THE PRODUCTION OF "DELAYED TYPE" CUTANEOUS HYPERSENSITIVITY TO HUMAN DONOR LEUKOCYTES AS A RESULT OF THE REJECTION OF SKIN HOMOGRAFTS*

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A large body of evidence relates the immune response accompanying the rejection of a skin homograft to the type of immunologic reaction which manifests itself in guinea pigs and human beings as hypersensitivity of the "delayed type" (1). A classic example of this reaction is the delayed type of inflammation and swelling that results from the intradermal injection of tuberculin into a sensitized recipient. Of interest in the homograft reaction is the fact that this tuberculin reactivity can be transferred from one animal to another by means of sensitized lymphoid cells as can transplantation immunity (1). In further support of the analogy between these two types of immunity, Brent, Brown and Medawar have recently shown in guinea pigs that the rejection of a skin homograft is accompanied by a delayed type of cutaneous hypersensitivity to donor leukocytes administered either as a suspension of living cells or as a cell-free extract (2). The reaction elicited by the intradermal injection of donor antigen is called by them "the direct reaction." A similar type of reaction reaching its maximum somewhat more slowly and declining more slowly than the direct reaction is produced by the intradermal injection of cells from the lymph nodes of the sensitized homograft recipient into its donor. This response they call "the transfer reaction." The present experiment reports extension of these observations to humans following the production of transplantation immunity.

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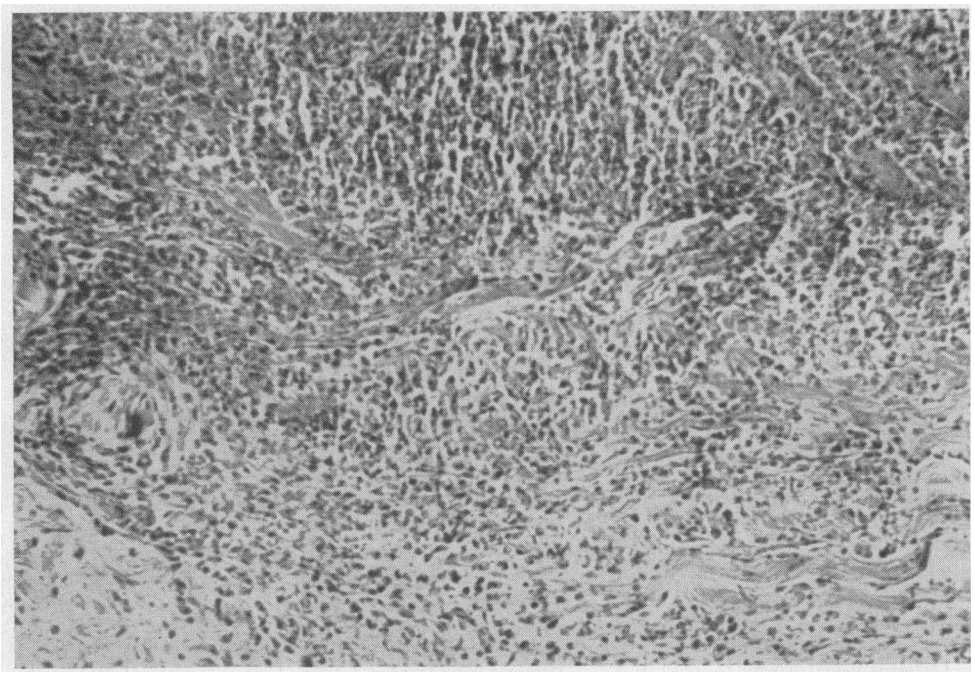
METHODS

