ANALYSIS OF SOME MECHANISMS OF ANTIGEN-SPECIFIC SUPPRESSION OF THE IMMUNE RESPONSE

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It was shown previously that when mice are immunized with sheep's red blood cells (SRBC) T suppressors, which specifically depress the primary immune response of intact recipients, appear in the spleen [2, 8]. Extract of immune spleen cells (ISC) also was found to have an antigen-specific immunosuppressive action [4]. It was decided to study some of the mechanisms of the suppressive effect of ISC and an extract of them.

In the investigation described below dependence of the suppression effect on the time of injection of the ISC or their extract after immunization was studied and the target cells for the suppressive action were identified.

EXPERIMENTAL METHOD

Male CBA/Lac mice, from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, aged 3-4 months were used. The ISC were isolated on the 14th day after immunization of the donors of the SRBC in a dose of 10^9. Since it has been shown that mature suppressor cells in this particular model are resistant to the action of cyclophosphamide (CP) in a dose of 200 mg/kg [3], 3.5-4.5 h before isolation of the cells the donors were treated with this compound in the above dose. The resulting cells (ISC-CP) were used in a series of experiments as suppressor cells. ISC extract was obtained as described previously [4], using ISC in a concentration of 2×10^8 cells/ml. In adoptive cell transfer experiments the volume of ISC extract was 0.5 ml per recipient. Bone marrow cells were isolated by washing out of the tibia and fibula, followed by filtration through a Kapron filter. Thymus cells were isolated by means of a homogenizer and then filtered and washed 3 times in medium No. 199. Immune B cells were obtained by treating the ISC with anti-T-serum. The conditions for obtaining the serum and for treatment of the cells were described previously [1, 2].

Spleen cells of "T-mice," obtained on the 7th day after intravenous injection of 5×10^7 thymus cells of syngeneic animals into recipients lethally irradiated in a dose of 900 R and after intraperitoneal immunization with SRBC in a dose of 5×10^8, were used as cells enriched with immune T-dependent lymphocytes. In the experiments with adoptive cell transfer the recipients were mice treated with CP (200 mg/kg). ISC or their extract were injected intravenously. The animals were immunized intraperitoneally and the dose of SRBC was 5×10^8. The immune response was assessed on the 5th day after immunization from the number of antibody-forming cells in the spleen of the mice, determined by the local hemolysis in agar method [5].

EXPERIMENTAL RESULTS

In one series of experiments dependence of the suppression effect on the time of injection of ISC or their extract after immunization was investigated. In one group of experiments ISC were transferred to intact syngeneic recipients on the day of immunization, 1 day or on the 4th day after injection of the antigen. In the other group of experiments the effect of ISC extract was studied on the immune response of normal spleen cells transplanted into syngeneic recipients treated with CP. The ISC extract was injected into the recipients at different times after transplantation of 2×10^7 cells and immunization.

The experimental results (Fig. 1) showed that by the 4th day after immunization the spleen cells of intact mice had become resistant to the suppressor action of ISC or their extract.

The different sensitivity of different stages of immunogenesis to the suppressor effect necessitated a study of the comparative sensitivity of the primary and secondary immune response to suppression. Spleen cells from intact mice or from donors immunized 14 days before removal of the cells with SRBC in a dose of 10^6 or 10^9 were used in the experiments of this series. Syngeneic animals treated with CP served as recipients. ISC-CP were used as suppressor cells, and in some experiments an extract of ISC was used.

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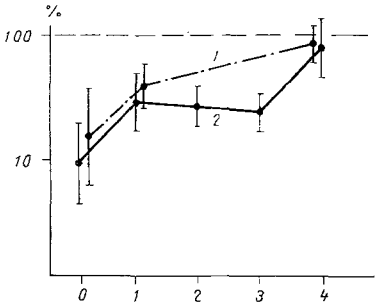

Fig. 1. Dependence of suppression effect on time of injection of ISC or their extract after immunization: 1) 5×10^7 ISC (mice receiving 5×10^8 SRBC used as control); 2) extract of ISC (syngeneic animals receiving 2×10^7 normal spleen cells on day of immunization, and treated with CP, used as control). Abscissa, time of injection of ISC or their extract (days after immunization); ordinate, immune response of recipients (in % of control).

TABLE 1. Comparative Sensitivity of Spleen Cells of Immune and Intact Donors to Suppressive Action of Suppressor Cells or of Their Facor

Donors of spleen cells	Dose of SRBC for immunization of donors	Source of suppressive action	Number of antibody-forming cells in spleen				
			experiment 1	experiment 2	experiment 3	experiment 4	experiment 5
Normal	—	—	7 499 (6 039—9 311) $n=5$	2 518 (1 271—4 989) $n=4$	2 138 (1 132—4 036) $n=5$	5 200 (2 767—9 772) $n=4$	1 986 (1 524—3 475) $n=5$
	—	ISC-CP	—	102 (42—248) $n=5$	567 (437—738) $n=5$	916 (632—1 327) $n=5$	151 (73—315) $n=4$
	—	Extract of ISC	2 455 (1 415—4 256) $n=5$	—	—	—	—
Immune	10^6	—	127 600 (84 920—191 900) $n=5$	31 990 (23 010—44 460) $n=4$	—	—	—
	10^6	Extract of ISC	63 390 (21 730—184 900) $n=5$	7 278 (4 246—12 470) $n=5$	—	—	—
	10^9	—	—	—	1 079 (632—1 841) $n=6$	1 225 (573—7 379) $n=4$	2 113 (773—5 781) $n=5$
	10^9	ISC-CP	—	—	1 138 (628—2 061) $n=4$	1 169 (721—1 897) $n=6$	1 151 (366—3 614) $n=5$

Legend. Here and in Table 2, geometric mean values and confidence intervals at $P < 0.05$ level are given.

