A METHOD OF OBTAINING SPECIFIC ANTICANCER SERA

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 9,
pp. 95-98, September, 1963

In order to obtain specific antitissue sera, some investigators have immunized animals with untreated tissues or their fractions, and also with tissues subjected to physico-chemical treatment [3,7,10]. Others have used methods that exhaust the nonspecific antibodies outside the organism of the immune animals, combining the obtained polyvalent antiserum with tissues corresponding to the nonspecific antibodies, or with extracts of these tissues "loaded" on various adsorbents [1,2,5,6,8]. Recently, an attempt was made to use the method of artificially acquired tolerance as a means of obtaining monospecific sera against normal and malignant tissues [4,9]. However, up until now no simple method has been found for obtaining monospecific antisera.

In microbiological practice, a phenomenon is known wherein, in the process of immunizing animals with microbial antigens, there occurs an inhibition of antibody formation or a reduction in its titer. Working on obtaining sera against tumor and normal human tissue from large and small animals, we sometimes observed this phenomenon, apparently associated with the injection of an amount of tissue antigen that was elevated for the given animal, while with smaller doses of the same antigen we noted good antibody production in them.

We attempted to use the capacity of the organism to inhibit the production of antibodies upon injection of a large amount of antigen as a means of elevating the specificity of the obtained antisera.

EXPERIMENTAL METHOD

As the antigen for immunization, we used an aqueous-saline extract of the tissues. To obtain an extract of tumor tissue, or a mixture of the latter with normal tissue, we cut it into fine pieces with scissors, added distilled water (10 ml of water per 1 gram of tissue), and then groud it all up in a homogenizer for 15 min. The obtained homogenate was allowed to stand overnight in a refrigerator. On the following day, after centrifuging for 5 min at 1000-1500 rpm, sodium chloride (0,85%) was added to the centrifugate. The protein concentration was determined in the resultant aqueous-saline extract, according to the nitrogen content.

Rabbits were immunized over a couse of 4-6 cycles. Each cycle consisted of a 3-day period of antigen injection with a subsequent 4-day intervening period. At the beginning, the antigen was injected intraperitoneally, and in the last 1-2 cycles, intravenously. Immunization of the rabbits was carried out with a serological control, for which the antibody titer in the blood of the experimental animals was investigated before immunization, before the third and fifth cycles, and then on the eighth-tenth day after the last injection of antigen. During the course of immunization, each animal received approximately 240 mg of antigen protein.

The experimental rabbits were divided into 3 groups. Rabbits of the first group were immunized only with the aqueous-saline extract of tissue from human stomach cancer. In the first two cycles of the rabbits in the second group, they were immunized in the same fashion as the first group, with aqueous-saline extract of tissue from human stomach cancer; in the following cycles, they received only extract of a mixture of tissues from cancerous and normal human stomach (in a ratio of 1:7). In the third group of animals, the antigen consisted of only aqueous-saline extract of a mixture of cancerous and normal tissue (1:7). These quantities of tumor and normal tissue were selected by us because at a ratio of 1:5, the rabbits produced more antibodies against the normal tissue.

Antibody Titer in the Sera of Rabbits, Established by the Method of Complement Fixation

Serum dilution	Rabbit No. 2 (first group)			Rabbit No. 16 (first group)			Rabbit No. 19 (second group)			Rabbit No. 27 (second group)			Rabbit No. 30 (third group)			Rabbit No. 35 (third group)		
	test antigens used in the reaction																	
	SC	NS	NSp	SC	NS	NSp	SC	NS	NSp	SC	NS	NSp	SC	NS	NSp	SC	NS	NSp
1:10	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:20	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:40	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++++	++++
1:80	++++	+++	++++	++++	+++	+++	+++	+++	++	+++	++	++	+++	+++	++++	++++	+++	++++
1:160	++++	+	++++	+	++++	++	++	H	++	H	±	+	+	+	++	+++	H	+

Note. The reactions were set up with corresponding controls for the serum, antigens, complement, and hemolytic system.
Symbols: SC) stomach cancer; NS) normal stomach; NSp) normal spleen; H) hemolysis.

EXPERIMENTAL RESULTS

The table shows the results of the investigation on the sera of two rabbits from each group, since the antibody titers of the animals in each group were similar.

The work was carried out on 15 rabbits, but the data presented in the table are corroborated by the results of other experiments, where the animals were immunized according to the schema of the first or second group.

The data in the table are evidence that the differences in antibody titer for the rabbits of the first, second, and third groups are insignificant. The rabbits of the first group often produced somewhat more antibody (by 1-2 dilutions) against normal tissue than against the cancer. In the animals of the second and third groups, the antibody titer against normal and tumor tissues was basically the same.

We have still not succeeded in completely suppressing antibody production against the investigated normal human tissues in animals of the second and third groups, using the method of increasing by 7 times the immunizing-antigen concentration of the extracted substances from the tissue of normal stomach, as compared with the aqueous-saline extract of the tissue from human stomach cancer. At the same time, this significant predominance of antigen from the normal organ, in the majority of cases, does not lead to an elevation of the antibody titer against normal tissue. This affords a basis for postulating that further significant change in the quantitative relationship of the extract of cancer and normal tissue, tending toward even greater increase of the latter, may lead to suppression of antibody production against normal tissue.

SUMMARY

An attempt was made to apply the known phenomenon of inhibited antibody production associated with antigen overdosage as a method of increasing the specificity of anticancer sera. In order to depress the production of noncancer antibodies, and to raise the titer of antitumor anitbodies, rabbits were immunized with a mixture of aqueous-saline extracts obtained from the tissue of human stomach cancer and from normal stomach tissue, at first in the ratio of 1:5 and then 1:7. In the first case, the production of noncancer antibodies was greater. Although no complete depression of antibody production against the investigated normal human tissue was achieved in the second ratio, in the majority of cases such immunization did not lead to the rise of the titer of noncancer antibodies over that of the anticancer ones. In the author's opinion, further increase in the amount of extract or fractions from normal tissue used in the immunizing antigen may possibly effect a greater depression on the production of antibodies against normal tissues, and increase the anticancer sera specificity.

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