Journal of Clinical Investigation
Vol. 41, No. 12, 1962

CROSS-REACTIONS TO SKIN HOMOGRAFTS IN MAN *

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(Submitted for publication June 6, 1962; accepted August 15, 1962)

A considerable array of evidence has been secured to suggest that the tempo and intensity of the host's immune response to tissue transplants are conditioned by the degree of histocompatibility existing between the individuals studied (1–4). Although this subject has received a great deal of attention in animal species, it has not been studied extensively in man (5–7).

The present report is concerned with the influence of genetic disparity upon the fate of skin homografts in man. The results of a 9-year study of 147 skin homografts performed in a group of unrelated normal subjects are presented and discussed in the light of observed variations in the individual specificity of the rejection reaction.

MATERIALS AND METHODS

Selection of skin homograft donors and recipients. The subjects were students and house officers of the Third and Fourth Medical and Surgical Divisions (New York University) at Bellevue Hospital, New York. The donors were individuals known not to transmit serum hepatitis on the basis of their previous record of skin or blood donations at Bellevue Hospital.

Technique of grafting and of graft observation. The skin specimens were removed from the donors under 1 per cent procaine local anesthesia, and anchored to the host bed with 5-0 interrupted nylon sutures. The grafts were full-thickness specimens 11 mm in diameter. They were placed on the anterior surface of the forearm of the recipient. Pressure dressings were applied and changed 2 days later. On the fourth day, they were replaced by Band-aids.

The grafts were observed daily after the second day with a Bausch and Lomb dissecting stereomicroscope. Magnifications of 19.5, 45, and 90 times were employed to examine the graft surface and permit the accurate visualization of blood flow in the superficial blood vessels of the graft (8).

Criteria for the determination of homograft rejection. The objective determination of the time of onset of homograft rejection was based upon the results of gross and stereomicroscopic observations. The graft surface capillaries were studied daily by the method of Taylor and Lehrfeld (9, 10) adapted to man in the course of an initial study of the rejection of human skin homografts (8). This method permits the accurate visual determination of successful graft vascularization, as well as of the graft rejection changes. The criteria for graft rejection include: *1*) cessation of blood flow and thrombosis of the graft vessels, *2*) cyanosis and edema of the graft, and *3*) development of erythema and induration around the graft. The subsequent escharification of the graft confirms the diagnosis of rejection.

These criteria have been applied to first-set as well as to the accelerated homograft rejection reaction (11). In the case of the white graft reaction (12), the distinctive appearance of the graft permits ready recognition of this type of response. The white graft is noted for its lack of vascularization, dead-white color, and its evolution into a distinctive tan-colored eschar. The appearance of the latter is quite different from that of the black eschar associated with first-set or accelerated rejection.

RESULTS

The range of skin homograft reactivity in man

The application of a skin homograft from one individual to another, i.e., a first-set graft, results in its initial vascularization and growth. This is followed 8 to 10 days later by an abrupt cessation of blood flow in the graft vessels, multiple thromboses and hemorrhages, and the eventual change of the graft into a black eschar (8). This event is associated with the development in the host of a systemic state of altered reactivity to subsequent homografts from the same donor.

It is of considerable importance to note that the recipient's response to a second graft from the same

* Supported in part by U. S. Public Health Service Grant E-1254 (C6) and by Training Grant E.T.S. 2E-5, both from the National Institute of Allergy and Infectious Diseases, Bethesda, Md.; and in part by a grant from the John A. Hartford Foundation, Inc.

† Work done on U. S. Public Health Service Research Career Development Award AI-K3-6, 602 (Department of Surgery).

‡ Work done on U. S. Public Health Service Research Career Development Award GM-K3-15, 491-C2 (Department of Medicine).