Nineteen human volunteers received full thickness skin grafts from 19 donors. Grafts were placed on the upper extremity along with a control autograft. Eight recipients received two grafts and the remainder, one graft. The donor and recipient pairs varied from totally nonrelated individuals to parent and child and closely related dizygotic twins. The time of beginning of gross rejection of the skin grafts, judged by edema and by loss of capillary blanching, ranged from 6 to 26 days. Following the rejection of one or both homografts, intradermal tests were performed in the recipients employing leukocytes harvested from the peripheral blood of the donor. Twenty-five to 30 ml of venous blood was collected in a syringe containing 1 ml (10 mg) of heparin (Liquaemin, Organon) and 1.5 ml of a 20 per cent solution of dextran with an average molecular weight of 188,000.[1] A cork was placed on the needle of the syringe, the syringe inverted and blood allowed to sediment at 4° C. When adequate sedimentation had occurred, a plasma and white cell layer remaining at the top of the sedimented column of blood was expressed slowly through the needle and the plasma and white cell-rich plasma were harvested separately. Donor white cells (average dose, two million cells), donor plasma and donor red blood cells were injected intradermally as were similar quantities of autologous white cells, plasma and red cells. In seven experiments, sonic lysate of donor cells was also injected. This lysate was prepared by the method of Billingham, Brent and Medawar (3), who showed it to contain antigenic material active in accelerating the rejection of transplanted skin. In all cases white cells, plasma and red cells from nondonor humans were also injected. As controls, 51 patients with various forms of disease were injected with autologous and homologous white cells prepared in a similar fashion (4). The reactions were observed at 1, 8, 24 and 48 hours. The criterion for a positive result was an erythematous area with at least a 10 mm area of induration, graded separately by two observers. Characteristically, a positive reaction began at 8 to 12 hours, was maximal at 24, and began to fade at 36 hours. The

[1] Grade H, A. K. Larus Company, Bethlehem, Pa.; lot no. H12757.



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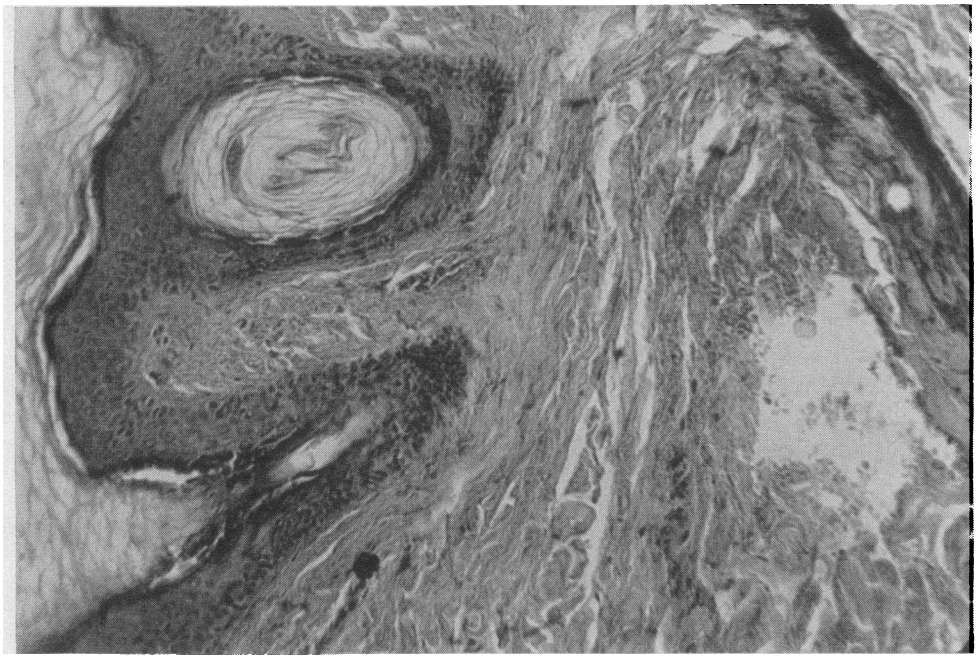

B

FIG. 1. HISTOLOGICAL SECTION OF SKIN BIOPSY FOR "POSITIVE" INTRADERMAL TEST AFTER INJECTION OF DONOR LEUKOCYTES. A. This field represents the deeper dermis; the cellular reaction seems more intense here than in the upper dermis, although the predominant cell is the neutrophilic polymorphonuclear cell. There are also moderate numbers of mononuclear cells and occasional eosinophils. This is the distribution of cells seen in a delayed hypersensitivity reaction at 24 hours (hematoxylin and eosin; magnification × 400). B. Skin-epidermis and upper dermis of skin site shown at 24 hours after inoculation of autologous buffy coat. Little nuclear debris is present and no intact leukocytes are noted. The defect in the lower field is incidental to the inoculation and contains fragmented collagen and occasional erythrocytes (hematoxylin and eosin; magnification × 400).

