Prevention of Staphylococcal Bacteriophage Activity by Antigen A Precipitins in Human Sera

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Antigen A precipitins in human sera prevented plaque formation and propagation of staphylococcal bacteriophages. Over 20% of total IgG was removed from human sera by absorption with staphylococci containing antigen A. The specific precipitating antibody in rabbit antisera formed lines of identity with antigen A precipitins in lower dilutions of human sera but formed lines of nonidentity with antigen A precipitins in higher dilutions of the same sera, suggesting both specific and non-specific antigen A precipitins in human sera. The specific and non-specific antigen A precipitins in human sera may prevent the *in vivo* activity of staphylococcal bacteriophages which have been demonstrated previously in animals whose sera do not contain either specific or non-specific antigen A precipitins.

In previous studies (14), human sera prevented propagation of several staphylococcal bacteriophages upon their host staphylococcal strains. Sera did not neutralize phages, interfere with adsorption of bacteriophages to their propagating strains, or inhibit growth of the propagation strains.

Since these studies were published, several previously unrecognized antigens of coagulase-positive staphylococci have been described. Human sera contain precipitins against many of these antigens (1, 2, 6-9, 12, 13, 15).

In the present studies, the presence of staphylococcal precipitins in human sera was correlated with the ability of sera to prevent propagation of staphylococcal bacteriophages. Sera were absorbed to remove precipitins formed with Jensen's protein antigen A, teichoic acids, and the type-specific antigens. Only sera from which antigen A precipitins were removed permitted propagation of staphylococcal bacteriophages.

In addition, these studies not only support previous reports (4) that antigen A nonspecifically precipitates large quantities of IgG from human sera but also suggest that part of the precipitates are specific antigen-antibody reactions.

MATERIALS AND METHODS

The methods used for propagation of staphylococcal bacteriophages, for plaque counts, and for studying the effect of sera on bacteriophage propagation have been published previously (14, 16).

Antibodies against Jensen's protein antigen A,

staphylococcal teichoic acids, and type-specific antigens were detected in Ouchterlony plates (12) or gel-diffusion tubes (8). The supernatant fluids obtained after ultrasonic treatment of a 20% cell suspension of Cowan I staphylococci were used as antigens in Ouchterlony plates (12). These supernatant liquids contained protein antigen A, teichoic acids, and type-specific antigens. The antigen in gel-diffusion tubes was polysaccharide, separated by continuous-flow electrophoresis from Cowan I supernatant fluids (8). The final antigen concentration was equivalent to 1.5 μ g of teichoic acid per ml. Gel-diffusion tubes detected the low concentrations of teichoic acid antibody in normal human sera which cannot be detected by Ouchterlony plates.

Sera were absorbed by incubation at 37 C for 1 hr and at 4 C overnight with one-third to one-fifth the volume of packed staphylococcal cells. Staphylococci were harvested from overnight cultures on Trypticase Soy plates and were washed once with normal saline before absorption of sera.

Staphylococcal strains Wood 46 and Cowan I were obtained from Jay Cohen, National Communicable Diseases Center, Atlanta, Ga. Bacteriophages and their propagating strains were obtained from John Blair, Hospital for Joint Diseases, New York, N.Y.

Concentrations of IgG, IgA, and IgM were measured by radial-diffusion methods (3), using Immuno-plates from Hyland Laboratories, Los Angeles, Calif.

RESULTS

Human sera consistently prevented propagation of bacteriophages 3b, 47, and 80 on their propagating strains. Inhibition of phage multiplication could be demonstrated either by the

failure of an increase in plaque counts of staphylococcal bacteriophages incubated with propagating strains and human sera in liquid media (Fig. 1) or by the failure of plaque formation when known amounts of plaque-forming particles were added to semisolid agar containing propagating strains and different concentrations of human sera (Fig. 2).

The staphylococci used for absorption contained different staphylococcal antigens; thus it was possible to selectively remove precipitins against teichoic acids, precipitins against teichoic acids and antigen A, or precipitins against teichoic

acids, antigen A, and type-specific antigens (Table 1).

After absorption with propagating strains 3b, 47, or 80, all detectable precipitins were removed and sera no longer prevented propagation of bacteriophages 3b, 47, or 80. Absorption with Cowan I staphylococci removed teichoic acid precipitins detected in Preer tubes and antigen A precipitins detected in Ouchterlony plates; sera absorbed with Cowan I staphylococci did not prevent bacteriophage multiplication (Fig. 3). However, absorption with Wood 46 staphylococci removed teichoic acid precipitins but not antigen A precipitins; sera absorbed with Wood 46 staphylococci still prevented phage multiplication.

No antigen A precipitins were detected in sera from unimmunized rabbits, but, after immunization with several different strains of coagulase-positive staphylococci, antigen A precipitins were demonstrated in Ouchterlony plates at maximal

FIG. 1. Plaque counts after incubation of bacteriophages 3b, 47, or 80 with their propagating strains in liquid media with different concentrations of a human serum. Phage inoculum 10^2 to 10^3 plaques per ml.

