INFECTION AND IMMUNITY, May 1992, p. 1957–1962
0019-9567/92/051957-06$02.00/0

Vol. 60, No. 5

Mouse Antibody to Phosphocholine Can Protect Mice from Infection with Mouse-Virulent Human Isolates of *Streptococcus pneumoniae*

DAVID E. BRILES,[1,2,3]* COLYNN FORMAN,[1] AND MARILYN CRAIN[1,2]

Departments of Microbiology,[1] Pediatrics,[2] and Comparative Medicine,[3] University of Alabama at Birmingham, Birmingham, Alabama 35294

Received 13 December 1991/Accepted 28 February 1992

Previous studies have demonstrated that mouse antibodies to phosphocholine (PC) can protect mice against fatal infection caused by several, but not all, mouse-virulent laboratory strains of *Streptococcus pneumoniae*. Because the pneumococcal strains used in previous studies had been mouse passed and were propagated for many years outside of humans, it was not known whether antibody to PC would be able to protect mice against *S. pneumoniae* freshly isolated from humans. In the present study, we examined the ability of an immunoglobulin G (IgG) monoclonal antibody (MAb) to PC to protect against infections in mice caused by 14 pneumococcal strains of capsular types 3, 4, 6A, and 6B. Nine of these strains were selected as the most virulent strains for mice from a group of 69 fresh clinical isolates. Five were mouse-passed laboratory strains. Mouse IgG3 MAb to PC was able to exhibit protective effects (survival or increased time to death) against infection with virtually all of the strains injected intravenously and against infection with 70% of the strains injected intraperitoneally. The protective effects of antibody to PC appeared to be partially dependent on capsular type. MAb to PC was most effective against capsular type 3 strains and least effective against type 4 strains. With type 3 and type 4 strains, MAb to PC could frequently protect against larger numbers of CFU injected intravenously than intraperitoneally. For capsular type 6A and 6B strains the reverse was true.

It is been long recognized that antibodies to capsular polysaccharides can be efficacious in the prevention of serious pneumococcal infection (3,5). In the 1920s and 1930s it was also recognized that somatic antigens of pneumococci could elicit protective immune responses (20, 23). In spite of the development of a pneumococcal vaccine based on the capsular polysaccharides (3) and the susceptibility of pneumococci to penicillin (19), pneumococcal infection has remained serious in both developed and underdeveloped countries (33, 36). The renewed awareness of the continuing morbidity and mortality resulting from pneumococcal infections (4, 33) has encouraged many laboratories to extend earlier studies of pathogenic mechanisms of pneumococci and host defenses against pneumococcal infection.

These studies have included investigations of the ability of somatic antigens to elicit protection (2, 7, 15, 27, 29, 31). Mice can be protected from fatal pneumococcal infection with mouse monoclonal antibodies (MAb) to the phosphocoline (PC) and non-PC determinants of pneumococcal teichoic acids (9, 11, 14, 15, 28, 30, 39, 43). Antibodies to PC isolated from human serum have been shown to protect mice against fatal type 3 *Streptococcus pneumoniae* infection (11). Human C-reactive protein, which also reacts with the PC determinant of pneumococcal teichoic acids, has also been shown to extend the life of mice infected with pneumococci (11, 25, 32, 44).

Antibodies to other somatic antigens can also protect against pneumococcal infection. Mouse MAb reactive with pneumococcal surface protein A (PspA) can protect mice against otherwise fatal infection with *S. pneumoniae* (11, 29, 31). Immunity to the pneumococcal enzymes pneumolysin and neuraminidase have been shown to extend the life of infected mice (7, 27, 35).

The most protective mouse antibodies to PC are of the T15 idiotype (12, 41). Among T15 antibodies to PC, the immunoglobulin G (IgG) MAb were more protective against fatal sepsis than those of the IgM isotype. IgA antibodies were not protective (9). T15 IgG MAb of the IgG1, IgG2b, IgG2a, and IgG3 subclasses all exhibited similar protective capacities (11, 13, 14). It has been shown that antibody to PC can protect mice against fatal pneumococcal infection following intraperitoneal (i.p.) or intravenous (i.v.) challenge (11, 39, 42). It was demonstrated that MAb to PC could protect against three of three capsular type 3 strains, a single type 4 strain, and a single type 6A strain. Protection was not observed against individual type 1, type 5, and type 6B pneumococcal strains (39, 43). The discovery that antibodies to PC could protect against pneumococcal infection (9) was intriguing because PC is present on all pneumococci (17).

