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TOLERANCE TO A XENOGRAFT IN AN ADOPTIVE SYSTEM

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Induction of tolerance to xenografts is a complex task, for rejection of such grafts usually takes place very acutely. Methods of successful transplantation of allogeneic organs and tissues are in most cases unsuitable for a xenogeneic system. Positive results in this direction have been obtained with the aid of cyclosporin A, whole-body lymphocidal irradiation, and certain other procedures [3, 6-9]. In 1980, the writers developed a method of inducing tolerance to xenogeneic antigens with the aid of cyclophosphamide, including thymectomy and injection of a massive dose of xenogeneic splenocytes into the animals. Treatment of this kind resulted in prolonged (over 5 months) survival of a transplanted neonatal August rat heart in mice [4, 5].

The aim of the present investigation was to develop a method of adoptive transfer of tolerance to xenogeneic antigens in order to study the specificity of such tolerance and the mechanisms maintaining it.

EXPERIMENTAL METHOD

Animals (male and female) of the following inbred lines were used: CBA ($H\text{-}2^k$) and (CBA × C57BL/6)F_1 ($H\text{-}2^{k/b}$) hybrid mice, and August (RI^c) rats. Erythrocytes from various species of animals — sheep, rat, and goose (SRBC, RRBC, and GRBC respectively) were used as the test antigens.

To induce tolerance, thymectomy was performed on adult mice and 3-4 weeks after the operation an intravenous injection of 1.2×10^8 rat splenocytes was given, followed 24 h later by an intraperitoneal injection of 200 mg/kg of cyclophosphamide (CP), dissolved in distilled water immediately before injection.

In the case of adoptive transfer of tolerance the recipients were irradiated from a ^{60}Co source in a dose of 9.5 Gy, which was followed by an intravenous injection of 2×10^7

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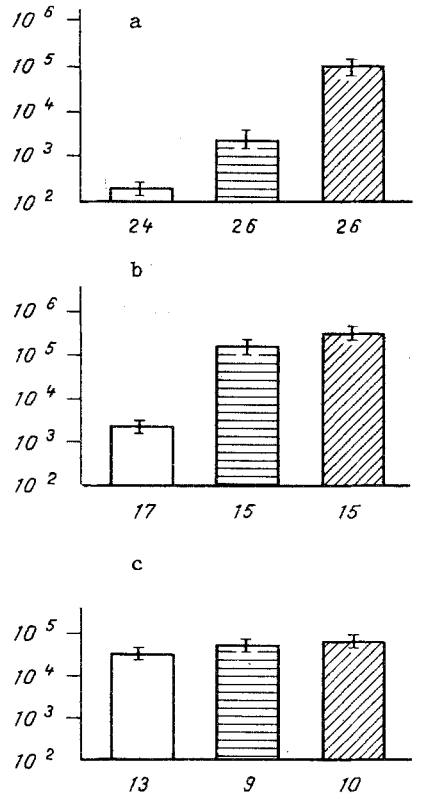

Fig. 1. Immune response to RRBC (a), SRBC (b), and GRBC (c) and suppressor activity of splenocytes of mice tolerant to rat xenogeneic antigens, 10 days after induction of areactivity. Abscissa, number of mice; ordinate, number of AFC per spleen. Unshaded columns (group 1); transfer of 2×10^7 splenocytes from tolerant donors; horizontal shading (group 2): transfer of 5×10^7 splenocytes of tolerant donors together with 2×10^7 splenocytes of intact donors; oblique shading (group 3): transfer of 2×10^7 splenocytes from intact donors. Test red blood cells (2×10^7) were injected mixed with spleen cells on the day of transfer. After 4 days mice were given intraperitoneal injection of 5×10^8 red blood cells of the same species. Number of AFC in recipients' spleen determined on 8th day after transfer by local hemolysis in gel method.

splenocytes from tolerant donors. Together with the splenocytes the animals each received 2×10^7 RRBC, SRBC, or GRBC. Four days after adoptive transfer the irradiated mice received a further intraperitoneal injection of 5×10^8 of the corresponding red blood cells, and 4 days later, the number of hemolysin-synthesizing cells in the spleen was determined by the local hemolysis in gel method.

Besides the test antigen, irradiated animals in the control groups received an injection of 2×10^7 splenocytes from intact syngeneic donors or from syngeneic thymectomized mice, treated with 200 mg/kg CP (TE + CP mice). In some experiments, to detect the suppressor activity of splenocytes of tolerant mice they were injected into irradiated recipients together with intact syngeneic splenocytes in the ratio of $5 \times 10^7 : 2 \times 10^7$.

To abolish tolerance and study the nature of the suppressor activity of splenocytes of tolerant mice, anti-I-J^k-serum, generously provided by A. S. Apt (Central Research Institute of Tuberculosis, Ministry of Health of the USSR), was used. The serum was injected intravenously into tolerant donors in two doses each of 30 µl: on day −2 and on day 0, 4 h before transfer of the cells. Normal mouse serum was injected into control tolerant mice by the same schedule.

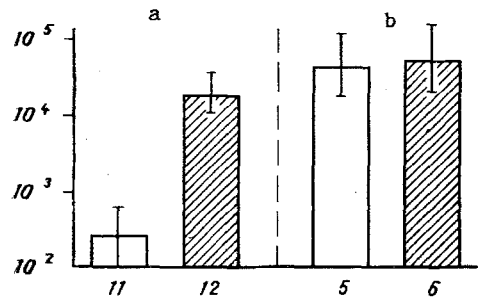

Fig. 2. Analysis of specificity of tolerance to xenogeneic antigens 30 days after tolerogenic treatment in an adoptive system. a) Response to RRBC, b) response to SRBC. Unshaded columns — group 1: transfer of 2×10^7 spleen cells from tolerant donors; oblique shading — group 2: transfer of 2×10^7 spleen cells from intact donors. Remainder of legend as to Fig. 1.

