CUTANEOUS HYPERSENSITIVITY REACTIONS TO CELLULAR ISOANTIGENS IN RATS*

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(Received for publication 8 May 1967)

In some species, sensitivity to homografts may be demonstrated by means of delayed cutaneous reactions of the tuberculin type. These reactions have been studied extensively—first in guinea pigs (1), then in rabbits (2) and man (3), and more recently in hamsters (4) and dogs (5). For reasons which are obscure, rats have been regarded as unsatisfactory subjects for expressing transplantation immunity in this useful manner. However, Flax and Waksman (6) and others[1] have established the rat's ability to mount delayed cutaneous reactions to purified protein derivative (PPD), bovine gamma globulin, and other defined antigens. To elucidate this apparently paradoxical situation, a systematic study has been carried out to determine the capacity of various types of homologous cellular inocula to incite cutaneous responses in normal or specifically sensitized rats, making use of isogenic strains.

In the experiments reported here, it has been shown that rats are capable of displaying cutaneous reactivities resembling those obtained in other species. An attempt has been made to define the role of factors such as immunogenetic disparity, circulating isoantibodies, and host participation in the development of the cutaneous lesions.

Materials and Methods

Experimental Animals.—The rats employed belonged to domestically maintained sublines of the isogenic Lewis, Fischer, and DA strains. Lewis and Fischer animals are of albino phenotype, and DA rats are of agouti phenotype. These three strains differ from each other at numerous histocompatibility loci (7). However, at the important Ag-B locus, the Lewis and Fischer strains appear to be identical, having the Ag-B,1 determinant allele whereas the DA strain possesses the Ag-B,4 allele.[2] Like the H-2 alleles in the mouse, these Ag-B alleles were originally detected as red cell isoantigens. Subsequently, they were also shown to determine

* This work was supported by U. S. Public Health Service Grant AI-07001-01. Dr. Streilein is a Pennsylvania Plan Scholar.

[1] Streilein, J. W., and S. Zakarian. Study of delayed cutaneous hypersensitivity reactions to lyophilized tubercle bacilli, PPD, and bovine gamma globulin in guinea pigs, rats, and hamsters. In preparation.

[2] Palm, J. Personal communication.

strong histocompatibility antigens. After the rejection of a homograft bearing these antigens, circulating isoantibodies usually appear in the host's serum that are both cytotoxic and hemagglutinating. By employing appropriately absorbed antisera in genetically defined backcross populations of rats, Palm (8) and others (9) have been able to demonstrate four alleles at the Ag-B locus distinguished by antigenic specificities designated Nos. 1, 2, 3, 4.

Lymph Node Cell Suspensions.—These were prepared according to the method of Billingham and Silvers (10). Cells for inoculation were suspended at suitable concentrations in Hanks' balanced salt solution.

Sensitization of Animals.—Sensitization was carried out by grafting with homologous skin. Following the rejection of these grafts, each animal received a booster injection intradermally of 30–50 $\times$ 10^6 lymphoid cells of the same genetic origin as the skin homograft.

Isoantisera.—These were prepared by boosting specifically sensitized animals with homologous lymphoid cells on three successive weeks. 1 wk after the final inoculation, blood was removed by cardiac puncture. Serum was obtained and tested for its lymphocytotoxic and hemagglutinating activity.

Lymphocytotoxicity tests were performed by mixing suspensions of lymph node cells with appropriate dilutions of antiserum in the presence of fresh normal guinea pig serum as a source of complement (11). The cells were incubated for 30 min at 37°C and then scored microscopically, using trypan blue dye exclusion as the criterion for their viability. Each serum used in these experiments had a cytotoxic titer of 1:80 or greater.

Hemagglutination was performed according to the method of Palm (12). Erythrocytes were washed free of plasma and then suspended in saline containing 1% polyvinylpyrrolidone. These cell suspensions were then mixed with heat-inactivated isoantiserum and incubated at room temperature for 2 hr. Following brief centrifugation (200 *g* for 90 sec), the sedimented cells were swirled up with saline. Readings were made macro- and microscopically on each test.[3]

Skin Testing and Scoring.—Rats to be skin tested were anesthetized with chloral hydrate (13) and their abdominal skin clipped and shaved between xiphoid and pubic symphysis. Standard 0.1 ml volumes of cell suspensions were inoculated intradermally via a #27-gauge hypodermic needle. Each host received from 4 to 14 separate inoculations in the prepared skin, at least four replicates of each type of inoculum being administered in each experiment.

Skin tests were read at 16, 24, 48, 72, 96, and 120 hr after inoculation with the animals under ether anesthesia. Reactions were scored on a 0 to 4+ scale based on amount of edema and induration (14). Scores for replicates of each inoculation type at the various times were averaged.

Whole-Body X-Irradiation.—Irradiation was carried out with a 200-kv machine. Animals to be irradiated were placed at a distance of 62 cm from the source and received the prescribed number of rads through a ½ mm aluminum filter.

EXPERIMENTS AND RESULTS

Delayed cutaneous hypersensitivity reactions in guinea pigs and hamsters may be incited under a variety of circumstances, depending upon (*a*) the source

[3] When DA rats are immunized with Lewis tissue antigens, cytotoxic and hemagglutinating antibodies appear. With one rare exception (and this may be dependent upon the method of immunization), these antibodies are specific for the antigen of the Ag-B,1 allele. Although the antisera utilized in these experiments to detect the Ag-B phenotype have not been compared directly with Palm's, it seems highly likely that they contained only Ag-B specificity since (1) erythrocytes from 50% of a DA $\times$ (DA $\times$ Lewis)F_1 backcross population were agglutinated by this serum, suggesting the detection of a single antigen; and (2) skin grafts from these same animals were uniformly rejected in 11 days or less by DA hosts.

of cells in the inoculum, (*b*) the extant state of specific immunity of cell donor and/or host, and (*c*) the immunogenetic disparity between donor and host. The capacity of rats to manifest the following types of skin reactions were evaluated: *direct reactions*, *transfer reactions*, *normal lymphocyte transfer reactions*, and *lymphocyte supplemented skin reactions*. Each of these reactivities will be defined in the section with which it is related. Lymphocytes may perform two entirely different functions in the skin reactivities under study, i.e., they may serve as vehicles of transplantation isoantigens or as immunologically competent cells. Consequently, F_1 hybrids derived from donor-host strain matings have been utilized when appropriate to allow separate evaluation of donor and host components.

