Peptide Mimic of Phosphorylcholine, a Dominant Epitope Found on *Streptococcus pneumoniae*

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Received 23 May 2000/Returned for modification 3 July 2000/Accepted 20 July 2000

Even in the age of antibiotics, *Streptococcus pneumoniae* causes significant morbidity, especially in the young, the elderly, and the immunocompromised. While a carbohydrate-based vaccine exists, it is poorly immunogenic in the at-risk populations. In mice, antibodies directed against phosphorylcholine (PC), an epitope present on the cell wall C polysaccharide of all pneumococcal serotypes, protect against infection. However, PC itself is a poor vaccine candidate. We report here peptide mimics of PC based on the anti-idiotypic interaction of T15 anti-PC antibodies. T15 antibodies, the dominant and protective idio type induced in mice by PC immunization, self-associate via a 24-amino-acid region in the PC binding site (ASRNKANDYTTEYSASVKGRFIVS; peptide 1). Peptide 1 has been shown to bind in the PC binding site. We demonstrated that amino acid sequences derived from peptide 1 starting at amino acid 9, 11, or 13 inhibit PC binding. Therefore, we immunized mice with bovine serum albumin (BSA) conjugates of peptide 1 or either of two selected 12-mers. The 12-mer peptides were not immunogenic. Mice immunized with peptide 1-BSA developed an anti-PC response consisting mainly of immunoglobulin G1 and expressed the T15 heavy chain. Nonetheless, neither BALB/c nor CBA/N mice were protected from lethal pneumococcal infections by immunization with peptide 1-BSA. Preliminary data suggest that peptide 1-BSA is not able to elicit the canonical T15 light chain, explaining the absence of protection. This idio type-derived mimotope of PC is a useful tool for understanding immunologic cross-reactivity and learning to design T-cell-dependent vaccines for *S. pneumoniae*.

Streptococcus pneumoniae is a major infectious agent in humans and a significant cause of morbidity in the young, the elderly, and the immunocompromised (14, 16). Despite antibiotics, mortality due to pneumococcal bacteremia remains high (15). Of increasing concern is the growing number of antibiotic-resistant organisms among clinical isolates (3). Pneumovax, a polysaccharide vaccine for *S. pneumoniae*, does not elicit a B-cell memory response and is poorly immunogenic in those most at risk of serious disease (38, 42). Furthermore, there are at least 90 different serotypes of *S. pneumoniae*, and most of the protective anti-capsular polysaccharide antibodies are specific for a single serotype (20, 30). In contrast, the cell wall polysaccharide is relatively invariant across serotypes (43). Antibodies directed against phosphorylcholine (PC), a major epitope on the cell wall polysaccharide, protect against pneumococcal infections in mice (6, 23); the role of anti-PC antibodies in humans, however, remains controversial (13).

A growing number of reports describe the use of peptide mimics of carbohydrates and other nonprotein molecules in generating an immune response (2, 19, 21, 39, 40, 45, 48). In general, two approaches have been used to identify peptide mimics; peptide mimotopes either have been deduced from the interactions between anticarbohydrate antibodies and monoclonal anti-idiotypic antibodies or have been discovered by screening phage-displayed peptide libraries with monoclonal anticarbohydrate antibodies. Exploiting idio type:anti-idio type interactions to determine potential peptide mimics is attractive since the existence of an idio typic interaction demonstrates that the antibody of interest is capable of interacting with both

the cognate (nonprotein) antigen and proteins; however, only a subset of anti-idiotypic antibodies carry an internal image. Westerink et al. modeled an idio typic interaction with an antimeningococcal antibody in order to design a peptide mimic of meningococcal carbohydrate (22, 48). Mice immunized with this peptide mimic were protected from bacterial infection, proving that an idio typic interaction can be reduced to a peptide.

Immunization of BALB/c mice with PC induces one major idio type, T15. T15 antibodies have the interesting and unique property of self-association. The self-association site has been shown to span a 24-amino-acid region in the PC binding site (ASRNKANDYTTEYSASVKGRFIVS; peptide 1). Kang et al. demonstrated that peptide 1 inhibited PC binding by binding in the antigen binding site; thus, peptide 1 was considered a potential peptide mimic of PC (24). We tested a nested set of 12-mer peptides (peptides 2 to 8, spanning amino acids 1 to 12, 3 to 14, 5 to 16, 7 to 18, 9 to 20, 11 to 23, and 13 to 24, respectively) for their ability to block binding of PC by a panel of anti-PC monoclonal antibodies in order to determine the minimal peptide size and the specific 24-mer peptide residues needed for mimicry. C-terminal peptides (i.e., peptides 6, 7, and 8, beginning at amino acids 9, 11, and 13, respectively) were shown to bind at or near the PC binding site.

