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Monoclonal Antibodies That Recognize a Common Pneumococcal Protein with Similarities to Streptococcal Group A Surface Glyceraldehyde-3-Phosphate Dehydrogenase

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Monoclonal antibodies (MAbs) against clinical isolates of *Streptococcus pneumoniae* were produced in a search for common pneumococcal proteins. One of the fusions generated two MAbs, 174,B-8 (immunoglobulin G2a) and 177,D-8 (immunoglobulin G1), which by Western blotting (immunoblotting) stained with a main band of 40 kDa found in all isolates of *S. pneumoniae* examined. Cross-reactivity studies with streptococci other than pneumococci revealed very weak or moderate reactions with the MAbs. The 40-kDa protein was isolated by immunoaffinity chromatography and subsequent preparative Western blotting. N-terminal amino acid sequencing showed 90% amino acid sequence homology with a surface-located glyceraldehyde-3-phosphate dehydrogenase from *Streptococcus pyogenes*. This protein has also been reported to exhibit binding to mammalian proteins such as fibronectin, which may serve as host receptors. The epitopes for MAbs 174,B-8 and 177,D-8 reacting with the pneumococcal analog were not accessible to antibody binding in live bacteria but were exposed after heat killing. The MAbs showed negligible cross-reactions with *S. pyogenes*.

Infections caused by *Streptococcus pneumoniae* throughout the world remain a major cause of morbidity and mortality, in particular among infants and the elderly. Capsular polysaccharides are essential virulence factors and are used for classification of pneumococci into 90 types (13). The cell wall polysaccharides (C-Ps) containing the immunologically dominant phosphorylcholine epitope is common to all pneumococcal serotypes. When mice are immunized with pneumococci, one of the most prominent antibody responses is to this phosphorylcholine determinant (20). There are therefore few reports on monoclonal antibodies (MAbs) against pneumococcal capsular polysaccharides and protein antigens. This may also partly explain why only a few proteins from this gram-positive bacterium are characterized (for reviews of proteins, see references 2 and 6).

The pneumococcal vaccine currently used is composed of unconjugated, capsular polysaccharides from 23 types of *S. pneumoniae*. Whereas this vaccine has been shown to be protective in adults, it is of limited use in children and the elderly (for a review, see reference 2). These populations are poor responders to polysaccharide antigens because they do not stimulate T helper cells (27). The immunogenicity of the polysaccharides can be increased by covalently linking them to a carrier protein. The success of the *Haemophilus influenzae* type B conjugates has greatly stimulated interest in developing conjugate vaccines against other capsulated bacteria, including *S. pneumoniae*. Since only a few heterologous proteins like diphtheria or tetanus toxoids have been used as carriers in humans, there is a risk that anticarrier antibodies induced by one conjugate will suppress the response to the other (4). There is thus a great interest in finding pneumococcal proteins which can be used as carriers for capsular polysaccharides. New pneumococcal vaccine strategies also include the use of proteins as protective immunogens in their own right. Among the protein vaccine candidates are autolysin (16), neuraminidase (17), pneumococcal surface adhesin A (PsaA) (24), pneumococcal surface protein A (PspA) (31), and pneumolysin (1).

We have immunized mice with different clinical isolates of *S. pneumoniae*; after fusion of spleen cells with myeloma cells, the supernatants were first screened with the strain used for immunization and C-Ps. Antibodies that reacted with the immunogen but did not stain with C-Ps were then tested by Western blotting (immunoblotting) to select those reacting with epitopes expressed on proteins. From these experiments, we identified two MAbs that recognize a common pneumococcal 40-kDa protein.