In spite of the studies described above, the degree to which antibodies to PC might contribute to resistance against pneumococci in general was not known. The studies with the type 1 and type 6B strains utilized only an IgM MAb to PC (39). Whether protection against these strains might have been observed with a more protective IgG MAb to PC (9, 28) was not known. Most of the studies that have examined the protective effects of antibody to PC have used X-linked immunodeficient (XID) mice, raising the possibility that antibody to PC may be more important in these unusual mice than in immunologically normal animals. Furthermore, the strains used in previous studies were all mouse-passed laboratory strains selected for their ability to cause fatal bacteremia in mice. Since most pneumococcal strains are not highly virulent in mice (10) it was possible that mouse passage might select for traits not commonly expressed in fresh human isolates. If mouse passage selected for traits that increased the likelihood that the strains would be susceptible or resistant to the protective effects of antibody to PC, then the results observed with mouse-passed strains might not be representative of human isolates in general.

In the present study, we evaluated the ability of IgG3 MAb

* Corresponding author.

TABLE 1. Ability of antibody to PC to protect against individual pneumococcal isolates injected i.v.

Strain	Type	Origin of isolate (site[b]/diagnosis[c])	Buoyant density[d]	Median day of death						LD_{50} in BALB/c mice
				CBA/N (300 CFU)			BALB/cJ (10^6 CFU)			
	Caps/PspA[a]			No antibody	anti-PC	P[e]	No antibody	anti-PC	P[e]	
ATCC 10813	3/PA3	mp	1.013	2 (0)[f]	>21 (100)	0.0005	2 (0)	>21 (75)	0.025	$<10^2$
A66	3/PA13	mp	1.019	2 (10)	>21 (100)	0.0005	2 (0)	>21 (100)	0.025	ND[g]
EF10197	3/PA18	N/C	1.016	2 (17)	>21 (100)	0.01	3 (0)	>21 (75)	0.0005	10^3
WU2	3/PA1	mp	1.016	2 (0)	>21 (100)	0.025	2 (0)	>21 (100)	0.025	10^5
BG7649	4/PA9	B/S	1.026	2 (0)	5.5 (0)	0.025	2.5 (0)	3.5 (0)	NS	
BG9739	4/PA26	B/S	1.028	2 (0)	2 (0)	NS	3 (0)	14.5 (50)	0.0005	$<10^2$
EF3296	4/PA20	N/O	1.029	2 (0)	3 (0)	0.01	3 (13)	3 (0)	NS	$<10^2$
L81905	4/PA23	B/P	1.023	—[h]	—		2 (0)	2 (0)	NS	$<10^2$
BG5-8A	6A/PA0	N/U	1.022	5.5 (0)	5.5 (0)	NS	—	—		10^3
DBL6A	6A/PA19	mp	1.021	9 (0)	20 (50)	0.0025	4 (0)	9 (0)	0.05	$<10^2$
BG25-9	6B/PA0	N/C	1.033	2 (0)	>21 (100)	0.025	—	—		ND
BG7322	6B/PA24	B/O	1.019	3 (0)	14.5 (25)	0.001	—	—		ND
BG9163	6B/PA21	B/O	1.031	—	—		4 (21)	16 (50)	0.01	$<10^2$
DBL1	6B/PA0	mp	1.030	6.5 (0)	>21 (83)	0.0025	6 (23)	11 (42)	0.05	$<10^2$

[a] Capsular type/PspA type; all PspA types are designated by the prefix PA.
[b] Site of isolation of pneumococcal isolate. B, Blood; N, nasopharynx; C, cerebrospinal fluid; mp, mouse passed.
[c] Clinical diagnosis. C, carriage; M, meningitis; O, otitis media; P, pneumonia; S, septicemia; U, upper respiratory infection; mp, mouse passed.
[d] The density of rough *S. pneumoniae* Rx1 is 1.058.
[e] Statistically different from no antibody at P less than the value shown; NS, not significant at $P < 0.05$. Statistics calculated by two-sample rank test and expressed as one-tailed P values.
[f] Numbers in parentheses indicate percent mice alive at 21 days postinfection.
[g] ND, not done.
[h] —, Strain was not highly virulent in the mouse strain infected (less than 76% mortality) (10), and a protection study was not conducted.

to PC to protect against fatal infections caused by type 3, 4, 6A, and 6B pneumococci. Nine recent human isolates of *S. pneumoniae* virulent for mice and five mouse-passed laboratory strains were examined. The nine recent human isolates were selected as the most virulent strains for mice from 69 clinical isolates of type 1, 3, 4, 6A, 6B, 14, 19, and 23 pneumococci (10). Immunologically normal BALB/cJ mice and immune deficient (XID) CBA/N mice were used in these experiments because they represent extremes of resistance and susceptibility to pneumococcal infection (10, 14).

