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Effect of Antibody Concentration on Opsonic Requirements for Phagocytosis *in Vitro* of *Streptococcus pneumoniae* Types 7 and 19 (41543)

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Abstract. We investigated the effect of the concentration of type-specific antibody to pneumococcal polysaccharide (PPS) on opsonic requirements for phagocytosis *in vitro* of *Streptococcus pneumonia* types 7 and 19. We measured the uptake by human neutrophils of radiolabeled *S. pneumoniae* opsonized with either complement-intact, complement-depleted (by heat inactivation), or alternative complement pathway-activated (by magnesium dichloride–ethylene glycol tetraacetic acid (MgEGTA) chelation) immune sera with varying concentrations of antibody from individuals immunized with polyvalent PPS vaccine. Increased opsonization was found with increasing concentrations of type-specific antibody in the sera. Higher concentrations of antibody were required to opsonize type 7 than type 19 bacteria, both in the presence and absence of complement activity. Type 19 bacteria were more efficiently opsonized via the alternative complement pathway than type 7. For both types, antibody and the alternative complement pathway provided most of the opsonic activity in sera with lower concentrations of type-specific antibody. At high antibody concentrations, effective opsonization occurred in heat-activated sera, indicating the requirement for complement could be overcome with sufficient amounts of antibody alone.

In spite of antimicrobial treatment, infections with *Streptococcus pneumoniae* remain a major cause of morbidity and mortality (1). Because of their polysaccharide capsules, most pneumococci are resistant to phagocytosis unless serum opsonins make them susceptible to ingestion by phagocytic cells (2). Previous studies have demonstrated that the critical opsonins for *S. pneumoniae* are type-specific antibodies to pneumococcal polysaccharide (PPS), and components of the classical and alternative complement systems (3–9). We have used a functional phagocytic assay which measures the uptake of radiolabeled *S. pneumoniae* (7, 8) to determine the effect of PPS antibody concentration on opsonization requirements for phagocytosis of *S. pneumoniae* types 7 and 19.

Materials and Methods. *Sera.* Six normal adult volunteers were immunized with 14-valent PPS vaccine (Pneumovax-MSD). Two weeks following immunization, serum was obtained and stored in aliquots at −70°. Antibody concentrations were measured by radioimmunoassay (10) (performed by Dr. G. Schiffman, Downstate Medical Center, Brooklyn, N.Y.) and by enzyme immunoassay (11) in our laboratory. Intact complement activity of the frozen aliquots was confirmed by measuring normal hemolytic complement (CH50) of ≥25 units in sera from each individual (performed by University of California, San Francisco clinical laboratories). Sera from these different individuals were utilized as either complement-intact immune serum (antibody plus classical and alternative complement pathways), serum heat-inactivated at 56° for 60 min (antibody alone), or serum chelated with a final concentration of 10 m*M* ethylene glycol tetraacetic acid with 2.5 m*M* magnesium dichloride (MgEGTA) (antibody plus alternative complement pathway) (5–9, 12). Loss of complement activity following heat inactivation was confirmed by CH50 assay. No significant differences in uptake were found between sera heat-inactivated at 56° for 30 min, 56° for 60 min, or 53° for 90 min (data not shown). Preservation of alternative complement pathway activity in MgEGTA-chelated serum was confirmed by demonstration of activated Factor B by immunoelectrophoresis following incubation of serum with opsonized zymosan (12).

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Bacteria. Types 7F and 19F *S. pneumoniae* were chosen for study because they are among the types which cause significant disease (13), and because previous studies have shown they differ in their opsonic requirements (5, 8, 9). Lyophilized type 7F and 19F bacteria were obtained from American Type Culture Collection (Rockville, Md.). They were initially grown in brain heart infusion overnight at 37° in a humidified incubator with 5% CO_2. After purity checks (plating on blood agar, and Quelleng capsular swelling reaction with type-specific anti-PPS antiserum), the bacteria were frozen in aliquots at −70° in Hanks balanced salt solution with 10% glycerol and 10% bovine serum albumin. The bacteria were mouse passaged every 2 months to maintain virulence.

