IMMUNOLOGIC REACTIONS IN ANIMALS WITH TUMORS

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The sera of normal rats and of rats with tumors induced by a carcinogen or arising as a result of hormonal disturbances react in low titers with antigens of auto-, iso-, and homologous normal organs. Serum of healthy rats reacts more weakly with isologous tumor tissue than with normal organs of the same origin. Serum of rats with tumors reacts twice to three times more strongly with antigens of an autologous tumor than with antigens of corresponding normal organs.

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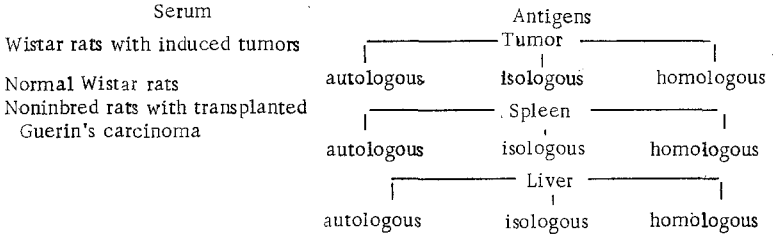
The object of this investigation was to determine antitumor antibodies under conditions in which immunologic differences between the tumor and normal tissues would not prevent estimation of their antitumor specificity [1, 2, 6, 9, 12-15].

EXPERIMENTAL METHOD

The main experiments were carried out on Wistar rats with tumors induced by a carcinogen (DMBA) or by artificially produced hormonal disturbances* under the conditions of a single autochtonous host. This completely ruled out the influence of factors connected with transplantation immunity. Analogous experiments were carried out on normal rats of the same genetic line (control No. 1) and on noninbred rats with a transplanted Guerin's carcinoma (control No. 2). Besides investigations of the same animal (autologous conditions), a series of crossed reactions was carried out between serum and antigens belonging to experimental and control rats, i.e., under isologous (Wistar rat with tumor—normal Wistar rat) and homologous (Wistar rat—noninbred rat) conditions. Hence, in the course of the investigation, in each experiment "autoimmune" and crossed reactions were studied in three animals with antigens of tumors and of normal tissues (liver, spleen, sometimes ovary) of each of them. The scheme of the experiment is as follows:



In every case rats of the same weight, sex, and age were used in the control and experimental series.

The presence of antibodies was determined from results of the complement fixation reaction in Ioffe's modification in the cold with serum in dilutions of 1, 2, 4, 8, 16, 20, 40, 80, 160, 320, and 640 times. Antigens of tumors and normal tissues (individual) were obtained by the method described by V. Ya. Felem [10]. The results were read from the last dilution of serum in which complete inhibition of hemolysis was observed (++++). The results were subjected to statistical analysis to obtain the mean titers, which were calculated as the geometric mean of the titers of individual reactions of each type [7].

*Transplantation of the ovary into the spleen of castrated rats by Biskind's method; for brevity tumors induced in this way are described as Biskind's model.

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TABLE 1. Mean Titers of Antibodies in CFR in the Cold in Experiments with DMBA Sarcoma (I) and Biskind's Method (II)

	Animals from which sera obtained	Antigen									
		tumor			spleen			liver			ovary (isogenous)
		autogenous	isogenous	homogenous	autogenous	isogenous	homogenous	autogenous	isogenous	homogenous	
I	Male Wistar rats with DMBA sarcoma	1:52,8±2,2		1:21±1,9	1:23±1,3 $P<0,0001$	1:20±1,4	1:18,3±1,8	1:15±1,1 $P<0,0001$	1:19±1,2	1:10,6±1,9	
	Normal rats with Wistar rats		1:11±1,6	1:11±1,6	1:20±2,1 $P>0,05$	1:21±2±±1,4	1:15±1,4	1:8,5±1,7 $P>0,05$	1:40±1,5	1:12±1,6	
		1:139±1,7	1:38±1,2		1:63±1,8	1:113±1,6	1:80	1:153±14	1:25,3±5,1	1:16±1,6	
II	Female Wistar rats with Biskind's tumor	1:35±1,4		1:9,7±1,7	1:17,5±2,7 $P<0,0001$	1:20±1,6	1:15,4±1,4	1:10±1,5 $P<0,0001$	1:17,6±2,6	1:14,7±1,3	1:12 $P<0,0001$
	Normal female Wistar rats		1:9,1±1,9	1:11,5±1,7	1:21,3±2,1 $P>0,05$	1:40±1,5	1:28±2,1	1:10±1,6 $P>0,05$	1:9±1,3	1:15,9±1,8	1:110±1,7 $P<0,0001$
	Noninbred rats with Guerin's tumor	1:16±1,3	1:18,6±2,1		1:17,9±3,2	1:14,2±1,4	1:2,4±1,7	1:8±1,8	1:6±1,6	1:6,3±2,3	

