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Research Article

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A MODEL SYSTEM FOR DETERMINING HISTOCOMPATIBILITY IN MAN *

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Of the many factors that may be significant in permitting successful homotransplantation of tissue and organs, there is little question that the genetic relationship of the donor to the recipient will always be of prime importance. In the laboratory animal, there are many methods of producing permanent or markedly prolonged survival of homografts when major histocompatibility gene loci are the same. In every instance, however, when there is a major genetic incompatibility between donor and recipient, a much more intensive attack is required to accomplish any prolongation of homograft survival.

In the human, this is equally true. Where sublethal irradiation and uremia are the two major variables affecting homografts, the only permanently successful kidney transplant that we have been able to perform has been between nonidentical twins of the same sex (1). These men had 26 identical blood groups, and there was a 26-day survival of skin on the healthy donor from the sick recipient. There is little doubt that the inherent compatibility in this donor-recipient pair was as significant for success as the other maneuvers that were carried out. We believe that a constant search for methods of assessing histocompatibility in humans must accompany the extensive efforts to permit homograft survival in the animal.

Working with normal human volunteers over the past few years, our clinic has demonstrated that *a*) skin homograft rejection will induce a delayed intradermal sensitivity not only for the donor leukocytes, but also for nonspecific leukocytes (2); *b*) preimmunization with peripheral leukocytes from a single donor would permit "accelerated" and even "white-graft" rejections of skin from nonspecific donors (3); and *c*) uniform production of "white grafts" could be accomplished if successive grafts between the same donor-recipient pair were placed at 14-day intervals (4). Many other investigators, notably Rapaport and Converse (5, 6), have provided basic information necessary for this study.

We have attempted to analyze a given donor-recipient pair for any evidence of compatibility based on the concepts above. The tissue antigens that the donor-recipient pair share are qualitatively measured by evaluating the degree of sensitization that a skin graft from one member of the pair will provoke in an indifferent individual previously immunized against the other member of this pair. Since this is a search for any evidence of "closeness" between people who are obviously different and in whom immunosuppressive treatment would be necessary for any sort of prolonged homograft survival, the use of an indifferent recipient is essential, as cross-grafting between the two members of the donor-recipient pair in either direction is of little use. Skin transplanted from the donor to the intended recipient would sensitize this individual so that even minor degrees of incompatibility would be exaggerated, while grafts from the recipient to the intended donor would only reveal the major incompatibility factors and eliminate the usefulness of the study. Thus, for example, if two individuals have in their complement of transplantation antigens several that are shared and many others that are completely unrelated, it is probable that when a graft between these two is placed, the usual first-set rejection will result, and no evidence of the shared antigens will be apparent. With a third party as an indifferent

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recipient, only the shared antigens of the donor-recipient pair will influence the rejection pattern of the second graft, since exposure of this third party to antigens from one member of the pair will set up a state of immunity that will influence the pattern of rejection of skin from the second. Complete genetic similarity of the donor-recipient pair (same individual) (4, 6) will produce a "white-graft" rejection in the second graft. It is to be expected that identical twins will do the same, and that with a lesser degree of similarity, an "accelerated" rejection would result. Predictably, there will be many gradations in the severity of the "accelerated" rejection. If there is no antigenic overlap in the pair of individuals being studied, a "first-set" rejection should be produced.

MATERIALS AND METHODS

Donor-recipient pairs of several different combinations were chosen to explore the validity of such a model system. Circular, full-thickness skin grafts measuring about 2 to 2.5 cm in diameter were used on all occasions. All were carried out under local anesthesia by the same surgeon, and the grafts were meticulously sewn in place with interrupted sutures of 5-0 nylon or silk. A stent of fine mesh gauze was used as a pressure dressing for 6 days to provide maximal protection for the grafts. There were no surgical failures. The donor skin was always obtained from the inner side of the upper arm, and the donor site was closed primarily after undermining the skin edges. The grafts were usually placed on the forearm of the indifferent recipient, although occasionally the upper arm was utilized. The three individuals involved were the donor-recipient pair being evaluated and a third party referred to as the indifferent recipient. In Figure 1, Donor A and Donor B represent the two members of the donor-recipient pair. It is the genetic closeness of the two individuals comprising the donor-recipient pair that is being evaluated by this test system. On day 1, skin was grafted from one member of the pair (the intended recipient in all kidney transplant cases) to the indifferent recipient, and on day 15, a skin graft was placed on the indifferent recipient from the other member of the pair. The second graft was placed adjacent to the first graft on all occasions except one, when it was placed on the opposite arm.

