AN ANALYSIS OF THE GENETIC REQUIREMENTS FOR DELAYED CUTANEOUS HYPERSENSITIVITY REACTIONS TO TRANSPLANTATION ANTIGENS IN MICE*

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(Received for publication 29 September 1969)

Delayed inflammatory reactions that follow the intracutaneous inoculation of antigen into specifically sensitized animals of certain species, especially guinea pigs, have become one of the cornerstones upon which the modern concept of cellular immunity is based. In transplantation immunology, cutaneous hypersensitivity reactions have been adapted to study the initiation of homograft sensitivity in vivo (1), to assess the degree of immunogenetic disparity between recipients and graft donors (2–5), and to discriminate between normal and sensitized lymphoid cell populations (6). These studies have been conducted principally in guinea pigs (6), hamsters (7), and man (8), and to a lesser extent in rabbits (6, 9), rats (10), and dogs (11), and have provided considerable insight into the cellular events underlying the immunologically based cutaneous inflammatory response. But since appropriate, genetically defined stocks of these species are not generally available, the genetic requirements for the development of these reactivities are poorly understood.

If skin reactivities could be studied in mice, the broad array of well-defined isogenic strains available, together with our detailed knowledge of the histocompatibility systems in this species, should facilitate an analysis of the genetic requirements for delayed cutaneous reactivity to transplantation antigens. Although, with the aid of special techniques, the capacity of mice to express certain cellular immunities, including transplantation immunity, in terms of delayed skin reactions has been demonstrated by a few investigators (12–15), most students of delayed hypersensitivity still regard these animals as unfavorable subjects for study (6, 16).

The purpose of this communication is, first, to present evidence that mice can consistently express the three defined types of delayed cutaneous reactivity to transplantation antigens—i.e. "direct reactions", "immune lymphocyte transfer reactions", and "normal lymphocyte transfer reactions"—and, second, to present the results of a study of the immunogenetic contexts in which these reactivities are expressed.

* This work was supported by United States Public Health Service Grant AI 07001 and by a Pennsylvania Plan Award.

‡ Dr. Streilein is a Markle Scholar in Academic Medicine.

Materials and Methods

Experimental Animals.—Mice of the following isogenic strains were obtained from the Jackson Laboratory, Bar Harbor, Maine: A/J, CBA/J, C3H/J, BALB/c/J, C57BL/6J, and DBA/2J. Where required, the F_1 and R_2 progeny were obtained from appropriate matings set up in our animal colony. Adult animals, 2–4 months of age, were used throughout.

Lymph node cell suspensions were prepared in Hanks' balanced salt solution, according to the procedure of Billingham and Silvers (17).

Skin Testing and Scoring of Reactions.—Prospective subjects for skin testing were anesthetized with chloral hydrate administered intraperitoneally; their dorsal skin was closely clipped and then depilated with Nair (Carter Products, New York). Test inocula, which comprised 10×10^6 lymph node cells in a standard volume of 0.03 ml, were injected into the dermis via No. 30 gauge hypodermic needles. Each host received three or four injections distributed over the prepared area. With the exception of experiments carried out in R_2 animals (see below), all of the animals in a panel to be skin tested were of the same sex, usually male. Lymph node cell donors were exclusively male.

The intensities of cutaneous reactions incited were assessed at 24 hourly intervals, according to the following arbitrary scale: 0, no response; 1+, barely perceptible swelling at the site; 2+, swelling, 3–4 mm in diameter, site soft to palpation; 3+, swelling 5 mm or more in diameter, and firm; 4+, large reaction with indurated core. The scores recorded in the various tables are the means of at least three independent replicate tests on a single animal. In general, the various reactions developed their peak intensities by 48 hr though a few peaked at 24 hr.

Skin grafting was performed and the survival times of the homografts assessed according to standard procedures described by Billingham and Silvers (18). All the skin tests were performed and scored by Dr. Streilein independently. To avoid bias, as far as possible, not until all the data for a particular experiment were complete were they compared and evaluated.

Hemagglutination.—In the C3H and DBA/2 combination, the *H-2*^{d} allele was identified serologically by use of specific isoantibody in the dextran–human serum test of Gorer and Mikulska (19).

Hemolytic complement determinations were made using sheep erythrocytes coated with complete rabbit anti-sheep erythrocyte antibody. Fresh serum from A strain mice was added, followed by the addition of the test serum. Since serum from A mice provides all components of the complement system, except C5 (20), any hemolysis resulting from this incubation was taken to mean that the test serum contained significant levels of C5.

Types of Delayed Cutaneous Reactions Used to Study Transplantation Immunity

The Direct Reaction.—This is analogous to the classic tuberculin reaction. A host specifically sensitized against the transplantation antigens of a given donor strain, usually by means of a skin homograft, is challenged intradermally with the same antigens in the form of living cells (usually lymphoid cells or leukocytes, for the sake of convenience) or an extract prepared therefrom. Sensitivity on the part of the host is revealed by the development of an inflammatory lesion at the inoculation sites within 24–48 hr.