MATERIALS AND METHODS

Bacterial strains. The bacteria examined are described in Table 1. Most of the organisms were from human clinical isolates, and some were obtained from the American Type Culture Collection, Rockville, Md., or National Collection of Type Cultures, Central Public Health Laboratory, London, England. Pneumococcal strains of the different capsular types included in the 23-valent vaccine (Pneumovax; Merck, Sharp & Dohme, West Point, Pa.) were typed by the capsular reaction test with rabbit antisera purchased from Statens Seruminstitut (SSI), Copenhagen, Denmark. Differentiation of types within groups was carried out at SSI. This institute also supplied us with *Streptococcus suis* type 8 SSI 14636 and *Escherichia coli* U5/91. *Mycobacterium leprae* was from M. J. Colston, National Institute for Medical Research, London, England; a *Mycobacterium bovis* BCG strain was also used. *Archaeoglobus fulgidus* VC-16 (28) was obtained from Torleif Lien, Institute of Microbiology, University of Bergen, Bergen, Norway.

Polysaccharides. Purified C-Ps from *S. pneumoniae* was a gift from Jørgen Henrichsen, SSI.

Production of MAbs. As antigens, we used different heat-inactivated (30 min at 60°C) and sonicated pneumococcal strains. Six-week-old BALB/c mice were immunized intraperitoneally with a suspension containing 50 μg of protein in 0.25 ml of phosphate buffered saline (PBS) mixed with 0.25 ml of Freund's incomplete adjuvant and given a booster injection of the same mixture 2 weeks later. Four months later, and 4 days before fusion, each mouse was given intravenously the antigen in PBS. Spleen cells were fused with NSO myeloma cells by standard methods. Mouse splenocytes were used as feeder cells.

Cell culture supernatants were screened doubly by enzyme-linked immunosorbent assay (ELISA) (see below) against the immunizing pneumococcal strain and against C-Ps. Those positive only to the heat-killed pneumococci were checked against strips with bacterial proteins from the immunizing strain and, as a negative control, *H. influenzae*, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and electrotransferred to nitrocellulose papers (see below). Interesting hybridoma cells were cloned by limiting dilution with

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TABLE 1. Western blot reactivities of MAb 174,B-8

Source of antigen (*n*)	Staining[a]
Eubacteria	
Streptococci	
S. pneumoniae (40)	++
S. suis type 8 SSI 14636	+
S. mitis (4)	+
S. bovis 971/92	+
S. milleri (2)	+
S. sanguis (4)	+
S. zooepidemicus 44/93	+
S. salivarius 56/93	(+)
S. pyogenes ATCC 12353	(+)
S. agalactiae 1010/90	(+)
S. equisimilis C 74	(+)
Other	
Staphylococcus aureus ATCC 12598[b]	−
H. influenzae (7)	−
Neisseria meningitidis (12)	−
E. coli U5/41	−
Salmonella typhimurium 1406 strain 9	−
Brucella melitensis M16	−
Micrococcus luteus FH-Ba 2029	−
B. stearothermophilus ATCC 7953	−
Enterococcus faecalis ATCC 19433	−
Enterococcus faecium ATCC 19434	−
M. bovis BCG	−
M. leprae	−
Archaebacterium	
A. fulgidus VC-16	−
Eukaryotes	
Mouse splenocytes (1)	−
Human lymphocytes (1)	−
Human erythrocyte ghosts (3)	−

[a] ++, strong staining; +, moderate staining; (+), very weak staining; −, no staining.

[b] Some other bands were seen in this region. They were also detected with MAbs against irrelevant antigens.

Hybridoma Enhancing Supplement (Sigma Chemical Co., St. Louis, Mo.) instead of feeder cells. Isotyping of MAbs in cell culture medium was performed in ELISA with heat-killed pneumococci as the coating antigen (see below), using a kit (catalog no. 93-6550) from Zymed Laboratories Inc., San Francisco, Calif.

Protein assay. For determination of protein concentrations, aliquots of the bacteria were dissolved in 0.5 M NaOH. Lowry's method (19) with bovine serum albumin as the standard was used.

ELISA measurements. Flat-bottom microtiter plates (MaxiSorp; Nunc A/S, Roskilde, Denmark) were coated with heat-killed and sonicated pneumococci, 25 μg/ml in PBS and 50 μl per well. Washing and incubations with antibodies, enzyme, and substrate were performed as previously described (15).

