INVESTIGATION OF THE DELAYED-TYPE HYPERSENSITIVITY REACTION TO TRANSPLANTATION ANTIGENS IN MICE WITH INDUCED TOLERANCE TO ALLOGENEIC AND XENOGENEIC GRAFTS

I. V. Lyadova, I. Yu. Chernyakhovskaya, and V. Golan'

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Transplantation of foreign organs and tissues induces a complex chain of reactions in the recipient, which lead ultimately to rejection of the graft. Until recently the main role in graft rejection has been ascribed to cytotoxic lymphocytes (CTL) [6, 13]. Now, however, this view has been questioned [8, 12]. It has been shown by adoptive cell transfer [11] that a subpopulation of T lymphocytes, enriched with cells with the Lyt 1+,2−phenotype (in which, consequently, precursors of CTL were absent), restored transplantation immunity just as effectively in "B mice" as did unfractionated T cells. Several other investigations also indicated that cells not belonging to the CTL population, but possibly effectors of delayed-type hypersensitivity (DTH), participate in rejection of allografts [7, 9].

In connection with these facts it was decided to study the presence or absence of DTH to transplantation antigens in animals with induced tolerance to a graft. Such investigations have so far been conducted in very small numbers and mainly during tolerance to tumor grafts. The data thus obtained on correlation between the intensity of the DTH reaction and survival of the grafts, are ambiguous [14, 15].

The aim of this investigation was to study DTH to the donor's antigens in mice with induced tolerance to an allogeneic and xenogeneic grafts of the neonatal heart, and also to investigate the mechanisms of regulation of this reaction.

EXPERIMENTAL METHOD

Male mice of inbred lines CBA, C57B1/6, BALB/c, and DBA/2, male August rats, and guinea pigs obtained from nurseries of the Academy of Medical Sciences of the USSR were used in the experiments.

Suspensions of allogeneic and xenogeneic spleen cells, obtained by the method described previously [1, 3], were used as antigens.

DTH was determined by the method developed by the writers [1, 3]: CBA mice were sensitized by subcutaneous injection of 10^7 allogeneic spleen cells in a volume of 0.2 ml or of 5×10^6 xenogeneic spleen cells in Freund's complete adjuvant (from Calbiochem, USA), in a volume of 0.1-0.2 ml. DTH was tested after 5 days by subcutaneous injection of the antigen (5×10^6 allogeneic or xenogeneic spleen cells in 0.05 ml) into the footpad of the right hind limb. The same number of syngeneic spleen cells was injected into the left foot. The thickness of the feet was measured with an MK-025 mm micrometer with an accuracy of 0.01 mm. The intensity of the reaction was estimated from the difference between the edema measured in the experimental (right) and control (left) limbs in millimeters. Mice receiving only a test injection of the antigen without sensitization served as the control.

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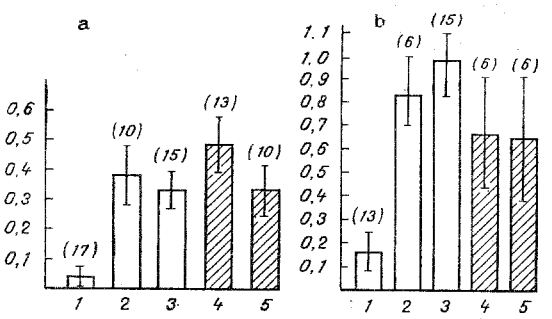

Fig. 1. DTH reaction in CBA mice, tolerant to alloantigens of C57B1/6 mice (a) and to xenoantigens of August rats (b). 1,4) Tolerant mice; 2) mice thymectomized and receiving CP; 3, 5) intact mice. Unshaded columns – DTH to donor's antigens; a) to C57B1/6 alloantigens, b) to August rat xenoantigens; shaded columns – DTH to foreign antigens; a) to BALB/c alloantigen, b) to guinea pig xenoantigens. Here and in Fig. 2: abscissa, groups of animals; ordinate, intensity of DTH reaction (in mm); number of animals given in parentheses.

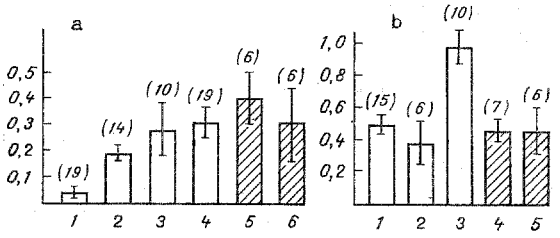

Fig. 2. Transfer of suppressors of afferent phase of DTH from CBA mice tolerant to allogeneic antigens of C57B1/6 mice (a) and to xenogeneic August rat antigens (b). CBA recipients received an intravenous injection of spleen cells from the following donors: 1, 2, 5) tolerant (2 – cells were treated with ATG or a-Thy-1.2. antibodies and complement); 3) thymectomized and receiving CP; 4, 6) DTH in intact mice. Unshaded columns – DTH to donor's antigens: a) to C57B1/6 alloantigens, b) to August rat xenoantigens; shaded columns – DTH to foreign antigens: a) to DBA/2 alloantigens, b) to guinea pig xenoantigens.