The Immune Lymphocyte Transfer Reaction.—Although similar in appearance to the direct reaction, the immune lymphocyte transfer reaction differs from it by virtue of the direction of the immunological attack involved. In this case, lymphoid cells from a specifically sensitized donor are injected intradermally

into the skin of a normal recipient against whose transplantation antigens this sensitivity is directed. In this fashion, local adoptive transfers of the immune state are affected, the reactions themselves being local graft-vs.-host responses on the part of the injected, sensitized lymphoid cells.

The Normal Lymphocyte Transfer Reaction.—This type of reaction takes place when suspensions of immunologically competent lymphoid cells derived from normal, unsensitized donors are injected into the skins of normal homologous hosts. Within 24 hr, a cutaneous inflammatory reaction generally develops, attains its maximum intensity between 48 and 72 hr, and then fades, occasionally to be followed by a recrudescence of reactivity after the fifth day. Analyses have revealed that these reactions are initiated by local graft-vs.-host activity on the part of immunologically competent donor cells directed against alien cellular transplantation antigens, which confront them in the host's skin. However, normal homologous hosts, which are immunologically competent, are capable of recognizing the foreign antigens of the putative attacking cells and make their own cellular contribution to the escalating cutaneous fray, converting it from a one-way reaction into a two-way reaction. Pure one-way normal lymphocyte transfer reactions can easily be obtained by experimental artifice, e.g. by irradiating normal homologous hosts, or by employing appropriate genetically tolerant F_1 hybrid animals as hosts.

Scoring of cutaneous reactions has been carried out in guinea pigs by measuring the diameters of both erythema and induration, and these values have been fitted to an arbitrary scale of 0–4+ units. In hamsters and rats, erythema only fitfully reflects the intensity of the cutaneous response, so that the investigator has to rely principally upon the more subjective criteria afforded by the degree of edema and induration present in the lesions, also expressed on a 0-4+ scale.

EXPERIMENTS AND RESULTS

A series of pilot experiments were conducted at the outset to determine whether mice could display delayed cutaneous hypersensitivity reactions to transplantation antigens under any circumstances and, if so, to describe the gross appearance and time course of these lesions. Mice of the A strain served as hosts for cutaneous inocula containing 10 million specifically immune lymphoid cells derived from CBA strain donors which had previously rejected A strain skin (see Fig. 1). Within 8–12 hr of inoculation, edema appeared locally, and by 24 hr impressive inflammatory reactions had developed. At this time, the lesions were quite firm on palpation, the induration measuring 4–5 mm in diameter. In the 10 hosts that made up the test panel, erythema rarely accompanied the cutaneous reactions, and so a scoring system was adopted in which only the degrees of induration and edema were taken into account. Between 24 and 48 hr the reactions remained intense and unchanged,

but by 72 hr the inflammation had subsided considerably, leaving very little substantive reaction at 96 hr.

Reduction of dosage of immune CBA cells inoculated into A strain hosts, from 10 to 5 million per inoculum, resulted in a diminution of the intensities of the immune lymphocyte transfer reactions incited. At the 10×10^6 cell dose level, control inoculations of CBA anti-A cells into CBA hosts, and of

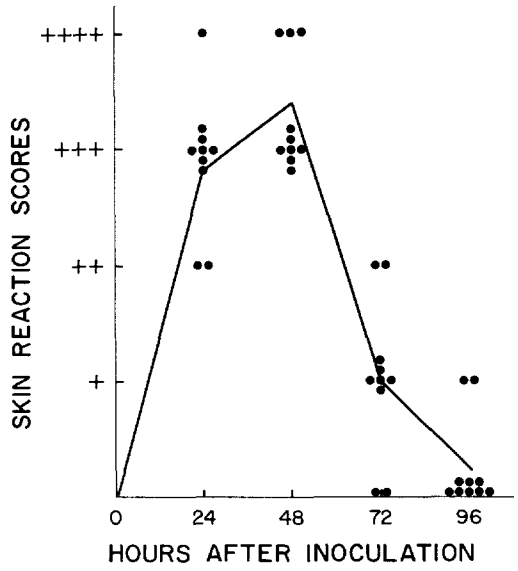

FIG. 1. Immune lymphocyte transfer reactions in A strain hosts inoculated with lymph node cells from specifically sensitized CBA strain donors. Inocula contained 10×10^6 CBA lymph node cells/0.03 ml. Each point represents the mean score of three skin reactions per host.

A lymphoid cells into A hosts, uniformly failed to incite inflammatory reactions of any significance. However, increasing the dosage of cells per inoculum, from 10 to 20 or 40 million cells, frequently did result in the appearance of rather intense nonspecific reactions.

