THE BACTERIAL INDUCTION OF HOMOGRAFT SENSITIVITY

II. Effects of Sensitization with Staphylococci and Other Microorganisms*

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Group A streptococci of Lancefield Types 4, 5, 6, 11, 12, 14, and 49 have been shown to induce in guinea pigs a state of altered reactivity to skin homografts indistinguishable in its gross and histologic manifestations from the response observed in this species following sensitization with homologous tissues (1, 2). This observation may provide an approach to further studies of tissue responses to bacterial antigens (3–9). Its significance is limited, however, by the absence of data on the distribution in other microorganisms of the factor(s) in streptococci concerned with sensitization to skin homografts.

The present report evaluates the ability of staphylococci and a variety of other microorganisms to induce in guinea pigs a state of altered homograft reactivity similar to that resulting from sensitization with homologous tissues and Group A streptococci. In the course of this study, 291 guinea pigs were pretreated with 3 strains of staphylococci, 8 Lancefield Groups of streptococci, and 14 other Gram-positive, Gram-negative, and Acid-fast bacteria. Recipients sensitized with *Staphylococcus aureus* and *Staphylococcus albus* rejected first-set skin homografts as white grafts or in an accelerated fashion. None of the other microorganisms tested was active in this respect. Results of this study are described and discussed in the light of their pertinence to the heterologous nature of antigens related in their biologic effects to tissue transplantation antigens.

Materials and Methods

Experimental Animals and Operative Technique.—Outbred male guinea pigs of the Hartley strain, fed on a standard Purina pellet diet were used. The animals were 2 to 4 months of age

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and weighed 250 to 350 gm. Methods of grafting, graft observation, and criteria for the determination of homograft rejection were described in the adjoining report (2). Gross, stereomicroscopic, and histologic examination constituted the basis for the determination of the type of skin homograft response observed.

Bacterial Strains and Method of Sensitization.—

Gram-positive bacteria: Staphylococcus aureus (phage type 80/81), *Staphylococcus aureus* (New York Hospital 6-phage type 80/81), and *Staphylococcus albus* (Pringle strain) were supplied by Dr. Stephen Morse of The Rockefeller University. Streptococcal strains from the Rockefeller University stock included: Group B (OR90), Group C (C74), Group D (D74), Group E (K129), Group G (D166B), Group H (F90A), Group L (D167A), and Group O (B357). Rockefeller University strains of pneumococci of Type II (D39), Type II rough (R36A), and Type III (A66) were also used. Strains of *Corynebacterium xerosis* and *Bacillus subtilis* were obtained from Mr. Louis Garmise, of the Department of Microbiology, New York University School of Medicine.

Gram-negative bacteria: Strains of *Escherichia coli, Aerobacter aerogenes, Salmonella typhimurium*, and *Proteus vulgaris* were supplied by Dr. Russel Schaedler of the Rockefeller University. Strains of *Neisseria catarrhalis* and *Haemophilus influenzae* were obtained from Mr. Louis Garmise.

Mycobacteria: A virulent strain of heat-killed *Mycobacterium tuberculosis* was provided by Dr. Martin H. Flax of the Department of Bacteriology and Immunology of the Harvard Medical School. In addition, Complete BactoAdjuvant, (Difco Laboratories, Inc., Detroit), containing heat-killed H37Ra human *Mycobacterium tuberculosis*, was utilized in a second group of guinea pigs.

Method of Preparation.—With the exception of mycobacteria, bacterial strains were grown in Wannamaker's dialysate medium (10) as described in the adjoining report (2). Pneumococci, streptococci, and *Corynebacterium xerosis* were heat-killed and prepared for injection as described previously (2). Staphylococci and Gram-negative bacteria were heat-killed by incubation at 60°C for 1 hour. The determination of dry weights and of preparation of suspensions for injections has been described (2).

The Flax strain of mycobacteria was resuspended in media 199 and Freund's incomplete adjuvant, and 5 mg of the microorganisms were injected into each recipient. Complete bactoadjuvant, containing *Mycobacterium tuberculosis* in concentrations of 1 mg/ml was injected in volumes of 0.2 ml into each foot-pad of the recipients.

EXPERIMENTAL RESULTS

Effects of Sensitization with Staphylococci.—Seventy-six guinea pigs were sensitized with three strains of staphylococci. Thirty-eight animals received suspensions of *Staphylococcus aureus* (NYH 6); 10 animals were treated with another strain of *Staphylococcus aureus*, and 28 guinea pigs were injected with *Staphylococcus albus* (Pringle strain). As noted in Table I, 62 of 76 recipients gave altered homograft responses, consisting of 59 white grafts, and 3 accelerated rejections at 4 to 5 days. The remaining 14 grafts were rejected in first-set fashion, at 7 to 12 days. There was no significant difference in the ability of the staphylococcal strains employed to induce altered homograft responses in the recipients.

