TRANSFER OF DELAYED HYPERSENSITIVITY TO SKIN HOMOGRAFTS WITH LEUKOCYTE EXTRACTS IN MAN *

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There is general agreement that the rejection of foreign homologous tissues applied as homografts to genetically unrelated individuals is accomplished by an immunological mechanism undertaken by the host (1–5). It is not yet clear, however, whether the highly specific immune mechanism evoked and the consequent allergic inflammatory response resulting in tissue destruction are mediated by serum antibody of the classical type or by a factor or factors intimately bound to cells of the leukocyte series (6–8). The preponderance of evidence accumulated to date has implicated the latter mechanism (9–14), although there is evidence to suggest a role for serum antibody, either as a contributing ancillary agent of tissue destruction (4, 15–17), or as a paralled but unrelated immunological event (1, 18, 19).

Much interest has converged upon the outcome of this issue, since any efforts designed to ameliorate or abolish an immunological response with this high degree of specificity are logically dependent upon the precise definition of the specific antigen or antigens that induce it and the specific antibody or antibodies that mediate it.

The most convincing evidence for a cellular effector mechanism has accrued from the successful transfer of accelerated homograft rejection of tumors by Mitchison (9, 18, 19) and skin by Billingham, Brent and Medawar (10, 11) in mice, and similar transfer of delayed tissue reactivity to homograft antigens in the guinea pig by Brent, Brown and Medawar (12). This application of the cellular transfer system to the study of homograft hypersensitivity in animals has done much to bring the mechanism mediating homograft hypersensitivity in close analogy to that shown earlier by Landsteiner and Chase (20) and by Chase (21) to mediate contact chemical and bacterial hypersensitivities of the delayed type.

In the human subject, the application of the cellular transfer system to the study of bacterial (22–25), fungal (26), viral (27, 28), and contact chemical (25, 29) hypersensitivities of the delayed type has resulted in additional insight into the immunity mechanisms mediating this general type of altered tissue reactivity. The unique feature of this system in man, in contrast to animal species, is the finding that extracts of leukocytes are as effective as intact viable cells in the transfer of delayed hypersensitivity (30–34). This observation has afforded an opportunity to attempt analysis of the mechanisms involved and identification of the factor or factors (transfer factor) in sensitive human leukocytes concerned with the transfer of delayed hypersensitivity. The precise biochemical or immunological nature of transfer factor is not known; however, certain properties of the biologically active principle have been described (35–37).

Briefly, transfer factor obtained from minute quantities of peripheral blood leukocytes (0.01 to 0.5 ml.) endows the recipient with the specific sensitivity (ies) of the donor within a matter of hours; the transferred sensitivity is generalized and may endure for as long as one to two years. The success of transfer is governed by the degree of sensitivity of the donor, the volume of leukocytes used and an unknown contribution of the recipient. Leukocytes from nonsensitive individuals are ineffective in this regard. Transfer factor has been shown unable to cross the species barrier;

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it resists freezing and thawing and treatment with the enzymes desoxyribonuclease (DNA-ase), ribonuclease (RNA-ase) or trypsin. Transfer factor interacts with but is not neutralized by antigen, and conventional antibody is not detectable in donor leukocyte extracts nor in recipient's sera or skin reactivity following transfer; it may be readily liberated from sensitive leukocytes into cell-free supernatant solutions (22, 23, 26, 30, 31, 38).

In the present paper, we report on the transfer of homograft hypersensitivity (accelerated homograft rejection) in man by means of an injection of DNA-ase-treated leukocyte extracts obtained from the peripheral blood of specifically sensitized donors.

MATERIALS AND METHODS

Basic plan of experiments

1) Skin grafts from Subject A were used to sensitize Subject B. *2*) Anti-A leukocytes were obtained from B; an extract was prepared, and injected into C. *3*) Sensitivity transferred to C was measured by the rejection period of a *test* graft (skin from A), as compared to that of a *control* graft (skin from D) from a subject unrelated to the experiment. *4*) A positive result was scored when the test graft was rejected in an accelerated fashion (4 to 6 days) while the control graft was rejected as a first set graft (10 to 12 days).

Sensitizing material and method of sensitization

Under local anesthesia the donor site was incised with a circular trephine (11 mm. diameter) and a full-thickness skin graft excised (39). The graft was placed on gauze saturated with normal saline and the undersurface freed of subcutaneous tissue. A graft bed area, identical in size, was prepared in similar fashion in the recipient upon the radial aspect of the volar surface of the forearm. Upon cessation of bleeding and capillary oozing in the recipient bed, the graft was inserted into the defect and its edges approximated to the recipient skin with interrupted (5–0 nylon) sutures. The area was covered with a layer of fine nylon mesh and a pressure dressing applied. Alternate sutures were removed on the third day and the remaining sutures on the fourth day.

Schedule of sensitization of leukocyte donors

Two schedules of leukocyte donor sensitization were employed, differing from each other in the time interval between application of the sensitizing skin grafts. *1*) The second set, third set and fourth set grafts from the same skin donor were applied two to three weeks after the occurrence of accelerated rejection of the preceding skin graft. This resulted in the accelerated rejection of each skin graft applied after the first set; *2*) the second set, third set and fourth set skin grafts were applied seven days after the rejection of the preceding graft. This schedule resulted in the occurrence of a "white graft" reaction (*i.e.*, vascular connections between graft and host did not occur) in each graft applied after the first set (40, 41).

Criteria of homograft rejection

Two methods were used routinely to determine the onset of the rejection period in all skin grafts.