These preliminary observations indicated that significant and readily observable delayed cutaneous hypersensitivity reactions can be procured with the CBA-A murine strain combination, and encouraged the extension of the study to other strain combinations and to other types of reactivities.

The Normal Lymphocyte Transfer Reaction.—The capacity of lymph node cell suspensions to incite NLT[1] reactions in a variety of genetically different

[1] *Abbreviations used in paper:* ILT, immune lymphocyte transfer; MST, median survival time; NLT, normal lymphocyte transfer.

donor-host combinations was studied. Each host was challenged at three or four different sites with a standard inoculum of 10×10^6 donor cells dispensed in 0.03 ml as before. The mean peak reaction scores listed for each donor-host combination represent observations on more than 30 individual test sites distributed on at least 10 different hosts. The range of histoincompatibilities involved included differences at many loci, exclusive or inclusive of the important *H-2* locus, and the trivial difference afforded by the Y-linked (*H*-Y) factor in C57 mice.

The findings summarized in Table I indicate that NLT reactions develop (i.e. attain 1+ or greater intensity) only where donor and host differ at the *H-2* locus in addition to other "minor" loci. Even in the CBA and C3H combi-

TABLE I

Normal Lymphocyte Transfer Reactions Incited by Intracutaneous Inocula of 10 × 10^6 Homologous Lymph Node Cells

Donor	*H-2* genotype	Host	*H-2* genotype	No. of animals tested	Distribution of mean peak reaction scores*					Cumulative mean peak reaction score
					4+	3+	2+	1+	0	
BALB/c	d/d	C3H	k/k	20	1	2	7	8	2	1.6+
C3H	k/k	BALB/c	d/d	13	1	2	4	5	1	1.6+
BALB/c	d/d	(BALB/c × C3H)F_1	d/k	14	1	0	2	8	3	1.1+
C3H	k/k	(BALB/c × C3H)F_1	d/k	12	0	0	0	2	10	0.1+
CBA	k/k	A	a/a	15	0	0	6	7	2	1.3+
A	a/a	CBA	k/k	13	0	0	0	0	13	0.0+
A	a/a	C3H	k/k	12	0	0	0	0	12	0.0+
C3H	k/k	A	a/a	11	0	1	2	7	1	1.3+
CBA	k/k	C3H	k/k	12	0	0	0	3	9	0.2+
C3H	k/k	CBA	k/k	11	0	0	0	1	10	0.1+
C3H	k/k	(C3H × CBA)F_1	k/k	8	0	0	0	0	8	0.0+
C57BL/6 female		C57BL/6 male		10	0	0	0	0	10	0.0+

* Mean peak reaction scores were obtained by averaging the maximum scores of the three individual skin reactions per animal.

nation, where the additive effects of several non-*H-2* incompatibilities bring about the rejection of exchanged skin homografts in about 15 days, normal lymphocyte transfer reactions failed to materialize. Indeed, significant cutaneous reactions did not always occur even when the strains being tested did differ at the *H-2* locus. C3H lymphoid cells regularly incited positive reactions when injected into A strain skin, but in the reciprocal, A strain cells were quite ineffectual. A possible hint toward explaining this discrepancy is afforded by the fact that the $H\text{-}2^a$ allele of the A mouse determines a wide array of antigenic specificities, eight of which are alien to C3H mice, whereas, with the exception

of a single relatively weak specificity (No. 32), all the antigenic specificities determined by the *H-2*^{k} allele of C3H mice are also determined by the *H-2*^{a} allele (21).

With the BALB/c and C3H combination, NLT reactions took place with both donor-host combinations. However, when genetically tolerant (C3H × BALB/c) F_1 hybrid hosts were employed, reactivity was incited by inocula of BALB/c origin, but not by inocula from the C3H strain. Whether or not this observation has as its theoretical basis the hemagglutinogenic differences between these strains at the *H-2* locus is uncertain.

TABLE II

Direct Reactions Incited by Intracutaneous Inocula of 10 × 10^6 *Homologous Lymph Node Cells into Specifically Sensitized Hosts*

Donor	Host	No. of animals tested	Distribution of mean peak reaction scores*					Cumulative mean peak reaction score
			4+	3+	2+	1+	0	
A	CBA anti-A	6	0	0	4	2	0	1.7+
CBA	A anti-CBA	8	0	0	2	2	4	0.7+
C3H	A anti-C3H	9	0	0	1	4	4	0.7+
C3H	CBA anti-C3H	7	0	0	3	3	1	1.2+
(C3H × CBA)F_1	CBA anti-C3H	8	0	0	2	4	2	1.0+
(C3H × CBA)F_1	C3H anti-CBA	8	0	1	2	3	2	1.2

* Mean peak reaction scores were obtained by averaging the maximum scores of the three individual skin reactions per animal.

