ANTIGENIC FEATURES CHARACTERISTIC FOR LIPID–POLYSACCHARIDE COMPLEXES IN THE TISSUES OF OLD ANIMALS

PRELIMINARY COMMUNICATION

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In studies concerning the antigenic features, connected with the animal's age, of blood serum and aqueous saline extracts of animal tissues, which studies were carried out by the method of anaphylaxis and desensitization with the specific antigen, we were able to establish [5] that antigens prepared from the tissue of old animals preserved in the main the antigenic properties of analogous tissues in younger individuals, but at the same time acquired additional antigenic features specific for older animals. In addition to the method of anaphylaxis with desensitization we made attempts to establish antigenic features connected with the age in the blood serum and aqueous saline extracts from the tissues of old animals by the method of the complement fixation test (CFT). To this purpose we immunized rabbits with the antigens enumerated above in the hope of detecting in the immune serum of these rabbits by means of the CFT method the antibodies specific for antigens present in the tissues of old animals. Our hopes, however, were not fulfilled; in these experiments the CFT showed precisely the organ-specific character of the antigens and completely failed to detect the age-specific features in those antigens in which these features could be clearly differentiated with the aid of the reaction of anaphylaxis and desensitization. From this fact it followed that the CFT was unsuitable to detect age-specific features in such complex preparations (containing numerous antigens) as blood serum of animals and aqueous saline extracts of animal tissues.

In view of this fact we decided in subsequent experiments to study only age-specific antigenic features of lipid-polysaccharide complexes (LPC) from the tissues of old animals by means of the CFT.

METHODS AND RESULTS

The LPC were obtained from the blood serum of cows and aqueous saline extracts from the organs and tissues of white mice by the method of Boivin and Mesrobeanu, adapted for our special purposes. The blood serum was added to a cooled 0.25 N solution of trichloroacetic acid in a proportion of 1:4 and aqueous saline extracts of homogenized rat tissue (one part homogenate to two parts normal saline) in a proportion of 1:2. The extraction was continued for 16-18 hours in a refrigerator (2-4° C). The dialysis of supernatant was carried out in cellophane bags. Then the preparation was condensed at 75° C-80° C to one-fifth of the original volume. After addition of 0.85% solution of sodium chloride one half of the condensed preparation was sterilized in fractions for three days for one hour daily at 80° C and the other half in an autoclave for 30 min at 120° C.

The preparations were investigated with regard to the presence of protein. Samples investigated with 20% sulfosalicylic acid or 10% trichloroacetic acid gave in all cases a negative result. The biuret reaction gave in a number of cases a pink-violet or blue-violet stain. Taking into account the fact that the methods of protein de-

tection enumerated above are insufficiently sensitive, although they are generally accepted, we are not justified in assuming that the LPC fractions obtained by us were completely free of protein, the more so as in the opinion of the majority of contemporary immunologists the antigenic function of any complex can be based only on substances of protein nature.

TABLE 1

The Polysaccharide and Total Lipid Content of the Preparations

Date of preparation	No. of preparation and age of rats used	Polysaccharide content (in mg per ml)		Total lipid content (in mg per ml)
		preparation not treated in autoclave	autoclaved preparation	
December, 1957	No. 27, 2 years	0.368	–	–
	No. 28, 6 months	0.324	–	–
March, 1958	No. 29, 21 months	0.373	–	27.20
	No. 30, 4 months	0.320	–	40.67
June, 1958	No. 32, 2 years	0.308	0.260	35.21
	No. 33, 2 years	0.300	0.255	–
	No. 34, 4 months	0.260	0.222	41.90
	No. 35, 4 months	0.300	0.254	–
March-April, 1959	No. 36, 2 years, 2 mos.	0.340	–	29.58
	No. 37, 4 months	0.270	–	40.68
	No. 38, 4 months	0.340	–	–
July, 1959	No. 39, 4 months	0.238	0.203	44.37
	No. 40, 2 years, 2 mos.	0.240	0.203	34.51