Based on these data, we immunized mice with peptide 1, 7, or 8 coupled to bovine serum albumin (BSA). Peptide 7- and peptide 8-BSA failed to elicit anti-PC antibodies. In contrast, the anti-PC antibodies elicited by peptide 1-BSA expressed the canonical T15 heavy chain but did not appear to express the canonical T15 light chain, thus demonstrating that peptide 1-BSA elicited an idio typic profile different from that elicited by PC-BSA. PC-BSA and peptide 1-BSA elicited antibodies of different isotypes. Peptide 1-BSA elicited a significant anti-PC immunoglobulin G (IgG) response consisting of mainly IgG1

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and some IgG_{2a}; no IgM nor IgG3 activity was noted. In contrast, PC immunization elicits mainly IgM, IgG3, and some IgG1 (8, 11). Interestingly, mice immunized with peptide 1-BSA were not protected from lethal pneumococcal infection even though anti-PC antibodies expressing the T15 heavy chain were present. The inability of peptide 1-BSA to elicit the canonical T15 light chain may account for this lack of protection. This idotype-derived mimotope of PC will serve as a lead compound for the development of protective, T-cell-dependent vaccines for *S. pneumoniae* and other PC-containing pathogens and will be a useful tool for gaining an understanding of both immunologic cross-reactivity and the structural requirements for immune protection.

MATERIALS AND METHODS

Peptides with N-terminal acetates and C-terminal amides were synthesized by Research Genetics (Huntsville, Ala.). BSA, glutaraldehyde, and PC chloride were purchased from Sigma (St. Louis, Mo.). PC-BSA was synthesized according to the method of Chesebro and Metzger (7). Mice were purchased from Jackson Laboratory (Bar Harbor, Maine). Secondary antibodies were purchased from Sigma, Southern Biotech (Birmingham, Ala.), or Zymed (South San Francisco, Calif.). Rat anti-T15 monoclonal antibodies T139 and TC54 were generous gifts from Matthew Scharff.

Conjugation. BSA (5 mg) was dissolved in a 0.1 M sodium citrate solution (pH 5.5; 500 μ l) and mixed with peptide (1, 7, or 8; 5 mg) in 0.1 M sodium citrate (pH 5.5; 500 μ l) to provide a BSA:peptide ratio of 1:25 (for peptide 1) or 1:50 (for peptides 7 and 8). Glutaraldehyde (0.1%) was added, and the solution was incubated for 1 h at room temperature. The reaction mixture was dialyzed against phosphate-buffered saline (PBS) for 5 days at 4°C.

Immunizations. Members of groups of 6-week-old female BALB/c or CBA/N mice (Jackson Laboratories) were initially immunized with 100 μ g of the peptide- or PC-BSA conjugate, or with BSA alone, in complete Freund's adjuvant H37Ra (DIFCO); for the booster immunizations, performed on day and day 42, incomplete Freund's adjuvant was used. The mice were bled before each immunization, 2 weeks after the final immunization, and 1 week before pneumococcal infection.

Antibody purification. The day 57 postimmunization sera from peptide-BSA-immunized mice were pooled, diluted with an equal volume of phosphate buffer (0.1 M, pH 8), and batch adsorbed with PC-Sepharose (Pharmacia, Piscataway, N.J.). Bound antibodies were eluted with PC chloride (10 mM in Tris-buffered saline) and dialyzed against PBS overnight at 4°C to remove bound PC.

The non-PC-binding fraction (i.e., the supernatant from the PC-Sepharose) was batch adsorbed to protein G-Sepharose (Pharmacia). Bound antibodies were eluted with 0.5 M glycine buffer (pH 3) containing 0.15 M NaCl for 5 min and added to one-half volume of Tris buffer (2 M, pH 8).

