REJECTION OF AUTOGRAFTS CAUSED BY SENSITIZATION BY CERTAIN BACTERIAL ANTIGENS

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Rejection of a skin autograft takes place in CC57Br mice sensitized by streptococcal, staphylococcal, and yeast antigens if grafting is carried out 6-19 days after the end of the sensitization course.

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Certain bacterial antigens have now been shown to possess common properties with the antigens present in animal and human tissues. This similarity has been established between antigens of group A streptococci and mammalian heart and kidney tissues, and also between type IV pneumococci and human group A blood. A number of investigators [1, 2, 4] have demonstrated a connection between sensitization with bacterial antigens resembling tissue antigens and lesions of the corresponding organs (the heart in rheumatic fever, the kidneys in nephritis, for example). The state of sensitization is accompanied both by the production of circulating antibodies against the related antigens and by the development of hypersensitivity of delayed type, the cell reaction which leads to considerable tissue damage. Exposure to antigens similar to those of the tissue thus induces a state of autoaggressiveness. An important model of induced autoaggressiveness is given by stimulation of rejection of an autograft by a strong transplantation antigen [3, 7]. Rejection of a homograft may, in turn, be accelerated by injection of cultures of group A streptococci or of Staphylococcus aureus into the recipient before grafting.

In the present investigation the possibility of rejection of an autograft as a result of injection of several bacterial antigens was studied.

EXPERIMENTAL METHOD

Experiments were carried out on CC57Br mice weighing 15 g. Autografting was carried out by Billingham's method [6] by transplanting a piece of skin from the lower part of the back to its upper part. The bacterial antigens used were suspensions of bacteria in 0.87% NaCl solution killed by heating to 58° three times (streptococci of groups A and C; Staphylococcus aureus; Escherichia coli; Shigella shigae; Salmonella typhimurium; Pneumococcus type I; Candida albicans; a formalin-killed polyvalent brucellosis vaccine obtained from Khar'kov Biological Preparations Factory, batch 94, dated August 3, 1965). In addition, a suspension of dried Mycobacterium tuberculosis cells and endotoxin obtained from Salmonella typhi cells by Boivin's method were injected.

Before the autografting operation the mice received three intraperitoneal injections of bacterial antigen at intervals of 24 h. At the first injection 10^7 bacterial cells (estimated by turbidity) were injected, the dose at the second injection was 10^8, and at the third injection $15 \cdot 10^7$ bacterial cells, while in special experiments the number of bacterial cells was increased to $5 \cdot 10^7$, $15 \cdot 10^8$, and $30 \cdot 10^8$ cells per injection, respectively. Endotoxin was injected intraperitoneally in three doses of 10, 15, and 20 μg. Freund's complete adjuvant was injected subcutaneously three times on alternate days and intradermally in a dose of 0.15 ml, the content of M. tuberculosis cells in the adjuvant being 2 mg/ml. On the 8th day after the last injection, autografting was performed. Control mice received the same volume of physiological saline (0.15 ml), in which antigens of the experimental animals were suspended, while the control of action of the complete adjuvant took the form of injection of incomplete adjuvant.

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TABLE 1. Effect of Bacterial Antigens on Rejection of Autograft

Type of antigen	Number of mice			
	experiment		control	
	total	with rejection of graft	total	with rejection of graft
Group A streptococcus . .	30	25	30	0
Group C streptococcus . .	41	36	46	8
Staphylococcus aureus . . .	40	31	40	3
C. albicans	40	31	40	8
E. coli	20	2	20	2
S. shigae	40	5	40	5
S. typhimurium	20	1	20	0
Endotoxin	40	5	40	4
M. tuberculosis (bovine type)	40	9	40	4
Complete adjuvant	14	1	14	2
Pneumococcus type I . . .	40	4	40	2
Polyvalent brucellosis vaccine	20	0	20	0

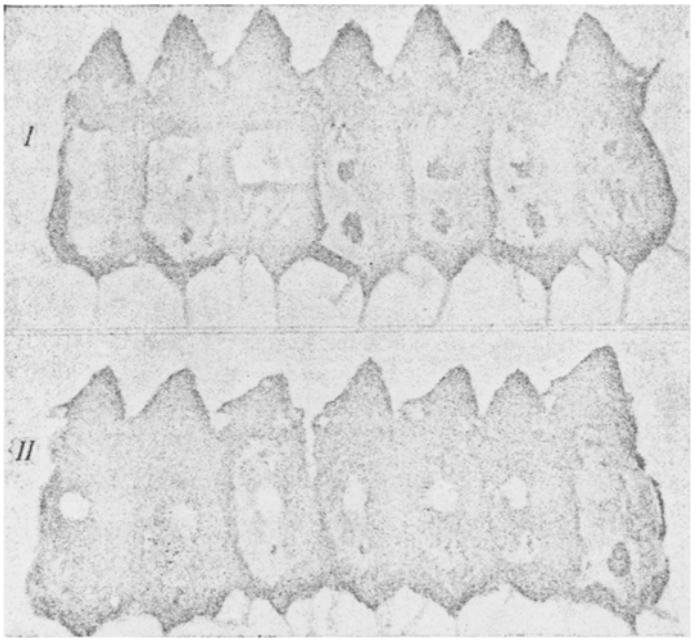

Fig. 1. Rejection of graft after preliminary sensitization of mice with vaccine from S. aureus. I) Experimental animals; II) controls. The graft in the upper part of the experimental animals' backs is being rejected. In the control mice the graft survived. Scar changes are visible in the lower part of the back of the experimental and control animals at the place from which the graftwas taken.

