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RESISTANCE OF RATS VACCINATED WITH BCG TO TUMOR GROWTH AFTER ADMINISTRATION OF CYCLOPHOSPHAMIDE AND DESENSITIZATION

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Acceleration of growth of tumors was shown to take place after implantation of tumor cells not only in the early stages of sensitization of the animal with BCG (previously published data), but also in the late stages. Cyclophosphamide abolished this effect, but desensitization carried out after administration of cyclophosphamide restored it. It is concluded from the data on the connection between allergy to tuberculin and the state of the mechanisms of immunologic protection of the animal that tumor growth can be stimulated by procedures abolishing the state of inhibition of immunologic mechanisms.

KEY WORDS: Allergy; desensitization; cyclophosphamide; tumors.

BCG vaccine, in conjunction with other methods of treatment and, in particular, with chemotherapy, is widely used in clinical oncology. However, according to some investigators [12, 13] no convincing evidence of its beneficial effect on antitumor resistance has yet been obtained. Since the functional capacity of the immunologic system of the body is reduced in the presence of malignant neoplasms, the view has been expressed that there is no future for active immunotherapy in clinical oncology [6]. Attempts have recently been made to use in oncology methods based on removal of the components of reactivity which adversely effect the immunologic defence of the body [2, 11].

At the same time it has been shown that the resistance of the organism to tumors is always higher in the late stages after administration of BCG either per se or as a component of Freund's complete adjuvant than in the early stages (at the height of development of allergy), and may exceed its initial level [4]. The resistance of the body to tumors, in the presence of the immunologic reaction to BCG vaccine, can also be raised by removal of the allergic component of reactivity [2]. This can be explained on the grounds that as a result of desensitization the ability of the organism to carry out protective immunologic reactions is increased [1, 5, 9, 10].

However, it is not yet known whether the resistance of the body is increased in the late stages after administration of BCG vaccine as a result of the development of malignant cells which have already appeared, or what importance must be attached to the fact that during the immunologic response to BCG the animal was subjected to the action of cytotoxic drugs and, in particular, cyclophosphamide. These are important problems in connection with metastasization and recurrence of malignant neoplasms. We likewise have no information on whether the danger of immunologic potentiation of tumor growth exists when the components of reactivity adversely influencing immunologic protective reactions are excluded.

The investigation described below was carried out to study these matters.

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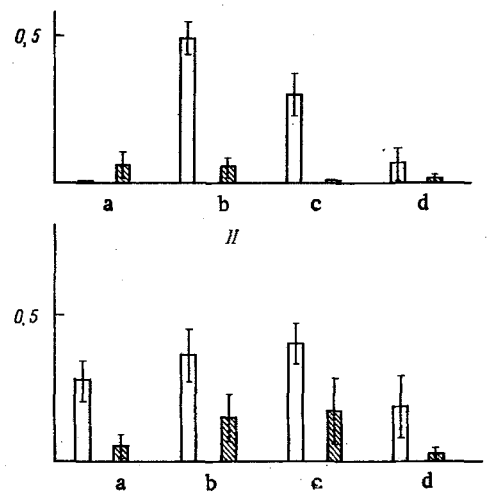

Fig. 1. Intensity of specific and nonspecific skin reactions in different groups of experimental rats. I) Allergic reactions to tuberculin; II) reactions to antigen from allogeneic liver. Abscissa: a) control, b) sensitization with BCG, c) sensitization with BCG plus cyclophosphamide; d) sensitization with BCG + cyclophosphamide + desensitization; ordinate, intensity of reactions (in points). Unshaded columns give data after 24 h, shaded columns data after 48 h.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 4 months. The experimental animals were divided into the following groups: 1) control; 2) sensitized to tuberculin; 3) treated with cyclophosphamide against a background of sensitization; 4) treated with cyclophosphamide after sensitization and then desensitized.

The animals were sensitized by the formation of a focus of *Mycobacterium tuberculosis* in the lungs by the method described by the writers previously [2]. Administration of cyclophosphamide was started 25 days, and desensitization 41 days after the formation of the sensitizing focus. Cyclophosphamide was injected intraperitoneally in 1 ml physiological saline in a dose of 20 mg/kg daily for 4 days. Desensitization was combined — specific and nonspecific. Specific desensitization was carried out with dry purified tuberculin (DPT). For nonspecific antiallergic desensitization a preparation made by the same method as DPT [15] from allogeneic muscle tissue was used (conventionally called MDPT). Preliminary experiments on 140 guinea pigs showed that this preparation inhibits general and local allergic reactions in animals sensitized with BCG and with antigens of animal origin. A solution of the contents of one ampul (50,000 tuberculin units) in 1 ml was taken as the original (100%) concentration of DPT, and a solution of 5 mg of the dry preparation in 1 ml was taken as the 100% concentration of MDPT. A combined solution of these two preparations was injected intraperitoneally in a dose of 1 ml twice a week, starting with a 0.78% solution of each of them, in a concentration which doubled with each injection until the original concentration was reached. On the day of ending of desensitization, tuberculin tests were carried out in the region of the footpads (the method was described previously in [2]). Meanwhile, skin tests with a saline extract of rat liver containing 20 mg dry residue in 1 ml were carried out in the region of the opposite footpad. A Walker's carcinosarcoma obtained from the Laboratory of Tumor Strains, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, was transplanted into all the animals 22 days after the end of desensitization and 82 days after the formation of the sensitizing focus. For this purpose, tumor tissue, finely minced in physiological saline in the ratio 1 : 5, was injected subcutaneously in a dose of 0.5 ml into the thigh. The volume of the tumors was judged from the product of their dimensions in three mutually perpendicular directions. The volume of the tumors was calculated on the 3rd, 6th and 10th days after transplantation. The results were subjected to statistical analysis.