TABLE 2

Complement Fixation Titers in Experiment No. 2

Antigens and doses used for the immunization of rabbits	Rabbit No.	Antigens used in the CFT in a dilution of 1:25								Control sera
		No. 23, from the blood serum of a heifer				No. 24, from the blood serum of old cows				
		dilution of rabbit serum								
		1:10	1:25	1:50	1:100	1:10	1:25	1:50	1:100	
No. 23, LPC from the serum of 3-yr-old heifers; 1st dose: 6 ml; subsequent doses: 3.5 ml ea.	1	+	H	H	H	+	H	H	H	H
	2	+±	±	H	H	+	H	H	H	H
	3	+	H	H	H	+	H	H	H	H
No. 24, LPC from the serum of three 11-13 yr-old cows; 1st dose: 5 ml; subsequent doses 3 ml ea.	4	+++	++	+	H	++++	++++	+++	++	H
	5	+	±	H	H	+++	++	+	±	H
	6	±	H	H	H	+++	++	+	H	H
	7	+	H	H	H	+++	++	+	±	H
Antigen control		Hemolysis								

Note: The insufficiently high antigen and antibody titer can be explained (as subsequently established) by a certain loss of the active principle from the antigens in the process of their preparation (too long dialysis in cellophane bags). H = hemolysis.

In some preparations we were able to establish the presence of polysaccharides by the method of Siebert and Etno and of lipids by the method recommended by D. L. Ferdman and E. F. Sopin [10]. The results of these estimations are set forth in Table 1. From the data set forth in Table 1 the following conclusions can be drawn:

TABLE 3

Complement Fixation Titers in Experiment No. 6

Antigens (LPC) used for the immunization of rabbits	Dose of polysaccharides, ml/ml, first and subse. doses	Rabbit no.	Antigens used in the CFT in a dilution of 1:5												Control sera
			no. 34 + 35 (from young rats)						no. 32 + 33 (from old rats)						
			autoclaved			not autoclaved			autoclaved			not autoclaved			
			dilutions of rabbit serum												
			1 : 10	1 : 25	1 : 50	1 : 10	1 : 25	1 : 50	1 : 10	1 : 25	1 : 50	1 : 10	1 : 25	1 : 50	
No. 34; LPC from three 4-month-old rats, autoclaved	8.1 : 1.47 and 4 : 0.74	1	+++	+	H	+++	+	H	++	+	H	+++	+	H	H
		2	H	H	H	+	H	H	H	H	H	+	H	H	H
No. 34, not autoclaved	7.8 : 1.5 and 4:0.76	3	H	H	H	++++	+++	+	+	H	H	++	+	H	H
		4	H	H	H	++++	+++	+	H	H	H	++	+	H	H
No. 35; LPC from two 4-month-old rats, autoclaved	7 : 1.28 and 3 : 0.55	5	H	H	H	+±	H	H	+	H	H	+	H	H	H
		6	H	H	H	++	+	H	+	H	H	+	H	H	H
No. 35; not autoclaved	7 : 1.4 and 3 : 0.6	7	H	H	H	+±	+	H	H	H	H	++	H	H	H
		8	H	H	H	+	±	H	H	H	H	±	H	H	H
No. 32; LPC from two 2-year-old rats, autoclaved	7 : 1.47 and 3 : 0.63	9	H	H	H	+	H	H	+++	++	+	++++	++++	+++	H
		10	H	H	H	±	H	H	++±	+±	±	++++	+++	+	H
No. 32; not autoclaved	6 : 1.44	11	±	H	H	+	H	H	+±	±	H	++++	++++	+++	H
No. 33; LPC from two 2-year-old rats, autoclaved	9 : 1.44 and 4 : 0.64	12	++	H	H	++	+	H	++++	+++	++	++++	++++	++	H
		13	H	H	H	+	H	H	++	+	±	++++	++	±	H
No. 33; not autoclaved	7.6 : 1.5 and 3.5 : 0.69	14	H	H	H	+++	+±	H	+++	+	H	+++	+++	++	H
		15	H	H	H	++±	+	H	H	H	H	++±	+	H	H
Antigen control				H			H			H			H		

1) The quantity of polysaccharides in all preparations not treated in an autoclave varied within a range of 0.238-0.373 mg per 1 ml; 2) treatment in the autoclave apparently decreased the polysaccharide content of the preparations; 3) the total lipid content of the preparations reached 27.20-44.37 mg%; 4) in preparations from the tissues of old animals the lipid content proved to be in all cases somewhat lower (27.20-35.21 mg%; on the average 31.62 mg%) than in preparations from the tissues of young animals (40.68-44.37 mg%; on the average 41.91 mg%).