ELISAs. For enzyme-linked immunosorbent assays (ELISAs), microwells were coated with antigen overnight at 4°C, using a 20- μ g/ml solution of PC-BSA or BSA or a 5- μ g/ml solution of C polysaccharide (Statens Serum Institut, Copenhagen, Denmark). The T15-positive monoclonal antibodies PC2 (μ , γ 2a, and γ 2b), PC1.4.1 (γ 1), and M4.37 (γ 3) were used to generate standard curves. Isotype-specific or total IgG goat anti-mouse secondary antibodies were used for ELISA development. Peptides were coated at a concentration of 10 μ M, and peptide DRIPMDYWGQGSVTSS was used as a control.

Wells were washed with PBS-0.05% Tween 20 and blocked with Blotto (5% milk powder in Tris-buffered saline) for 1 h at 37°C. Dilution buffer (1% BSA-0.05% Tween 20-PBS) was used to block C-polysaccharide-coated plates. Pre-immunization sera from groups of mice were pooled together. Sera were preincubated in 5% BSA for 1 h at room temperature and then serially diluted 1:2 into ELISA wells containing 5% BSA prior to incubation for 2 h at 37°C. Secondary antibodies conjugated with alkaline phosphatase were used at appropriate dilutions and incubated for 1 h at 37°C. ELISA wells were developed with *p*-nitrophenyl phosphate, and the absorbance of each well at 405 nm was determined. The optical densities (ODs) of BSA-coated wells were subtracted from the ODs of PC-BSA-coated wells to account for any residual BSA-binding activity.

For C polysaccharide binding activity, postimmunization sera from mice immunized with peptide 1-, peptide 7-, or peptide 8-BSA were pooled together, tested for their ability to bind to microtiter wells coated with C polysaccharide, and arbitrarily assigned a value of 100 U/ml. Sera from individual mice were initially diluted 1:50 and then serially diluted 1:3 in order to determine the amount of C polysaccharide binding activity compared to that of the pooled postimmunization sera.

For determination of T15 anti-PC activity, rat anti-T15 monoclonal antibodies (T139 or TC54) were added after incubation of sera in PC-BSA-coated wells; the plates were then incubated for 1 h at 37°C. PC1.4.1 was used to generate standard curves. Bound rat anti-T15 was detected with biotinylated goat anti-rat antibody (heavy and light chains) followed by an alkaline phosphatase-streptavidin conjugate.

TABLE 1. IC₅₀s of PC and potential peptide mimics of PC^a

Anti-PC monoclonal antibody	IC ₅₀ (mM) of:				PC
	Peptide				
	1 ^b	6 ^c	7 ^d	8 ^e	
M4.37.3 (γ3)	NI ^f	2.5	8.8	0.9	0.9
PC2 (μ)	1.7	NI	2.7	5	0.03
T15 (α)	0.62	5.4	0.32	<0.625	0.5
180.2E3.4 (γ1)	NI	5	<0.125	0.125	0.4

^a 12-mer peptides beginning at amino acid positions 1, 3, 5, and 7 were tested and did not reach 50% inhibition even at a 10 mM peptide concentration. IC₅₀, 50% inhibitory concentration.

^b The sequence of peptide 1, N terminus to C terminus, is ASRNKANDYT TEYSASVKGRFIVS.

^c Amino acids 9 to 20.

^d Amino acids 11 to 22.

^e Amino acids 13 to 24.

^f NI, no inhibition; 50% inhibition was not achieved.

Opsonization. Serum samples were threefold serially diluted in opsonization buffer (1.2 mM CaCl₂, 0.5 mM MgCl₂, 0.1% gelatin, and 10% fetal bovine serum in Hanks' balanced salt solution) in round-bottom microwells (Costar, Cambridge, Mass.). Ten microliters (about 100 CFU) of a pneumococcus strain SP85 (serotype 6A) culture was added to each of the wells, and the plates were incubated for 15 min at 37°C. Ten microliters of rabbit complement (Pelfreeze) and 40 μ L of a HL-60 cell suspension (differentiated to granulocytes with dimethyl formamide; 10⁷ cells/ml in opsonization buffer [9]) were added to each well, and the plates were incubated at 37°C for 45 min. The plates were subsequently incubated at 37°C in a candle jar for 7 h, and the resulting colonies were counted. The opsonization titer was the reciprocal of the final serum dilution in the well which gave a 50% reduction of colonies.