Histologic study of white grafts and accelerated rejections in this group of animals indicated no differences from the microscopic pattern observed in

guinea pigs pretreated with Group A streptococci or with homologous skin. White graft reactions in recipients of heat-killed staphylococci (Figs. 1 to 4) had the appearance of an avascular, diffusely eosinophilic graft, covered with a thin and necrotic epidermis, resting upon the mononuclear cell "black line" of Bauer (11). There were varying degrees of polymorphonuclear infiltration, particularly in the subepidermal layer (2). A number of mixed white grafts was observed. These were characterized by more intense polymorphonuclear leucocyte infiltration, and by partial vascularization of the deeper layers of the graft dermis (2) (Figs. 1 to 4).

TABLE I

Homograft Response in Guinea Pigs Sensitized with Staphylococcus Aureus and Staphylococcus Albus

Staphylococcal strain used in pretreatment	No. of animals studied	Response of recipients to skin homografts		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
Staphylococcus aureus, New York Hospital-6 Strain Phage type 80/81	38	5	2	31
Staphylococcus aureus, Phage type 80/81	10	2	0	8
Staphylococcus albus, Pringle strain	28	7	1	20

Total number of animals pretreated with staphylococci, 76.
First-set responses elicited, 14.
Accelerated rejections or white graft reactions elicited, 62.

Effects of Sensitization with Lancefield Groups Other Than Group A Streptococci.—Ninety-two guinea pigs were pretreated with streptococci of eight different Lancefield Groups (Groups B, C, D, E, G, H, L, and O). As noted in Table II first-set homograft responses were observed in 92 consecutive instances. Homograft suvivals extended from the 6th to the 16th postoperative day, with a mean survival time of 8.46 days. There were no white grafts or accelerated rejections. The histologic features of homograft rejection were similar to those noted in untreated animals (11).

Effects of Sensitization with Other Gram-Positive Bacteria.—Table III summarizes the homograft responses of 51 guinea pigs to 4 strains of pneumococci, including Types II, III, and XIV, and a rough strain of pneumococcus Type II, as well as to strains of *Corynebacterium xerosis* and *Bacillus subtilis*. There were 50 first-set rejections in this group, with graft survivals extending from the 6th to the 15th postoperative day (mean survival time = 8.80 days). In 19 recipients of a suspension of pneumococcus Type III, there were 18 first-set rejections and one accelerated rejection on the 5th postoperative day. No other accelerated

TABLE II

Homograft Response in Guinea Pigs Sensitized with Streptococci of Lancefield Groups Other than Group A

Streptococcal group used in pretreatment	No. of animals studied	Response of recipients to skin homografts		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
Group B	7	7	0	0
Group C	14	14	0	0
Group D	14	14	0	0
Group E	5	5	0	0
Group G	19	19	0	0
Group H	23	23	0	0
Group L	4	4	0	0
Group O	6	6	0	0

Total number of animals studied, 92.
First-set responses elicited, 92.
Accelerated rejections or white graft reactions elicited, 0.

TABLE III

Homograft Response in Guinea Pigs Sensitized with Other Gram-Positive Bacteria

Gram-positive bacteria used in pretreatment	No. of animals studied	Response of recipients to skin homografts		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
Pneumococcus Type II	7	7	0	0
Pneumococcus Type III	19	18	1	0
Pneumococcus Type XIV	5	5	0	0
Pneumococcus, rough strain	6	6	0	0
Corynebacterium xerosis	7	7	0	0
Bacillus subtilis	7	7	0	0

Total number of animals studied, 51.
First-set responses elicited, 50.
Accelerated rejections, 1.
White graft reactions, 0.

rejections or white graft reactions were observed. Histologic study of the homograft reactions in this group of animals showed the patterns of homograft rejection previously described (2).

Effects of Sensitization with Gram-Negative Bacteria.—Forty-one guinea pigs were injected with heat-killed *Escherichia coli, Aerobacter aerogenes, Salmonella*

TABLE IV

Homograft Response in Guinea Pigs Sensitized with Gram-Negative Bacteria

Bacterial strain used in pretreatment	No. of animals studied	Response of recipients to skin homografts		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
E. Coli	9	9	0	0
A. Aerogenes	6	6	0	0
S. typhimurium	7	7	0	0
P. vulgaris	7	7	0	0
N. catarrhalis	6	6	0	0
H. influenzae	6	6	0	0

Total number of animals studied, 41.
First-set responses elicited, 41.
Accelerated rejections or white graft reactions elicited, 0.