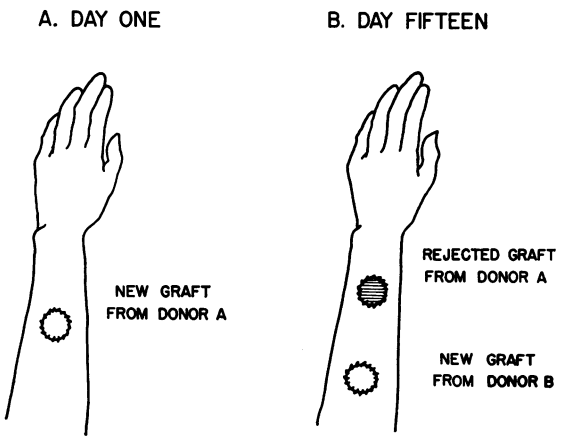

FIG. 1. EXAMPLE OF A DONOR-RECIPIENT PAIR. On day 1, the indifferent recipient receives a homograft from one member of the donor-recipient pair (Donor A). Two weeks later, a graft is placed from the second member of the pair (Donor B) to the same indifferent recipient. The type of rejection that the second graft exhibits provides the information as to the genetic relationship between the two members of the donor-recipient pair.

Full-thickness biopsies were taken of the first graft between 7 and 14 days. A biopsy was always taken of the second graft on day 6, and it was on the basis of this biopsy and of the gross appearance of the graft that the decision was made as to the type of homograft rejection. The criteria for a gross and microscopic determination of "white-graft," "accelerated," and "first-set" rejection have been adequately described (7).

Obviously, the antigenic configuration of the indifferent recipient is a major factor; the closer the antigenic resemblance to the donor, the greater the discriminatory power. Consanguinity also increases the likelihood of greater antigenic compatibility. We have taken both of these factors into consideration in our choice of the indifferent recipients. Of the 20 pairs of grafts we have placed, five were to a parent of one or both of the donor-recipient pair, with varying degrees of consanguinity within the donor-recipient pair. Results obtained with seven pairs of identical twins, three pairs of dizygotic twins, two pairs of siblings, five pairs of parent-child relationships, and three pairs of unrelated individuals will be reviewed.

In most instances, grafts were also placed from one member to the other of the donor-recipient pair (Table I). Two of these donor-recipient grafts (no. 12 and 15) could not be included in this presentation because the recipient was being treated with chemotherapeutic agents, and the criteria for graft rejection were not felt to be comparable.

In this series of patients, all but one of the identical twin donor pairs and pairs 18, 19, and 20 in Table I were donor and recipient of renal homografts. Table II demonstrates how this study has been adapted as a method of selecting the best possible donor of a kidney for a given sick recipient from among individuals already screened by other parameters. The indifferent recipient was immunized by the potential recipient of the renal homograft, and 2 weeks later, skin grafts from the selected donors were placed on the indifferent recipient as described above. Differential rejection patterns were determined by gross and microscopic study of these grafts 6 days later. The patients in Table II are an additional group to those in Table I.

