ACCELERATED SKIN GRAFT REJECTION IN HUMANS PRE-IMMUNIZED WITH HOMOLOGOUS PERIPHERAL LEUKOCYTES *

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(Submitted for publication May 18, 1961; accepted August 17, 1961)

Delayed intradermal sensitivity to homologous white blood cells has been described in humans rejecting full thickness skin homografts (1). The reaction at the site of the challenge leukocyte was greatest at 18 to 24 hours against the cell suspensions obtained from the skin donor. It was noted, however, that lesser reactions were consistently elicited to similarly prepared and simultaneously injected leukocytes obtained from donors other than the sensitizing skin donor. There was no recipient response to injections of autologous leukocytes, homologous erythrocytes or homologous plasma. Biopsy sections taken at peak reaction times were similar to early tuberculin responses. It was postulated that the skin erythema and induration were a manifestation of an antigen-antibody reaction, in which antibodies developed against the skin homograft were capable of also reacting with shared antigen(s) within or upon the peripheral white blood cell. To explain the lack of specificity of the skin response, it was assumed that there is a distribution of common transplantation antigens in the human, as has been shown by Berrian and Jacobs in the mouse (2). The present experiment supports this supposition and suggests that transplantation immunity in the human is not as specific a phenomenon as has been previously proposed (3).

* The investigation was supported in part by the United States Army Medical Research and Development Command, Department of the Army, under Contract no. DA-49-007-MD-429, and in part by the National Heart Institute, United States Public Health Service (H-444-C11).

† This work was performed during the author's tenure as an American Heart Research Fellow.

‡Fellow of the National Foundation.

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MATERIALS AND METHODS

Eighteen ambulant adult volunteers received full thickness skin homografts on the medial surface of the upper arm, after inoculation with homologous leukocytes. All of the subjects had negative past histories for malaria, jaundice and syphilis. In addition to the physical examination, they were subjected to the usual screening procedures required for donors to a hospital blood bank. The subjects had never received blood transfusions and were free of diseases known to alter immunologic reactivity. ABO and CDE blood groups were determined prior to immunization (Table I). Donor leukocytes were harvested from freshly drawn peripheral venous blood by a dextran sedimentation technique (4). The leukocytes employed were harvested prior to administration. The time lag before injection was related to preparation and varied from slightly under 1 hour to a maximum of 3 hours. In each instance the white blood cells were resuspended in donor plasma, in a total volume of 3 ml. The final sediment was counted and measured and an aliquot was chosen so that 3 ml (30 injections) contained the desired dose. Duplicate chamber counts of the leukocyte yield enabled adjustment of the volume of inoculum so that the predetermined number of white blood cells could be administered. The total number of leukocytes injected varied from 2×10^7 to 48×10^7 (Table II). Subjects 1 and 2 received four subcutaneous doses of 12×10^7 leukocytes at 7-day intervals for a total dose of 48×10^7 leukocytes. On Day 29 of the experiment, 1 day after the last injection, these subjects were grafted with skin from the leukocyte donor and from an unrelated person. As a control of surgical technique, an autograft was placed in close proximity to the two homografts. Because subcutaneous immunization failed to induce accelerated graft rejection, the experimental protocol was changed for Subjects 3 through 16. These volunteers received, as their immunizing dose, 30 intracutaneous leukocyte injections on the back and thighs. Each injection contained, in a volume of 0.1 ml, one-thirtieth of the total leukocyte dose which varied from 2×10^7 to 30×10^7 (Table II). Six days after the multiple intracutaneous inoculations, donor and nonspecific skin grafts were placed as in Subjects 1 and 2 (Figures 1 and 2).

TABLE I

Race, sex and donor blood types in 18 patients immunized with leukocytes

Patient no.	Recipient			Leukocyte donor			Nonspecific donor		
	Race	Sex	Blood type	Race	Sex	Type	Race	Sex	Type
1	N	F	A+	N	M	B+	N	F	A+
2	N	M	B+	N	F	A+	N	F	A+
3	W	F	O+	W	M	B+	W	F	B+
4	W	M	B+	W	F	O+	W	F	B+
5	W	M	A+	W	M	O+	Not done		
6	W	M	O+	W	M	A+	Not done		
7	W	M	A+	W	M	B−	W	M	O+
8	W	M	B−	W	M	A+	W	M	O+
9	W	M	O+	W	M	O+	W	M	O+
10	W	M	O+	W	M	O+	W	M	O+
11	W	M	A+	W	M	A−	W	M	O+
12	W	M	A−	W	M	A+	W	M	O+
13	W	M	A+	W	M	A−	W	M	O+
14	W	M	A−	W	M	A+	W	M	O+
15	W	M	A+	W	M	O+	W	M	O+
16	W	M	O+	W	M	A+	W	M	O+
17	W	M	B−	W	M	A+	W	M	O+
18	W	M	A+	W	M	B−	W	M	O+

