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DELAYED-TYPE HYPERSENSITIVITY REACTION OF MICE TO XENOGENEIC RAT ANTIGEN

I. V. Lyadova and I. Yu. Chernyakhovskaya

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Xenogeneic transplantation into an adult animal induces a whole range of reactions leading to gradual destruction of the grafted tissue. In the modern view, cytotoxic T lymphocytes, T effector cells, and also delayed-type hypersensitivity (DTH) antibodies may participate in graft rejection [3, 8, 11, 12]. Depending on the nature of the graft, the method of transplantation (with vascular anastomoses or by free grafting), and also the level of sensitization of the recipient, each of the above components of the immune response may play a more or less important role in rejection. Evidence of the important role of DTH reactions in allograft rejection has recently been published [6, 7, 10]. The mechanisms of rejection of xenogeneic grafts have received much less study. Their interpretation is important in connection with the prospects for the use of xenografts in medical practice.

A method of induction of tolerance in adult mice to allogeneic and xenogeneic grafts of neonatal heart has recently been suggested in the writers' laboratory [2, 4, 5]. During a study of the immunologic status of animals tolerant to an allograft, depression of DTH to the corresponding alloantigens was observed [1]. A similar investigation conducted on animal tolerance to a xenograft was difficult, because no method of determining DTH against transplantation antigens of xenogeneic origin has yet been developed.

The aim of this investigation was to devise a scheme for induction of DTH to xenogeneic lymphocytes in mice.

EXPERIMENTAL METHOD

Male CBA mice of inbred lines, August rats, and guinea pigs aged 2-3 months, obtained from the nurseries of the Academy of Medical Sciences of the USSR-were used.

Suspensions of lymphocytes from the lymph nodes and spleen of mice, rats, and guinea pigs were prepared in medium 199 with the addition of anitbiotics (penicillin 100 U/ml, streptomycin 100 μg/ml and HEPES (0.005 M), filtered through Kapron filters, and washed by centrifugation at 200g for 10 min. The residue was resuspended and diluted to the required concentration.

To induce DTH, a scheme including sensitization of CBA mice followed by subcutaneous injection of the reacting dose of antigen, was adopted. The antigen was a suspension of splenic lymphocytes from August rats or guinea pigs. In preliminary experiments the mice were immunized intraperitineally with various doses of antigen (5×10^6, 10^7, 5×10^7 cells) and the test injection was given after 5, 7, 11, 14, or 21 days. Later, to sensitize the mice, 5×10^6 xenogeneic cells in Freund's complete adjuvant (FCA) were injected subcutaneously and the reaction was tested 5 days later. In all cases, to test the reaction, 5×10^6 xenogeneic spleen cells in a volume of 0.05 ml were injected into the plantar surface of the right hind limb, and the same number of syngeneic spleen cells was injected into the left hind limb. The reaction was recorded 24 h later as the difference between the volume of edema of the right and left limbs, measured with and MK-0-25 mm micrometer with an accuracy of 0.01 mm. A difference of 0.1 mm was taken as 1 unit of reaction.

In experiments to study adoptive local transfer of DTH, a suspension of lymphocytes was

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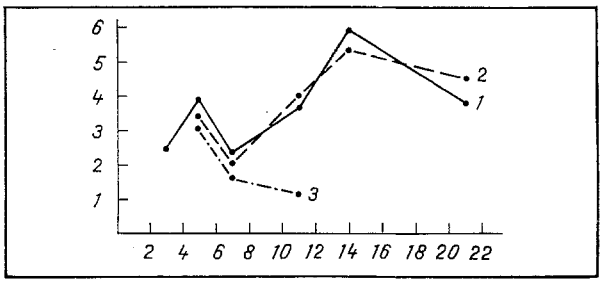

Fig. 1. Effect of conditions of induction on intensity of reaction to xenogeneic antigens. Abscissa, day of testing reaction relative to day of sensitization; ordinate, reaction (in DTH units). Mice were sensitized intraperitoneally with 5×10^6 (1), 10^7 (2), and 5×10^7 (3) xenogeneic spleen cells.

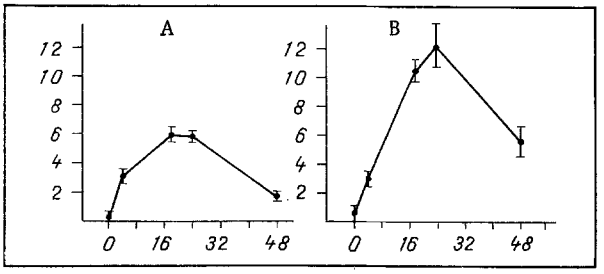

Fig. 2. Time course of development of hypersensitivity to xenogeneic antigens. Abscissa, time (in h) from reacting injection until reading of result; ordinate, reaction (in DTH units). A) intraperitoneally with 5×10^6 August rat spleen cells; B) subcutaneously with 5×10^6 August rat spleen cells in FCA.

obtained from the donors on the 5th day after sensitization. The reacting dose of antigen was injected beforehand into the plantar surface of the right hind limb of the syngeneic recipients, and this was followed 3 h later by injection of 5×10^6 viable donor's lymphocytes in 0.05 ml. In both cases 5×10^6 lymphocytes from intact CBA mice (in volume of 0.05 ml) were injected into the left (control) limb. The reaction was read 24 h after transfer.

