

SKIN HOMOGRAFTS AND IMMUNOLOGICAL ACTIVITY IN RABBITS

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It is well known that homografting is associated with the development in the recipient of immunity to the foreign antigenic donor tissue. Most investigators consider that the development of transplantation immunity is of the nature of a delayed allergic sensitivity reaction and that it is brought about chiefly by the cellular (lymphocytic) elements [6, 11].

The problem of the involvement of humoral antibodies in the destruction of the homologous transplant remains debatable. In experiments on mice of pure lines it has been convincingly shown that homografts of normal tissues invariably give rise to an accumulation in the blood of the recipient of isoantibodies consisting of complete or incomplete hemagglutinins, leucoagglutinins, hemolysins, or cytotoxins [3, 7, 12]. The appearance of incomplete antibodies has been noted as early as the 3-5th day after transplantation, i.e., 1-2 days before the beginning of the graft had taken.

At the same time the number of such investigations on rabbits is comparatively small and in most cases all that has been done has been to discover complete or incomplete hemagglutinins in the blood of the operated animals [1, 2].

The present investigation consists of a study of the changes of the serum indices of acquired immunity and of nonspecific resistance developed by rabbits against skin homografts.

EXPERIMENTAL METHOD

Homo- and autografts of thin cutaneous fragments measuring 3.5×3.5 cm were made on the lateral aspect of the back*. Estimates of the viability of the graft were based chiefly on its external appearance (color and elasticity), and on thermocouple measurements of the temperature of the grafted skin and surrounding tissue.

Before the operation and 3, 7, 10, 14, 21, 28, and 35 days after it blood was collected from the rabbits for study of the number of leucocytes and erythrocytes, hemoglobin, osmotic resistance of erythrocytes, and to obtain serum.

The serum was used for immunological reactions for the estimation of complete or incomplete hemagglutinins, leucoagglutinins, hemolysins, complement-fixation and precipitation of antibodies, the titer of the complement and of lysozyme was determined. The lysozyme content was established by the greatest dilution of the serum which would allow complete lysis of a suspension of 1 billion *Micrococcus lysodeicticus* after a 3 hour incubation at 37°.

In some of the rabbits we tested the phagocytic activity of the neutrophils with respect to *Staphylococcus aureus*.

EXPERIMENTAL RESULTS

The work was carried out on 26 male chinchilla rabbits weighing 2.5-3 kg.

In 24 of them skin homografts were made, and two received an autograft of the same size (control).

The mean survival time of the cutaneous homografts was 11.5 ± 0.3 days. The autografts remained viable for the whole period of observation.

*The operation for transplantation of the skin was made together with M. E. Cherkasova.

In studying the condition of the peripheral blood during the first days after the operation, in all animals we found an increased number of leukocytes, and a shift of the count to the right. By the seventh day the number of leukocytes had returned to the original value, and by the 14-21st day after homografting in most of the animals the indices of leukocytosis were markedly increased. At this time a moderate reduction in the number of erythrocytes in the circulating blood could be observed and their osmotic resistance was increased; however a test with anti-globulin (Coomb's test) gave a positive result.

Apparently a positive Coomb's reaction in rabbits after skin homografting does not indicate any reaction of the graft against the recipient [8] but is the result of toxic damage to the erythrocytes of the host by products of the protein disintegration of the graft, or else it is an auto-aggressive influence of antibodies formed after the operation.

In a study of sera of experimental animals it has been shown that homografting skin in rabbits brings about the consistent appearance of humoral isoantibodies which interact with donor erythrocytes and leukocytes. The most consistent finding in our experiments was the appearance of incomplete serum hemagglutinins, which we found in the blood of all animals after homografting. The incomplete antibodies first appeared in the sera on the 7-10th day after the operation, and at this time they were present in the greatest amount; subsequently their titer was reduced, but they were present in very low concentration up to the 35th day after the operation.

We made a parallel and comparative study of the methods proposed for the detection of incomplete serum agglutinins. For this purpose we used the indirect Coomb's test, agglutination of erythrocytes previously treated with trypsin, agglutination in a serum-albumin medium, and Vinner's blocking test. By means of the indirect Coomb's test we were able to detect the presence of incomplete antibodies in far higher dilutions than was possible with other methods.