To radiolabel the bacteria, they were grown overnight in a synthetic medium lacking thymidine (14) with added tritiated methylthymidine (specific activity 20 Ci/mmol, New England Nuclear Corp., Boston, Mass.). The radiolabeled bacteria were washed three times with calcium and magnesium-free phosphate-buffered saline (PBS) with centrifugation at 1600*g* for 15 min, and adjusted spectrophotometrically to approximately 2×10^8 bacteria/ml in PBS (optical density of 0.180 at 620 nm, confirmed by microscopic counts of stained, diluted bacteria).

Opsonization. For most experiments, 100 μl of either complement-intact or heat-inactivated immune serum, 100 μl of bacteria (approximately 2×10^7), and 50 μl of PBS were added to individual polypropylene vials (Biovials, Beckman Instruments, Irvine, Calif.). For experiments with MgEGTA-chelated serum, 100 μl of immune serum was incubated at ambient temperature for 10 min with 25 μl of 50 m*M* EGTA–12.5 m*M* $MgCl_2$ (final EGTA concentration 10 m*M*, $MgCl_2$ concentration 2.5 m*M*) followed by addition of 100 μl of bacteria and 25 μl of PBS. The total volume of the opsonic mixture was 250 μl, and the percentage serum in it 40%. In some experiments, the percentage serum concentration was changed by varying the amount of serum added and adjusting the PBS added appropriately. The bacteria were opsonized in the individual vials for 60 min on a rotator at 37° in an incubator with 5% CO_2. No significant differences were found in uptake of bacteria opsonized with immune sera for 15, 30, or 60 min (data not shown); 60 min was chosen to allow adequate time for alternative complement pathway activation (5). Then the bacteria were washed by adding 3 ml of PBS to each vial, and the vials centrifuged at 1600*g* for 15 min. The supernatants were discarded and the washed, opsonized bacteria resuspended in 100 μl of Hanks balanced salt solution with 0.1% gelatin (gel–HBSS).

Neutrophils. Blood from a single healthy individual was drawn into a heparinized syringe (10 units/ml), and one-third volume of 4.5% dextran in PBS added. The blood was allowed to sediment for 30 min and then the neutrophil-rich plasma was drawn off. A cell pellet was obtained by centrifuging the plasma at 120*g* for 10 min. The red cells in the pellet were lysed by adding 5 ml of 0.8% ammonium chloride for 5 min. The remaining cells, over 80% neutrophils, were washed three times with PBS with 0.2% glucose and 1 unit heparin/ml, and resuspended to a concentration of 4×10^6/ml in gel–HBSS.

Phagocytosis. Five hundred microliters of the neutrophil mixture (2×10^6 cells) was added to the washed, opsonized bacteria (2×10^7 bacteria) in each vial. The total volume of the phagocytic mixture was 600 μl, and the bacteria:neutrophil ratio was approximately 10:1. Preliminary experiments demonstrated this ratio to be optimal for demonstrating opsonic differences (data not shown). Each sample was run in quadruplicate so that leukocyte-associated and total radioactive counts could be determined in duplicate.

To allow phagocytosis, the vials were rotated at 37° for 30 min in an incubator with 5% CO_2. Experiments showed no significant further increase in uptakes at 45 or 60 min (data not shown). Phagocytosis was stopped by adding 3 ml of iced PBS. To determine leukocyte-associated counts, two of the four vials were centrifuged at 120*g* for 5 min at 4° and the supernatant discarded. The cell pellet containing neutrophils and associated bacteria was washed an additional two times with iced PBS to remove non-leukocyte-associated bacteria. Phase contrast microscopy demonstrated most of the leukocyte-associated bacteria to be ingested rather than merely adherent; this was the case when the bacteria

were opsonized with either complement-intact or heat-inactivated immune sera. To obtain total counts, the other two vials were centrifuged once at 1600*g* for 15 min which pelleted all bacteria and leukocytes.