These findings suggest that an essential prerequisite for the development of an NLT reaction is a difference between donor and host with respect to more than one of the specificities determined by the *H-2* locus. Where F_1 hybrid hosts are employed, NLT reactions only appear when the inoculated cells are confronted by alien *H-2* specificities of the "strong" or hemagglutinogen type. Of possible relevance here is the fact that F_1 cells appear to be inferior to parental cells with respect to their capacity to incite transplantation immunity, possibly because fewer determinants are expressed on their plasma membranes (22).

The Direct Reaction.—Several strain combinations were utilized to determine whether mice could display direct reactions as an expression of extant transplantation immunity. As the results in Table II indicate, although positive cutaneous responses followed the injection of lymphoid cells bearing antigens to which the murine hosts were sensitive, the lesions were unimpressive. Indeed, even when donor and host differed at the *H-2* locus, the intensity of the reaction never exceeded 2+. This finding places the mouse at variance with the

guinea pig and hamster, but is quite consistent with experience in the rat, dog, and rabbit, species in which the direct reaction has been described as attenuated. The explanation for this observation may rest with unsuspected immunogenetic considerations, or with isoantibodies to transplantation antigens which may influence the characteristics of the direct reaction. These possibilities are now under investigation.

The Immune Lymphocyte Transfer Reaction.—In the experiments now to be described, lymphocytes from *presensitized* donors were transferred intracutaneously into normal hosts, using donor-host combinations representing minor

TABLE III

Immune Lymphocyte Transfer Reactions Incited by Intracutaneous Inocula of 10 × 10^6 Lymph Node Cells from Donors Presensitized against Tissue Antigens of the Host Strain

Donor	Host	No. of animals tested	Distribution of mean peak reaction scores*					Cumulative mean peak reaction score
			4+	3+	2+	1+	0	
BALB/c anti-C3H	C3H	13	6	3	2	2	0	3.0+
C3H anti-BALB/c	BALB/c	9	5	1	1	2	0	3.0+
BALB/c anti-C3H	(BALB/c × C3H)F_1	7	1	3	1	1	1	2.3+
C3H anti-BALB/c	(BALB/c × C3H)F_1	9	0	1	6	1	1	1.8+
CBA anti-A	A	10	1	7	2	0	0	2.9+
A anti-CBA	CBA	9	2	3	2	1	0	2.4+
A anti-C57BL/6	(A × C57BL/6)F_1	11	2	5	3	1	0	2.8+
C3H anti-DBA/2	(C3H × DBA/2)F_1	11	4	3	3	0	0	2.9+
C3H anti-CBA	(C3H × CBA)F_1	6	0	0	2	3	1	1.1+
CBA anti-C3H	C3H	6	0	1	2	2	1	1.5+
C57BL/6 female anti-male	C57BL/6 male	12	0	0	5	4	3	1.2+

* Mean peak reaction scores were obtained by averaging the maximum scores of the three individual skin reactions per animal.

to major degrees of histoincompatibility. F_1 hybrid hosts were used in some experiments to ensure that only one-way reactions—i.e. donor against host—could take place. As the results summarized in Table III show, reactivity was incited by the cellular inocula in every combination tested, including the injection of C57BL/6 female node cells into isogenic males, where only the weak Y antigen is involved. As one might have anticipated, the intensities of ILT reactions were greater than those of NLT reactions with the same donor-host combinations. Furthermore, it will be noted that strong ILT reactions developed in some genetic contexts where no perceptible NLT reactions were incited.

These findings suggest that if the method of donor sensitization remains

constant, the intensity of an ILT reaction is determined not only by the state of host reactivity or immunological state (i.e., normal or tolerant), but also by the degree of immunogenetic disparity between the host and the donor of the immune node cells.

Comparison of Intensities of ILT Reactions with Skin Graft Survival Times.—Series of tests using different donor-host inbred strain combinations do not afford a satisfactory means of determining the extent to which the intensity of an ILT reaction is a valid expression of the degree of histoincompatibility between host and lymphoid cell donor. So far as experimental animals are concerned the conventional and most exacting procedure for comparing immunogenetic histocompatibility barriers entails determination of the survival times of skin homografts. Short survival times are indicative of greater degrees of incompatibility than longer survival times.

