SPECIFIC SUPPRESSION OF THE IMMUNE RESPONSE IN THE ADOPTIVE TRANSFER SYSTEM

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Injection of spleen cells (SC) of syngeneic animals immunized with large doses of sheep's red cells (SRBC) into intact mice led to marked specific suppression of the immune response of the recipients. The highest suppressive activity was shown by SC taken from donors on the 14th day after intraperitoneal injection of SRBC. The SC of intact animals and of mice receiving a preliminary injection of rat red cells did not affect the immune response of the intact recipients on immunization with SRBC. Treatment of the immune SC with anti-T serum (ATS) or anti-B globulin (ABG) and complement considerably reduced or completely abolished the suppressive activity. Injection of a mixture of two suspensions of immune SC, one treated with ATS and the other with ABG, into intact recipients did not lead to suppression of the immune response. It is postulated that the suppressor cells in this particular model are T lymphocytes, expressing common antigens or antigens cross-reacting with B cells.

KEY WORDS: suppression of immune response; T lymphocytes; antigens of T and B lymphocytes.

The process of immunogenesis is due to interaction between different subpopulations of immunocompetent cells [3]. Thymus-dependent lymphocytes (T cells) are able to regulate antibody synthesis by B cells [8]. One way of regulation of immunogenesis is through depression of the immune response by suppressor cells. The existence of such cells has been shown in various models [4, 7]. In particular, it has been shown that after immunization of mice with sheep's red cells (SRBC) cells capable when transplanted into intact animals of specifically suppressing the immune response appear in the mice [11].

The object of this investigation was to determine optimal conditions for manifestation of the suppression phenomenon and also to attempt to describe the characteristics of suppressor cells.

EXPERIMENTAL METHOD

Experiments were carried out on adult (weighing 18-25 g) male C3H/He and DBA/2 mice and (CBA × C57BL/6)F_1 hybrids and also on C3H/He male mice from the Stolbovaya nursery. The general scheme of the experiments was as follows. The mice donating the spleen cells (SC) were immunized by intraperitoneal injection of SRBC (different doses). At various times after immunization the spleens of the animals were taken and a cell suspension prepared from them; after washing twice in medium No. 199 with antibiotics on the centrifuge, the suspension was injected intravenously into intact syngeneic mice. After 20-30 min the recipients of the immune spleen cells (ISC) received an intraperitoneal injection of SRBC in a dose of 2×10^8. Five days later, the number of 19S-antibody-forming cells (AFC) in the spleens of the mice was determined by the local hemolysis in gel method [6]. In the experiments to study the specificity of the phenomenon, rat red cells (RRBC) were used. In some experiments rabbit antiserum against mouse T lymphocytes (ATS) and antiglobulin against B lymphocytes (ABG), prepared as described previously [1], were used. Control experiments demonstrated that the preparations used were sufficiently effective and specific.

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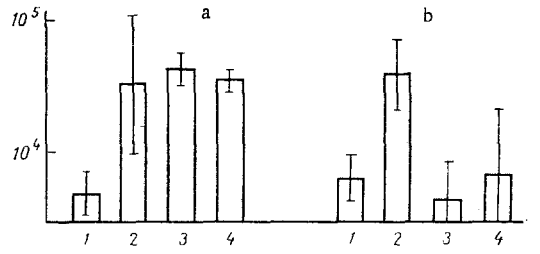

Fig. 1

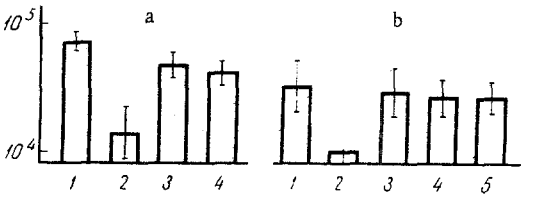

Fig. 2

Fig. 1. Specificity of phenomenon of suppression of immune response. Here and in Fig. 2, ordinate: number of AFC in spleen: a) number of AFC determined in reaction with SRBC in mice immunized with 2×10^8 SRBC after injection of 5×10^7 ISC (1), 5×10^7 spleen cells of mice immunized with RRBC (2), 5×10^7 spleen cells from intact mice (3), and in mice not receiving spleen cells (4); b) number of AFC determined in reaction with SRBC (1, 2) and RRBC (3, 4) in mice immunized with a mixture of 2×10^8 SRBC and 2×10^8 RRBC after injection of 5×10^7 ISC (1, 3) or not receiving spleen cells (2, 4).

Fig. 2. Effect of ATS and ABG on suppressive activity of ISC: a) (CBA × C57BL/6)F_1 hybrid mice; b) DBA/2 mice. 1) Intact mice; 2) mice receiving 2×10^7 ISC, treated with complement only; 3) mice receiving 2×10^7 ISC, treated with ATS and complement; 4) mice receiving 2×10^7 ISC, treated with ABG and complement; 5) mice receiving mixture of 2×10^7 ISC, treated with ATS and complement, and 2×10^7 ISC treated with ABG and complement. All mice immunized with 2×10^8 SRBC.

EXPERIMENTAL RESULTS

In the experiment to reproduce the phenomenon of suppression of the immune response, 50 million ISC from donors killed on the 14th day after immunization with SRBC in a dose 3×10^9 were injected intravenously into intact recipient C3H/He mice. Under these conditions a high degree of depression of the number of AFC in the recipients of the ISC was observed. In the control (injection of 2×10^8 SRBC alone) the number of AFC was 33,810 (from 28,080 to 40,700) per spleen, compared with 1652 (1097-2486) after injection of ISC and SRBC. Subsequent experiments showed that the suppression phenomenon is also reproduced after immunization of donors with a smaller dose of SRBC and, for that reason, in most cases (excluding special experiments) the donors of the ISC were immunized with SRBC in a dose of 5×10^8.