The pellets from all vials were each resuspended in 200 μl distilled water and vortexed to lyse the cells; 3 ml scintillation fluid (Aquasol, New England Nuclear Corp., Boston, Mass.) was then added. The samples were counted with a Beckman LS 7000 scintillation counter. The bacterial uptake was calculated as

$$\% \text{ uptake} = \frac{\text{cpm in } 120 \times \text{g pellet (leukocyte-associated counts)}}{\text{cpm in } 1600 \times \text{g pellet (total counts)}} \times 100.$$

Total counts were always greater than 5000 cpm.

Data analysis. The mean counts from duplicate vials on a single day were used to calculate the bacterial uptake produced by an opsonic source (immune, heat-inactivated, or MgEGTA-chelated sera from a single individual). For most experiments, sera were assayed on at least 3 different days, and means and standard deviation determined from the uptake values for each day. The mean coefficient of variation for the assay was 22.4% between different days. An unpaired *t* test was used to calculate differences between mean uptake values.

Results. *Sera.* Antibody concentrations in postimmunization sera, as determined by radioimmunossay and enzyme immunoassay, are listed in Table I.

Effect of antibody concentration on bacterial uptake. The degree to which the concentration of type-specific PPS antibodies affects opsonization of *S. pneumoniae* types 7F and 19F in complement-intact immune serum was assessed in two ways. First, varying percentages of immune serum from a single individual were used to opsonize the bacteria. Because the opsonic mixture volume was constant, diluting the serum resulted in varying concentrations of antibody available for opsonization, although complement components were also diluted.

TABLE I. ANTIBODY CONCENTRATIONS IN POSTIMMUNIZATION SERA

Serum sample[a]	Type 7F RIA[b] (ng AbN/ml)	Type 7F EIA[c] (OD)	Type 19F RIA (ng AbN/ml)	Type 19F EIA (OD)
1	0	0	0	0
2	239	0.23	14	0.24
3	1989	0.37	139	0.53
4	3156	0.78	48	0.13
5	>5000	0.93	708	0.85
6	>5000	0.98	886	0.79
7	>5000	0.99	1036	0.87

[a] Healthy adults were immunized with polyvalent pneumococcal polysaccharide vaccine, and serum samples obtained 2 weeks later. Sample 1 was from a patient with hypogammaglobulinemia.

[b] Antibodies to types 7F and 19F pneumococcal polysaccharides were measured by radioimmunoassay, and the results expressed as ng antibody nitrogen/ml.

[c] IgG antibodies to types 7F and 19F pneumococcal polysaccharides were measured by enzyme immunoassay, and the results expressed as the mean optical density from triplicate wells. The serum was diluted 1:100, and alkaline phosphatase-conjugated anti-human IgG was utilized in the assay.

In a representative experiment, type 7 bacteria were opsonized with 20, 40, and 60% immune serum containing 1989 ng antibody nitrogen (Ab N)/ml representing 5, 10, and 15×10^{-6} ng Ab N/bacterium, respectively. Increased bacterial uptake was measured with increasing antibody concentration in the opsonic mixture (solid bars, Fig. 1). A similar experiment was performed with type 19 bacteria using 20, 40, and 60% immune serum containing 47 ng Ab N/ml of type 19 PPS antibodies corresponding to 0.12, 0.24, and 0.35×10^{-6} ng Ab N/bacterium, respectively. Again, increased bacterial uptake was seen with increasing amounts of type-specific antibody (solid bars, Fig. 1). Comparing types 7 and 19, more antibody/bacterium was required for phagocytosis of type 7 bacteria than for type 19.