Protection assay. WU-2, a virulent type 3 strain of *S. pneumoniae* (a gift from D. Briles), was used in the protection assays. Bacteria were streaked out on a blood agar plate (Becton Dickinson, Franklin Lakes, N.J.) and incubated for 18 h at 37°C in a 5% CO₂ atmosphere. An inoculum broth culture was prepared by incubating 5 to 10 colonies in Todd-Hewitt broth until the OD at 620 nm was 0.25. One-milliliter aliquots were frozen and stored at -70°C until needed. The inoculum broth was thawed and diluted in Todd-Hewitt broth for challenge. BALB/c and CBA/N mice, either naïve or immunized, were challenged intraperitoneally (i.p.) with 100 or 10 CFU, respectively. The number of CFU in the inoculum was confirmed by plating on blood agar plates.

RESULTS

C-terminal portions of a T15-derived peptide inhibit binding of PC to a panel of anti-PC monoclonal antibodies. Antibodies expressing the T15 idotype dominate the response to PC (28). These antibodies have been shown to self-associate (25). Kang et al. demonstrated that a 24-mer peptide (ASRN KANDYTTEYSASVKGRFIVS; peptide 1) spanning a portion of CDR2 and FR3 of the H chain bound at or near the PC binding site, suggesting that it might be a potential mimic of PC (24). To test this hypothesis and to determine the minimum size and specific amino acid residues needed for mimicry, we tested a nested set of 12-mer peptides (peptides 2 to 8, spanning amino acids 1 to 12, 3 to 14, 5 to 16, 7 to 18, 9 to 20, 11 to 22, and 13 to 24, respectively) for their ability to inhibit binding of PC by a panel of anti-PC monoclonal antibodies. Three of the anti-PC monoclonal antibodies, PC1.4.1 (γ 1), PC2 μ (μ), and M4.37.3 (γ 3), have the canonical T15 sequence, with M4.37.3 differing from the germ line sequence at four amino acid residues (29, 44). A fourth monoclonal anti-PC antibody, 180.2E3.4 (γ 1; a gift from J. L. Claffin) is of the M603 type and expresses the T15 heavy chain but not the light chain (1).

Peptide 1 as well as three C-terminal peptides, peptides 6, 7, and 8, inhibited binding of antibody to immobilized PC-BSA (Table 1). That these peptides also inhibited the binding of 180.2E3.4 (T15 negative) to PC-BSA suggests that they are potential PC mimics and not just T15-specific binding site

FIG. 1. Peptide 1-BSA induces an anti-PC IgG response. Preimmunization sera from groups of mice were pooled, and postimmunization sera from individual mice were titrated on microtiter wells coated with PC-BSA. The amount of anti-PC antibody was determined from standard curves generated by using the T15-expressing anti-PC IgG1 monoclonal antibody PC1.4.1 and a goat anti-mouse total IgG secondary antibody PC1.4.1 and a goat anti-mouse total IgG secondary antibody. Symbols represent postimmunization sera of individual mice. A Mann-Whitney U test returned a *P* value of 0.0396 for peptide 1-BSA-immunized mice compared to BSA-immunized mice. d, day.

reagents. N-terminal peptides did not inhibit binding of antibody to PC, suggesting that the N terminus of peptide 1 does not make essential contacts with the antibody combining site. The T15 sequence of M4.37.3 contains three point mutations, and 180.2E3.4 does not have the T15 light chain. These sequence differences may lead to unfavorable interactions with the N-terminal portion of peptide 1 and thereby account for their lack of binding. The observation that peptide 6 inhibits binding of antibody to PC while peptide 5 does not suggests that the Gly and Arg at positions 19 and 20 play an important role in antibody recognition of the peptide mimics.

Immunization of mice with T15-derived peptide mimics of PC. Based on the inhibition data, we immunized BALB/c mice with a BSA conjugate of peptide 1, 7, or 8, PC-BSA, or BSA. The use of different secondary antibodies allowed a quantitative determination of relative amounts of anti-PC antibody within an assay but not a comparison of absolute amounts between different assays. Neither peptide 7-BSA nor peptide 8-BSA was immunogenic (data not shown). Peptide 1-BSA induced an IgG PC-binding response significantly higher than that caused by BSA alone (Fig. 1). The antibodies induced by peptide 1-BSA also bound cell wall C polysaccharide, thus demonstrating their antibacterial activity (Fig. 2) (23, 43).

To confirm that the anti-PC antibodies were cross-reactive with peptide, we purified the PC-binding fraction from serum of peptide 1-BSA-immunized mice. The anti-PC antibodies from the peptide 1-BSA-immunized mice bound peptide 1 at 550 ng/ml, whereas the non-PC-binding antibodies had to be eight times more concentrated (2.5 μg/ml) before any peptide binding could be detected (Fig. 3). Thus, the anti-PC antibodies cross-reacted with peptide; presumably these cross-reactive antibodies were induced by molecular mimicry.

