IMMUNOCHEMICAL PROPERTIES OF VARIOUS FRACTIONS OF CANCEROUS AND NORMAL HUMAN TISSUES

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In our earlier investigations of quantitative serological reactions, we studied the immunochemical properties of saline and trichloroacetic extracts of cancerous and normal human tissues as well as their fractions: proteins, polysaccharides, lipids and residual antigen. In a previous study [1, 2] it was shown that the protein fraction and the residual antigen are serologically inactive, whereas the polysaccharide and lipid fractions possess activity. Moreover, the polysaccharide fraction of the "complete" antigen possesses the same serological activity as the original "complete" antigen; the polysaccharide fraction of the saline extract is 10-200 times more active than the original extract. "Complete" antigen is 10-90 times more active than the saline extract of the same tissues.

Thus, extraction with trichloracetic acid and acid hydrolysis of tissues lead, as it were, to concentration of serologically active substances.

In this study we investigated the antigenic properties of these serologically active fractions of cancerous and normal tissues of the human stomach.

EXPERIMENTAL METHOD

Seven rabbits were immunized with complete antigen obtained from human tissues by extraction with trichloracetic acid, according to Boiven's method; four of these were immunized with complete antigen obtained from cancerous stomach tissue, and 3 – with complete antigen of normal tissue. A total of four injections of antigen, one every other day, was given in the ear vein in amounts depending on its protein content. The amount of protein given was increased with each consecutive injection by 25-30%. A total of 3 mg of protein was administered. On the 7th and 11th day after the final injection blood was withdrawn for tests. The antibody titer was 1:10, 1:20. A month later these rabbits were reimmunized. Antigen was injected intravenously every third day for a total of 5 injections. The total amount of protein injected during the immunization was 16.4 mg. Blood was withdrawn on the 8th and 11th days after the final injection. The antibody titer was 1:80, 1:160.

Polysaccharide fractions representing polysaccharide – protein mixture were obtained with Morgan's method [3] by oxygen hydrolysis of saline extracts of cancerous and normal stomach tissues. Eight rabbits were immunized: four with the polysaccharide fraction of cancerous stomach tissue and four – of normal tissue. The plan of immunization and the quantity of protein administered were the same as in immunization with complete antigen.

Polysaccharide fractions were found less antigenic as compared to trichloroacetic extracts: their antisera had an antibody titer of 1:20 and 1:40. Thus, both complete antigen and polysaccharide fraction of cancerous and normal tissues of the human stomach possess antigenic properties. The lower antigenicity of the polysaccharide fractions can probably be explained by the intense alteration, in the course of obtaining the fraction

TABLE 1

Determination of Cancerous Specificity in Complete Antigen, Polysaccharide and Lipid Fractions of Cancerous Tissue of the Human Stomach

Animal No.	Sensitization		Demonstration of similarity among antigens						Demonstration of dissimilarity among antigens					
			testing for completeness of desensitization			resolving injection			testing for completeness of desensitization			resolving injection		
	antigen	dose of protein in mg	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg (for lipids — mg dry wt)	reaction	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg	reaction
1	Saline extract of cancerous tissue of the human stomach	2	Saline extract of normal tissue of human stomach	4	—	Trichloracetic acid extract of cancerous tissue of the human stomach, complete antigen	4	++(+)	Trichloracetic extract of cancerous tissue of the human stomach	4,5	—	Saline extract of cancerous tissue of the human stomach	4	+
2		2		4	—		4	++		4,5	—		4	±
3		2		4	—		4	+(+)		4,5	—		4	+
4		2		4	—		3,5	+++		4,5	—		4,5	+
5 Control		.		.	.		4	—		.	.		.	.
1		2		4	—	Polysaccharide fraction of saline extract of cancerous tissue of the human stomach	4	+++	Polysaccharide fraction of saline extract of cancerous tissue of the human stomach	4,5	—		4	—
2		2		4	—		4	++		4,5	—		4	+
3		2		4	—		4	+++		4,5	—		4	+
4		2		4	±		4	+++		4,5	—		4	±
5 Control		.		.	.		4	—		.	.		.	.
1		2		4	—	Lipid fraction of saline extract of cancerous tissue of the human stomach	30	++	Lipid fraction of saline extract of cancerous tissue of the human stomach	30	—		4	±
2		2		4	—		30	+(+)		30	—		4	+
3		2		4	—		30	++		30	—		4	+
4		2		4	—		30	++		30	—		4	+
5 Control		.		.	.		30	—		.	.		.	±
Control		.		4	—		.	.		.	.		.	
Control		.		.	.		.	.		.	.		4	—

Remarks: The same and identical cancerous human tissue was used as material in the preparation of sensitizing and resolving antigens.

