10. H. Friedman, Transplantation, 11, 288 (1971).
11. J. T. Harrington, Cell Immunol., 24, 195 (1976).
12. M. Nabholz, J. Vives, H. M. Young, et al., Eur. J. Immunol., 4, 378 (1974).
13. W. Newman, S. Gordon, U. Hämmerling, et al., J. Immunol., 120, 927 (1978).
14. M. Röllinghoff and H. Wagner, Eur. J. Immunol., 5, 875 (1975).
15. N. Thompson, A. Dimitriu, and J. Hamburger, Cell Immunol., 12, 247 (1974).
16. R. E. Tigelaar and R. M. Gorczynski, J. Exp. Med., 140, 267 (1974).

INDUCTION OF T-SUPPRESSORS DURING IMMUNIZATION WITH ALLOGENEIC SPLEEN CELLS IN THE MOUSE H-2 SYSTEM

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The nature of suppressor cells contained in a suspension of splenic lymphocytes immunized with allogeneic spleen cells and inhibiting activation of DNA synthesis in mixed lymphocyte cultures was studied. The suppressor cells were resistant to mitomycin C and carrageenin, were not inactivated by treatment with rabbit anti-B- and anti-Ig or mouse antibodies (anti-Mls serum) against B-lymphocytes, in the presence of complement, but were eliminated by rabbit antilymphocytic globulin and also by antibodies against T-lymphocytes (rabbit ATG and anti-θ-serum). The T-suppressors studied were concentrated in the large lymphocyte fraction in a ficoll gradient. Blocking of activation of DNA synthesis by these cells has a well-marked nonspecific component.

KEY WORDS: mixed lymphocyte culture; T-suppressors; activation of DNA synthesis; carrageenin.

Immunization of mice with allogeneic spleen cells or tumor cells leads to the formation of suppressor cells of macrophagal and T-cell origin, which block activation of DNA synthesis in a one-way normal mixed lymphocyte culture (MLC) [7, 13]. The writers previously showed that suppressors can be induced by immunization with an allogeneic tumor not consisting of T-cells [6].

In the investigation described below the conditions of induction of T-suppressors during intravenous immunization of mice with allogeneic spleen cells and certain properties of T-suppressors of this type were studied.

EXPERIMENTAL METHOD

B10.D2 (H-2^d) or BALB/c (H-2^d) mice were immunized by a single intravenous injection of 90 million allogeneic spleen cells from C57BL/10 (H-2^d) (abbreviation B10) or C57BL/6 (H-2^b) mice. In the control, 90 million syngeneic spleen cells were injected. Lymphocytes were obtained from the spleen on the 4th day after immunization. The MLC test was carried out in a modification [3] of the micro method in [10], by incubating mixtures of lymph node cells with irradiated (Co, 1500 rad) allogeneic spleen cells and, in the control, syngeneic spleen cells, in No. 3040 Microplates (Falcon Plastics). The samples were applied to filters after culture for 112 h and 16 h after addition of 1 μCi ^{3}H-thymidine. Incorporation of thymidine was determined in a scintillation β-spectrometer.

To determine suppressor cell activity normal reacting lymphocytes were mixed with immune cells in the ratio of 1.5 : 1. To maintain a constant density in the culture, the corresponding number of normal lympho-

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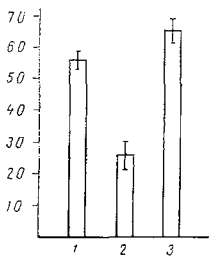

Fig. 1

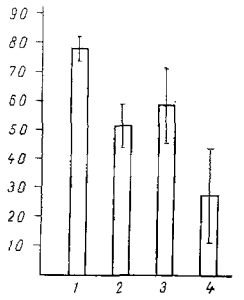

Fig. 2

Fig. 1. Fractionation of suppressor cells in a linear ficoll gradient (results of four experiments). 1) Unfractionated lymphocytes; 2) mixture of fractions I and II; 3) fraction III. Ordinate, percentage inhibition.

Fig. 2. Blocking activation of DNA synthesis by B10.D2 anti-B10 immune lymphocytes (results of three experiments). Stimulators: 1) B10; 2) DBA/2; 3) CBA; 4) B10.A. Ordinate, percentage inhibition of DNA synthesis in MLC.

TABLE 1. Blocking Activation of DNA Synthesis in MLC by Immune Lymphocytes Treated with Various Preparations

Preparation	No. of experiments	Inhibition of incorporation of ^{3}H-thymidine into MLC	
		untreated lymphocytes	treated lymphocytes
MC	3	66,3±11,8	63,8±4,3
NMS	5	75,5±3,3	69,2±6,9
Anti-$\theta^1/_3$	5	79,6±4,7	17,6±5,8*
Anti-$\theta^1/_9$	5	79,6±4,7	33,3±7,0*
Anti-$\theta^1/_{27}$	2	75,5±7,0	70,5±4,0
Anti-$Mls^1/_3$	2	76,0±8,0	74,5±11,5
ALG	3	67,0±5,4	16,6±3,3*
ATG	2	68,0±9,0	26,5±7,5*
ABG	2	68,0±9,0	66,5±3,5
Anti-Igl/3	2	80,5±9,0	77,5±7,0
Carageenin	3	81,3±7,4	70,0±12,0

