IMMUNOCHEMICAL STUDY OF COMPLEX ANTIGENS FROM HUMAN TISSUES

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Progress in problems of non-infectious immunology — tissue compatibility, cancer immunology, immunology of embryogenesis etc. — requres a thorough research of the immunochemistry of the antigenic composition of human tissues. Compared with our knowledge of bacterial antigens, however, the information on the antigenic structure of human tissues is very sparse. Nevertheless, in a number of works [1, 6-10, 12, 13, 15, 16, 18] it has been shown that serologically active polysaccharide complexes can be isolated from various tissues of man and animals.

The aim of the present work was to isolate from tumor and normal human tissue an antigenically active complex similar to that found in gram-negative bacteria and in tissues of animal origin, and to study its serological and chemical properties.

METHODS OF INVESTIGATION

We extracted a complex antigen from human tissues with trichloroacetic acid, according to A. Boivin's method [14]. Tissues from normal human stomach and spleen, and also of human stomach cancer, were homogenized in distilled water in proportions 1 : 5 and then treated with an equal volume of 0.5 N trichloroacetic acid for three hours in a refrigerator. The homogenate was centrifuged, the deposit discarded, and the supernate subjected to dialysis through cellophane for 24 hours against tap water and for 24 hours against distilled water in the cold. The dialyzate was then precipitated in the cold with 5 volumes of alcohol during the night. The deposit was separated by centrifugation and dissolved in water. The obtained solution of 'complete' antigen was opalescent, gave positive Molisch and Trommer reactions and negative biuret and trichloroacetic acid reactions.

For a comparison of serological activity we prepared parallel samples of saline extracts from the same tissues. For these the tissue was ground with glass sand and, physiological saline was added in proportions 1 : 10. The extraction was carried out in the cold. The solution was then centrifuged and the centrifugate taken as the saline extract.

In the obtained saline and trichloroacetic extracts of the human tissues, dried by the lyophil method, we determined the nitrogen content according to Conway, phosphorus by the Fiske-Subarrow method, and reducing substances by the Hagedorn-Jensen method. The percentage proportions of nitrogen, phosphorus and reducing substances present in the complex antigens which we investigated were quite close to those found in the chemical examination of antigens of pathogenic microbes and higher animals [2, 3, 7-9, 17]. We might just draw attention to the lower polysaccharide content of antigens of animal and human origin as compared to microbial antigens.

RESULTS OF INVESTIGATIONS

We determined the serological activity of the complex antigens of human organs by the classical complement fixation test (CFT), and also by a quantitative 50% titer CFT, developed in the laboratory of M. Heidelberger [19, 20] and somewhat modified by A. P. Konikov [5]. The complete antigens, isolated from normal and tumor human tissue, were found to be 10-90 times more serologically active than the saline extracts.

TABLE 1

Percentage Relations of Fractions of Complete Antigen

Object of investigation	Fractions				Source
	polysaccharide	lipid	protein	low-molecular substances	
Shiga's dysentery bacillus	50-55	9-12	17-20	14	V. Morgan, S. Partridge [17]
Paratyphoid bacillus	30.04	10.38	12.38	45.53	I. I. Dubrovskaia, A. N. Bitkova, V. S. Gostev [3]
Typhoid bacillus	53.8	6.44	21.14	9.1	L. N. Mekhedov
Tularemia microbe	36.7-50.2	32.4-37.8	5-7.7	11.7-18.2	G. K. Shipitsina [11]
Human tissues: stomach cancer	39.7	31.9	6.9	18	N. M. Mazina
Human tissues: normal stomach	50	39.3	10.7	14.3	N. M. Mazina
Human tissues: normal spleen	49.9	20.7	8.5	14.6	N. M. Mazina
Guinea pig tissues	39	9	16	36	A. M. Kuzin, I. S. Bulanovskaia, A. M. Rykaleva, N. N. Kuzina [7].

For a more thorough research of the serological and chemical properties, the obtained saline and trichloroacetic extracts were fractionated, according to V. Morgan [17], into separate components by a 5-hour hydrolysis with 0.1 N acetic acid solutions. As we already know, complex bacterial antigens in these conditions are split up into 4 components: protein, specific polysaccharide, lipid and residual antigen. We succeeded also in separating these components from the hydrolysis products of the 'complete' antigens and saline extracts of human tissues. By extraction of the hydrolyzate with ether the lipid was isolated; on centrifuging the water phase the protein fraction was precipitated; the polysaccharide fraction in the centrifugate was precipitated with alcohol, and the fourth fraction — residual antigen — remained in solution in the alcohol after all these processes. Table 1 shows the percentage yield of the fractions of 'complete' antigens of various origin, according to the works of various authors, and also our data, obtained by fractionation of the complete antigen of human tissues.

TABLE 2

Chemical Characterization of Saline Extract, Complete Antigen and Their Polysaccharide Fractions

Test-antigens	Content as percent of absolutely dry antigen (fraction)					
	total nitrogen		reducing substances calculated as glucose		phosphorus	
	stomach cancer	normal stomach	stomach cancer	normal stomach	stomach cancer	normal stomach
Saline extract	10	13	2.8-3.5	1.4-3	0.45-0.66	0.4-0.8
Complete antigen	3.1-4	2.3-4.3	6.8-12.8	5.2-9.6	0.48-0.54	0.71
Polysaccharide fractions: from saline extract	4.1	3.6	6-8.5	8.5	0.26	0.23
Polysaccharide fractions: from complete' antigen	3.3	4	21-26.2	24	—	—

The results given in Table 1 show the rather close percentage relations of the individual fractions in complete antigens of microbial, as well as of animal and human origin. We will just note the higher percentage content of the lipid component in tissues of human origin and in the tularemia microbe.