Two rat anti-T15 monoclonal antibodies were used to determine the amounts of T15 anti-PC antibody induced by the mimotopes (12). T139 recognizes the canonical T15 heavy- and light-chain combination (S107V1-DFL16.1-J1; V_K22-J_K5), whereas TC54 recognizes the canonical T15 heavy chain alone.

The rat anti-T15 monoclonal antibodies were used to determine the amount of T15 activity captured on PC-BSA-coated microtiter wells. The anti-PC response induced by peptide 1-BSA resulted in expression of the T15 heavy chain (Fig. 4). The amount of TC54-positive anti-PC in sera of peptide 1-BSA-immunized mice was significantly larger than that in sera of BSA-immunized mice (*P* = 0.0171 by using analysis of variance). There was no increase in T139 anti-PC antibody, suggesting that peptide 1-BSA induces the T15 canonical heavy chain but not the canonical light chain (data not shown). The PC-purified antibodies were tested for T15 expression by coating microtiter wells with these antibodies and using the rat anti-T15 monoclonal antibodies to determine the amount of T15 expression. Three percent of the PC-purified antibodies expressed either the T15 heavy chain or the T15 light chain (data not shown).

The anti-PC response elicited by PC itself is mainly IgM and IgG3 with some IgG1 (11). The isotype profile of the anti-PC response elicited by peptide 1-BSA consisted of mainly IgG1 (Fig. 5), with only one mouse producing IgG2a anti-PC activity (data not shown). Very little IgG2b activity was observed, and no IgG3 or IgM anti-PC activity was detected (data not shown). Thus, peptide 1-BSA, unlike PC-BSA, elicits a PC binding response with an isotype profile that suggests the presence of T-cell help.

Immunization of CBA/N mice with peptide 1-BSA. CBA/N mice carry the X-linked immunodeficiency defect and do not respond to T-cell-independent type II antigens (41). As a result, they do not mount a T15 response to PC immunization (33). CBA/N mice were immunized with peptide 1-BSA in order to determine if peptide 1 was capable of overcoming the immune defect in these mice and eliciting a PC binding response similar to that seen in BALB/c mice. Peptide 1-BSA induced IgG anti-PC activity but no expression of the T15 heavy chain (data not shown).

PC-binding antibodies elicited by peptide-BSA conjugates neither opsonized bacteria nor were protective. None of the

FIG. 2. Peptide mimics of PC induce a C polysaccharide binding response. See Materials and Methods for an explanation of the binding units. Symbols represent individual mice. d, day.

sera taken from BALB/c mice on day 63 following three immunizations with any of the peptide- or PC-BSA conjugates was able to opsonize *S. pneumoniae* (data not shown). Serum from mice immunized with PC-BSA does not always opsonize bacteria in vitro even though the mice are protected from lethal pneumococcal infection. The bacterial strain used for the opsonization experiments is an encapsulated strain that does not contain PC in its capsular polysaccharide. Therefore, it is likely that the PC epitopes on C polysaccharide were unavailable for antibody binding. Neither BALB/c nor CBA/N mice immunized with peptide 1-BSA were protected from lethal pneumococcal infection (data not shown). Thus, peptide 1-BSA elicits PC-binding antibodies that are not protective. The inability of peptide 1-BSA to elicit the canonical heavy- and light-chain combination may explain why this PC-binding response fails to protect mice from bacterial challenge. It has been demonstrated that T15-positive antibodies of all isotypes are protective against pneumococcal infections (4, 5), while alterations of the canonical T15 idiotypic have been shown to decrease affinity for PC as well as protective potential (26, 35).

