NATURE OF SUPPRESSOR CELLS BLOCKING ACTIVATION OF DNA SYNTHESIS IN MIXED CULTURES OF NORMAL LYMPHOCYTES

B. D. Brondz, E. Ya. Khachikyan, and A. V. Karaulov

UDC 612.112.94:612.398.145.1

A further study was made of the nature of suppressor cells contained in a lymphocyte suspension from mice immunized with an allogeneic tumor and inhibiting activation of DNA synthesis in mixed cultures of normal lymphocytes. Suppressor cells, resistant to mitomycin C and to irradiation, are not inactivated by treatment with sera against θ-antigen and immunoglobulins in the presence of complement, are adsorbed on plastic, and are eliminated by carrageenan in vitro. The macrophagal nature of the suppressor cells is suggested.

KEY WORDS: mixed lymphocyte culture; suppressor cells; activation of DNA synthesis; carrageenan.

Injection of allogeneic spleen [15] or tumor [4, 6] cells into mice induces the formation of suppressor cells (SC), which block activation of DNA synthesis in a one-way normal mixed lymphocyte culture (MLC) that serves as a model of immunologic identification in vitro. Since SC differ in their properties from killer T-cells, inhibition of activation of DNA synthesis in MLC is not due to the cytotoxic effect of the immune lymphocytes relative to stimulators of this reaction [5, 6].

The object of this investigation was to continue the study of the properties of SC, for which purpose they were subjected to the action of sera against θ-antigen and immunoglobulin (Ig), mitomycin C (MC), carrageenan, irradiation, and adsorption on plastic.

EXPERIMENTAL METHOD

B10.D2 ($H\text{-}2^d$) mice were immunized by single subcutaneous injections at five different points of sarcoma MCh11, induced in C57BL/10 ($H\text{-}2^b$) mice (subsequently abbreviated to B10). Immune lymphocytes were obtained from the regional lymph nodes 8 days after immunization. The reaction in MLC was set up in the modification of Brondz et al. [4] of the micromethod of Glick et al. [11], by incubating mixtures of lymph node cells with irradiated (cobalt source, 1500 rad) allogeneic (syngeneic in the control) spleen cells in No. 3040 microdisks (from Falcon Plastics, USA). The samples were transferred to the filters after culture for

TABLE 1. Absence of Effect of Sera against θ-Antigen (Anti-θ) and against Immunoglobulins (Anti-Ig) on Blocking of Activation of DNA Synthesis by Immune Lymphocytes in MLC*

№ expt.	Reacting lymphocytes				
	normal	immune + normal (1:2.5)†			
		untreated	NMS‡	anti-θ	anti-Ig
1	29,9±1,3	16,4±1,4	13,4±2,1	19,6±1,9	—
2	23,4±1,9	6,6±0,6	6,6±0,1	7,6±0,4	—
3	17,1±0,6	9,8±0,8	8,6±0,1	8,9±1,5	9,0±0,5
4	15,8±1,5	5,1±0,4	4,8±0,5	—	7,7±0,2

*Numbers denote index of stimulation of DNA synthesis in MLC (M ± m).
†Only immune lymphocytes were treated (dilution of sera 1:3). $P < 0.01$ compared with normal lymphocytes alone in all cases.
‡Normal mouse serum.

Laboratory of Immunochemistry and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 325-328, April, 1979. Original article submitted May 3, 1978.

