MECHANISM OF SUPPRESSION OF IMMUNOLOGIC MEMORY FORMATION

D. Ya. Aleinik, V. M. Pisarev,
and L. A. Pevnitskii

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It was shown previously that injection of sheep's red blood cells (SRBC) in a high dose into mice causes the generation of suppressor cells, which suppress both the primary immune response [4, 12] and also immunologic memory formation [1]. An extract of immune spleen cells (ISC) of mice immunized with SRBC specifically inhibited the primary immune response of spleen cells [6]. The targets for the action of the suppressor mediator of ISC in this case were B cells [5].

By contrast with suppression of the primary immune response, suppression of immunologic memory formation under the influence of ISC extract is antigen-nonspecific in character [2].

In the present investigation, in order to study the mechanisms of suppression of immunologic memory formation, an attempt was made to discover whether suppression of the primary immune response and of immunologic memory formation is brought about by the same product of ISC, and which cells are targets for the action of the suppressor mediator of ISC.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA/Lac Sto mice weighing 18-30 g. The immune response of the spleen cells of the mice was investigated in an adoptive transfer system. Mice treated with cyclophosphamide in a dose of 200 mg/kg were used as recipients. The primary immune response was evaluated on the 5th day after transfer of $5 \cdot 10^7$ normal spleen cells (NSC) and SRBC or of rat's red blood cells (RRBC) in a dose of $5 \cdot 10^8$. To investigate immunologic memory formation, recipients treated with cyclophosphamide were given an injection of a mixture of $5 \cdot 10^7$ NSC with 10^6 SRBC or RRBC. On the 7th day the animals were reimmunized with the antigen used for primary immunization, and on the 4th day the height of the immune response was tested. In some experiments splenic B cells from donors immunized with 10^6 SRBC 7 days before isolation of the cells were used. To obtain a suspension enriched with B cells, the spleen cells were treated with horse globulin against mouse T cells (batch No. 526, Department of Immunology, G. N. Gabrichevskii Moscow Research Institute of Epidemiology and Microbiology) by the method described previously [3] in a dilution of 1:5.

An extract of ISC containing suppressor factor was obtained as described previously [6] on the 14th-15th day after immunization of the donors with SRBC in a dose of $2 \cdot 10^9$. The ISC extract was absorbed twice with heterologous RBC [6]. To study the primary immune response, the ISC extract was injected intravenously mixed with NSC, after which the recipients were immunized intraperitoneally. To study immunologic memory formation the recipients were given the ISC extract on the day after injection of ISC and antigen. The dose of extract corresponded to $15 \cdot 10^7$ ISC disintegrated by ultrasound.

To assess immune T cell function the property of T cells, primed by SRBC, of preserving their helper activity after irradiation [8] was utilized. Mice treated with cyclophosphamide were injected with NSC and 10^6 SRBC; 7 days later the mice were irradiated in a dose of 900 R on the "Stebel'-3A" apparatus and given an injection of immune B cells. Some mice received extract of ISC 24 h after transplantation of NSC and SRBC. The results were read on the 5th day after transfer of B cells and reimmunization. The ability of T cells irradiated *in vivo* to cooperate with B cells was assessed as the "cooperative number" [11], i.e., as the number

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TABLE 1. Effect of Absorption of ISC Extract with Specific Antigen on Activity of Suppressor Factor During Primary Immune Response and Immunologic Memory Formation

Extract of ISC	Primary immune response to injection of antigen		Secondary immune response to injection of antigen	
	SRBC	RRBC	SRBC	RRBC
Control (extract not injected)	48 080 34 910—66 070 (12)	24 660 19 100—31 840 (12)	57 540 37 330—88 720 (13)	21 680 16 900—27 730 (12)
Unabsorbed extract	1 778 1 365—2 323 (12)	20 280 15 240—26 980 (12)	6 353 5 309—7 603 (13)	3 855 2 985—4 977 (13)
Extract absorbed with SRBC	49 430 34 590—70 630 (11)	23 390 18 370—29 720 (13)	45 710 37 840—56 750 (13)	17 380 13 240—22 800 (11)

Legend. Here and in Tables 2 and 3 geometric mean numbers of AFC in spleen and confidence intervals are given. Number of animals shown in parentheses.

