6. L. Ya. Shipova, N. A. Kharkovskaya, and N. B. Ladygina, Éksp. Onkol. (1983).
7. F. F. Becker, R. A. Fox, K. M. Klein, et al., J. Natl. Cancer Inst., 46, 1261 (1971).
8. F. F. Becker, K. M. Klein, and S. R. Wolman, Cancer Res., 33, 3330 (1973).
9. G. Jones and W. H. Butler, in: Mouse Hepatic Neoplasia, Amsterdam (1975), p. 21.
10. Y. Koike, Y. Suzuki, A. Nagata, et al., Histochemistry, 73, 549 (1982).
11. N. Inui, S. Takayama, and N. Kawabara, J. Natl. Cancer Inst., 47, 47 (1971).
12. G. L. Plincler, G. Vlahakis, K. H. Kortright, et al., Eur. J. Cancer, 17, 1241 (1981).
13. L. Sternberger, Immunocytochemistry, New York (1974).
14. H. F. Stich, J. Natl. Cancer Inst., 24, 1283 (1960).
15. J. M. Ward, D. G. Goodman, R. A. Squire, et al., J. Natl. Cancer Inst., 63, 849 (1979).

RATIO BETWEEN PROLIFERATING AND QUIESCENT SPLEEN CELL POPULATIONS DURING DEVELOPMENT OF RAUSCHER LEUKEMIA AND AFTER LOADING OF MONONUCLEAR PHAGOCYTES WITH COLLOIDAL GOLD

R. P. Alekhina, V. M. Bukhman, A. V. Likhtenshtein, and V. S. Shapot*

UDC 616.155.392-022:578.825.24]-07:616.411-018.15-02:615.31:546.59

KEY WORDS: mononuclear phagocytes, NPC chromatography, Rauscher leukemia, colloidal gold.

The ratio between the pools of proliferating and quiescent cells is one of the most important critical factors determining the sensitivity of normal and tumor tissues to most cytostatic agents [2]. Correlation between these parameters and the sensitivity of target cells to killer cells, which is evidently connected with modulation of antigens and cell membrane receptors [10, 11], likewise is becoming increasingly evident.

The writers demonstrated previously that a temporary change in functional activity of the mononuclear phagocyte system (MPS) caused by parenteral injection of particles of inert colloids leads to modification of the toxic and antitumor effects of cytostatics: methotrexate, ftorafur,† sarcolysin [3, 9]. In particular, injection of colloidal gold or carbon particles potentiated the antitumor effect of methotrexate on developing Rauscher leukemia. The mechanism of this effect is not clear. It may perhaps be explained by changes in proliferation of the tumor cells connected with disturbance of MPS function. Macrophages are known to be secretory cells, and among the biologically active compounds which they secrete, the so-called monokines, there are factors which affect proliferation of other cells, including hematopoietic cells [7].

To test this hypothesis experiments were carried out, the results of which are described below. In most experiments a technique of nucleoprotein-celite chromatography (NPC chromatography) was used to analyze the ratio between pools of proliferating and quiescent cells; this method enables the proliferative status of the cell population to be assessed [5, 6]. DNA in proliferating cells is very firmly bound with proteins and a high concentration of LiCl and urea (4 M and 8 M respectively) and heating to 96°C are necessary for its elution. When the cells change to the resting state, 1.5 M LiCl and 3M urea at 4°C are sufficient to dissociate the DNA-protein complex [8].

*Corresponding Member, Academy of Medical Sciences of the USSR.
†5-Fluoro-1-(tetrahydro-2-furyl)uracil (translator).

Laboratory of Tumor Biochemistry, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Sector of Principles of Cell Proliferation, Research Institute for Biological Testing of Chemical Compounds, Ministry of the Medical Industry of the USSR, Kupavna, Moscow Region. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 6, pp. 713-715, June, 1984. Original article submitted July 10, 1983.