SDS-PAGE and immunoblotting. The bacterial suspensions in PBS were boiled for 5 min with sample buffer containing 2-mercaptoethanol. The mycobacteria were first sonicated for 20 min in ice water. Samples with 7 μg of protein were placed in each well formed by a 15-tooth comb with a Bio-Rad (Richmond, Calif.) Mini-Protean Slab Cell apparatus. SDS-PAGE was performed with stacking and separating gels containing 4 and 15% acrylamide, respectively. The separated proteins were electrotransferred to a nitrocellulose paper (pore size, 0.2 μm). The MAbs were used as diluted cell culture media, and as the secondary antibody we used peroxidase-conjugated rabbit immunoglobulins to mouse immunoglobulins (dilution, 1:1,000; Dakopatts A/S, Glostrup, Denmark). The Rainbow protein molecular weight markers were obtained from Amersham International plc, Amersham, England.

Dot blot assay. The bacterial species examined were from stationary-phase cultures. They were heat killed and spotted to nitrocellulose membranes as previously described (15). Some experiments were also performed with live, log-phase pneumococci. The primary antibodies were used as diluted cell culture media, and bound MAbs were detected with peroxidase-conjugated antibodies (see above).

Solubilization of bacterial proteins. Bacteria from stationary growth phase were harvested by centrifugation, washed three times in PBS, and killed by heating for 5 min at 100°C. The washing and heating were omitted in the last experiments. The bacteria were dissolved in 0.5% sodium deoxycholate (DOC) in 0.05 M Tris buffer containing 2 mM EDTA (pH 8.6) for about 1 h at room temperature. The detergent was obtained from Merck, Darmstadt, Germany. Nondissolved materials were removed by centrifugation. Affinity purification (see below) in the presence of DOC was performed without success. The proteins in the DOC extract were therefore precipitated by adding ethanol to a concentration of 80%. DOC itself is soluble in ethanol. The precipitate was isolated by centrifugation and suspended in 1% Triton X-100 (Sigma) in PBS. Nondissolved materials were removed by centrifugation.

Immunoaffinity purification of bacterial proteins and sequencing. One MAb (174,B-8) was purified from ascitic fluid by affinity chromatography on protein A-Sepharose (Pharmacia Biotech, Uppsala, Sweden). The MAb was then coupled to CNBr-activated Sepharose (Pharmacia Biotech). The gel contained about 4 mg of protein per ml. The 1% Triton extract was run first through a Sepharose 6B column for removal of nonspecifically binding proteins and then through the column with immobilized MAb 174,B-8. The column was first washed with Triton X-100, but at a concentration of 0.1%, followed by PBS without detergent. This washing procedure was chosen because Triton X-100 has a high A_{280} and hence would interfere with protein monitoring during elution of bound proteins with 4 M guanidine-HCl in PBS. The fractions were dialyzed against PBS and concentrated by vacuum filtration. The eluted proteins were separated by SDS-PAGE, using a comb for preparative electrophoresis, and electroblotted onto a polyvinylidene difluoride membrane (0.45-μm pore size; Millipore, Bedford, Mass.). Strips were cut off and probed with MAb 174,B-8, and the rest of the membrane was stained with 0.025% Coomassie brilliant blue in methanol. After destaining in 50% methanol, bands reacting with the MAb were identified by the strips stained with MAb and then cut off for microsequencing. Automatic Edman degradation was performed on an Applied Biosystems model 477A protein sequencer equipped with an on line model 120A phenythiohydantoin-derived amino acid analyzer.

Sequence alignment. Searches in the Swiss-Prot protein sequence data bank (3) and sequence alignment were done by using the University of Wisconsin sequence analyses package (7).

Enzyme activity. The assay for glyceraldehyde-3-phosphate dehydrogenase (GADPH) activity was carried out as described by Ferdinand (8), with GADPH from *Bacillus stearothermophilus* (Sigma) as a positive control.

