tumor. With the development of a new local blood supply beneath RC an abundant vascular network is available for providing the tumor with a constant supply of nutrients [6].

Injection of Cytosar 2 days before irradiation enables a large dose of irradiation to be used [9], and under these conditions of ID, growth of the BRO melanoma takes place more rapidly. The tumor grew faster still when implanted into C57BL/6 mice, which are less resistant to irradiation. These results suggest that the degree of growth of the BRO melanoma depends on the degree of immunodepression.

The absence of any marked signs of rejection during the period of observation, up to and including the 14th day after transplantation, supports the view that BRO cells possess an intrinsic immunodepressive effect [2].

The results described above show that a human tumor can undergo continuous and reproducible passage in irradiated immunocompetent mice. The use of a simple method of immunodepression and of the known method of tumor implantation beneath the renal capsule make it possible to grow certain rapidly proliferating human tumors without the use of nude mice or of more complicated conditions of immunodepression.

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IMMUNOMODULATING ACTIVITY OF EXOGENOUS CERULOPLASMIN IN MICE WITH EXPERIMENTAL TUMORS

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The development of immunodepression is an essential manifestation of the systemic action of a tumor on the host. Restoration of disturbed activity of the immune system with the aid of various immunomodulators is an important step in the treatment of tumors. Data on the immunopotentiating and antitumor action of ceruloplasmin (CP), a blood plasma protein which performs several important biochemical functions in the body, are of great interest [2, 7-9, 11]. However, information on the immunomodulating properties of CP during growth of tumors is

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definitely insufficient and contradictory. In the investigation described below an attempt was therefore made by means of in vivo tests to assess quantitatively the immunologic changes taking place in animals in response to CP and to analyze the possible contribution of the immunomodulation thus revealed to the antitumor effect of CP.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 mice weighing 20-22 g. The tumor model was a metastasizing Lewis lung carcinoma (3LL), transplanted by intramuscular injection of $2 \cdot 10^5$ cells into the animal's thigh. CP was obtained at the Kiev Bacterial Preparations Factory, Ministry of Health of the USSR, from waste arising in the production of gamma-globulins (the fraction of betaglobulins and lipoids of retroplacental human blood) and had the following parameters: mol. wt. 12.4 kD, E_{610}/E_{280} 0.035, corresponding to 90-95% purity of the preparation. CP in doses of 1-100 mg/kg body weight was injected intraperitoneally once every 3 days [1]. The effect of the preparation in the course of tumor growth was judged by the times of appearance of a tumor nodule at the site of inoculation of the 3LL cells (latent period) and the degree of inhibition of tumor growth, expressed in per cent [1]. The proliferative response of the lymphocytes was induced by injection of T-(concanavalin A) and B- (dextran sulfate) cell mitogens into the hind footpads of the animals. Concanavalin A (con A, "Sigma," USA) and dextran sulfate ("Loba Chemie," Austria) in a dose of 100 μg were injected 2 days before radiometric recording of the proliferative response. Lymph nodes regional relative to the site of injection of the mitogen (popliteal) were removed, cell suspensions were prepared, and the average number of cells in the lymphoid organ calculated for a group (3-5 mice). Next, ^{3}H-thymidine was added to primary cultures containing $1 \cdot 10^6$ cells in 1 ml of culture medium. The incorporated label was transferred to filters by the standard method. The organ index of incorporation of the labeled precursor, characterizing the overall magnitude of the proliferative response in the lymph node tested, was calculated by multiplying the cell density and specific incorporation of ^{3}H-thymidine [3, 5, 6]. The effect of CP was estimated by the index of modulation (IM) of the proliferative response, namely the ratio of the difference of the organ indices of incorporation in the control and the experiment to its value in the control, expressed as a percentage. The immune response to sheep's red blood cells (SRBC) was determined as the number of antibody-forming cells (ABFC) in the spleen by the method of local hemolysis in agarose [9]. In the local adaptive delayed-type hypersensitivity (DTH) test, the results were read after 24 h by weighing the foot of the experimental and intact limbs of the mice and determining the increase in mass of the limbs (in mg) [10, 12]. The negative control in this case consisted of intact syngeneic animals receiving an injection of SRBC and splenocytes from unimmunized mice, whereas the experimental group consisted of mice with Lewis carcinoma, receiving CP.

EXPERIMENTAL RESULTS

The use of a combination of immunologic tests in vivo showed that CP possesses marked immunomodulating properties. For instance, in a study of the effect of CP on the proliferative response induced by polyclonal mitogens (Fig. 1) it was shown that CP significantly activates the T-cell system. After a single injection of CP in doses of 1 and 40 mg/kg, IM of the response to con A reached 80.7 and 73.1% respectively. The positive immunomodulating action of CP under the conditions of this model was significantly weakened by an increase in the dose of CP to 100 mg/kg: IM was only 19.8%. CP was able to activate the proliferative response also to the B-cell mitogen dextran sulfate, when injected in doses of 1 and 40 mg/kg. However, the level of positive modulation was significantly lower than in the response to con A, and reached a peak of 13.8% with a dose of CP of 40 mg/kg. Increasing the dose of CP to 100 mg/kg revealed a significant weakening of the proliferative response (IM = −25.6%) compared with the control animals. Thus injection of CP in doses of 1-40 mg/kg potentiates the functional activity of both T-cell and B-cell components of the immune system. Increasing the dose of CP to 100 mg/kg, however, led to the appearance of a tendency toward inhibition of the functional activity of the animals' immune system.