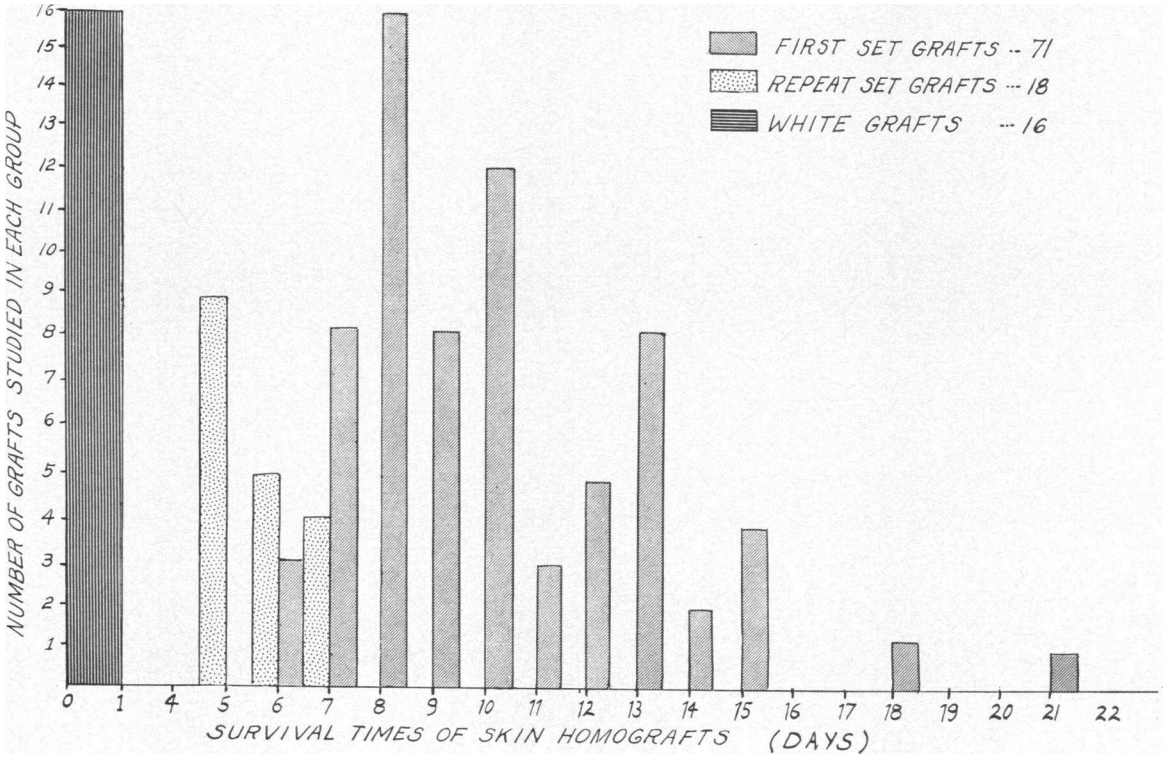
donor is conditioned by the time interval allowed between rejection of the first graft and application of the second-set graft. This is a factor which may also have a bearing upon the behavior of subsequent grafts from the same donor, regardless of the variations in the time intervals employed after rejection of the second-set graft. If the second-set graft is applied to the recipient within 1 to 5 days after first-set graft rejection, the white graft reaction occurs. When a latent period of longer duration, from 10 to 26 days, is allowed between first-set rejection and application of the second-set graft, the accelerated rejection reaction is elicited. This response differs from the avascular white graft reaction in its initial vascularization, which is followed by hemorrhagic necrosis on the fourth or fifth day after transplantation (11, 12).

In those instances where the second-set graft has undergone accelerated rejection and a latent period of 12 to 26 days has been observed, any further repeat-set grafts from the same donor have also undergone accelerated rejection. If a second-set graft application resulted in a white graft and a latent period of 1 to 5 days was observed after the rejection of each preceding graft, further repeat-set grafts also became white grafts (12).

The survival times of 105 skin homografts performed in a random population of normal human recipients are presented in Table I. Reactions in 71 first-set grafts, 18 repeat-set grafts (second-, third-, or fourth-set), and 16 white grafts were observed. The survival time of first-set grafts ranged from 6 to 21 days, with a median survival time of 8 to 11 days. No first-set graft was rejected before the sixth postoperative day. This permits the definition of accelerated rejection to be applied to any homograft reaction occurring before the sixth day. Second-, third-, and fourth-set skin homografts applied to a recipient from the same donor 10 to 26 days after rejection of the preceding graft had a median survival time of 4.7 days in the eighteen subjects studied. None of the grafts was rejected before the fourth day. In only four instances did repeat-set grafts survive until the seventh day. The white graft reaction

TABLE I

Patterns of behavior of skin homografts in human recipients



was elicited in sixteen consecutive instances. The survival times of the white grafts are listed as zero in Table I. This refers to the complete avascularity of these grafts, as compared with the first-set and repeat-set homografts, which are fully vascularized by the third day after transplantation.

