two HL-A subloci determined antigenic incompatibilities, and only 3 percent of the genes provided for donor-recipient identities. The number of donor-recipient identities was therefore 12 times greater in group A (that is, the grafts accorded the longest survival times) than in group B (the grafts which underwent early rejection). These studies have also yielded evidence that the currently known HL-A antigens may be roughly equipotential in their immunogenicity (*4*).

Review of the observed ratio of identities to incompatibilities (ID/IN) in donors and recipients of 76 renal transplants obtained from donors that were living and related and from cadaver donors (*8*) has yielded results that paral-

Table 1. Calculation of net histocompatibility ratios (NHR) for human transplantation. Donor and recipient are compared at two known HL-A subloci.

Situation 1	Situation 2
All antigens determined by four genes at the two subloci have been detected	Not all of the antigens determined at each of the four genes at the two subloci have been determined
a) Four antigens have been detected in each subject	a) Unknown antigens not yet detected at that sublocus
or	*or*
b) Less than four antigens have been recognized but the subjects are homozygous at the sublocus in question on the basis of family studies	b) Less than four antigens have been detected in a subject where homozygosity at any one given sublocus cannot be confirmed by family studies
There are clear-cut identities (ID) and incompatibilities (IN) at each sublocus	There may be some clear-cut identities (ID) and incompatibilities (IN) at each sublocus
	But also
	Each PR situation may be either an ID or an IN between donor and recipient
NHR = ¼ × (total ID/total IN)	NHR = ¼ × (avg. of total ID/total IN) taking each PR into consideration as either an ID or an IN

Table 2. Possible net histocompatibility ratios (NHR) when all possibilities of donor-recipient antigen identities or incompatibilities at each sublocus are known (situation 1). NHR = ¼ (ID/IN).

Donor-recipient compatibility		Values	
ID	IN	(ID/IN)	NHR
4	0		1.00
3	1	3/1	0.75
2	2	2/2	0.25
1	3	1/3	0.08
0	4		0

lel those noted with skin allografts. Fifty of 56 (89 percent) renal allografts functioning uneventfully for at least 6 months after transplantation occurred in donor-recipient situations where the number of identities was equal to or greater than the number of incompatibilities at the two known HL-A subloci. In contrast, only 7 of 20 renal transplants (35 percent) that had undergone immunological rejection were performed under such conditions—that is, there were more incompatibilities than identities between donor and recipients in this group of subjects. These data suggested the potential of a numerical estimate or ratio of donor-recipient compatibility.

Each HL-A sublocus is theoretically capable of determining a variety of different serological relations in any given donor-recipient pair, resulting in a large number of possible combinations for the two subloci. The situation is complicated also by the probable existence of a number of still undetected alleles, each of which may constitute an identity or incompatibility. The possible degrees of donor-recipient compatibility could be expressed by a net histocompatibility ratio (NHR) based on identity (ID) or incompatibility (IN), or both, at each of the four genes of the two currently known HL-A subloci of donor and recipient. Two key assumptions are made. First, that the HL-A antigens are equipotential in their immunogenicity, and, second, that there are no inactive alleles in the HL-A system.

When two individuals are compared on the basis of HL-A phenotypes, a variety of possibilities may occur. In the first instance, illustrated in situation 1, Table 1, currently available typing serums permit detection of all of the antigens determined at the two HL-A subloci. Either four different antigens have been detected in each individual or, in those cases where less than four antigens were found, studies of families have shown that this particular subject was homozygous at one or both subloci. In such cases, the NHR would equal the total number of identities divided by the incompatibilities, or ID/IN. Donor-recipient compatibility may therefore occur in four different ways. In the first instance, the four HL-A antigens of donor and recipient are identical; the same degree of compatibility occurs when the donor is homozygous for an antigen present in the recipient, the second antigen governed by this sublocus being different (this is equivalent to an impossibility of incompatibility at that sublocus and is considered as an identity). In this instance, the "gross ratio" is defined as 4. In order to convert this value to a range of zero to unity, it is divided by 4, yielding a value of 1 for donor-recipient situations of full compatibility. In the second instance, three antigens may be identical and one is incompatible. In the third instance, there may be two identities and two incompatibilities. Finally, there may be only one identity and three incompatibilities. In these three cases, the NHR will be 0.75, 0.25, and 0.08, respectively. In those cases where there is no actual or potential situation of identity between donor and recipient, that is, where there is zero compatibility, the NHR equals zero.

