11. J. A. M. Kerchaert, G. J. van den Berg, and F. M. S. Hofnius, J. Immunol., 113, 1801 (1974).
12. M. Schlesinger and J. Iron, Science, 164, 1412 (1963).
13. D. Steinmuller, Transplantation, 26, 2 (1978).
14. I. A. Ramshow, P. A. Bretscher, and C. R. Parish, Eur. J. Immunol., 7, 180 (1977).
15. M. Segre, E. Toweb, and D. Segre, Cell. Immunol., 91, 443 (1985).

HUMORAL MECHANISM OF INHIBITION OF IMMUNOREACTIVITY OF MOUSE LYMPHOCYTES IN VITRO BY MASTOCYTOMA P815 CELLS

A. É. Medvedev

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KEY WORDS: tumor cells, immunosuppressor factors, suppressors.

Immunosuppression in the wide sense of the term is one of the most important problems in antitumor immunity. The "escape" of tumor cells (TC) from "surveillance" of the immune system can be attributed to at least two causes. First, TC may inhibit the activity of effector antitumor lymphocytes. We know that lymphocytes which infiltrate a tumor are characterized by sharply reduced functional activity [7, 8]. Second, during growth of a syngeneic tumor in vivo suppressor cells which prevent generation of cytotoxic T lymphocytes [1, 6, 10, 12] and inhibit their function [3, 9] are activated. Evidence has been obtained that TC produce immunosuppressor factors (ISF) which directly inhibit the activity of effector lymphocytes [2, 5, 11, 13] and (or) activate suppressor T cells [4].

This paper describes an attempt to assess the ability of mastocytoma P815 cells and a supernatant of their culture fluid (SN) to inhibit proliferation of the splenocytes of DBA/2 mice in the blast transformation reaction (BTR) and in mixed lymphocyte culture (MLC) and to study the effect of preliminary treatment of splenocytes with SN on their functional activity.

EXPERIMENTAL METHODS

Male DBA/2 (H-2^d) and C57Bl/6 (B6, H-2^b) mice aged 2-4 months were used. The mastoma P815 was maintained in vitro. To obtain the SN, P815 cells were cultured for 72 h, after which the SN was collected by centrifugation and kept at −20°C. The following culture medium (CM) was used for the functional tests: RPMI 1640 (Flow Laboratories, England), with the addition of L-glutamine (2 mM), HEPES-buffer (25 mM, Flow Laboratories), inactivated human group IV serum (2.5%), 2-mercaptoethanol ($5 \cdot 10^{-5}$ M; Serva, West Germany), and gentamicin (50 μg/ml). The BTR was carried out by culturing DBA/2 spleen cells ($250 \cdot 10^3$ per well) with concanavalin A (5 μg/ml, Flow Laboratories) in round-bottomed 96-well micropanels (Linbro, England) for 72 h at 37°C in an atmosphere with 5% CO_2. To set up the MLC the cells were cultured in flat-bottomed 96-well micropanels (Flow Laboratories) for 5 days at 37°C in an atmosphere with 5% CO_2, with a ratio of responding cells (DBA/2 splenocytes; $250 \cdot 10^3$ per well) to stimulators (B6 spleen cells treated with mitomycin C) of 1:3. As the third component in BTR and MLC, P815 cells treated with mitomycin C (DBA/2 splenocytes in the control) or different dilutions of SN were added. The cells were treated with mitomycin C in a dose of 50 μg/ml at 37°C for 40 min, and then washed 3 times. ^{3}H-Thymidine (specific activity 24 Ci/mmole, 1 μCi per well) was added to the BTR 6 h before the end of culture. To study cell proliferation in MLC, 1 μCi of ^{3}H-thymidine (specific activity 4 Ci/mmole) was added to each well 18 h before the end of culture. The samples were harvested on glass fiber filters (Flow Laboratories) by means of a harvester, and radioactivity was measured on a β-spectrometer. Preliminary treatment of the DBA/2 mouse spleen cells with SN was carried out for 2 h at 37°C (dilution of SN 1:1, pretreatment with CM in the control) was carried out for

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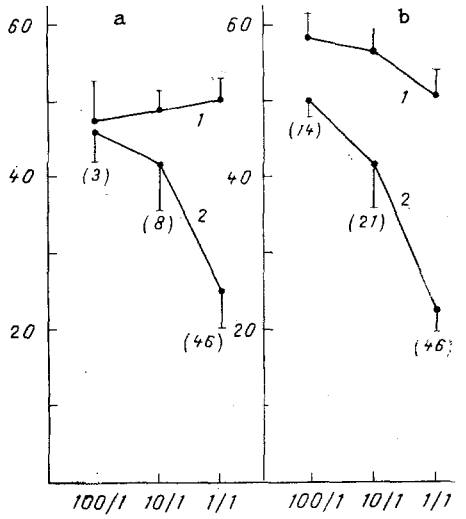

Fig. 1. Inhibition of proliferation of DBA/2 mouse splenocytes on addition of P815 cells to MLC (a) and BTR (b). Abscissa, ratio of responders to cells added as the third component; ordinate, incorporation of ^{3}H-thymidine (in cpm × 10^3). 1) Splenocytes of DBA/2 mice; 2) P815 cells. Numbers in parentheses denote II (in %). Results (M ± m) of one typical experiment given.