TABLE V

Homograft Response in Guinea Pigs Sensitized with Mycobacteria

Type of mycobacterial preparation used in pretreatment	No. of animals studied	Response of recipients to skin homografts		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
Bacto adjuvant, complete, Difco Laboratories* (*Mycobacterium tuberculosis*, strain H37A)	26	26	0	0
Mycobacterium tuberculosis, heat-killed‡	5	5	0	0

Total number of animals pretreated with Mycobacteria, 31.
First-set responses elicited, 31.
Accelerated rejections or white graft reactions, 0.

* Difco Laboratories standard preparation of bacto-adjuvant, containing 1 mg/ml of heat-killed tubercle bacili; injected in volumes of 0.2 ml into each of the four foot-pads of the recipient animals 2 weeks prior to challenge with a first-set skin homograft.

‡ Kindly supplied by Dr. M. H. Flax.

typhimurium, *Proteus vulgaris*, *Neisseria catarrhalis*, and *Haemophilus inflenzae*. Results of this experiment are summarized in Table IV. There were 41 consecutive first-set homograft responses. Graft survivals extended from the 6th to the 13th postoperative day (mean survival time = 9.58 days). There were no accelerated rejections or white grafts in this group of recipients. The histologic patterns of rejection observed were indistinguishable from those noted in untreated animals (11).

Effects of Sensitization with Mycobacteria.—Table V summarizes the response of 31 guinea pigs to sensitization with mycobacteria, including 26 recipients of complete (H37Ra) bacto-adjuvant and 5 recipients of a suspension of another virulent strain of heat-killed tubercle. There were 31 first-set graft rejections in this group. Survival times extended from the 7th to the 15th postoperative day, with a mean survival time of 9.52 days. There were no white grafts or accelerated rejections. The histologic features of graft rejection in this group were similar to those noted in other first-set homograft responses.

Comparison of First-Set Skin Homograft Survival Times in Control Guinea Pigs and in Animals Pretreated with Bacterial Suspensions which Failed to Induce

TABLE VI

First-Set Skin Homograft Responses in Control and in Unsensitized Test Guinea Pigs

Mode of pretreatment	No. of animals	Range of survival time	Mean survival time
		days	*days*
Untreated control guinea pigs	102	6–13	8.10
Control guinea pigs treated with media 199, Wannamaker's media and/or incomplete Freund's adjuvant	51	6–14	8.98
Streptococcal groups B, C, D, E, G, H, L, O	92	6–16	8.46
Gram-positive bacteria other than streptococci or staphylococci	51	6–15	8.80
Gram-negative bacteria	41	6–13	9.58
Mycobacteria	31	7–15	9.52

Homograft Sensitivity.—Table VI summarizes the behavior of skin homografts in recipients of bacterial suspensions which failed to induce homograft sensitivity, and compares it to responses obtained in control guinea pigs. The latter group included untreated guinea pigs, and animals pretreated with bacteria-free reagents (2). The mean survival time of first-set skin homografts in 102 untreated guinea pigs was 8.10 days. A slight increase in mean survival times was observed in the other groups studied. Pretreatment with streptococci other than Group A resulted in a mean survival time of 8.46 days. Recipients of other Gram-positive and of Gram-negative microorganisms had first-set homograft mean survival times of 8.80 and 9.58 days, respectively. Administration of mycobacteria resulted in a mean homograft survival time of 9.52 days. Pretreatment of guinea pigs with bacteria-free control reagents resulted in a mean survival time of 8.98 days. With the exception of one recipient of pneumococcus

Type III cells, in whom a skin homograft was rejected at 5 days, there were no white grafts or accelerated rejections in this group.

DISCUSSION

Results of this study (Table VII) indicate that *Staphylococcus aureus* and *Staphylococcus albus* share with Group A streptococci the ability to induce altered reactivity to skin homografts in the guinea pig. The ability of staphylococci to induce homograft sensitivity is of interest in view of the association

TABLE VII

The Bacterial Induction of Homograft Sensitivity in the Guinea Pig—Summarized Results

	No. of animals studied	Types of homograft reactions observed		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
Mode of pretreatment				
Group A streptococci (2)	181	40	42	99
Group B, C, D, E, G, H, L, O streptococci	92	92	0	0
Staphylococci	76	14	3	59
Other Gram (+) bacteria	51	50	1	0
Gram. (−) bacteria	41	41	0	0
Mycobacteria	31	31	0	0
Control study group				
Untreated	102	102	0	0
Bacteria-free control emulsions	51	51	0	0

Total number of animals studied, 625.

of this micoorganism with other altered states of mammalian tissue reactivity (18, 19). It is also relevant to recent reports of *in vitro* (20) and *in vivo* (21) cross-reactions between streptococcal and staphylococcal antigens. Such cross-reactions lend support to the possibility that the bacterial induction of homograft sensitivity in the guinea pig may be an expression of the presence of similar or identical antigens in bacterial groups and in mammalian tissues (1, 2).