The broad range of survival times of first-set homografts noted in Table I prompted a review of the donor-recipient combinations used. As noted in Table II, twelve donors had been paired with twelve different groups of recipients. The number of recipients in each group varied from two to eight. In the groups consisting of only two or three recipients of grafts from one donor, the graft survival times exhibited a narrow range, e.g., 10 to 13 days. As the number of recipients in each group increased, the range of survival times of grafts from one donor applied to each recipient also increased, e.g., 6 to 19 days. This widening of the range of survival times may be a function of the increasing number of individuals studied in each series. Firm conclusions, however, as to the meaning of this finding will have to await the accumulation of more information of this type.

TABLE II

Range of individual donor compatibility with multiple recipients with first-set grafts

Donor	Recipient	First-set survival time
		days
CAR	1) GEO	8 to 9
	2) CAS	9
JAF	1) BRI	7
	2) ROB	8
ABR	1) BAR	10
	2) JOS	13
	3) RUB	13
NIG	1) PAC	9 to 10
	2) MAR	13
	3) HAI	12
ROS	1) AZZ	13
	2) SUT	10
	3) DOU	12
PAL	1) BRA	11
	2) BUR	10
	3) FLE	10 to 11
RUB	1) DER	7
	2) GRA	14
	3) ATK	21
FRA	1) BRA	11
	2) GAN	10
	3) BAS	10
	4) BOW	12
PAU	1) MCK	9 to 10
	2) SMI	8
	3) ROS	12
	4) PAL	10
	5) FOL	7 to 8
ROB	1) JAF	8
	2) CAR	11
	3) RUB	7
	4) WES	8
	5) SHE	13
NAM	1) BRO	8
	2) MCC	12
	3) GOR	13 to 14
	4) MEL	15
	5) KEL	9
	6) FEN	8
MAT	1) ELS	9
	2) REN	6 to 7
	3) NEW	6 to 7
	4) GOU	8
	5) OBR	8
	6) FOL	10
	7) RUB	15
	8) BOW	18 to 19

The isolated finding that one first-set graft persisted for 21 days in one donor-recipient pair (RUB to ATK) may be the result of the chance selection of highly compatible subjects.

Experimental evaluation of the specificity of skin homograft rejection in man

The individual specificity of skin homograft rejection was studied in human subjects sensitized by two different techniques: subjects were sensitized *1*) by several successive grafts from the same donor, with the induction of accelerated rejection of each of these grafts, and *2*) by a first-set graft from the "test" donor, with deliberate induction of a white graft reaction by the application of a skin graft from the same donor 1 to 5 days after first-set rejection. When the last graft from the sensitizing or test donor was applied, the recipients also received skin grafts from other unrelated donors, i.e., first-set grafts to serve as controls.

1. Cross-sensitization resulting from the induction of accelerated rejection. Each of two recipients received two successive grafts from donor NIG. The first-set graft from donor NIG survived for 13 days in one recipient, MAR, and for 12 days in the other recipient, HAI. Fifteen days after rejection of the first-set grafts, each recipient was grafted with a second-set graft from donor NIG. In both subjects, the grafts were rejected 4 to 5 days later. Twenty-four hours after second-set rejection, the 2 recipients received *a*) a third-set graft from the original test donor NIG, and *b*) six first-set grafts obtained from six unrelated donors. In this experiment, therefore, the specificity of sensitization to grafts from donor NIG in subjects MAR and HAI was tested by the

TABLE III

Specificity of sensitization induced by the accelerated rejection reaction

Recipient	No. of grafts from sensitizing donor NIG	Fate of first-set skin grafts applied to recipient after sensitization	
		Donor of graft	Survival time
			days
MAR	3	ABR	8 to 9
		PAC	8 to 9
		BAR	5 to 6*
		ARO	5*
		SHE	5*
		CAS	8 to 9
HAI	3	PAU	7 to 8
		MAT	7
		RUB	9
		BOW	9 to 10
		FRA	9 to 10
		ROB	10

* Accelerated rejection reaction.

application of a third-set graft from NIG, as well as by the application of first-set grafts from a total of twelve additional donors. The results of this experiment are outlined in Table III.

