DELAYED-TYPE HYPERSENSITIVITY REACTION TO ALLOANTIGENS IN MICE

I. Yu. Chernyakhovskaya, I. V. Lyadova, and L. N. Fontalin

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Evidence confirming classical ideas of a common mechanism of delayed-type hypersensitivity (DTH) and graft rejection [2, 4, 5], is constantly being published. In this connection the study of the pattern of development of DTH to tissue-compatibility antigens under conditions of tolerance to an allograft is of definite interest.

The undertaking of such investigations, however, is made difficult by the absence of a generally accepted technique of DTH determination to alloantigens, and, in particular, in mice. Most workers have succeeded in inducing DTH to alloantigens in mice only after preliminary administration of cyclophosphamide (CP) to the animals [3, 8]: CP is known to have the property of eliminating suppressor cells. Such a technique, while facilitating the development of the reaction, at the same time modifies the initial level of immunoreactivity of the animals substantially.

This paper describes an attempt to develop a scheme of inducing DTH to alloantigens in mice without administering CP to the animals.

EXPERIMENTAL METHOD

Experiments were carried out on male mice of inbred lines CBA (haplotype $H-2^k$), C57BL/6 (haplotype $H-2^b$), and BALB/c (haplotype $H-2^d$), aged 2-3 months.

To prepare suspensions of lymphoid cells from lymph nodes or the spleen they were homogenized in medium No. 199 with the addition of antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin) and HEPES (0.005 M), filtered through kapron filters, and centrifuged for 10 min at 1200 rpm, after which the cell residue was resuspended.

To induce DTH a scheme including intraperitoneal sensitization of CBA mice followed by subcutaneous injection of the reacting dose of antigen was used. A suspension of spleen cells from C57BL/6 mice was used as the antigen. In preliminary experiments the mice were immunized with different doses of antigen ($5 \cdot 10^6$, 10^7, or $5 \cdot 10^7$ allogeneic spleen cells) and the test injection of antigen was given after 3, 4, 5, 7, or 11 days. In all the subsequent experiments an injection of 10^7 cells was given and the reaction was tested 5 days later. To do this, $5 \cdot 10^6$ allogeneic spleen cells in a volume of 0.05 ml were injected into the right hind foot pad, and the same number of syngeneic spleen cells was injected into the left foot pad. The reaction was read after 24 h as the difference between the amount of edema in the right and left paws, by measuring the thickness of the paws with an MK 0-25 mm micrometer with an accuracy of 0.01 mm. A difference of 0.1 mm was taken as the unit of reaction.

In the adoptive transfer of DTH experiments lymphoid cells were obtained from donors on the 5th day after sensitization. For intravenous transfer $8 \cdot 10^7$ viable cells from donors' lymph nodes were injected into syngeneic intact recipients. The reacting injection of antigen was given immediately after.

For local DTH transfer, both the reacting dose of antigen and the donors' lymphoid cells ($5 \cdot 10^6$ spleen or lymph node cells in a volume of 0.05 ml) were injected subcutaneously into the right hind foot pad of syngeneic intact recipients with an interval of 3 h. The same number of lymphoid cells from intact CBA mice was injected into the control (left) foot pad.

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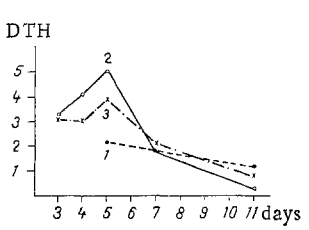

Fig. 1

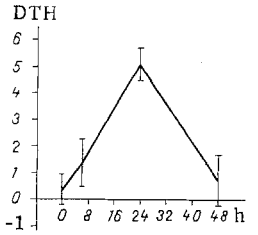

Fig. 2

Fig. 1. Effect of conditions of induction on intensity of DTH reaction to alloantigens. Abscissa, day of testing reaction relative to day of immunization; ordinate, reaction (in DTH units). Number of allogeneic spleen cells used for immunization was $5 \cdot 10^6$ (1), 10^7 (2), and $5 \cdot 10^7$ (3).

Fig. 2. Dynamics of development of hypersensitivity to alloantigens. Abscissa, time (in h) from time of reacting injection to recording of reaction; ordinate, intensity of reaction (in DTH units).

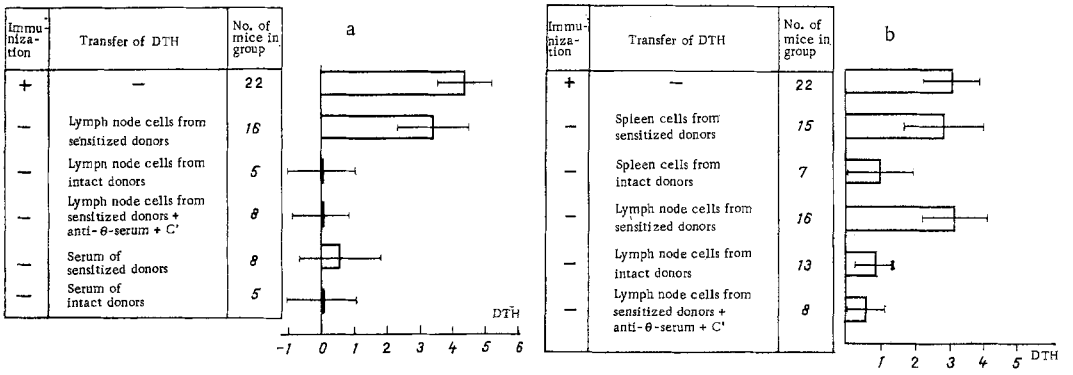

Fig. 3. Transfer of DTH to alloantigens to syngeneic intact receipients: a) intravenous transfer, b) local transfer. Reaction expressed in DTH.