All grafts were full thickness skin removed under local anesthesia from the medial aspect of the upper arm and sutured into the host bed with interrupted 5-0 nylon sutures. The wounds were covered with a nonadherent plastic dressing which facilitated frequent inspection, photography and biopsy. In the first 6 patients studied, all of the grafts were 2.5 cm^2. From the data obtained in this group, and from another concurrently studied series (5), it was believed that sufficient information could be gained from a smaller graft to warrant a reduction in size to 1.0 cm^2. The excellent autograft survival in the earlier volunteers caused us to discontinue simultaneous control autografts in Subjects 7 through 18. Material was obtained by biopsy of the graft only and not of the graft bed. In all cases two biopsies were taken at Days 6 or 7 and another on Day 9 or 10. This allowed a mi-

TABLE II

Type of rejection in 18 patients preimmunized with homologous leukocytes

Subject	No. of leukocytes administered $\times 10^7$	Route of injection	Rejection pattern	
			Leukocyte donor graft	Nonspecific*
1	48.0	Subcutaneous	First set	First set
2	48.0	Subcutaneous	First set	First set
3	10.0	Intracutaneous	White graft	Accelerated
4	7.2	Intracutaneous	Accelerated	First set
5	6.5	Intracutaneous	Accelerated	Not done
6	9.0	Intracutaneous	White graft	Not done
7	20.0	Intracutaneous	Accelerated	First set
8	20.0	Intracutaneous	White graft	Accelerated
9	20.0	Intracutaneous	White graft	White graft
10	20.0	Intracutaneous	Accelerated	First set
11	12.0	Intracutaneous	Accelerated	First set
12	12.0	Intracutaneous	White graft	White graft
13	2.0	Intracutaneous	Accelerated	Accelerated
14	2.0	Intracutaneous	First set	First set
15	30.0	Intracutaneous	White graft	White graft
16	30.0	Intracutaneous	Accelerated	Accelerated
17	12.0	Intracutaneous	White graft	Accelerated
18	12.0	Intracutaneous	White graft	Accelerated

* In two instances, the nonspecific graft was an unusually prolonged first set rejection. In Subjects 11 and 14 active rejection was observed to commence by Day 21 after grafting.

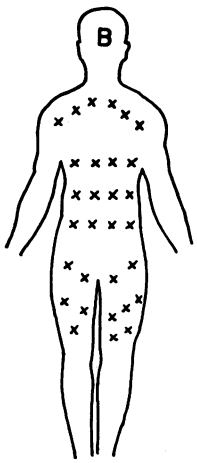

FIG. 1. APPROXIMATE LOCATION OF THE MULTIPLE INTRADERMAL LEUKOCYTE INJECTIONS EMPLOYED TO IMMUNIZE SUBJECTS 3 TO 18. Subject B was intradermally inoculated with leukocytes of Subject A at 30 sites (total volume of inoculum is 3 ml, containing 2 to 30×10^7 leukocytes).

croscopic assessment of the type of rejection; in two cases showing slow rejection, biopsies also were taken on Days 17 and 19, respectively.

Grading of the rejection process

In order to assess properly the changes in altered skin grafts, a series of unmodified first set, accelerated, and white graft rejections was carefully studied (5), by daily inspection and biopsy. A brief summary of the criteria

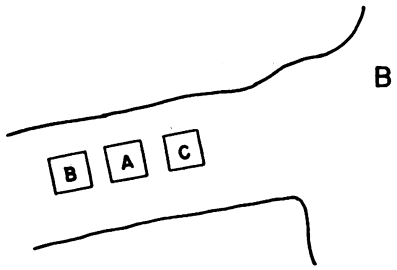

FIG. 2. LOCATION OF DONOR AND NONSPECIFIC SKIN GRAFTS. Six days after multiple intradermal leukocyte injections, Subject B was challenged with full thickness skin grafts from Subjects A and C. B, a control autograft, was also placed. Only the first 6 subjects were given autograft controls. The nonspecific homograft was omitted in Subjects 5 and 6.

used to determine the type of rejection encountered may be subdivided into two sections.

Gross findings. Typical of the first set rejection is early (2 to 3 days) vascularization, detectable by a pink coloration, blanching when pressure is applied, and brisk bleeding upon incisional biopsy. Thereafter, the first set rejection and the autograft are indistinguishable until the sixth to seventh day when the homograft assumes a cyanotic color, fails to blanch on pressure, and has obvious interstitial edema. During the next 5 to 9 days, the now avascular homograft gradually changes into a crusty black scab (Figures 3 and 4).

