Complement Fixation by Tuberculopolysaccharide.* (22622)

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The sera of rabbits sensitized by repeated injections with BCG have a high content of antibodies to tuberculoprotein and tuberculopolysaccharide, as can be detected by the precipitin reaction. These sera have been shown in an earlier study(1) also to give high complement-fixation titers with PPD-S, but not with polysaccharide. However, it appeared that the addition of polysaccharide was able to inhibit the complement fixation produced by the PPD-S. It was the object of this investigation to study the mechanism of this inhibition.

Materials and methods. The protein antigen used in this study was PPD-S, a protein fraction isolated from tuberculin culture filtrate after autoclaving. It contained 5.9% polysaccharide. This preparation was adopted as the international standard for purified tuberculins and has been extensively studied (2,3). It proved to be one of the most effective antigens for complement fixation and was used in the previous study(1). Polysaccharide I had been isolated(4) from heated tuberculin filtrate of a human strain of tubercle bacilli (DT), and contained only 0.24% nitrogen. The sera were taken from rabbits used in previous experiments(1). The rabbits had been sensitized (Table I) by repeated injections with heat-killed or live tubercle bacilli or protein fractions. Blood had been taken at frequent intervals and the sera were tested for antibodies by the precipitin reaction. Complement-fixation tests were made by the block titration method in which all dilutions of the antigen were titrated against all dilutions of the serum, using a previously standardized amount of amboceptor and complement and a constant quantity of sheep cells. The sera were inactivated at 56°C for 20 minutes. Every dilution of serum and every dilution of antigen was included in the controls, and in this way it was shown that the antigens used (PPD-S and polysaccharide I) were not anticomplementary, since they by themselves fixed no complement at any dilution. The serum, antigen and complement were incubated in some cases for a half hour at 37°C, and in others kept overnight at 4°C, and then the red blood cell-amboceptor suspension was added. This mixture was then incubated at 37°C for a half hour and placed in the cold room overnight to settle before reading the degree of hemolysis in each tube. The results were plotted, as shown in the curves: each curve encompasses the block of tubes showing no hemolysis, *i.e.*, where complement was fixed by the antigen-antibody system under investigation.

Results. Complement fixation by tuberculoprotein PPD-S and tuberculopolysaccharide I. High complement-fixation titers occurred in anti-BCG sera with the antigen PPD-S (Table I).

The polysaccharide, on the other hand, fixed no complement during incubation at 37°C for a half hour with anti-BCG sera, even though the sera were shown by the precipitin reaction to contain antibodies to polysaccharide. However, if the reaction took place while standing at 4°C overnight, before the amboceptor-blood-cell system (Amb-rbc.) was added, then it was obvious that the polysaccharide did fix considerable complement in those sera containing antibodies to polysaccharide. On the other hand, very little or none was fixed in the case of sera which did not contain antibodies to polysaccharide. This shows that the reaction with polysaccharide was specific. Rice(5) and Eagle(6) both showed that the complement-fixing system becomes more sensitive at low temperature.

The fact that no complement was bound by

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TABLE I. Complement Fixation and Precipitin Titers.

Rabbits sensitized with	Complement fixation titers						Precipitin titers							
	37°C			4°C			PPD-S				Polys.			
	PPD-S	Polys.	PPD-S + polys.	PPD-S	Polys.	PPD-S + polys.	50	10	5	1	50	10	5	1
BCG (dead)	192	0	0	512	128	24	6+	5+	3+	±		3+	4+	6+
	512		0		256	64	6+	5+	3+	±	+	5+	5+	6+
BCG	256	8	48		128		6+	5+	3+	±	+	5+	5+	6+
"	256	0	96				5+	4+	3+	+	2+	3+	4+	3+
"	32		0		0		+	±	±					
" + polys.	64		16		6		5+	3+	3+					
" "	64		64		0		3+	4+	2+	+				
" + protein A	64		0		8, 12		4+	2+	±					
Ravenel				48	0	24	3+	+	+				±	
PPD-S	48		48				4+	2+	+	±				
Protein A	32		16				5+	4+	3+	2+				
" A	32		48				5+	5+	4+	3+				
" C	96		96				3+	+	+	±				

