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SUPPRESSOR ACTIVITY OF SPLEEN CELLS IN DRUG-INDUCED IMMUNOLOGIC TOLERANCE

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Tolerance to sheep's red blood cells (SRBC) was induced in (CBA × C57BL/6)F_1 mice by a single intraperitoneal injection of 6×10^9 SRBC followed by injection of 100-200 mg/kg cyclophosphamide 44-46 h later. Spleen cells of tolerant mice, obtained at various times (12-26 days) after induction of tolerance, when injected into intact syngeneic recipients, did not depress their immune response to SRBC. Unlike intact mice, tolerant mice were unable to produce suppressor cells after a single immunization with SRBC. Only if three additional injections of large doses (6×10^9) of SRBC were given to the tolerant mice did their spleen cells acquire the ability to inhibit the immune response on injection into normal mice. It is postulated that the absence of suppressor cells on induction of immunologic tolerance by means of cyclophosphamide is due to clonal elimination. Suppressor cells may arise in tolerant animals under the influence of intensive antigenic stimulation, leading to deepening of the state of tolerance as a result of additional injections of SRBC.

KEY WORDS: *immunologic tolerance; cyclophosphamide; suppressor cells.*

Recent investigations have shown that suppressor cells play a role in the mechanism of some forms of immunologic tolerance [6, 10]. Suppressor T cells have also been found in drug-induced tolerance, although attempts by some workers to detect their presence were unsuccessful [5, 7, 9].

It was shown previously that during immunization of mice with a sufficiently high dose of sheep's red blood cells (SRBC) T suppressors inhibiting the immune response of intact syngeneic recipients appeared among their spleen cells (SC) [3, 11].

The object of this investigation was to study suppressor activity of mouse SC in tolerance induced to SRBC with the aid of cyclophosphamide (CP).

EXPERIMENTAL METHOD

Experiments were carried out on adult (CBA × C57BL/6)F_1 male mice weighing 22-28 g (from the Stolbovaya nursery, Academy of Medical Sciences of the USSR). Tolerance was induced by intraperitoneal injection of 6×10^9 SRBC followed 44-46 h later by injection of

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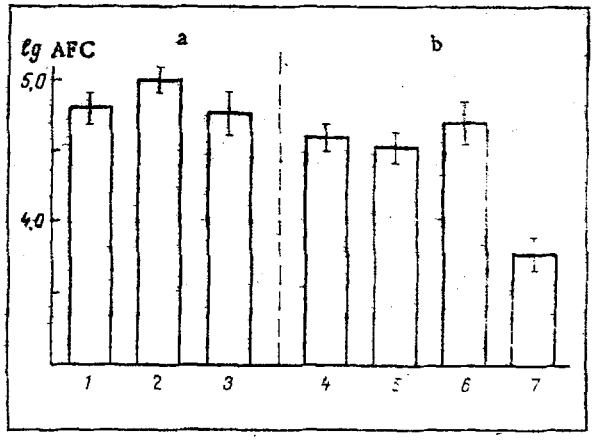

Fig. 1. Ability of SC of tolerant animals to suppress immune response of intact mice. a) SC obtained 12 days after induction of tolerance; b) SC obtained 14 days after test injection of SRBC into tolerant animals. 1, 4) Intact immunized mice (control I); 2) mice receiving SC from donors treated with CP only (control II); 3) mice receiving SC from tolerant donors; 5) mice receiving SC from tolerant donors 26 days after induction of tolerance; 6) mice receiving SC from tolerant donors receiving test injection of SRBC. 7) Mice receiving SC from immune donors. Dose of CP used to induce tolerance 100 mg/kg. Number of SC injected 5×10^7. Ordinate, here and in Figs. 2 and 3: log of number of antibody-forming cells (AFC) in spleen.

TABLE 1. Effect of SC of Tolerant Mice on Immune Response of Normal SC in Adoptive Transfer (geometric mean and confidence intervals at $P \leq 0.05$ level)

Mice donating spleen cells	Number of recipients	Number of antibody-forming cells in spleen of irradiated recipients
Intact	10	7079 (4416±11350)
Tolerant	11	188 (128±277)
Tolerant with additional injection of SRBC	10	51 (19—138)
Intact + tolerant	8	8851 (5358±14620)
Intact + tolerant, receiving additional injection of SRBC	8	6324 (3698±10810)
No cells injected	6	13 (9—17)

100-200 mg/kg CP. Some mice were later given three further intraperitoneal injections, each of 6×10^9 SRBC, at intervals of 4-5 days.