0007-4888/79/8704-0329$07.50

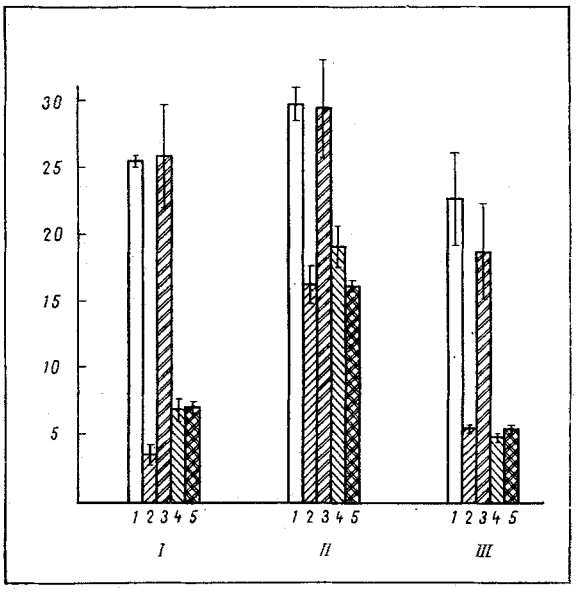

Fig. 1

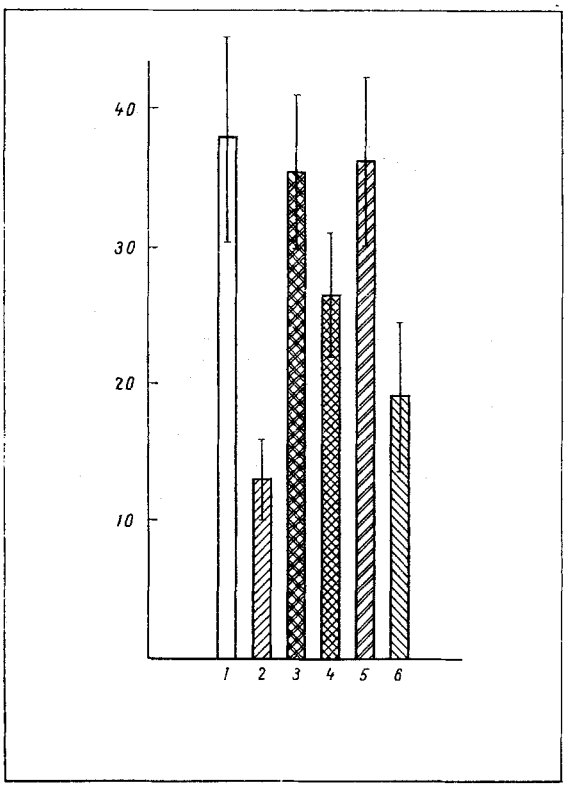

Fig. 2

Fig. 1. Effect of mitomycin C and irradiation on blocking of activation of DNA synthesis by immune lymphocytes in MLC. Ordinate — IS of normal B10.D2 lymphocytes (1) and a mixture of them (1.5:1) with immune B10.D2 anti-B10 immune lymphocytes (2), with normal lymphocytes treated with MC (3), with immune lymphocytes treated with MC (4), and with immune lymphocytes irradiated in a dose of 3000 rad (5). I, II, III) No. of experiment.

Fig. 2. Effect of carrageenan and adherent cells on activation of DNA synthesis in MLC (results of five experiments). Ordinate, IS of normal lymphocytes (1) and mixture of them (1.5:1) with immune B10.D2 anti-B10 lymphocytes (2), normal (3) or immune lymphocytes treated with carrageenan (4), or adherent normal (5) or immune cells (6).

112 h, and 16 h after addition of 1 μCi thymidine-^{3}H. Incorporation of thymidine-^{3}H was determined in a Mark II scintillation β-spectrometer (USA). The index of stimulation (IS) of DNA synthesis was estimated as the ratio between incorporation of thymidine-^{3}H, in cpm, into allogeneic MLC and incorporation into syngeneic MLC. To determine activity of SC, normal reacting lymphocytes were mixed with immune in the ratio of 2.5:1 or 1.5:1. To maintain a constant density in the culture, the corresponding number of normal lymphocytes was added in the control series. The cytotoxic reaction of antibodies in the presence of guinea pig complement, selected for nontoxicity, was tested in Brondz's [1] modification of the method of Gorer and O'Gorman [12]. AKR anti-C3H anti-θ-serum [14] killed 35% of lymph node cells in dilutions up to 1:256. Rabbit antiserum against mouse Ig was obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology and adsorbed 3 times with sarcoma SaI cells [3]. The cytotoxic index of the antibodies of this serum in the reaction with spleen cells was about 0.4 in dilutions to 1:32. Treatment of the cells with antisera: $2 \cdot 10^7$ lymphocytes were incubated in 1 ml antiserum for 30 min at 20°C and, after centrifugation, were incubated again in 1 ml guinea pig complement (1:3) for 1 h at 37°C, after which they were washed 3 times in medium No. 199 with 20% bovine serum and 0.005 M HEPES buffer. In the control, cells were treated with culture medium and also with normal mouse serum and complement. After treatment the suspensions were adjusted to equal concentrations of living cells. For treatment with carrageenan (Marine Colloids, Springfield) $5 \cdot 10^6$ cells were incubated for 4 h at 37°C with periodic shaking in 1 ml medium No. 199, containing 0.01 M HEPES

