THE USE OF IMMUNOTHERMISTOGRAPHY AS A METHOD OF DETECTING HUMORAL ANTIBODIES FOLLOWING ALLOGRAFTING OF SKIN AND CORNEA IN RABBITS

M. M. Kapichnikov, A. M. Nogaller, L. G. Levkoeva, L. F. Lazarenko, and A. K. Saakov

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Humoral antibodies were investigated in 43 rabbits, after allografting of the skin and cornea, by the method of immunothermistography, based on changes in the thermal conductivity of the medium as a result of the antigen-antibody reaction. Within 3-5 days after skin grafting antibodies were detected in the blood serum and their titer rose steadily. Repeated skin grafting at first led to a decrease in the concentration of antibodies, but after the 8th-12th day their titer increased. Antibodies disappeared after 2-3 months in most animals. Similar results were obtained after corneal grafting. A parallel was observed between the thermistographic data and Hoigne's reaction, and also the dynamics of the complement titer. The results showed that immunothermistography is capable of showing the presence of humoral antitissue antibodies at an early stage of the rejection reaction. The method is highly sensitive and technically easy to perform.

KEY WORDS: *immunothermistography; antibodies; allografting.*

It is now a firmly established fact that humoral antibodies are formed as hemagglutinins, precipitins, lymphocytotoxins, etc., in response to antigens of various allografts [3-5, 14, 15]. However, the dynamics of formation of these antibodies and their discovery in the early stage of formation of transplantation immunity have so far been inadequately studied. Methods widely used in transplantation practice by no means always demonstrate in sufficiently good time the presence of antibodies formed in the recipient in response to transplantation antigens. Moreover, antibodies such as hemagglutinins, lymphocytotoxins, etc., as a rule are detected after the graft rejection reaction is complete [7, 8, 14, 15]. Sensitive methods of detecting humoral antibodies in recipients have assumed great importance, in conjunction with other approaches to the solution of a very important problem in clinical transplantology, namely the early diagnosis of the allograft rejection reaction.

Data in the literature on the role of humoral antibodies in the graft rejection reaction are highly contradictory [1, 3-5, 7, 13]. The degree of correlation between the serum antibody titer and the intensity of the graft rejection reaction is not sufficiently clear. Most workers consider that cellular responses play the most important role in graft rejection and that factors of humoral immunity play only a subsidiary role [1, 2]. To some extent this may depend on the low sensitivity of the methods used to detect circulating antibodies. Recently immunothermistography has attracted

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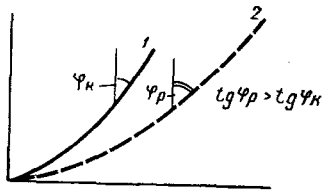

Fig. 1

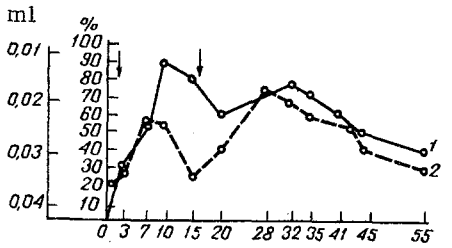

Fig. 2

Fig. 1. Graph showing results of immunologic reaction by immunothermistography: 1) control, 2) reaction. Abscissa, change in thermal resistance; ordinate, time of recording.

Fig. 2. Titer of complement and humoral antibodies from results of thermistography after skin grafting (at time shown by arrow). 1) Data of thermistography; 2) complement titer. Abscissa, days after transplantation; ordinate, complement titer (in ml) and percentage of positive thermistograms.

attention as a method of determining autoantibodies. This method records the immunologic antigen-antibody reaction on the basis of changes in the thermal conductivity of the medium by means of microthermistors [6, 9, 10, 11]. The very first experimental studies and clinical observations on bone tissue homografting showed that even very small quantities of freely circulating antibodies can be detected by immunothermistography [9, 12]. The method of immunothermistography has not been used to determine humoral antibodies after allografting of skin and cornea.

The object of this investigation was to detect humoral antibodies by immunothermistography at various times after primary and repeated allografting of skin and cornea in rabbits.