Designations: — absence of reaction; + momentary scratching of the nose; ++ strong scratching, ruffling of fur, sneezing, dyspnea, cough; +++ same, but more marked, elimination of urine and feces; ++++ convulsive jumping, convulsions usually terminating in death of the animal; dot — no injection; ± doubtful reaction.

The designations of desensitizing injections are omitted.

TABLE 2

Determination of Cancerous Specificity in Polysaccharide and Lipid Fractions of Cancerous Tissue of the Human Stomach

Animal No.	Sensitization		Second desensitization			Testing completeness of desensitization			Resolving injection		
	antigen	dose of protein in mg (for lipids mg of dry weight	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg	reaction	antigen	dose of protein in mg	reaction
1	Polysaccharide fraction of saline extract of cancerous tissue of the human stomach	2	Saline extract of normal tissue of the human stomach	1,5	+++	Saline extract of normal tissue of the human stomach	4	—	Saline extract of cancerous tissue of the human stomach	4	++
2		2		1,5	++		4	±		4	+++
3		2		1,5	+		4	—		4	++
4		2		1,5	++		4	—		4	++
1	Lipid fraction of saline extract of cancerous tissue of the human stomach	30		1,5	++		4	—		4	+
2		30		1,5	+		4	—		4	++
3		30		1,5	++		4	—		4	++
4		30		1,5	±		4	—		4	±
Control (2 aminals)		.		.	.		4	—		.	.
		.		.	.		.	.		4	—

Remarks: The same and identical cancerous human tissue was used as material in the preparation of sensitizing and resolving antigens.
Designations: — absence of reaction; + momentary scratching of nose; ++ strong scratching, ruffling of fur, sneezing, dyspnea, cough; +++ same, but more marked elimination of urine and feces; ++++ convulsive jumping, convulsions usually terminating in death of the animal; dot — no injection; ± doubtful reaction. The designations of desensitizing injections are omitted.

(hydrolysis, treatment with ether, etc.), in their protein component which is mainly responsible for the antigenicity of the preparation.

In a later study we investigated the presence of cancerous specificity in the complete antigen and the polysaccharide and lipid fractions of the saline extract of cancerous tissue of the human stomach by making use of the anaphylactic reaction with desensitization.

Twelve guinea pigs weighing 280-400 g were sensitized by hypodermic injections of saline extracts of cancerous stomach tissue. The amount of antigen administered was based on its protein content, which was determined by Conway's method. Lipids were weighed when dry. Desensitization was produced 21 days later with a saline extract of normal stomach. On the day before, all pigs were desensitized with an intraperitoneal injection of antigen from normal stomach in the amount equal to three times the sensitizing dose. On the day of the experiment desensitization was accompanied by intracardiac injection every $1\frac{1}{2}$ - 2 hours until complete absence of reaction to the administration of antigen. For the resolving injection the animals were divided into 3 groups of 4 pigs each. The pigs in group I were given complete antigen from cancerous stomach tissue as the resolving antigen; the pigs in group II – polysaccharide fraction; the pigs in group III – lipid fraction.

The results of the experiments are given in Table 1.

As shown in Table 1, the pigs responded with 2-3 plus reaction in all instances. On the basis of data obtained, one may think that complete antigen, polysaccharide and lipid fractions of cancerous tissue participate in determining cancerous specificity. In order to determine whether or not all cancerous specificity is removed by lipid, polysaccharide and "complete" antigen, separately administered, the experimental animal was given an intracardiac injection of saline extract of cancerous stomach tissue, after examining the completeness of desensitization in relation to these fractions. The pigs responded with a weak reaction. Apparently the saline extracts contain substances other than lipid, polysaccharide and complete antigen responsible for cancerous specificity.

