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Prevention of Bacteriophage Adsorption to *Staphylococcus aureus* by Immunoglobulin G

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Normal human and rabbit sera when incubated with *Staphylococcus aureus* inhibit the adsorption of bacteriophages. The bacteriophage adsorption was also inhibited by separated normal immunoglobulin M (IgM), $F(ab')_2$, and Fab-fragments of IgG. No inhibition was obtained with myeloma IgG or Fc-fragments of normal human and rabbit IgG. The results indicate that the serum inhibition of bacteriophage adsorption to *S. aureus* is not due to a binding of IgG to protein A on the surface of *S. aureus*.

Protein A has been shown to be associated with the cell surface of most *Staphylococcus aureus* strains (9, 10, 14, 19, 22). In a previous report from this laboratory cell-bound protein A was demonstrated to inhibit the adsorption of bacteriophages to *S. aureus* (16). However, no inhibiting effect of soluble protein A on adsorption of bacteriophages was obtained. It was concluded that protein A as a surface component rendered the bacteria more resistant to adsorption of staphylococcal typing phages by masking the phage receptor sites on the cell wall mucopeptide and teichoic acid (2, 3, 16, 18).

It has been reported that protein A precipitins in normal serum prevent phage propagation (13). Protein A is characterized by its reaction with the Fc-part of immunoglobulin G (IgG) in normal serum. In the present study the effect of IgG on phage adsorption is investigated. The paper presents evidence of an inhibiting effect of normal human and rabbit serum on adsorption of phages to *S. aureus*. The active part of IgG is located in the Fab-fragment.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* Cowan I and 8325 N were used. Two protein A-deficient mutants of Cowan I (NG 274 and NG 316) and a protein A-rich mutant (NG 11) and a protein A-negative mutant (NG 143) of 8325 N were isolated after treatment with nitrosoguanidine (NG) and have been described earlier (6, 16).

Phages. Staphylococcal bacteriophage 80 from the standard typing set and phage 80α obtained from R. P. Movick were used in this study.

Adsorption of phages in the presence of serum and serum fractions. Bacteria were grown in Trypticase soy broth (TSB) and were harvested at the beginning of the log phase. To 0.5 ml of serial twofold dilutions of serum or serum fractions in TSB 0.25 ml of the bacterial suspension (10^8 to 2×10^8 colony forming units [CFU]/ml) was added. After 10 min of preincubation at 37 C, 0.25 ml of phage suspension was added to give a multiplicity of infection (MOI) of 0.1 to 1.0, and calcium was added to a final concentration of 0.004 M. Free phages were measured after adsorption for 10 min (16).

Sera. Anti-staphylococcus serum was obtained by weekly serial intravenous injections of heat-killed bacteria in rabbits by methods earlier described (5).

Preparation of human IgM. Normal human IgM was separated by chromatography on a Sephadex G-200 column and further purified by chromatography on a DEAE-cellulose column as earlier described (5). In immunoelectrophoresis, a single line was detected against rabbit anti-human plasma protein serum and rabbit anti-human IgM serum (Behring Werke AG).

Preparation of human and rabbit IgG. Ammonium sulfate precipitation followed by chromatography on DEAE-Sephadex or -cellulose columns was used for separation of pooled normal human and rabbit IgG and human myeloma IgG as earlier described (5, 7). All immunoglobulin preparations were dialyzed against phosphate buffered saline (PBS) before use in phage adsorption experiments.

$F(ab')_2$-fragments of IgG. Rabbit IgG was digested according to Nisonoff (15) as described earlier (8). The $F(ab')_2$-fragments were further purified by chromatography on a Sephadex G-150 column equilibrated with 0.02 M sodium phosphate buffer, pH 7.4, containing 0.3 M NaCl (1). In immunoelectrophoresis a single line was formed against donkey anti-rabbit plasma protein serum and goat anti-rabbit γ-globulin serum (Behring Werke AG).