In the first half of our study we examined the ability of IgG3 MAb to PC to prevent fatal infection following i.v. inoculation of all 14 isolates. Parenteral inoculation of mice with most virulent pneumococci results in blood bacteremia and generalized sepsis. We felt that i.v. challenge would avoid potential complications that might arise from bacteria growing in sites not exposed to the filtering action of the reticuloendothelial system.

Since i.p. infections have classically been used in mouse protection studies, 12 of the isolates were also studied by using i.p. challenge so that we could compare results obtained by the i.v. and i.p. routes. We previously observed that passive MAb to PC could protect against type 3 strain WU2 whether the pneumococci were injected by the i.p. or i.v. route (11).

MATERIALS AND METHODS

Pneumococcal strains. Fourteen capsular type 3, 4, 6A, and 6B strains of pneumococci were used in this study. Nine of the 14 strains were the most virulent strains identified among 69 recent fresh human isolates of pneumococci (10). All of these strains killed greater than 76% of CBA/N or BALB/cJ mice infected with 300 or 10^6 CFU, respectively. Five laboratory strains (DBL1, serotype 6B; DBL6A, type 6A; A66, type 3, ATCC 10813, type 3; and WU2, type 3) that had been mouse passed and were already known to be virulent in mice (10, 15, 42, 43) were also examined. Determination of capsular and PspA types for these strains was described previously (10, 18). Two strains (DBL1 and BG25-9) were capsular type 6B and PspA type PA0. Their DNA was cut with *Hin*dIII and examined by Southern blotting with a full-length *pspA* probe at a stringency of about 98% (37) to see if the strains might be members of the same clone. Detected restriction fragments of BG25-9 were 3.5 and 4.6 kb long, and those of DBL1 were 3.0 and 4.0 kb long. Thus, on the basis of capsular type, PspA type, and Southern blotting, the strains were all observed to be different and were assumed to be clonally distinct (Table 1). The buoyant densities of these pneumococci was determined in a previous study (10) by a procedure modified from that of Hakansson et al. (22). Comparisons of density among strains of the same capsular type should be related to the degree of encapsulation (10, 22). However, since the extent of hydration is dependent on the composition of the capsule and not just the amount of polysaccharide, differences in density of strains of different capsular types may not necessarily be an indication that the strains differ in the amount of capsular polysaccharide.

Pneumococci for injection were prepared as previously described by growth in Todd-Hewitt medium containing 0.5% yeast extract. Pneumococci were harvested in late log phase and suspended in lactated Ringer's solution. Numbers of bacteria injected were estimated by A_{420} and confirmed by plating (15).

Mouse protection experiments. Female 6- to 8-week-old BALB/cJ and CBA/N mice were obtained from Jackson Laboratory, Bar Harbor, Maine. Mice were kept in our animal facilities for 2 to 4 weeks prior to use. Except as indicated, groups of 4 to 15 mice were infected i.v. or i.p. with 0.2 ml of lactated Ringer's solution containing the indicated numbers of pneumococci 1 h after i.p. injection of

20 μg of the IgG3 anti-PC MAb 59.6C5 (1, 11) or sterile saline. Deaths were recorded daily for a period of 21 days. All mice alive at the end of this period were considered to have survived the challenge infection. Fifty percent lethal dose (LD_{50}) determinations were made by first injecting small numbers of mice with 5, 50, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, or 10^8 *S. pneumoniae* cells and monitoring the mice for 21 days. Next, groups of at least five mice were injected with the two doses adjacent to the suspected LD_{50}. LD_{50} values were determined graphically by the method of probits from the percentages of mice surviving at the doses just above and just below the LD_{50} (8).

Statistics. Comparisons of the time of death of groups of mice were made using the two-sample rank test (45). The *P* values were calculated by using a one-tailed table since we were testing a previous hypothesis that antibody to PC protected mice. In no case did anti-PC antibody decrease the time to death. Comparisons of protection against death used the chi-square test corrected for continuity by the method of Cochran (45).

RESULTS

i.v. route of challenge. The ability of IgG3 MAb to PC to protect against i.v. pneumococcal infection was examined by using both CBA/N and BALB/cJ mice. CBA/N mice were injected with 300 CFU. BALB/cJ mice were injected with 10^6 CFU to compensate for their greater resistance relative to that of CBA/N mice to pneumococcal infection (10, 14, 15, 43). Not all 14 pneumococcal strains were highly virulent in both mouse strains, and as a result not all of the 14 strains were tested in each of the mouse strains. Passive protection with MAb to PC increased the percentage of mice surviving and/or the median survival time of mice infected with most of the 14 strains (Table 1). For 10 of the 12 strains used to infect CBA/N mice and 6 of the 9 strains used to infect BALB/cJ mice, the increase in median survival time was statistically significant. LD_{50}s were determined for nine of the strains. For eight of them the LD_{50}s were observed to be $\leq 10^3$ CFU. For five of these eight strains, MAb to PC resulted in an increase in the LD_{50} of at least 1,000.