Preliminary experiments indicated that the minimum standard inoculum dosage required to evoke direct reactions in sensitized rats was 40 million cells, whereas 10 million lymphoid cells are adequate for this purpose in guinea pigs (1). With this high dosage of lymphoid cells, control inocula of isologous cells on occasion incite the development of inflammatory lesions. The results of all experiments in which control inocula incited nonspecific responses were therefore disregarded.

In rats, delayed cutaneous hypersensitivity reactions tend to reach peak intensity between 18 and 48 hr and, with the exception of *transfer reactions*, fall off sharply thereafter. At the time of maximal response, the lesions are only faintly erythematous, being characterized by considerable edema and a striking degree of induration. The most intense reactions culminate in ulceration.

Direct Reactions.—When cellular isoantigens in the form of viable lymphocytes from a donor of one strain are injected into the skin of a host rat, guinea pig, or hamster of a different strain previously sensitized to the first, a delayed inflammatory reaction ensues at the inoculation site. This reaction appears to be primarily a local host-versus-graft reaction and, as such, can be utilized to determine qualitatively the host's preexistent state of specific hypersensitivity.

When Lewis rats sensitized to DA tissues (Lewis-anti-DA) were challenged with a standard inoculum of 40×10^6 DA lymphocytes, inflammatory reactions developed at the test sites, reached a peak intensity of 3.75+ at 48 hr, and then gradually dissipated (Fig. 1). Although it seems reasonable to assume that this reactivity is primarily a measure of the sensitized Lewis host's ability to react with the DA antigens, it may have another component. The inoculated DA cells are immunologically competent and are thus capable of initiating in their own right a local graft-versus-host reaction. This complication was obviated by using (DA $\times$ Lewis)F_1 hybrid animals as the source of DA cellular antigens. These cells are genetically incapable of reacting against Lewis antigens. The 2.75+ reactions incited by the inoculation of hybrid cells into Lewis-anti-DA hosts (Fig. 1 *a*) can be ascribed in their entirety to host-versus-graft reactivity. The time course of these reactions differed from that of the direct

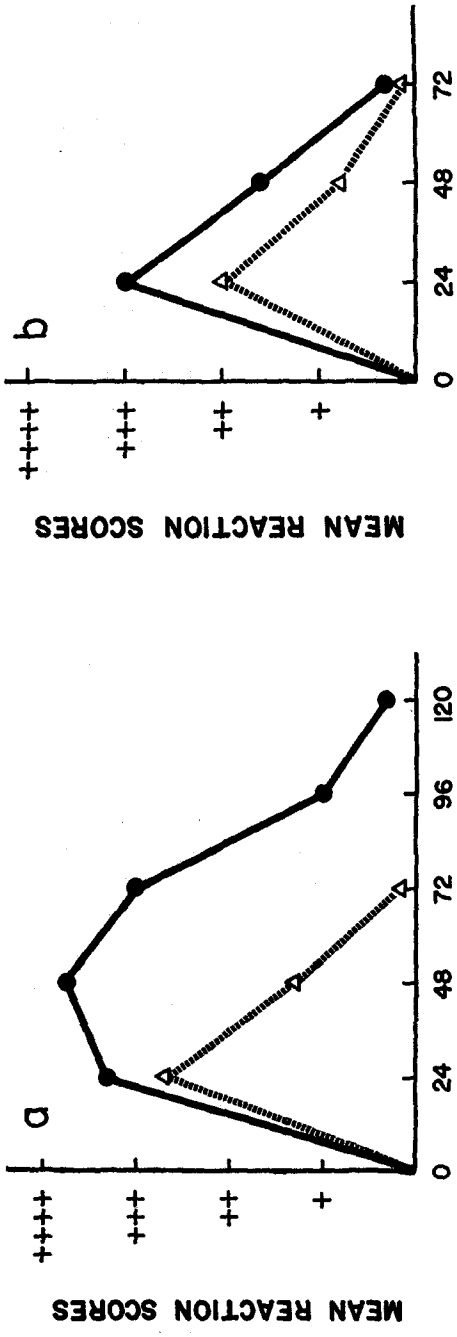

FIG. 1. Direct reactions. (*a*) Incited by DA lymphoid cells ●—●; and (DA × Lewis)F_1 lymphoid cells △---△; in Lewis hosts sensitized against DA tissue antigens. (*b*) Incited by Fischer lymphoid cells ●—●; and (Lewis × Fischer)F_1 lymphoid cells △---△; in Lewis hosts sensitized against Fischer tissue antigens. Each inoculum contained 40 × 10^6 cells.

reactions incited by the inoculation of DA lymphoid cells, since the reactivity had all but disappeared at 72 hr, whereas reactions incited by DA lymphoid cells persisted beyond 96 hr.

To determine whether a difference at the Ag-B locus is essential for the occurrence of direct reactions, Ag-B compatible Lewis and Fischer rats were used. As illustrated in Fig. 1 *b*, direct reactions do occur but are much weaker than in the DA and Lewis combination. In fact, the time courses of the reactions were similar irrespective of whether F_1 hybrid or homologous-strain cells were inoculated into specifically presensitized hosts. This finding is consistent with the observation that, when donor and host are compatible at the Ag-B locus, cells from unsensitized donors do not incite graft-versus-host reactions (15).