TABLE I

Results in patients who rejected skin homografts

		Number of grafts (one)	(two)	
Recipients		19	11	8
Positive reaction to intradermal leukocytes	Donor cells > nondonor cells			16
	Donor cells < nondonor cells			3
	Autologous cells			2
Donor lysate	Positive	5		
	Negative	2		

volume of suspended cells was adjusted to 0.1 ml in a tuberculin syringe. Injection of this amount of material may rise to an immediate bleb of 8 to 10 mm, which gradually fades over a period of 6 to 8 hours and is characteristically accompanied by little induration. Only these reactions which *increased* from the twelfth to the twenty-fourth hour were characterized as positive.

RESULTS

The 51 control patients injected with homologous white cells [discussed in a previous paper from this laboratory (4)] failed to react to them. The results of the patients who had rejected skin homografts are summarized in Table I. In 16 instances, the reaction to donor cells was greater than that to nondonor cells, although both were positive by our criterion. In three instances, nondonor cells gave rise to a greater reaction than that caused by specific donor cells. Two individuals showed a positive reaction to their own white cells, but in no instance was the reaction greater than that to donor or nondonor homologous cells. There were no positive reactions to donor red cells or to plasma alone. In five instances, injection of the water-soluble sonic lysates of donor cells induced a positive reaction which was uniformly less pronounced than that to fresh living cells. In all cases wherein reaction to donor lysate was positive, reactions to lysate of nondonor homologous cells were also positive. In two instances, lysate of donor cells failed to cause a positive reaction, whereas whole living cells induced a positive reaction. Histological sections of biopsies through a "positive" reaction and a negative control are shown in Figure 1.[2]

DISCUSSION

The significance of the positive intradermal tests depends largely upon the negative results in the control studies. In our control series, a group of 51 patients with various diseases, including active rheumatic fever, showed negative reactions to both autologous and homologous leukocytes similarly prepared in doses ranging from three to nine million cells per dose. Higher doses than this, even of autologous cells, may give positive skin reactions in normal individuals. Lawrence (5) has reported a higher incidence of positive skin reactions than that found in the present investigation in control patients challenged with homologous leukocytes intradermally. His method of preparation, however, was different from ours in that bovine fibrinogen was employed as the sedimenting agent, and the dosage of leukocytes in each intradermal site was not ascertained. Moreover, the total dosage of leukocytes for each recipient was governed by the requirements for the transfer of delayed sensitivity to bacterial antigens. Holman (6) found positive reactions to the intradermal injection of white cells in one of six patients with rheumatic fever and in two of four patients with coronary occlusion. Six normal individuals and ten other patients with various diseases had negative reactions. However, Holman found positive reactions in two of five patients with rheumatoid arthritis. Two of seven patients with rheumatoid arthritis whom we tested had positive reactions. This latter result is not surprising in view of the positive reactions to the intradermal injection of autologous and homologous leukocytes in patients with disseminated lupus erythematosus (4) and of the immunologic "overlap" between the two diseases. Holman's technique differed from ours in using homogenates of autologous cells rather than whole cells, and this fact might explain in part the difference from our results. It can only be re-emphasized, therefore, that under the particular circumstances of our experiment, the 51 control patients showed negative reactions to the intradermal injection of homologous leukocytes.

This work appears to confirm in humans the results Brent and associates obtained in the guinea pig (2). In our work, however, only the "direct reaction" was tested. The number of positive reactions with nondonor homologous cells suggests that the reaction is not entirely specific for donor tissue. This finding is contrary to the results in the single human reported by Martin, Waite and

[2] Dr. Gustave J. Dammin has kindly prepared and described the histological material.