TABLE I

Rejection patterns of skin homografts from the second member of each donor pair 6 days after their placement on the indifferent recipient

Donors	Sex	Donors' genetic relation	Recipient's genetic relation	Rejection type		Interdonor graft survival
				Gross study	Microscopic study	
1. J.A.	F	Ident. twins				
J.A.	F		Father	White-graft	White-graft	Permanent
2. J.A.	F	Ident. twins				
J.A.	F		Mother	White-graft	White-graft	Permanent
3. R.F.	M	Ident. twins				
R.F.	M		Father	White-graft	White-graft	Permanent
4. J.H.	M	Ident. twins				
L.H.	M		Unrelated	White-graft	White-graft	Permanent
5. F.F.	M	Ident. twins				
H.F.	M		Wife	White-graft	White-graft	Permanent
6. F.F.	M	Ident. twins				
H.F.	M		Nephew	White-graft	White-graft	Permanent
7. R.H.	M	Ident. twins				
J.H.	M		Unrelated	White-graft	White-graft	Permanent
8. J.H.	M	Unrelated				
E.M.	M		Unrelated	Accel.	Accel.	Not done
9. A.H.	M	Nonident. twins				
A.H.	F		Unrelated	Accel.	Accel.	16 days
10. M.B.	F	Nonident. twins				
M.A.	F		Unrelated	White-graft	White-graft	14 days
11. E.A.	F	Nonident. twins				
H.C.	M		Unrelated	1st-set	1st-set	7 days
12. M.B.	F	Mother-daughter				
L.Z.	F		Unrelated	White-graft	Very accel.	Not done
13. R.S.	F	Brother-sister				
C.J.	M		Unrelated	Accel.	Accel.	10 days
14. L.B.	F	Father-daughter				
L.B.	M		Mother	Accel.	Accel.	13 days
15. A.P.	M	Unrelated				
R.H.	M		Unrelated	Accel.	Accel.	Not done
16. A.M.	M	Father-son				
D.M.	M		Mother	Accel.	Accel.	Not done
17. A.M.	M	Father-son				
D.M.	M		Wife	Accel.	Accel.	Not done
18. E.M.	M	Brothers				
P.M.	M		Unrelated	White-graft	Very accel.	11 days
19. F.C.	F	Mother-son				
S.C.	M		Uncle	White-graft	Very accel.	18 days
20. C.R.	F	Unrelated				
J.C.	M		Wife	Accel.	Accel.	Not done

RESULTS

In Table I can be seen the results of the 20 pairs of skin grafts placed upon the indifferent recipients as well as the findings upon cross-grafting between the donors and recipients within the pair. The gross and microscopic evaluation of the grafts must enter into the final decision as to the type of rejection. The correlation between the clinical and histological grading of the test grafts has been excellent.

All pairs of identical twins produced "white grafts" as predicted. Pairs 1 and 2 were the same set of twins, but two different indifferent recipients were used. The grafts to their mother were

TABLE II

Three examples of the use of this skin grafting procedure to aid in the selection of a potential donor

Kidney recipient	Indifferent recipient	Potential donor	Sex	Relation	Rejcetion type	
					Gross study	Microscopic study
1. H.R.	Unrelated	R.R.	F	Wife	Slightly accel.	Slightly accel.
		L.R.*	M	Brother	Slightly accel.	Slightly accel.
		A.Z.	F	Unrelated	Slightly accel.	Slightly accel.
2. G.B.	Wife	D.L.*	M	Unrelated	Highly accel.	Highly accel.
		N.L.	F	Unrelated	1st-set	Slightly accel.
3. L.U.	Unrelated	E.U.*	M	Father	White-graft	White-graft
		H.U.	M	Uncle	Accel.	Accel.

* Donor chosen for transplantation.

placed side by side on the forearm; those to the father were placed on the opposite arm. Donor pairs 5 and 6 were also a single pair of identical twins with simultaneous grafts performed on two indifferent recipients. Since grafts from the primary recipient and the donor were rejected as "white grafts," it is concluded that the sex and varying antigenic configuration of the indifferent host do not affect the result and that the immunity of the sensitizing graft is systemic and not just a local phenomenon.

Of particular importance were donor pairs 7 and 8. These grafts were performed simultaneously with the same indifferent recipient, and the initial graft was from one of the identical twins. The second grafts then came from the other member of the twin pair (no. 7) and an unrelated donor (no. 8). The findings of a "white-graft" with pair 7 and an "accelerated" rejection with pair 8 attests to the discriminatory capabilities of this model system.

