SECRETION OF A SUPPRESSOR FACTOR BY MOUSE LYMPHOCYTES ON CONTACT WITH SYNGENEIC ERYTHROCYTES

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UDC 612.112.94.017.1:612.111

During incubation of spleen cells from (CBA × C57BL)F_1 mice immunized with rat erythrocytes *in vitro* with syngeneic erythrocytes a suppressor factor inhibiting the immune response to sheep's erythrocytes is secreted. On intravenous immunization the factor is secreted by thymus and spleen cells, but not by bone marrow or lymph node cells. The factor is probably antigen-nonspecific and is secreted by nonspecific T suppressors on contact with syngeneic erythrocytes.

KEY WORDS: immune response; suppressor factor; thymocytes.

During incubation of spleen cells of immune mice *in vitro* with syngeneic erythrocytes a factor or factors possessing suppressor activity are secreted into the medium [1]. Secretion of suppressor factors takes place only when spleen cells of immune mice are used.

The aim of the present investigation was to study the specificity of this factor with respect to antigen and to study the secretion of this factor by cells of different organs of the immune system.

METHODS

(CBA × C57BL)F_1 mice were immunized intravenously with sheep's erythrocytes. The factor to be investigated was injected intravenously immediately after it had been obtained 3 days later. The mice were killed after 16-18 h and the number of plaque-forming cells (PFC) determined in the spleen by Jerne's method [3]. The factor was obtained by the method described previously [1].

RESULTS

It is concluded in many surveys on T suppressors that there are two types of T suppressor cells: antigen-specific and antigen-nonspecific; which secrete antigen-specific and antigen-nonspecific factors, respectively. Since in the system used in the present investigation the factor was secreted, not as the result of antigenic stimulation of suppressor cells, but in response to contact with syngeneic erythrocytes, it would be logical to suggest that the factor secreted was antigen-nonspecific. To test this hypothesis the following experiments were carried out. Mice were immunized with sheep's erythrocytes (SRBC) in a dose of $100 \cdot 10^6$ cells per mouse. On the 3rd day the test factor was injected, and the number of PFC in the spleen was counted 16-18 h later. The donors of the factor were (CBA × C57BL)F_1 mice immunized with SRBC (group 1), with erythrocytes from "August" rats (group 2), and with erythrocytes from BALB/c mice (group 3). In all cases the factor was obtained 3 days after immunization. The experimental results are given in Table 1.

The data in Table 1 show that after injection of the factor obtained from donors immunized with rat erythrocytes and also with SRBC into mice the level of the immune response was lower in both cases by 58%. Immunization of the donors of the factor with rat erythrocytes, which do not possess cross antigens with SRBC, thus leads to secretion of a factor inhibiting the immune response to SRBC by the spleen cells of these mice. This suggests that the factor secreted by the spleen cells in response to contact with syngeneic erythrocytes is antigen-nonspecific.

Department of Immunology, Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 11, pp. 576-578, November, 1979. Original article submitted February 5, 1979.

0007-4888/79/8811- 1315$07.50

TABLE 1. Effect of Immunization of Donors of Spleen Cells by Different Antigens on Suppressive Activity of Test Factor

Source of erythrocytes used to immunize donors	Number of erythrocytes injected	Level of immune response in test mice (number of PFC per spleen)
Sheep	100.10^6	20 810±225
		$P<0,01$
Rats	100.10^6	19 344±3511
		$P<0,01$
BALB/c mice	250.10^6	25 651±5650
		$P>0,05$
Control		46 910±7881

Legend. P given relative to control.

TABLE 2. Effect of Supernatants Obtained on Incubation of Different Lymphocytes with Syngeneic Erythrocytes on Level of Immune Response

Dose of antigen (SRBC)	Source of cells studied	Level of immune response (number of PFC per spleen)
30.10^6	Thymus*	1513±438
		$P>0,05$
	Thymus	479±88
		$P<0,01$
	Bone marrow	2464±706
		$P>0,05$
	Spleen	2610±801
		$P>0,05$
	Lymph nodes	2060±382
		$P>0,05$
	Control	2348±295
100.10^6	Thymus*	8636±844
		$P>0,05$
	Thymus	3671±672
		$P<0,05$
	Bone marrow	6829±1501
		$P>0,05$
	Spleen	1993±500
		$P<0,01$
	Lymph nodes	5682±1020
		$P>0,05$
	Control	7821±554

Legend: 1) experiments referred to in Tables 1 and 2 were carried out on different batches of animals.
2) Groups of mice receiving supernatant obtained after incubation of cells without addition of syngeneic erythrocytes marked by asterisks.
3) P given relative to control.

Investigations of lymphocyte subpopulations have shown that ability to respond to contact (to form rosettes) with syngeneic erythrocytes is a feature predominantly or exclusively of T cells [2, 4]. This was confirmed by the results now obtained. On treatment of spleen cells with anti-T serum the number of rosettes formed was reduced from 12% in the control to 2% in the experiment, and after treatment with anti-B serum from 12 to 9%. Hence it follows that the suppressor factor was probably secreted by T cells. This was confirmed by the results given in Table 2.

It will be clear from Table 2 that in response to a dose of antigen of $30 \cdot 10^6$ SRBC, injection of factor obtained after incubation of thymus cells with syngeneic erythrocytes reduced the level of the immune response by 80%. The supernatant of bone marrow, spleen, and lymph node cells incubated with syngeneic erythrocytes did not affect the level of the immune response. When the antigen was used in a dose of $100 \cdot 10^6$ cells the immune response was inhibited by factor obtained after incubation of spleen and thymus cells with syngeneic erythrocytes by 75 and 52%, respectively. The supernatant of bone marrow and lymph node cells did not depress the immune response. It follows from these results that the suppressor factor in this particular system is secreted by thymocytes. The results also suggest that with an increase in the dose of antigen migration of thymocytes capable of secreting the suppressor factor into the spleen is increased. This probably leads to the appearance of the ability of the spleen cells to secrete the factor and to a decrease in the ability of thymus cells to secrete this factor when the dose of antigen was increased from $30 \cdot 10^6$ to $100 \cdot 10^6$.

It can tentatively be suggested that thymocytes can secrete the suppressor factor even without contact with syngeneic erythrocytes. However, as Table 2 shows, the supernatnat obtained after incubation of thymocytes without the addition of syngeneic erythrocytes did not lower the level of the immune response appreciably. To sum up the results as a whole, it can be concluded that the suppressor factor or factors are secreted in response to contact between thymocytes and syngeneic erythrocytes and that they are not antigen-specific. The responsible cells are thus nonspecific T suppressors which, on contact with syngeneic cells, secrete a factor which inhibits the immune response.

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