Dilutions of immune serum resulted in dilution of complement components as well as antibody. To study the effect of varying antibody concentrations on opsonization with constant complement concentrations, we obtained immune sera from six normal adults 2 weeks after immunization with 14-valent PPS vaccine. Because these individuals had

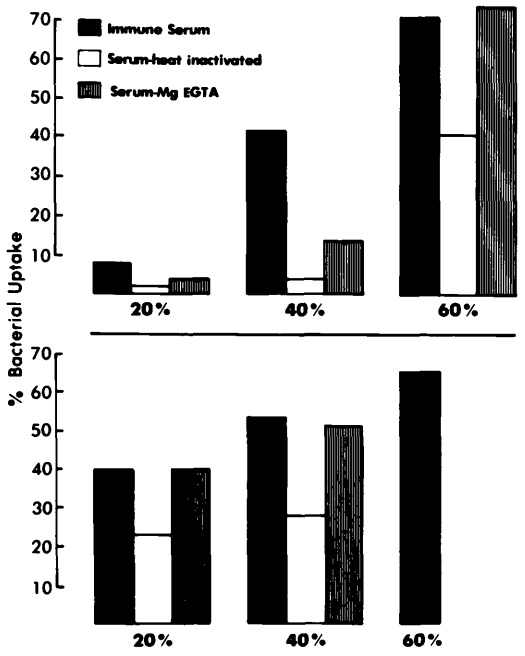

FIG. 1. Effect of serum opsonizing concentration on bacterial uptake. Radiolabeled type 7F *S. pneumoniae* were opsonized with complement-intact (solid bars), heat-inactivated (open bars), or MgEGTA-chelated (striped bars) immune sera (1989 ng Ab N/ml) at concentrations of 20, 40, and 60%, and the percentage bacterial uptake by human neutrophils at 30 min measured (upper section). Radiolabeled type 19F *S. pneumoniae* were similarly opsonized in immune serum (47 ng Ab N/ml) from another individual and the uptake measured (lower section).

varying antibody responses to immunization, sera with different type-specific antibody amounts were available and utilized in further experiments at 40% concentration. Serum from a patient with pan-hypogammaglobulinemia (IgG = 25 mg/dl, IgM = 14 mg/dl, no type 7 or 19 antibodies by radioimmunoassay) was also studied.

For type 7, bacterial uptake was ≤10% when complement-intact immune serum containing no or minimal type-specific antibody (239 ng Ab N/ml, 1.2×10^{-6} ng Ab N/bacterium) was utilized for opsonization (solid bars, Fig. 2). Moderate uptake (40%) occurred with immune serum containing 1989 ng Ab N/ml or 10×10^{-6} ng Ab N/bacterium. The greatest uptake (65%) was measured with immune serum containing 3156 ng Ab N/ml or 15.8×10^{-6} ng Ab N/bacterium, with only minimal additional increases in uptake using immune serum with higher antibody concentrations. A relatively linear relationship between antibody concentration and uptake of opsonized type 7 bacteria was found measuring these and other serum samples (correlation coefficient = 0.91) (Fig. 3).

For type 19, bacterial uptake occurred with complement-intact immune serum containing as little as 14 ng Ab N/ml (0.07×10^{-6} ng Ab N/bacterium) of type-specific antibody (solid bars, Fig. 4). Increased uptake was noted with higher concentrations of type 19 anti-