1. Microscopic appearance of graft rejection. The method of stereomicroscopic observation of the graft capillaries devised by Taylor and Lehrfeld (42) and adapted to man has been used throughout this study; it has been described in detail elsewhere (39). With this method, the ultimate macroscopic rejection of the skin graft is predicted by the microscopic changes, which include cessation of capillary blood flow, intracapillary thrombosis and extravasation of blood into the graft. This method was employed from the third postoperative day forward and served also to indicate the onset of blood flow in the capillaries of homografted skin, as a critical measure of satisfactory union between host and graft.

2. Gross appearance of graft rejection. The gross characteristics of graft rejection were regularly preceded by the microscopic findings described above, and consisted of graft cyanosis, hemorrhage and swelling, with an area of surrounding erythema and induration in the recipient skin, the graft ultimately undergoing necrosis and escharification.

The criteria for graft rejection therefore included: *1*) development of erythema and induration around the graft; *2*) cyanosis and edema of the graft; *3*) cessation of blood flow and capillary thrombosis in the graft vessels. The subsequent development of an eschar, and its sloughing, confirmed the diagnosis of graft rejection in each case.

Using these criteria, it had been determined earlier, in an experimental study of the homograft rejection phenomena in human skin (39), that the average time of rejection of first set grafts is 10 to 12 days, whereas a second graft from the same donor to the same recipient, if applied two to three weeks later, will be rejected in an accelerated fashion (four to six days), as a manifestation of actively acquired homograft sensitivity, specifically directed against the tissues of that particular donor (40).

Preparation of leukocyte extracts

Leukocytes were isolated from the peripheral blood of sensitized donors by a modification of the usual technique (22, 23, 30) which has been described in detail elsewhere (26). Briefly, 400 ml. of whole blood was collected from each donor, through a cation-exchange resin chamber, into a plastic Fenwal bag to which was added 100 ml. of a Seitz-filtered distilled water solution containing 0.05 Gm. per ml. bovine fibrinogen (Fraction I, Armour). The contents were mixed, and the bag was suspended in a 37° C. incubator for 30 to 45 minutes; the sedimented erythrocytes were siphoned off, and the plasma supernatant suspension of leukocytes was collected in conical-tip centrifuge tubes graduated in 0.01

TABLE I

Homograft rejection patterns of leukocyte donors sensitized for transfer experiments

Skin donor	Skin recipient WBC donor	Time of rejection of first set grafts	Interval between grafts	Time of rejection of second set grafts	Interval between grafts	Time of rejection of third set grafts	Interval between grafts	Time of rejection of fourth set grafts
		days	*days*	*days*	*days*	*days*	*days*	*days*
Dav	Jos (D1)	15–16	50	4				
Mat	Bow (D2)	18–19	18	5–6				
Mat	Rubn (D3)	13–15	22	6–7				
Mat	Fol (D4)	9–10	27	5–6				
Abr	Jos (D5)	11–12	17	7–10				
Abr	Bar (D6)	11–13	20	7–9				
Cox	Wal (D7)	9	22	4	26	4	22	4
Fol	Jos (D9)	8–10	23	5–6	22	4–5	17	4–5
Jos	Fol (D10)	5–6	28	6–7	21	4	18	5–6
Car	Cas (D8)	7–8	7	0*	7	0*	7	0*

* White graft reaction.

ml., and centrifuged at 2,500 rpm for 15 minutes. The volume of packed leukocytes per tube was recorded, the supernatant plasma pipetted off and the leukocytes aspirated with a Pasteur pipette. The pooled leukocytes were resuspended in sterile, pyrogen-free nomal saline, and complete leukocyte disruption was accomplished by alternate rapid freezing (95 per cent alcohol-dry ice) and thawing (37° C. water bath) for 7 to 10 cycles. The leukocyte extract was treated with a solution containing 1 mg. per ml. crystalline pancreatic desoxyribonuclease (Worthington) in the presence of Mg^{++} before injection into the recipient.

EXPERIMENTAL AND RESULTS

Selection of skin donors and recipients

Skin donors and recipients were selected from a stable population of healthy volunteers who were pedigreed blood donors known not to transmit homologous serum hepatitis. These individuals were either physicians on the house staff of the Third (N. Y. U.) Medical or Surgical Divisions of Bellevue Hospital, or students at the New York University College of Medicine. The recipients of skin grafts were also selected with regard to the above pedigree, since following sensitization to the respective skin grafts, they would in turn become the donors of the leukocytes used for transfer purposes.

Selection of recipients of leukocyte extracts

This group of volunteers was identical to that described above with the exception that there was no necessity for the recipient to be a pedigreed blood donor. In addition to the physicians and medical students who participated in this study, six patients on the Fractures Service also served as recipients of transfers (see Table IV).

Results of sensitization experiments

The responses of each individual who served as a leukocyte donor in the transfer experiments to the initial sensitizing (first set) and subsequent (second set, third set and fourth set) skin grafts from the same source are summarized in Table I.

It may be seen that the onset of the rejection of first set grafts varies from as early as five to six days in one pair of individuals to 18 to 19 days in another pair. The onset of accelerated rejection varies from 7 to 10 days at its latest to four days at its earliest. The average time of onset for the rejection of first set grafts was established to be 8 to 12 days, and four to six days for the accelerated rejection of subsequent grafts. This is based upon earlier (39–41) as well as current observations.