 0007-4888/84/9706-0790$08.50

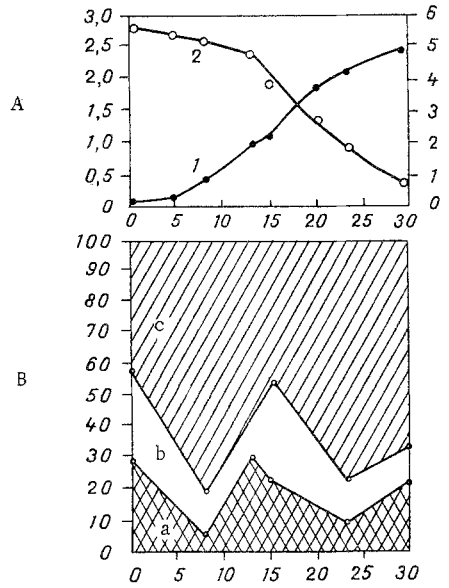

Fig. 1

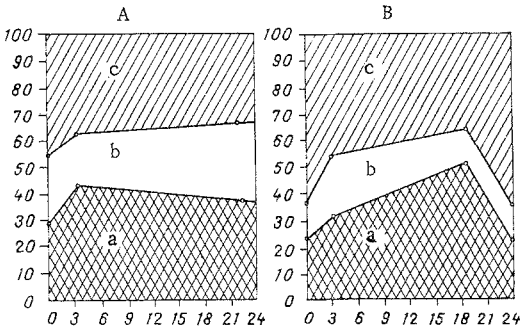

Fig. 2

Fig. 1. Changes in weight of spleen, radioactivity (10^6 cells), and ratio between DNA from NPC chromatograms in fractions differing by strength of bond with proteins, during development of Rauscher leukemia. A: 1) Weight of spleen; 2) radioactivity of DNA. B: fractions of gradient: a) salt, b) intermediate, c) high temperature. Mice received injections of 100 μ Ci[^{3}H]thymidine daily on 5th, 6th, and 7th days of development, followed by 50 μCi on alternate days. Abscissa, days of development of Rauscher leukemia; ordinate: A (on left) weight of spleen, in mg, (right) radioactivity of DNA $\times 10^3$, B) radioactivity of DNA (in per cent).

Fig. 2. Dependence of DNA distribution from NPC-chromatograms in fractions differing by strength of binding with proteins, on duration of action of colloidal gold preparation. A) Normal mice, B) mice with developing Rauscher leukemia (15th day). Fractions of gradient: a) salt, b) intermediate, c) high temperature. Mice of all groups received injections of [^{3}H]thymidine in a dose of 300 μCi 23 h (A) and 24 h (B) before sacrifice. Colloidal gold preparation (1.5 mg per animal) injected 4 and 23 h (A) and 3, 19, and 24 h (B) before sacrifice. Abscissa, time (in h); ordinate, radioactivity of DNA (in per cent).

EXPERIMENTAL METHOD

Male (C57B1/6j $\times$ DBA/2)F_1 mice aged 2–3 months, from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. Leukemia was induced by intraperitoneal inoculation of 10^7 leukemic spleen cells on day 0. In the experiments of series I proliferation of spleen cells was studied during the development of leukemia, in series II after a single intraperitoneal injection of colloidal gold [9] on day 0 in a dose of 1.5 mg per mouse. Mice of all the groups compared were sacrificed simultaneously, and gold was injected beforehand. [^{3}H]Thymidine was injected into the mice 24 or 1.5 h before sacrifice in a dose of 300 μCi per animal (specific activity 23 Ci/mmole). In some experiments [^{3}H]thymidine was injected during development of leukemia in doses of 100 or 50 μCi. The relative DNA content in the chromatographic samples was determined by measuring [^{3}H]thymidine incorporated into the cells. Radioactivity was counted in a Triton–toluene system on a Mark III counter (Nuclear Chicago).

Unfractionated cell lysates were used as the original material. For this purpose mouse spleens were homogenized at 4°C in a lightly ground Potter–Elvehjem homogenizer in TKM buffer (0.25 M KCl, 0.005 M $MgCl_2$, 0.025 M Tris-HCl, pH 7.6), containing 1 mM phenylmethylsulfonyl fluoride of 0.1% diethyl pyrocarbonate. The number of cells was then counted in a Goryaev's chamber. The cells (1×10^6 to 2×10^6) were then lysed in a Dounce homogenizer in 3 ml of a 1% solution of Triton X–100, made up in TKM buffer, with inhibitors of nucleases and proteases.