The immune response of the animals was assessed from the number of direct plaque-forming cells in the spleen on the fourth day after intravenous test injection of 5×10^8 SRBC [8]. In experiments with adoptive transfer of SC the recipients were irradiated in a dose of 900 R on the Stebel'-3A apparatus (dose rate 900 R/min) 4-6 h before transfer of the cells.

Statistical analysis of the results was carried out by Student's t test.

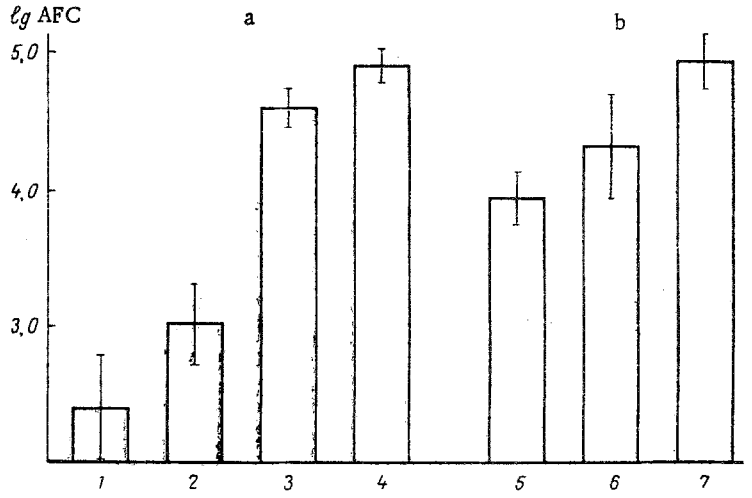

Fig. 2. Effect of additional injections of SRBC on state of tolerance. a) Investigation 3 weeks, b) 9 weeks after induction of tolerance. 1, 5) Tolerant mice receiving additional injections of SRBC; 2, 6) tolerant mice; 3, 7) mice receiving CP only (control I); 4) intact mice (control II).

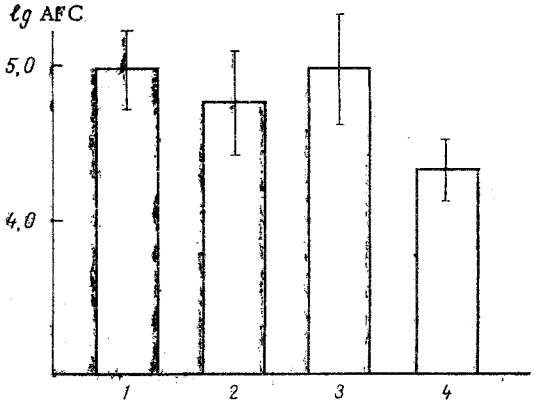

Fig. 3. Suppression of immune response of intact mice by SC from tolerant animals receiving additional injections of SRBC. 1) Intact immunized mice (control I); 2) mice receiving SC from animals treated with CP only (control II); 3) mice receiving SC from tolerant animals; 4) mice receiving SC from tolerant animals with additional injection of SRBC.

EXPERIMENTAL RESULTS

In the experiments of series I the ability of SC of the tolerant mice to affect the immune response of intact syngeneic recipients was assessed. The latter received an injection of SC from tolerant donors killed either 12 days after the induction of tolerance (Fig. 1a) or receiving a test injection of SRBC at that time and killed 14 days therafter (Fig. 1b). As Fig. 1 shows, in both cases the immune response of the mice was the same as in the animals of the control groups. By contrast with this, SC taken from donors on the 14th day after immunization with SRBC in a dose of 5×10^8 had marked suppressive action on the immune response of the intact recipients.

The results of these experiments thus showed absence of suppressor cells in tolerant mice in this particular system and they also indicated that suppressor cells cannot be induced in such animals by a single immunization.

In the experiments of series II the immunologic status of tolerant mice receiving additional injections of SRBC was studied. The results given in Fig. 2 show that the state of "nonresponsiveness" to SRBC was considerably intensified as a result of the additional injections, in agreement with earlier findings [1].

To determine any suppressor cells which might be present in these mice the following experiments were carried out. In the first variant, SC of intact mice together with SC obtained from tolerant animals or from tolerant mice receiving additional injections of SRBC were transplanted into irradiated recipient mice. The results (Table 1) show that cells of tolerant animals did not suppress the immune response of normal SC in adoptive transfer. In other experiments SC taken from mice of the following groups were injected intravenously into intact unirradiated mice: 1) tolerant mice, 2) tolerant mice receiving additional injection of antigen, 3) mice receiving CP only. The cells were taken 3 weeks after induction of tolerance. The cells were transplanted without antigen, and 3 weeks after transplantation the recipients were immunized with $5 \cdot 10^8$ SRBC. As Fig. 3 shows, injection of SC from tolerant donors receiving additional injections of SRBC into intact recipients led to 75% suppression of the immune response. Transfer of SC from other groups of donors did not affect the immune response of the mice.