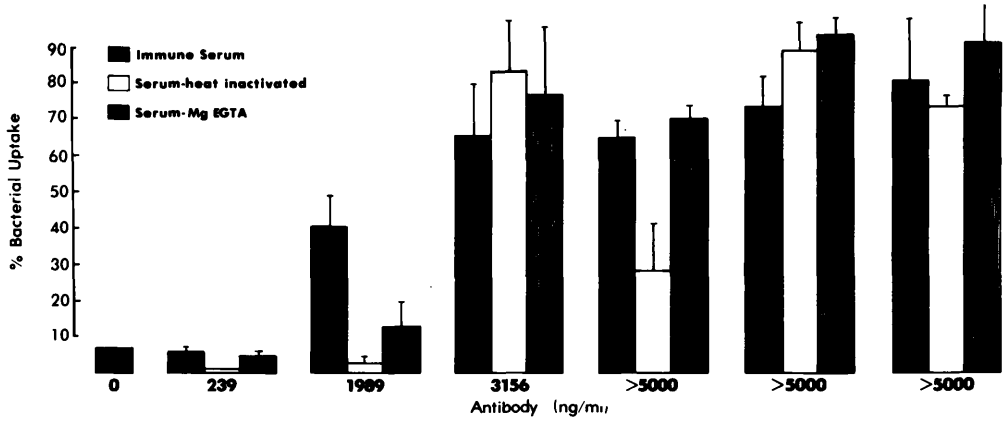

FIG. 2. Effect of antibody concentration on bacterial uptake of type 7F *S. pneumoniae*. Radiolabeled bacteria were opsonized with complement-intact (solid bars), heat-inactivated (open bars), or MgEGTA-chelated (striped bars) immune sera at 40% concentration from different individuals immunized with polyvalent pneumococcal polysaccharide vaccine, and the percentage bacterial uptake by human neutrophils measured at 30 min. Concentrations of antibody in the immune sera are given on the abscissa as ng Ab N/ml. Values for percentage bacterial uptake are expressed as mean ± 1SD for samples done in duplicate on at least 3 different days.

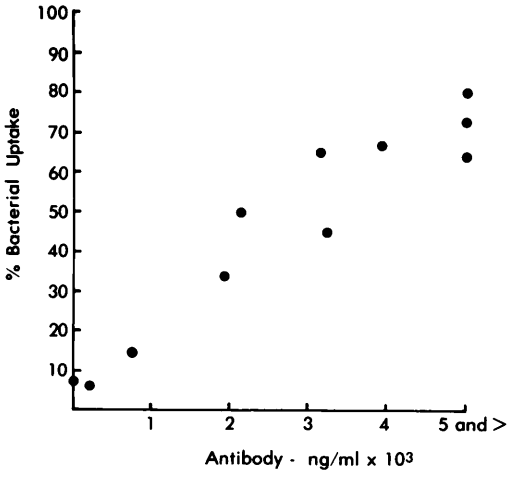

FIG. 3. Bacterial uptake correlates with concentration of type-specific antibody. Type 7F *S. pneumoniae* were opsonized with complement-intact immune sera at 40% concentration from different individuals immunized with polyvalent pneumococcal polysaccharide vaccine, and the percentage bacterial uptake by human neutrophils at 30 min measured. Bacterial uptake values are expressed as a function of ng Ab N/ml in the sera for type 7F antibodies.

body. The greatest uptake (67%) was noted at antibody concentrations of 708 ng Ab N/ml (3.5×10^{-6} ng Ab N/bacterium) or greater.

Thus, utilizing complement-intact immune sera from different individuals with varying concentrations of type-specific antibody to opsonize *S. pneumoniae* types 7 and 19, we found (1) increased opsonization, as assessed by bacterial uptake, with increasing antibody concentrations, and (2) differences in opsonization requirements for types 7 and 19, with type 7 requiring about five times more antibody than type 19 for maximal uptake.

Effect of heat inactivation on bacterial uptake. Immune sera were heat inactivated at 56° for 60 min to inactivate both the classical and alternative complement pathways. These sera were utilized to opsonize types 7 and 19 bacteria to determine the effect of antibody alone, without complement activity, on bacterial uptake. For type 7, dilutions of a single heat-inactivated immune serum containing 1989 ng Ab N/ml resulted in negligible uptake at 20 and 40% serum concentrations; however, at 60% (15×10^{-6} ng Ab N/bacterium) the uptake with heat-inactivated serum was about half that of complement-intact immune serum (open bars, Fig. 1). For type 19, there was moderate (≥20%) although reduced uptake using dilutions of heat-inactivated serum at concentrations as small as 20% (0.12×10^{-6} ng Ab N/bacterium). For both types, uptakes with complement-intact sera were higher than with heat-inactivated sera, indicating a contribution of complement to opsonic activity.