A white graft, by contrast, never establishes graft-bed vascular anastomosis, the total lack of blood supply being responsible for its peculiar pallor. In time, the white

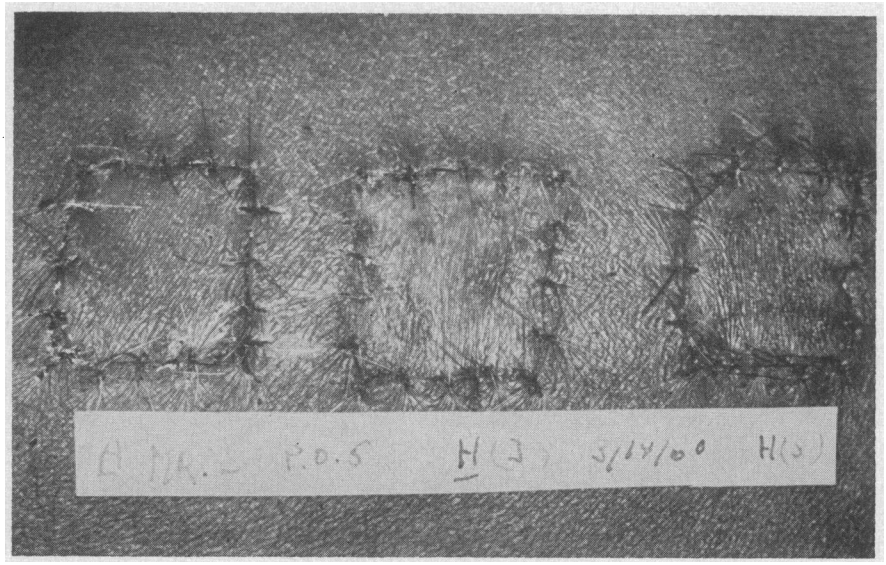

FIG. 3. PHOTOGRAPH OF AUTOGRAFT (LEFT), LEUKOCYTE DONOR SKIN (CENTER) AND NONSPECIFIC SKIN (RIGHT), ON THE FIFTH POSTGRAFTING DAY IN SUBJECT 2. The photograph demonstrates no discernible difference between the auto- and homografts. In this case, subcutaneous leukocyte injections failed to induce homograft immunity.

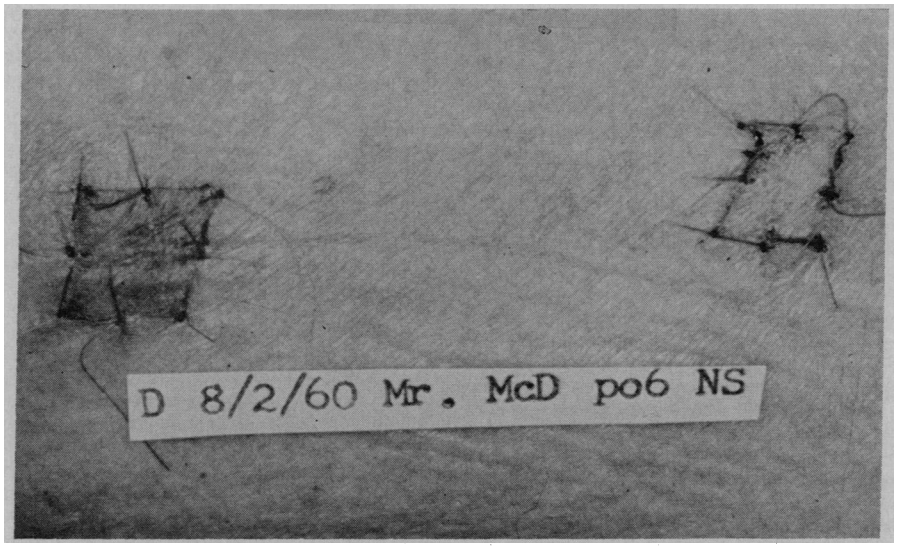

FIG. 4. SUBJECT 10. The leukocyte donor graft (left) is edematous and cyanotic by the sixth postgrafting day, indicating accelerated rejection. The nonspecific graft (right), placed simultaneously 6 days after intradermal leukocyte immunization, resembles the surrounding host skin and is undergoing a first set rejection.

graft appears to melt away as a result of a sterile proteinolysis, although its color remains pale gray as new epithelium granulates in from the surrounding bed margins. Both its striking color and its failure to bleed when incised easily identify the white graft (Figure 5).

The accelerated or second set rejection pattern rapidly recapitulates the events of a primary rejection. After transient but clear early vascularization, cyanosis and edema are discerned by the fourth postgrafting day. By the eighth day the florid rejection is completed and a hard scab remains (Figures 4 and 6).