and 400 μg/ml carrageenan [13]. For treatment with MC, $5 \cdot 10^6$ cells were incubated for 30 min at 37°C with periodic shaking in 0.5 ml medium No. 199 containing 50 μg/ml of MC. After treatment the cells were washed 3 times. To study inhibition of the reaction in MLC by adherent cells, 10^6 lymph node cells from normal or immune mice were introduced into wells on the microdisks (see above). After incubation for 2 h the nonadherent cells were removed, the wells were washed twice, mixtures of reacting cells of normal lymph nodes with irradiated stimulators were added, and subsequent treatment of the MLC was carried out as indicated above.

EXPERIMENTAL RESULTS

On addition of a fraction of immune lymphocytes to normal reacting lymph node cells inhibition of activation of DNA synthesis in MLC amounted in different experiments to between 47 and 88%, in agreement with data published previously [4]. Addition of immune lymphocytes to normal was accompanied by an increase in the incorporation of thymidine-^{3}H in the control (syngeneic) mixtures, and this was connected with the greater number of DNA-synthesizing cells in the suspension of immune than of normal lymphocytes. This difference between the controls itself led to a decrease in the value of IS. To abolish this effect, DNA synthesis in immune lymphocytes added to normal was inhibited by treating them beforehand with MC, which led to equalization of thymidine-^{3}H incorporation in all the controls.

It follows from Fig. 1 that treatment of the immune lymphocytes with MC did not affect their suppressor activity, in agreement with results obtained in other systems [10, 15]. Irradiation of the suspension of immune lymphocytes in doses of up to 3000 rad likewise had no significant effect on their suppressor activity (Fig. 1), evidence of the radioresistance of the suppressor cells.

Treatment of the immune lymphocytes with anti-θ or anti-Ig sera and complement likewise had no effect on their ability to block DNA synthesis in MLC (Table 1). On the contrary, treatment of the immune lymphocytes with carrageenan reduced the blocking effect. Abolition of the blocking of activation of DNA synthesis was not the result of the nonspecific action of carrageenan, for treatment of normal lymphocytes with carrageenan and their subsequent addition to normal lymph node cells did not affect IS in MLC (Fig. 2). Cells of the suspension of immune lymphocytes adherent to the plastic caused blocking of activation of DNA synthesis in MLC. Conversely, the same cells from a suspension of normal lymphocytes did not have this action (Fig. 2).

The results given are evidence that blocking of activation of DNA synthesis in MLC is due to the active action of a suppressor cell population contained in the fraction of immune lymphocytes. They are evidently neither T- nor D-lymphocytes, for they are not inactivated by sera against θ-antigen or Ig. The radio-resistance and MC-resistance of the suppressors studied suggest that they are macrophages. This suggestion was confirmed by the following facts. 1) Blocking the reaction in MLC was not abolished by preliminary treatment of the immune lymphocytes with carrageenan, which selectively eliminates macrophages without affecting the activity of T- and B-cells [13]. 2) Cells blocking activation of DNA synthesis in MLC are adsorbed on the surface of the plastic.

The ability of macrophages to block activation of DNA synthesis in MLC has been demonstrated during growth of a tumor in a syngeneic system of virus [9] or carcinogenic [8] origin. Conversely, immunization of mice with allogeneic cells as a rule leads to generation of T-suppressors [15], but this does not rule out the possibility of the joint participation of macrophages and T-cells in the blocking of immunity. For instance, suppressor activity of macrophages, revealed after immunization of rats with large doses of serum proteins, may turn out to be the result of activation of T-suppressors [7]. The possibility cannot be ruled out that in our system suppressor activity of the macrophages was induced by T-suppressors.

The authors are grateful to N. N. Medvedev for providing the inbred lines of mice and to G. N. Vornakova for expert technical assistance. The research was partly supported by the World Health Organization.