The extent of protection appeared to be related to the capsular type of the isolate (Table 1). The MAb to PC always protected mice infected with type 3 strains against death. With the capsular type 4 strains, passive MAb to PC generally either had no measurable effect or resulted in only a slight increase in survival time. The only exception was infection of BALB/cJ mice with type 4 strain BG9739, when half of the anti-PC antibody-treated mice lived. The effects of MAb to PC on the survival of mice infected with type 6A and 6B pneumococci were variable and frequently intermediate to the results obtained with type 3 and type 4 pneumococci. There was no significant difference in the ability of MAb to PC to protect against recent clinical isolates versus mouse-passed laboratory strains of the same capsular type. In no case was treatment with anti-PC MAb associated with higher mortality or more rapid death of the infected groups of mice.

Nine of the strains were tested in both CBA/N and BALB/cJ mice. For six strains (ATCC 10813, A66, EF10197, WU2, DBL6A, and DBL1) MAb to PC resulted in increased survival time and protection against death in both inbred lines of mice. For two strains (BG7649 and EF3296) passive MAb to PC was protective in CBA/N but not in BALB/cJ mice, and for one strain (BG9739) it was protective in BALB/cJ but not in CBA/N mice (Table 1).

TABLE 2. Protective effects of antibody to PC in BALB/cJ mice challenged i.v. with pneumococci

Strain	Capsular type	PspA type	Median day of death (300 CFU)[a]	
			No antibody	Anti-PC (20 mg)
EF3296	4	20	2.2 (13)[b]	4[c] (20)
L81905	4	23	2.5 (0)	>30[c] (57)
DBL6A	6A	19	10 (0)	14.5[c] (0)
BG9163	6B	21	13.5 (0)	>30[c] (75)

[a] Data based on four to six animals per group.
[b] Numbers in parentheses indicate percent alive at 21 days postinfection.
[c] Significant increase in days to death or survival with antibody at $P < 0.025$ by two-sample rank test.

Relationship between virulence after i.v. injection and protective effects of anti-PC antibody. Since the isolates most difficult to protect against were among the strains with the lowest LD_{50}s in BALB/cJ mice, it was possible that our failure to protect against some of the *S. pneumoniae* strains was because they were more virulent than the strains we could protect against. Since several of the strains used to infect BALB/cJ mice were virulent at doses far less than 10^6 CFU, we examined the possibility that even strains we could not protect against when injected into BALB/cJ mice at 10^6 CFU would be affected by MAb to PC when injected into mice at lower numbers. To test this, BALB/cJ mice were infected with 300 CFU of four strains whose LD_{50}s were less than 100 CFU (Table 2).

One strain examined was type 6A strain DBL6A, which when injected at a dose of 10^6 CFU had killed anti-PC antibody-protected mice but only after an anti-PC-mediated extension of survival of about 5 days. When only 300 CFU of DBL6A was injected, anti-PC MAb still failed to protect against death and the anti-PC-mediated extension of time to death was still only about 5 days. Another strain examined was type 6B strain BG9163, against which MAb to PC had protected only half of the mice infected with 10^6 CFU. Antibody to PC provided complete protection against death following injection of 300 CFU of BG9163. The two remaining strains were type 4 isolates that had completely resisted the protective effects of MAb to PC when 10^6 CFU was injected into BALB/cJ mice. Antibody to PC provided complete protection against death caused by infection with 300 CFU of one of the type 4 strains and significantly extended the time to death of mice infected with the other.

Protection against i.p. infection. Experiments using i.p. challenge were performed with BALB/c mice and 12 pneumococcal strains. These strains had been identified previously as being virulent when injected i.p. into BALB/cJ mice (10). Because the i.v. injection experiments discussed above had revealed strong effects of challenge dose on the ability of anti-PC MAb to protect, the i.p. studies included challenge with a range of doses and LD_{50}s were determined in the presence and absence of MAb to PC. For 9 of the 12 strains, administration of MAb to PC was associated with an increase in the LD_{50} (Table 3). For eight of these strains the protective effects of MAb to PC were statistically significant. For six of the strains, the increase in the LD_{50} was 100-fold or more.

For capsular type 3 and type 4 strains the protective effects of anti-PC MAb were more apparent with i.v. (Tables 1 and 2) than i.p. (Table 3) challenge. With all three capsular type 3 strains injected by both the i.v. and i.p. routes we

TABLE 3. Ability of antibody to PC to protect BALB/cJ mice against *S. pneumoniae* injected i.p.