Determinations were made of nitrogen, phosphorus and reducing substances in the polysaccharide fractions.

A chemical characterization of the saline extracts, trichloroacetic extracts, and their polysaccharide fractions is given in Table 2.

We did not discover any regular relationship between the nitrogen content, reducing substances and cancer specificity. Both the saline and trichloroacetic extracts, as well as their polysaccharide fractions, contained approximately equal amounts of nitrogen, phosphorus and reducing substances, independent of their tissue origin, normal stomach or stomach cancer.

We determined the serological activity of the obtained fractions in a 50 % titer CFT. Two fractions were found to be serologically active; the ether-soluble (lipid) and the alcohol-precipitated (polysaccharide). The protein fraction and residual antigen were serologically inactive. The study of the serological activity of the lipid fraction will be dealt with in a special paper.

Table 3, compiled from several records, compares the serological activity of trichloroacetic and saline extracts, and their polysaccharide fractions. The figures indicate the amount of antigen in γ, specifically fixing in the reaction at least one unit of complement.

TABLE 3

Comparison of Serological Activity of Complete Antigens and Saline Extracts Isolated from Human Tissues, and of Their Hydrolysis Products

Test-antigens		Test-antigens from tissues		
		stomach cancer	normal stomach	normal spleen
Saline extract		14.1	15	25.2
Fractions	protein	—	—	—
	polysaccharide	0.05	0.09	2.6
	low-molecular	—	—	—
Complete antigen		0.39	0.17	6.9
Fractions	protein	—	—	—
	polysaccharide	0.35	0.138	0.12
	low-molecular	—	—	—

NOTE: The figures denote the amount of antigen in gammas, fixing at least one unit of complement.

As Table 3 shows, the polysaccharide fractions and the original 'complete' antigens have almost equal serological activity, except for the spleen polysaccharide, where the serological activity is markedly higher; on the other hand, the polysaccharide fractions isolated from saline extracts are considerably more active than the original extracts. While 14 or 15 or 25 γ of the original saline extract fixes 1 unit of complement a much smaller amount — 0.05 or 0.09 or 2.6 γ — of the polysaccharide fraction does this, i.e. it is 280 or 160 or 10 times more serologically active.

Thus, from cancer and normal human stomach tissue a complex antigen was obtained, which in chemical respects is quite similar to the complex antigens isolated by other authors from gram-negative microbes and from animal tissues. It should be mentioned that the trichloroacetic extract of the human tissue which we investigated has a higher content of the lipid component than the complete antigens of bacteria and animals obtained by other authors; the one exception is the tularemia antigen (G. K. Shipitsina [11]). The lipid-richness of the human tissues in our investigation can be explained by the fact that, in order to avoid excessive damage to the native properties of the tissues, we did not subject them to a preliminary defatting.

The complex antigens which we isolated were found to be more active serologically than saline extracts of the same tissues. It is possible that the treatment of the tissue with trichloroacetic acid leads to the precipitation of proteins which are superfluous as regards serological activity, and to the liberation of serologically active groups in the complex antigens.

The polysaccharide component of the 'complete' antigen is equal in serological activity to the original complex antigen. Obviously the further division does not result in a concentration of the serological activity in the polysaccharide fraction. On the contrary, the isolation of the polysaccharide fraction from saline extracts leads to a considerable increase of serological activity in this fraction, which is probably also due to its liberation from ballast proteins and the exposure of serologically active groups.

The protein fractions were found to be serologically inactive, which by no means indicates their secondary role in the creation of immunological specificity. It is obvious that in obtaining this fraction (hydrolysis, solution in alkali, neutralization with acid, treatment with ether etc.) the proteins were greatly altered; hence they were serologically inactive towards serum obtained from animals immunized with unaltered protein. The alcohol-soluble fraction — residual antigen — was also serologically inactive, which can be explained by the low-molecular nature of the substances composing it.

Using a 50 % titer CFT we were able in the present work to determine quantitatively the serological activity of the original extracts as well as their separate fractions independent of their anti-complementary properties. The increased serological activity of human tissue antigens on fractionation with trichloroacetic acid or on weak acid hydrolysis of saline extract is of immunological interest, since it is by extraction with trichloroacetic acid (A. Boivin [14]) and acid hydrolysis (N. I. Kovaleva [4]) that active bacterial antigens are prepared.

I express my deep gratitude to my principal, Prof. V. S. Gostev, for proposing the subject and for his constant guidance.

SUMMARY

Immunochemical properties of trichloroacetic and saline extracts of the human tissues, as well as their "polysaccharide" fractions, were investigated. The polysaccharide fractions of the total antigen have the same serological activity as the original antigen. Polysaccharide fractions of the saline extracts are on the contrary 10 to 200 times more active than the original extracts. Extraction by trichloroacetic acid and acid hydrolysis of the tissues results in concentration of the serologically active substances.

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