To remove T lymphocytes from the cell suspension, rabbit gamma-globulin against mouse T lymphocytes (ATG: titer 1:160), obtained in the writers' laboratory, was used. Lymphocytes in a concentration of 2.5×10^7 cells/ml were incubated in medium 199 containing ATG (in a dilution of 1:10) for 30 min at room temperature, with constant mixing, after which rabbit complement was added and the mixture incubated a further 30 min at 37°C. The cell suspension thus treated was filtered through cotton wadding, centrifuged twice at 200 g for 10 min each time, after which the cells were resuspended and the number of viable cells in the suspension counted.

The numerical results were subjected to statistical analysis by Student's t test and are presented in the form $M \pm m$. Each group included at least 7 mice.

EXPERIMENTAL RESULTS

Optimal conditions of induction of DTH to xenogeneic antigens were determined in the experiments of series I. After intraperitoneal sensitization of the mice with different doses of antigen (5×10^6, 10^7, and 5×10^7 xenogeneic cells) and testing the reaction at different

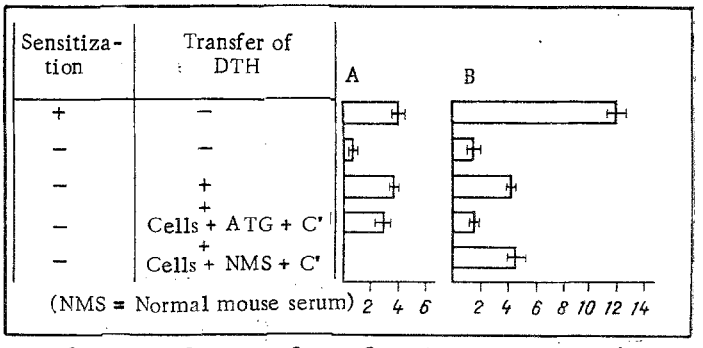

Fig. 3. Local transfer of DTH to xenogeneic antigens into intact recipients. Reaction expressed in DTH units. Reaction was transferred locally with 5×10^6 lymph node cells from donors sensitized 5 days before transfer; A) 5×10^6 August rat spleen cells injected intraperitoneally; B) 5×10^6 August rat spleen cells in FCA injected subcutaneously.

times, two peaks of response were discovered: on the 5th day in animals of all three groups and on the 14th day in mice sensitized with 5×10^6 and 10^7 cells (Fig. 1). The reaction lasted longer (up to the 21st day) in these two groups of mice than in mice sensitized with the larger dose 5×10^7 cells), in agreement with views regarding the character of development of DTH reaction [9]. Dependence of the level of response on the sensitizing dose and the times of testing, which we observed, is in agreement with data in the literature [13], according to which two peaks of response also were observed when AKR mice were sensitized intraperitoneally with lymphocytes from WKA rats (10^7): 5 and 10 days after sensitization.

On the basis of these results the conditions for induction of DTH for the subsequent experiments were chosen: sensitization with 5×10^6 cells, test injection 5 days later.

To study the nature of the reaction observed, the necessity for sensitization for its development was established, its time course studied, and the possibility of transfer to intact recipients was investigated with the aid of lymphocytes from sensitized donors.

It was found that when the reacting dose of antigen was injected into intact, unsensitized mice, the reaction did not develop.

To study the time course of the reaction it was recorded immediately after the test injection and against after 4, 17, 24, and 45 h. A positive reaction was observed as early as after 3-5 h, and its intensity increased a little toward 24 h (Fig. 2A).

The reaction could be transferred locally to intact recipients with the aid of lymph node cells from sensitized donors; however, the treatment of these cells with ATG and complement, removing the T lymphocytes, did not completely abolish transfer of the reaction (Fig. 3A). Similar results were obtained when the reaction was studied on the 14th day after sensitization of mice with the same dose of antigen 5×10^6 cells, data not shown).

On the basis of these results it was suggested that the reaction was complex in nature and consisted of two components: the DTH reaction and a reaction of immediate type. Evidence in support of the latter was given by the presence of xenoagglutinins in the serum of the sensitized mice, detectable in the hemagglutination test with August rat erythrocytes (titers on the 5th day exceeded 1:32, on the 14th day exceeded 1:512).

To shift the reaction toward DTH, attempts were made to replace intraperitoneal sensitization by subcutaneous, and also to sensitize the mice by injecting xenogeneic cells in FCA. This last modification of the scheme for inducing DTH proved to be successful: the intensity of the reaction was increased and its maximum shifted toward 24 h after testing (Fig. 2). Lymph node cells from donors sensitized by this method transferred the reaction into syngeneic intact recipients ($P < 0.01$), and removal of T lymphocytes from the suspension before transfer abolished this effect (Fig. 3).

In experiments to assess the specificity of the reaction, suspensions of spleen cells from August rats and guinea pigs were used as the antigen. In mice sensitized with guinea pig spleen cells a reaction was observed only when testes by the same antigen, and none was

observed during testing with rat spleen cells (the level of reaction was 1.22±0.03 and 0.11± 0.03 respectively). The level of response to a test injection of guinea pig antigen in mice sensitized with August rat spleen cells was higher than the degree of edema of the limb observed in unsensitized mice (0.48±0.07 and 0.24±0.04 respectively, $P < 0.01$), but lower than DTH to the specific antigen (0.76±0.08, $P < 0.05$). In the writers' view, these results indicate the existence of cross-reacting antigens and they are not evidence against the immunologic specificity of the test reaction.

The suggested scheme is thus capable of inducing a reaction with the basic characteristics of DTH, and can be used to study the immune response to transplantation antigens of xenogeneic origin.

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