Strain	Capsular type	PspA type	Log_{10} LD_{50}[a]		Log_{10} CFU injected	Median no. of days to death[a]		No. alive/total[a]		P[b] (+ vs −)
			−	+		−	+	−	+	
ATCC 10813	3	3	<1	4	2	1.5	>21	2/12	4/6	0.05
					6	1.5	2	0/4	0/4	
A66	13	13	<2	4.5	2	2	>21	0/9	8/9	0.001
					6	2	2	0/5	0/4	
EF10197	3	18	<1	4.4	2	2	>21	0/9	9/9	0.001
					4	2	>21	2/12	5/6	0.05
					6	2	2.5	0/4	1/4	
WU2	3	1	2	5.2	2	12[c]	>21	2/4	4/4	
					4	3	>21	4/13	10/12	0.025
					6	2	2.5	0/4	0/4	
BG9739	4	26	<2	<2	2	2	2	0/2	0/6	
EF3296	4	20	<1	1	2	2	3	0/4	1/4	
L81905	4	23	<2	<2	2	2	2	0/4	0/4	
DBL6A	6A	19	<2	>4	2	6	>21	3/9	6/11	
					4	13	>21	1/9	7/9	0.005
BG25-9	6B	0	6	>8	2	12	>21	1/5	4/4	0.05
					6	13	>21	3/9	6/7	0.05
BG7322	6B	24	<2	>6	2	7	>21	0/5	4/4	0.025
					6	3	>21	1/6	5/6	0.05
BG9163	6B	21	2–7[d]	>7	2	5.5	>21	1/4	4/4	0.05
					6	10	>21	3/7	7/7	0.025
DBL1	6B	0	2–6[d]	>6	2	13	>21	3/7	4/5	
					6	20	>21	2/4	4/6	

[a] Without (−) and with (+) antibody to PC.
[b] Difference is survival of mice with and without antibody to PC at P less than the value shown. Statistics calculated by Cochran-corrected chi-square test. Where no P value is shown the result was not statistically significant ($P > 0.05$).
[c] Of the four mice in this group, two died by day 3 and two were alive at day 21.
[d] About 50% of the infected mice died over the range of doses indicated.

were able to observe protection against death with higher numbers of CFU injected i.v. than i.p. For the three capsular type 4 strains injected by both routes there was no evidence of anti-PC antibody protection with i.p. challenge, whereas MAb to PC protected against death or extended survival when mice were infected i.v. with the same three strains. The opposite was seen with the type 6A and 6B strains: MAb to PC appeared to be more protective when injections were given i.p. rather than i.v. With i.v. challenge, MAb to PC caused some increase in the number of survivors of 6A and 6B infections but generally only extended days to death, whereas with i.p. challenge MAb to PC protected mice from fatal infection with four of the five strains examined.

Differences in amount of capsule do not explain the differences in resistance to the protective effects of MAb to PC. The specific gravity of pneumococci is largely dependent on the amount of hydration of the capsule, which for any capsule type is a function of the amount of capsular polysaccharide (10, 22). We have found no associations between the specific gravity of the individual strains (Table 1) and the ability of MAb to PC to protect against fatal infection by either the i.p. or i.v. route (Tables 1 and 3).

Lack of association between protective effects of anti-PC antibody and disease of patients. We knew the disease diagnosis of the patients and the tissue source (Table 1) for the nine recent isolates described in Tables 1 and 3. No association was observed, however, between the abilities of the isolates to resist the protective effects of MAb to PC and either the tissue sources or the disease diagnoses of the patients from which the isolates were obtained.

DISCUSSION

Antibody to PC was able to enhance the survival of mice infected with most of the pneumococci tested. Its effects were dependent on route of infection and capsular type, as well as on unknown variables associated with individual strains. Of the four capsular types studied, type 3 pneumococci were most readily protected against death and type 4 pneumococci were the most refractory to the protective effects of MAb to PC. We were able to protect against higher numbers of type 3 and type 4 pneumococci when they were injected i.v. than when they were injected i.p. The opposite was observed with type 6A and 6B pneumococci: protection against death was more readily demonstrated following i.p. rather than i.v. challenge.

No differences were observed between the ability of MAb to PC to protect against infection with laboratory strains and clinical isolates of the same capsular types. By combining the present results with the results of previous studies that examined individual laboratory strains (11, 15, 29, 39, 43), a broader picture of the protective effects of antibody to PC is obtained. Protection with MAb to PC has been observed with strains of capsular types 2, 3, 4, 6A, 6B, and 27. In the case of type 4 strains the protective effects of anti-PC antibody were observed only with low numbers of CFU injected i.v. No protection has been observed against a single type 5 strain (39, 43). C-reactive protein, which recognizes the PC epitope of pneumococcal teichoic acids, has been shown to protect against type 3, 4, and 27 pneumococci (11, 25, 32, 44). In type 27 pneumococci, the PC is a component of the capsular polysaccharide (6). In all of the other capsular types, the capsular polysaccharide does not

contain PC (26) and the protective antibodies must react with the PC on the teichoic and lipoteichoic acids of the pneumococci (16, 17).