Accordingly, an experimental design was employed which enabled the survival times of skin homografts to be compared with ILT reactions scored as independent measures of the *same* degree of histoincompatibility. Genetically heterogeneous populations of mice were produced by back-crossing $(P_1 \times P_2)F_1$ hybrids with one of their parental strains, say P_1. Such animals will express all the transplantation antigens of *this* parent, and a variable number of the transplantation antigens of the other parental strain, P_2, as dictated by the law of independent assortment. The immunogenetic disparities to be measured would be those existing between host animals of the isogenic P_1 strain and animals of the segregating R_2 population. The mean survival time, in days, of two skin grafts surgically removed from each R_2 mouse and transplanted to two normal parental strain (P_1) recipients[2] provided the conventional indication of the degree of histoincompatibility involved. 1–2 wk later, each R_2 animal was inoculated intradermally with lymphoid cells from P_1 donors specifically sensitized by means of skin homografts against the full spectrum of P_2 alien antigens. The intensity of the resultant immune lymphocyte transfer reaction was then scored.

In the first experiment carried out, $(CBA \times A)F_1$ hybrids were mated with A strain animals to produce back-cross mice assorting only for the CBA antigens. 2 wk after skin had been grafted from each R_2 to a pair of normal A strain hosts, these donors were challenged intradermally at three different sites, with 10 million A anti-CBA lymph node cells. The results of this experiment (Table IV) reveal *no* correlation between the skin graft survival times and the corresponding ILT reaction scores! During the course of the experiment it was noted that 6 of the 20 animals showed erythematous reactions, while the re-

[2] Two parental strain recipients were tested rather than one, partly as a protection against the death of an animal, and partly to see whether there was any significant variation in the survival times of the grafts.

sponses of the remainder were devoid of an erythematous component. One hypothesis advanced to account for this interesting but baffling observation was based upon the knowledge that the fifth component of complement is genetically absent from A strain mice, but present in CBA mice (20). It was postulated that the development of erythema at a skin test site might depend upon complement activity, and since the C5 allele would assort independently from most histocompatibility genes in this strain combination, it would be

TABLE IV

Comparison of Immune Lymphocyte Transfer Reaction Scores and Skin Homograft Survival Times for (A × (A × CBA)F_1)R_2 Mice

Survival times of R_2 skin grafts on A strain hosts		Immune lymphocyte transfer reactions incited by A anti-CBA lymph node cells in R_2 animals: distribution of mean peak reaction scores*				
Graft observation	No. of grafts rejected per day	4+	3+	2+	1+	0
days						
11	1			1		
12	1			1		
13	5		1111	1		
14	5		111	1	1	
15	9		111	1111	1	1
16	6				111	11
17	3		1		11	
18	2		1		1	
19	3					111
20	0					
21	3		11		1	
22	0					
23	0					
24	1				1	

* Each mean peak reaction score is presented on a horizontal level consistent with the survival time of its corresponding skin homograft. The MST of (A × CBA)F_1 skin grafted to a panel of 10 A hosts was 10.6 ± 0.9 days.

expected to be present in approximately 50% of the animals with positive skin reactions. To test this hypothesis, hemolytic complement levels were determined on fresh serum samples from 18 of the back-cross animals. However, no correlation was demonstrable between the gross appearance of the cutaneous reactions incited in these animals and the presence or absence of the fifth component of complement.

A hint toward explaining these finds was forthcoming subsequently when skin tests were carried out for quite another reason in host mice that had been segregated for sex. It was observed that whereas the ILT reactions incited in the skins of *female* hosts were uniformly of the erythematous type, the reac-

tions expressed by male hosts were devoid of erythema. In the light of this finding, the original back-cross data set out in Table IV were examined to see if there was any relationship between sex of the host and the presence or absence of erythematous reactions. Table V records, for each R_2 animal, the presence or absence of erythema at the skin test sites, the ability or otherwise of its serum to lyse antibody-coated red cells (C5 activity), and its sex. It will be noted that in every instance in which a host expressed an erythematous reaction it was a female.[3]

TABLE V

Characterization of (A × (A × CBA)F1)R2 Mice with Regard to Sex, Presence of Erythema at Skin Test Site, and Presence of Hemolytic Complement Activity in Serum

R_2 animal No.	Sex	Erythematous skin reactions	Serum hemolytic activity
1	F	+	−
2	F	+	+
3*	F	−	−
4	M	−	−
5	M	−	−
6	F	+	−
7	F	+	+
8	M	−	−
9	M	−	−
11	M	−	+
12	M	−	−
13	M	−	+
14	M	−	−
16	M	−	+
17	M	−	−
18	F	+	−
19	F	+	+
20	M	−	−

* This animal failed to exhibit any cutaneous response. Erythema would only be expected to accompany positive inflammatory reactions.

With this information, the original ILT reaction data of Table IV were reanalyzed separately according to the sex of the R_2 animals (see Table VI). Comparison of graft survival times with the ILT reaction scores for *male* hosts revealed a linear correlation, indicating that the greater the histoincompatibility as evidenced by skin homograft survival times, the more intense the cutaneous reaction. The regression coefficient calculated for this association was highly significant by Student's t-test ($P < 0.001$).

[3] One female R_2 animal had no reaction at the skin test sites, and consequently could express no erythema.