Results of transfer experiments

A. Use of one or of two consecutive skin homografts to sensitize the leukocyte donor.

1. Systemic transfer. The graft was applied at a site remote from the injection of the leukocyte extract.

Systemic transfer was used exclusively in this report, except in the experiments detailed in Section 2 below. It consists of the intradermal and subcutaneous injection of sensitized leukocyte extract into the skin overlying the shoulder of the

TABLE II

*Effects of systemic transfer from leukocyte donor sensitized with one or two grafts**

Leukocyte donor	Sensitization of leukocyte donor	Type of transfer	Time of transfer	Material used for transfer	Dosage WBC	Recipient No.	Day of graft rejection: Test graft	Day of graft rejection: Control graft	Interpretation of result
					ml.				
D1	1 Graft	Systemic	13 Days before grafts applied	WBC extract	0.5	R1	11 11	9 9	Negative
						R2	12 12	Poor take 9	Negative
D2	2 Grafts	Systemic (pooled WBC first and second set rejection)	5 Days before grafts applied	WBC extract	1.28	R3	11 11	8 8	Negative
D3	2 Grafts	Systemic (pooled WBC first and second set rejection)	5 Days before grafts applied	WBC extract	1.06	R4	10 10	7 7	Negative

* Skin donor	Skin recipient WBC donor	WBC recipient	Test graft donor	Control graft donor
Dav	Jos (D1)	McK (R1)	Dav	Mar
		Mar (R2)	Dav	McK
Mat	Bow (D2)	Rob (R3)	Mat	Jaf
Mat	Rubn (D3)	Bri (R4)	Mat	Jaf

nonsensitive recipient, followed at a later date by the application of a *test* and a *control* graft on each forearm.

The results of this type of experiment are summarized in Table II.

In the first instance, only one graft was used to sensitize the leukocyte donor. DNA-ase-treated extract was prepared from leukocytes obtained at the height of rejection of this graft, and injected into the shoulder of each of two nonsensitive recipients. Thirteen days after transfer, two test grafts and two control grafts were applied to the forearms of each recipient. It may be seen that, in this experiment, there is little difference between the rejection period of the test grafts (11 and 12 days, respectively) and that of the control grafts (nine days). *The results of this attempt at transfer of homograft sensitivity are interpreted as negative.*

In the second instance, an initial and a second set homograft from the same subject were applied to sensitize the leukocyte donor. The DNA-ase-treated extract was prepared by pooling leukocytes isolated at the height of the rejection periods of the initial graft and of the second set graft. The pooled leukocyte extract was divided into equal aliquots and injected into the skin of the shoulders of each of two recipients. Five days after transfer, two test and two control grafts were applied to the forearms of each recipient. Here again, there was little difference between the rejection period of the test grafts (11 and 10 days, respectively) and that of the control grafts (eight and seven days, respectively). *The results of this attempt at transfer of homograft sensitivity are interpreted as negative.*

2. Local technique of transfer. Leukocyte extracts were injected concentrically around test and control grafts.

In this series of observations, advantage is taken of the technique of Stetson and Demopoulos (15, 16) (in animals) of transfer of homograft sensitivity with serum. The present application is based on earlier findings in the transfer of tuberculin type allergy (22, 23) whereby the juxtaposition of antigen and transfer factor increases the sensitivity of the test system so as to allow a 10-fold reduction in the dosage of leukocytes ordinarily required to effect transfer by the systemic technique. In the local technique, the materials

(DNA-ase-treated leukocyte extracts, serum) to be tested for their capacity to transfer homograft sensitivity are injected around but not into test and control grafts, in halo fashion. The donor of the sensitive leukocyte extracts was sensitized with a first and a second set graft, as above, and the leukocytes were collected at the height of each rejection period, and pooled. Serum was obtained from the same donor at each of these bleedings, and was also pooled. The control donor of nonsensitive leukocyte extracts received no treatment.

Each of six nonsensitive individuals received a test and a control graft on each forearm. Three days later, when flow was established in each of the grafts, a concentric circle was formed in the recipient's forearms, about 20 mm. from the edges of each test and control graft, by the injection of either sensitive leukocyte extract or sensitive serum from the same donor, or nonsensitive leukocyte extracts prepared from a control donor.

The results of this experiment are summarized in Table III.

It may be seen that injection of sensitive leukocyte extracts into the recipient's forearm around test and control grafts in each of four recipients resulted in the accelerated rejection of the test grafts only, at the fourth to sixth day after grafting, while the control grafts survived for 8 to 10 days before rejection. It is notable that these effects upon the test grafts occurred within 24 to

TABLE III

*Effects of local transfer from leukocyte donor sensitized with two grafts**

Leukocyte donor	Sensitization of leukocyte donor	Type of transfer	Time of transfer	Material used for transfer	Dosage injected around each graft site	Recipient No.	Day of graft rejection: Test graft	Day of graft rejection: Control graft	Interpretation of results
					ml.				
D4	2 Grafts	Local (pooled WBC first and second set rejection)	3 Days after grafts applied	Sensitive WBC extract†	0.22	R5	4–5	8	Positive
				Sensitive serum†	1.0		10	8	
D5	2 Grafts	Local (pooled WBC first and second set rejection)	3 Days after grafts applied	Sensitive WBC extract†	0.2	R6	4	10	Positive
				Sensitive serum	1.0	R7	10		Control
				Nonsensitive WBC extract	0.2		10		
D6	2 Grafts	Local (pooled WBC first and second set rejection)	3 Days after grafts applied	Sensitive WBC extract†	0.17	R8	5–6	10	Positive
				Sensitive WBC extract†	0.17	R9	5–6	10	Positive
				Sensitive serum	1.0	R10	10		Control
				Nonsensitive WBC extract	0.20		10		

* Skin donor	Skin recipient WBC or serum donor	WBC or serum recipient	Test graft donor	Control graft donor
Mat	Fol (D4)	Jaf (R5)	Mat	Rob
Abr	Jos (D5)	Pau (R6)	Abr	McK
		Tay (R7)	Abr	
			Abr	
Abr	Bar (D6)	McK (R8)	Abr	Pau
		Pac (R9)	Abr	Nig
		Lit (R10)	Abr	
			Abr	

† Injected around test and control graft, respectively.

