

## IMMUNOLOGICAL STUDY OF HUMAN THYROID TUMORS

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By the complement fixation test (CFT) antigens not present in healthy adult and embryonic human tissues were found in six of 17 malignant tumors of the human thyroid gland. Immunological variants of these antigens were found in individual patients. The specificity of the antigenic determinants of thyroid tumors is evidently determined by substances of protein nature.

KEY WORDS: *tumor antigen; carcinoma of the human thyroid gland.*

The use of immunological methods in clinical medicine for the diagnosis, treatment, and prevention of malignant diseases in man calls for a profound knowledge of the antigenic composition of tumors. However, there is as yet no general agreement regarding one of the fundamental problems of human tumor immunology: the existence of specific antigenic substances for tumors; hence the need for further investigations in order to discover such antigens.

Immunological mechanisms are known to play an important role in the pathogenesis of several thyroid diseases. A possible role for them has also been demonstrated in thyroid carcinoma [7, 11]. Nevertheless, the problem of antigenic changes in the tissues of the gland during the development of a malignant growth in it has received insufficient study.

In tumors in this situation the content of organ-specific antigen of the thyroid gland (thyroglobulin) has been found to be reduced [1, 2, 8, 9] or to disappear completely [2]. This process correlates with the morphological structure of the carcinomas and the degree of their malignancy [1, 9]. Thyroid tumors also lose another organ-specific antigen of the normal gland — the microsomal antigen [10, 11]. Furthermore, antigens not characteristic of the normal thyroid gland, but characteristic of other healthy human organs, are also found in tumor tissues [6, 8].

The presence of specific antigenic substances for tumors has not yet been demonstrated in thyroid carcinoma. The presence of such antigens in malignant cells can only be conjectured on the basis of isolated reports of serological differences between tumor and certain normal tissues of the human adult or embryo [6, 8, 12].

In this investigation an attempt was made to find antigens characterizing the immunological specificity of malignant tumors of the human thyroid gland.

## EXPERIMENTAL METHOD

Carcinomas of the thyroid gland with histological confirmation of the diagnosis were obtained after operations from the P. A. Gertsen Moscow Oncologic Research Institute, for which the authors are grateful to V. Ya. Rogal'skii. Various tissues from healthy men dying accidentally and from human fetuses were used as the control.

Comparative antigenic analysis of the malignant and normal tissues was carried out with the aid of the complement fixation test (CFT) at 37°C, using fresh guinea pig complement.

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Rabbit immune sera obtained against two thyroid carcinomas (a tumor from patient B., a metastasis of a papillary cystadenocarcinoma in a lymph node, and a tumor from patient K., a primary solid carcinoma with amyloidosis) were used in the experimental series and organ-specific sera against normal human thyroid gland and spleen as the control. For immunization with saline extract of the corresponding tissue (1:10-1:20) three injections of the antigen were given intravenously each week for 4 weeks.

Cross-reacting antibodies were removed from the immune sera by adsorption with a homogenate of native adult or fetal lung tissue.

Saline extracts from native tissues and also from tumor tissues treated with formalin (in a dilution of 1:8), exposed to a high temperature (100°C for 30 min), and extracted with alcohol or ether [5], were used as antigens for the CFT. All antigens were titrated in the presence of complement and used in the test in the maximal dose not fixing complement.

The tumor antigens also were compared in crossed tests in which the immune sera were absorbed by homogenates of native tumor tissues.

#### EXPERIMENTAL RESULTS

The results of the serological study of the absorbed sera obtained during the crossed tests by the CFT are given in Table 1. Clearly neither anticancer serum reacted with antigens from healthy adult or fetal human tissues. A clear positive reaction (with serum in a dilution of 1:160) was observed between each of the anticancer sera and the corresponding tumor antigen. Antiserum against the tumor from patient B. in addition fixed complement to some degree in the presence of antigen from the tumor from patient K. also.

Similar results were obtained in the absorption experiments also. Antibodies from the immune serum of patient K. were extracted by a homogenate of the tumor from patient K. but not from patient B., whereas antibodies from the serum against the tumor from patient B. were exhausted by homogenates of both tumors.

Table 1 also shows that the organ-specific serum against healthy human spleen used as the control fixed complement only with antigen from the spleen and did not react with antigens from the other healthy human organs or from the tumors. Immune serum against normal thyroid gland detected the corresponding organ-specific antigen not only in the tissue of the healthy gland but also, to some extent, in the tissues of the carcinomas, in agreement with the incomplete loss of organ-specific thyroid antigens in carcinoma of this organ observed by other workers [1, 2, 8, 9].

It can be concluded from these experiments that tissues of thyroid carcinomas exhibit immunological specificity, that the tumor of patient K. contained two antigens (antigenic determinants), whereas the tumor from patient B. contained one antigen, present in neither case in healthy human adult or fetal tissues. One of the antigenic components contained in the tumor from patient K., differed immunologically from the antigen of the tumor from patient B., whereas the second antigen of the tumor from patient K. was serologically identical with the antigen of the tumor from patient B.

When saline extracts of 15 other samples of tumor tissues from patients with carcinoma of the thyroid gland were tested with the available anticancer sera, in two cases a component identical with the antigen of the tumor from patient K. was found (in serum diluted 1:160 and 1:40), and in two cases an antigen identical with the antigen present in the carcinoma from patient B. was found (1:40). The remaining 11 tumors gave a negative result in the CFT.

Analysis of the results shows that the ability of the tumor extract to react or not to react is independent of the histological structure of the tumor. For example, the tumor from patient B. used for immunization was a capillary cystadenocarcinoma. The antiserum obtained, as pointed out above, reacted with extract from this same tumor, but did not react with antigens of two other tumors of similar histological structure, but on the other hand, it fixed complement with antigens from the tumor from patient K. and two other carcinomas with different structure.

These experiments thus showed not only antigenic differences between malignant thyroid tissues and normal tissues of the human adult and fetus, but also the existence of immunological variants of antigens of tumors in this situation in different individuals, in agreement with earlier results obtained during the study of malignant tumors arising in other organs [4].

TABLE 1. Serological Specificity of Human Thyroid Carcinomas

Antigens from native tissues	Immune sera			
	against tumor from patient K.	against tumor from patient B.	against normal thyroid gland	against normal spleen
Carcinomas				
from patient K.	160	160	40	—
from patient B.	—	160	160	—
Healthy adult human tissues				
thyroid	—	—	160	—
liver	—	—	—	—
kidney	—	—	—	—
spleen	—	—	—	—
lung	—	—	—	160
brain	—	—	—	—
skeletal muscle	—	—	—	—
Human fetal tissues	—	—	—	—
thyroid	—	—	—	—
liver	—	—	—	—
spleen	—	—	—	—
lung	—	—	—	—
brain	—	—	—	—
intestine	—	—	—	—
heart muscle	—	—	—	—

Legend. Reciprocals of antibody titers in CFT with corresponding antigens given. Dilution giving positive reaction of ++ or higher taken as titer of serum. —) Negative result of CFT. Initial dilutions of sera 1:20.

Antigens of the thyroid carcinomas studied, as the results of these experiments showed, were thermolabile, insoluble in alcohol and ether, and destroyed by treatment with formalin. Consequently, the specificity of the antigenic determinants of thyroid tumors is evidently determined by substances of protein nature, by contrast with the specific antigens of tumors in other situations, which are glycolipid in nature [3].

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