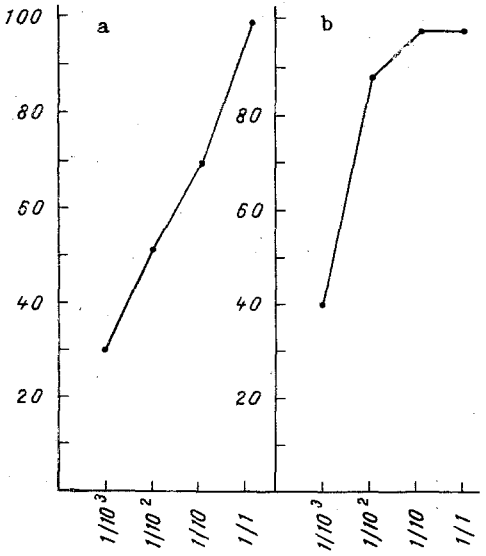

Fig. 2. Inhibition of immunoreactivity of DBA/2 mouse spleen cells on addition of P815 cells to MLC (a) and BTR (b). Abscissa) dilution of SN; ordinate) II (in %). Results of one typical experiment given.

2 h at 37°C, followed by washing 3 times. The proliferative activity of the pre-treated cells was estimated by using them as responding cells in MLC. To study the suppressor activity of these cells they were treated with mitomycin, washed 3 times, and added to MLC as the third component (ratio of responders to suppressors 1:1). The inhibition index (II) was calculated by the equation:

$$II = (A - B/A) \cdot 100\%,$$

TABLE 1. Effect of Pretreatment of DBA/2 Mouse Lymphocytes with SN of P815 Mastocytoma Cells on Their Functional Activity (M ± m)

Group No.	Pretreatment of responders	Suppressors	Pretreatment of suppressors	Incorporation of ^{3}H-thymidine, cpm	II, %
1	—	—	—	14952±4024	—
2	CM	—	—	21096±576	—
3	SN	—	—	2901±570	87
4	—	+	CM	10369±1955	—
5	—	+	SN	6454±1716	34

Legend. Absence (−) or presence (+) of pretreatment of cells or their addition to MLC.

where A denotes incorporation of ^{3}H-thymidine on addition to MLC of splenocytes pretreated with CM; B denotes incorporation of ^{3}H-thimidine on addition of splenocytes pretreated with SN to MLC.

EXPERIMENTAL RESULTS

In the first stage the ability of P815 cells and the SN of their culture fluid to inhibit proliferation of DBA/2 mouse splenocytes in functional tests was estimated. It will be clear from Fig. 1 that on the addition of P815 cells, treated with mitomycin C (DBA/2 mouse spleen cells in the control) to BTR and MLC as the third component, dose-dependent inhibition of splenocyte proliferation was observed in the experimental samples. The degree of inhibition was most marked when the ratio of lymphocytes to TC was 1:1 (II = 46%). Culture medium obtained from P815 cells also inhibited the immunoreactivity of DBA/2 mouse spleen cells in both reactions. This inhibition was inversely proportional to the dilution of SN (Fig. 2).

In the next series of experiments DBA/2 splenocytes were pretreated with SN in a dilution of 1:1 (pretreatment with CM in the control) for 2 h, which was followed by washing and testing of the functional activity of the cells. As Table 1 shows, DBA/2 splenocytes pretreated with SN, had sharply reduced proliferative activity in MLC (II = 87%) compared with cells pretreated with CM (groups 2 and 3). This effect may depend on activation of suppressor cells. To test this hypothesis, suppressor activity of lymphocytes pretreated with SN was tested. On the addition of these splenocytes as the third component in MLC, inhibition of proliferation of the responding cells was observed (II = 34%) by comparison with the control culture (addition of spleen cells pretreated with CM; groups 4 and 5).

Comparison of our results with data in the publications cited above revealed much stronger inhibitory activity of ISF of P815 cells. In the present experiments these substances inhibited immunoreactivity of the lymphocytes even in a dilution of $1/10^3$-$1/10^4$. It has been shown in the literature that factors of others TC "work" in a dilution of 1/10-$1/10^2$. According to our own data, significantly shorter pretreatment of lymphocytes with ISF of P815 cells (2 h) is required to induce their suppressor activity than is mentioned in the literature for factors of other TC (6-48 h) [4].