DISCUSSION

We have demonstrated that peptide 1, a 24-mer peptide derived from the heavy-chain CDR2 and FR3 (24), as well as C-terminal peptides 6, 7, and 8 (i.e., amino acids 9 to 20, 11 to 22, and 13 to 24) were able to inhibit the binding of anti-PC monoclonal antibodies to PC-BSA. Thus, all were potential mimics of PC. Earlier, Lai et al. reported the inhibition of PC binding to antibodies using peptides derived from the heavy-chain CDR1 (27). These authors concluded that the peptides they tested inhibited the binding of PC in a nonspecific manner since several control peptides of similar size also inhibited PC binding. The peptides used in our work have related sequences and are of similar size yet exhibited different abilities to inhibit PC binding. Thus, these CDR2-derived peptides interact with the anti-PC antibodies specifically. We do not know why the

pattern of peptide inhibition differed for each of the antibodies. It may be that peptide 1 has more secondary structures such that it binds less well in the antigen binding site. It is also tempting to speculate that the difference is due to isotype because PC2 and T15 have identical variable-region sequences (29, 44), a fact demonstrated by using anti-idiotypic reagents. Anti-idiotypes have been reported to display differential reactivity with T15 antigens depending on the heavy-chain isotype (34). If isotypes display subtle differences in antigen binding, it

FIG. 3. PC affinity purification of postimmunization anti-peptide 1-BSA serum isolates antipeptide activity. Binding to the cognate peptide or a control peptide (DRIPMDYWGQTSVTSS) was tested at a 1:500 dilution of whole serum. Affinity-purified antibodies were used at 550 ng/ml; non-PC-binding antibodies were tested at 550-ng/ml and 2.5 μ g/ml. Error bars represent standard deviations. ■, peptide control (1 μ M); ■, peptide 1 (1 μ M). d, day.

FIG. 4. Peptide 1-BSA induces the T15 canonical heavy chain. Peptide 1-BSA induces more TC54 reactivity than BSA. The preimmunization sera from groups of mice were pooled together. Symbols represent postimmunization sera of individual mice.

may help to explain the isotype restriction that is seen in the anti-PC response (11).

Westerink et al. were able to model a peptide mimotope of *Neisseria meningitidis* group C based on an idiotypic interaction (48). Several groups have used phage-displayed peptide libraries to isolate peptide mimics of bacterial carbohydrates, with various degrees of success. Pincus et al. and Grothaus et al. isolated peptides that elicit antibacterial responses directed against group B streptococci and *N. meningitidis* group A, respectively (17, 40). Phalipon et al. isolated 19 peptide mimics of *Shigella flexneri* lipopolysaccharide, of which only four elicited lipopolysaccharide-binding antibodies (39). De Bolle et al. and Moe et al. isolated peptide mimics of *Brucella* spp. and *N. meningitidis* group B, respectively, which elicited only very weak antibacterial responses (10, 32). Valadon et al. isolated peptide mimics of *Cryptococcus neoformans* which elicited the

correct idotype but did not bind *C. neoformans* (46). Thus, binding alone does not guarantee that a peptide mimic will successfully elicit the desired immune response.

Peptides 7 and 8 bound at or near the PC binding site with affinities similar to that of peptide 1, but they did not induce a similar PC-binding response. It may be that peptides 7 and 8 occupy only part of the PC binding site, enough to inhibit PC binding but not enough to be a mimotope.

Peptide 1-BSA elicits the canonical heavy chain but not the canonical light chain, suggesting an interaction with the heavy chain alone. Valadon et al. have reported analogous results for a peptide mimic of *C. neoformans* (46). A crystal structure of an anticryptococcal monoclonal antibody bound to the peptide mimic revealed that the peptide occupied only part of the binding site, making contacts mainly with the heavy chain (49). Similarly, anti-idiotypic monoclonal antibodies raised against

FIG. 5. The anti-PC response induced by peptide 1-BSA consists of IgG1. Preimmunization sera from groups of mice were pooled, and postimmunization sera from individual mice were titrated on microtiter wells coated with PC-BSA. The amount of anti-PC antibody was determined from standard curves generated by using the T15-expressing anti-PC IgG1 monoclonal antibody PC1.4.1 and a goat anti-mouse IgG1 secondary antibody. The symbols represent postimmunization sera of individual mice.

an anti-*Brucella* carbohydrate monoclonal antibody fail to elicit a carbohydrate binding response (50). Young et al. suggest that the anti-idiotypic monoclonal antibodies do not mimic the carbohydrate because they do not fill the carbohydrate binding site of the original antibody (50). A crystal structure of a monoclonal anti-PC antibody complexed with one of the peptide mimics would provide information about the molecular interactions between the antibody and the peptide mimics. Moreover, an anti-PC antibody:peptide mimic structure would provide information on how to modify the peptide in order to elicit a protective anti-PC response.