McCullough (7). These authors produced a delayed cutaneous reaction of hypersensitivity to viable donor leukocytes by their intradermal administration to a patient with hypogammaglobulinemia, 250 and 266 days after transplantation of lymph nodes from the donor. In their case, injection of autologous leukocytes caused no reaction, and a similar result was obtained with an equal number of leukocytes derived from three normal nondonors. The number of leukocytes employed, however, was only one-third of the number used in our experiments. Brent and coworkers (2) mention in the discussion of their work with guinea pigs that the reaction "depends upon the sensitization toward antigens present in animals whose tissues provoke the state of sensitivity in the first instance." They go on to say that the presence of these antigens in the donor does not imply that they are absent from any other guinea pig chosen at random from a heterogeneous stock. Since our human patients were certainly "heterogeneous stock," it is not surprising that this reaction is not entirely "individual specific." Actually, in the work reported by Brent and associates, the intradermal reaction was uniformly positive for the donor strain but was not tested against leukocytes from other strains of guinea pigs from which a cross reaction might have been obtained (2). The lack of individual specificity for the homograft donor is of particular interest as it relates to the rejection of homografts by man. The actual rejection of cutaneous homografts appears to be a highly individual, specific phenomenon in both purebred strains of mice and in man. The previous rejection of a skin graft in both species will result in an immunity which causes the accelerated rejection of a second graft from the same donor. However, preliminary results from our laboratory suggest that previous immunization of humans by the intradermal injection of donor leukocytes results not only in accelerated rejection of skin from the specific donor but of skin from nondonors. This fact, plus the results mentioned above, suggests that immunization with peripheral leukocytes is not as specific as immunization by transplantation of skin. This fact may be due perhaps to a difference in antigenic structure between skin and leukocytes or possibly to the degradation of their antigenic structure by processing of the leukocytes. The lack of consistency in our results might also be explained by the fact that the antigens in our studies were peripheral leukocytes, a heterogeneous cell population, probably of more varying antigenicity when compared with the more homogeneous cell population of the lymph nodes and spleen preparations of Brent and co-workers. Our preliminary results with extracted antigen differ from Brent's in that the reactions were considerably weaker and less specific. Brent states that "extracted splenic antigen gives more regular results in the direct reaction than living donor cells and is to be preferred on all counts except speed and convenience of preparation" (2). In Brent's experiments with guinea pigs, the cell-free antigen extracted from 50 mg of splenic tissue elicited a result comparable to that of 12 million living cells. In our experiments, however, with a much larger subject (man) only two million cells were used. In the work of Billingham and associates, the antigenicity of the cell-free extract was only 1/100 as potent per milligram of nitrogen as that of living cells (3). The antigenic material in our lysate preparation must have been considerably less potent for the recipient than that used in Brent's experiments. In part, therefore, our divergent results with a limited number of injections of lysate may be accounted for by differences in dosage and by the effect of the method of the preparation upon the potency of the material. This idea is supported by our observations that, where injection of lysate gave a positive reaction, the injection of living cells was invariably positive and that, in two instances in which the injection of lysate was followed by a negative reaction, the living cells gave a positive reaction.

The relation of the mechanism of the rejection of the skin homograft and that of delayed hypersensitivity has been the subject of some speculation. It is possible that a weak state of immunity results in the rejection of a first set homograft and in the production of a delayed type of intradermal sensitivity, whereas a hyperimmune state associated with circulating serum antibodies may result in the direct or "Arthus" phenomenon (1) and in the nonvascularized "white graft" in the case of skin transplantation (8). It is possible that both these mechanisms may simply be variants of the same immune response differing only

in the quantitative aspect. If this is true and if both the classical homograft rejection and the delayed type of cutaneous sensitivity depend upon contact of antigen with a previously sensitized lymphocyte, the delay in both instances may represent time for liberation of antigen (in the case of the injected intradermal leukocytes) plus the accumulation of "sensitized" lymphocytes from the circulating lymphocyte population. One other point might be included in comparing the present results to animal work insofar as an analogy between homograft immunity and other types of "delayed sensitivity" is concerned. Intravenous injection of antigen does not give rise to delayed hypersensitivity, whereas the intradermal or subcutaneous injection of antigen does. Similarly, the placing of a skin homograft, which is essentially a subcutaneous antigen, causes accelerated rejection of a subsequent homograft from the same donor; whereas prior intravenous injection of epidermal cells causes, if anything, an increase in the survival time of subsequently transplanted skin (9). It is of interest in this regard that among our 51 controls were 10 patients who had received multiple transfusions presumably containing white blood cells and that none of these recipients of intravenous white cells had a subsequent positive cutaneous reaction to the intradermal injection of leukocytes.

Brent and associates have suggested that the delayed type hypersensitivity reaction may be common to the tuberculin test, to the homograft reaction, and to the autoimmune diseases. Such a disease is disseminated lupus erythematosus in which circulating antibodies are found which react with the patient's own nuclear material. If such nuclear material were injected intradermally, therefore, one might expect a similar type of delayed hypersensitivity. This finding has proved to be the case (4).

SUMMARY

The immunity occasioned by the rejection of a skin homograft in man is manifested by delayed intradermal sensitivity following injection of donor white blood cells. Unlike the immunity occasioned by the rejection of a previous skin homograft itself, under the conditions of our experiments, this reaction is not specific for donor leukocytes but may be elicited by the injection of nondonor leukocytes into a sensitized recipient.

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