Utilizing heat-inactivated immune sera with varying antibody concentrations from different individuals we found minimal uptake (≥20%) when sera with low antibody concentrations were used to opsonize either types 7 or 19. At high antibody concentrations, however, bacterial uptake with heat-inactivated

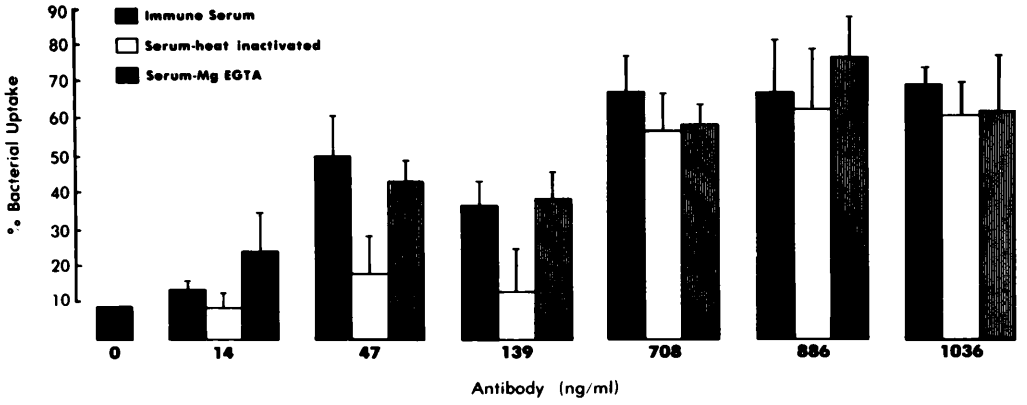

FIG. 4. Effect of antibody concentration on bacterial uptake of type 19F *S. pneumoniae*. Same as Fig. 2, except type 19F bacteria were utilized, and the amounts of antibody given on the abscissa are ng Ab N/ml for type 19F antibodies.

sera equaled that of complement-intact immune sera. For types 7, three of four heat-inactivated sera with high antibody concentrations (≥3156 ng Ab N/ml or 15.8×10^{-6} ng Ab N/bacterium) resulted in bacterial uptake equal to that of the same sera with intact complement activity (open bars, Fig. 2). For type 19, all three heat-inactivated sera with high antibody levels (≥708 ng Ab N/ml or 3.5×10^{-6} ng Ab N/bacterium) had equal opsonic activity to that of the same complement-intact sera (open bars, Fig. 4).

Thus, utilizing heat-inactivated immune sera we found (1) less antibody was required for opsonization of type 19 than type 7; (2) complement activity contributed to opsonization of types 7 and 19, especially when lesser concentrations of type-specific antibody were present; and (3) opsonization equal to that with antibody and complement could be achieved with antibody alone at high concentrations of type-specific antibody.

Effect of MgEGTA chelation on bacterial uptake. MgEGTA chelation blocks the classical complement pathway but allows activation of the alternative pathway, permitting measurement of the opsonic capacity of the alternative complement pathway in the presence of antibody (5–9, 12). Using dilutions of chelated immune sera to opsonize type 7 bacteria, there was minimal uptake at 20 and 40% serum concentrations, but uptake equal to that with the same complement-intact immune serum at 60% (striped bars, Fig. 1). At all dilutions, uptake with chelated serum was greater than with heat-inactivated serum, indicating a contribution of the alternative complement pathway to opsonic activity. For type 19, uptake with chelated immune serum was equal to that with complement-intact immune serum at both 20 and 40% concentrations, and greater than that with heat-inactivated serum, also suggesting alternative complement pathway activity.

Similar results were obtained using Mg-EGTA-chelated sera with varying concentrations of antibody from different individuals. For type 7 bacteria there was minimal uptake using chelated sera containing small amounts of antibody (1989 ng Ab N/ml (10×10^{-6} ng Ab N/bacterium) or less) (striped bars, Fig. 2), suggesting a role for antibody in alternative complement activation with type 7. Uptake with chelated sera was greater than that with heat-inactivated sera, indicating an alternative complement pathway contribution to opsonic activity. At higher antibody concentrations, chelated sera provided opsonic activity equal to or greater than the same complement-intact or heat-inactivated immune sera. With type 19, bacterial uptake using chelated sera was equal to or greater than that with complement-intact immune sera regardless of antibody concentration (striped bars, Fig. 4). Uptake with three different chelated sera between 14 and 139 ng Ab N/ml was significantly ($p \leq 0.03$) greater than that with the same sera which had been heat inactivated, indicating the importance of alternative complement pathway activity for opsonization within this lower antibody range.