Transfer Reactions.—These reactions are incited by inoculating specifically sensitized lymphoid cells into the skins of animals against which the sensitivity is directed. It is assumed that the transferred cells recognize the foreign isoantigens in their host's skin and, reacting against them, initiate an inflammatory response similar to the direct reaction. In Figs. 2 *a*, and 2 *b*, results of experiments using various donor/recipient combinations are presented. Where Ag-B locus disparities are involved, transfer reactions are quite intense, reaching 3–4+ by 48 hr. As with the direct reaction, transfer reactions are much weaker where donor and recipient are alike at the Ag-B locus. This is exemplified by the results obtained with the Lewis-Fischer combination (Fig. 2 *b*).

Of particular interest is the observation that, even when F_1 hybrids were employed as hosts, transfer reactivity persisted well beyond 96 hr when there was an Ag-B locus disparity.

Normal Lymphocyte Transfer Reaction.—Lymphoid cells from a normal, unsensitized donor of one strain inoculated into the skin of a normal, unsensitized homologous recipient may also provoke a delayed inflammatory reaction. Usually this is of lesser intensity than direct or transfer reactions. It has been suggested that the inoculated lymphoid cells undergo primary sensitization in the host's skin, initiating a local graft-versus-host reaction (16). If the recipient is genetically and immunologically competent, it may of course mount a compounding host-versus-graft reaction at the inoculation site. Consequently, the resulting gross lesion may be the sum total of these responses; and interpretation of host or donor contribution is hazardous unless either can be evaluated singly. By employing appropriate F_1 hybrid animals as recipients, it was possible to evaluate the donor component of these reactions. Lymphocytes from Lewis donors characteristically incited 2–2.5+ reactions in the skins of (DA × Lewis) F_1's (Fig. 3 *a*). On the other hand, barely perceptible lesions resulted when (Lewis × Fischer) F_1 hybrid hosts were inoculated with lymph node cells from either of their parental strains (Fig. 3 *b*). As might be expected, Lewis lymphoid cells inoculated into DA recipients (and vice versa) evoked intense reactions (Fig. 3 *a*). Lewis cells inoculated into Fischer rats' skins incited similar

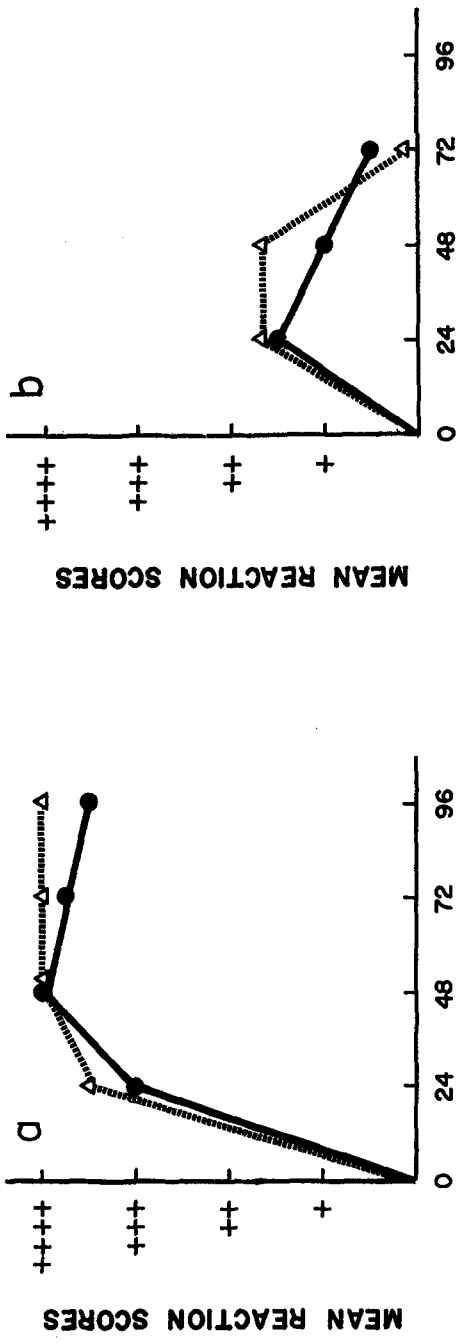

FIG. 2. Transfer reactions. (*a*) Incited in Lewis hosts ●—●; and (DA × Lewis)F_1 hosts△—△; by inoculation of lymphoid cells from DA donors sensitized against Lewis tissues. (*b*) Incited in Fischer hosts ●—●; and (Lewis × Fischer)F_1 hosts △—△; by inoculation of lymphoid cells from Lewis donors sensitized against Fischer tissues. Each inoculum contained 40×10^6 cells.