RESULTS

Dot blot and Western blot reactivities of MAbs. Mice were immunized with different clinical isolates of *S. pneumoniae* for production of MAbs against proteins. The hybridoma cell culture supernatants were initially doubly screened in ELISA against the strain used for immunization and C-Ps. Those positive against *S. pneumoniae* and negative against C-Ps were expanded and retested in immunoblotting. One fusion performed after immunization with a type 23F strain resulted in MAbs 174,B-8 (immunoglobulin G2a) and 177,D-8 (immunoglobulin G1). The MAbs stained by Western blotting with a main band of 40 kDa (Fig. 1). The epitopes were found in all pneumococcal strains ($n = 40$) analyzed by Western blotting and all ($n = 60$) examined by dot blotting with heat-killed *S. pneumoniae*. Streptococci other than pneumococci showed very weak or moderate cross-reactivities to the two MAbs (Fig. 1 and Table 1). The epitopes were not expressed on other gram-positive or gram-negative eubacterial organisms. Furthermore, the epitopes were not present in the archaebacte-

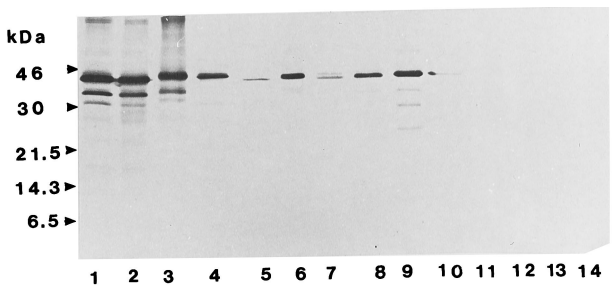

FIG. 1. Western blots with bacteria probed with MAb 174,B-8 used as cell culture medium diluted 1:500. Lanes: 1 to 3, three different strains of *S. pneumoniae*; 4, *S. bovis* 971/92; 5, *S. salivarius* 56/93; 6, *S. mitis* 44/89, 7, *S. pyogenes* ATCC 12353; 8, *S. sanguis* 11/88; 9, *S. zooepidemicus* 44/93; 10, *S. agalactiae* 1010/90; 11, *E. coli* U5/41; 12, *H. influenzae* NCTC 8466; 13, *B. stearothermophilus* ATCC 7953; 14, *M. bovis* BCG.

```
                1       5       10      15      20
Strpn 40 kDa    V V K V G I N G F G ? I G ? L A F D ? I Q N V
Strpy GADPH     - - - - - - - - - - R - - R - - - R R - - - I
Strpy plr       - - - - - - - - - - R - - R - - - R R - - - I
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FIG. 2. Comparison of the N-terminal amino acid sequence of *S. pneumoniae* (Strpn) 40-kDa protein with those of GADPH (22) and plasmin receptor (plr) (18) from *S. pyogenes* (Strpy). The pneumococcal 40-kDa protein was isolated by immunoaffinity chromatography on a column with MAb 174,B-8 bound to CNBr-Sepharose. The eluted proteins were separated by SDS-PAGE and electrotransferred onto a polyvinyidenedifluoride membrane. A strip of the membrane was cut off and probed with MAb 174,B-8. The rest of the membrane was stained with Coomassie brilliant blue. A strip containing the 40-kDa band was cut off and subjected to amino acid microsequencing.

rium or in mammalian cells analyzed. The patterns of reactivities were similar for the two MAbs, except in the case of the *S. suis* strain, which stained with MAb 174,B-8 and not with MAb 177,D-8.

Purification and N-terminal amino acid sequence. The 40-kDa protein was isolated from a pneumococcal strain by immunoaffinity chromatography, and the N-terminal amino acid sequence was determined. Comparison with the sequences in the Swiss-Prot data bank showed up to 60% homology to the enzyme GAPDH. Using this as a key word for searches in literature databases, we found that the N-terminal amino acid sequence for a major surface protein on group A streptococci had been determined and found to have GAPDH activity (22). Eighteen of 20 determined residues in the pneumococcal 40-kDa protein were identical to those reported for the streptococcal protein (Fig. 2), giving an identity of 90%. The deduced amino acid sequence from nucleotide sequencing of a plasmin receptor from group A streptococci also showed 90% homology with the pneumococcal protein (18) (Fig. 2).

Enzyme activity. The purified 40-kDa protein did not show GADPH activity. One aliquot of the positive control enzyme from *B. stearothermophilus* was therefore treated with the detergents DOC and Triton X-100 and with guanidine-HCl as used for the isolation of the 40-kDa protein. This treatment destroyed the enzyme activity.