It follows from the results that cells of mastocytoma P815 secrete humoral factors suppressing the immunoreactivity of mouse lymphocytes in MLC and BTR, and facilitating activation of suppressor cells.

LITERATURE CITED

1. H. D. Bear, Cancer Res., 46, 1805 (1986).
2. A. Fontana, H. Hengartner, N. de Tribolet, and E. Weber, J. Immunol., 132, 1837 (1984).
3. S. Fujimoto, T. Matsuzawa, K. Nakagawa, and T. Tada, Cell. Immunol., 38, 378 (1978).
4. A. Keong and A. R. Rabson, Cancer Immunol. Immunother., 15, 178 (1983).
5. A. Keong, J. Herman, and A. R. Rabson, Cancer Immunol. Immunother., 15, 183 (1983).

6. J. G. Levy, T. Maier, and D. G. Kilburn, J. Immunol., 122, 766 (1979).
7. S. Miescher, T. L. Whiteside, S. Carrel, and V. von Fleidner, J. Immunol., 136, 1899 (1986).
8. B. Mukherji, S. A. Wilhelm, A. Guha, and M. T. Ergin, J. Immunol., 136, 1888 (1986).
9. D. Naor, Adv. Cancer Res., 29, 45 (1980).
10. R. J. North and J. Bursuker, J. Exp. Med., 159, 1295 (1984).
11. J. A. Roth, E. A. Grimm, R. K. Gupta, and R. S. Ames, J. Immunol., 128, 1955 (1982).
12. F. Takei, J. G. Levy, and D. G. Kilburn, J. Immunol., 116, 288 (1976).
13. J. Werkmeister, R. Zbroja, W. McCarthy, and P. Hersey, Clin. Exp. Immunol., 40, 168 (1980).

ELIMINATION OF DEPOSITS OF IMMUNE COMPLEXES FROM THE KIDNEYS OF AUTOIMMUNE NZB/N MICE BY INJECTION OF PERFUSATE OF A HETEROLOGOUS SPLEEN

A. B. Tsypin, V. A. Nasonova,
L. A. Vedernikova, L. N. Pleskovskaya,
L. V. Beletskaya, T. A. Ryazantseva,
and B. M. Manuilov

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The treatment of nephritis accompanying systemic lupus erythematosus (SLE), despite the extensive use of immunosuppressants, still remains a difficult problem because of the unremitting progression of the kidney lesion. New Zealand mice (NZB/N), which spontaneously develop an autoimmune disease similar to human SLE [7, 10], constitute an irreplaceable model for the development of new methods of its treatment. Methods of treatment of NZB/N mice, including a special diet or administration of corticosteroids, cytostatics, monoclonal antibodies, and so on [6, 8, 9, 11], can arrest the development of the disease only in the preclinical stage and are ineffective if the autoimmune process is well advanced.

The aim of this investigation was to study the possibility of influencing the late stage of development of the autoimmune process by intravenous injection of a solution obtained during perfusion of an isolated hog spleen (perfusate) intravenously into NZB/N mice in which the disease was of long duration. The starting point of the research was the fact that the mechanism of phagocytosis, responsible for the elimination of circulating immune complexes (CIC) from the body, is inhibited in New Zealand mice [7, 10], just as it is in patients with SLE [2, 4]. According to the results of the writers' previous investigations [3, 5], splenic perfusate can considerably increase phagocytosis by increasing the metabolic activity of neutrophils and macrophages.

EXPERIMENTAL METHODS

Experiments were carried out on 103 male NZB/N mice aged 6 and 10 months. The experimental group consisted of 54 and the control group of 49 mice. Animals of the experimental group were given an intravenous injection of 0.2 ml of splenic perfusate every 2-3 days for eight injections. Mice of the control group received 0.2 ml of isotonic buffer solution (pH 7.4) by the same schedule. All procedures connected with obtaining the perfusate were carried out under sterile conditions. The spleen was taken from a healthy animal (pig), the artery and vein were cannulated, after which the vascular bed of the spleen was washed free from blood with physiological saline. The spleen was then perfused in a closed circuit with 100 ml of isotonic buffer solution (pH 7.4) for 45 min, with the temperature of the perfusate 37°C. The perfusate was oxygenated under a pressure of 2 atm, and its pH was corrected to normal values with Tris-buffer. The rate of perfusion was 20-25 ml/min. The necessary conditions were maintained for normal viability of the spleen, as shown by the arteriovenous

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