EFFECT OF FREUND'S COMPLETE ADJUVANT IN THE PRECANCER PERIOD ON CHEMICAL CARCINOGENESIS

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Freund's complete adjuvant, when administered in the precancer period to Wistar rats by injection into the plantar pad, reduces the resistance of the animals to the carcinogenic action of 20-methylcholanthrene.

The writer has shown that if Freund's adjuvant was given 10 days before or 10 days after administration of the carcinogen, it accelerated tumor formation in intact Wistar rats and in similar rats previously vaccinated with BCG [3]. Similar results have been obtained in experiments to study virus carcinogenesis [7]. The phenomenon has been provisionally explained by the development of autoimmune processes in the body.

It may be considered that the immunologic mechanisms of protection against tumors are manifested most strongly when the tumor cells are just beginning to form and the tumor itself cannot yet be defined, i.e., before these mechanisms have been suppressed by the process of neoplasia. It is then that a change in the immunologic status of the organism must have the strongest effect on its resistance to tumors.

The object of this investigation was to study mechanisms of carcinogenesis with the aid of Freund's complete adjuvant.

EXPERIMENTAL METHOD

Experiments were carried out on 49 sexually mature Wistar rats aged 8 months. All the animals received a subcutaneous injection of 3 mg 20-methylcholanthrene in apricot oil, in a concentration of 1 mg/0.1 ml, into the right thigh. Freund's complete adjuvant was injected 80 days later into 16 rats in a dose of 0.03 ml into the left plantar pad, the same dose of adjuvant was injected subcutaneously into the left scapular region, and 17 animals were left as the control. During the experiment, some animals in each group were eliminated for various reasons as described in the analysis of the results. Freund's adjuvant was prepared as described previously [3]. The time interval between injections of carcinogen and adjuvant was chosen as 80 days because the acute phase of the reaction to the adjuvant, taking place 13 days after its injection and continuing for up to 30 days [10], would then coincide with the precancer period, associated with the formation of tumor cells. The normal time of appearance of tumors in control animals following administration of the carcinogen by the method described [3], was accepted in this investigation.

To detect tumors the animals were examined after 3.5, 4.5, 5, 5.5, and 6.5 months. A tumor was considered to have developed at a particular time if the nodule at the sight of injection of the carcinogen had attained a size of 5 ×5 ×5 mm. All the tumors were investigated histologically.

EXPERIMENTAL RESULTS

A marked inflammatory reaction developed 1-2 h after injection of Freund's adjuvant into the limb of the experimental animals. On the 10th-14th day this reaction reached its maximum development, when the

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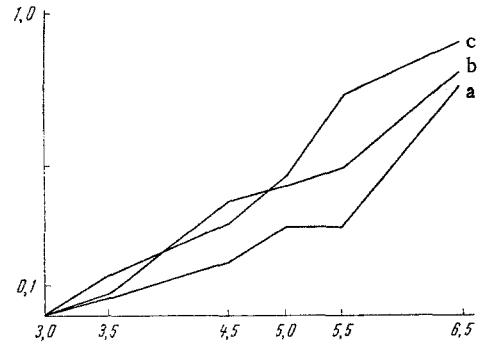

Fig. 1. Dynamics of tumor yield: a) control animals; b) rats receiving adjuvant subcutaneously; c) rats receiving adjuvant into plantar pad. Abscissa, time (in months); ordinate, yield of tumors in relative units (ratio between number of animals with tumors and total number of rats in corresponding group).

volume of the limb was increased by 3-4 times because of of inflammatory edema and infiltration. An area of subcutaneous infiltration ranging in size from a lentil to a hazel nut was observed at these times after subcutaneous injection of the adjuvant.

Polyarthritis developed only in animals receiving the adjuvant by injection into the plantar pad. The polyarthritis was noted on the 14th day in 4 animals, and after 1 month in 8 of the 16 rats of this group.

No tumor was observed 3 months after injection of the carcinogen in any of the rats of the control or experimental groups. The first tumor appeared after 3.5 months (Fig. 1). In rats receiving adjuvant into the plantar pad, a significantly higher yield of tumors was found after 5.5 months ($P < 0.01$). At other times, differences between the yield of tumors in the groups of animals receiving adjuvant into the plantar pad, and at all times in the group of animals receiving adjuvant subcutaneously into the scapular region, compared with the control animals were not statistically significant ($P > 0.05$).

No statistically significant correlation could be found between the development of polyarthritis by the animals and lowering of their resistance to tumors in the group of rats receiving adjuvant into the plantar pad.

Histological examination of tumors developing in animals receiving adjuvant into the plantar pad revealed a shift toward malignancy compared with the tumor in the control animals. Polymorphocellular sarcomas were predominant in the former, and spindle-cell or spindle-cell tumors with polymorphism in the latter. In the number of malignant forms, tumors developing in animals receiving adjuvant subcutaneously occupied an intermediate position.

The results indicate that after injection of Freund's complete adjuvant into the plantar pad, resistance to growth of tumors is reduced in the precancer period.

These results, like those obtained after injection of the adjuvant into the plantar pad 10-12 days before administration of the carcinogen [3], can be explained by changes in reactivity toward predominance of autoimmune processes. Investigations have shown that Freund's complete adjuvant, even if given without endogenous tissues, gives rise to such changes in reactivity [2, 5, 6, 8, 9]. On the other hand, the increase in content of autoantibodies against the body's own tissues may evidently lower their resistance to carcinogenic action. This is confirmed by results obtained by Grachenova and Bershtein [4], who observed a decrease in resistance of the corresponding organs to growth of tumors after treatment with specific organotropic serum.

After subsutaneous administration of adjuvant, the increase in antibody content is less marked than after its administration in other ways [11]. The possibility is therefore not ruled out that the absence of a significant increase in tumor yield in the present experiments in rats receiving adjuvant subcutaneously into the scapular region may be explained by inadequate stimulation of autoantibody formation.

It can be assumed that changes in reactivity following administration of Freund's complete adjuvant, which are generally considered to promote increased resistance to tumor development (for example, by stimulating macrophagal and lymphoid responses [1, 12]) are in competition with changes in reactivity promoting a decrease in resistance (autoimmune processes). Under the conditions of the present investigation, the effect of the latter is predominant.

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