LITERATURE CITED

1. B. D. Brondz, Byull. Éksp. Biol. Med., No. 5, 64 (1964).
2. B. D. Brondz, Folia Biol. (Prague), 14, 115 (1968).

3. B. D. Brondz and I. F. Kotomina, Byull. Éksp. Biol. Med., No. 9, 69 (1973).
4. B. D. Brondz, E. Ya. Khachikyan, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., No. 2, 189 (1978).
5. E. Ya. Khachikyan, B. D. Brondz, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., No. 2, 189 (1978).
6. B. F. Argyris, Cell Immunol., 28, 390 (1977).
7. J. A. Bash, A. M. Singer, and B. H. Waksman, J. Immunol., 116, 1350 (1976).
8. A. E. Eggers and J. R. Wunderlich, J. Immunol., 144, 1554 (1975).
9. B. R. Fernbach, H. Kirchner, G. D. Bonnard, et al., Transplantation, 21, 381 (1976).
10. H. Folch and B. H. Waksmann, J. Immunol., 113, 127 (1974).
11. J. L. Glick, C. Lockwood, J. Williams, et al., Transplantation, 18, 86 (1974).
12. P. A. Gorer et al., Transplant. Bull., 3, 142 (1956).
13. W. W. Lake, D. Bice, H. J. Schwartz, et al., J. Immunol., 107, 1745 (1971).
14. A. E. Reif and J. M. V. Allen, J. Exp. Med., 120, 41 (1964).
15. R. R. Rich and S. S. Rich, J. Exp. Med., 140, 158 (1974).

ELECTRON-MICROSCOPIC STUDY OF CONJUGATIVE PLASMIDS OF SEROLOGICALLY TYPED STRAINS OF *Escherichia coli* AP1

A. P. Kalyuzhnaya, N. I. Strizhov, N. I. Matvienko, and V. P. Shchipkov

UDC 576.851.48.098.396.332

Plasmid DNAs isolated from cells of *E. coli* AP1 were studied by electron microscopy. Two plasmid DNAs (FBI-1 and FBI-2) with molecular weights of $35.9 \pm 0.5 \times 10^6$ and $51.5 \pm 0.6 \times 10^6$ daltons respectively were identified.

KEY WORDS: plasmid DNA; electron microscopy; molecular weight.

Cells of serologically typed strain *Escherichia coli* AP1 contain transmissive F-like conjugation factor, previously described as plasmid FB1 [2]. During investigation of the physicochemical properties of the DNA of this plasmid by sedimentation in a neutral glycerol gradient and electrophoresis of its fragments in agarose gel, results were obtained which suggested that a complex consisting of two different plasmids exists in the cells of this strain [1].

TABLE 1. Length of Outlines of Plasmid DNA of *E. coli* AP1 Cells and Their Molecular Weight

No. of specimen	Length of outline of first plasmid	Length of outline of ColE1 plasmid DNA, cm	Molecular wt. of first plasmid DNA (× 10^6 daltons)	No. of specimen	Length of outline of second plasmid DNA, cm	Length of outline of ColE1 plasmid DNA, cm	Molecular weight of second plasmid DNA (× 10^6 daltons)
1	109,0	12,7	36,0	1	162,5	13,0	52,5
2	107,0	13,0	34,6	2	155,0	12,6	51,5
3	114,0	12,8	37,4	3	168,0	13,0	54,2
4	118,0	13,0	38,1	4	162,5	14,0	48,6
5	111,0	12,1	38,5	5	164,0	14,0	49,4
6	95,0	12,1	32,9	6	160,0	12,6	52,5
7	106,0	13,5	33,0				
8	106,0	12,8	34,2				
9	114,0	13,5	35,5				
10	118,0	13,1	37,9				
11	104,0	13,0	34,6				
12	124,0	14,0	37,2				
13	120,0	13,8	36,5				

Department of Biology and General Genetics, P. Lumumba Peoples' Friendship University, Moscow. Laboratory of Genetics of Viruses, Institute of Biochemistry and Physiology of Microorganisms, Academy of Sciences of the USSR, Pushchino-on-Oka. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 328-330, April, 1979. Original article submitted July 4, 1978.

 0007-4888/79/8704-0332$07.50