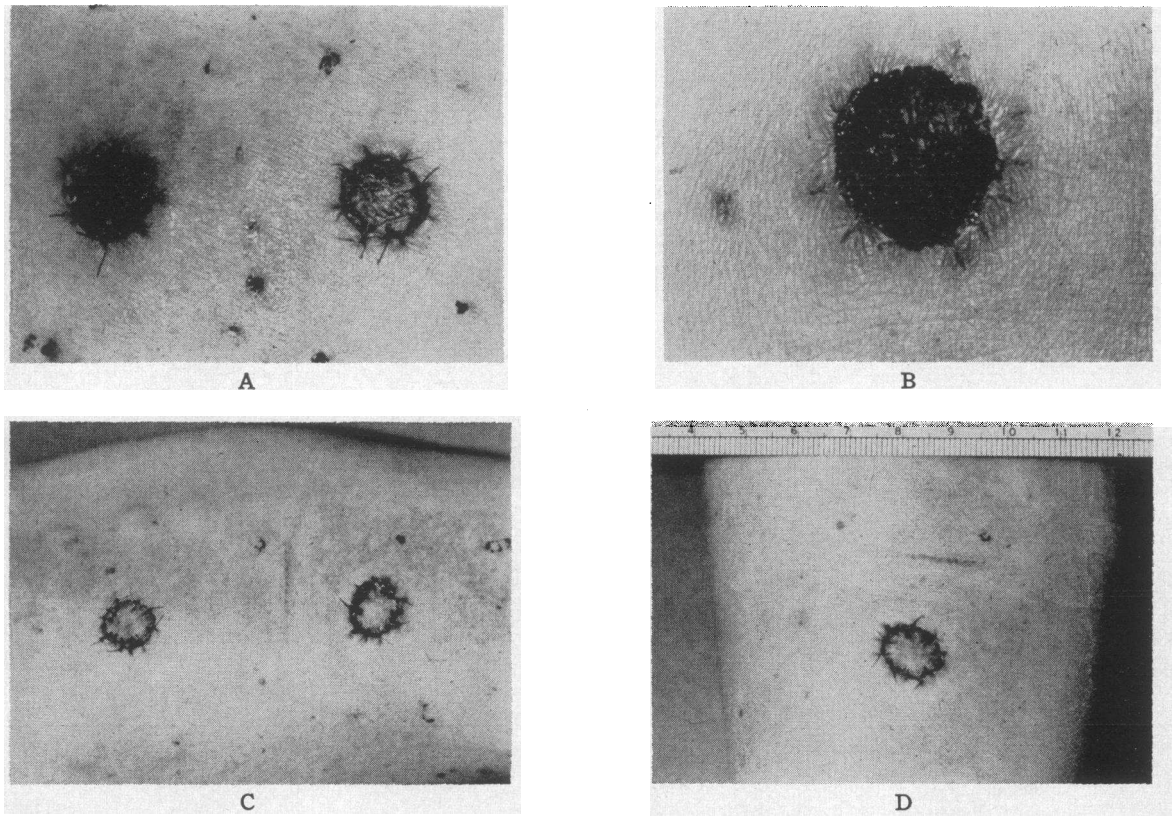

FIG. 1. LOCAL TRANSFER OF HOMOGRAFT HYPERSENSITIVITY WITH LEUKOCYTE EXTRACTS BUT NOT WITH SERUM FROM SENSITIZED DONOR [RECIPIENT NO. 5 (JAF)—TABLE III]

A. Left forearm—illustrating hemorrhagic appearance of test homograft (left) undergoing accelerated rejection and normal appearance of control homograft (right)—five days after application of both grafts and 48 hours after infiltration of sensitive leukocyte extract around both grafts. **B.** Close-up view of fully rejected test homograft in **A** above, six days after application of graft and 72 hours after transfer. **C.** Right forearm—illustrating normal appearance of test homograft (left) and control homograft (right) three days after application of grafts and immediately after infiltration of sensitive serum around both grafts. **D.** Close-up view of normal appearance of the test homograft in **C** above, four days after application of graft and 24 hours after serum transfer.

48 hours following the injection of sensitive leukocyte extracts.

The injection of sensitive serum or of nonsensitive leukocyte extracts around test grafts in this fashion had no effect upon their survival time (which was identical to that of the control grafts, *i.e.*, 10 days).

An example of this type of local transfer of homograft sensitivity is illustrated in photographs of the test and control grafts in Recipient No. 5 (R5) of Table III (see Figure 1). *The results of this attempt at local transfer of homograft sensitivity are interpreted as positive.*

B. Use of four consecutive skin homografts to sensitize the leukocyte donor. In this series of experiments, the donor of leukocytes was sensitized by a series of skin grafts from the same individual, applied on four successive occasions; each graft after the first underwent an accelerated rejection reaction. At the height of the fourth set rejection reaction, leukocytes were obtained, DNA-ase-treated leukocyte extracts were prepared, and injected into the nonsensitive recipient. In the experiments to follow, the leukocyte extract was injected into the shoulder of the recipient either 8 or 11 days before, or three days after application of test and control homografts to the respective recipients' forearms.