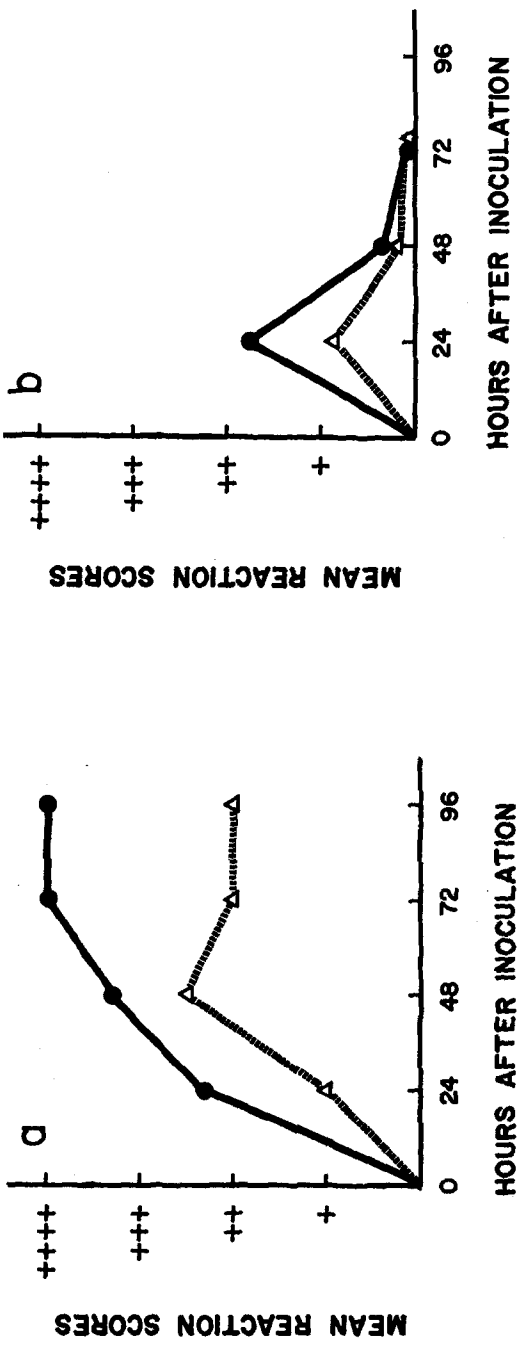

FIG. 3. Normal lymphocyte transfer reaction. (*a*) Incited by inoculations of Lewis lymphoid cells into DA hosts ●—●; and (DA × Lewis)F_1 hosts △—△. (*b*) Incited by inoculation of Fischer lymphoid cells into Lewis hosts ●—●; and into (Lewis × Fischer)F_1 hosts △—△. Each inoculum contained 40×10^6 cells.

though less intense reactions. Unlike the DA and Lewis combination, however, the resulting lesions faded quite promptly after the initial flare at 24 hr (Fig. 3 *b*).

Influence of X-Irradiation on Host's Capacity to Sustain Cutaneous Reactions.—In an effort to define more precisely the extent of host participation in these delayed skin reactions, experiments were carried out in which the host was exposed to 1500 R whole-body irradiation prior to skin testing. As shown by the accompanying Fig. 4, this dose of X-irradiation is sufficient to obliterate virtually the entire circulating lymphocyte population of a rat. Irradiated and lymphopenic animals were challenged intracutaneously 24–28 hr after X-

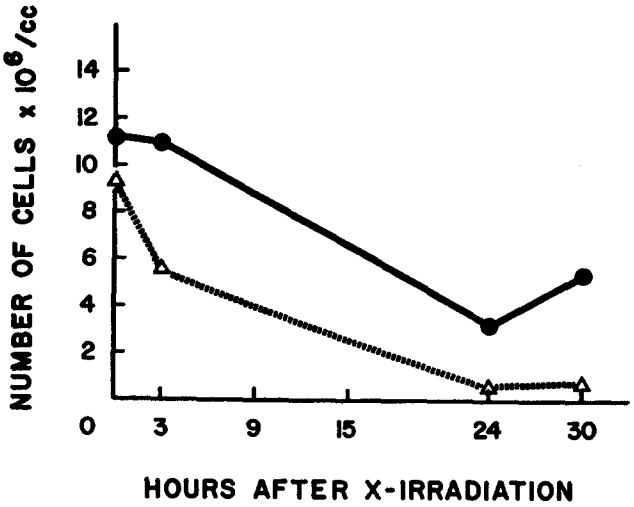

FIG. 4. Peripheral blood leukocyte response of Lewis rats exposed to 1500 R whole-body X-irradiation. Total leukocytes ●—●; total lymphocytes △---△.

irradiation. These irradiated hosts generally died within 96 hr after being irradiated.

(*a*) *Effect on direct reactions:* Irradiation of sensitized Lewis-anti-DA animals severely depressed their capacity to give direct reactions following intracutaneous challenge with (DA × Lewis)F_1 lymphoid cells (Fig. 5 *a*). An attempt to "reconstitute" this reactivity by mixing F_1 cells with normal Lewis lymph node cells failed, although mixing specifically sensitized Lewis-anti-DA lymph node cells with F_1 cells led to full restitution of the skin reactivity in irradiated hosts.

(*b*) *Effect on transfer reactions:* Despite whole-body irradiation of (DA × Lewis)F_1 hosts, transfer reactions were incited by subsequent inoculation of 40×10^6 Lewis-anti-DA lymphoid cells, reaching 3.5+ intensity. This level of

reactivity is similar to that reached in unirradiated F_1 hybrids (Fig. 5 *b*). However, the time courses of these reactions in normal and irradiated hosts differed: after peaking at 24 hr, the transfer reactions in irradiated hosts subsided rapidly, whereas those induced in unirradiated hosts persisted beyond 96 hr.

(*c*) *Effect on normal lymphocyte transfer reactions:* Lethal X-irradiation of (DA × Lewis)F_1 hybrid animals totally prevented the subsequent development of a *normal lymphocyte transfer reaction* following the inoculation of normal Lewis lymphoid cells (Fig. 5 *c*). Attempts to restore the reactivity by adding F_1 lymphoid cells to the Lewis cell inoculum were unsuccessful.

Lymphocyte-Supplemented Skin Reactions.—It was possible to increase the intensity of a normal lymphocyte transfer reaction incited by Lewis lymph node cells injected into the skins of (DA × Lewis)F_1 hybrids by the simple expedient of adding F_1 lymphoid cells directly to the Lewis cell inoculum. The intensity and time course of the resulting lesions resembled those of transfer reactions incited by inocula of Lewis-anti-DA cells in F_1 hybrid skin (Fig. 6). "Lymphocyte-supplemented" skin reactions have previously been studied in rats, hamsters, and guinea pigs[1] and are of particular interest because of the nature of their time course. An accelerated development of these reactions might have been expected. However, when F_1 cellular antigens were added to the Lewis node cell inoculum, cutaneous reactions developed no more swiftly than when Lewis-anti-DA lymphoid cells were injected into F_1 hybrid skin.

Evaluation of the Transfer Reaction as a Means of Predicting Histocompatibility.—It has been assumed that those factors responsible for the development of delayed cutaneous hypersensitivity reactions are similar or identical to the isoantigenic factors responsible for the rejection of tissue homografts. This assumption has given rise to at least two histocompatibility matching tests based on cutaneous reactivities (16, 17). To determine whether, in the rat, there is in fact a relationship between the intensity of a skin reaction, such as a transfer reaction, and the homograft-induced immunity responsible for it, experiments were carried out based on the hypothesis that the intensity of transfer reactions should correlate inversely with the survival times of skin homografts exchanged between donor and host.