In order to finally settle the question of participation of the serologically active polysaccharide and lipid fractions in the production of cancerous specificity, use was made of anaphylactic reaction with desensitization under somewhat different circumstances so that one could at the same time evaluate the sensitizing capacity of these fractions.

Guinea pigs weighing 280-400 g were sensitized with polysaccharide and lipid fractions of cancerous tissue of human stomach. On the 21st day the animals were found to be sensitive to the cancerous as well as normal component of the tumor: on their desensitization by the introduction of saline extracts of normal tissue of the human stomach, the pigs responded with an anaphylactic reaction.

After desensitization, a saline extract of cancerous stomach tissue was used as a resolving antigen. The results of the experiments are shown in Table 2.

As shown in Table 2, upon sensitization of guinea pigs with polysaccharide and lipid fractions of cancerous tissue and after desensitization to normal tissues, the animals respond to the introduction of saline extract of cancerous stomach tissue with a marked anaphylactic reaction.

Thus, the lipid and polysaccharide fractions investigated not only participate in the production of cancerous specificity, but are also capable of sensitizing animals to cancerous and normal human tissue. Antigenicity of the polysaccharide – its capacity to sensitize pigs and to produce antibodies in rabbits – is easily explained. The polysaccharide fraction obtained with Morgan's method contains 3-4% of nitrogen, apparently, due to protein admixture. This cannot be said of the lipid fraction. When 20 mg of lipid fraction was burned we were unable to detect nitrogen with Conway's microtechnic. Despite this, the fraction sensitizes pigs to cancerous human tissues. Apparently, this occurs due to microadmixture of protein which is undetectable by chemical methods but is detectable by such a highly sensitive reaction as the anaphylactic reaction with desensitization, or because the given fraction actually does not contain protein but when introduced into the organism combines with its proteins and becomes antigenic.

In order to become convinced that the antigenicity of the polysaccharide fraction as well as the complete antigen depends on the presence of protein, we studied the amino acid content of these fractions by the method of distributive chromatography on paper. The solvent used was a mixture consisting of butanol, acetic acid,

and water (40 : 10 : 50). As a result of chromatographic examination 19 amino acids were identified in the preparations: cystine, lysine, histidine, arginine, aspartic acid, serine, glycine, hydroxyproline, glutamic acid, threonine, α-alanine, trolin,* tyrosine, aminobutyric acid, methionine, valine, phenylaline, isoleucine, lucine. The preparations investigated did not differ so far as qualitative content of amino acids was concerned, but only by the intensity of color spots was it possible to conclude that various preparations contained different amounts of amino acids.

The presence of such a large variety of amino acids in the fractions studied testifies to the fact that protein is one of their components which, apparently, determines their antigenic properties.

Therefore, this project demonstrates that the complete antigen and the polysaccharide fraction of the saline extract of cancerous human tissues possess antigenicity.

Thus, anaphylactic reaction with desensitization demonstrates the presence of cancerous specificity in lipid and polysaccharide fractions and in the complete antigen. It is possible that specificity of cancerous human tissues is determined by a combination of substances – polysaccharides and lipids along with proteins.

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SUMMARY

Complete antigen obtained according to Boiven's method, and polysaccharide fractions of saline extracts of cancerous and normal tissues of the human stomach possess antigenic properties. The presence of a protein component in these fractions was established by the method of chromatography on paper. Existence of cancerous specificity in lipid and polysaccharide fractions and in complete antigen of human tumor tissue was demonstrated by the reaction of anaphylaxis with desensitization.

LITERATURE CITED

[1] N. M. Mazina, Biull. Eksptl. Biol. i Med. 45, 2, 98-102 (1958).**

[2] N. M. Mazina, Biull. Eksptl. Biol. i Med. 45, 5, 74-79 (1958).*

[3] W. T. J. Morgan and S. Partrige, Biochem. J., 1940, 34, 169.

*As in original.

**Original Russian pagination. See C. B. Translation.