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EFFECT OF SOME POLYSACCHARIDES IN CYCLIC NUCLEOTIDE LEVELS AND PHOSPHODIESTERASE ACTIVITY IN ORGANS OF MICE WITH LEWIS' LUNG CARCINOMA

I. G. Veksler and S. G. Antonenko

UDC 616.24-006.6-092.9-085.331:
577.114.5/-036.8-07:616
008.93:577.123.3

KEY WORDS: polysaccharides; cyclic nucleotides; metastasizing Lewis' lung carcinoma; thymus; spleen.

The problem of restoring disturbed activity of the immune system is an important factor in the treatment of several diseases accompanied by immunosuppression or disturbance of immune surveillance. In the combination treatment of malignant neoplasms great attention is paid to the use of various immunomodulators, which weaken immunosuppression and effectively enhance the body's immune response to the tumor [1, 5, 6]. According to some workers [3, 8, 9] the action of immunomodulators and other physiologically active substances is exerted through a system of universal cellular mediators, namely cAMP and cGMP. Much evidence has been obtained as a result of attempts to determine the role of cyclic nucleotides and enzymes responsible for their formation (adenylate cyclase, guanylate cyclase) and degradation (phosphodiesterase) in various pathological processes, including malignant growth.

This paper describes the results of a study of the effect of the bacterial polysaccharide prodigiosan and the yeast cell membrane biopolymer zymosan on the absolute and relative levels of cAMP, cGMP, and cAMP-dependent phosphodiesterase (PDE) in the thymus, spleen, and lungs of healthy mice and of mice with metastasizing Lewis' lung carcinoma.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL mice weighing 20-23 g. Lewis' lung carcinoma was transplanted intramuscularly into the hind limb in a dose of $2 \cdot 10^5$ tumor cells. A readily palpable tumor developed at the site of implantation after 9-12 days and metastases of this tumor in the lungs were found after 9-21 days. The test substances were injected intraperitoneally into the experimental animals on the 14th day, in 0.5 ml of physiological saline and in the following doses: zymosan (from Tallin Pharmaceutical Chemical Factory) 25 mg/kg, prodigiosan (from the Department of Microbiology, Central Postgraduate Medical Institute, Moscow) 2.5 mg/kg. Both substances, according to data obtained previously [4], effectively raised the levels of several immunologic parameters in the above doses. Animals of the control group (transplantation of the tumor) received physiological saline in a volume of 0.5 ml at the same time. All the animals were killed on the 21st day. The tumor at the site of implantation was weighed, the number of metastases in the lung and the mean number of metastases in the group were determined, the number of animals without metastases was counted, and the cAMP and cGMP concentrations and PDE activity were determined in tissue from the thymus, spleen, and lung. The cyclic nucleotide concentration was determined by radioimmunoassay using standard kits from Amersham Corporation (Great Britain). Radioactivity of the samples was studied in an SL-30 liquid scintillation counter (Intertechnique, France). PDE activity was determined by paper chromatography, using 8-^{3}H-cAMP as the substrate [11]. Values obtained on intact mice of the same strain, sex, and age were used as normal values. The numerical results were subjected to statistical analysis with calculation of the level of significance (P) and the coefficient of correlation (r).

R. E. Kavetskii Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 605-607, November, 1985. Original article submitted November 18, 1984.

 0007-4888/85/0011-1576$09.50

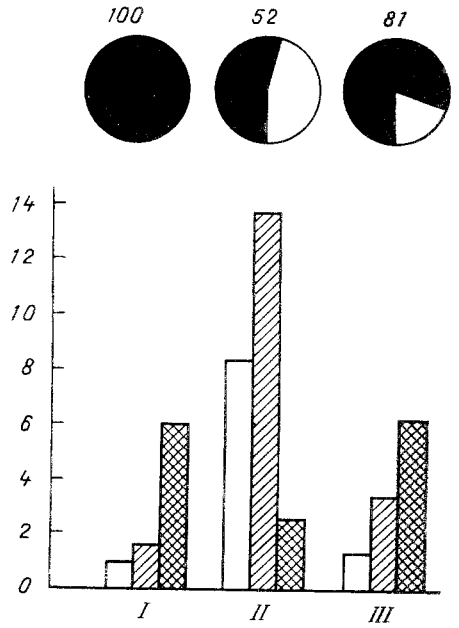

Fig. 1. Relationship between number of metastases formed and change in cAMP/cGMP ratio in organs of mice with Lewis' carcinoma after administration of polysaccharides. Ordinate, ratio cAMP/cGMP. I) Control, II) zymosan, III) prodigiosan. Unshaded columns) thymus, oblique shading) spleen, cross-hatching) lung. Pie charts indicate number of metastases in lung (in %).