The differences in the protective effects of MAb to PC are probably not differences in the ability of antibodies to PC to bind pneumococci in the presence of different capsules. Earlier studies showed no effect of capsule type on the binding of MAb to PC and no relationship between the amount of anti-PC MAb bound and the ability of the strain to resist the protective effects of MAb to PC (14, 43). Although the reason that antibodies to PC show different abilities to protect against different capsular types is not known, one possibility is that complement fixed by antibodies binding the teichoic acids of some strains may be more exposed to phagocytes by some capsular types than others. Another possibility is intrinsic differences in the abilities of the different capsular types to bind active complement components or inhibitors of complement activation.

The present study revealed no association between protective effects of MAb to PC and the degree of encapsulation (as measured by buoyant density) of the mouse-virulent strains. Thus, the virulence of the strains tested was probably not limited by their amounts of capsule. Presumably, differences in noncapsular pneumococcal virulence factors (possibly including PspA, pneumolysin, or neuraminidase) are able to have an effect on the relative ability of antibodies to PC to protect against different strains of the same capsular type.

Past observations that MAb to PC can be protective against pneumococcal infection (9, 11, 14, 15, 28, 30, 39, 43) have not been universally accepted (24, 34, 38), and not all studies have reported a protective effect of antibodies to PC (5, 34, 40). We suspect that the failure of some studies to detect protective effects of antibodies reactive with PC is largely because higher concentrations of antibody to PC than antibody to capsule are required for protection (9, 11, 28, 39, 42) and antibody to PC results in much slower blood clearance of *S. pneumoniae* than does antibody to capsule (11, 28). The reason that anti-PC antibodies are generally less protective than antibodies to the capsule is thought to be because the PC determinants are largely buried by capsular polysaccharides (38, 43). Unlike antibodies that react with the pneumococcal capsule, antibodies to PC are not very effective in a blood bactericidal assay (where neutrophils effect antibody-dependent killing of pneumococci), even though the same antibodies can protect against death (11). We have interpreted this to mean that the killing mechanisms mediated by antibodies to PC are much more efficient in vivo than in vitro (11).

In mice it is clearly possible to produce nonprotective antibodies to PC. Murine antibodies to PC exist in three major idiotype families (T15, M603, and M511) that are distinguished by the V_L region used in the formation of the antibody binding sites (1, 21). Only antibodies of the T15 idiotype are highly protective (12). Since it is clear that not all antibodies reactive with PC are protective, it seems quite possible that not all species would necessarily make antibodies to PC that could protect against pneumococcal infection.

Although antibodies to PC are not as protective on a weight basis as antibodies to capsule (9, 28), antibodies to PC present in normal mouse sera have been shown to be a major factor in the resistance of mice to infection with pneumococci (9). Antibodies to PC may be able to retard pneumococcal infections long enough to permit the host to make an effective immune response to other pneumococcal antigens or to permit a physician to administer antibiotics. Whether antibodies to PC actually play such a role in humans is not known. The single demonstration that naturally occurring human antibody to PC can protect against pneumococcal infection revealed that the isolated antibody was only about 1/10 as protective as the mouse IgG antibody to PC (11). The isotype of the human antibody to PC was not determined, and it is not known whether humans can make binding sites for PC that result in antibodies as protective as mouse IgG antibodies of the T15 idiotype.

ACKNOWLEDGMENTS

We are grateful to Zhengfang Liang for assistance with pneumococcal typing and to Barry Gray for many helpful discussions.

This study was supported by National Institutes of Health grants AI13357, AI21548, and HD17812.