Fab and Fc-fragments of IgG. Rabbit IgG was digested with papain (Worthington Biochemical Corp.) according to Porter (17) by using 1 mg of enzyme per 100 mg of protein. By chromatography on a carboxymethyl (CM)-cellulose column at pH 5.5 Fab and Fc peaks were separated and identified by immunoelectrophoresis against anti-rabbit γ-globulin serum.

Human IgG was digested with papain (1 mg of enzyme per 100 mg of protein) according to Porter (17). After incubation at 37 C for 30 min iodoacetamide was added to a final concentration of 0.05 M, the pH was adjusted to 8.0, and the digestion mixture was incubated at room temperature for 30 min. After dialysis against 0.05 M sodium phosphate, pH 8.0, and 0.0002 M EDTA Fab and Fc-fragments were separated on a DEAE-Sephadex column equilibrated with the same buffer. The Fab-fraction was first eluated with 0.05 M sodium phosphate, pH 8.0, after which the Fc-fraction was eluated with a gradient from 0.05 M sodium phosphate, pH 8.0, to 0.2 M sodium phosphate, pH 8.0. The immunoglobulin fragments were characterized by immunoelectrophoresis against rabbit antihuman IgG serum, rabbit anti-IgG/Fab-serum and rabbit anti-IgG/Fc-serum (Behring Werke AG).

RESULTS

Phage adsorption in the presence of human and rabbit serum. The effect of normal human serum on the quantity of phage adsorbed to either *S. aureus* Cowan I or a protein A-deficient mutant, NG 316, is shown in Fig. 1. When a low concentration of serum was present the mutant NG 316 adsorbed the phages more effectively than the protein A-rich Cowan I. However, a higher concentration of serum (25%) markedly inhibited adsorption of phage 80 by mutant NG 316 so that little difference could be detected between this protein A-deficient mutant and the protein A-rich Cowan I. In all experiments done serum also slightly decreased the adsorption of phages to Cowan I. Inhibition of phage adsorption by serum was consistent in all experiments. When NG 316 was preincubated with serum for 10 min at 37 C and then washed in TSB the same inhibition of phage adsorption was obtained as with serum present during adsorption. In other control experiments serum was adsorbed with heat-killed NG 316 for 1 h at 37 C and then used in phage adsorption experiments. Serum adsorbed with NG 316 had no inhibitory effect on phage adsorption. In another set of experiments phages were preincubated with serum or TSB for 10 min at 37 C and then washed in TSB by centrifugation for 2 h at 120,000 × *g*. No difference in infectivity and in adsorption to *S. aureus* could be detected between phages preincubated with serum or TSB. These results show that the serum activity is directed against the bacteria and not the phage.

Serum when incubated with *S. aureus* 8325 N and a protein A-rich mutant of 8325 N (NG 11) also inhibited the phage adsorption to those strains. The inhibitory effect was more pronounced on the protein A-poor wild-type 8325 N than the protein A-rich mutant NG 11. A similar inhibitory effect on bacteriophage adsorption to *S. aureus* was obtained with pooled rabbit serum as with pooled human serum.

Phage adsorption in the presence of IgG and IgM. Figure 2 shows a representative phage

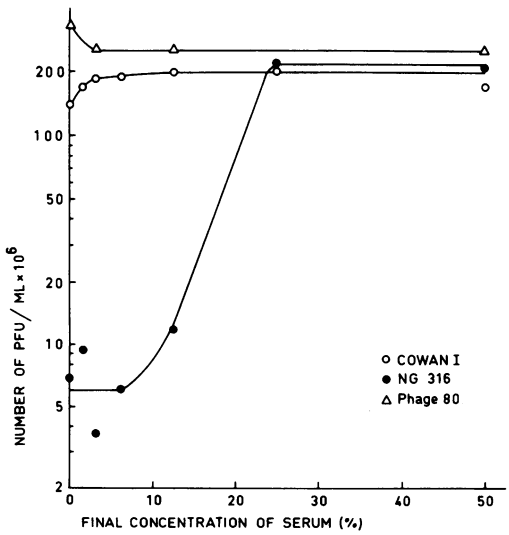

FIG. 1. *Inactivation of phage 80 after 10 min of incubation with S. aureus Cowan I and with a protein A-deficient mutant (NG 316) in the presence of normal human serum in different concentrations. MOI is 0.1.*