The cell lysate was mixed with celite 545, suspended in TM buffer, and applied to a constant-temperature column cooled beforehand to 4°C. DNA was eluted from the nucleoprotein–celite column as described previously [5]: initially in a linear concentration gradient of LiCl and urea (from TM buffer to 4 M LiCl, 8 M urea in

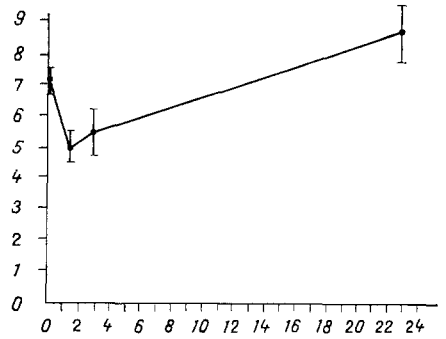

Fig. 3. Changes in specific radioactivity of DNA in Rauscher leukemia, depending on duration of action of colloidal gold. Mice of all groups received an injection of 300 μCi of [^{3}H]thymidine 1.5 h before sacrifice on 15th day of development of Rauscher leukemia. Colloidal gold preparation injected 1.5, 3, and 23 h previously. Vertical lines show standard deviation. Abscissa, time (in h); ordinate, specific radioactivity of DNA (cpm × 10^6).

TM buffer at 4°C) to elute DNA bound relatively weakly with proteins, then in a temperature gradient from 4°C to 96°C, using a solution of 4 M LiCl and 8 M urea made up in TM buffer as the eluate, to elute DNA most firmly bound with proteins. DNA fractions eluted in a salt gradient (salt fraction) and in temperature gradients from 4 to 70°C (intermediate fraction) and from 70 to 96°C (high temperature fraction) were collected separately.

EXPERIMENTAL RESULTS

It will be clear from the data given in Fig. 1A that the development of leukemia is characterized by negative correlation between the increasing weight of the spleen and [^{3}H]thymidine incorporation (calculated per 10^6 cells). Among the various possible causes of this phenomenon, a reduction in the proliferative pool and differences in the character of recirculation of the cells dependent on the stage of the process can be distinguished. Fluctuating changes in the ratio between pools of proliferating and quiescent spleen cells were observed: In the normal spleen proliferating cells constitute about 40% of all cells, by the 8th day their fraction has doubled, by the 13th–15th days it has fallen again to its original level, after which it again rises sharply until the 23rd day, and falls until the 30th day (Fig. 1b). These changes take place against the background of steady development of splenomegaly and, in our opinion, they reflect quantitative and qualitative changes in the cell composition of the spleen – the principal organ affected in Rauscher leukemia. By the 8th day proliferation of malignant erythroblasts in the spleen, which normally is a lymphoid organ, leads to the appearance of detectable macroscopic colonies, and by the second week the spleen again becomes a more homogeneous organ, for most of its cells are malignant erythroblasts. Intensive proliferation later begins again in the spleen, this time of cells of the lymphoid and granulocytic series [1]. This wave of proliferation can be regarded as connected with the leukemoid response of the host to leukemia. Changes taking place in Rauscher leukemia can be used as a model of various clinical phases of development of human leukemia. They can be used for the experimental study of therapeutic measures, taking the pathogenesis of development of leukemia into account [4].

Data showing changes in the ratio between pools of dividing and quiescent cells during the 24-h period after loading of MPS with colloidal gold when the spleen was in the two relatively homogenous states (normal and on the 15th day of development of leukemia) are illustrated in Fig. 2. This procedure clearly caused roughly the same kind of response in the normal lymphoid and the malignant erythroblastic cell populations in the spleen. After a reduction of the fraction of proliferating cells, restoration of the dividing pool of leukemic cells was observed. The same changes, but less marked, were observed in the cell population of normal spleen.

Injection of colloidal gold led to a transient decrease in the number of cells in the S phase and to a decrease in the rate of DNA synthesis. The rate of DNA synthesis then increased, and by 24 h it was higher than the initial level (Fig. 3).