Calculation of the NHR provides an approach to the problem created by the fact that it has not yet been possible to identify all of the alleles determined by the two HL-A subloci. As a result, an individual may not have any detectable antigens at one sublocus, possibly leading to one, or even two, donor-recipient incompatibilities at that sublocus. In other cases, if only one antigen is detected at a sublocus and family studies are not feasible, it may not be clear whether this situation constitutes homozygosity at this sublocus or whether it is due to the presence of a second unknown allele at this sublocus. This type of situation may potentially constitute either an identity or an incompatibility. The present calculations are based upon the hypothesis that such a potential relation (PR) between donor and recipient may have as many chances of being an identity as an incompatibility. The NHR will therefore represent the average of the individual NHR's, as calculated on the basis of each of the possibilities of identity or of incompatibility for this undetected antigen.

Table 3. Ranks of donor-recipient compatibility (DRC) for organ transplantations based upon net histocompatibility ratios (NHR).

Rank	DRC from comparison of HL-A antigens at two subloci*			NHR
	ID	IN	PR	
	Current acceptable donor-recipient compatibility (NHR = 0.50 to 1)			
1	4	0	0	1.00
2	3	0	1	0.88
3	3	1	0	0.75
4	2	0	2	0.66
5	1	0	3	0.53
6	2	1	1	0.50
	Contraindication to transplantation (NHR = 0 to 0.36)			
7	1	1	2	0.36
8	0	1	3	0.27
9	2	2	0	0.25
10	1	2	1	0.17
11	0	2	2	0.11
12	1	3	0	0.08
13	0	3	1	0.04
14	0	4	0	0

* A theoretical situation which has not yet been encountered and is not listed here is: 0 ID–0 IN and 4 PR.

The validity of this calculation is supported to a certain extent by the fact that the sum of the known gene frequencies at each sublocus has been found to be 0.70 and 0.80, respectively (*3*). One exception to the interpretation of the observed serological results is the specific situation where the recipient is positive for two known antigens at one sublocus, while the donor has no detectable alleles at that same sublocus. Here, in view of the concept that inactive antigens do not occur in the HL-A system, at least one of the two potential situations is likely to constitute an incompatibility, and the formula employed for calculation of donor-recipient compatibility at that sublocus should be 1 IN and 1 PR, and not 2 PR, since one of the two situations is an incompatibility.

The proposed calculation of NHR's implies that there may be only a comparatively small number of unknown antigens at the two HL-A subloci. Indeed, if the number of such alleles were much larger, they would be more likely to constitute incompatibilities, rather than identities. The NHR therefore gives such situations less weight than they might have if a large number of unknown alleles actually did exist at each sublocus. It must be emphasized that the proposed evaluation of donor-recipient compatibility is based on our present knowledge and is intended to facilitate application to the clinical situation. The proposed formula is likely to gain increasing validity as the number

of potential or unknown situations dwindles, and it has the virtue of remaining applicable as additional subloci of the HL-A system become known. For example, if three subloci governing equipotential antigens were to be considered, the NHR value would be the ratio ID/IN divided by 6 (2 alleles at each sublocus); with four subloci, it would be divided by 8.