RESULTS

The reactions to tuberculin and tissue antigen of the desensitized animals (group 4) were significantly less marked than those of the rats of groups 2 and 3 (Fig. 1). The decrease in the intensity of the reactions to tuberculin in animals treated with cyclophosphamide compared with the animals of group 2 was not significant ($P > 0.05$). A characteristic feature of the reactions to tuberculin in the rats of all groups was their almost complete disappearance after 48 h.

TABLE 1. Growth of Tumors in Sensitized Rats, Rats Sensitized and Treated with Cyclophosphamide, and Rats Treated with Cyclophosphamide and then Desensitized

Group of animals	Procedure	Number of animals	Volume of tumors, cm^3		
			3rd day	6th day	10th day
1	Control	20	0,36±0,02	26,5±2,07	42,9±2,51
2	Sensitization	24	0,73±0,14 $P<0,01$	31,2±3,07 $P>0,2$	45,6±1,93 $P>0,3$
3	Sensitization + cyclophosphamide	26	0,37±0,07 $P>0,8$ $P'<0,02$	21,4±1,64 $0,1>P>0,05$ $P'<0,01$	34,2±2,02 $P<0,01$ $P'<0,001$
4	Sensitization + cyclophosphamide + desensitization	26	0,46±0,09 $P>0,2$ $P'>0,1$ $P''>0,4$	23,0±1,96 $P>0,1$ $P'<0,03$ $P''>0,5$	45,41±2,31 $P>0,5$ $P'>0,8$ $P''<0,001$

Legend. P) probability of no difference in growth of tumors relative to control group of rats; P') the same compared with sensitized animals; P") the same compared with sensitized animals treated with cyclophosphamide.

Growth of the tumors was more rapid in the sensitized animals than in the control and slower in the sensitized animals treated with cyclophosphamide. The differences in the rate of tumor growth in the animals of groups 2 and 3 were highly significant (Table 1). Desensitization after treatment with cyclophosphamide did not delay growth of the tumors. Conversely, at the final time of observation (the 10th day) the dimension of the tumors in the rats of this group were significantly larger than those of the animals in group 3 (Table 1).

In the present experiments the resistance of the animals to tumor development (from implanted tumor cells) was reduced. Practically every person is known to be sensitized with *M. tuberculosis*. The results of the present experiments suggest that the state of allergy to tuberculin arising as a result of this sensitization, at least under certain conditions, can contribute to the development of tumors. The increased reactivity in animals sensitized to tuberculin to allogeneic tissue antigen is noteworthy. The same result was obtained by the writers previously during a study (in the early stages after sensitization with BCG) of the local reaction to tumor material used for transplantation [3]. This phenomenon is possibly connected with the enhancement of nonspecific autoimmune processes, which according to some workers [4, 8, 14] may play an important role in the pathogenesis of malignant neoplasms in the sensitized organism.

After a course of cyclophosphamide injections the resistance of the animal sensitized with BCG to tumor development, as the results described above show, was significantly increased and remained high for at least 25 days. This result is evidently connected with the fact that the effect of cyclophosphamide *in vivo* persists for a long time [7].

It must be emphasized that cyclophosphamide delayed the development of tumors not only in animals sensitized to tuberculin (in that case the differences were particularly well marked), but also in control rats. Consequently, the results confirm that cyclophosphamide can be used to prevent recurrence of malignant tumors and also in allergic states.

However, desensitization after cyclophosphamide treatment did not lead to any further increase in antitumor resistance. On the contrary, under these circumstances, growth of the tumors was more rapid. This result can be attributed to the fact that desensitization leads to some degee of activation of immunologic reactivity (or certain of its components), when depressed by cyclophosphamide, thus creating conditions for the development of the phenomenon of immunologic potentiation of tumor growth (immunologic "autopotentiation").

Consequently, when not only passive or active nonspecific immunotherapy, but also methods deblocking the immunologic system are used in oncology, there is the risk of inducing the phenomenon of acceleration of tumor development. This result is also very likely to occur in organisms affected with tumors and not treated with cytostatics, for the immunologic system may be pathologically changed and depressed as a result of the malignant neoplasm itself. Activation of the immunologic system by deblocking procedures may thus be sufficient to produce manifestation of the phenomenon of potentiation of tumor growth but insufficient for producing an antitumor effect.

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