A backcross (R_2) population of rats was obtained by mating (DA × Lewis) F_1 hybrids with DA-strain animals. Since the offspring possess the full spectrum of DA transplantation isoantigens, they are genetically tolerant of grafts of DA lymphocytes (as intracutaneous inocula). However, the skins of these R_2 animals will express alien Lewis transplantation isoantigens to a variable degree dictated by the genetic law of independent assortment. Consequently, any delayed inflammatory reaction that results from the intracutaneous inoculation of DA-anti-Lewis lymphoid cells into R_2 hosts must be the result of a one-way immunologic attack on the part of the inoculated cells against foreign Lewis-strain antigens confronting them in the host's skin.

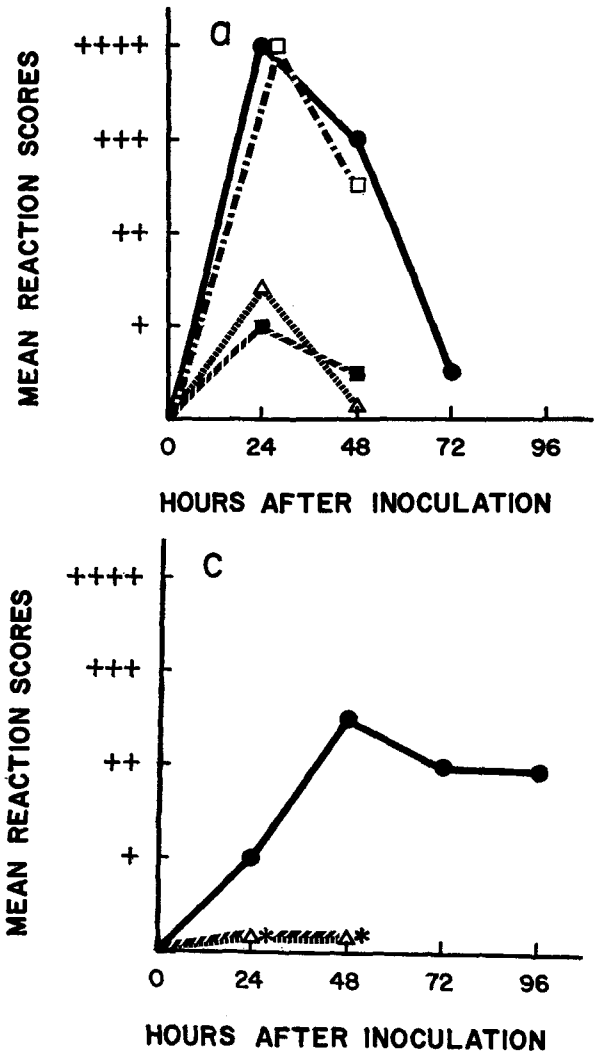

FIG. 5. Effect of whole-body X-irradiation on cutaneous reactivities. (*a*) Direct reactions in Lewis hosts sensitized with DA tissue antigens; (Lewis × DA)F_1 lymphoid cells inoculated into unirradiated hosts ●—●; and into irradiated hosts △---△. Each inoculum contained 40 × 10^6 cells. (DA × Lewis)F_1 + Lewis lymphoid cells ■---■; and (DA × Lewis)F_1 + Lewis-anti-DA lymphoid cells □---□; inoculated into irradiated hosts. Each inoculum contained 20 + 20 × 10^6 cells. (*b*) Transfer reactions incited by lymphoid cells from Lewis donors sensitized to DA tissue antigens; inoculated into unirradiated (DA × Lewis)F_1 hosts ●—●; and irradiated (DA × Lewis)F_1 hosts △---△. Each inoculum contained 40 × 10^6 cells. (*c*) Normal lymphocyte transfer reaction incited by Lewis lymphoid cells in unirradiated (DA × Lewis)F_1 hosts ●—●; and irradiated (DA × Lewis)F_1 hosts △---△. Each inoculum contained 40 × 10^6 cells. Mixtures of (DA × Lewis)F_1 + Lewis lymphoid cells in irradiated hosts ✳----✳. Each inoculum contained 20 + 20 × 10^6 cells.

In the first phase of the experiment, a skin graft was removed from the abdomen of each of 33 R_2 rats and transplanted to a normal DA host. The survival time of each R_2 graft on its DA host was a measure of the immunogenetic disparity between the donor animal and the DA strain. After the donor site had healed, each R_2 animal was challenged by intradermal inoculation, in four replicates, of 40 × 10^6 DA-anti-Lewis lymphoid cells. A summary of the R_2 skin homograft survival times and the scores of these transfer reactions in the skins of the R_2 donor animals at initial peak reactivity and at the secondary

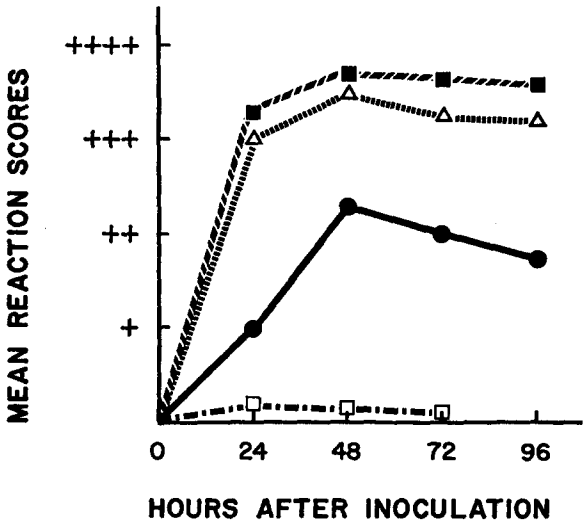

FIG. 6. Lymphocyte-supplemented skin reactions in (DA × Lewis)F_1 hosts incited by Lewis lymphoid cells ●—● (40 × 10^6); Lewis + (DA × Lewis)F_1 lymphoid cells △---△; and Lewis-anti-DA + (DA × Lewis)F_1 lymphoid cells ■---■. 20 + 20 × 10^6 cells per inoculum. (DA × Lewis)F_1 lymphoid cells □---□.

peak at 96 hr is presented in Table I. The Ag-B phenotype of each R_2 animal as determined by hemagglutination is also listed in this table.