Legend. MC) Mitomycin C; NMS) normal mouse serum. *) Differences from control significant ($P < 0.01$).

ocytes was added in the control series. To determine suppressor cell activity, the percentage of inhibition of activation of DNA synthesis was calculated by the formula $[(a-b)/b] \times 100$, where a and b represent incorporation of ^{3}H-thymidine (in cpm; mean of three parallel determinations) into allogeneic MLC of the control and experimental series respectively. The cytotoxic test with antibodies of homologous sera in the presence of guinea pig and rabbit complements, selected for nontoxicity, was carried out in modification [1] of the method in [11]. AKR anti-C3H anti-θ-serum killed 48% of lymph node cells in a dilution of up to 1 : 32. Serum against β-lymphocyte antigen – the product of the MLS (minor lymphocyte stimulation) locus – was obtained by immunization of C3H mice with CBA spleen and lymph node cells [15]. This serum did not kill CBA thymocytes and, when mixed in a final dilution of 1 : 2 with anti-θ-serum in a final dilution of 1 : 4 the cytotoxic index (CI) against CBA spleen cells was 0.87 (when tested separately, CI of the anti-θ-serum was 0.35 and of the anti-Mls-serum 0.53). Summation of the CI indicates that anti-Mls serum in fact reacts only with B-lymphocytes.

Rabbit antiserum against mouse Ig, obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology and absorbed with sarcoma Sa 1 cells [2] reacted with spleen cells in a dilution of up to 1/32 with a CI of about 0.4. Rabbit antilymphocytic globulin (ALG) and globulins against mouse T- and B-lymphocytes (ATG and ABG) were obtained, absorbed, and tested in the Department of Immunology, Institute of Experimental Medicine, Ministry of Health of the RSFSR [5]. During treatment of the cells with anti-θ, anti-Mls, and anti-Ig antisera, 20×10^6 lymphocytes were incubated in 1 ml serum for 30 min at 20°C and, after centrifugation, they

were reincubated in 1 ml of 1 : 3 nontoxic guinea pig complement or 1 : 20 nontoxic rabbit complement (treatment with anti-Mls) for 1 h at 37°C, followed by washing 3 times. After treatment with ALG, ATG, and ABG, 0.05 ml antigen (protein concentration 2.5 mg/ml) and 0.05 ml nontoxic rabbit complement (1/2) were added to the lymphocyte suspension (2×10^7 cells suspended in 0.9 ml medium No. 199). The mixture was incubated for 30 min at 37°C and then washed 3 times. Next, 5×10^6 cells were treated with carrageenin (400 μg/ml) for 4 h at 37°C with periodic shaking in 1 ml medium No. 199 containing 0.01M HEPES. Cells (5×10^6) were treated with mitomycin C (MC) (50 μg/ml) in 0.5 ml medium No. 199. After these treatments the cells were washed 3 times. Fractionation of the cells in a linear ficoll gradient was carried out by the method in [9], after which the top two fractions, containing fewer than 10% of large lymphocytes, were pooled and washed 3 times to remove ficoll simultaneously with the lower fraction, containing up to 60% of large lymphocytes. To study the specificity of action of the suppressors, spleen cells from CBA ($H\text{-}2^k$), DBA/2 ($H\text{-}2^d$), and B10.A ($H\text{-}2^a$) cells were added to the mixture of normal B10.D2 anti-B10 lymphocytes as stimulators.

EXPERIMENTAL RESULTS

On the addition of the fraction of immune lymphocytes to normal reacting lymph node cells inhibition of activation of DNA synthesis in MLC was 65 ± 2.4% (23 experiment), and on addition of the fraction of spleen cells from mice immunized with syngeneic spleen it was 24.0 ± 2.4% (13 experiments). Addition of immune lymphocytes to the normal cells was accompanied by an increase in incorporation of ^{3}H-thymidine in the control syngeneic mixtures, evidently because of the higher content of cells synthesizing DNA in the immune lymphocytes than in the controls. To equalize ^{3}H-thymidine incorporation in the syngeneic mixtures of control MLC, the immune lymphocytes were treated beforehand with MC. This treatment had no effect on suppressor activity (Table 1).

As Table 1 shows, treatment of the immune lymphocytes with anti-θ-serum and also with ALG and ATG in the presence of complement considerably reduced the blocking effect. Conversely, the same treatment with antibodies against B-cells (ABG, anti-Ig serum, and mouse anti-Mls serum), and also with carageenin, had no effect on the ability of the lymphocytes to block DNA synthesis in MLC. These results indicate that most of the blocking of activation of DNA synthesis in MLC was connected with the action of T-suppressor cells, whereas B-cells and macrophages were much less active.

The results of fractionation of the spleen cells show that the blocking activity of a mixture of fractions I and II is reduced whereas activity of fraction III is increased compared with unfractionated spleen cells (Fig. 1). The most active T-suppressors are thus evidently large lymphocytes.