the polysaccharide at 37°C, whereas high titers were given by PPD-S, shows that the antigen in PPD-S responsible for the fixation was not the small amount (5.9%) of polysaccharide present in this antigen, but rather the protein. In fact, it was pointed out(1) that one of the tuberculin proteins, designated as C protein, and which forms a large part of PPD-S, gave as high or higher complement-fixing titers than did PPD-S, and this protein had only 1.1% carbohydrate. The amount of polysaccharide present in PPD-S, and which presumably is the same as polysaccharide I, would be effective merely as an inhibitor to the fixation at 37°C or, at 4°C would slightly increase the titer to the polysaccharide deliberately added to the system. It could not account for the other maximum titer at a lower (1:6400) dilution (curve 2, Fig. 1), which was presumably due to the protein.

It is noteworthy that the range of antigen dilution at which complement fixation was evident was very different for the 2 antigens, PPD-S and polysaccharide. For example, PPD-S fixed complement at 37°C maximally at a dilution of 1:6400 to 1:25,600, whereas polysaccharide fixed complement only at 4°C maximally at a dilution of about 1:220,000 to 1:27,000,000. The two reactions could, therefore, be easily distinguished from each other, as shown in the diagrams of their block titrations (Fig. 1 and 2). A parallel distinction was noted between the ranges of dilution for the precipitin reaction of the protein antigen-antibody system (PPD-S-AAb) and that of the polysaccharide-antigen-antibody system (Polys.-AAb) (Table I). For example, the greatest precipitate occurred with 50 γ PPD-S antigen, whereas with the polysaccharide one γ or less gave the heaviest precipitate, but both of these reactions took place at 37°C. Evidence that they really are different reactions is found in the fact that the characters of the 2 antigen-antibody precipitates were different(1).

Mutual effect of protein and polysaccharide in the complement-fixation reaction. If protein and polysaccharide were mixed in the ratio of 1:7 for use as antigen, the complement-fixation titer to PPD-S in serum containing

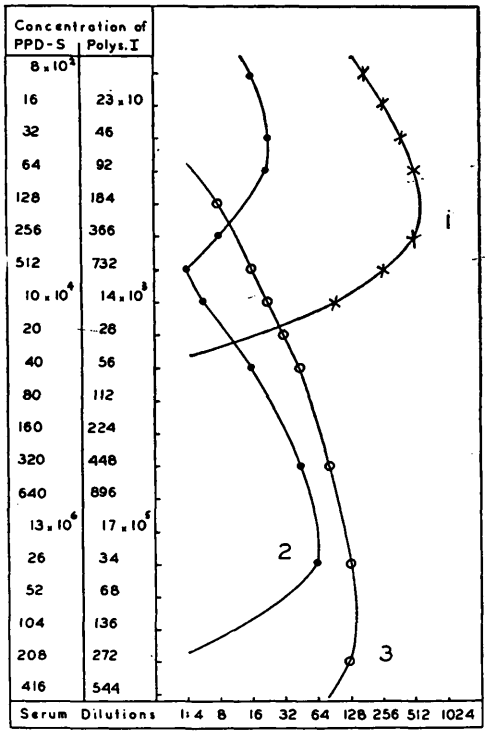

FIG. 1. Block titration diagrams of complement fixation at 4°C by: Curve 1 (×———×) PPD-S antigen-antibody system, curve 2 (●——●) PPD-S + polysaccharide antigen-antibody systems, curve 3 (○———○) polysaccharide antigen-antibody system. Areas encompassed by the curves include tubes showing no hemolysis.

antibodies to both protein and polysaccharide was lower than if the protein alone was used as the antigen, and in many cases it even became zero. This was true if the complement-fixation test was made in the usual way of incubating at 37°C. However, if the fixation took place at 4°C overnight, then it was obvious that both the protein-antigen-antibody system and the polysaccharide-antigen-antibody system fixed complement. Two titer maxima appeared at different antigen dilution ranges (Fig. 1, curve 2). Moreover, the sum of the amounts (indicated by serum titers) of the antibodies to protein, PPD-S, (upper part of curve 2) and to polysaccharide (lower part of curve 2) which fixed all the complement, was equal to the amount of antibody to protein which was required when the protein-antigen-antibody-system was present alone (curve 1).