A comparison of the R_2 skin homograft survival times with the Ag-B phenotypes of the donor animals indicates that grafts from animals expressing the Lewis Ag-B,1 allele were rejected by their DA hosts within 11 days—usually by the 8th day. With two exceptions (Nos. 13, 17), grafts from R_2 animals homozygous for the DA Ag-B,4 allele were not rejected until the 12th day or later.

The initial peak transfer reaction scores, attained within 24–48 hr, showed only a very poor inverse correlation with the homograft survival times. Of the 18 R_2 animals bearing the Lewis Ag-B,1 antigen, the mean initial peak transfer reaction score was 3.5+ and skin grafts from these animals were consistently rejected by their DA hosts within 11 days or less. The 15 R_2 animals which

TABLE I

Comparison of Survival Times of Skin Homografts from (DA × Lewis) F_1 × DA Backcross Rats on Normal DA Hosts with (a) the Peak Intensities of Transfer Reactions Incited in Skins of the Backcross Donors by Inoculation of DA-Anti-Lewis Lymphoid Cells, and (b) the Ag-B Phenotype of each R_2 Rat

R_2 animal number	Survival time of R_2 skin on DA hosts	Intensity of transfer reaction incited by inoculation of DA-anti-Lewis lymphoid cells into R_2 animals*		Ag-B phenotype
		Initial peak‡	96–120 hr peak	
	(*days*)			
5	27	3	1	4/4
25	21	2	0	4/4
9	20	4	2	4/4
21	16	3	1	4/4
3	15	3	0	4/4
15	15	2	0	4/4
30	15	2	0	4/4
12	14	3	0	4/4
35	13	3	0	4/4
38	13	3	0	4/4
18	12	2	1	4/4
19	12	2	0	4/4
33	12	3	0	4/4
1	11	4	4	1/4
28	11	4	4	1/4
32	11	4	4	1/4
13	10	2	0	4/4
17	9	3	1	4/4
4	9	4	3	1/4
39	9	4	2	1/4
7	8	3	1	1/4
31	8	3	1	1/4
34	8	4	2	1/4
29	8	2	2	1/4
22	8	3	2	1/4
37	8	2	2	1/4
16	8	3	2	1/4
36	8	3	3	1/4
11	8	4	3	1/4
26	8	3	3	1/4
40	8	4	3	1/4
6	8	4	4	1/4
14	8	4	4	1/4

* Average scores of 3–4 replicates: 0–<1 = 0; 1–<2 = 1; 2–<3 = 2; 3–<4 = 3.

‡ Initial peak skin reactivity invariably was attained between 24 and 48 hr.

were homozygous at the Ag-B locus for the DA allele gave a mean initial peak transfer reaction score of 2.5 and, despite the considerable range of survival times of their grafts on DA hosts (9–27 days), there was no discernible correlation between transfer reaction scores and graft survival times.

However, when transfer reactions in these backcross animals were followed beyond 48 hr, an interesting phenomenon was observed. With the exception of two animals (Nos. 7, 31), every R_2 animal bearing the Lewis Ag-B antigen displayed a transfer reaction that underwent a second flareup of reactivity that reached peak intensity at 96 to 120 hr after inoculation. In only one instance did such a recall of reactivity occur when the recipient was homozygous for Ag-B, 4 (No. 9). Histologic evaluation of these lesions at 120 hr after inoculation revealed numerous mitotic figures, whereas few cells in mitosis were seen during the initial phase of these cutaneous reactions.

Effect of Isoantiserum on Development of Cutaneous Reactions in Rats.—Although little controversy exists concerning the predominant role of lymphocytes as effectors in the various manifestations of transplantation immunity, considerable uncertainty exists concerning the role of isoantibodies whose appearance frequently accompanies the development of cellular homograft immunity. The antigens determined by several histocompatibility loci in the rat may induce the production of specific cytotoxic isoantibodies in homologous hosts. Sera containing these antibodies were tested in experiments designed to determine the extent to which these humoral agents might influence the cutaneous reactivities under analysis:

(*a*) (DA × Lewis)F_1 hybrid lymphoid cells were suspended in either heat-inactivated normal Lewis or Lewis-anti-DA serum. Inoculations of 40×10^6 cells per 0.1 ml were made intracutaneously into normal Lewis rats. Routinely, the cells suspended in the specific isoantiserum incited larger reactions than those suspended in normal Lewis serum. In this situation, the important control—inoculation of F_1 cells mixed with either type of serum into normal F_1 hybrids—incited no significant reaction (Fig. 7 *a*). This indicated that the cytotoxicity of the serum alone could not be held responsible for the lesions observed in Lewis hosts.

(*b*) Normal DA rats were injected intraperitoneally with 2 cc of normal DA or DA-anti-Lewis isoantiserum at 24 and again at 2 hr *before* intradermal challenge with (DA × Lewis)F_1 or Lewis lymphoid cells. Significant intensification of the resulting cutaneous lesions occurred in the isoantiserum-treated hosts (Fig. 7 *b*).

(*c*) DA lymphoid cells were suspended in normal DA, normal Lewis, or Lewis-anti-DA serum. 40×10^6 cells/0.1 ml were then inoculated into the skins of normal DA recipients. No significant reactions appeared at any of the inoculation sites.