All the reactions observed in the female members of the test panel had been assigned 2+ or 3+ scores and showed no correlation with the graft survival times. Whatever the basis of this influence of sex on the expression of ILT

TABLE VI

Comparison of Immune Lymphocyte Transfer Reaction Scores and Skin Homograft Survival Times for $(A \times (A \times CBA)F_1)R_2$ *Mice*

Sex	Survival times of R_2 skin grafts on A strain mice		Immune lymphocyte transfer reactions incited by A anti-CBA lymph node cells in R_2 animals: distribution of mean peak reaction scores*				
	Graft observation	No. of grafts rejected per day	4+	3+	2+	1+	0
	days						
Males	13	3		11	1		
	14	4		11	1	1	
	15	6		11	11	1	1
	16	5				111	11
	17	2				11	
	18	1				1	
	19	3					111
	20	0					
	21	1				1	
	22	0					
	23	0					
	24	1				1	
Females	11	1			1		
	12	1			1		
	13	2		11			
	14	1		1			
	15	3		1	11		
	16	0					
	17	1		1			
	18	1		1			
	19	0					
	20	0					
	21	2		11			

* Mean peak reaction scores were obtained by averaging the maximum scores of the three individual skin reactions per animal.

reactions, it was clearly a critically important factor to be heeded when employing these reactions as "tools" in transplantation immunology. With female hosts the observer might be unduly influenced by the erythematous component, giving the reactions higher scores than warranted by the edema and induration. The experiments now to be described were carried out to evaluate this pos-

sibility and to expand the evaluation of the intensity of the ILT reaction as a quantitative expression of histoincompatibility.

Three different back-cross populations were produced: C3H × (C3H × DBA/2)F_1, A × (A × BALB/c)F_1, and A × (A × C57BL/6)F_1; skin grafting tests were performed and appropriate lymphoid cells were inoculated to incite ILT reactions, as before. However, for skin testing purposes, males and females were scored separately and pains were taken to disregard the erythema-

TABLE VII

A. Comparison of Immune Lymphocyte Transfer Reaction Scores and Skin Homograft Survival Times for (C3H × (C3H × DBA/2)F_1)R_2 Mice

Sex	Survival times of R_2 skin grafts on C3H strain hosts		Immune lymphocyte transfer reactions incited by C3H anti-DBA/2 lymph node cells in R_2 animals: distribution of mean peak reaction scores*				
	Graft observation	No. of grafts rejected per day	4+	3+	2+	1+	0
	days						
Females	8	3	*11*‡		*1*		
	9	3	1	*11*			
	10	6	1		*1*111	1	
	11	5			*11*	111	
	12	1			1		
Males	8	1		*1*			
	9	5		*1*11	*11*		
	10	3			11	1	
	11	3				111	

* Each mean peak reaction score is presented on a horizontal level consistent with the survival time of its corresponding skin homograft. The median survival time of (C3H × DBA/2) F_1 skin grafted to a panel of 10 C3H hosts was found to be 8.5 ± 0.58 days.

‡ An italic number indicates an R_2 animal of genotype d/k as determined by hemagglutination.

tous component of the cutaneous response in the latter. The mean ILT reaction score, together with the corresponding skin graft survival times for the animals in each of the three different back-cross populations tested, are presented in Table VII A–C.

The findings sustain the following conclusions.

1. For each back-cross population tested, irrespective of sex, there was a very obvious correlation between the degree of histoincompatibility indicated by the skin grafting tests and the intensity of the ILT reactions. Regression coefficients were calculated for the results for each panel, and in all three cases, by the *t*-test, the correlation was highly significant ($P < 0.001$).

2. Of the three different R_2 parental strain combinations evaluated, the

C3H and DBA/2 combination exhibited the greatest degree of histoincompatibility as reflected by the fact that the median survival time (MST) of (C3H × DBA/2)F_1 skin on C3H hosts was 8.5 ± 0.58 days. It will be noted that none of the back-cross grafts survived longer than 12 days, and ILT reactions were

TABLE VII—*Continued*

B. Comparison of Immune Lymphocyte Transfer Reaction Scores and Skin Homograft Survival Times for (A × (A × C57BL/6)F_1)R_2 Mice

Sex	Survival times of R_2 skin grafts on A strain hosts		Immune lymphocyte transfer reactions incited by A anti-C57BL/6 lymph node cells in R_2 animals: distribution of mean peak reaction scores*				
	Graft observation	No. of grafts rejected per day	4+	3+	2+	1+	0
	days						
Females	8	6		1111	11		
	9	7		111	1111		
	10	2		1	1		
	11	7			11	1	1111
	12	1				1	
	13	0					
	14	3					111
	15	1					1
Males	8	1		1			
	9	2		1	1		
	10	4		111	1		
	11	3		11			1
	12	2			1	1	
	13	9			111	111	111
	14	1				1	
	15	2				11	
	16	0					
	17	1				1	
	18	0					
	19	0					
	20	1				1	

* The MST of (A × C57BL/6)F_1 skin grafted to a panel of 10 A strain hosts was 10.8 ± 0.52 days.

incited in every case (scores ⩾ 1+). Furthermore, only with this combination did some of the cutaneous responses attain a 4+ intensity, and in six animals epidermal necrosis took place at the test sites. Necrosis was not observed at the test sites in animals of the other series.