FIG. 2. Plaque counts of bacteriophages 3b, 47, or 80 in semisolid agar containing different concentrations of a human serum.

TABLE 1. *Antigens of staphylococci*^a

Strain	Type-specific
3b	$a_5bc_p hk$
80	$a_5bc_p hk$
47	$a_5bc_p n$
Cowan I	a_2k_2m
Wood 46	i_2

^a All strains contained α - and β -N-acetylglucosamine forms of teichoic acid, and all strains except Wood 46 contained antigen A.

FIG. 3. Inhibition of propagation of phage 3b after overnight incubation by unabsorbed serum or by the same sera absorbed with Wood 46 staphylococci. No inhibition of propagation occurred with sera absorbed with Cowan I staphylococci or with 3b staphylococci. Initial plaque counts, 10^2 /ml.

dilutions of sera ranging from 1:16 to 1:256. The antigen A precipitins in rabbit antisera formed lines of identity with antigen A precipitins in the lower dilutions of human sera but formed lines of nonidentity with antigen A precipitins in higher dilutions of the same sera (Fig. 4a, b). Dilutions of rabbit antisera did not form lines of "nonidentity" with undiluted rabbit antisera (Fig. 5).

In sera from three subjects, the concentrations

of IgG after absorption with Cowan I staphylococci were 21, 26, and 45% lower than the concentrations after absorption with identical volumes of Wood 46 staphylococci (Table 2). Antigen A precipitins were detected at a 1:16 dilution of all three sera before absorption but were not detectable in undiluted sera after absorption with Cowan I staphylococci. After absorption with Wood 46 staphylococci, antigen A precipitins were still detected at 1:16 dilutions of sera. There were no significant differences in the concentrations of IgA or IgM in sera after absorption with

FIG. 4a. Lines of identity of antigen A precipitins in an undiluted rabbit antiserum (R) and in undiluted, 1:2, and 1:4 dilutions of a human serum. C: supernatant fluid of Cowan I staphylococci after ultrasonic vibration.

FIG. 4b. Lines of nonidentity of antigen A precipitins in an undiluted rabbit antiserum (R) and in 1:16 and 1:32 dilutions of a human serum. C: supernatant fluid of Cowan I staphylococci after ultrasonic vibration.

FIG. 5a. Lines of identity of antigen A precipitins in an undiluted rabbit antiserum (R) and in undiluted, 1:2, and 1:4 dilutions of the same antiserum. C: supernatant fluid of Cowan I staphylococci after ultrasonic vibration.

FIG. 5b. Lines of identity of antigen A precipitins in an undiluted rabbit antiserum (R) and in 1:16 and 1:32 dilutions of the same antiserum. C: supernatant fluid of Cowan I staphylococci after ultrasonic vibration.

TABLE 2. *Comparison of immunoglobulin content of three human sera after absorption with Wood 46 or Cowan I staphylococci*

Subject	Absorbed with	Amt of immunoglobulin (mg/ml)		
		IgM	IgA	IgG
1	Wood 46	.21	1.8	6.1
	Cowan I	.19	1.4	3.4
2	Wood 46	.86	1.7	8.8
	Cowan I	.81	1.7	6.9
3	Wood 46	.52	3.4	8.8
	Cowan I	.50	3.4	6.5

Cowan I staphylococci as compared to sera absorbed with Wood 46 staphylococci.

DISCUSSION

Jensen first demonstrated that precipitins against antigen A were detectable in all human sera tested in Ouchterlony plates (6). He also showed this antigen was present in almost all coagulase-positive staphylococci but not in coagulase-negative strains. In subsequent studies, it was found that the concentrations of precipitins did not increase after infections with coagulase-positive staphylococci (7, 12).

Several other somatic antigens of coagulase-positive staphylococci are also detectable by precipitin methods, including the group teichoic acids and several type-specific antigens. Antibodies against these antigens are rarely detectable with unconcentrated sera from patients without infections in Ouchterlony plates, but they may be demonstrated by quantitative precipitin methods (15), by gel-diffusion tubes (7, 8), or by using concentrated human sera in Ouchterlony plates (1). During the course of staphylococcal diseases, the concentrations of antibodies increase, and specific precipitin lines are frequently detectable with unconcentrated sera in Ouchterlony plates (1, 8, 13).

Antibodies against any of these antigens might have been responsible for the previous observations that human sera prevented propagation of several staphylococcal bacteriophages on their propagating strains. In this study, it was demonstrated by selective absorption of sera that antigen A precipitins prevented phage multiplication but teichoic acid or type-specific precipitins did not.

Since human sera which prevented propagation of staphylococcal bacteriophages did not prevent adsorption of phages and did not neutralize phages (14), the reaction between human sera and antigen A presumably prevents penetration

of phage particles by mechanisms as yet unexplained.