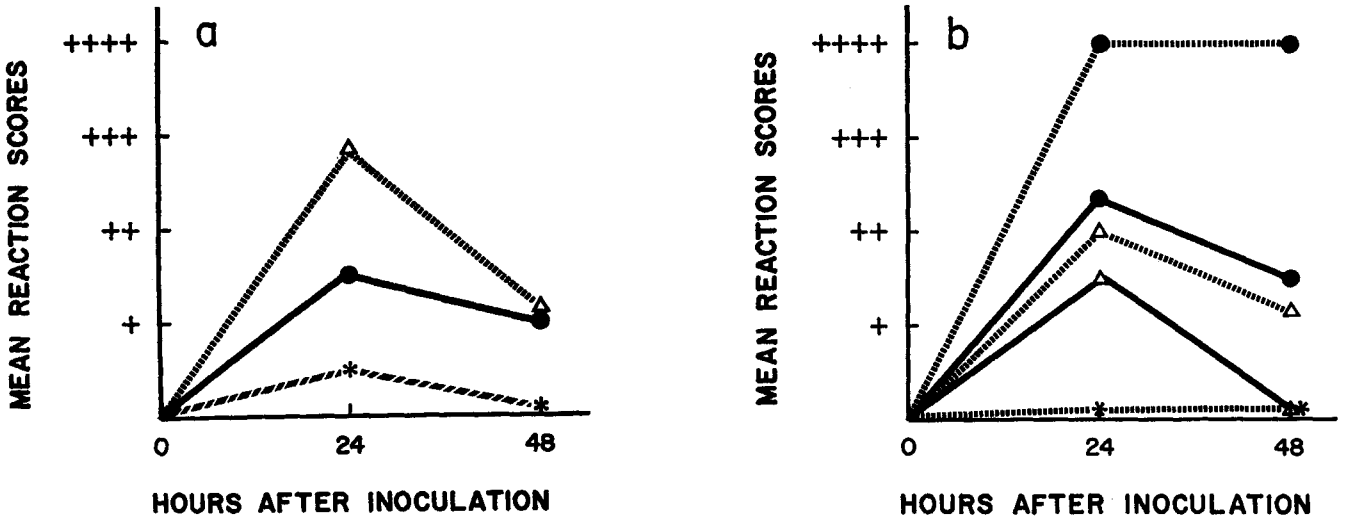

FIG. 7. Isoantibody-supplemented skin reactions. (*a*) Effect of addition of isoantiserum to cellular inocula. (DA × Lewis)F_1 lymphoid cells in normal Lewis serum ●—●; (DA × Lewis)F_1 lymphoid cells in Lewis-anti-DA serum △---△; (DA × Lewis)F_1 lymphoid cells in Lewis-anti-DA serum, inoculated into (DA × Lewis)F_1 hosts ✳----✳. (*b*) Cutaneous reactions in DA hosts pretreated with

Cellular inocula	Normal DA serum	DA-anti-Lewis serum
Lewis lymphoid cells	●—●	●----●
(DA × Lewis)F_1 lymphoid cells	△—△	△----△
DA lymphoid cells	✳—✳	✳----✳

DISCUSSION

Rats are capable of displaying all the various cutaneous hypersensitivity reactions to cellular transplantation antigens that have been described in other mammalian species. However, whereas in guinea pigs, rabbits, hamsters, and man there is considerable erythema associated with these reactions, in the rat, as in the dog, the erythematous component is trivial or absent. Furthermore, for reasons yet to be elucidated, the dosage of cells required to incite significant cutaneous reactivity in rats is very much higher than in these other species.

The present experiments, carried out with isogenic strains and their F_1 hybrids, have corroborated and amplified observations made by others working with different species.

The capacity of parental-strain lymphoid cells to incite a normal lymphocyte transfer reaction on inoculation into an F_1 hybrid host and the inability of lymphoid cells from a hybrid donor to initiate an NLT reaction on injection into the skin of a parental-strain recipient sustain the thesis that the initial component of this reaction is graft-versus-host in nature. The finding that intradermal inoculation of Fischer rats with Lewis lymphocytes, or vice versa, incited barely perceptible lesions, whereas prominent lesions were evoked when lymphoid cell inocula were exchanged between DA rats, on the one hand, and Lewis or Fischer rats, on the other hand, suggests that in this species a difference between cell donor and host at the Ag-B locus may be mandatory for the development of significant NLT reactions (15).

That the direct reaction is primarily an expression of the host's sensitivity or immunity followed from the observation that cells from appropriate F_1 hybrid donors were capable of inciting these reactions following their inoculation into specifically sensitized parental-strain animals. As in the case of NLT reactions, direct reactions were much more intense when there was disparity between donor and recipient at the Ag-B locus than when they were compatible at this locus.

Unlike the situation in hamsters (4), transfer reactivity in rats was found not to be perceptibly depressed by prior high-dosage X-irradiation of the host. The inability of heavily irradiated hamsters to sustain transfer reactions has been tentatively ascribed to depletion of mononuclear cells from the peripheral blood, precluding their participation in the development of the lesions—possibly by migrating into inoculation sites and supplementing the antigenic stimulus afforded by the "native" skin cell population and/or acting as a source of a pharmacologically active mediator, such as LNPF (lymph node permeability factor) (18). The ability of an irradiated rat to manifest transfer reactivity may be due to the fact that its skin is antigenically more effective than that of the irradiated hamster. However, another factor which may contribute to the apparent difference in cutaneous reactivity of irradiated hosts in these two

species may be the much larger numbers of sensitized lymphoid cells which were necessary to incite transfer reactions, even in normal rats.

The finding that the Ag-B locus is crucially important in determining the occurrence and magnitude of the various cutaneous reactions studied is consistent with the observations of other workers. Elkins and Palm (15) found that local graft-versus-host reactions develop at the site of inoculation of parental-strain lymphocytes beneath the renal capsules of adult F_1 hybrid hosts only if there is an Ag-B incompatibility between donor and host. Silvers, Wilson, and Palm (19) have recently reported that, in mixed-lymphocyte cultures from rats, disparity at this locus is mandatory for the occurrence of significant new DNA synthesis. Thus it appears that rat lymphocytes are stimulated to high degrees of mitotic activity when confronted by alien Ag-B antigens whether the milieu is culture medium, or the kidney, and perhaps also the dermis. This proliferative activity may be analogous to that postulated to occur in the second phase of the NLT reaction occurring in the skins of host guinea pigs whose own immunologic response has been blunted by prior whole-body irradiation (20).