Earlier work by McNamara et al. used 4C11, a monoclonal anti-idiotypic antibody raised against a monoclonal anti-PC antibody, coupled to keyhole limpet hemocyanin to induce a protective PC-binding response in mice (31). Approximately 90% of the PC-binding antibodies were T15 positive. Peptide 1 was derived from a different idiotype interaction and has no sequence similarity to 4C11. Immunization with peptide 1-BSA elicited an anti-PC response without induction of canonical T15 antibodies. Only the T15 heavy chain occurred in the response. These antibodies failed to protect mice from a lethal *S. pneumoniae* infection. Bacterial challenge of BALB/c mice occurred on day 170 after the total amount of anti-PC antibody, as well as T15-positive anti-PC, had peaked, as seen in Fig. 1; however, the amount of anti-PC antibody present was still within the range of reported protective anti-PC titers (6, 47). Moreover, sera taken on day 63 (when the anti-PC titer was high) from mice immunized with each of the peptide-BSA conjugates failed to opsonize bacteria in vitro. Thus, the lack of protection afforded by peptide 1-BSA is likely due to a failure to elicit the protective idiotype rather than to a low anti-PC titer (18). This is consistent with previous data suggesting that the heavy-chain CDR3 sequences affect, or the associated light chain determines, bacterial specificity (18).

It is interesting that the anti-PC antibody does bind C polysaccharide despite its failure to protect mice against bacterial infection. It has previously been reported that both protective and enhancing antibodies may occur in an immune response and that these antibodies differ with respect to the antigenic epitopes to which they bind (36). It has also been reported that monoclonal antibodies directed against the same epitope of cryptococcal polysaccharide may differ in their ability to protect mice against a lethal cryptococcal infection (37, 51–53). Understanding the nature of a protective epitope and a protective antibody is complex and a major challenge in vaccine development.

PC is considered to be a T-cell-independent type II antigen and elicits mainly IgM, IgG3, and some IgG1, even though all isotypes of anti-PC antibodies have been shown to be equally protective (5). The IgG anti-PC response elicited by peptide 1-BSA consisted of mainly IgG1; no IgG3 or IgM activity was detected. The presence of IgG1 anti-PC antibodies suggests that peptide 1-BSA elicits T-cell help, perhaps through T-cell recognition of variable-region epitopes. Thus, the peptide mimics may induce antipneumococcal responses in children and other populations whose members do not respond well to T-cell-independent vaccines such as Pneumovax.

Our work proves that peptides can mimic PC, a small non-peptide epitope found on several major pathogens. It will be important to gain further understanding of the role of both idiotype and the isotype in a protective anti-PC response. It is clear that one can develop peptide mimics of PC, but the idiotype and isotypic characteristics and protective potential elicited will differ.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI31473 (M.H.N.) and AI42997 (B.D.). S.L.H. is the recipient of a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada.

We thank Barbara Birshtein, Matthew Scharff, and Czeslawa Kowal for useful discussions and comments.