EXPERIMENTAL RESULTS

The cAMP level in the thymus, spleen, and lungs of healthy C57BL mice was 315.0 ± 1.8, 378.0 ± 23.6, and 199 ± 16.9 pmoles/g wet weight of tissue respectively, which exceeded the cGMP concentration in these organs by 15.8, 15.7, and 4.7 times. Under the influence of the polysaccharides the cAMP and cGMP levels in the organs of healthy mice showed no significant changes with the exception of the cAMP level in the thymus. However, whereas after administration of zymosan the cAMP concentration in the thymus rose (on average by 26%; $P < 0.01$), after injection of prodigiosan there was a small decrease in the cAMP concentration in that organ (on average by 9%; $P < 0.05$). Activity of cAMP-dependent PDE in the thymus, spleen, and lungs of normal animals varied from 9.3 ± 0.3 to 14.0 ± 0.2 nmoles/mg protein/min. Changes in PDE activity under the influence of zymosan, incidentally, were opposite in direction to changes in the cAMP level. In all probability elevation of the cAMP level by the action of zymosan was due to inhibition of activity of cAMP-dependent PDE, which in turn indicates the possible point of application of the biological action of this polysaccharide and agrees with observations made by other workers [7].

The cAMP level in mice with developing lung metastases (on the 21st day) was 14 times lower in the spleen compared with its level in healthy animals, 4.8 times lower in the lung tissue ($P < 0.01$), and 1.8 times lower in the thymus ($P < 0.05$). The intracellular cAMP concentration in these mice was almost 6 times lower than normally in the lung tissue ($P < 0.01$), substantially unchanged in the spleen, and sharply increased (tenfold) in the thymus ($P < 0.01$). After administration of the polysaccharides to mice with Lewis' carcinoma partial recovery of the cAMP level was observed in the thymus and spleen, evidently due to activation of phosphorylation of membrane, cytoplasmic, and nuclear proteins. After injection of zymosan, there was a simultaneous decrease in the cGMP concentration in the spleen by 4.5 times ($P < 0.01$) with a tendency toward normalization of the cAMP level in the thymus. Prodigiosan caused no statistically significant changes in the cAMP level in the organs investigated compared with the control.

In the modern view [2, 8, 10], cell growth and tissue proliferation are controlled by the balance and ratio between the intracellular cAMP and cGMP levels (cAMP/cGMP). It was therefore interesting to compare data on the effect of polysaccharides on metastasization of Lewis' lung carcinoma with changes in the cAMP/cGMP ratio in these same animals. It will be

clear from Fig. 1 that the cAMP/cGMP ratio in the control was sharply reduced for the thymus and spleen but increased for lung tissue with metastases present. After injection of prodigiosan these cAMP/cGMP ratios differed very little from those in the control. Prodigiosan reduced the number of formed metastases in the lungs by only 19% ($P < 0.05$). The changes observed in the cyclic nucleotide levels in the thymus and spleen under the influence of zymosan were accompanied by an increase in the cAMP/cGMP ratio, but the number of metastases formed in the lungs was reduced on average by 48% ($P < 0.01$).

After administration of zymosan close negative correlation ($r > -0.7$) was found between the increase in the cAMP/cGMP ratio and a decrease in the number of lung metastases, whereas after administration of prodigiosan, only weak negative correlation was found ($r < -0.3$). By changing the ratio between the cyclic nucleotides in the immunocompetent organs in a certain way and creating a favorable immune background, zymosan thus evidently increases the resistance of the body to the development of metastases. This hypothesis is supported by our previous data [4] showing that zymosan acts mainly on T-cell immunity whereas prodigiosan acts mainly on the humoral immune response. Characteristically bacterial polysaccharides, of which prodigiosan is one, which are mitogens for B lymphocytes, induce a sharp rise of the intracellular cAMP level in mouse spleen cultures [12]. Considering these data and the results of the present experiments showing a marked increase in the cAMP concentration in the immunocompetent organs of animals with Lewis' carcinoma, including after administration of prodigiosan, it can be postulated that the action of bacterial lipopolysaccharides is largely connected with inhibition of activity of cAMP-dependent PDE.

The preparations of polysaccharide nature studied in these experiments thus have different effects both on the intracellular cAMP and cGMP concentrations in the organs of mice with Lewis' carcinoma and on the formation of metastases of this tumor in the lungs. In all probability it is not the absolute concentrations of cyclic nucleotides that are of decisive importance in the formation of resistance of the animal to the development of metastases, but the ratio between them. Determination of the cAMP/cGMP ratio in the thymus and spleen, in our opinion, can be used as an additional test for screening new antitumor immunomodulators.

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