In this R_2 panel, back-cross animals bearing the *H-2* allele of the DBA/2 strain (genotype, *H-2*^{d} *H-2*^{k}) were identified by hemagglutination. It will be noted that 9 of 12 animals which rejected their R_2 grafts within 9 days had

been confronted with the strong DBA/2 *H-2*^{d} allele, whereas of the 18 animals whose grafts lived for 10–12 days, only three were confronted by this allele, indicating a strong positive correlation between the presence of this allele in a graft and fast rejection. However, prompt rejection of grafts of genotype

TABLE VII—*Continued*

C. Comparison of Immune Lymphocyte Transfer Reaction Scores and Skin Homograft Survival Times for (A × (A × BALB/c) F_1) R_2 Mice

Sex	Survival times of R_2 skin grafts on A strain hosts		Immune lymphocyte transfer reactions incited by A anti-BALB/c lymph node cells in R_2 animals: distribution of mean peak reaction scores*				
	Graft observation	No. of grafts rejected per day	4+	3+	2+	1+	0
	days						
Females	8	1		1			
	9	3		1	11		
	10	4		1	11	1	
	11	1			1		
	12	9			11111	1111	
	13	4			1	111	
	14	2				1	1
	15	2				1	1
	16	2				11	
Males	9	2		1		1	
	10	2		1	1		
	11	5		111	1	1	
	12	4		11	1	1	
	13	1				1	
	14	3			11		1
	15	0					
	16	0					
	17	0					
	18	0					
	19	0					
	20	2					11
	21	2					11

* The MST of (A × BALB/c)F_1 skin grafted to a panel of 10 A hosts was 12 ± 2.65 days.

H-2$^{k/k}$ was also associated with 3+ ILT reaction scores, indicating that *H-2* incompatibility is not a prerequisite for development of these cutaneous lesions. It is interesting to note that with five of the six animals in which epidermal necrosis developed at the ILT reaction sites, *H-2* incompatibility did prevail.

3. The MSTs of (A × C57BL/6)F_1 grafts and of (A × BALB/c)F_1 skin grafts on A strain hosts were 10.8 ± 0.52 days and 12 ± 2.65 days, respectively, suggesting that weaker histocompatibility barriers are involved here

than with the DBA/2 to C3H combination—a conclusion borne out by the finding that ILT reactions failed to develop in many of the animals of the two former back-cross populations, and positive reactions were less intense than with C3H and DBA/2.

Unfortunately, it is difficult to identify by a hemagglutinating procedure the BALB/c and C57BL/6 alleles of the *H-2* locus on an A strain background. Consequently, it was not possible to evaluate the influence of differences at this locus on the incitement of ILT reactions.

TABLE VIII

Compilation of Experience with Immune Lymphocyte Transfer Reactions in Various R_2 Populations of Mice

R_2 animals tested	MST*	Immune lymphocyte transfer reaction scores‡									
		4+		3+		2+		1+		0	
	days										
A and CBA	15 ± 1.2	0	0	6	0	4	0	3	7	2	4
A and BALB/c	12 ± 1.2	0	0	9	0	14	3	8	8	0	7
A and C57BL/6		0	0	14	0	10	5	1	10	1	11
Female	9.5 ± 1.3										
Male	11.8 ± 2.7										
C3H and DBA/2	9.5 ± 0.65	4	0	6	0	9	3	3	6	0	0
Totals		4	0	35	0	37	11	18	31	3	22

* Median survival time of skin from different R_2 animals grafted onto normal parental strain hosts.

‡ The members of each R_2 panel are distributed under appropriate columns headed by the different reaction score levels. Within each column, the numbers to the right indicate those animals whose skin grafts survived longer than the MST for that combination, while those to the left indicate those animals whose skin grafts were rejected on or before the MST.

To achieve a common ground for comparison of the experience with ILT reactions in the various R_2 populations, median survival times were calculated for skin grafts from R_2 donors on parental strain hosts. These are presented in Table VIII, where ILT reaction scores are arranged according to their respective MST. Using the MST as an arbitrary dividing line, ILT reaction scores corresponding to skin grafts surviving up to and including that day have been placed on the left, while ILT reaction scores from donors whose grafts were rejected after that day are listed on the right. It can readily be seen that an ILT reaction score of 3+ or 4+ was uniformly correlated with a graft survival less than, but never longer than, the MST. Conversely, 19 of 22 animals with ILT reaction scores of 0 donated skin grafts that survived longer than the MST. The table also indicates that the majority of 2+ scores represented

short-lived grafts, while most of the 1+ scores indicated grafts surviving longer than the MST.