TABLE 2. Effect of Absorption of ISC Extract by Rat and Human Red Blood Cells on Activity of Suppressor Factor During Immunologic Memory Formation

Extract of ISC	Secondary immune response to antigen	
	SRBC	RRBC
Control (extract not injected)	47 470 39 810—56 620 (13)	17 990 12 300—26 330 (10)
Unabsorbed extract	3 536 1 919—6 516 (8)	1 596 813—3 133 (11)
Extract absorbed with RRBC	4 560 2 642—7 852 (7)	2 350 1 062—5 200 (11)
Extract absorbed with HRBC	6 950 5 082—9 484 (9)	2 080 1 452—2 985 (8)

of antibody-forming cells (AFC) which, in these experiments, was the difference between the number of AFC in the experimental group (irradiated immune mice receiving B cells) and in the two control groups (immune irradiated mice and normal irradiated mice, receiving B cells only).

The level of the immune response in all experiments was estimated as the number of AFC in the recipients' spleen, which was determined by the method of local hemolysis in gel [9]. The results were subjected to statistical analysis with calculation of the geometric mean number of AFC and condidence intervals at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of absorption by heterologous RBC, including specific antigen (SRBC) on suppressor activity of the ISC extract was studied (Tables 1 and 2). As will be clear from Table 1, absorption of the ISC extract with SRBC completely abolished its suppressor activity as regards both the primary immune response and during immunologic memory formation. Absorption with foreign antigens (RRBC) and with human red blood cells (HRBC) had no significant effect on activity of the ISC extract (Table 2).

It can be concluded from these results that suppressor factor of ISC extract, inhibiting both types of immunologic response, carries an antigen-specific receptor for SRBC. One and the same factor is evidently responsible for the specific and antigen-nonspecific immunosuppressive effect.

In series II an attempt was made to discover the target cells for the ISC suppressor factor during immunologic memory formation. For this purpose the helper ability of T cells, preimmunized *in vivo* and subjected to the action of ISC extract, was studied.

TABLE 3. Effect of ISC Suppressor Factor on Cooperative Function of T Cells

Group No.	NSC + SRBC (day 0)	ISC extract (day +1)	Immune B cells (day +7)	SRBC (day +7)	Experiment 1		Experiment 2	
					number of AFC in spleen	"cooperative number"	number of AFC in spleen	"cooperative number"
1	+	—	—	+	10 020 7 274—13 810 (7)	—	5 105 2 755—9 458 (5)	—
2	—	—	+	+	9 705 7 194—13 090 (6)	—	4 385 3 096—6 212 (7)	—
3	+	—	+	+	78 160 70 470—86 700 (6)	58 435	50 930 40 690—63 750 (6)	41 440
4	+	+	+	+	19 820 15 410—25 480 (4)	95	3 069 1 993—4 726 (6)	0

Legend. Mice in groups 1, 3, and 4 received cyclophosphamide in a dose of 200 mg/kg on "day 0." On day +7 mice of all groups were irradiated. Dose of NSC and of immune B cells was $5 \cdot 10^7$. Dose of SRBC for primary and reimmunization 10^6.

The results of two experiments are given in Table 3. It will be clear from Table 3 that the "cooperative number," characterizing helper activity of the T cells, for mice not receiving ISC extract (group 3) was considerably higher than for recipients receiving it (group 4). Consequently, T helper activity was virtually absent after injection of ISC extract. It can therefore be concluded that splenic T cells responsible for immunologic memory formation become sensitive to the nonspecific suppressor effect of ISC factor 24 h after immunization with SRBC in a priming dose.

These results suggest that besides the antigen-binding receptor, suppressor factor contains a structure causing inhibition of generation of memory T cells. The nonspecific action of the factor may perhaps be mediated through activation of T-cells of the suppressor cycle, i.e., of second and third order T suppressors [7]. The latter prevents release of mediators facilitating the development of immunologic memory through their effect on proliferation or differentiation of T cells after priming. The possibility cannot be ruled out that one such mediator is interleukin-2, release of which is disturbed by the action of suppressor factors inhibiting the immune response and proliferative response of spleen cells to mitogens [10]. However, the direct action of antigen-specific suppressor factor on a hypothetical acceptor, which is expressed on the cell surface of the T helper cells 24 h after priming may also be postulated.

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