Under the present experimental conditions, Gram-positive bacteria other than staphylococci, as well as Gram-negative and acid-fast microorganisms, did not sensitize guinea pigs to skin homografts. Reports that Group C streptococci are frequent inhabitants of guinea pig tissues (12, 13) have suggested that streptococcus-induced homograft rejection may be an expression of a pre-

existing state of hypersensitivity. The inability of Group C streptococci to induce homograft sensitivity militates against this possibility. When coupled with other studies of streptococci (14–17) it may also provide a guide to the localization of the factor(s) in Group A streptococci responsible for the induction of homograft sensitivity. Reports of the immunologic identity of Group A and Group C cell walls (with the exception of a single amino-sugar determinant) by Krause and McCarty (14, 15) and Araujo and Krause (16), and Freimer's (17) demonstration of immunological differences between Group A and Group C cytoplasmic membranes, may be of particular relevance to this question.

The failure of other bacterial strains, and particularly of *Mycobacterium tuberculosis*, to induce homograft sensitivity under the experimental conditions described, is of interest. It indicates that homograft responses induced by Group A streptococci and by staphylococci are not the result of the type of general hyperreactivity observed in other animal species by Old, Clarke, and Benacerraf (22), Balner, Old, and Clarke (23), and by Vitale and Allegretti (24) following BCG vaccination. Gram-negative bacteria tested in the course of this study did not induce accelerated homograft rejection. Skin homografts applied to the recipients were, however, accorded a slight increase in survival time. This observation is compatible with the report of Miller, Martinez, and Good (25) on the attempt to inhibit homograft rejection responses in mice by the use of Gram-negative antigens.

The induction of homograft sensitivity in the guinea pig by bacterial cells may be an expression of the presence in such microorganisms of antigens related in their biological effects to tissue transplantation antigens. The finding of such activity in 7 types of Group A streptococci and in three strains of staphylococci supports the possibility that, as has been noted in other heterologous systems (26, 27), the antigens concerned with the bacterial induction of homograft sensitivity may be ubiquitous in nature.

A correlation between the presence or absence of cross-reacting antigens and the response of mammalian hosts to microorganisms was first suggested by Rowley (28), and was recently reviwed by Rowley and Jenkin (29). The experimental approach described in the present report offers a potential means for further investigation of this question. Its value is limited, however, by the absence of information on the intracellular localization of the factor(s) in streptococci and staphylococci responsible for the induction of homograft sensitivity in the guinea pig. Studies concerned with this problem, and extension of this observation to other mammalian species, are currently in progress.

SUMMARY

Heat-killed strains of *Staphylococcus aureus* and *Staphylococcus albus* can induce in guinea pigs a state of altered reactivity to skin homografts which is in-

distinguishable from that which results from sensitization with homologous tissues or Group A streptococci. Challenge of suitably prepared recipients with first-set skin homografts obtained from unrelated randomly selected donors elicits white graft reactions or accelerated rejection of such grafts.

Other bacteria tested included Lancefield streptococcal groups B, C, D, E, G, H, L, and O, pneumococcus Types II, III, XIV and a rough strain, *Corynebacterium xerosis, Bacillus subtilis, Escherichia coli, Aerobacter aerogenes, Salmonella typhimurium, Proteus vulgaris, Neisseria catarrhalis, Haemophilus influenzae,* and two human virulent strains of *Mycobacterium tuberculosis.* None of these microorganisms was active in the induction of homograft sensitivity in the guinea pig. Pretreatment of recipients with Gram-negative bacterial suspensions was associated with a slight increase in the mean survival time of first-set skin homografts.

Results of this study suggest the presence in staphylococci, as well as in Group A streptococci, of antigens related in their biologic effects to tissue transplantation antigens.