Comparison of the survival times of skin homografts from individual members of a genetically defined population of [DA × (DA × Lewis)F_1] backcross rats on DA hosts with (*i*) the intensities and time courses of transfer reactions incited by inoculation of DA-anti-Lewis lymph node cells into the various skin graft donors, and (*ii*) the cellular expression (or its lack) of the Lewis Ag-B,1 antigen in the latter animals has led to the following conclusions.

(*a*) The R_2 animals may be divided into two distinct categories on the basis of the survival times of their individual skin homografts on normal DA hosts: All except two grafts rejected before 12 days came from R_2 animals bearing the Lewis Ag-B,1 antigen in addition to the DA Ag-B,4 antigen. Longer survival times occurred exclusively when grafts were obtained from R_2 animals homozygous for the DA Ag-B,4 allele.

(*b*) With few exceptions, R_2 animals whose skin was rejected in 11 or less days by DA hosts also developed a recrudescence of the cutaneous transfer reaction incited by DA-anti-Lewis lymphoid cells indicated by a second peak score of 2+ or greater at 96–120 hr after inoculation. The great majority (16 of 18) of these same R_2 animals bore the Ag-B,1 allele, alien to the inoculated DA cells.

(*c*) The initial peak intensity of transfer reactions evoked by DA-anti-Lewis lymph node cells in the skins of R_2 animals bore no consistent relationship with either homograft survival time or presence of the Lewis Ag-B allele. Only when these reactions achieved an initial peak intensity of 4+ did they predictably indicate a graft survival of 11 days or less and disparity at the Ag-B locus.

The reason for the lack of any correlation between skin graft survival times and initial peak transfer reactivity is unclear. Experimental evidence is not

available that would indicate whether the antigens responsible for the rejection of a skin graft are operative to the same degree in grafts of other tissues, e.g., cutaneous inocula of lymphoid cells. A tradition has emerged in which skin homograft survival times have been employed as the measure of immunogenetic disparity. It may be that this "tyranny of the skin homograft" (21) will prove to be unwarranted in light of future evidence. The relative importance of certain antigens may not be revealed by simple skin grafting. Silvers et al. (19) have shown that, if two rats share the same Ag-B allele but differ sufficiently at other histocompatibility loci so that exchanged skin homografts are rejected in 10 to 11 days, the survival time of exchanged skin grafts can be considerably prolonged by doses of immunosuppressive agents scarcely able to prolong graft survival between animals disparate at the Ag-B locus.

It is of considerable interest that specific isoantiserum, under appropriate circumstances, can be shown to play a role in the development of delayed cutaneous hypersensitivity reactions. However, the evidence is overwhelming that the rejection of skin homografts is initiated and carried out by lymphoid cells (22, 23). With the exception of a few reports (24, 25), attempts to obtain accelerated rejection of skin grafts by the transfer of isoimmune serum have been unsuccessful (26, 23). Yet, the experiments reported here indicate that specific isoantiserum can bring about a cutaneous inflammatory response resembling direct and transfer reactions under conditions suggesting that specific immunity is responsible. One obvious interpretation of this observation is that the antiserum, being cytotoxic as determined in vitro, is simply annihilating the inoculated cells at the challenge site. Pharmacologically active substances (e.g., LNPF) released from within these cells may be the mediators of the inflammatory response. Such a model, however, does not account for the lack of inflammation at sites where cells isologous to the recipient were inoculated with cytotoxic isoantiserum. Perhaps, if appropriate conditions prevail, antibody plays a synergistic role with lymphoid cells in transplantation immunity, enhancing the potentialities of the cellular component as has been suggested by Batchelor and others (27).

SUMMARY

Rats have been shown to be capable of displaying the various kinds of delayed cutaneous hypersensitivity reactions attributable to transplantation immunity previously described in guinea pigs, hamsters, rabbits, dogs, and man. The rat is unlike most other species in that much larger numbers of lymphoid cells are needed to incite these cutaneous reactions.

With direct, transfer, or normal lymphocyte transfer reactions, the cutaneous responses were greater when donor and recipient differed at the Ag-B histocompatibility locus than when donor and recipient shared the same Ag-B alleles.

An experiment was performed in which adult rats of a genetically defined

backcross population, resulting from matings between DA and (DA × Lewis) F_1 hybrid rats, were inoculated intradermally with lymph node cells from DA rats sensitized against tissues from Lewis-strain donors. Some of the R_2 animals gave a biphasic transfer reaction with peak reactivities occurring first at 48 hr and recurring at 96–120 hr, while the others lacked this second component. Hemagglutination tests revealed that the R_2 rats giving the biphasic response possessed the Ag-B,1 antigen, which is also present in Lewis rats, whereas rats which gave monophasic reactions were homozygous for the Ag-B,4 antigenic determinant which is present in the DA strain. This suggested that the recall flare at 96–120 hr reflects proliferative activity on the part of the inoculated cells confronted by the disparate Ag-B isoantigen in the host's dermis. Skin homografts from R_2 animals bearing the Ag-B,1 antigen were uniformly rejected by DA hosts in 11 days or less, while grafts from backcross animals homozygous for the Ag-B,4 antigen usually lived longer, being rejected in 9 to 27 days.

Evidence is also presented which suggests that specific isoantibodies may act synergistically with immune lymphocytes to bring about cutaneous inflammatory reactions in the rat.

We wish to thank Dr. Norman Anderson and Dr. Joy Palm for helpful criticism in the preparation of this manuscript. Dr. Willys K. Silvers and Dr. Darcy B. Wilson kindly provided breeding nuclei for the Lewis and DA strain rats, respectively. Without the invaluable assistance of Miss Siraik Zakarian and Mr. George Sawchuck, this work would not have been possible.

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