Microscopic correlation. No distinction between autograft and first set homograft biopsy material is possible for the first 6 days. Further sections of autografts progressively display a gradual return to the appearance of normal skin. Increasing numbers of perivascular lympho-

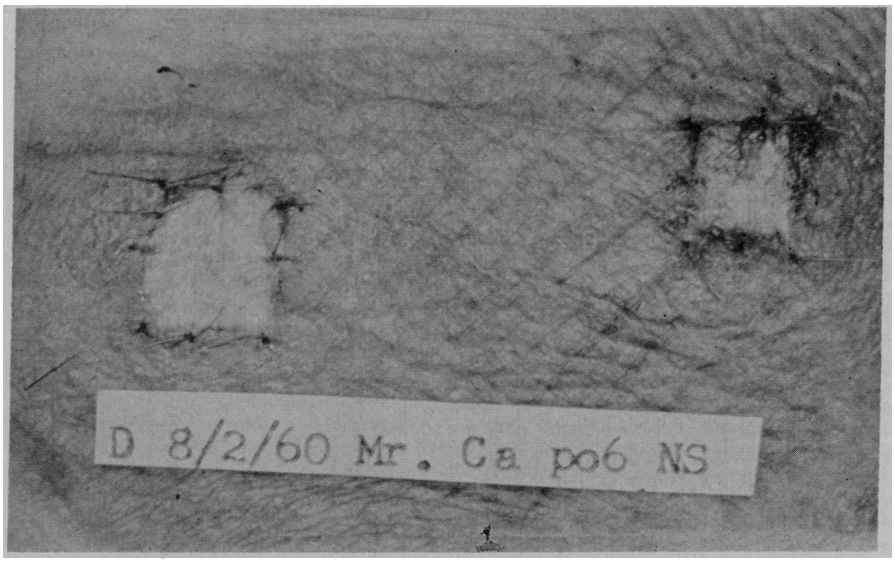

FIG. 5. SUBJECT 8. This subject evinced white graft rejections of leukocyte donor skin (left), and nonspecific skin (right), after intracutaneous preinjection with homologous leukocytes 6 days before grafting. The white graft is interpreted as a manifestation of a very intense transplantation immunity.

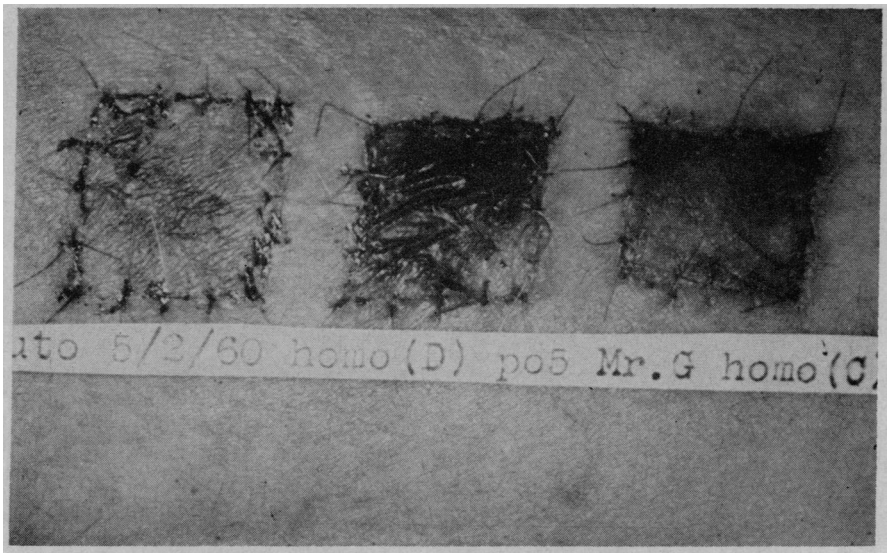

FIG. 6. SUBJECT 4. After the intracutaneous inoculation of 7.2×10^7 leukocytes 6 days prior to grafting, Subject 4 rejected both the leukocyte donor skin (center) and nonspecific control graft (right) in an accelerated fashion. Cyanosis and interstitial edema are present in both homografts while the autograft (left) appears healthy, with a well healed biopsy site in the upper left-hand corner. By Day 5, easy distinction can be made between accelerated and first set rejections (see Figure 3).

cytes, gradually extending into the dermis and epidermis, represent the primary rejection on the seventh to eighth day (Figures 7 and 8). Extensive vascular thrombosis supervenes on the eleventh or twelfth day. Symbolic of a white graft type of rejection is an early polymorphonuclear cell permeation of the dermis and epidermis of the totally avascular graft (Figure 9). The accelerated or second set rejection has a blood supply for only 2 to 3 days. The skin biopsies of grafts undergoing accelerated rejection showed no vascular thrombosis. Presumably the vessels at the junction of graft host tissues are primarily involved, but in this study this junctional area was not examined, since it would have required local anesthetic for each biopsy procedure. There is only slight cellular infiltration prior to the death of the graft on the seventh or eighth day (Figure 8). Patterns intermediate