1. Relationship between the homograft rejection period and the capacity of leukocyte extracts to

TABLE IV

*Relationship between homograft rejection period and the capacity of leukocyte extracts to transfer hypersensitivity**

Leukocyte donor	Time of WBC collection	Method of transfer	Material used for transfer	Dosage WBC	Recipient No.	Day of homograft rejection: Test graft	Day of homograft rejection: Control graft	Interpretation
				ml.				
D7	At height of rejection of fourth graft	Systemic: 8 days before grafts	WBC extract	0.52	R11	4 4	11 11	Positive
		Systemic: 3 days after grafts	WBC extract	0.52	R12	4–5	8	Positive
	11 Days after above bleeding	Systemic: 11 days before grafts	WBC extract	0.50	R13	8	8	Negative
				0.50	R14	9	9	Negative
		Systemic: 3 days after grafts	WBC extract	0.50	R15	10 11	14 14	Negative
				0.50	R16	8 7	15 15	Questionable positive

* Skin donor	Skin recipient WBC donor	WBC recipient	Test graft donor	Control graft donor
Cox	Wal	McC (R11)	Cox	Nam
		Bro (R12)	Cox	Nam
		Fen (R13)	Cox	Nam
		Kel (R14)	Cox	Nam
		Gor (R15)	Cox	Nam
		Mel (R16)	Cox	Nam

transfer homograft hypersensitivity. The DNA-ase-treated leukocyte extracts used in this experiment were obtained from the same sensitized donor at two distinct periods: *1*) at the height of the fourth set accelerated homograft rejection period and *2*) 11 days after that period. Extract secured at each period was injected into the shoulder of a nonsensitive recipient, either 8 or 11 days before, or three days after the application of a test and control graft. The results are summarized in Table IV.

It may be seen that when leukocyte extracts obtained at the height of the accelerated rejection period of the fourth set graft were injected into the shoulder of a nonsensitive recipient eight days before the application of test and control grafts, this resulted in the selective accelerated rejection of the two test grafts at four days, while the two control grafts were unaffected and survived for 11 days. Extract of leukocytes obtained at the same bleeding were injected into the shoulder of a nonsensitive recipient three days after test and control grafts had been applied (at a time when blood flow was established in each graft). Within 24 hours after transfer the test graft underwent accelerated rejection (at the fourth day post-grafting) while the control graft survived for eight days.

The leukocyte extracts subsequently obtained from the same donor (D7) 11 days after the height of his fourth set rejection period were incapable of causing the accelerated rejection of test grafts in each of four additional recipients. This incapacity was evident whether the leukocyte extracts were injected 11 days before or three days after the application of test and control grafts.

With reference to Recipient No. 16 (R16), it should be mentioned that survival times of eight and seven days for test grafts as against 15 days for control grafts suggest a positive result. If it is indeed a positive result, it is surely a weak and questionable one, and further bears out the negative results achieved with the same leukocyte extracts, as an indication of the rapid decay in the capacity of Donor No. 7 (D7) leukocytes to transfer systemic sensitivity. It has not been given equal weight with other positive results, since the

response of the recipient to active sensitization by the graft itself may begin as early as the seventh day. It is for this reason that we have restricted the critical period for acceptance of a positive effect of transferred sensitivity to four to six days.

It is suggested from this experiment that an optimal time for securing leukocytes from sensitized donors capable of transferring sensitivity is at the height of the rejection period of the fourth set graft. These results also suggest a transient endowment of the leukocytes with the capacity to transfer sensitivity, when the donor is sensitized in this fashion.

2. Failure to transfer homograft sensitivity when the leukocyte donor is sensitized with three successive "white grafts." Since the schedule of sensitization employed above (*i.e.,* in which two to three weeks are allowed to pass between rejection of the last and application of the next successive graft) requires several months, an effort was made to sensitize leukocyte donors over a shorter period. To accomplish this, the interval between rejection of the preceding graft and application of the next successive graft was shortened to seven days.

It was observed, however, that when second, third and fourth grafts were applied in this fashion, the sequence of initial vascularization (three days) followed by accelerated rejection (four to six days) did not occur. Instead, each graft after the first was not vascularized, and remained white and opaque from the time of application. This has been termed the "white graft" reaction, and its occurrence and possible significance have been described elsewhere (40, 41).

Leukocytes and erythrocytes in plasma were obtained from a donor sensitized in this fashion, at the height of the fourth "white graft" reaction. The DNA-ase-treated leukocyte extracts were injected into the shoulder of four nonsensitive recipients and an equivalent amount of erythrocytes and plasma was similarly injected into an additional nonsensitive recipient. Each recipient had received a test and control graft on each forearm four days before the attempts at transfer were made. The results of this experiment are summarized in Table V.

TABLE V

Effects of systemic transfer from leukocyte donor sensitized with "white grafts" *

Leukocyte donor	Type of transfer	Time of transfer	Material used for transfer	Dosage WBC	Recipient No.	Day of homograft rejection: Test graft	Day of homograft rejection: Control graft	Interpretation of results
				ml.				
D8	Systemic	4 Days after grafts applied	Sensitive WBC extract	0.6	R17	7	9	Negative
			Sensitive WBC extract	0.59	R18	7	6	Negative
			Sensitive WBC extract	0.63	R19	6†	6†	Negative
			Sensitive plasma and RBC	2.5 0.6	R20	9	8	Control
			Sensitive WBC extract	0.59	R21	9	8	Negative

* Skin donor	Skin recipient WBC or control plasma donor	WBC or plasma recipient	Test graft donor	Control graft donor
Car	Cas	Els	Car	Mat
		New	Car	Mat
		Ren	Car	Mat
		Gou	Car	Mat
		O'Br	Car	Mat

† Technically unsatisfactory graft application.