The fact that with optimal dosage of CP it is possible to obtain positive immunomodulation was confirmed also in the other model systems which we used. For instance, injection of CP in a dose of 40 mg/kg led to significant potentiation of the cooperative humoral immune response to SRBC (Table 1) and of the intensity of the DTH reaction induced by the same antigen. All these data, obtained on intact mice, led to the conclusion that the CP

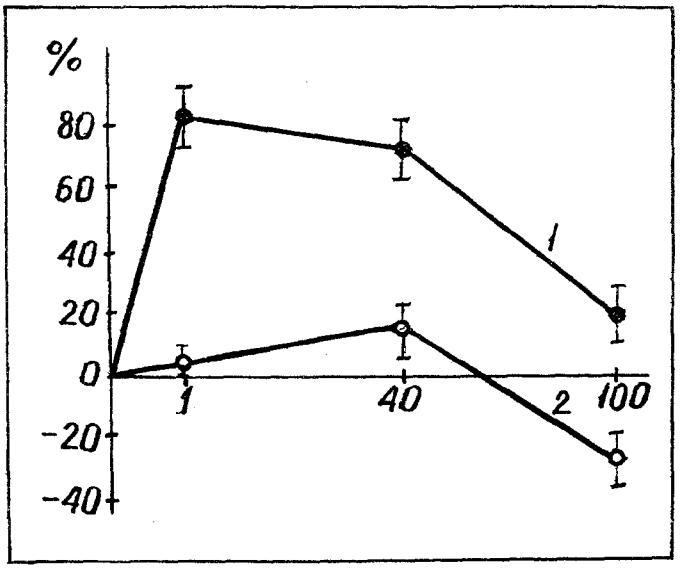

Fig. 1. Effect of ceruloplasmin on proliferative response of lymphocytes induced by concanavalin A (1) and dextran sulfate (2). Abscissa, dose of CP (in mg/kg); ordinate, IM (in %).

TABLE 1. Effect of CP on Number of ABFC and on Formation of DTH to SRBC in Tumor-Bearing Mice (M ± m)

Parameters	Control groups of animals		Time of investigation of animals with Lewis carinoma, days					
	intact	receiving CP	7		15		30	
			CP (−)	CP (+)	CP (−)	CP (+)	CP (−)	CP (+)
ABFC × 10^3	38,3±4,6	41,9±2,1	22,5±2,1	29,4±1,2	7,5±1,1	10,9±1,3	2,5±0,5	2,4±0,4
DTH								
Increase in mass of limbs, mg	32,4±0,63	47,8±2,1	28,8±2,5	31,8±1,0	22,2±1,5	27,3±2,0	15,6±1,2	19,8±1,5
	Positive control							

Legend. CP (+) indicates that CP was injected 1, 4, 7, 10, 13, and 16 days after inoculation of tumor cells; CP (−) indicates that no injection of CP was given.

preparation which we used can stimulate immunogenesis both at the level of induction of proliferation of immunocytes and at the level of their functional differentiation. This evidently was explained by the complex character of the immunomodulating action of CP, affecting systems of both T- and B-lymphocytes.

The results provided a basis for arguing that the immunopotentiating properties of CP can also be manifested during tumor growth. In fact, we obtained a positive effect of CP during growth of a metastasizing carcinoma 3LL when injected in a dose of 40 mg/kg. In this case lengthening of the latent period of tumor development by 5 days ($p < 0.05$) and inhibition of tumor growth by the 18th day after inoculation by 31% ($p < 0.05$) were observed.

The results of immunologic monitoring correlated with these results. For instance, on the 7th day after injection of tumor cells the decrease in the number of ABFC was less marked in the group of tumor-bearing animals receiving CP. A significant difference between these groups was also recorded on the 15th day, although in this case there was a distinct general tendency toward inhibition of the humoral immune response, which is characteristic of progressive tumor growth [4]. The time course of the changes was basically similar also in the DTH test (Table 1). Thus the results obtained in the in vivo system are evidence that CP delays, and up to a certain degree prevents the manifestation of the immunodepressive action of the growing tumor. This conclusion, which we draw from a model of the highly malignant metastasizing Lewis carcinoma, confirms previous findings, obtained on other models of tumor growth, indicating the antitumor effect of exogenous CP. Using completely different approach techniques, Itoh and co-workers [7, 8] clearly demonstrated in tests in vitro that CP activates not only the T helper-cell population, but virtually all the cell components directly involved in the elimination of tumor cells from the body: specific killer

T cells, effectors of antibody-dependent cytotoxicity, and mononuclear phagocytes. Since activated macrophages, together with natural killers, constitute the first line of nonspecific defense against malignantly transformed cells, lengthening of the latent period of appearance of tumors, which we noted in the present experiments, can be explained by the ability of CP to activate precisely this cell population.

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INHIBITION OF REACYLATION OF PHOSPHOLIPIDS DURING OXIDATIVE DAMAGE TO TUMOR CELLS

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Tumor cells are exposed to oxidative stress in the tumor-bearing host during interaction with neutrophils and macrophages, producing active forms of oxygen [1], or when acted upon by chemotherapeutic agents capable of forming free radicals [9]. Oxidative action on mammalian cells is accompanied by considerable destructive changes in the plasma membrane, such as increased permeability for high-molecular-weight substances [8], disturbance of transmembrane ionic currents [2], and reduction of flowability [4]. We know that the structural and functional properties of biological membranes are maintained by a strictly definite composition of the phospholipids, and an excessive content of derivatives such as lysophospholipids leads to destabilization of the lipid bilayer of the membranes and, as a result, to death of the cell [11]. A possibility that lysophosphatidylcholine accumulates in membranes during their oxidation has been demonstrated [12]. Lysophospholipids are reduced to diacyl-glycerol phospholipids by specific acyl-CoA-lysophospholipid acyltransferases (ACLAT) [10]. It can therefore be postulated that oxidative cell damage will be accompanied by disturbance of repair processes in cell membranes and, in particular, of phospholipid reacylation.

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