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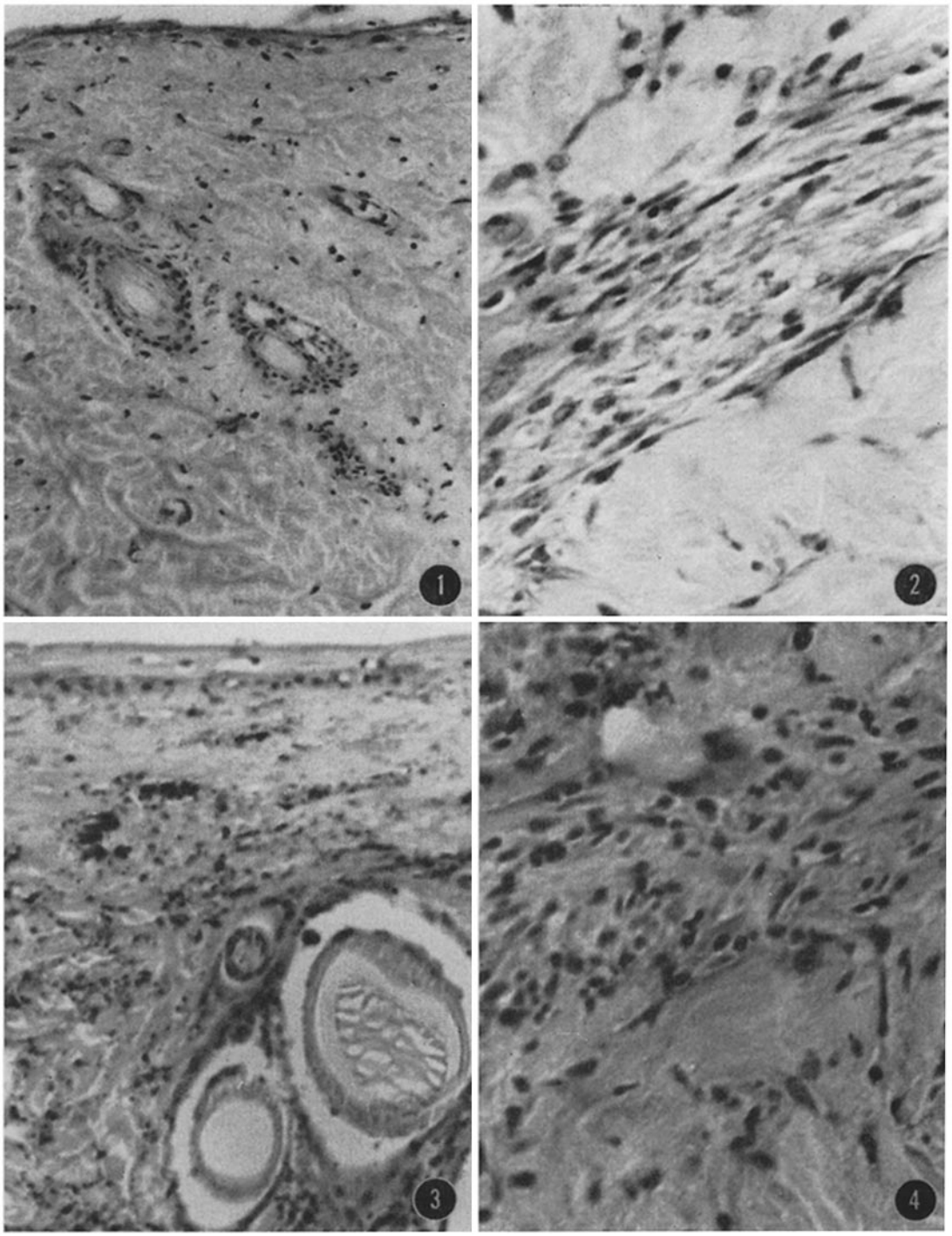
EXPLANATION OF PLATE 55

FIG. 1. White graft reaction induced in a guinea pig after sensitization with *Staphylococcus aureus*. Section taken on the 5th postoperative day, illustrating thinning and necrosis of the epidermis, avascularity of the graft, and disorganization of the dermal elements. × 100.

FIG. 2. White graft reaction induced in a guinea pig after sensitization with *Staphylococcus aureus*. Section taken on the 5th postoperative day, illustrating the network of mononuclear, spindle-shaped cells found at the base of the graft (black line of Bauer). × 250.

FIG. 3. Mixed white graft reaction induced in a guinea pig after sensitization with *Staphylococcus aureus*. Section taken on the 5th postoperative day, illustrating thinning and necrosis of the epidermis, disorganization of dermal elements, multiple hemorrhages and cellular infiltrates. × 100.

FIG. 4. Mixed white graft reaction induced in a guinea pig after sensitization with *Staphylococcus aureus*. Section taken on the 5th postoperative day, illustrating the presence of mononuclear, spindle-shaped cells at the base of the graft (black line of Bauer). × 250.



(Rapaport and Chase: Bacterial induction of homograft sensitivity. II)