It may be seen that neither the putatively sensitive leukocyte extract nor the erythrocytes and the plasma obtained from the same donor had any effect on the survival of test as compared to control grafts.

It is concluded that the schedule of sensitization by means of skin grafts cannot be hurried. The results suggest that the accelerated rejection reaction is superior to the "white graft" reaction as a means of sensitizing the leukocyte donor.

3. Corroboration of the efficacy of four successive grafts and accelerated rejection as a means of sensitizing the leukocyte donor. Following the results described in Section 2 above, the next donor of leukocytes was sensitized exactly as described under Section *1* above (*i.e.,* by a series of four successive grafts, the latter three being applied at intervals of two to three weeks after rejection of the preceding graft, and each undergoing accelerated rejection). The DNA-ase-treated extract was prepared from leukocytes obtained at the height of accelerated rejection of the fourth set graft. The leukocyte extract was injected into the shoulders of four nonsensitive recipients. Eight days following transfer, a test and control graft were applied to both forearms of each recipient. The results of this experiment are summarized in Table VI.

It may be seen that in each recipient, the test graft was rejected in an accelerated fashion 24 hours after transfer (and four days postgrafting) while the control grafts did not undergo accelerated rejection. It should be noted that technical difficulties at the time of grafting contributed to the rejection of the control graft in Recipient No. 25 (R25) at seven days.

From these results, it is concluded that homograft sensitivity is amenable to transfer with leukocyte extracts when the latter are obtained from an adequately sensitized donor. Examples of this type of transfer are illustrated in Figure 2.

C. Summary of results of transfer experiments. The results of attempts at transfer of homograft sensitivity are listed in Table VII.

It may be seen that neither one nor two successive grafts are sufficient to endow the leukocyte extracts of donors sensitized in this fashion with the capacity to transfer homograft sensitivity *systemically.* However, two successive grafts are sufficient to sensitize donor leukocyte extract to such an extent as to allow a *local* transfer of sensitivity. Serum obtained from the same sensitive donors, as well as leukocyte extracts obtained from nonsensitive donors, are incapable of transferring homograft sensitivity, even when favored by the highly sensitive technique of local transfer.

It is also noted that systemic homograft sensitivity can be transferred by leukocyte extracts if a dosage of four successive grafts is used to sensitize the leukocyte donor, and each graft after the first has undergone accelerated rejection.

The effects of transfer of local or of systemic sensitivity to recipients bearing vascularized grafts appear promptly within 24 to 48 hours following transfer. When graft application follows the transfer of sensitivity, the test graft is seen to be vascularized by the third day and to undergo accelerated rejection within 24 hours after this event.

From one group of our observations, it would appear that the capacity of the sensitized donor's leukocytes to transfer sensitivity is short-lived. From another group of observations, it would also appear that graft rejection via the "white graft" reaction does not serve as an efficient stimulus for sensitizing the leukocyte donor to the extent necessary to effect a successful transfer of sensitivity with leukocyte extracts.

DISCUSSION

The rapid onset of accelerated rejection of test grafts 24 to 48 hours after transfer, in subjects bearing fully vascularized test and control grafts, may be taken as good evidence of a specific passive transfer of sensitivity. The prompt effects achieved by this method of transfer exclude the participation of antigen in the results observed. However, the accelerated rejection of test grafts placed upon recipients eight days after transfer implies a more intimate participation of the recipient in the inauguration of the mechanism of rejection. This is suggested by the fact that in each of such instances, the test graft became vascularized, and flow between host and graft was established usually 24 hours before the onset of accelerated rejection. Inherent in the design of the latter type of transfer experiment is the remote possibility that antigen, however minute in quantity, may be carried over in the sensitized leukocyte extract used to transfer sensitivity. If

TABLE VI

*Effects of systemic transfer from leukocyte donor sensitized with four grafts via accelerated rejection reaction**

Leukocyte donor	Type of transfer	Time of transfer	Material used for transfer	Dosage WBC	Recipient No.	Day of homograft rejection: Test graft	Day of homograft rejection: Control graft	Interpretation of result
				ml.				
D9	Systemic	8 Days before grafts applied	WBC extract	0.7	R22	4	11	Positive
				0.7	R23	4	8	Positive
D10	Systemic	8 Days before grafts applied	WBC extract	0.9	R24	4	13	Positive
				0.9	R25	4	7†	Positive

* Skin donor	Skin recipient WBC donor	WBC recipient	Test graft donor	Control graft donor
Fol	Jos (D9)	Car (R22)	Fol	Rob
		Wes (R23)	Fol	Rob
Jos	Fol (D10)	She (R24)	Jos	Rob
		Rub (R25)	Jos	Rob

† Partial separation from bed on third day due to unsatisfactory technique.

this possibility is proved, then the possibility of active sensitization in the recipient of this method of transfer will have to be excluded. Experiments are planned to assess the capacity of DNA-ase-treated leukocyte extracts in this regard.