DISCUSSION

The MAbs 174,B-8 and 177,D-8 identified a common 40-kDa pneumococcal protein by Western blotting. N-terminal amino acid sequencing of the common pneumococcal protein isolated by immunoaffinity chromatography showed sequence homology to GAPDH, a key enzyme involved in glucose metabolism. GAPDH is composed of identical subunits of 37 kDa (9, 12), which is in agreement with our finding of 40 kDa by SDS-PAGE. The lack of GADPH activity of the 40-kDa protein is most likely due to the isolation conditions with use of two detergents and guanidine-HCl. Analogous treatment of GADPH from *B. stearothermophilus* destroyed the activity.

The highest sequence homology (90%) was found to be a streptococcal (*S. pyogenes*) GAPDH which was reported to be surface located (22). The glycolytic enzyme GAPDH usually has an intracellular location, but GADPH has also been identified on the surface of other cells such as *Schistosoma mansoni*, an invasive parasite (11). Another surface protein from *S. pyogenes*, a plasmin receptor (18), also showed 90% amino acid sequence homology to the pneumococcal protein described here. The genes encoding the plasmin receptor have been sequenced, and the deduced amino acid sequence revealed a significant similarity to bacterial GAPDH (18). These two *S. pyogenes* proteins described by different investigators could be the same protein because a comparison of the reported surface GAPDH sequences in the N-terminal region from positions 1 to 39 shows 97% sequence homology to the plasmin receptor.

This surface-located *S. pyogenes* GAPDH is suggested to contribute to the organism's invasiveness by its ability to capture plasmin (18) and the binding of proteins such as fibronectin (22), a protein which has been suggested to play an important role in the adherence of *S. pyogenes* to host epithelial cells (21, 26). The streptococcal surface GAPDH has also been reported to function as an ADP-ribosylating enzyme which may enable communication between host and parasite during infection (23). The MAbs described here with specificity for the pneumococcal analog did not recognize live bacteria, which could indicate that this GAPDH is located intracellularly. However, as the antigen for MAb production, we used heat-killed *S. pneumoniae*. This treatment might have exposed epitopes of a surface-located GAPDH which are not accessible for antibody binding in an intact organism.

GAPDH is present in both prokaryotes and eukaryotes. All examined pneumococci showed strong reactions with the MAbs. None of the gram-negative eubacteria nor the only archaebacterium examined stained with the MAbs. All analyzed mammalian cells, including human erythrocyte ghosts, were also nonreactive with the MAbs. In erythrocytes, 60 to 70% of the enzyme activity is membrane bound and constitutes about 5 to 7% of the total membrane proteins (14). The epitopes for the MAbs are therefore assumed to be outside the coenzyme-binding domains and the catalytic domains of GAPDH.

Very weak or moderate cross-reactions were seen with the examined streptococci other than pneumococci. Despite the high N-terminal amino acid sequence homology demonstrated here between the *S. pyogenes* and *S. pneumoniae* GAPDH proteins, the MAbs showed negligible cross-reactions by Western blotting. The data thus indicate that the epitope must be outside this region.

The bacteria staining with the MAbs are mainly commensal and potentially pathogenic streptococcal organisms on the mucosal surface of, for example, the upper respiratory tracts of humans and animals (Table 1). Of the moderate cross-reacting streptococci, some strains of *Streptococcus mitis* have been reported to possess phosphorylcholine, which also is an antigen common to all *S. pneumoniae* strains (25, 29). The cross-reacting *S. suis* strain of capsular type 8 has not been isolated from humans (10). It is a pig pathogen with a capsule similar to those of pneumococci of serotypes 19A and 19F (10).