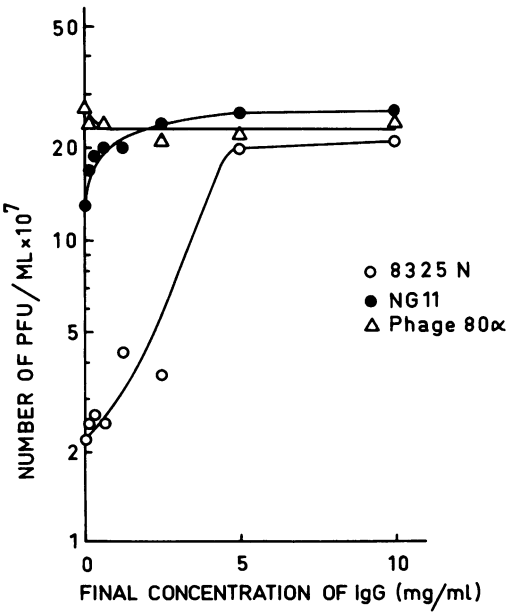

FIG. 2. *Effect of human IgG on adsorption of phage 80 α to S. aureus 8325 N and a protein A-rich mutant of 8325 N (NG 11) during 10 min of incubation. MOI is 0.1.*

adsorption experiment with *S. aureus* 8325 N and phage 80α in the presence of normal human IgG. As seen in the figure, normal human IgG had a pronounced inhibiting effect on phage adsorption to the protein A-poor wild-type 8325 N. Identical results were obtained when rabbit IgG was used instead of human IgG. However, two different preparations of human myeloma IgG, subgroup I, did not show any inhibiting effect on adsorption of phage to either Cowan I, 8325 N or derived protein A mutants. Purified normal human IgM had a similar inhibition on phage adsorption to *S. aureus* as normal IgG. The effect of separated IgG and IgM corresponded to the total inhibiting effect of unfractionated serum.

These results indicate that the serum inhibition of bacteriophage adsorption to *S. aureus* is not due to common structures of IgG or IgM but to specific sites in the F(ab')-fragments of the IgG- or IgM-molecules. Further support for this concept was obtained by using IgG from rabbit antiserum against *S. aureus* NG 316 in phage adsorption experiments. There was at least a 10-fold stronger inhibiting effect with anti-NG 316-IgG compared with normal rabbit IgG.

Effect of IgG-fragments on phage adsorption. As seen in Fig. 3, purified $F(ab')_2$-fragments from rabbit IgG inhibit the adsorption of phage 80 to Cowan I and protein A-deficient mutant NG 316 in the same way as intact IgG. The $F(ab')_2$-fragments had the most pronounced effect on mutant NG 316. At a concentration of 6 mg of $F(ab')_2$-fragments per ml little difference in adsorption was detected between the protein A-deficient mutant and the protein A-rich wild type. The same inhibiting effect on adsorption of phages to *S. aureus* was obtained when Fab-fragments of human IgG was included in the adsorption system instead of $F(ab')_2$-fragments of rabbit IgG (Fig. 4). The Fc-fragments of human IgG had no effect on phage adsorption to *S. aureus*. As seen in Fig. 4, there is more than one hundred-fold difference between the wild-type Cowan I and the protein A-deficient mutant NG 316 in number of unattached phages independent of the concentration of Fc-fragments. Rabbit Fab- and Fc-fragments of IgG behaved similarly to human Fab- and Fc-fragments.