TABLE 1. Abolition of Tolerance and Suppressor Activity of Cells of Tolerant Donors with the Aid of Anti-I-J^k-serum (M ± m)

Group No.	Preliminary treatment of tolerant donors	Donors of splenocytes		Number of recipients	Number of AFC to RRBC per spleen
		tolerance	control		
1	NMS	$2 \cdot 10^7$	—	17	214 2,337±0,114
2	Anti-I-J^k	$2 \cdot 10^7$	—	11	5 853 3,767±0,102
3	—	—	$2 \cdot 10^7$ TE + CP	5	6 698 3,826±0,083
4	NMS	$5 \cdot 10^7$	$2 \cdot 10^7$ Intact	9	1 717 3,234±0,135
5	Anti-I-J^k	$5 \cdot 10^7$	$2 \cdot 10^7$ Intact	13	12 046 4,082±0,122
6	—	—	$2 \cdot 10^7$ Intact	9	21 348 4,329±0,100

Legend. Anti-I-J^k-serum and normal mouse serum (NMS) injected intravenously into tolerant mice in two doses, each of 30 µl, on day −2 and day 0, 4 h before transfer of the cells. Simultaneously with spleen cells, the irradiated recipients also received 2×10^7 RRBC intravenously, on day +4 they received 5×10^8 RRBC intraperitoneally, and on the 8th day after transfer the number of AFC in the recipient's spleen was determined by local hemolysis in gel. The number of AFC per spleen in groups 5 and 6 did not differ significantly. The experiments were carried out 30 days after induction of tolerance.

EXPERIMENTAL RESULTS

Tolerance to rat antigens in the adoptive system was studied 10 days and 1 month after its creation. For this purpose splenocytes of tolerant mice were transferred into lethally irradiated syngeneic recipients together with RRBC. Spleen cells of tolerant mice did not synthesize hemolysins to RRBC at either time (Table 1, group 1). Splenocytes of control (intact or TE + CP mice) were able to respond to RRBC in adoptive transfer (Table 1, groups 3 and 6; Fig. 1). Areactivity of the splenocytes in the adoptive system 1 month after induction of tolerance was strictly specific in character: in the absence of a response to RRBC the response to SRBC was preserved at the normal level (Fig. 2). In the early stages (after 10 days) the immune response was inhibited both to SRBC and to RRBC, while the normal level of response to GRBC was preserved (Fig. 1).

Cells of tolerant mice specifically depressed the response of splenocytes of intact donors to RRBC at both times of the investigation (10 days and 1 month; see Table 1, groups 4 and 6; Fig. 1a), but did not lower the level of response to SRBC and GRBC (Fig. 1b, c). Two injections of anti-I-J^k-serum into tolerant donors caused loss of suppressor activity of the splenocytes (Table 1, groups 4 and 5) and complete recovery of the immune response up to the level of response of the spleen cells of control TE + CP mice (Table 1, groups 2 and 3). The results are evidence that suppressor activity was due to T lymphocytes, carrying the I-J^k

marker. According to our preliminary data, these suppressors are resistant to the action of CP: injection of 100 mg/kg CP 2 days or 1 day before adoptive transfer did not abolish the suppressor activity of splenocytes from tolerant mice.

Helper T cells are known to cross-react to mammalian erythrocytes [1]. The weakened immune response not only to RRBC, but also to SRBC, which we observed on the 10th day after induction of tolerance, may therefore be explained as the result of inactivation or deletion of helper T cells. Since suppression of the immune response by splenocytes of the tolerant mice was strictly specific at both times of testing, it can be concluded that the specificity of suppressor T cells is narrower than that of helper T cells involved in the response to xenogeneic antigens. These data showing more specificity of suppressor T cells are in agreement with results obtained by other workers [1, 2].

It can be concluded from the results of these investigations of tolerance to xenogeneic antigens that immunologic tolerance in adult animals in the early stages after tolerogenic treatment is maintained by two mechanisms: by highly specific suppressor T cells and by a clonal deficit of helper T cells, which exhibit weaker specificity. Later, $I\text{-}J^+$ suppressor T cells evidently become the factor determining maintenance of areactivity.

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REGULATION OF IMMUNITY OF MICE TO TUBERCULOSIS BY GENES OF THE H-2 COMPLEX

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Genetic control of susceptibility and resistance to virulent pathogens, including to *Mycobacterium tuberculosis* and *M. bovis*, in inbred mice has been shown to be polygenic in character [2, 7, 8]. Besides the Bog-Ity-Lsh gene (genes), mapped in mouse chromosome 1 [5, 11, 12], and other genetic loci determining the level of natural resistance of mice to these pathogens, an important role in the regulation of immunity to tuberculosis is played by genes of the H-2 complex, which evidently determine the acquired immune response to infectious antigens [4]. Data obtained in recent years by T-lymphocyte cloning show that the immune response to antigens of intracellular agents, including mycobacteria, is restricted by products of genes in the I region of the H-2 complex [6, 9]. Meanwhile the involvement of H-2 genes in the formation and regulation of immunity to tuberculosis in vivo has virtually not been investigated and there are no reliable estimates of the function of the IR-genes of the H-2 complex in determination of the level of the immune response to tuberculosis and of resistance to the disease.

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