REFERENCES

1. **Andres, C. M., A. Maddalena, S. Hudak, N. M. Young, and J. L. Claflin.** 1981. Anti-phosphocholine hybridoma antibodies. II. Functional analysis of binding sites within three antibody families. J. Exp. Med. **154**:1584–1598.
2. **Au, C. C., and T. Eisenstein.** 1981. Nature of the cross-protective antigen in subcellular vaccines of *Streptococcus pneumoniae*. Infect. Immun. **31**:160–168.
3. **Austrian, R.** 1979. Pneumococcal vaccine: development and prospects. Am. J. Med. **67**:547–549.
4. **Austrian, R., and J. Gold.** 1964. Pneumococcal bacteremia with special reference to bacteremic pneumococcal pneumonia. Ann. Intern. Med. **60**:759–776.
5. **Avery, O. T., and W. F. Goebel.** 1933. Chemoimmunological studies of the soluable specific substance of pneumococcus. I. The isolation and properties of the acetyl polysaccharide of pneumococcus type 1. J. Exp. Med. **58**:731–755.
6. **Bennett, L. G., and C. T. Bishop.** 1977. Structure of the type XXVII Streptococcus pneumoniae (pneumococcal) capsular polysaccharide. Can. J. Chem. **55**:8–16.
7. **Berry, A. M., J. Yother, D. E. Briles, D. Hansman, and J. C. Paton.** 1989. Reduced virulence of a defined pneumolysin-negative mutant of *Streptococcus pneumoniae*. Infect. Immun. **57**:2037–2042.
8. **Boyd, W. C.** 1966. Fundamentals of immunology, 4th ed., p. 773. Interscience Publishers, New York.
9. **Briles, D. E., J. L. Claflin, K. Schroer, and C. Forman.** 1981. Mouse IgG3 antibodies are highly protective against infection with *Streptococcus pneumoniae*. Nature (London) **294**:88–90.
10. **Briles, D. E., M. J. Crain, B. M. Gray, C. Forman, and J. Yother.** 1992. Strong association between capsular type and mouse virulence among human isolates of *Streptococcus pneumoniae*. Infect. Immun. **60**:111–116.
11. **Briles, D. E., C. Forman, J. C. Horowitz, J. E. Volanakis, W. H. Benjamin, Jr., L. S. McDaniel, J. Eldridge, and J. Brooks.** 1989. Antipneumococcal effects of C-reactive protein and monoclonal antibodies to pneumococcal cell wall and capsular antigens. Infect. Immun. **57**:1457–1464.
12. **Briles, D. E., C. Forman, S. Hudak, and J. L. Claflin.** 1984. The effects of idiotype on the ability of IgG_1 antiphosphorylcholine antibodies to protect mice from fatal infection with *Streptococcus pneumoniae*. Eur. J. Immunol. **14**:1027–1030.
13. **Briles, D. E., C. Forman, S. Hudak, and J. L. Claflin.** 1984. The effects of subclass on the ability of anti-phosphocholine antibodies to protect mice from fatal infection with *Streptococcus pneumoniae*. J. Mol. Cell. Immunol. **1**:305–309.
14. **Briles, D. E., J. Horowitz, L. S. McDaniel, W. H. Benjamin, Jr., J. L. Claflin, C. L. Booker, G. Scott, and C. Forman.** 1986. Genetic control of susceptibility to pneumococcal infection. Curr. Top. Microbiol. Immunol. **124**:103–120.
15. **Briles, D. E., M. Nahm, K. Schroer, J. Davie, P. Baker, J. Kearney, and R. Barletta.** 1981. Antiphosphocholine antibodies found in normal mouse serum are protective against intravenous infection with type 3 *Streptococcus pneumoniae*. J. Exp. Med. **153**:694–705.

