COMPLEMENTARY NATURE OF RECEPTOR STRUCTURES OF BACTERIOPHAGES AND SENSITIVE BACTERIAL CELLS

V. I. Ioffe*, K. M. Rozental' and A. A. Totolyan

UDC 576.858.9.095.38

The hypothesis that interaction between bacteriophage and sensitive cell is determined by their complementary structure was investigated experimentally. Experiments were carried out with antiphage serum, whose antibodies were regarded as complementary in structure to phage (homologous to the surface of the microorganism), and with antibacterial serum, the specific globulins of which must be complementary to the surface antigens of the bacteria (homologous to phage). Specific interaction was established between the antibacterial and corresponding antiphage sera, supporting the hypothesis.

* * *

One of the microbiological processes involving two partners is interaction between phage and sensitive cells. It may be postulated that this interaction is based on the existence of mutually complementary receptor structures on the surfaces of the partners. Such a hypothesis can be tested by various methods, including serologic tests. If the hypothetical complementary structure is associated with the presence of definite determinant groups in the partners, the corresponding antisera must react with these groups and also with each other.

The object of this investigation was to test experimentally the hypothesis that interaction between phage and sensitive cell is determined by the complementary structure of receptors. Specific globulin of antiphage serum was regarded as complementary in structure to phage, and therefore structurally homologous to the surface of the cell with which the particular phage can react. On the other hand, the specific globulins of antibacterial serum must be structurally complementary to the surface antigens of the cell, and to some extent homologous to phage antigens.

EXPERIMENTAL METHOD

Experiments were carried out with sera against streptococcal, staphylococcal, and enterophages and with the corresponding antibacterial sera. To obtain antiphage sera temperate streptococcal phages 9 and 10, staphylococcal phages 3A, 55, and 77, and enterophages T4r and $\phi \times 174$ were used. Rabbits were immunized for 7-9 weeks (for streptococcal phages) and 2-3 weeks (for the rest). The phages were injected intravenously and subcutaneously twice a week in doses of 5 ml. All the antiphage sera in a dilution of 1:100 possessed high neutralizing ability against the homolgous phage for 5-15 min.

Antistreptococcal sera were prepared by immunizing rabbits with killed vaccines from strains of the group A:1H (a reference strain for phages) and strain 53. Sera were prepared in the same way against staphylococcal strains 3A and 5/2. To obtain antisera against Escherichia coli strains B and C, living vaccines were used. The titers of the antibacterial sera in the agglutination reaction varied from 1:400 to 1:1600.

Interaction of the test sera with one another was studied by the complement fixation reaction, using the method of back titration of complement. The antibacterial sera was diluted from 1:10 to 1:80, and the antiphage sera from 1:15 to 1:135. Mixtures were prepared from each dilution of both sera in equal volumes (0.4-0.6 ml of each). In the control mixtures, one of the sera (antiphage or antibacterial) was replaced by a heterologous serum. An equal volume of complement diluted 1:5-1:8 was added to each system. The tubes

*Corresponding Member, Academy of Medical Sciences of the USSR.

Department of Microbiology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 8, pp. 85-88, August 1968. Original article submitted October 30, 1965.

TABLE 1. Interaction between Antipneumococcal Sera and Serum Against C-Reactive Protein in the Complement Fixation Reaction

Type of serum and dilution	Antipneumococcal serum								Antistreptococcal serum			
	№ 44				№ 67							
	1:10	1:20	1:40	1:80	1:10	1:20	1:40	1:80	1:10	1:20	1:40	1:80
Serum against C-reactive protein												
1:15	+++	+++	+++	+++	++	++++	+++	++++	—	—	—	—
1:45	+++	+++	+++	++	++	++	++	+ ++	—	—	—	—
1:135	+++	±	±	±	+++	++	—	—	—	—	—	—
Serum against horse serum												
1:15	—	—	—	—	·	·	·	·	·	·	·	·
1:45	—	—	—	—	·	·	·	·	·	·	·	·
1:135	—	—	—	—	·	·	·	·	·	·	·	·

A dot (·) means no experiment performed.

TABLE 2. Interaction between Antibacterial and Antiphage Sera

Type of antibacterial serum	Strain used for preparing serum	Serum against enterophages			Serum against staphylococcal phages				Serum against streptococcal phages			
		T4r	T4r	φ×174	3A	3A	55	77	10	10	9	9
Against E. coli	B	+	+	—	—	—	—	—	—	—	—	±
Ditto	C	+	+	++	—	—	—	—	—	—	—	±
Antistaphylococcal	3A	—	—	—	++	++	+	+	—	—	—	±
Ditto	5/2	—	—	—	++	+++	++	+++	—	—	—	—
Antistreptococcal	1H	—	—	—	—	—	—	—	+	+	+++	++
Ditto	53	±	±	±	±	±	—	—	—	+	+	±

were left overnight at 4°. Next day the titer of free complement was determined, by withdrawing 0.3, 0.2, and 0.1 ml from each mixture respectively into three tubes, equalizing the volumes in the tubes by adding 0.1 and 0.2 ml physiological saline to the last two tubes, and adding 0.2 ml of hemolytic system to each. The results were read after complete hemolysis had occurred in the control at 37°. The following scheme was used: with complete inhibition of hemolysis in the tube with the smallest volume of test mixture (0.1 ml) and incomplete hemolysis in the next tube (0.2 ml) the reaction was assessed as +, with complete inhibition of hemolysis in the tube with two volumes of test mixture (0.2 ml) as ++, and with inhibition of hemolysis in the tube with three doses of test mixture (0.3 ml) as +++ or as ++++.

EXPERIMENTAL RESULTS

The possibility of interaction between two antisera, based on the complementary structure of the specific globulins, was first demonstrated in a model system of antipneumococcal serum and serum against C-reactive protein. Antistreptococcal serum and serum against C-reactive protein, and antipneumococcal serum and serum against horse protein acted as controls (Table 1).

A negative result was obtained in both control systems when tested in different relative proportions. Meanwhile, in the experimental system definite inhibition of hemolysis was observed, indicating interaction between the antipneumococcal serum and serum against C-reactive protein in the complement fixation test.

The results of the experiments to study interaction between antibacterial sera and homologous and heterologous antiphage sera are given in Table 2. They are aggregated results, taking into account the results of experiments with different dilutions of the test sera.

As Table 2 shows, definite positive results were observed only during interaction between sera in homologous systems, and they were almost completely absent in experiments with heterologous systems. Sera against staphylococci, for example, fixed complement in the presence of sera against staphylococcal phages and did not react with sera against enterophages and streptococcal phages. Of the antistreptococcal sera the best results were obtained with serum against strain 1H, to which the streptococcal phages were most adapted.

The results thus demonstrated a well marked specific interaction between antibacterial and corresponding antiphage sera. Hence it follows that thd reaction took place only on account of the specific globulins of the test sera, and that these globulins and the corresponding receptor groups of the partners possess a complementary structure.

The results described are evidence of species specificity. The problem of differentiation within the limits of the species requires further study.