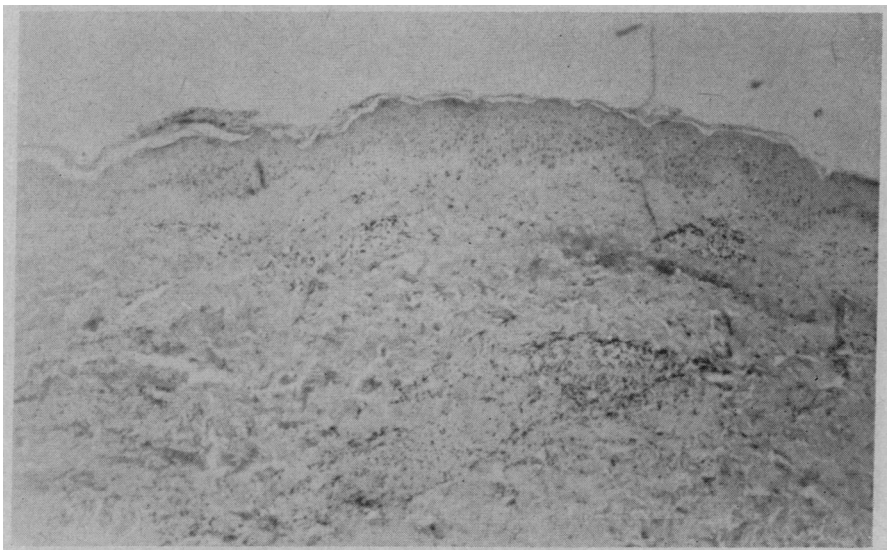

FIG. 7. SUBJECT 7. The earliest sign of primary or first set rejection is perivascular lymphocytic infiltration, here observed on the sixth postoperative day in the nonspecific graft placed on Subject 7 (H&E, × 55).

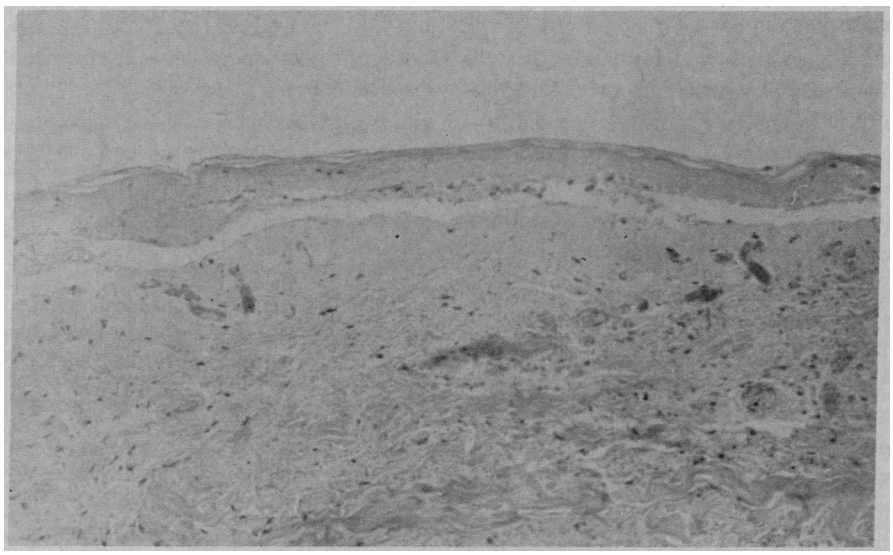

FIG. 8. SUBJECT 4. Contrast the first set rejection shown in Figure 7 with the features of accelerated rejection noted in this biopsy of the nonspecific graft placed on Subject 4 also taken on the sixth postgrafting day. A dead epidermis, dilated blood vessels and the absence of cellular infiltration connote an accelerated rejection (H&E, × 55).

between accelerated and white graft have been observed twice. In these grafts, the gross appearance determined the category ascribed.

RESULTS

There were no technical failures in any of the grafts in this series. Technical failure of a graft is usually due to an underlying hematoma. The graft is raised from the bed and the clot is discovered on biopsy. The graft undergoes escharification and does not have the appearance of a "white graft," which always remains flat. On microscopic section the polymorphonuclear infil-