Table 3 summarizes the degrees of compatibility (NHR values) possible between donor and recipient. Studies designed to correlate ranks of histocompatibility with the observed results of renal transplantation indicate that the most favorable results occur within NHR values of 0.5 to 1; these situations were found most frequently in transplants from a living, related donor. Fair results have been obtained with NHR values of 0.27; in contrast, donor-recipient compatibility situations where the NHR was less than 0.25 have regularly been associated with a high incidence of renal transplant failure, regardless of whether the organ was from a living, related donor or from a cadaver donor (*8*).

Although the NHR is evidently a simplification of an extremely complex situation, it may be useful as a statistical guide for the rapid choice of optimum organ donors and recipients within a given population. However, the successful use of NHR values is predicated on the determination of the individuals' HL-A phenotypes by laboratories with the serological capability of detecting all of the alleles known at the two HL-A subloci (*9*). It is only under such conditions that the sum of the *known* gene frequencies at each sublocus (0.70 and 0.80, respectively) can be invoked to support the validity of the proposed calculations. This validity will increase in direct proportion to the number of new HL-A alleles detected in the future.

Note added in proof: Recent observations suggest that the term "subloci" might better be substituted by "loci" of the HL-A system.

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Drosophila melanogaster: Identity of Male Lipid in Reproductive System

Abstract. *A lipid found exclusively in the ejaculatory bulb of adult male* Drosophila melanogaster *has been identified as* cis-*vaccenyl acetate. Identification is based on spectral comparisons with a synthetic sample.*

A new lipid that was detected in the ejaculatory bulb of adult male *Drosophila* (*1*) has been identified.

Gram quantities of male and female *D. melanogaster* (7 days old) were extracted with a mixture of chloroform and methanol (2:1). The crude lipid extract was freed of more polar components by column chromatography on silica gel. The fraction containing the lipid was eluted with a mixture of hexane, diethyl ether, and acetic acid (85:15:1). Further purification was achieved with preparative thin-layer chromatography on silica gel G. The male lipid was finally isolated by gas chromatography (1.5-m column packed with 15 percent diethyleneglycol succinate on Chromosorb W). The lipid is the major component of this fraction and is chromatographically identical with material from isolated tissue.

The mass spectrum indicates that the lipid has a molecular weight of 310. High-resolution mass spectrometry gave the elemental formula $C_{20}H_{38}O_2$ for the molecular ion. The major peak in the high mass region is an ion at mass to charge (*m/e*) 250 (M-60), suggesting loss of the fragment CH_3COOH. The relatively simple infrared spectrum shows an ester carbonyl absorption at 1750 cm^{-1} and a weak band at 724 cm^{-1}, suggesting the presence of a *cis* double bond. The nuclear magnetic resonance (NMR) spectrum showed a triplet at 4.80 τ, and another at 6.10 τ, each corresponding to two protons. These absorptions would correspond respectively to the two vinyl protons of the double bond and two protons on the carbon bearing the acetoxy group. In addition, there is a sharp singlet for the methyl protons of the acetoxy group at 8.22 τ and the terminal methyl protons appear at 9.17 τ.

These data suggest that the lipid is the acetate of an unsaturated C_{18} alcohol. To establish the position of the double bond, the ester was converted to the corresponding diol with OsO_4. This diol was then converted to the ditrimethylsilyl ester, which was analyzed by mass spectrometry (*2*). The major fragments appeared at *m/e* 187 and 301, which would place the double bond between carbon atoms 11 and 12 of the alcohol chain. The final structure would then correspond to *cis*-vaccenyl acetate [*cis*-$CH_3(CH_2)_5CH = CH(CH_2)_{10}OCOCH_3$], which is present in one other insect source, the hair pencil secretion of the male butterfly *Lycorea ceres ceres* (*3*). A synthetic specimen was accordingly prepared from *cis*-vaccenic acid by methylation, reduction, and acetylation (*3*). The synthetic material proved identical to the male lipid on infrared, NMR, and mass spectral comparison, and showed an identical retention time on gas-liquid chromatography.

The physiological function of this lipid is not yet established. Since the lipid is transferred to females during mating (*1*), its function may involve some aspect of reproduction.

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