To remove T lymphocytes from the suspension of lymphoid cells, rabbit γ-globulin against mouse T lymphocytes (ATG), obtained from the laboratory of Immunologic Tolerance, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, or monoclonal anti-Thy-1.2. antibodies (a-Thy-1.2.), obtained from the Institute of Molecular Genetics, Czehcoslovak Academy of Sciences, Prague, were used. The dilution of ATG used to treat the cells was 1:15, and that of a-Thy-1.2. was 1:100. The method of treating the cells was described in detail previously [3].

Tolerance was induced in adult CBA mice by the method suggested previously [4, 5]: the animals were thymectomized and 3 weeks later they were given an intravenous injection of 10^8 viable spleen cells from C57B1/6 mice (for induction of tolerance to alloantigens) or from August rats (for induction of tolerance to xenoantigens). After 18-20 h the animals were given an intraperitoneal injection of cyclophosphamide (CP) in a dose of 200 mg/kg body weight. As was shown previously [4, 5] mice receiving this kind of tolerogenic treatment are tolerant and do not reject grafts of neonatal heart of the donor line for a long

time. Thymectomized mice, receiving CP simultaneously with the experimental animals, served as the control.

The experimental results were subjected to statistical analysis by Student's t test. Arithmetic mean values and confidence intervals at the P = 0.05 level are given in Figs. 1 and 2.

EXPERIMENTAL RESULTS

It was shown previously that a cutaneous reaction of DHT to transplantation antigens is induced in mice by injection of allogeneic or xenogeneic spleen cells. The reaction thus induced is specific relative to the antigen used for sensitization, it is maximal 24 h after testing, and is mediated by T lymphocytes [1, 3]. The technique of determination of DTH developed by the writers previously was used in the present investigation.

In the experiments of series I DTH was determined to allogeneic and xenogeneic antigens in mice receiving tolerogenic treatment. It will be clear from Fig. 1 that induction of tolerance in CBA mice to allogeneic antigens of C57Bl/6 mice was accompanied by loss of the animals' ability to form DTH to the donor's antigens. The reaction to allogeneic antigens of mice of a third line (BALB/c) was preserved. In mice tolerant to xenogeneic August rat antigens, absence of DTH to the donor's antigens also was observed while the reaction to foreign xenogeneic antigens was preserved. In thymectomized mice receiving CP, reactions to both allogeneic and xenogeneic antigens were indistinguishable from the reactions of intact animals to these antigens (Fig. 1).

Thus complete tolerogenic treatment of adult mice which, as was shown previously, leads to long survival of allo- and xenogeneic grafts of the neonatal heart [4, 5], induces also a state of areactivity, revealed by the DTH reaction.

The observed areactivity could be connected both with deletion or inactivation of the clone of immunocompetent cells and with the action of active suppresor mechanisms. In the experiments of series II the presence of suppressors of the afferent phase of DTH in tolerant animals was investigated. For this purpose 5×10^7-8×10^7 viable spleen cells from tolerant donors were injected into intact CBA mice, and 3 h later the recipients were sensitized for induction of DTH.

Injection of spleen cells of mice tolerant to alloantigens into normal recipients caused depression of DTH to alloantigens of C57Bl/6 mice (donor's antigens, see Fig. 2). Treatment of the cells with a-Thy-1.2. and complement, leading to removal of T lymphocytes, abolished the observed effect. The suppression was specific in character, as shown by preservtion of the response of mice of the third line (DBA/2) to alloantigens.

Spleen cells of mice tolerant to xenogeneic rat antigens suppressed the afferent phase of DTH partially in the recipients. The suppression was specific, but unlike that associated with tolerance to alloantigens, it was not abolished by treatment of the cells with ATG and complement (Fig. 2).

The writers showed previously the cells suppressing the efferent phase of DTH are present in the spleen of mice tolerant to alloantigens [2]. The suppressors were specific and belonged to the class of T lymphocytes. In the present investigation, the model described previously [2] was used to investigate the presence of suppressors of the efferent phase of DTH in mice tolerant to xenogeneic antigens. In none of the six animals investigated could suppressors of the efferent phase be discovered.

Thus in mice tolerant to allogeneic antigens suppressors acting on both phases of DTH (afferent and efferent) and belonging to the class of T lymphocytes were discovered. The specificity of the suppressors, and also their absence in thymectomized mice and mice receiving CP, suggest that intravenous injection of a massive dose of allogeneic cells, used during induction of tolerance, is essential for their formation. This hypothesis is in agreement with data in the literature on the induction of suppressors of DTH by injection of large doses of antigen into mice, and in particular by intravenous injection of allogeneic spleen cells [10].

By contrast with tolerance to alloantigens, tolerance to xenoantigens was accompanied by the formation of suppressors of only the afferent phase of DTH, and these suppressors, moreover, were resistant to treatment with ATG and complement. It is possible that suppres-

pressor activity of the cells in this case was connected with production of blocking antibodies by them. This is shown by our data on depression of the DTH reaction in recipients after preliminary injection of serum of donors tolerant to xenogeneic antigens (data not given), and also the presence of hemagglutinins and cytotoxins in that serum [5].

Thus suppressors of DTH are present in mice exhibiting tolerance to both allogeneic and xenogeneic antigens, but they differ in certain characteristics. The suppressors thus found evidently participate in maintenance of the state of tolerance to allogeneic and xenogeneic grafts. Meanwhile their role at the stage of establishment of tolerance is unknown and requires further investigation.

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