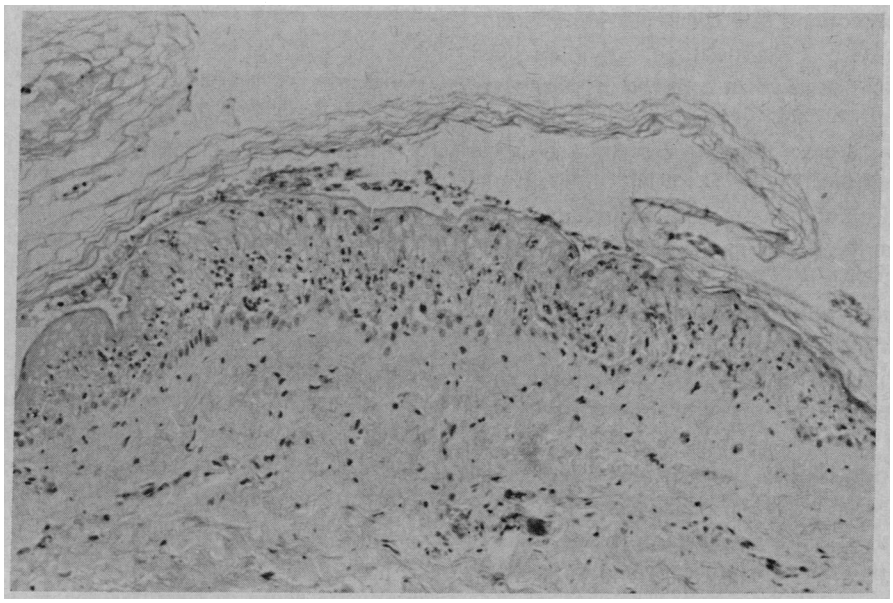

FIG. 9. SUBJECT 3 ON SIXTH POSTGRAFTING DAY; SPECIFIC GRAFT. Diagnostic of a white graft rejection is the early death of the epidermis coupled with dense diffuse infiltration with polymorphonuclear leukocytes (H&E, × 110).

trate, typical of a white graft does not occur in the graft which has been a technical failure because of separation from its bed by a hematoma. The subjects withstood the stresses of multiple inoculations and subsequent skin grafting without untoward event. None of the volunteers suffered temperature elevation, regional adenopathy or wound infection. The third and fourth subcutaneous injections in Subjects 1 and 2 caused the formation of hot sterile abscesses which proved painful for several days although surgical drainage was not necessary. An uncomfortable, local stinging sensation, lasting about 5 minutes, usually followed the intracutaneous inoculations.

Subcutaneous immunization. Repeated subcutaneous injections of a total of 48×10^7 leukocytes prior to grafting with leukocyte donor and nonspecific skin failed to elicit accelerated rejections of any of the homografts placed on Subjects 1 and 2. The gross signs of a first set rejection were apparent in the leukocyte donor skin on Subject 1 on Day 10, and in the nonspecific skin on Day 8. Similarly, Subject 2 evinced a primary rejection of leukocyte specific skin on Day 9, and of nonspecific skin on Day 8. In both instances, the autografts survived normally.

Intracutaneous immunization. Of the 15 patients pretreated with intradermal homologous leukocytes, all but 1 had altered immunologic rejections of subsequent full thickness skin homografts. Illustrative of the modified responses were the white grafts observed in 8 of the 16 leukocyte donor skin grafts in this group. Accelerated second set rejections were noted in 7 of the leukocyte donor skin grafts (Table II). One patient experienced an unmodified first set rejection of the skin graft from the donor of the pregrafting leukocytes. In 14 instances an additional homograft was placed from a donor whose leukocytes had not been used to presensitize the recipient. This nonspecific skin graft was placed at the same time as the graft from the donor whose leukocytes had been injected 6 days previously. Of these 14, white grafts occurred 4 times, accelerated rejections 6 times, and primary or first set rejections were noted in 4 subjects. Examples of each of the rejection patterns encountered are included in Figures 3–9.

There was no obvious correlation between the ABO and CDE blood groups of donor and recipient and the type of rejection response encountered. Thus, O positive patients immunized with O positive leukocytes developed white graft rejections as did recipients who had major blood group incompatibility with their respective leukocyte and skin donors.

DISCUSSION

The pioneer studies of Medawar incontrovertibly established the immunologic basis of homograft rejection (6). It is now known that the ability to reject a second homograft in an accelerated fashion is a fundamental biological process found in all species studied. Early experiments suggested that the homograft reaction is an individual specific response (6). Medawar was able to immunize an animal against skin homografts by the prior injection of blood leukocytes (7), and Billingham, Brent and Medawar showed that second set rejections followed preimmunization of the host with liver, spleen or kidney cell suspensions (8). These same workers later demonstrated that cells totally disintegrated by ultrasonication retained their power to elicit skin transplantation immunity in mice (9).