DISCUSSION

The experiments described clearly demonstrate that mice, like other mammalian species evaluated to date, are fully able to display the vicissitudes of homograft immunity by means of delayed cutaneous hypersensitivity reactions. In general, these findings confirm the cumulative experience derived from the study of these reactivities in other species. However, because of the employment of a variety of defined immunogenetically disparate, isogenic murine strains, some of the results have provided fresh insights into the genetic and physiological requirements for hypersensitivity responses to transplantation antigens in the skin.

For example, it has been found that a difference between donor and recipient of an intracutaneous lymphoid cell inoculum at the important *H-2* histocompatibility locus is an essential prerequisite for a normal lymphocyte transfer reaction to develop. Lymphocytes from normal donors inoculated into the skins of hosts which confront them with antigens determined by a multiplicity of H loci, *excluding* the *H-2*, invariably failed to elicit NLT responses. Indeed, some of the present observations suggest that NLT reactions are even more exacting in their immunogenetic requirements: not only do they require *H-2* differences, but *H-2* differences with respect to component antigenic specificities that are strongly hemagglutinogenic. If subsequent work sustains this thesis, it will indicate a degree of immunological precision unsuspected among the cellular immunities, as they are studied in vivo.

It is well established that an essential condition for the development of systemic graft-vs.-host reactions, such as runt or transplantation disease, in addition to well-studied local manifestations of graft-vs.-host reactivity, such as splenomegaly, hepatomegaly, and local renal lesions, is that the putative attacking donor lymphoid cells must be confronted by alien antigens determined by the *H-2* locus, or its equivalent in other species (23–25). However, if these cells are provided by a specifically presensitized donor, graft-vs.-host reactions are capable of developing in the absence of a difference at the major locus. The present findings indicate that so far as mice are concerned, the NLT and ILT reactions as special types of graft-vs.-host reactivity closely resemble other, more conventional forms of graft-vs.-host reactivity with respect to the genetic conditions for their occurrence. A critical test of this thesis will be to determine whether the presence of differences with respect to certain potent *H-2* component specificities are as important for conventional graft-vs.-host reactions as they have proved to be for the NLT reaction in the mouse.

With a surprising degree of consistency, it has been found that the intensities of ILT reactions paralleled the immunogenetic disparity between donor and host

as revealed by the criterion of skin homograft survival times. An indication of the sensitivity of the ILT reactions as a means of detecting histocompatibility differences is afforded by the fact that significant levels of reactivity occurred when the antigenic stimulus was as trivial as the *H-Y* locus, i.e. when C57BL/6 males were inoculated intracutaneously with specifically sensitized cells from females of the same strain.

Perhaps the most important, and certainly the least expected, observation was that erythema regularly accompanied delayed skin reactions only in female mice. Similarly procured reactions in male skins were consistently devoid of erythema, although comparable degrees of edema and induration were achieved at the inoculation sites. The simplest hypothesis to explain this singular observation implicates the physiological and pharmacological actions of the various steroid sex hormones, and experiments bearing on this question are under way. It is likely, however, that the most important conclusion from these findings is that, immunological considerations aside, nonspecific host factors may exert a significant influence on the intensity and evolution of the inflammatory reaction that ultimately appears at the skin test site.

SUMMARY

The experiments reported herein provide ample evidence that mice, like most other mammalian species, are capable of displaying readily observable and reproducible delayed cutaneous hypersensitivity reactions indicative of transplantation immunity. By employing a variety of genetically defined strains, it has been shown that a genetic requirement for the development of a positive normal lymphocyte transfer reaction in mice is a difference between host and cell donor at the *H-2* locus. By contrast, the immune lymphocyte transfer reaction consistently reflected the full range of histoincompatibility, both inclusive and exclusive of the *H-2*. It was incidentally discovered that erythema regularly accompanied delayed cutaneous reactions in the skins of female mice, whereas no local redness accompanied their counterparts in male skins. The influence of cutaneous erythema on the scoring of delayed skin reactions is discussed.

We are indebted to Dr. Willys K. Silvers for his intellectual and material support of this project; he graciously provided many animals from his colony for some of the experiments. Dr. Joy Palm very kindly performed the hemagglutination tests used to determine certain *H-2* phenotypes, and in addition provided invaluable assistance with the preparation of this manuscript. Without the excellent and faithful technical aid of Mrs. Joan Streilein and Mr. George Sawchuck, this work could not have been accomplished.

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