EXPERIMENTAL METHOD

A full-thickness allograft of the skin (6 X 4 cm) was transplanted in rabbits, at a primary operation on the back, and 15 days later the operation was repeated on the concha auriculae (2 X 2.5 cm). Corneal transplantation was carried out on the rabbits after a preliminary chemical burn. The times of rejection of the graft were determined on the basis of clinical evidence and the degree of opacity of the transplanted cornea. To determine antibodies by immunothermistography, the modified (by B. I. Levkoev) instrument described by Krishtal' and Kamenskaya [9] was used. For the skin grafting operations, an extract of the lymph glands or skin of the donor rabbits was used as antigen. For corneal grafting the antigen consisted of an extract of the donor's and burned corneas. Serum of an intact rabbit was used in every case as the control. The thermistograms were recorded for 7-10 min. The presence or absence of an immunologic response was judged from the gradient of the thermistograms in the experimental and control series. If the ratio between the tangent of the angle of slope in the control and the experimental records was greater than 1, the reaction was taken to be positive (Fig. 1). In the case of corneal transplantation, antibodies in the blood serum were detected by Hoigne's turbidity test, but in rabbits undergoing skin grafting the complement titer was studied. Tests were carried out at intervals on 43 rabbits and altogether 420 sera were investigated.

EXPERIMENTAL RESULTS

Before transplantation of skin or cornea no antitissue antibodies were present in the blood serum of any of the animals.

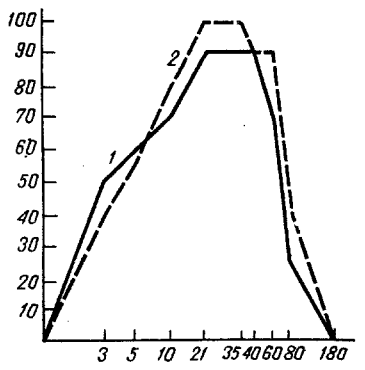

Fig. 3. Results of thermistography and Hoigne's test after corneal grafting: 1) results of thermistography; 2) of Hoigne's test. Abscissa, days after grafting; ordinate, percentage of positive reactions.

After primary skin allografting the presence of antibodies was discovered in the serum by immunothermistography in several animals as early as on the third day. On the 5th-7th day antibodies were found in half of the animals, and by the 10th day in the overwhelming majority of experimental rabbits (Fig. 2). These antibodies remained in the blood at a high level until the time of regrafting of skin, i.e., until the 16th day. After regrafting of the skin, as the results of thermistography showed, a marked decrease in the blood antibody concentration occurred by the 3rd-5th day. This could depend on the binding of freely circulating antibodies by antigens of the graft. Later, from the 8th-12th day after regrafting of the skin the blood antibody titer rose, but from the 16th day after regrafting of the skin or 1 month after the primary grafting the intensity of the reaction as a rule began to fall. The complementary activity of the serum was raised in all animals starting from the 3rd day after primary skin grafting. After the second skin homografting operation a more marked increase in serum complementary activity was observed, and it remained at this level for 2 weeks. The complement titer then gradually returned to its initial value. The complement titer showed no significant change in the control animals.

Antibodies against corneal tissue were detected in animals with bilateral burns of the eyes by means of Hoigne's microprecipitation test and by immunothermistography. On the 3rd day after burning, autoantibodies were demonstrated in one-third of the animals both by Hoigne's test and by immunothermistography.

From the 3rd day after corneal allografting antibodies against antigens of the donor's cornea were detected in half of the animals both by immunothermistography and by Hoigne's test. By the 5th-7th day the reactions were positive in two-thirds of the animals. By the 21st day after homografting antibodies against donor's cornea were discovered in nearly all animals tested and their titer was maximal by the end of the third week. The antibody titer remained at this level until 35-40 days after corneal grafting. The intensity of the reactions then fell and by the end of the 4th-6th month they became negative. As Fig. 3 shows, close correlation exists between the two reactions. Antibodies against donor's cornea were discovered in the recipients' blood serum with the graft in different conditions, whether translucent, cloudy, or opaque. However, with the graft in translucent and cloudy states the antibodies appeared later (10th-14th day after the operation), their titer was lower, and by the 6th month the antibodies had completely disappeared from the serum. With the graft in an opaque condition the reactions were positive or strongly positive. The reaction to antigen from the burned cornea was weaker than when soluble antigens from normal cornea were used.

The results of these investigations suggest that the method of immunothermistography can be used successfully to detect tissue antibodies during allografting and to assess the state of the recipient's sensitization to transplantation antigens. The increase in the complementary activity of the recipients' serum after skin allografting can evidently be explained by the rise in the level of globulin fractions in the blood, possibly in connection with the increased intensity of their production by lymphocytes. The presence of correlation between the indices of immunothermistography and of Hoigne's test confirms the role of detection of allergic antibodies (of the reagin type) in the assessment of immunologic changes taking place in response to transplantation antigens. Immunothermistography is technically easier, more accurate, and more sensitive than many other serologic tests and, in particular, the determina-

tion of lymphocytotoxins and hemagglutinins [14].

It can be concluded that immunothermistography is a useful method for detecting the beginning of the immunologic graft rejection reaction.

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