In some experiments T lymphocytes were removed from the suspension of donors' lymphoid cells, by the use of anti-Θ-serum obtained in the writers' laboratory by the usual technique [7]. Lymphoid cells in a concentration of $2.5 \cdot 10^7$/ml were incubated in medium containing anti-Θ-serum (in a dilution of 1:10) for 30 min at room temperature with constant mixing, after which freshly obtained rabbit's complement was added and the mixture was incubated for a further 30 min at 37°C. After centrifugation twice (10 min at 1200 rpm) the cells were resuspended and the number of viable cells in the suspension was determined.

The results were subjected to statistical analysis by Student's t test. Arithmetic mean values with confidence interval at the $P < 0.05$ level are shown in Figs. 1–3.

EXPERIMENTAL RESULTS

In the experiments of series I optimal conditions for induction of DTH to alloantigens were determined. As Fig. 1 shows, in mice sensitized with allogeneic cells in a dose of $5 \cdot 10^6$, DTH was not observed regardless of the time at which the test injection of antigen was given (5th, 7th, of 11th day). Immunization of the mice with the larger dose of antigen (10^7) led to the development of hypersensitivity. A further increase in the dose of antigen used for immunization ($5 \cdot 10^7$) was not accompanied by enhancement of the reaction. In the last two groups of animals (receiving 10^7 or $5 \cdot 10^7$ cells) the reaction was positive as early as on the 3rd day, it increased in intensity until the 5th day, and then gradually decreased until the 11th day. The maximal reaction was observed in mice immunized with 10^7 cells and receiving the reacting injection 5 days later. This scheme was therefore chosen for the subsequent experiments.

Because there is no generally accepted method of determining DTH to transplantation antigens, it had to be confirmed that the reaction observed is in fact related to DTH. For this purpose the necessity for sensitization in order that the reaction should develop was determined, and the specificity of the reaction and the dynamics of its development were studied.

It was shown that after injection of the reacting dose of antigen into intact, unimmunized mice, no reaction developed. To study the specificity of the reaction, CBA mice were immunized with spleen cells from C57BL/6 mice. Spleen cells from C57BL/6 or BALB/c mice were used for the test injection. A reaction was observed only in the first case.

To investigate the dynamics of development of the reaction, it was recorded immediately after test injection of the antigen and again 4–6, 24, and 48 h later. The degree of edema at the site of injection of the antigen was found to increase gradually until 24 h, and then to decrease until 48 h (Fig. 2).

The reaction was thus strictly specific for the antigen used for immunization and maximal 24 h after injection of the reacting dose of antigen. These data are in full agreement with existing views on the character of DTH reactions.

One of the main characteristics of DTH is its realization by T lymphocytes and, consequently, the possibility of adoptive transfer to syngeneic intact recipients by means of lymphocytes of sensitized donors. Unlike the reactions of immediate type, DTH is not connected with antibody formation and cannot be transferred passively by means of serum of sensitized donors.

The reaction studied could be transferred adoptively to syngeneic intact recipients by injecting lymph node cells from the donors into them intravenously. Removal of T lymphocytes from the suspension before transfer abolished the observed effect. It was impossible to transfer the reaction by means of serum (0.5 ml) from the same donors (Fig. 3a).

It was thus shown that the reaction induced by the scheme suggested above is in fact DTH: It is specific, realized by T lymphocytes, not transferred by serum, and maximal in intensity 24 h after injection of the reacting dose.

To assess the role of different populations of lymphoid cells in DTH a model of local transfer of the reaction may be useful (Fig. 3b). The advantages of this over the intravenous method is that a much smaller number of of donor's lymphocytes can be used ($5 \cdot 10^6$ compared with 10^8). According to the observations of Marchal et al. [6] local transfer of even one sensitized lymphocyte can lead to the development of DTH.

We studied various schemes of local transfer of DTH to alloantigens. The most effective model was that used previously by Chernousov and Yurin [1] to study DTH to sheep's red blood cells. A reacting injection of antigen was given to intact recipients, and 3 h later, $5 \cdot 10^6$ lymph node cells from sensitized donors were injected. Spleen cells may be used for the same purpose, but the effectiveness of transfer is lower in this case (Fig. 3b).

The model of local DTH transfer may prove useful for determining the immunologic status of animals, in particular when the mechanisms of formation and maintenance of tolerance are studied.

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