6. B. V. Polushkin and S. M. Gordienko, Vopr. Onkol., No. 7, 51 (1980).
7. V. I. Pytskii, Immunologiya, No. 3, 82 (1980).
8. J. M. Cavaillon, J. P. Dandeu, S. Mecheri, et al., Int. Arch. Allergy, 75, 113 (1984).
9. J. Herman and A. R. Rabson, Clin. Exp. Immunol., 57, 380 (1984).
10. K. Kumagi, K. Itoh, S. Hinuma, et al., J. Immunol. Metho, 19, 17 (1979).
11. S. G. Mizel and D. Mizel, J. Immunol., 126, 834 (1981).
12. T. Timonen, J. R. Ortaldo, and R. B. Herberman, J. Exp. Med., 153, 569 (1981).

STIMULATING EFFECT OF A POLYION OF CYTOLYTIC T LYMPHOCYTE PRODUCTION IN CELL CULTURE IN VITRO

I. G. Derevyanchenko, S. N. Bykovskaya, R. I. Ataullakhanov, and R. M. Khaitov

UDC 612.112.94.017.1.014.46: 615.276.4/-084

KEY WORDS: immunostimulator, cytolytic T lymphocytes, proliferation of lymphocytes.

The immunostimulating effect of polyions on antibody formation has been studied in detail [2, 3, 6]. The cellular and molecular mechanisms of this phenomenon have been investigated during synthesis of protective antibodies in response to microbial [7] or viral [8] antigens under natural conditions in vitro, and also in cultures of lymphoid cells in model experiments in vitro [5, 9]. The action of polyions potentiating T-cell responses has been studied [7]. On the whole, however, the effect of polyionic immunostimulators on immunity mediated by effector T lymphocytes has been inadequately studied. Yet as we know, these cells are one of the most important factors in antitumor immunity.

The aim of this investigation was to study the action of the polyionic agent vegetan on the formation and functioning of cytolytic T lymphocytes (CTL) in cell culture.

EXPERIMENTAL METHOD

Mice of lines BALB/c ($H\text{-}2^d$), C3H ($H\text{-}2^k$), and (CBA × C57BL)F_1 hybrids aged 8-12 weeks were studied. Mouse splenocytes were suspended in medium RPMI 1640, to which were added 10% embryonic calf serum, 2 mM L-glutamine, 5 mM HEPES, 30 μM 2-cercaptoethanol, and 100 U each of penicillin and streptomycin. The cells were cultured in 96-well plastic plates (Falcon Plastics, USA) at 37°C in an atmosphere of 5% CO_2 for 3-5 days.

CTL were obtained in mixed lymphocyte culture (MLC) by the method in [10]. For this purpose, 2×10^6 splenocytes from BALB/c mice were incubated for 5 days with 10^6 or 10^5 C3H mouse spleen cells, previously irradiated in a dose of 1000 R.

The cytolytic activity of cells from MLC was determined by using L-fibroblasts of the $H\text{-}2^k$ haplotype from mice syngeneic with C3H as the target cells. A suspension of 10^6 L-cells in 1 ml was incubated in the presence of ^{51}Cr (100 μCi/ml) for 45 min at 37°C. The L cells were then washed 3 times by centrifugation in Hanks' solution and introduced at 37°C into flat-bottomed plates (Falcon Plastics) at the rate of 4×10^4 cells per well in a volume of 100 μl. Lymphocytes from a 5-day MLC were added for 3 h with lymphocyte–target ratios of 10:1, 5:1, and 2:1 respectively. The degree of cytolysis was estimated from the specific outflow of ^{51}Cr from the target cells into the incubation medium. For this purpose the cell-free fluid was transferred into plastic ampules for measurement of radioactivity in a γ-spectrometer (Nuclear Chicago, USA). The percentage of specific outflow of chromium was calculated by the equation:

$$\text{Cytolysis} = \frac{\text{Experiment} - \text{control}}{\text{Total lysis} - \text{control}} \times 100\%.$$

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'nois Biologii i Meditsiny, Vol. 102, No. 9, pp. 327-329, September, 1986. Original article submitted February 14, 1986.

 0007-4888/86/0009-1248$12.50

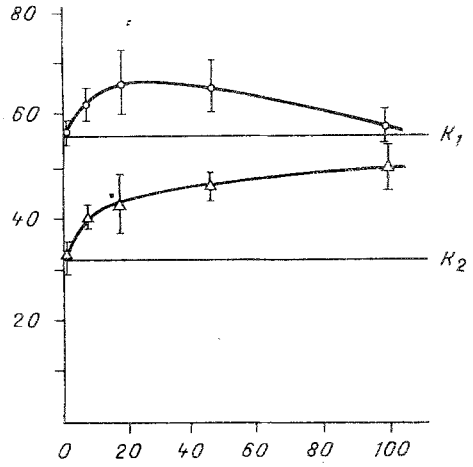

Fig. 1. Stimulation of killer T cell production by vegetan in MLC in vitro. Abscissa, concentraton of vegetan in MLC (in μg/ml); ordinate, cytolysis (in %). Values for cytolysis given were obtained during combined culture of cytotoxic lymphocytes from optimal (1) or weakly immunogenic MLC (2), containing different concentrations of vegetan, with target cells, K_1 and K_2. Levels of cytolytic activity of control cells from optimal and weakly immune MLC respectively, without vegetan.