Therefore, measuring uptake with Mg-EGTA-chelated serum we found (1) type 19 bacteria were more efficiently opsonized via alternative complement pathway than type 7, especially at low antibody concentrations; (2) at low antibody concentrations the alternative pathway played a major role in opsonization of both types 19 and 7; and (3) the opsonic requirement for complement could be overcome by high amounts of antibody alone for both types.

Discussion. In opsonization of *S. pneumoniae*, type-specific antibodies to PPS and C3b attach to the bacteria (2–4). The presence of these molecules on the bacterial surface promotes adherence and ingestion of the organism by phagocytic cells, which have receptors for C3b and the Fc portion of certain classes of immunoglobulin. Classical complement pathway activation requires the presence of antibody, whereas the alternative pathway can be activated by the pneumococcus alone, probably via the teichoic acid constituent of its cell wall (15). The presence of antibody enhances activation of the alternative pathway, however (15, 16).

Various methods have been used to assess the opsonic requirements in man for different *S. pneumoniae* serotypes. These studies have not directly investigated the effect of antibody concentrations on opsonic requirements. Using a complement consumption assay, Fine showed that some *S. pneumoniae* types activated the alternative pathway in the absence of antibody (types 7, 12, 14, and 25), some other types could do so only if antibody was present (types 3, 4, and 8), and type 1 was

unable to do so (5). The concentration of type-specific antibody in the pooled sera studied was not quantitated.

Utilizing a chemiluminescence assay, Matthay *et al.* reported reduced opsonization of types 7, 14, and 19 with heat-inactivated immune serum, and a marked reduction with serum from which most type-specific antibody had been absorbed with the type-specific organism (9). The alternative complement pathway, assessed with MgEGTA-chelated serum, resulted in opsonic activity equal to that of immune serum for type 19, and intermediate activity for types 7 and 14. The chemiluminescence response, measured in sera of four individuals with varying type 19 antibody concentrations (0 to 765 Ab N/ml), was greater in those sera with higher antibody amounts. However, the relative effect of increasing antibody concentration on complement requirements was not studied.

Giebink and co-workers utilized pooled sera in defining opsonic requirements for several *S. pneumoniae* types with a radiolabeled bacterial uptake assay (7, 8). Their pooled sera contained 214 ng Ab N/ml of type 7, 668 Ab N/ml of type 18, 363 ng Ab N/ml of type 23, and 92 ng Ab N/ml of type 6. In dilution experiments, they found a serum concentration of 10% was adequate to result in uptakes of ≥40% for these types. With MgEGTA-chelated serum, type 18 bacteria, for which the pooled serum contained the highest antibody concentration, was opsonized at a 10% serum concentration, while types 6, 7, and 23 required 20, 40, and 60% serum, respectively, for ≥10% uptake. Markedly decreased opsonization was noted utilizing heat-inactivated serum for all except type 18, for which the serum contained the highest type-specific antibody concentration.

In the present study, we employed a modification of the method of Giebink *et al.* (7, 8) to investigate the effect of antibody amounts on opsonic requirements for types 7 and 19. Radiolabeled *S. pneumoniae* were opsonized with complement-intact, heat-inactivated, or MgEGTA-chelated sera from different individuals immunized with PPS vaccine, and the bacterial uptake by human neutrophils measured. It should be pointed out that the post-immunization sera utilized in our experiments may have contained different classes or quality of antibody than that in sera from nonimmune individuals. Furthermore, even after immunization, the quality of antibody produced by different individuals may vary. Our uptake results are not directly comparable to those of Giebink *et al.* (7, 8) because of differences in the bacteria:neutrophil ratio, duration of bacterial opsonization, and other assay conditions.