EXPERIMENTAL RESULTS

When antigens of streptococci, staphylococci, and C. albicans were used, gradual rejection of the autograft was observed in the animals of the experimental series (Table 1, Figs. 1 and 2). In the first two days after operation the graft in its outward appearance was indistinguishable from normal skin. Starting with the third day, multiple tiny hemorrhages appeared in the graft (often nearer to its border), and these subsequently merged to form large foci of bleeding. After the 6th-7th day the graft became necrotic and by the 10th-12th day it was completely destroyed. At the site of the graft a scab was formed and this later dropped off. The place of the graft was clearly defined by a scar. With an increase in the doses of microorganisms, the time of onset of this effect and the external appearance of graft rejection were unchanged.

No antibodies against the antigens used were discovered in the serum of the animals either in the CFR or in Boyden's reaction, or by staining by Coons' indirect method, when streptococcal vaccines were used at the moment of autografting. With a considerable increase in the dose of bacterial cells injected ($5 \cdot 10^8$ cells, five injections at intervals of four days) and when autografting was performed on the 17th day after the last injection, the autograft was not rejected although complement-fixing antibodies against streptococcal vaccine were found in the animals' serum in a titer of 1 : 10-1 : 20. Injection of streptococcal vaccines in lower doses than those used in the main experiment did not cause rejection of the graft transplanted at the same time as in the main experiment.

In a special series of experiments to study the relationship between rejection of the autograft and the time elapsing between the last injection and the operation, rejection was observed when the grafting was performed from the 6th to the 19th day after the last injection. Autografts transplanted sooner or later than these times survived.

At least three possible causes may be suggested for rejection of the autograft. First is the toxic action of disintegration products of the injected bacterial antigens on the graft. This hypothesis is in conflict with the absence of rejection in early periods (until the 6th day after the last injection of antigen) and also with survival of the graft on the 17th day after intensive immunization, when the total amount of disintegration products of the bacterial vaccines was greater than from the 6th to the 19th days when the basic doses of antigen were used. Second is the effect of circulating antibodies common to streptococci or another of the antigens used and the tissue on the graft. Evidence against this hypothesis is given by the fact that the

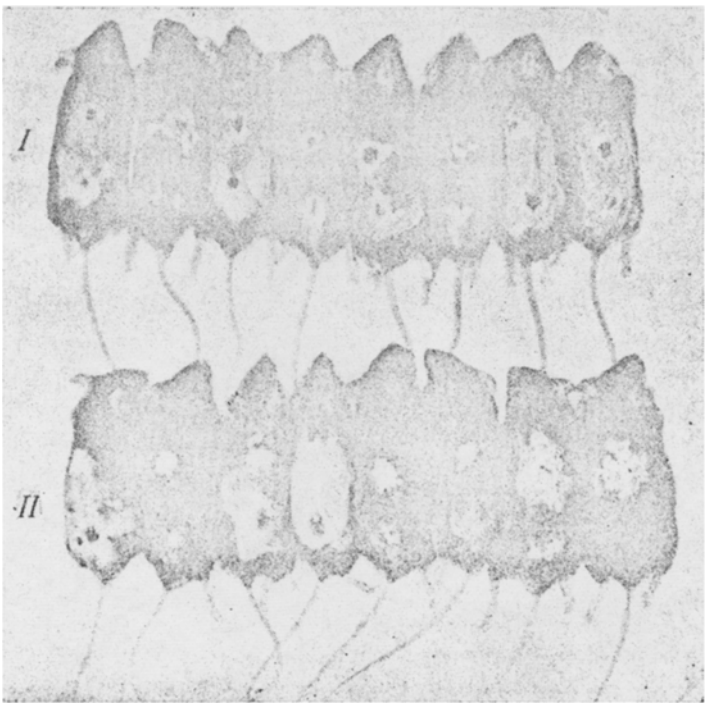

Fig. 2. Rejection of the graft under the influence of preliminary sensitization of mice with vaccine of C. albicans. Legend and explanation as in Fig. 1.

autograft took in the presence of antistreptococcal antibodies in a titer of 1 : 20 and was rejected in their absence. Third is the action of sensitized immunocompetent cells, acquiring autoaggressive properties as a result of injection of antigens closely related to mouse tissue antigens on the graft. The action of trauma inflicted during transplantation on the tissue may facilitate the manifestation of the autoaggressive effect of sensitized cells. We have no objective evidence against this last hypothesis. It appears the most probable, although its confirmation requires further experiments involving the active transfer of ability to cause rejection of the autograft by means of sensitized donor's lymphoid cells.

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