The requirement of four successive grafts to

TABLE VII

Summary of results of transfer of specific sensitivity to skin homografts

Mode of sensitization and time of collection of WBC from donor	Method of transfer	Material used for transfer	Results of transfer: No. of recipients	Results of transfer: No. of positive transfers
First set rejection	Systemic	Sensitive WBC extract	2	0
Second set rejection	Systemic	Sensitive WBC extract	2	0
First and second set rejection	Local	Sensitive WBC extract	4	4
		Nonsensitive WBC extract	2	0
		Sensitive WBC extract on control graft	4	0
		Sensitive serum on test graft	3	0
Fourth set rejection	Systemic	Sensitive WBC extract	6	6
11 Days after fourth set rejection	Systemic	Sensitive WBC extract	4	0
5 Days after fourth set "white graft" application	Systemic	Sensitive WBC extract	4	0
		Sensitive plasma and RBC	1	0

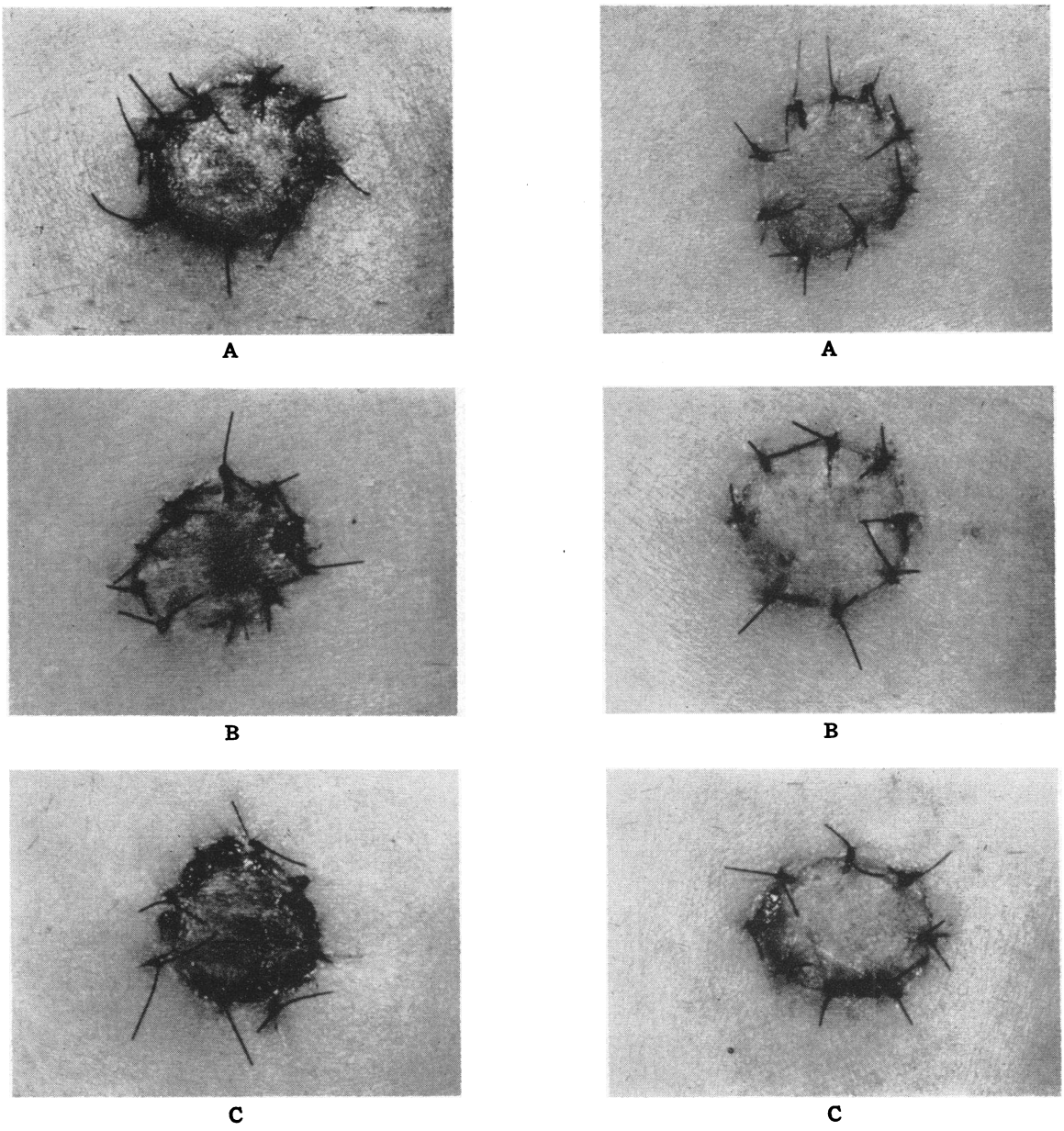

FIG. 2. SYSTEMIC TRANSFER OF HOMOGRAFT HYPERSENSITIVITY WITH LEUKOCYTE EXTRACTS OBTAINED FROM SENSITIZED DONOR—TABLE VI

A. Recipient No. 22 (Car), **B**. Recipient No. 23 (Wes) and **C**. Recipient No. 24 (She)—illustrating in each recipient the hemorrhagic appearance of the test homograft (left) undergoing accelerated rejection and normal appearance of the control homograft (right)—four days after application of each graft and 12 days after transfer to each recipient, respectively.

sensitize the donor's leukocytes to the extent that a systemic transfer of sensitivity is possible suggests that the application of orthotopic skin homografts is not the most efficient means of sensitization for this purpose. Another indication of the critical importance of quantitative variables in governing the success of attempts at transfer may be gained from the observations made employing the local technique of transfer. In this instance, only two successive grafts were sufficient

to endow leukocytes with the capacity to transfer *local* but not *systemic* sensitivity. It may be that, for convenience and ease of interpretataion of results, this method of transfer is to be preferred over the systemic transfer of homograft sensitivity. However, in a preliminary study of this kind, there are good theoretical reasons for also having successfully effected the systemic transfers.