DISCUSSION

In a report by Smith and White (20) it was demonstrated that human serum prevented propagation of staphylococcal phages upon their host staphylococcal strains. In a subsequent study (13) the presence of staphylococcal precipitins in normal human serum was correlated with the ability of serum to prevent propagation of staphylococcal bacteriophages. Protein A (antigen A) precipitins in serum were shown to prevent plaque formation and propagation of staphylococcal bacteriophages. Sera

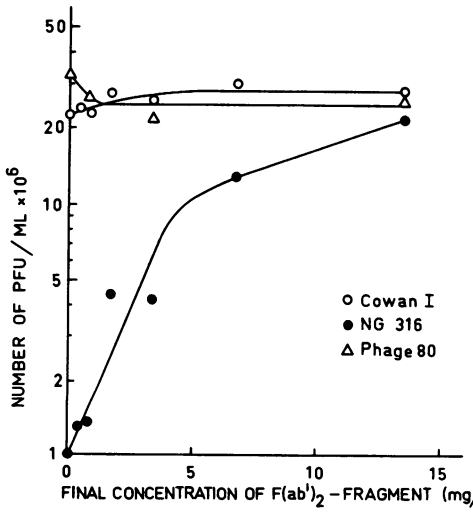

FIG. 3. *Titer of phage 80 after 10 min of incubation with S. aureus Cowan I and with a protein A-deficient mutant (NG 316) in the presence of $F(ab')_2$-fragments of IgG from nonimmunized rabbits. MOI is 0.1.*

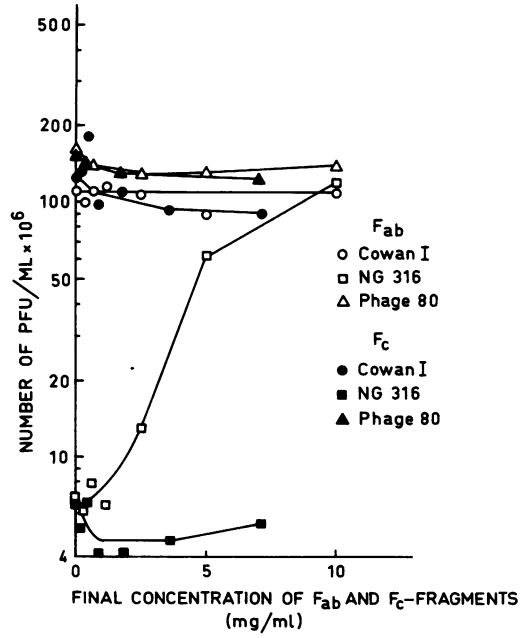

FIG. 4. *Inactivation of phage 80 after 10 min of incubation with S. aureus Cowan I and with a protein A-deficient mutant (NG 316) in the presence of Fab- and Fc-fragments of normal human IgG. MOI is 0.1.*

were adsorbed with staphylococci to remove precipitins formed with protein A, teichoic acids, and the type-specific antigens of *S. aureus*. Only sera from which protein A precipitins were removed permitted propagation of staphylococcal bacteriophages. However, it is now well known that protein A reacts with the Fc-part of mammalian IgG (7). It has been shown that adsorption with *S. aureus* can remove 90 to 100% of IgG in normal rabbit serum (5, 11). Thus it is likely that in the experiments with adsorption of serum with *S. aureus* by Martin and White (13) specific antibodies to other antigens of *S. aureus* were removed due to the protein A-Fc reaction.

The action of serum on propagation of staphylococcal phages has not been explained. Coagulase-positive staphylococci, such as the strains used in the present investigation, are not inhibited by serum as measured by colony counts (4). No direct effect of serum on phages could be demonstrated by Smith and White (20) or in this report. However, in this study a pronounced inhibiting effect of IgG on adsorption of phages to staphylococci is found. The experiments demonstrate an inhibition of phage adsorption by the Fab-part of IgG. No inhibiting effect was obtained with purified Fc-fragments or myeloma IgG known to react with protein A. It is possible that the inhibiting effect is caused by antibodies to teichoic acid present in normal serum (12). The antigenic determinant in teichoic acid is *N*-acetyl-glucosamine (21) which has been reported to be required for bacteriophage adsorption (2, 3). The true biological importance of the inhibition of phage adsorption by serum and IgG is not clear, but it may be one means by which staphylococcal bacteriophage multiplication, transduction, and lysis of staphylococci is prevented.

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