The findings noted in the three pairs of nonidentical twins are worthy of comment. Routine grafting from one twin to the other in the three pairs in no case demonstrated any significant prolongation of homograft survival; in fact, in pair 11 the rejection was definitely more rapid. This was corroborated by the findings of a "first-set" rejection when this pair was studied in our test system, indicating the least sharing of antigens. This was the only "first-set" rejection in our series. Only the pair of nonidentical twins that were of the same sex (no. 10) produced a "white graft" in the model system, despite an interpair graft rejection of 14 days.

The remaining donor pairs are really those of most practical and wide-spread interest because combinations of such individuals would be required for general use of homotransplantation. In this group too, many degrees of "accelerated" graft rejection were expected and were found.

Donor pair 12 was a mother and daughter combination and donor pair 19 was a mother and son combination. These have been referred to as compatible "genetic groupings" (8). In our experience, however, skin grafts from mother to child have shown no consistent increase in survival time. Although the gross appearance was that of a "white graft," the microscopic study demonstrated definite evidence of vascularization with patent vessels at the time of biopsy. The epidermis was dead, and it, as well as the dermis and hair follicles, showed moderate to mild infiltration of polymorphonuclear cells. This was interpreted as an "accelerated" type of graft rejection showing a high degree of immunity.

Donor pair 18 was two brothers who had all but two of 28 blood groups identical. They looked alike, but promptly rejected skin grafts from one to the other. Again, the gross appearance of the test-graft rejection in our system was that of the "white graft," a result suggesting a higher degree

of immunity. Histologic study, however, revealed that although most of the cellular elements of the graft were dead, a few cells in the basal layer were surviving. Epidermal separation had occurred above this layer, indicating that some attempt at vascularization had been made. This was supported by some dilated blood vessels in the dermis. There was little cellular infiltrate—only a few polymorphonuclear leukocytes in relation to the necrotic epidermis. This was again interpreted as representing evidence of marked immunity, although it could not be classified as a "white graft." These situations demonstrate the value of combined gross and microscopic study of the rejecting grafts. The other donor pairs produced "accelerated" rejections of intermediate degree with definite dermal vascularization (with or without hemorrhage), virtually no cellular infiltration, and associated epidermal death. This is, of course, the group with which we wish to enlarge our experience greatly, for only with a sufficient number of studies can any pattern or classification of individuals be delineated.

Donor pairs 16 and 17 were carried out in duplicate with the wife and mother of one of the members of the pair acting as the indifferent recipients. Although both responses could be considered to show "accelerated" rejection, the grafts on the mother were grossly more "accelerated" than those on the wife. Biopsies of the initial immunizing graft after 14 days looked very similar, but grossly the graft on the wife was much more viable.

Each of the three recipients in Table II received a renal homotransplant from the donor as indicated. Selection between possible donors already compatible by major blood groups was accomplished in two of the three cases as indicated in Table II. The renal homograft in L.U. was unsuccessful for technical reasons. Both in H.R., whose donor was his brother, and in G.B., whose donor was totally unrelated but showed a highly accelerated rejection on an indifferent recipient, the renal homografts are functioning well at the end of 3 and 2 months, respectively. Both of these recipients were conditioned with Imuran and azaserine therapy. The correlation of this skin-testing system and the success of the renal homograft cannot be made in this small number of cases because of variables such as the use of immunosuppressive therapy and the short time of follow-up.