Mechanism of binding of complement by protein and polysaccharide antigen-antibody systems. To study the difference between the binding of complement by the PPD-S-AAb and the Polys-AAb systems, the followng experiments were performed with the same serum; (1) BCG antiserum, PPD-S and complement were set up in a block titration arrangement in the usual manner, but the first antigen (PPD-S) was added in half the volume normally used. (The proper antigen dilution was in 0.05 ml instead of in 0.1 ml.) The tubes were held overnight at 4°C, then the respective serial dilutions of polysaccharide were added, also in half the usual antigen volume, in order to maintain a constant final volume. In all tubes a ratio of 1:7 of protein antigen to polysaccharide antigen was maintained. The dilutions of each antigen are shown at the left in Fig. 1 and 2. After a

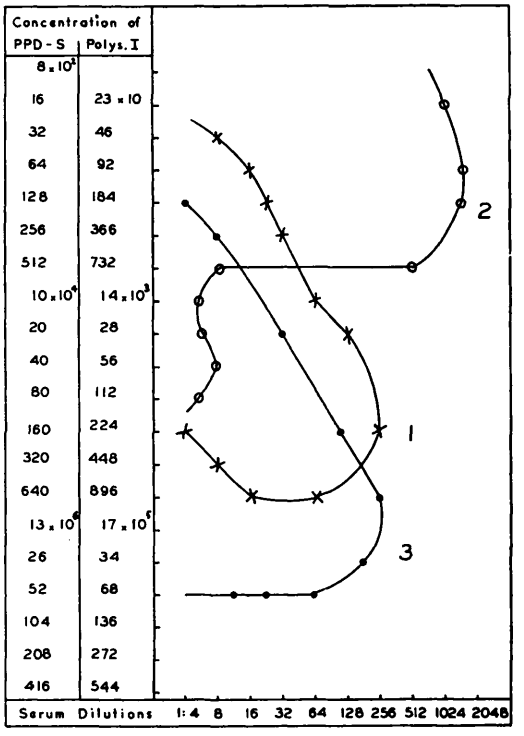

FIG. 2. Block titration diagrams of complement fixation at 4°C by: Curve 1 (×———×) polysaccharide antigen-antibody system alone, curve 2 (○———○) PPD-S added first and then polysaccharide, curve 3 (●———●) polysaccharide first and then PPD-S. Areas encompassed by the curves include tubes showing no hemolysis.

half hour incubation at 37°C the Rbc-Amb. system was added and the mixture was again incubated at 37°C. The resulting block titration diagram, (curve 2, Fig. 2) shows that considerable complement was fixed mainly in the dilution region where the PPD-S-AAb system was effective. A similar technic was used in which polysaccharide was first mixed with BCG antiserum and complement, and PPD-S was added on the following day. In this case (curve 3) considerable complement was also fixed, but this time mainly in the dilution where the Polys-AAb system was effective.

These 2 experiments showed that at 4°C both antigen-antibody systems were able to fix complement in a normal manner, and the system first exposed to the complement was the one effective in fixing it. Therefore, both systems would compete for the same complement if they are present simultaneously.

However, since the polysaccharide appeared to fix no complement at 37°C, but did cause a decrease in the titer given by PPD-S, the interpretation, in the light of the above experiments, might be as follows: The polysaccharide succeeded in holding its share of complement through the "first stage" of the reaction with an avidity which was sufficient to keep it from being fixed by the PPD-S-AAb system, but not sufficient at 37°C to hold it through the second and final stage of fixation, and so the complement was released from this system to the Amb-rbc. system when it was added. The final titer would, therefore, represent only that portion of the complement which had been held by the PPD-S-AAb system, and it would, therefore, appear that the polysaccharide had inhibited the complement fixation.

Summary. 1) High complement fixation titers with PPD-S were obtained with BCG antisera which showed high precipitating antibodies to PPD-S and to tuberculo-polysaccharide. 2) Polysaccharide fixed no complement even in these sera when incubation was carried out at 37°C. However, at 4°C fixation did take place. 3) When polysaccharide was mixed with PPD-S the complement-fixation titer was decreased from that obtained with the PPD-S alone, suggesting inhibition of fixation at 37°C. However, this decrease appeared to be due to fixation by polysaccharide in the first stage, followed by a probable release of the complement to the red-blood-cell-amboceptor system in the second stage.

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