One of the many great advantages of using animal species for a more precise demonstration of the transfer of homograft sensitivity has been the use of inbred strains. This is not possible in man; here, the tempo and intensity of the host's immune response to homografts, and the capacity to transfer such sensitivity, will parallel the degree of genetic disparity between the individuals studied. The greater the genetic differences (*i.e.,* the less the number of tissue transplantation antigens shared) between the individuals studied, the more abrupt and intense will be the reaction of one to a graft from the other. This consideration, coupled with the fact that the experimental demonstration of the individual specificity of homograft responses has not been as extensively studied in man (39–41, 43) as in animal species (4, 6, 7, 10, 11), introduces inescapable variables for the type of experimental observation reported in this study. Nevertheless, since the effects of transfer on the test grafts have been judged as significant only in terms of their relation to the behavior of control grafts on the same recipient, it is felt that the bearing of such variables on the observations reported, although not eliminated entirely, has been greatly diminished.

The observations reported here are in general agreement with Mitchison and Dube's (18, 19) findings on the transfer of tumor homograft sensitivity in mice, Billingham, Brent and Medawar's (10, 11) findings on the transfer of skin homograft sensitivity in mice and Brent, Brown and Medawar's (12) findings on the transfer of skin homograft sensitivity in the guinea pig. The effectiveness of peripheral blood leukocytes in human subjects is in contrast to the requisite for successful transfers in animal species that the leukocytes be obtained from lymph nodes draining the graft (10, 18, 19), or from the spleen (10) of the sensitive donor. The present demonstration of the critical role of timing in determining effective sensitization, transfer, and test of transferred sensitivity, suggests an approach to the resolution of this species difference.

The loss of capacity to transfer sensitivity in Donor No. 7 (D7), Table IV, suggests that sensitization and desensitization to skin homografts may proceed *pari passu,* and that the success or failure of transfer may depend upon which state is in the ascendancy at the time of leukocyte collection. A similar loss in the capacity of leukocytes to transfer delayed tuberculin hypersensitivity has been produced experimentally *in vitro* (38) and *in vivo* (44) in man following exposure to tuberculin and resultant desensitization of the leukocytes.

The property of DNA-ase-treated leukocyte extracts to transfer homograft sensitivity in man is a striking departure from the experience in animal species, where intact, viable cells are necessary for this effect (9–11). Such a species difference may be a reflection of yet another parameter of the greater relative ease with which man can become sensitized to a wide variety of foreign materials (bacteria, simple chemicals) (45). This is believed the more likely in respect to homograft sensitivity, since it has been shown earlier to be operative in the transfer of delayed bacterial (30, 31) and fungal (26) hypersensitivities. In the latter systems of delayed allergy, the efficacy of leukocyte extracts in the transfer of sensitivity has been repeatedly demonstrated (26, 30, 31, 38) and more recently confirmed elsewhere (32–34), in human subjects.

There has been no attempt to measure antibodies in the serum of recipients of transfer in this study, in view of the negative results of attempts at transfer with serum, and the failure to demonstrate conventional serum antibody following transfer of bacterial allergy in humans with leukocyte extracts (31) or viable cells (17, 25) when the leukocytes have been obtained from the *peripheral* blood.

The failure to transfer homograft sensitivity with extract obtained from "white graft" reactors suggests that this type of homograft response may not be mediated by "cell-bound" antibody.

It has been the purpose of this study to adapt the highly sensitive transfer system in human subjects to an evaluation of the possible mechanisms

of homograft rejection—an adaptation which broadens the biological properties of transfer factor and allows the use of extracts of leukocytes. The latter device gives promise of sorting out the antibody(ies) that mediates homograft rejection, the cells concerned in the process, and perhaps the antigen(s) of tissues concerned with the induction of this immune response. In any event, insofar as homograft sensitivity is amenable to transfer with extracts of leukocytes, an approach has been provided whereby the progress made with this system in humans in relation to delayed bacterial hypersensitivity can be applied directly to the study of the immunology and pathogenesis of the homograft rejection phenomenon, as a general biological prototype of altered tissue reactivity.

SUMMARY AND CONCLUSIONS

1. Skin homograft sensitivity (accelerated homograft rejection) has been transferred to nonsensitive recipients by means of an injection of desoxyribonuclease-treated leukocyte extracts prepared from sensitive donors: *a*) in four out of four consecutive attempts by employing a local technique of transfer and a dosage of two grafts to sensitize the leukocyte donor; *b*) in six out of six attempts by employing the systemic technique of transfer and a dosage of four grafts to sensitize the leukocyte donor.

2. A dosage of *one* or *two* grafts is not sufficient to sensitize the leukocyte donor so as to allow a systemic transfer of sensitivity.

3. A dosage of four successive grafts is sufficient to sensitize donor leukocytes so as to permit systemic transfer of sensitivity *only* if the repeat set grafts undergo accelerated rejection, and *not* if "white graft" reactions are produced.

4. Serum from sensitized donors or leukocytes from nonsensitive donors are incapable of transferring homograft sensitivity.

5. The results of transfer of homograft sensitivity by means of leukocyte extracts generally parallel those obtained in man following similar transfers of bacterial (tuberculin, streptococcal proteins, diphtheria toxoid) and fungal (coccidioidin) hypersensitivities of the delayed type. This finding fulfills a critical criterion relating delayed hypersensitivity of the tuberculin type to homograft sensitivity.

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