DISCUSSION

In proposing this model system for evaluating human histocompatibility factors, there are a number of points that need further substantiation. The question of whether or not reversal of the order of using the donor pair would make any difference has not been answered experimentally. Theoretically, the same shared antigenic determinants should be just as apparent when performing the test in either direction. The question of the genetic status of the indifferent recipient is more significant. His role is a very active one, since the test really assesses his ability to become specifically immunized. It is obvious that grafting must be performed on several indifferent recipients simultaneously to assure the validity of our assumptions. In the three instances where this was done, the results with the two identical twin pairs were the same, and so were those of pairs 16 and 17. These latter pairs should be qualified, however, in that the degree of "acceleration" was not the same in the two indifferent recipients. This supports the fact that adequate immunity must be developed in the indifferent recipient if this assay is to function properly. The choice of graft timing to produce a "white graft" if possible, as predicted by Marshall and associates (4), seemed to be correct in our experience. The choice of an interval of 6 days after placing the second test graft as the time to biopsy has proved successful, since histologic determinations at this time invariably showed good correlation with the gross appearance, and both observations yielded decisive evidence of the degree of acceleration of rejection. At 6 days, surgical trauma or failure is no longer a confusing factor, and "first-set" rejection reactions have not yet begun. An "accelerated" rejection is well established at this point, and the differentiation between "white grafts" and "accelerated" rejection can be made with fairly good assurances, at least histologically. It is probably valuable to obtain multiple biopsies at day 6, since there may be gradations of "accelerated" rejection in different areas of the graft. This would be true especially if some quantitation of the degree of acceleration of the rejection were to be attempted.

In donor pairs 12, 18, and 19, only by attempts at quantitation, and possibly by comparison with results of similar studies carried out in species where the genetic makeup is more thoroughly mapped, can it be known how far removed a highly "accelerated" rejection is from a "white graft" in terms of potency of unshared antigens. This raises the basic question of how a knowledge of antigenic sharing will permit successful homotransplantation to be carried out. Extensive studies in the mouse (9), where the relative strength of histocompatibility differences have been well worked out, have shown clearly that the production of tolerance to homografts requires less in the way of immunosuppressive measures as these antigenic differences decrease. It is to be hoped, therefore, that the delineation of similar quantitative differences in the putative donor and recipient of human organ transplants might be determined so that a donor might be selected, the survival of whose tissue in the recipient would require the minimum in immunosuppressive maneuvers. These facts emphasize the need for an approach to human tissue "typing."

Certainly, these studies quoted above support our previous findings and those of Rapaport and co-workers (10) that there is indeed a high degree of cross-reactivity in the heterogeneous human population. Sensitization of a recipient by skin grafts from one individual may immunize that recipient to tissue from other random donors.

The small series of nonidentical twins we have studied has already raised some controversial questions. These cannot be resolved without enlarging this group of donor pairs, which will be done. The fact that only in the pair of the same sex do we get a "white graft" raises the question as to whether the Eichwald-Silsmer effect is active here (11). They showed that the Y chromosome in mice determines an antigen to which female mice of most strains will react. Our inability to obtain prolonged survival of cross-grafts between nonidentical twins is contrary to other reports (12). Only in the nonidentical twins mentioned earlier and previously reported as a situation where renal homotransplantation was successful were we able to document prolongation of crossed skin homografts. The significance of such a finding in successful homotransplantation has already been discussed.

Dausset (13) has approached the problem of tissue typing and histocompatibility between donor and recipient by the technique of leukoagglutinins. Donor and recipient leukocytes are compared for similar reactions when incubated with sera known to contain leukoagglutinins for human leukocytes. Although it has been suggested that there is a parallel between human leukocyte groups elicited in this fashion and the survival of transplanted human kidneys (14), this has not been a constant experience (15). The advantage of a system of tissue typing based upon skin grafting is that the biologic activity of the test system is more closely related to the basic problem of transplantation. Preliminary work with a system of tissue typing in rabbits that parallels our technique has suggested that this might be a feasible procedure (16).

In the initial case in Table II, it was not possible to improve upon the selection of a donor on the basis of this test, and therefore the recipient's brother was used because of consanguinity. In Cases 2 and 3, however, a definite discrimination was apparent, and the donor was chosen on this basis. This was especially important in G.B., since neither donor had any relation to the recipient, yet there was a striking difference in their cross-reactivity with the recipient.

SUMMARY

A model system for studying histocompatibility in man has been presented that utilizes active immunization of an indifferent recipient as a means of evaluating the number of antigens shared by a prospective donor-recipient pair.

This system is designed to detect subtle genetic compatibility between two individuals that would certainly be missed if cross-grafting alone were carried out.

The practical utilization of such a system in the field of human homotransplantation has been presented.

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