THE USE OF SEROLOGIC REACTIONS FOR STUDYING IMMUNITY DURING SKIN HOMOGRAFTING IN RABBITS

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Numerous investigations have shown that the homotrasplatntation of tissues is accompanied by the appearance of specific antibodies in the recipient's body. However, the mechanism of the action of these antibodies on the graft and the dynamics of their accumulation in relation to the state of the graft are largely unknown. There is as yet no established method of investgation of the immunologic changes: some authors use serologic tests to study these problems, but opinions on their sensitivity and practical usefulness are still highly subjective, and this has hindered the study of the problem of transplantation immunity.

The object of the present investigation was to make a comparative study of the practical value of different methods of serologic analysis of transplantation immunity in order to recommend the most sensitive method and the one corresponding as closely as possible to experimental demands.

EXPERIMENTAL METHOD

Experiments were carried out on chinchilla rabbits weighing 2.5-3 kg undergoing transplantation of a full-thickness skin graft measuring 2.5×4 cm on the back or on the ears. The animals were subdivided into four groups, with 15 rabbits in each.

The rabbits of group 1 received skin autografts, and the animals of group 2—homografts; group 3 consisted of rabbits homografted twice at intervals of 45 days; group 4 included animals homografted 45 days after preliminary autografting. The serologic tests were carried out repeatedly: immediately befor transplantation, 5 days after transplantation of skin, and thereafter every three days throughout the period of observation.

Eight serologic tests were used: the hemagglutination reaction (HAR) with the donor's erythrocytes; the HAR with the donor's trypsinized erythrocytes; the indirect Coombs' test [3]; the turbidity test of Hoigne [5], as modified by Klemparskaya and Raeva [1]; Ouchterlony's precipitation reaction [6]; the passpassive hemagglutination reaction (PHAR) of Boyden [2]; Pettit's leukocyte lysis reaction [7]; and determination of the cytolymphotoxins by Gorer's method [4] as modified by Tarasaki [8].

In addition the changes in the protein fractions of all the recipients' sera were studed by electrophoresis.

As antigens in Hoigne's reaction, for Ouchterlony's precipitation reaction, and for the reactions of Boyden and Pettit, a lysate of the donor's blood cells was used.

EXPERIMENTAL RESULTS

In the rabbits of group 1, receiving skin autografts, as might be expected all the grafts survived and the serologic reactions revealed no antibodies in the serum.

In the rabbits of groups 2, 3, and 4, before homograftings all the serologic tests were negative. After grafting, starting on the 5th-12th day, in the great majority of recipients of these groups marked immunologic changes were found. Antibodies were not found in the serum of only two animals of group 2 and 1 rabbit of group 4, by any of the tests used, i.e., they were serologically inert. The homografts of all the snimals of these groups failed to take.

The sensitivity of the various serologic tests, so far as could be judged from the incidence of positive reactions and the time of their appearance, was identical. In all the series of experiments, the tests of

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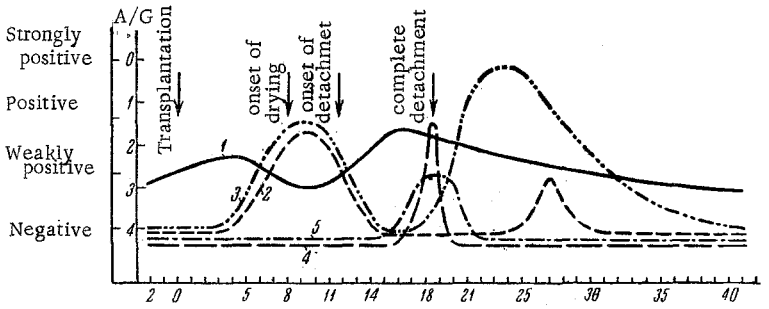

Dynamics of serologic changes after skin homografting in rabbits: 1) albumin-globulin ratio; 2) Boyden's reaction; 3) Hoigne's reaction; 4) Coombs' indirect test; 5) HAR. Ordinate—intensity of serologic reactions and albumin—globulin ratio (A/G); abscissa—days of experiment.

Hoigne and Boyden most frequently gave positive results. Somewhat inferior results were found with the HAR using donor's erythrocytes. These reactions demonstrated the appearance of antibodies at the earliest in some observations on the 5th-8th day after transplantation, but in a large proportion of the animals the immunologic changes did not appear until later—on the 11th-14th day.

The indirect Coombs' test, and the HAR with trypsinized donor's erythrocytes and with erythrocytes not treated with trypsin proved less sensitive as regards both the frequency of positive results and the time of their appearance. The Gorer-Tarasaki reaction, as these observations showed, is not suitable for the study of the serologic changes following tissue transplantation. The Ouchterlony precipitation test was negative in every case. Pettit's leukocyte lysis reaction gave very imprecise results, difficult to assess.

The immunologic response reaction varied in intensity in the different groups of experimental animals. The highest titers of the reactions were observed in the rabbits of group 3, undergoing repeated (twice) homografting.

The repeated antigenic stimulation evoked a more intensive antibody formation. The reaction to antigenic stimulation was somewhat less intensive in the rabbits of group 4, on which the homografting. However, it was more intensive than in the rabbits of group 2, undergoing a single homografting of skin. Although autografting, as the experiments on the rabbits of group 1 showed, did not cause antibody production, definite changes in the reactivity of the animal evidently took place, intensifying the subsequent reaction to antigenic stimulation. This was particularly noticeable from the results of Hoigne's, Boyden's and Coombs' tests.

The dynamics of the serologic changes in the homografted animals was not the same in all cases. However, in most animals the pattern was similar, as is clear from the figure, which summarizes the results of the serologic reactions in the rabbits undergoing a single homografting of skin in the dorsal region.

The figure shows that Hoigne's and Boyden's reactions yielded a curve with as a rule two maxima in the period of observation. The first maximum occurred 5-8 days after transplantation, after coinciding clinically with the onset of drying of the graft; the second rise of the curve appeared soon after detechment of the graft, on the average on the 18th day; the second maximum was observed between the 22nd and 27th days after skin grafting, and the rise of the curve of Hoigne's reaction as a rule occurred before the maximum of the increase in titer by Boyden's reaction.

The HAR and the indirect Coombs' test gave a curve with only one peak in these experiments, near the time of detachment of the graft. The changes in the serum protein fractions were on the whole parallel to those observed in the immunologic tests. As the figure shows, the decrease in the albumin/globulin ratio preceded the increase in the results of Hoigne's and Boyden's reactions.

The Gorer-Tarasaki test gave less demonstrative results (they are not shown on the figure), but here also a double fall in the cytotoxic index was seen: on the 9th-11th and the 20th-23rd days after the operation. The picture described was observed in the majority of animals, but in some of them a complete

correlation was not found between the state of the homograft and the dynamics of the increase in antibody titer. In a few cases detachment of the homograft took place against a background of complete serologic inertia. At the same time, cases were observed when antibodies appeared early and in high titers, and the state of the graft remained good for several days longer.

After detachment of the graft, antibodies were found for a long time (for 35-45 days by Hoigne's reaction).

It was thus found that Hoigne's and Boyden's reactions were the most sensitive of all the immunologic test used in the experiments and they can be recommended for the study of transplantation immunity.

None of the tests used in the investigation can be regarded as an absolutely reliable criterion during the investigations of the humoral changes arising after skin homografting, and when these problems are studied it is therefore desirable to use several reactions concurrently.

The dynamics of the changes in antibody titer are not a reliable indication of the state of the graft, because the relationship between these factors is subject to considerable individual variations.

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