The suppressor effect of SC from tolerant animals after additional injection of antigen in a high dose can thus be detected under certain conditions.

The results show that during induction of tolerance by means of a single injection of a high dose of SRBC and CP no suppressor cells are found in the spleen of the recipient mice. Moreover, production of suppressor cells likewise does not take place in tolerant mice if they are immunized with SRBC in a dose causing generation of suppressor cells in normal animals. This suggests that the process of clonal elimination, the basic mechanism of drug-induced tolerance, extends also to T suppressor cells proliferating after antigenic stimulation. It is interesting to note that suppressor cells already formed (14th day after immunization of normal mice with SRBC) are highly resistant to the action of CP [4].

The results of the present investigation agree on the whole with those indicating the absence of T suppressors in drug-induced tolerance to SRBC in mice [5, 7, 9]. At the same time, they contradict the evidence of the possible role of T suppressors in tolerance induced by means of CP to horse red blood cells [10]. The reason for this difference is not clear, but it can be tentatively suggested that it is based on the use of different antigens from mice of different lines, and also on differences in the method of administration of CP (Ramshaw et al. [10], in their investigations, injected CP subcutaneously in a dose of 100 mg/kg.

The suppressive effect of SC of tolerant mice receiving additional injections of SRBC, on transfer into intact recipients, can be interpreted in two ways. On the one hand it can be suggested that as a result of increased antigenic stimulation the production of blocking antibodies is intensified in the tolerant animals [2] and their formation by transplanted SC of these mice leads to depression of the recipients' response to SRBC. On the other hand, repeated injections of antigen may lead to the more rapid restoration of the suppressor function of spleen cells from the tolerant animals. In the writers' view this second explanation seems more likely, but further investigations are required to clear up this point.

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PREPARATION OF MONOSPECIFIC IMMUNOGLOBULIN AGAINST HUMAN LEUKOCYTIC INTERFERON

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Donkeys were immunized regularly at 7-day intervals with human leukocytic interferon, injected subcutaneously in a dose of 1600 units in 10 ml. Interferon-neutralizing antibodies were found in a titer of 1:128-1:256 in the sera of the animals after 38-40 immunizations. As a result of continued immunizations the titer of these antibodies rose considerably. Parallel tests revealed antibodies against components of the system in which the interferon was obtained. Donkey interferon plasma was prepared by plasmaphaeresis and an anti-interferon immunoglobulin was isolated from it by precipitation with ammonium sulfate at 50% saturation. Anti-interferon immunoglobulin was freed from contaminating antibodies by affinity chromatography on a combined immunosorbent.
KEY WORDS: *interferon; anti-interferon serum; monospecific immunoglobulin.*

Several recent investigations have yielded evidence of the antigenic properties of human interferons [2, 9, 10]. Preparation of antibodies against interferon presents the investigator with wide opportunities. To begin with, antibodies against interferon are necessary in order to study its antigenic properties and serologic specificity [7, 9]. Second, chromatographic separation of interferon and impurities by means of highly specific antibodies leads to a considerable increase in specific activity. Some investigations have already been carried out in this direction, on mouse [11, 12] and human [5, 6] interferons. The possibility of using immunologic methods to determine and titrate interferons is of great interest [15].

It has recently been shown that interferon is an important mediator of immunity [3, 13] and that, evidently, antibodies against interferon can be used to study the mechanism of formation of immunologic reactivity. The view has been expressed that hyperproduction of interferon *in vivo* leads to the development of autoimmune and allergic diseases [15]. In this connection antibodies against interferon may perhaps be used in clinical practice in order to produce immunodepression, and also as a component of the system of treatment of allergic and auto-immune diseases [4, 14, 15].

The object of the present investigation was to obtain a highly specific anti-interferon preparation (anti-interferon immunoglobulin) for immunologic and immunoclinical investigations and, in addition, to accumulate a sufficient amount of this material for clinical trials.

EXPERIMENTAL METHOD

Animals. Three donkeys each weighing 250-300 kg were used for immunization.

Interferon. Human leukocytic interferon with an activity of about 32 units/ml was provided by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

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