As Fig. 2 showed, the action of the suppressors lacked complete specificity: They inhibited activation of DNA synthesis, although to a lesser degree during the reaction for antigens differing in the H-2 system from the immunizing B10 cells. Blocking also was observed during stimulation of the reaction by DBA/2 cells, identical with the reacting B10.D2 cells with respect to the H-2 system, but differing from them in the Mls locus.

The results described above show that intravenous immunization with allogeneic spleen induces T-suppressors in mice which block activation of DNA synthesis in MLC; this blocking, moreover, has a marked nonspecific component. Since the effect is not abolished by treatment of the suppressors with mitomycin C, it is evident that DNA synthesis is not necessary for their action. The absence of complete specificity of action may be connected with the persistence of immunizing antigen in the spleen of the immunized mice, so that later it enters the culture along with the immune lymphocytes. Specific contact of the antigen with immune suppressors in MLC can induce nonspecific suppression of the functions of the other T-cells [16]. Another cause of the nonspecific component of suppression is the graft versus host reaction, which arises after intravenous injection of lymphocytes to allogeneic or semiallogeneic mice and is accompanied by the appearance of nonspecific suppressors [8]. The action of these mechanisms of nonspecific suppression may be weakened by the use of killed or irradiated allogeneic spleen cells for immunization. Separation of specific suppressors from nonspecific is possible because of their different sensitivity to irradiation [12], and also because of the presence of antigen-specific receptors [14]. This problem can be tackled by the promising method of adsorption and elution of lymphocytes on a target monolayer [4]. Investigations now in progress will enable specific T-suppressors to be concentrated and their receptors to be studied.

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LITERATURE CITED

1. B. D. Brondz, Byull. Éksp. Biol. Med., No. 5, 64 (1964).
2. B. D. Brondz and I. F. Kotomina, Byull. Éksp. Biol. Med., No. 9, 69 (1973).
3. B. D. Brondz, E. Ya. Khachikyan, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., No. 6, 723 (1977).
4. B. D. Brondz, S. G. Egorova, and I. F. Kotomina, Eur. J. Immunol., 5, 733 (1975).
5. N. A. Kraskina, in: Modern Methods of Experimental Immunology and Various Aspects of their Use [in Russian], Moscow (1976), pp. 5-12.
6. E. Ya. Khachikyan, B. D. Brondz, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., No. 2, 189 (1978).
7. B. F. Argyris and F. DeLustro, Cell Immunol., 28, 390 (1977).
8. R. Elie and W. S. Lapp, Cell Immunol., 34, 38 (1977).
9. W. H. Fridman, D. Fradelizi, A. Guimezanes, et al., Eur. J. Immunol., 7, 549 (1977).
10. J. L. Glick, C. Lockwood, J. Williams, et al., Transplantation, 18, 86 (1974).
11. P. A. Goger et al., Transplant. Bull., 3, 142 (1956).
12. R. J. Hodes, L. M. Nadler, and K. S. Hathcock, J. Immunol., 119, 961 (1977).
13. R. R. Rich and S. S. Rich, J. Exp. Med., 140, 158 (1974).
14. M. Taniguchi and J. F. A. P. Miller, J. Exp. Med., 146, 1450 (1977).
15. S. L. Tonkonogy and H. J. Winn, J. Immunol., 116, 835 (1976).
16. G. A. Truitt, R. R. Rich, and S. S. Rich, J. Immunol., 119, 31 (1976).

FORMATION OF HEMATOPOIETIC COLONIES OF DONOR'S TYPE IN THE BONE MARROW OF IRRADIATED CHICKS AFTER TRANSPLANTATION OF QUAIL YOLK SAC AND LIMB BUD CELLS INTO THEIR BONE MARROW

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Ability to form hematopoietic cell colonies from the yolk sac and limb bud of a quail embryo at the 60 h incubation stage (i.e., before establishment of a closed circulation) was studied in the bone marrow of sublethally irradiated 3-week-old chicks. The experimental results are based on the ability to distinguish between quail and chick cells by means of a natural marker (Feulgen-positive nucleolus). After transplantation of limb bud cells roughly 3 times more hematopoietic colonies were found to be formed than after transplantation of yolk sac cells of the quail embryo. With the dose of irradiation used, about 75% of exogenous (quail) and 25% of endogenous (chick) hematopoietic colonies were identified in the bone marrow.

KEY WORDS: bone marrow; hematopoietic colonies; quail embryonic yolk sac and limb bud.

The method of obtaining hematopoietic colonies in the bone marrow of lethally irradiated chicks after injection of chick embryonic bone marrow and yolk sac cells was first developed by Samarut and Nigon [3]. In their investigations the recipients were 3-week-old chicks, irradiated twice in doses of 750 and 970 R, and into which hematopoietic cells were injected intravenously 4 h after the second irradiation. On the 6th-10th day after injection of the cells, benzidine-positive macrocolonies were detected in the bone marrow. The clonal origin of these colonies was later established [4]. This method has not found widespread application, for the survival rate of the birds after this dose of irradiation is very low.

However, when modified, this method offers considerable opportunities for the study of the number of colony-forming units in presumptive anlagen of the hematopoietic organs in avian embryos.

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