EXPERIMENTAL RESULTS

The statistically treated results of experiments with both models of tumors are shown in Table 1. They can be summarized as follows. Sera of all the investigated animals reacted with antigens of normal organs—liver (mean 1:8-1:15) and spleen (1:17 - 1:23) as in autoimmune reactions. Their titers were not significantly dependent on presence or absence of a tumor or on its character, or on the line to which the animals belonged (Wistar or noninbred rats). Crossed reactions with antigens of normal organs (in isologous and homologous relationships) differed only slightly in mean titers from the corresponding "autoimmune" reactions and were largely independent of the presence or absence of a tumor in the animals. The autoimmune reactions with tumors depended on the nature of the tumors. In the case of a transplanted Guerin's tumor the titers of these reactions did not exceed those obtained with the corresponding normal organs. With the two models of primary tumors (DMBA sarcoma, Biskind's model) these reactions had mean titers of 1:52 and 1:35, i.e., 2 or 3 times higher than with normal organs of the same origin (difference statistically significant, $P < 0.0001$). In crossed reactions with tumor antigen this increased activity was not found in normal rats of the same genetic line or in rats with other tumors ($P > 0.05$). The mean titers in these cases corresponded to those obtained in reactions with normal organs of animals with the corresponding tumors and in autoimmune reactions with normal organs (or were even slightly lower).

Reactions with antigens of autologous tumors and normal tissues in animals with tumors have been described previously. If they are interpreted from the point of view of "induced" antibodies [8], i.e., associated with the presence of a tumor in the body, there is no need to discuss the existence of antibodies reacting with normal tissues or the antitumor specificity of antibodies reacting with tumor antigens. However, the results of the study of "autoimmune" reactions in genetically identical animals, but without tumors i.e., in normal animals, showed that in this case also reactions can be detected (and in the same titers) with antigens of the animals' own normal organs. It is difficult, therefore, to attribute the analogous reactions in animals with a tumor to antibodies "induced" by its presence.

Small but consistent differences in mean titers found in our experiments in reactions with the certain organs (spleen, liver), and their persistence in isologous and homologous reactions, evidently indicate the role of organ-specific antibodies in them. This hypothesis agrees with the conclusion drawn from their work by M. S. Lomakin and co-workers, namely that normal tissue antibodies giving a positive reaction with antigens from auto-, homo-, and heterologous tissues are present in the blood serum of normal rats and rabbits [4, 5]. In our experiments this was found to be true also of the sera of rats with various tumors.

It can thus be concluded that the blood of normal animals and of animals with tumors contains small amounts of natural antibodies possessing tissue specificity, capable of reacting with antigens of auto-, iso-, and homologous normal organs.

On the basis of this conclusion it is possible to compare reactions with antigens of normal tissues with reactions with tumor tissue in order to determine the antitumor specificity of the latter. In normal animals, in which autoimmune reactions with tumor antigen cannot of course be investigated, the concept of antitumor reactions can nevertheless be applied to the results of crossed experiments with isologous tumor antigens. The results of these experiments showed that serum of normal Wistar rats reacted with tumor antigens in very low titers (1:11, 1:9). Not only are these titers not higher than the titers of reactions with normal organs of the same origin as the tumor, but sometimes they are actually lower (just as Lomakin and co-workers found), i.e., they are determined by a certain fraction of the total tissue antigens. Hence it can be concluded that natural antibodies capable of reacting specifically with tumor tissue cannot be found in the blood of normal animals. In the presence of transplanted tumors (Guerin carcinoma) the titers of antibodies to them are the same as those for normal tissues of the same origin. Only in animals with induced tumors are the titers of antitumor antibodies higher (1:52, 1:35) than those of antibodies to normal tissues, even to those tissues sharing common antigenic properties to the greatest degree (spleen, 1:17-1:23, liver, 1:8-1:15) [3] and in which and from which they develop (spleen, 1:17-1:20; ovary in Biskind's model 1:12).* Consequently, interaction with tumor tissue in this case is associated not only with the presence of antigens common both to it and to normal organs, but also with a specific antitumor reaction. The fact that it is observed only in relation to an autologous tumor and is absent in crossed experiments (with Guerin carcinoma) is evidence of its auto(iso)immune character.

*The results obtained in investigations with ovarian antigen require further confirmation and special examination.

This last conclusion, deduction of which was the main purpose of this investigation, can thus be formulated as follows: the animal organism is capable of responding to the development of primary induced tumors by a specific autoimmune reaction, detectable by the presence of corresponding autoantibodies in the blood serum (in the CFR in the cold). The antitumor specificity of these autoantibodies is confirmed by the higher titers of reactions with the tumor than with normal tissues of the same origin and by absence of any such increase in genetically identical normal animals.

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