We have demonstrated increased opsonization, as assessed by bacterial uptake, with increasing concentrations of type-specific PPS antibody in the complement-intact serum used for opsonization. This is in agreement with previous studies (7–9). Minimal uptake was found using hypogammaglobulinemic serum with no PPS antibodies. The increase in uptake with antibody concentration was relatively linear for type 7 bacteria, and less so for type 19. We found greater concentrations of antibody were required for opsonization of type 7 than type 19 with complement-intact serum.

A major finding of this study is that at high concentrations of antibody the requirement for complement for opsonization of types 7 and 19 could be overcome. For type 7 this occurred at $\geq 15 \times 10^{-6}$ ng Ab N/bacterium, and for type 19 at $\geq 3.5 \times 10^{-6}$ ng Ab N/bacterium. At these high antibody levels, opsonization in heat-inactivated sera resulted in bacterial uptake equal to that of the same complement-intact immune sera in all but one sample tested. It should be noted that Giebink *et al.* found opsonic activity for only type 18 with heat-inactivated pooled sera, but it was for this type which their pooled sera contained the greatest concentration of antibody (7).

At lower antibody levels, complement activity was important for opsonization of both types 7 and 19 bacteria as demonstrated by differences found between uptakes in complement-intact versus heat-inactivated immune sera. In heat-inactivated sera, there was also increased opsonization with higher antibody concentrations, and type 7 required greater amounts of antibody for opsonization than type 19.

We have shown that type 19 bacteria were opsonized efficiently via the alternative complement pathway at all antibody concentrations tested, with uptake using MgEGTA-chelated sera equal to that with complement-intact sera. By comparing the uptake in MgEGTA-chelated sera to that in heat-inac-

tivated sera, we found the contribution of alternative complement pathway activity to opsonization was greater at lower concentrations of antibody. At high antibody concentrations, the presence or absence of alternative complement pathway activity did not appear to affect uptake.

With type 7, the presence of some antibody was required for uptake in MgEGTA-chelated serum. Although only a few samples with low antibody were measured, in all there were differences between MgEGTA-chelated and heat-inactivated serum, indicating an important role for alternative complement pathway activity. This was not the case in three out of four sera with high concentrations of antibody, where MgEGTA-chelated sera yielded uptakes equal to or greater than that in complement-intact or heat-inactivated sera. As with type 19, at high concentrations of antibody, it was antibody which provided virtually all the opsonic activity for type 7.

At least three conclusions concerning the study of host defenses against *S. pneumoniae* can be drawn from our results. First, heterogeneity exists among pneumococcal serotypes with respect to the antibody concentrations required for their effective opsonization. We found in a limited number of subjects that immunization with type 19 PPS, as part of the polyvalent vaccine, resulted in lower post-immunization antibody concentrations than type 7. Type 19 organisms, however, were more efficiently opsonized at lower antibody concentrations than type 7. Studies to determine any correlation between serotype pathogenicity, antibody response, and antibody requirements for opsonization would be desirable to determine whether this is coincidental for these two serotypes, or a more general phenomenon.

Second, complement requirements for *S. pneumoniae* opsonization may vary depending on the concentration of antibody in the sera. For example, we found opsonic activity in heat-inactivated immune sera to be equal to that in complement-intact sera when high, but not low concentrations of type-specific antibody were present. Therefore, one must take into account antibody concentration as a variable when studying complement requirements, and the results of previous studies which have not considered this variable should be reinterpreted.

Finally, although one cannot assume our *in vitro* results necessarily apply *in vivo*, our findings suggest that one might overcome opsonic defects for *S. pneumoniae* due to abnormal classical or alternative complement pathway activity by sufficiently increasing type-specific antibody concentrations. Such antibody levels were achieved for types 7 and 19 in three of six healthy individuals we immunized with polyvalent PPS vaccine. In sickle cell disease, frequent pneumococcal infections have been attributed to abnormal alternative complement pathway activity as well as to abnormal splenic function (17, 18). The efficacy of PPS vaccine immunization in sickle cell disease patients (19, 20) may result from raising type-specific antibody concentrations high enough to overcome the opsonic defect in alternative complement activity and/or splenic dysfunction (21).

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