The third-set grafts from NIG underwent accelerated rejection in both recipients.[1] Of the twelve first-set grafts, however, whose survival times were being compared with those of the NIG grafts, three grafts, from donors BAR, ARO, and SHE, underwent accelerated rejection on the fifth day, paralleling the behavior of the third-set grafts from NIG. This event suggested that the process of sensitization to NIG grafts had also sensitized this recipient to grafts from BAR, ARO, and SHE, i.e., to grafts from individuals to whom he had not been exposed previously. The remaining nine first-set grafts exhibited the usual type of first-set behavior, and were rejected within 7 to 10 days after transplantation.

2. Cross-sensitization resulting from the induction of the white graft reaction. The white graft reaction was induced in nine recipients by the application of a first-set graft from one donor, followed by a second-set graft from the same donor within 1 to 5 days after first-set rejection. At that time, the recipients were also grafted with skin from an unrelated donor. Table IV summarizes the results of this study. Five of the eight first-set, control grafts applied to recipients sensitized in this fashion underwent accelerated rejection instead of the first-set survival time usually accorded a graft from an unrelated individual.

An attempt to evaluate the possibility that cross-reactions in the recipients may have resulted from sensitization to closely related tissue transplantation antigens present in both the donors of the sensitizing grafts and the donors of first-set grafts was made in the experiment outlined in Table V. Three pairs of skin graft donors used in the white graft study (Table IV) were selected. In the case of the first pair of donors, FRA and BOW, sensitization of three consecutive recipients with

TABLE IV

Specificity of sensitization induced by the white graft reaction

Recipient	Donor of sensitizing grafts	No. of grafts from sensitizing donor	Fate of first-set skin grafts applied to recipient after sensitization	
			Donor of graft	Survival time
				days
SMI	PAU	2	FOL	6*
ROS	PAU	2	FOL	6*
PAL	PAU	2	FOL	9
BUR	PAL	2	CAR	8
FLE	PAL	2	CAR	9
BAS	FRA	2	BOW	6*
GAN	FRA	2	BOW	5*
BRA	FRA	2	BOW	6*

* Accelerated rejection reaction.

TABLE V

Evaluation of compatibility between members of three pairs of graft donors described in Table IV

Pairs of donors		Survival time of skin homografts exchanged between donors in each pair	
A	B	Graft A on Subject B	Graft B on Subject A
		days	*days*
FRA	BOW	12	14
PAL	CAR	8 to 9	
PAU	FOL	7 to 8	8 to 9

[1] It is not clear why the third-set grafts from NIG underwent accelerated rejection despite the short latent period of 1 day allowed after second-set rejection, which, in this instance, was an accelerated rejection reaction. One possible explanation may be that, once the second-set graft has evoked in the host one or the other type of altered response, i.e., accelerated rejection or white graft, further grafts from the same donor will elicit that same response regardless of subsequent variations in the latent periods.

grafts from FRA resulted in the accelerated rejection of first-set grafts obtained from BOW. This was not the case in the second pair donors, PAL and CAR, where sensitization of two recipients with grafts obtained from PAL did not result in the accelerated rejection of grafts obtained from CAR. In the third pair of donors, PAU and FOL, sensitization of three subjects with grafts obtained from PAU had resulted in the accelerated rejection of first-set grafts obtained from FOL in two recipients, but had no effect upon a similar graft placed upon a third recipient.

Skin homografts were exchanged between the members of each of these donor pairs. The grafts exchanged between the members of the first donor pair, FRA and BOW, survived for 14 and 12 days, respectively. A graft from PAL applied to CAR, in the second donor pair, survived for 8 to 9 days. Grafts exchanged between the members of the third donor pair, PAU and FOL, survived for 7 to 9 days.

In summary, grafts exchanged between one pair of donors who had succeeded in inducing the cross-reactions described in Table IV exhibited a somewhat prolonged survival time, when compared with the results obtained in the second group of donors, PAL and CAR. The significance, however, of this result as an indicator of shared tissue transplantation antigens is not supported by the results of cross-grafting the members of the third pair of donors, PAU and FOL. In this instance, although exposure to PAU grafts had cross-sensitized recipients to first-set grafts from FOL in two out of three subjects, the first-set grafts exchanged between PAU and FOL did not exhibit any prolongation of survival time.