Our preliminary efforts to locate human transplantation antigens within the circulating leukocyte were not successful. In several unreported experiments, volunteers repeatedly injected intramuscularly with donor whole blood evinced unmodified first set skin rejections. Likewise, Subjects 1 and 2 in this experiment had no gross or microscopic evidence of rejection acceleration subsequent to subcutaneous leukocyte pretreatment. The efficacy of intradermal immunization seen in our data offers further evidence of the variation of species response to the route of antigen administration. Thus, Billingham, Brent and Mitchison found all routes of administration of homologous cells tested in mice and guinea pigs immunologically equivalent in the induction of transplantation immunity, while the rabbit showed a markedly accentuated response to intradermal inoculation as compared with intravenous, subcutaneous or intraperitoneal routes (10).

A second explanation may in part account for failure of leukocyte administration by the subcutaneous route to sensitize the recipient.

The intradermally immunized patients exhibited considerably diversified rejection patterns. At

least two factors determine the intensity of homograft rejection: donor-recipient genetic dissimilarity and the immunizing dosage. Berrian and Jacobs were able to predict the timing of third strain skin homograft rejection in purebred mice following crossed immunization of two other pure strains with splenic cell suspensions (2). By repeated interstrain grafting they were able to calculate the number of transplantation antigens shared by donor and recipient and correlate the onset and intensity of rejection with the degree of genetic disparity of the engrafted pairs. Little is known concerning the number or distribution of human transplantation antigens (11). Merrill and co-workers reported the prolonged survival of a full thickness skin homograft placed on the upper arm of a fraternal twin who shared 23 blood groups with his brother-donor (12). Blood group antigens and transplantation antigens are not, however, inherited *pari passu*. Woodruff and Allan (13) have reported a normal first set rejection between two unrelated individuals with 10 identical blood group antigens. We have seen a normal first set rejection in skin transplanted from one nonidentical twin to another when all but 1 of 27 blood group antigens were identical. It is reasonable to assume that the different types of rejection encountered in several of our patients who received the same number of donor leukocytes was due to the heterogeneity of transplantation antigen distribution in man. In this light, the significance of the unusual prolongation of two nonspecific first set reactions (Table II) is unclear. A recent report by Rapaport, Thomas, Converse and Lawrence describes the prolongation of skin graft survivals in humans "typed" for similarity of transplantation antigens (14). The similarity of transplantation antigens in this instance was surmised when a skin graft from one member of the donor-recipient pair caused accelerated rejection of skin from the second member when an indifferent recipient was used for both grafts. The fact that skin from one accelerated the rejection of skin from another and that subsequently skin exchanged between the two, showed an unusually long survival, which was presumed to be due to a sharing of similar transplantation antigens. A similar observation was made by Kuss and LeGrain (15) in unrelated individuals between whom a kidney transplant with prolonged survival was subsequently done.

There is limited information concerning gradation of rejection intensity dependent upon increasing antigen dosage. Zotikov, Budik and Puza described shorter rejection times with increased size of rat homografts (16). They noted that as the size of the homograft was increased to greater than one-third of the recipient's total skin surface area, "immunoparalysis" with greatly prolonged graft survival was induced. No data pertinent to dose response are available for man. Our series is too small to allow statistical analysis although the data suggest that the degree of immunity evoked by intracutaneous leukocytes injections is, at least in part, dose related. None of the 4 subjects inoculated with less than 9×10^7 leukocytes responded with white grafts to either leukocyte donor or nonspecific challenge grafts, while 8 of 12 patients given at least 9×10^7 leukocytes developed white graft reactions in the graft from the leukocyte donor. Rapaport and associates believe the white graft to be "either a qualitatively different or a more intense form of immune response against foreign tissue perhaps mediated by serum antibody" (3). The importance of circulating antibody in homograft rejection is unclear (17, 18), and its contribution to the formation of the white graft is as yet unknown. If a dose-related antigenic response is present in man, as we believe, then this experiment lends credence to the concept that the white graft is an expression of a greater level of immunity than is an accelerated rejection.

To support this hypothesis further, it is noted that the intensity of rejection in the nonspecific grafts was in each case equal to or less than the degree of immunity displayed in the specific leukocyte donor skin. A white graft in the leukocyte donor graft was accompanied by either a white graft or an accelerated rejection in the nonspecific skin. Analogously, a specific accelerated rejection complemented either an accelerated or first set response in the nonspecific graft.

Therefore, the weaker reactions to nonspecific grafts would be in accord with partial sharing of transplantation antigens between leukocyte donor and nonspecific skin samples. This would also concur with Rapaport and co-workers' study of white graft production in which five of eight non-

specific grafts exhibited significantly reduced survival time (3).

The difficulties inherent in investigations of human transplantation immunity may be somewhat eased by additional attention to the circulating leukocyte. Lawrence, Rapaport, Converse and Tillett meticulously detected the presence of a "factor" capable of transferring homograft immunity in extracts of peripheral white blood cells (19). The data presented here indicate the presence within the same cell population of antigenic constituents competent to induce the known forms of skin homograft rejection.