The reaction patterns against different organisms were the same for MAbs 174,B-8 and 177,D-8, except in the case of the *S. suis* strain, which reacted with the former MAb but not with the latter. There might thus be some differences in the fine specificities of the two MAbs.

Various proteins have been suggested to be involved in the pathogenicity of *S. pneumoniae* and thus vaccine candidates. Autolysin-negative mutants have been shown to be less virulent than wild-type pneumococci, and immunization with autolysin (*N*-acetylmuramyl-L-alanine amidase) confers some protection against pneumococcal challenge in mice (5). The most important effect of amidase might be its role in mediating the release of pneumolysin (16), an intracellular protein belonging to the family of thiol-activated toxins. This protein is conserved among pneumococcal isolates. Immunization of mice with a toxoid of pneumolysin confers non-serotype-specific protection against *S. pneumoniae* (1). Shared among pneumococci is also a 37-kDa protein, PsaA, an adhesin which may be involved in the attachment or adherence to surfaces in the respiratory tract (24). PspA shows structural and antigenic variations between different *S. pneumoniae* strains (30). The

problems with antigenic variation might be overcome by using a vaccine with truncated PspA from several strains (31).

Work in progress involves immunoaffinity purification of the common pneumococcal 40-kDa protein so as to use it in experiments to evaluate a possible pathogenic role of the protein and thus whether it can serve as a vaccine component.

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REFERENCES

1. **Alexander, J. E., R. A. Lock, C. C. A. M. Peeters, J. T. Poolman, P. W. Andrew, T. J. Mitchell, D. Hansman, and J. C. Paton.** 1994. Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of *Streptococcus pneumoniae*. Infect. Immun. **62:**5683–5688.
2. **AlonsoDeVelasco, E., A. F. M. Verheul, J. Verhoef, and H. Snippe.** 1995. *Streptococcus pneumoniae*: virulence factors, pathogenesis, and vaccines. Microbiol. Rev. **59:**591–603.
3. **Bairoch, A., and B. Boeckmann.** 1991. The SWISS-PROT protein sequence data bank. Nucleic Acids Res. **19:**2247–2249.
4. **Barington, T., A. Gyhrs, K. Kristensen, and C. Heilmann.** 1994. Opposite effects of actively and passively acquired immunity to the carrier on responses of human infants to a *Haemophilus influenzae* type b conjugate vaccine. Infect. Immun. **62:**9–14.
5. **Berry, A. M., R. A. Lock, D. Hansman, and J. S. Paton.** 1989. Contribution of autolysin to virulence of *Streptococcus pneumoniae*. Infect. Immun. **57:** 2324–2330.
6. **Cundell, D., H. R. Masure, and E. I. Tuomanen.** 1995. The molecular basis of pneumococcal infection: a hypothesis. Clin. Infect. Dis. **21**(Suppl. 3)**:** S204–S212.
7. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
8. **Ferdinand, F.** 1964. The isolation and specific activity of rabbit-muscle glyceraldehyde phosphate dehydrogenase. Biochem. J. **92:**578–585.
9. **Fox, J. B., and W. B. Dandliker.** 1956. A study of some of the physical properties of glyceraldehyde-3-phosphate dehydrogenase. J. Biol. Chem. **218:**53–57.
10. **Gottschalk, M., J. Kolberg, N. Charland, and M. Jacques.** 1995. *Streptococcus pneumoniae* types 19A and 19F and *Streptococcus suis* capsular types 8 share common capsular epitopes. J. Clin. Microbiol. **33:**2492–2495.
11. **Goudot-Crozel, V., D. Caillol, M. Djabali, and A. J. Dessein.** 1989. The major parasite surface antigen associated with human resistance to schistosomiasis is a 37-kD glyceraldehyde-3P-dehydrogenase. J. Exp. Med. **170:**2065–2080.