DISCUSSION

The specificity of homograft rejection has undergone extensive investigation in animal species. The studies of Little (13), Snell (14, 15), Gorer (16, 17), Hauschka (18), and Medawar (19) have shown a direct correlation between the survival time of tissue homotransplants and genetically controlled histocompatibility determinants. The recent studies of Marshall, Friedman, Goldstein, Henry, Merrill, and Dammin (20, 21) have shown close similarities to exist between the morphologic events leading to homograft rejection in man and in experimental animals. With the possible exception, however, of some studies of the behavior of skin homografts in identical twins (22, 23) and in closely related individuals (6), there has been a paucity of data bearing directly on the role of individual specificity in human skin homograft reactions (24).

The present study suggests that the manifestations of homograft sensitivity in man may not necessarily follow the patterns of predictable individual specificity observed in grafts exchanged between lines of highly inbred animals of known genetic constitution. The broad variations observed in the length of survival of first-set skin homografts randomly exchanged between unrelated human subjects have prompted an evaluation of this problem in human recipients deliberately sensitized for this purpose by means of repeated skin homografts.

In eight out of twenty instances, sensitization with skin grafts from one individual has resulted in cross-sensitization to skin grafts obtained from other unrelated individuals. The possibility that this phenomenon may be an expression of common tissue transplantation antigens present in the individual donors studied was tested by exchanging skin homografts between such donors. Grafts exchanged between members of one donor pair who had caused cross-sensitization to occur survived for a longer time than a graft exchanged between members of a donor pair who had not induced such cross-sensitization. In another instance, however, where grafts were exchanged between members of a third pair of donors who had also induced cross-reactions to each other's grafts in two out of three recipients, a prolongation in survival time did not occur (see donors PAU and FOL in Tables IV and V).

The findings reported in this study, taken together, raise the possibility that unrelated human subjects may share tissue transplantation antigens. This interpretation is supported by the observations of Friedman, Retan, Marshall, Henry, and Merrill (25), who found that sensitization of human recipients with peripheral blood leukocytes may induce in these recipients a state of altered reactivity not only to the homologous donor's skin grafts, but also to skin grafts obtained from other unrelated individuals.

The conclusion that unrelated individuals may possess common transplantation antigens on the

basis of the results reported here is limited when viewed in the perspective of the total number of subjects in the present study. An alternative explanation, that hyperimmunization of recipients by repeated skin grafting may have blunted the capacity of the host to discriminate between closely related tissue transplantation antigens, is suggested by recent findings of cross-reactions and of competition of antigens observed in well-defined immunological systems. Maurer (26) has reported that previous injections of bovine serum albumin induce in rabbits the production of an antibody which cross-reacts with human serum albumin. Adler (27) has observed that the immunological response to one antigen can be impaired by the previous, simultaneous, or subsequent injection of one or more additional antigens. This kind of immune response to well-defined antigens could have a bearing on the host response to skin homografts under the experimental conditions employed in this study.

The approach to the selection of compatible donor-recipient combinations suggested by earlier studies in man (24, 28) and supported by some of the additional observations reported in this study has recently been applied by Kuss and Legrain (29) and by Mathe (30) to the problem of kidney homotransplantation in man. It may be pertinent, however, to recall that the selection of unrelated human subjects who may share transplantation antigens by the use of skin graft techniques is based upon a comparison of survival times of skin homografts in individuals who have been exposed to more than one skin graft. In such instances, the observed variations in survival times of skin homografts may be conditioned by immunological factors other than individual specificity. A clearer assessment of the relative roles of individual specificity and of other immunological factors in this problem awaits the characterization of the tissue transplantation antigens responsible for the induction of homograft sensitivity and of the host factor(s) concerned with this response.

SUMMARY

Variations in the specificity of skin homograft reactions have been studied in 147 normal unrelated human subjects. A primary exposure to a skin homograft from one individual is usually associated with the development in the recipient of generalized altered reactivity to subsequent skin grafts from the same individual, but not from other individuals. Depending upon the latent periods after the first graft, this altered reactivity is expressed in terms of accelerated rejection or of the white graft reaction when the subject is exposed to a second graft from the same donor.

Recipients hypersensitized with several skin grafts obtained from the same donor may respond with accelerated rejection when they are subsequently exposed to a graft obtained from another, unrelated individual. The occurrence of such cross-reactions suggests that unrelated human subjects may share tissue transplantation antigens. An evaluation of the contribution of hypersensitization to the occurrence of the cross-reactions observed in this study must await the identification and characterization of homograft antigens.

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