With the help of hemagglutination and hemolysis reactions which gave similar results antibodies to donor erythrocytes were found in 13 of the rabbits receiving homografts. Hemagglutinins and hemolysins were first found in the blood on the 10th day after grafting, by the 21st day their concentration had fallen considerably, and by the 28th the hemagglutination and hemolysis reactions were negative.

There were some animals (both in our experiments and in those reported by others [1]) in which the hemagglutination failed to reveal the presence of antibodies, but this result by no means indicates that they have undergone no immunological reorganization. It is known that by means of erythrocytes only antibodies to antigens of the system N-2 [7, 10] are revealed. When the recipient and donor differ with respect to isoantigens of other loci of tissue compatibility (N-1, N-3), a situation which is of course difficult to control in animals of impure strains, the erythrocytes do not contain transplantation antigens, and do not interact with the corresponding antibodies.

The view that leukocytes contain far more transplantation antibodies than do erythrocytes is well known [4], and is shown in particular by the fact that in homografting leucoagglutinating antibodies are present in a greater percentage of cases than are hemagglutinins. In our experiments, on the 14th day after homografting, leucoagglutinins were found in 19 rabbits, and their titers were higher than the titers of the antierythrocytic antibodies*.

After autografting skin no antibodies were found in the sera.

When the complement-fixation and precipitation in agar reactions were carried out with the sera of the experimental animals and with a saline extract of the skin of a healthy rabbit a negative result was obtained.

We studied the phagocytic activity of neutrophils in a comparatively small number of animals (12 rabbits), and the results must therefore be considered as preliminary. Increased phagocytic activity of leukocytes which showed up as an increase both in the number of active phagocytes and in the number of phagocytic microbial cells was found in the period following transplantation on two occasions, in the days immediately after the operation and on the 14th day. In the latter case the enhanced phagocytic activity of the neutrophils occurred at the same time as a secondary increase in the number of leukocytes in the circulating blood. A possible reason for the enhanced phagocytic activity of the neutrophils long after the operation may have been a greater opsonic index of the serum of these animals.

*Our method was to obtain a suspension of leukocytes from circulating blood by repeatedly suspending them in physiological saline. Because when this method of preparing a leucocytic suspension their spontaneous agglutination is frequently observed and that it is technically difficult to carry out the reaction the titer of the leucoagglutinins was not studied at all periods, but only at the time mentioned.

The lysozyme and complement contents, indices of nonspecific resistance, underwent characteristic changes in the blood of the homografted animals.

Immediately after the small increase in lysozyme activity of the blood observed 3-7 days after transplantation, on the 10-14th day there was a marked reduction in the amount of lysozyme in the serum of all the homografted rabbits. At this time no lysozyme could be found even in serum diluted 1:10, whereas its titer before the operation was 1:50-1:100. The reduction in the amount of lysozyme occurred at times at which the serum antibodies appeared. Similar changes were shown in the content of the complement in the serum, although in this case the changes were not so marked as in the case of lysozyme; the reduction of complement activity of the blood was found in 50% of the animals tested.

The reduction of the serum indices of nonspecific protective mechanisms in rabbits was transient, and by the 21-28th day after transplantation the indices returned to the level normal for the animal.

After transplantation of an autologous fragment of skin, we found no such changes in the indices of natural resistance.

At the present time we cannot give an exhaustive description of the mechanism of the reduction in the factors of nonspecific resistance in the period following homografting because this reduction may be the consequence either of inhibition of the products of the substances in the organism, or of a demand for them associated with the formation of the antigen-antibody complex, or finally it may be the result of them becoming directly bound during the destruction of the graft. In favor of the latter explanation there is evidence of observations of the *in vitro* action of lysozyme on transplantation antigens [5, 9].

SUMMARY

A study was made of the formation of serum isoantibodies and of the indices of nonspecific bodily resistance in rabbits after skin homografting. It was found that transplantation of a homologous skin fragment was consistently associated with the formation of complete and incomplete hemagglutinins, leucoagglutinins and hemolysins in the recipient's blood. At the time that the serum antibodies appear there is a phase of temporary reduction of nonspecific serum protection indices (lysozyme and complement).

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