The results of these experiments (Figs. 2 and 3) agree well with each other and can be interpreted as evidence of partial synchronization of the cells under the influence of loading of the MPS with colloidal gold.

Since colloidal gold has no direct effect on malignant erythroblasts, but is phagocytosed by macrophages, modifying their activity, the results indicate that proliferative activity of both normal and malignant hematpoietic cells depends on the state of the macrophages in vivo and, consequently, on the possibility of oriented regulation of the process by means of agents affecting MPS.

The method of determining the strength of DNA–protein interactions, developed as described above, is therefore useful for examination of several problems in experimental oncology. It can also evidently be used in clinical oncology for the study of samples of bone marrow, lymph node, and spleen cells and also of blood cells of patients with leukemias, containing a high proportion of blast cells.

LITERATURE CITED

1. M. F. Barkhotkina and V. M. Bukhman, Byull. Éksp. Biol. Med., No. 10, 472 (1980).
2. N. N. Blokhin, in: Chemotherapy of Malignant Tumors [in Russian], Moscow (1977), p. 3.
3. V. M. Bukhman, in: Experimental Aspects of Tumor Growth [in Russian], Moscow (1972), p. 37.
4. V. M. Bukhman, N. A. Brusentsov, B. S. Kikot', et al., Khim.-farm. Zh., No. 8, 58 (1981).
5. A. V. Likhtenshtein, M. M. Zaboikin, V. L. Moiseev, et al., Dokl. Akad. Nauk SSSR, 245, 1005 (1979).
6. A. V. Likhtenshtein, R. P. Alekhina, M. M. Zaboikin, et al., Vestn. Akad. Med. Nauk SSSR, No. 6, 50 (1980).
7. N. V. Medunitsin, V. I. Litvinov, and A. M. Moroz, Mediators of Cellular Immunity and Intercellular Interaction [in Russian], Moscow (1980).
8. N. I. S"yakste, M. M. Zaboikin, A. V. Likhtenshtein, et al., Mol. Biol., No. 6, 1321 (1981).
9. V. M. Bukhman (V. M. Buchman), R. M. Radzichovskaya, G. J. Svet-Moldavsky, et al., Biomedicine (Express), 19, 335 (1973).
10. N. V. Leneva and G. J. Svet-Moldavsky, J. Natl. Cancer Inst., 52, 699 (1974).
11. R. A. Lerner, N. B. Oldstone, and R. N. Cooper, Proc. Natl. Acad. Sci. USA, 68, 2584 (1971).

EFFECT OF SYNTHETIC β-CAROTENE ON CYTOLYTIC T LYMPHOCYTE FORMATION

S. A. Efimov, L. A. Vakulova,
A. N. Rytenko, G. I. Samokhvalov,
A. V. Sergeev, and I. M. Yakovleva

UDC 615.356:577.161.11].
015.4:612.112.94:017.4

KEY WORDS: vitamin A, β-carotene, cytolytic T lymphocyte, sarcoma 180.

The possibility of using vitamin A and its derivatives for the prevention and treatment of malignant neoplasms in man and animals has been reported several times in the recent literature [2, 3, 6]. The mechanism of the antitumor action of retinoids has not yet been studied. Meanwhile Lotan and Dennert [4] have shown that vitamin A, in doses giving a therapeutic effect, can stimulate the formation of cytolytic T lymphocytes (CTL) in vitro. These observations suggest that the antitumor action of vitamin A is connected with increased ability of the host to produce effector cells concerned with rejection of the tumor. The widespread use of vitamin A for the prevention and treatment of malignant tumors is restricted because this preparation, in therapeutic doses, has a toxic action associated with manifestations of hypervitaminosis. Accordingly the possibility of using β-carotene, a precursor of vitamin A which is nontoxic even in large doses, is particularly interesting.

The aim of this investigation was to study the effect of various doses of β-carotene on CTL formation in vitro, to determine the optimal dose and schedule of administration of this substance, and also to study its effect on growth of transplantable sarcoma 180 and the length of survival of the animals.

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 6, pp. 716-717, June, 1984. Original article submitted July 20, 1983.