16. **Briles, E. B., and A. Tomasz.** 1973. Pneumococcal forssman antigen. A choline-containing lipoteichoic acid. J. Biol. Chem. **248:**6394–6397.
17. **Brundish, D. E., and J. Baddiley.** 1968. Pneumococcal C-substance, a ribitol teichoic acid containing choline phosphate. Biochem. J. **110:**573–582.
18. **Crain, M. J., W. D. Waltman II, J. S. Turner, J. Yother, D. E. Talkington, L. M. McDaniel, B. M. Gray, and D. E. Briles.** 1990. Pneumococcal surface protein A (PspA) is serologically highly variable and is expressed by all clinically important capsular serotypes of *Streptococcus pneumoniae*. Infect. Immun. **58:** 3293–3299.
19. **Dowling, H. F., L. K. Sweet, J. A. Robinson, W. W. Zellers, and H. L. Hirsh.** 1949. The treatment of pneumococcic meningitis with massive doses of systemic penicillin. Am. J. Med. Sci. **217:**149–156.
20. **Felton, L. D.** 1937. Essential immunizing antigen of pneumococci. J. Bacteriol. **33:**335–337.
21. **Gearhart, P. J., N. D. Johnson, R. Douglas, and L. Hood.** 1981. IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. Nature (London) **291:**29–34.
22. **Håkansson, S., S. E. Holm, and M. Wagner.** 1987. Density profile of group B streptococci type III, and its possible relationship to enhanced virulence. J. Clin. Microbiol. **25:**714–718.
23. **Harley, D.** 1935. Species immunity to pneumococcus. Br. J. Exp. Pathol. **16:**14–20.
24. **Henrichsen, J., and C. H. Sorensen.** 1989. The role of antibodies to pneumococcal C polysaccharide in otitis media. Pediatr. Infect. Dis. J. **8:**S26–S27.
25. **Horowitz, J., J. E. Volanakis, and D. E. Briles.** 1987. Blood clearance of *Streptococcus pneumoniae* by C-reactive protein. J. Immunol. **138:**2598–2603.
26. **Lee, C.-J., S. D. Banks, and J. P. Li.** 1991. Virulence, immunity, and vaccine related to *Streptococcus pneumoniae*. Crit. Rev. Microbiol. **18:**89–114.
27. **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.
28. **McDaniel, L. S., W. H. Benjamin, Jr., C. Forman, and D. E. Briles.** 1984. Blood clearance by anti-phosphocholine antibodies as a mechanism of protection in experimental pneumococcal bacteremia. J. Immunol. **133:**3308–3312.
29. **McDaniel, L. S., G. Scott, J. F. Kearney, and D. E. Briles.** 1984. Monoclonal antibodies against protease sensitive pneumococcal antigens can protect mice from fatal infection with *Streptococcus pneumoniae*. J. Exp. Med. **160:**386–397.
30. **McDaniel, L. S., W. D. Waltman II, B. Gray, and D. E. Briles.** 1987. A protective monoclonal antibody that reacts with a novel antigen of pneumococcal teichoic acid. Microb. Pathog. **3:**249–260.
31. **McDaniel, L. S., J. Yother, M. Vijayakumar, L. McGarry, W. R. Guild, and D. E. Briles.** 1987. Use of insertional inactivation to facilitate studies of biological properties of pneumococcal surface protein A (PspA). J. Exp. Med. **165:**381–394.
32. **Mold, C., N. Nakayama, T. J. Holzer, H. Gewurz, and T. W. D. Clos.** 1981. C-reactive protein is protective against *Streptococcus pneumoniae* infection in mice. J. Exp. Med. **154:**1703–1708.
33. **Mufson, M. A., G. Oley, and D. Hughey.** 1982. Pneumococcal disease in a medium-sized community in the United States. JAMA **248:**1486–1489.
34. **Musher, D. M., A. J. Chapman, A. Goree, S. Jonsson, D. Briles, and R. E. Baughn.** 1986. Natural and vaccine-related immunity to *Streptococcus pneumoniae*. J. Infect. Dis. **154:**245–256.
35. **Paton, J. C., R. A. Lock, C.-J. Lee, J. P. Li, A. M. Berry, T. J. Mitchell, P. W. Andrew, D. Hansman, and G. J. Boulnois.** 1991. Purification and immunogenicity of genetically obtained pneumolysin toxoids and their conjugation to *Streptococcus pneumoniae* type 19F polysaccharide. Infect. Immun. **59:**2297–2304.
36. **Shapiro, E. D., A. T. Berg, R. Austrian, D. Schroeder, V. Parcells, A. Margolis, R. K. Adair, and J. D. Clemmens.** 1991. Protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N. Engl. J. Med. **325:**1453–1460.
37. **Sheffield, J. S., W. H. Benjamin, and L. M. McDaniel.** Detection of DNA in Southern blots by chemiluminescence is a sensitive and rapid technique. BioTechniques, in press.
38. **Sorensen, U. B. S., J. Blom, A. Birch-Andersen, and J. Henrichsen.** 1988. Ultrastructural localization of capsules, cell wall polysaccharide, cell wall proteins, and F antigen in pneumococci. Infect. Immun. **56:**1890–1896.
39. **Szu, S. C., S. Clark, and J. B. Robbins.** 1983. Protection against pneumococcal infection in mice conferred by phosphocholine-binding antibodies: specificity of the phosphocholine binding in relation to several types. Infect. Immun. **39:**993–999.
40. **Szu, S. C., R. Schneerson, and J. B. Robbins.** 1986. Rabbit antibodies to the cell wall polysaccharide of *Streptococcus pneumoniae* fail to protect mice from lethal challenge with encapsulated pneumococci. Infect. Immun. **54:**448–455.
41. **Vakil, M., D. E. Briles, and J. F. Kearney.** 1991. Antigen-independent selection of T15 idiotype during B-cell ontogeny in mice. Dev. Immunol. **1:**203–212.
42. **Wallick, S., J. L. Claflin, and D. E. Briles.** 1983. Resistance to *Streptococcus pneumoniae* is induced by a phosphocholine-protein conjugate. J. Immunol. **130:**2871–2875.
43. **Yother, J., C. Forman, B. M. Gray, and D. E. Briles.** 1982. Protection of mice from infection with *Streptococcus pneumoniae* by anti-phosphocholine antibody. Infect. Immun. **36:**184–188.
44. **Yother, J., J. E. Volanakis, and D. E. Briles.** 1982. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in mice. J. Immunol. **128:**2374–2376.
45. **Zar, J. H.** 1984. Biostatistical analysis, p. 718. Prentice-Hall, Inc., Englewood Cliffs, N.J.