The results of investigation of the specificity of the phenomenon are given in Fig. 1. As Fig. 1a shows neither spleen cells of intact mice nor spleen cells of animals immunized with RRBC in a dose of 5×10^8, when injected intravenously into intact recipients, followed by intraperitoneal injection of SRBC, reduce the immune response compared with mice receiving SRBC alone, whereas injection of ISC depresses the immune response to SRBC considerably (by 85%). It is clear from Fig. 1b that injection of ISC from animals immunized with SRBC into intact recipients, followed by immunization of these animals with a mixture of SRBC + RRBC, does not affect the number of AFC to RRBC compared with the control.

In the next series of experiments, carried out on (CBA × C57BL/6)F_1 hybrid mice, the effect of the dose of SRBC and the time elapsing after immunization of the donor mice on the ability of their spleen cells to suppress the immune response after transfer to intact recipients was studied. As Table 1 shows, mouse spleen cells at different times after immunization in doses of 5×10^8 and 5×10^9 had the ability to suppress the immune response of the intact recipients ($P < 0.001$ compared with the control); moreover, the ISC of donors taken on the 14th day after immunization had the highest suppressive activity. When spleen cells from mice immunized with 5×10^6 SRBC were used, only ISC from donors taken on the 14th day after immunization possessed the ability to reduce the immune response of the recipients ($P < 0.05$).

Effective suppression of the immune response in the (CBA × C57BL/6)F_1 hybrid mice and DBA/2 inbred mice was observed only after injection of ISC not treated with ATS or ABG (Fig. 2). In this series of experiments treatment of ISC from hybrid mice with ATS before transfer into intact syngeneic recipients considerably reduced the suppressive effect (Fig. 2a). ABG reduced the ability of the ISC to suppress the immune response by the same degree. However, neither of the preparations used completely abolished the suppressive power of the ISC of hybrid mice: The degree of residual suppression (after treatment with ATS or ABG) was low (two thirds of the control value), but was still significant ($P < 0.01$). Treatment of ISC from DBA/2 mice with ATS

TABLE 1. Effect of Dose of SRBC and Time after Immunization on Suppressive Activity of Spleen Cells of Immune Mice

Dose of SRBC	Time after immunization of mice donating ISC, days		
	7	14	28
5×10^6	93 110 ± 1,194 (n=6)	43 350 ± 1,377 (n=5)	93 330 ± 1,072 (n=6)
5×10^8	45 600 ± 1,096 (n=12)	10 190 ± 1,294 (n=11)	20 420 ± 1,189 (n=6)
5×10^9	39 990 ± 1,135 (n=9)	12 790 ± 1,189 (n=12)	21 780 ± 1,334 (n=6)
Control		92 900 ± 1,099 (n = 11)	

Legend. Geometric mean numbers of AFC in spleen with standard error given. Number of mice shown in parentheses.

or ABG led to complete loss of the ability of the ISC to suppress the immune response of intact syngeneic recipients (Fig. 2b). To discover whether this phenomenon of suppression is due to synergic participation of ISC subpopulations differing in their sensitivity to ATS and ABG, or whether one ISC subpopulation equally sensitive to both ATS and ABG is responsible for this effect, a mixture of two suspensions, one consisting of 20 million ISC treated with ATS and the other of 20 million ISC incubated with ABG, was injected into intact DBA/2 mice. The results showed that injection of this mixture of cells did not affect the immune response of the intact recipients to SRBC.

The results show that spleen cells of mice of different strains, immunized with SRBC, on adoptive transfer into intact syngeneic recipients inhibit the immune response of the latter to injection of specific antigen. This phenomenon was most marked when sufficiently high doses of antigen were used to immunize the mice donating the immune cells and when the spleen cells were taken 2 weeks after immunization.

When discussing the mechanisms of the phenomenon the first essential is to examine the possibility of depressive action of antibodies, notably IgG, formed by transplanted ISC in response to secondary stimulation by the antigen (regulation of feedback type) [2, 10]. However, this possibility is ruled out by the results of preliminary experiments which showed that treatment of the donors of the ISC with cyclophosphamide in doses virtually completely suppressing the secondary immune response to SRBC (both in situ and in adoptive transfer) did not significantly reduce the ability of these ISC to exert their suppressive effect. It can therefore be concluded that this phenomenon is based on cellular mechanisms. The sensitivity of suppressors of ISC to ATS suggests that immune mice contain T cells which participate in specific suppression of the immune response, confirming results obtained by other workers using the same model [11].

In the present writers' view, the most interesting fact is the sensitivity of cells participating in suppression not only to ATS, but also to ABG, revealed by this investigation. Since injection of a mixture of two suspensions of ISC, one treated with ATS and the other with ABG, into intact recipients did not affect the recipients' immune response, it can be presumed that this effect of suppression is due to one population of ISC sensitive to both ATS and ABG. It is stated in the literature that antigens similar to antigens of B cells can be expressed on the surface of activated mouse T cells [9] and cortisone-sensitive thymocytes of rats which, in all probability, participate in the suppression of the immune response [5]. The sensitivity of the suppressor cells to both ATS and ABG, as these experiments showed, possibly reflects this rule, namely the expression on T cells on antigenic structures common to them and B cells as a result of activation by erythrocytic antigen in vivo.

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