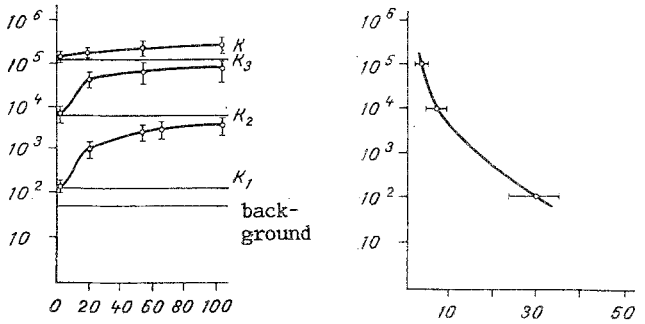

Fig. 2. Stimulating effect of vegetan on generation of antigen-dependent AFC in vivo depending on dose of the compound and of antigen (a) and levels of control response (without vegetan) in the experimental model used (b). a) Number of AFC in spleens of mice immunized with 2×10^6 SRBC (K_1); 2×10^6 SRBC + vegetan (1); 10^7 SRBC (K_2); 10^7 SRBC + vegetan (2); 10^8 SRBC (K_3); 10^8 SRBC + vegetan (3). Abscissa, dose of vegetan (in μg/mouse) injected intraperitoneally together with immunogen; ordinate, number of AFC specific for SRBC antigens formed in mouse spleen 4 days after immunization; b) coefficients of stimulation: ratio of number of AFC in experiments (vegetan + SRBC) to number of AFC in corresponding control (SRBC only). Abscissa, coefficient of stimulation of AFC production after injection of 100 μg vegetan together with different doses of SRBC; ordinate, number of AFC in control after injection of different doses of SRBC.

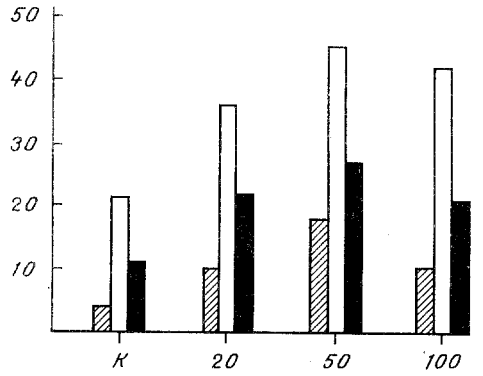

Fig. 3. Effect of vegetan on proliferative activity of cells. Abscissa, vegetan concentration (in μg/ml); ordinate, incorporation of ^{3}H-thymidine (in cpm) into monoculture (obliquely shaded columns), MLC (unshaded columns), and weakly immunogenic MLC (black columns). K) Control.

where experiment denotes radioactivity (in cpm) of cell-free fluid from wells containing CTL and target cells; control denotes radioactivity of liquid from wells containing target cells only; total lysis denotes radioactivity of liquid from wells containing target cells lyzed by detergent.

Two types of MLC were used: weakly immunogenic, in which the ratio between "reacting" and "stimulating" cells was 20:1, and optimal, in which this ratio was 2:1. The level of proliferation in monocultures and mixed cultures of lymphocytes was estimated from incorporation of ^{3}H-thymidine, reflecting the intensity of DNA synthesis [4].

A mother solution of vegetan was prepared by dissolving the dry substance in Hanks' solution or in medium RPMI 1640 and sterilized by passing through GSVP filters (Millipore, USA). The solution was kept at −20°C. Vegetan was added to the mono- and mixed cultures of lymphocytes in doses of 10 to 100 μg/liter. The total added volume did not exceed 1/10 of the volume of the culture.

The number of antibody-forming cells (AFC) was determined by the method in [5].

In all cases triplets of identical cell cultures were investigated in one experiment. The results of the measurements were subjected to statistical analysis by the usual method.

EXPERIMENTAL RESULTS

Active CTL, producing lysis of 50-55% of target cells, were formed in an optimal MLC during 5 days of culture. The formation of fewer (perhaps less active) CTL, which caused lysis of target cells amounting to about 30-35% of total lysis, was induced in a weakly immunogenic MLC.