12. **Harris, J. I., and R. N. Perham.** 1963. Studies on glyceraldehyde-3-phosphate dehydrogenases. Biochem. J. **89:**60p.
13. **Henrichsen, J.** 1995. Six newly recognized types of *Streptococcus pneumoniae*. J. Clin. Microbiol. **33:**2759–2762.
14. **Kant, J. A., and T. L. Steck.** 1973. Specificity in the association of glyceraldehyde 3-phosphate dehydrogenase with isolated human erythrocyte membranes. J. Biol. Chem. **248:**8457–8464.
15. **Kolberg, J., I. S. Aaberge, E. Jantzen, M. Løvik, G. Lermark, and T. Steen.** 1992. Murine monoclonal antibodies against pneumococcal capsular polysaccharide types 4, 8, 22F and 19A/19F. APMIS **100:**91–94.
16. **Lock, R. A., D. Hansman, and J. C. Paton.** 1992. Comparative efficacy of autolysin and pneumolysin as immunogens protecting mice against infection by *Streptococcus pneumoniae*. Microb. Pathog. **12:**137–143.
17. **Lock, R. A., J. C. Paton, and D. Hansman.** 1988. Comparative efficacy of pneumococcal neuraminidase and pneumolysin as immunogens protective against *Streptococcus pneumoniae*. Microb. Pathog. **5:**461–467.
18. **Lottenberg, R., C. C. Broder, M. D. P. Boyle, S. J. Kain, B. L. Schroeder, and R. Curtiss III.** 1992. Cloning, sequence analysis, and expression in *Escherichia coli* of a streptococcal plasmin receptor. J. Bacteriol. **174:**5204–5210.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
20. **McDaniel, L. S., and D. E. Briles.** 1986. Monoclonal antibodies against surface components of *Streptococcus pneumoniae*, p. 143–164. *In* A. J. L. Macario and E. C. de Macario (ed.), Monoclonal antibodies against bacteria. Academic Press, Inc., Orlando, Fla.
21. **Myhre, E. B., and P. Kuusela.** 1983. Binding of human fibronectin to group A, C, and G streptococci. Infect. Immun. **40:**29–34.
22. **Pancholi, V., and V. A. Fischetti.** 1992. A major surface protein on group A streptococci is a glyceraldehyde-3-phosphate-dehydrogenase with multiple binding activity. J. Exp. Med. **176:**415–426.
23. **Pancholi, V., and V. A. Fischetti.** 1993. Glyceraldehyde-3-phosphate dehydrogenase on the surface of group A streptococci is also an ADP-ribosylating enzyme. Proc. Natl. Acad. Sci. USA **90:**8154–8158.
24. **Sampson, J. S., S. P. O'Connor, A. R. Stinson, J. A. Tharpe, and H. Russell.** 1994. Cloning and nucleotide sequence analysis of *psaA*, the *Streptococcus pneumoniae* gene encoding a 37-kilodalton protein homologous to previously reported *Streptococcus* sp. adhesins. Infect. Immun. **62:**319–324.
25. **Sjögren, A. M., H. Holmberg, and A. Krook.** 1987. Etiologic diagnosis of pneumonia by antigen detection: crossreactions between pneumococcal C-polysaccharide and oral microorganisms. Diagn. Microbiol. Infect. Dis. **6:**239–248.
26. **Speziale, P., M. Höök, L. M. Switalski, and T. Wadström.** 1984. Fibronectin binding to a *Streptococcus pyogenes* strain. J. Bacteriol. **157:**420–427.
27. **Stein, K. E.** 1992. Thymus-independent and thymus-dependent responses to polysaccharide antigens. J. Infect. Dis. **165**(Suppl. 1)**:**S49–S52.
28. **Steiter, K. O.** 1988. *Archaeoglobus fulgidus* gen. nov.: a new taxon of extremely thermophilic Archaebacteria. Syst. Appl. Microbiol. **10:**172–173.
29. **Sørensen, U. B. S., and J. Henrichsen.** 1987. Cross-reactions between pneumococci and other streptococci due to C polysaccharide and F antigen. Infect. Immun. **25:**1854–1859.
30. **Talkington, D. F., D. C. Voellinger, L. S. McDaniel, and D. E. Briles.** 1992. Analysis of pneumococcal PspA microheterogeneity in SDS polyacrylamide gels and the association of PspA with the cell membrane. Microb. Pathog. **13:**343–355.
31. **Tart, R. C., L. S. McDaniel, B. A. Ralph, and D. E. Briles.** 1996. Truncated *Streptococcus pneumoniae* pspA molecules elicit cross-protective immunity against pneumococcal challenge in mice. J. Infect. Dis. **173:**380–386.

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