SUMMARY AND CONCLUSIONS

The intradermal inoculation of peripheral leukocytes prior to full thickness skin grafting induced white graft and accelerated rejection of first set homografts in the human. Simultaneously placed skin grafts obtained from patients other than the leukocyte donor were also rejected in an accelerated fashion, indicating that transplantation immunity in the human is not individual specific. It is postulated that the type of skin graft rejection encountered in this experiment was determined by both the dose of immunizing leukocytes administered and the genetic similarity between donor of immunizing leukocytes and donor of the nonspecific skin graft.

ACKNOWLEDGMENT

We are indebted to Professor Peter B. Medawar for his valuable comments pertinent to the experimental design; Mr. David McMakin offered tireless technical assistance; Dr. Thomas A. Warthin and Dr. Richard A. Warren kindly made available the facilities of the West Roxbury Veterans Administration Hospital where Mr. M. C. Cheney prepared several of the illustrations. Miss Audrey J. Clemens, R.N. handled with warmth and dignity many of the innumerable problems inherent in human experimentation.

REFERENCES

1. Merrill, J. P., Friedman, E. A., Wilson, R. E., and Marshall, D. C. The production of "delayed type" cutaneous hypersensitivity to human donor leukocytes as a result of the rejection of skin homografts. J. clin. Invest. 1961, **40**, 631.
2. Berrian, J. H., and Jacobs, R. L. Diversity of transplantation antigens in the mouse *in* Biological Problems of Grafting, F. Albert and P. B. Medawar, Eds. Oxford, Blackwell, 1959, p. 131.
3. Rapaport, F. T., Thomas, L., Converse, J. M., and Lawrence, H. S. The specificity of skin homograft rejection in man. Ann. N. Y. Acad. Sci. 1960, 87, 217.
4. Friedman, E. A., Bardawil, W. A., Merrill, J. P., and Hanau, C. "Delayed" cutaneous hypersensitivity to leukocytes in disseminated lupus erythematosus. New Engl. J. Med. 1960, **262**, 486.
5. Marshall, D. C., Friedman, E. A., Goldstein, D. P., Henry, L., Merrill, J. P., and Dammin, G. J. The rejection of skin homografts in the normal human subject. Parts I and II. J. clin. Invest. Accepted for publication.
6. Medawar, P. B. The behaviour and fate of skin autografts and skin homografts in rabbits (Report to War Wounds Committee of Medical Research Council). J. Anat. (Lond.) 1944, **78**, 176.
7. Medawar, P. B. Relationship between the antigens of blood and skin. Nature (Lond.) 1946, **157**, 161.
8. Billingham, R. E., Brent, L., and Medawar, P. B. The antigenic stimulus in transplantation immunity. Nature (Lond.) 1956, **178**, 514.
9. Billingham, R. E., Brent, L., and Medawar, P. B. Extraction of antigens causing transplantation immunity. Transplant. Bull. 1958, **5**, 377.
10. Billingham, R. E., Brent, L., and Mitchison, N. A. The route of immunization in transplantation immunity. Brit. J. exp. Path. 1957, **38**, 467.
11. Rogers, B. O. The genetics of skin homotransplantation in the human. Ann. N. Y. Acad. Sci. 1957, **64**, 741.
12. Merrill, J. P., Murray, J. E., Harrison, J. H., Friedman, E. A., Dealy, J. B., Jr., and Dammin, G. J. Successful homotransplantation of the kidney between nonidentical twins. New Engl. J. Med. 1960, **262**, 1251.
13. Woodruff, M. F. A., and Allan, T. M. Blood groups and the homograft problem. Brit. J. plast. Surg. 1953, **5**, 238.
14. Rapaport, F. T., Thomas, L., Converse, J. M., and Lawrence, H. S. Variations in individual specificity in human homograft reactions. Fed. Proc. 1961, **20**, 36.
15. Kuss, R., and Legrain, M. Homologous transplantations of the human kidney: Experience with four patients. Trans. Amer. Soc. artif. intern. Organs 1961, **7**, 116.
16. Zotikov, E. A., Budik, V. M., and Puza, A. Some peculiarities of the survival time of skin homografts. Ann. N. Y. Acad. Sci. 1960, **87**, 166.
17. Gorer, P. A. Some recent work on tumor immunity. Advanc. Cancer Res. 1956, **4**, 149.
18. Amos, D. B., and Wakefield, J. D. Growth of mouse ascites tumor cells in diffusion chambers. II. Lysis and growth inhibition by diffusible isoantibody. J. nat. Cancer Inst. 1959, **22**, 1077.
19. Lawrence, H. S., Rapaport, F. T., Converse, J. M., and Tillett, W. S. Transfer of delayed hypersensitivity to skin homografts with leukocyte extracts in man. J. clin. Invest. 1960, **39**, 185.