The results (Table 1) indicate that the immune response produced by hyperimmune cells is resistant to suppression. Spleen cells of mice immunized with SRBC in a dose of 10^6 proved to be less sensitive to suppression than normal cells.

These results having been obtained it was possible to carry out experiments to detect the target cells for suppressor action. For this purpose a cooperative system consisting of T and B cells of different origin was used. Mice treated with CP served as the recipients.

The results given in Table 2 show that the immune response effected by a mixture of thymocytes and immune B cells does not undergo suppression. It must be concluded that the resistance of hyperimmune spleen cells to suppression is linked with the insensitivity of the immune B cells to the action of T suppressors. It can consequently be postulated that in this system B cells are the target cells for suppressive action.

When the experimental results are discussed it must be noted that the conclusion that B cells are evidently the targets for suppressor action in this system is in harmony with data in the literature on effective absorption by B-, and not by T-cells, of the soluble factor inhibiting the immune response to SRBC [7].

TABLE 2. Determination of Target Cells for Suppressor Effect of ISC

Number of cells, millions						Number of antibody-forming cells in spleen of CP-treated recipients
BMC	B_{norm}	B_{imm}	T_{norm}	T_{imm}	ISC-CP	
20	—	—	40	—	—	1614 (1161—2244) $n=17$
20	—	—	40	—	20	330 (168—656) $n=16$
—	20	—	40	—	—	940 (532—1656) $n=15$
—	20	—	40	—	20	143 (72—282) $n=14$
20	—	—	—	40	—	1247 (647—2399) $n=8$
20	—	—	—	40	20	126 (57—279) $n=9$
—	—	20	40	—	—	675 (426—1069) $n=10$
—	—	20	40	—	20	344 (161—734) $n=14$
20	—	—	—	—	—	133 (42—395) $n=6$
—	20	—	—	—	—	71 (12—424) $n=12$
—	—	20	—	—	—	48 (31—75) $n=9$
—	—	—	40	—	—	59 (30—114) $n=8$
—	—	—	—	40	—	271 (143—515) $n=4$

Legend. BMC) Bone marrow cells; B_{norm}) normal spleen cells treated with anti-T serum; B_{imm}) ISC treated with anti-T serum; T_{norm}) thymocytes; T_{imm}) spleen cells of "T mice."

The results now obtained suggest that hyperimmunization of B cells confers resistance to reception of the suppressor signal on them. This resistance is perhaps the result of the existence of different subpopulations of normal B-cells, differing in their sensitivity to suppression. Immunization by antigen in a large dose may cause activation of T-suppressors which inhibit B cells sensitive to their influence. This leads to positive selection of B lymphocytes resistant to suppressor action, which will be responsible for the secondary immune response.

Evidence that this selective pressure exerted by T suppressors on B cells can exist in principle is given by the observations of Kipps et al. [6], who showed that after inhibition of activity of T suppressors the number and heterogeneity of clones of B cells producing antibodies against dinitrophenol are increased. The possibility cannot be ruled out that T suppressors, by inhibiting the activity of some clones of B cells, promote the expansion of others.

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ANTIGEN-INDUCED INCREASE IN THE NUMBER OF CELLS FORMING NONSPECIFIC IMMUNOGLOBULINS *in vitro*

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The appearance of large quantities of nonspecific immunoglobulins (NIG) [2] during immunization has not yet been satisfactorily explained. This problem assumes particular importance in connection with the clonal selection theory, according to which the action of an antigen is selective in character.

This paper describes a study of the formation of cells producing antibodies (AFC) and NIG (NIGFC) during culture of lymphoid cells *in vitro*.

EXPERIMENTAL METHOD

Spleen cells from C57BL/6 mice, intact or immunized 3-4 days before the experiment by intravenous injection of 5×10^8 sheep's red blood cells (SRBC), were used. Cell pools (from 4 to 5 spleens) were cultured for 1-4 days in a Mishell–Dutton system [8], in the modification described in [1, 4], in the presence of water-soluble SRBC antigen [11]. At the end of incubation the cells were washed 3 times with Eagle's medium containing 10% embryonic calf serum and used to determine the number of AFC [7] and of immunoglobulin-forming cells (IGFC) [9]. The number of NIGFC was calculated as the difference between the numbers of IGFC and AFC per 10^6 living cells.

The experimental results were subjected to statistical analysis with calculation of the arithmetic mean (M_A) and the standard error ($\pm m$). Each point represents the mean of 15-27 parallel cultures.

EXPERIMENTAL RESULTS

During culture of normal spleen cells for 1-4 days the mean survival rate of the cells until the end of incubation was 40%.

Addition of water-soluble SRBC antigen to the cell cultures induced a distinct immune response which reached a maximum on the 4th day. Besides an increase in the number of AFC in the cultures, the number of NIGFC also was increased. At the peak of the response (3rd day) the number of NIGFC was 25 times greater than initially (Table 1). The NIGFC/AFC ratio fell with an increase in the number of AFC in the samples.

It was shown previously [1, 5] that addition of antigen to a suspension of spleen cells from mice immunized 3-4 days previously with the same antigen causes an increase in the number of AFC, which is significantly greater than the number of AFC induced in cell suspensions from unimmunized animals. It was interesting to discover how preliminary immunization of animals *in vivo* would affect the increase in the number of NIGFC in cultures.

Experiments showed that under these circumstances many more NIGFC were formed than in cell suspensions from normal spleens (Table 1). The mean number of NIGFC reached 117,294/10^6 cells, but in one experiment the number of NIGFC was increased to 165,000 per 10^6 cells, i.e., to 16.5% of the total number of cells in culture, equivalent to 33%

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