Addition of vegetan to the optimal MLC led to a significant, but not very large, increase in cytolytic activity of the cells (Fig. 1). The action of vegetan was manifested more clearly under conditions of suboptimal stimulation in the weakly immunogenic MLC. In the presence of vegetan, lymphocytes from the weakly immunogenic MLC were able to induce lysis of target cells of the same intensity as TCL formed in response to the optimal antigenic stimulus. Cells activated by the polyion in a weakly immunogenic MLC induced lysis of target cells 1.3-1.7 times more strongly than in the control. The greatest effect was observed with vegetan in a concentration of 50-100 μg/ml. Marked stimulation took place if the preparation was present in MLC starting from time "0" for 5 days. Addition of vegetan actually during the cytolytic test did not lead to any appreciable modification of activity of the lymphocytes.

It was pointed out previously that in models of the optimally expressed immune response the stimulating effect of immunoadjuvants is as a rule less marked than in models of an attenuated immune response [6]. In other words, strengthening of a weak immune response was

more evident than strengthening of an already strong response, close in some cases to the strongest possible. This conclusion was drawn from a study of stimulation of antibody synthesis by synthetic polyions and membrane-active canalogens. In the present investigation we observed the same effect when studying the potentiating action of vegetan on generation of allospecific CTL (Fig. 1). In this connection it was interesting to compare the effect of vegetan on CTL production with its action on antibody production.

We induced immune reactions of different strengths in a model of primary antibody synthesis against antigens of heterologous red blood cells in vivo. For this purpose, (CBA × C56BL)F_1 hybrid mice were given an intraperitoneal injection of 10^6 to 5×10^8 sheep's red blood cells (SRBC). The number of AFC, reflecting the level of specific antibody production, was determined 4 days later in the spleens of the immunized mice (Fig. 2a). The immune system of the mice used virtually did not react to a single injection of 10^6 SRBC: each spleen contained about 40-50 AFC specific for antigens of SRBC. The same number of spontaneous AFC of this specificity were found in the spleens of intact mice. After immunization of the mice with 10^7 SRBC the immune response was significantly higher — up to 10^4 specific AFC. A dose of 10^8 SRBC evoked a near-maximal response (up to 1.5×10^5 cells). The action of vegetan was tested with three different doses of antigen (Fig. 2b). It was found that the polyion strengthed a very weak immune response to 2×10^6 SRBC on average 20-50-fold, a response of average intensity to 10^7 SRBC seven-eightfold, and a near-maximal response to 10^8 SRBC by 1.2-2 times. It will be clear from Figs. 1 and 2 that the immunostimulating action of vegetan is independent of the type of response activated (antibody synthesis or CTL generation), but it is inversely proportional to the strength of the immune response.

Previously it was shown on a model of antibody synthesis that the immunostimulating action of a synthetic polyelectrolyte or canalogen correlates also with weak mitogenic action [2]. They evidently induce transition of lymphocytes from the G_0 phase to the G_1 phase of the cell cycle. We were interested to study the mitogenic action of vegetan and also the possibility of nonspecific stimulation of CTL in lymphocyte monoculture.

The experiments demonstrated the marked mitogenic action of vegetan (Fig. 3). Incorporation of ^{3}H-thymidine into DNA of vegetan-activated cells was 1.5-2 times more intensive than incorporation in the control. This property distinguishes vegetan from most other polyions, which are weak mitogens [1, 6]. As will be clear from Fig. 3, vegetan considerably stimulated cell division not only in monoculture, but also in both types of MLC. The fact will also be noted that vegetan activated cell proliferation substantially in ordinary MLC, and in this case had only a weak stimulating action on CTL generation. It can be tentatively suggested that this substance stimulates proliferation not only of killer T cells, but also of other subpopulations of T lymphocytes.

LITERATURE CITED

1. R. I. Ataullakhanov, M. I. Gubarev, and V. V. Goncharov, Immunologiya, No. 2, 27 (1985).
2. R. V. Petrov, R. M. Khaitov, E. V. Kozhinova, and V. P. Evdakov, Tsitologiya, No. 10, 1172 (1975).
3. R. V. Petrov and R. M. Khaitov, Med. Ref. Zh., XXI, No. 7, 18 (1977).
4. R. V. Petrov, R. M. Khaitov, and R. I. Ataullakhanov, Immunologiya, No. 4, 27 (1982).
5. R. V. Petrov, R. M. Khaitov, and R. I. Ataullakhanov, Immunologiya, No. 5, 28 (1982).
6. R. V. Petrov, R. M. Khaitov, and R. I. Ataullakhanov, Immunogenetics and Artificial Antigens [in Russian], Moscow (1983).
7. R. V. Petrov, R. M. Khaitov, B. F. Semenov, et al., Zh. Mikrobiol., No. 12, 110 (1984).
8. R. V. Petrov, V. M. Zhdanov, V. A. Kabanov, et al., Dokl. Akad. Nauk SSR, 277, No. 3, 752 (1984).
9. R. M. Khaitov and R. I. Ataullakhanov, Immunologiya, No. 4, 30 (1982).
10. G. C. Cerrotini, H. D. Engers, H. R. McDonald, et al., J. Exp. Med., 140, 703 (1974).