Forsgren and Sjoquist (4) reported that the reaction between antigen A and human γ -globulin is not a true antigen-antibody reaction, based on the precipitation of 45% of a pooled normal IgG preparation by antigen A and the reaction of antigen A with myeloma IgG, H chains from both normal and myeloma IgG, and the Fc fragment of normal human IgG. In the present studies, excessively large quantities of IgG were removed after absorption of three sera with staphylococci to remove antigen A precipitins; our data are consistent with Forsgren and Sjoquist's conclusions of nonspecific precipitation. However, precipitins in rabbit sera were detected only after immunization with staphylococci. In addition, the precipitins present in rabbit antisera formed lines of identity with antigen A precipitins in lower dilutions of human sera, suggesting that a portion of the precipitins in human sera are specific antibody. The additional observations that lines of nonidentity were formed between rabbit antisera and higher dilutions of human sera suggest that two types of reactions occur between antigen A and human sera: (i) a specific antigen-antibody reaction, and (ii) a nonspecific precipitation of IgG by antigen A.

A considerable degree of heterogeneity of IgG is suggested by the reaction with antigen A. The concentrations of antigen A precipitins after absorption with staphylococci were reduced to less than one-sixteenth that before absorption, but the concentration of IgG was reduced only 21 to 45%. These observations are also consistent with those of Forsgren and Sjoquist that 45% of IgG was precipitated by antigen A, but 55% of IgG was not precipitated.

Staphylococcal bacteriophages transfer drug resistance in vitro and in infected mice (5, 10, 11). Unimmunized animals used in these studies usually do not possess either specific antibodies against antigen A or nonspecific precipitins. In human sera with high titers of antigen A precipitins, lytic activity of phages is prevented as shown in these investigations. Further studies are required to document that transduction can occur in the presence of specific antibodies or nonspecific precipitins before studies in animals can be applied to man.

The prevalence of precipitins against antigen A in human sera suggests that this is one means by which staphylococcal bacteriophage multiplication, transduction, and lysis of staphylococci may be prevented. In addition, specific phage neutralizing antibodies may occur after repeated exposure to specific staphylococcal bacteriophages.

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LITERATURE CITED

1. Daugharty, H., R. R. Martin, and A. White. 1967. Antibodies against staphylococcal teichoic acids and type-specific antigens in man. *J. Immunol.* **98**:1123-1129.
2. Daugharty, H., M. Shriver, and A. White. 1967. Serological activity of staphylococcal polysaccharide. *J. Bacteriol.* **93**:131-134.
3. Fahey, J., and M. Lawrence. 1963. Quantitative determination of 6.6 S γ -globulins, β_2 A-globulins and γ_1 -macroglobulins in human serum. *J. Immunol.* **91**:597-603.
4. Forsgren, A., and J. Sjoquist. 1966. "Protein A" from *S. aureus*. I. Pseudo-immune reaction with human gamma-globulin. *J. Immunol.* **97**:822-827.
5. Jarolmen, H., A. Bondi, and R. L. Crowell. 1965. Transduction of *Staphylococcus aureus* to tetracycline resistance in vivo. *J. Bacteriol.* **89**:1286-1290.
6. Jensen, K. 1958. A normal occurring staphylococcus antibody in human serum. *Acta Pathol. Microbiol. Scand.* **44**:421-428.
7. Martin, R., J. Crowder, and A. White. 1967. Human reactions to staphylococcal antigens. *J. Immunol.* **99**:269-275.
8. Martin, R. R., H. Daugharty, and A. White. 1966. Staphylococcal antibodies and hypersensitivity to teichoic acids in man. *Antimicrobial Agents and Chemotherapy*—1965, p. 91-96.
9. Martin, R., H. Daugharty, and A. White. Immunoglobulins of specific staphylococcal antibodies. *Pediatrics*, *in press*.
10. Mitsuhashi, S., H. Oshima, U. Kawaharada, and H. Hashimoto. 1965. Drug resistance of staphylococci. I. Transduction of tetracycline resistance with phage lysates obtained from multiple resistant staphylococci. *J. Bacteriol.* **89**:967-976.
11. Novick, R., and S. Morse. 1967. In vivo transmission of drug resistance factors between strains of *Staphylococcus aureus*. *J. Exptl. Med.* **125**:45-59.
12. Rinker, G., and A. White. 1965. Quantitative studies of antibodies against Jensen's antigen A in human sera. *Antimicrobial Agents and Chemotherapy*—1964, p. 672-676.
13. Singleton, L., G. Ross, and J. Kohn. 1964. Staphylococcal teichoic acid antibody in the sera of patients with burns. *Nature* **203**:1173-1174.
14. Smith, J., and A. White. 1960. Effect of human sera on the propagation of staphylococcal bacteriophages. *J. Bacteriol.* **80**:741-743.
15. Torii, M., E. Kabat, and A. Bezer. 1964. Separation of teichoic acid of staphylococcus aureus into two immunologically distinct specific polysaccharides with α and β -N-acetylglucosaminyl linkages respectively. *J. Exptl. Med.* **120**:13-29.
16. White, A., F. Foster, and V. Knight. 1959. Propagation of staphylococcal phages in liquid media. *Antibiot. Chemotherapy* **9**:81-86.