A. V. Nagornyi and E. F. Sergienko [7], V. N. Nikitin [8, 9] and others established that with increasing age the lipid content of the animal body increases; according to our findings, however, the lipid content proved to be lower in preparations extracted from the tissues of old animals than in similar preparations extracted from the tissues of younger individuals. We are inclined to explain this discrepancy in the results with the fact that we apparently obtained in our preparations only free lipids or lipids only loosely bound to proteins or nucleic acids in the lipoprotein complexes. In view of the fact, however, that with increasing age the intermolecular links in these complexes become more stable [9], it is quite logical to assume that the most solidly linked lipids which ought to be present in greater quantities in the tissues of old animals were precipitated with the proteins during the precipitation of the latter by trichloroacetic acid from the extract of the tissue homogenate. At the same time the tissues of older animals contained less free or loosely linked lipids – a fact reflected in our results.

The preparations described above were used to immunize rabbits; 2 weeks after the last injection the immune serum of these rabbits was used to establish – by means of the CFT – the antigenic features of the tissue LPC, connected with the age, in old animals. The antigens were injected into the ear vein in 4 periods of 3 days at an interval of 4 days after every third injection. The results of the experiments are presented in Tables 2 and 3.

Table 2 shows that the serum of the first 3 rabbits immunized with antigens prepared from the tissues of young animals gave about the same results in the CFT, both with antigen from the serum of heifers and antigen from the serum of old cows. This fact suggests that common antibodies are present against both antigens. A different picture could be observed in tests performed with the sera of the next 4 rabbits, which had been immunized with antigen prepared from the serum of old cows. Here the results obtained in the CFT with antigens prepared from the tissues of old animals were positive in much higher titers than with similar antigens prepared from the tissues of young individuals. This fact suggests that the blood of the above rabbits contained along with antibodies in common for both antigens other, additional immune bodies which were specific for the antigen prepared from the tissues of old animals.

Hence it follows that LPC prepared from the blood serum of old cows possesses – unlike the LPC prepared from the serum of heifers – additional antigenic properties, connected with changes due to age in the tissues of old animals.

In subsequent experiments LPC prepared from the organs and tissues of old and young white rats (males) served as antigen for the immunization of rabbits. To establish the influence of sterilization in the autoclave upon the activity of the preparations, part of the rabbits were immunized with preparations which had been autoclaved for 30 min at 120°C, and the other part with preparations exposed to fractional sterilization at 80°C. Table 3 shows the results of one of these experiments. The method used for the immunization of the rabbits was the same as in the Experiment No. 2 described above (see Table 2). In this experiment the first dose of each antigen was approximately twice as high as the subsequent 11 doses. The results of the CFT presented in Table 3 (similar to those in Table 2) show that the findings obtained in one set of two investigations on one and the same sera with one and the same antigen coincided as a rule. The findings presented in Table 3 warrant the conclusion that the CFT indices are in this experiment in principle the same as in Experiment No. 2. Similar results were obtained in a number of further experiments which will be reported in an additional publication. In all these experiments the CFT revealed more or less precisely age-specific antigen features of the LPC prepared from the tissues of old animals. Table 3 shows that sterilization in an autoclave slightly decreased the antigenic properties of the preparations.

It thus appears that the LPC prepared by us from the blood serum of cows and heifers and also from the organs and tissues of old and young rats were fully active antigens. They proved to be active not only in serological tests but also possessed immunogenic properties.

The LPC prepared from the tissues of old animals preserved to a greater or lesser degree the antigenic properties of the tissues in younger animals but at the same time possessed additional antigenic features, specific for their age and detectable only in the tissues of old animals.

Administration of lipid-polysaccharide complexes prepared from the tissues of old animals leads – in addition to the formation of antibodies against the LPC from the tissues of younger animals in certain quantities–also to the formation of antibodies against similar complexes occurring in the tissues of old animals, i.e., to the formation of "anti-old age" immune bodies.

SUMMARY

A number of experiments were conducted to detect the antigenic features of lipid-polysaccharide complexes in the tissues of animals of various age. The lipid-polysaccharide complexes from the animals' tissue are complete antigens; the complexes mentioned have specific antigenic peculiarities connected with age.

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