REFERENCES

- Andres, C. M., A. Maddalena, S. Hudak, N. M. Young, and J. L. Claffin. 1981. Anti-phosphocholine hybridoma antibodies. II. Functional analysis of binding sites within three antibody families. *J. Exp. Med.* **154**:1584–1598.
- Bottger, V., L. Peters, and B. Michael. 1999. Identification of peptide motifs for the fluorescein hapten binding of monoclonal antibody B13-DE1. *J. Mol. Recogn.* **12**:191–197.
- Breiman, R. F., J. C. Butler, F. C. Tenover, J. A. Elliott, and R. R. Facklam. 1994. Emergence of drug-resistant pneumococcal infections in the United States. *JAMA* **271**:1831–1835.
- Briles, D. E., C. Forman, S. Hudak, and J. L. Claffin. 1982. Anti-phosphorylcholine antibodies of the T15 idiotype are optimally protective against *Streptococcus pneumoniae*. *J. Exp. Med.* **156**:1177–1185.
- Briles, D. E., C. Forman, S. Hudak, and J. L. Claffin. 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.
- Briles, D. E., M. Nahm, K. Schroer, J. Davie, P. Baker, J. Kearney, and R. Barlett. 1981. Antiphosphocholine antibodies found in normal mouse serum are protective against intravenous infection with type 3 *Streptococcus pneumoniae*. *J. Exp. Med.* **153**:694–705.
- Chesebro, B., and H. Metzger. 1972. Affinity labeling of a phosphorylcholine binding mouse myeloma protein. *Biochemistry* **11**:766–771.
- Claffin, J. L., R. Lieberman, and J. M. Davie. 1974. Clonal nature of the immune response to phosphorylcholine. I. Specificity, class, and idiotype of phosphorylcholine-binding receptors on lymphoid cells. *J. Exp. Med.* **139**:58–73.
- Collins, S. J., F. W. Ruscetti, R. E. Gallagher, and R. C. Gallo. 1978. Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds. *Proc. Natl. Acad. Sci. USA* **75**:2458–2462.
- De Bolle, X., T. Laurent, A. Tibor, F. Godfroid, V. Weynants, J. J. Letesson, and P. Mertens. 1999. Antigenic properties of peptidic mimics for epitopes of the lipopolysaccharide from *Brucella*. *J. Mol. Biol.* **294**:181–191.
- Der Balian, G. P., J. Slack, B. L. Clevinger, H. Bazin, and J. M. Davie. 1980. Subclass restriction of murine antibodies. III. Antigens that stimulate IgG3 in mice stimulate IgG2c in rats. *J. Exp. Med.* **152**:209–218.
- Desaymard, C., A. M. Giusti, and M. D. Scharff. 1984. Rat anti-T15 monoclonal antibodies with specificity for VH- and VH-VL epitopes. *Mol. Immunol.* **21**:961–967.
- Dunlap, N. E., S. Ballinger, T. Reed, J. C. Christian, W. J. Koopman, and D. E. Briles. 1993. The use of monozygotic and dizygotic twins to estimate the effects of inheritance on the levels of immunoglobulin isotypes and antibodies to phosphocholine. *Clin. Immunol. Immunopathol.* **66**:176–180.
- Garcia-Leoni, M. E., E. Cercenado, P. Rodeno, J. C. Bernaldo de Quiros, D. Martinez-Hernandez, and E. Bouza. 1992. Susceptibility of *Streptococcus pneumoniae* to penicillin: a prospective microbiological and clinical study. *Clin. Infect. Dis.* **14**:427–435.
- Gillespie, S. H. 1989. Aspects of pneumococcal infection including bacterial virulence, host response and vaccination. *J. Med. Microbiol.* **28**:237–248.
- Gray, B. M., G. M. Converse, and H. C. J. Dillon. 1979. Serotypes of *Streptococcus pneumoniae* causing disease. *J. Infect. Dis.* **140**:979–983.
- Grothaus, M. C., N. Srivastava, S. L. Smithson, T. Kieber-Emmons, D. B. Williams, G. M. Carlone, and M. A. Westerink. 2000. Selection of an immunogenic peptide mimic of the capsular polysaccharide of *Neisseria meningitidis* serogroup A using a peptide display library. *Vaccine* **18**:1253–1263.
- Guo, W. X., A. M. Burger, R. T. Fischer, D. G. Sieckmann, D. L. Longo, and J. J. Kenny. 1997. Sequence changes at the V-D junction of the VH1 heavy chain of anti-phosphocholine antibodies alter binding to and protection against *Streptococcus pneumoniae*. *Int. Immunol.* **9**:665–677.
- Harris, S. L., L. Craig, J. S. Mehroke, M. B. Zwick, K. Kenar, E. J. Toone, N. Greenspan, F. I. Auzanneau, J. R. Marino-Albernas, B. M. Pinto, and J. K. Scott. 1997. Exploring the basis of peptide-carbohydrate cross-reactivity: evidence for discrimination by peptides between closely related anti-carbohydrate antibodies. *Proc. Natl. Acad. Sci. USA* **94**:2454–2459.
- Henrichsen, J. 1979. The pneumococcal typing system and pneumococcal surveillance. *J. Infect.* **1**(Suppl. 2):31–37.
- Hoess, R., U. Brinkmann, T. Handel, and I. Pastan. 1993. Identification of a peptide which binds to the carbohydrate-specific monoclonal antibody B3. *Gene* **128**:43–49.
- Hutchins, W. A., T. Kieber-Emmons, G. M. Carlone, and M. A. Westerink. 1999. Human immune response to a peptide mimic of *Neisseria meningitidis*

- serogroup C in hu-PBMC-SCID mice. *Hybridoma* **18**:121–129.
23. Jennings, H. J., C. Lugowski, and N. M. Young. 1980. Structure of the complex polysaccharide C-substance from *Streptococcus pneumoniae* type 1. *Biochemistry* **19**:4712–4719.
 24. Kang, C. Y., T. K. Brunck, T. Kieber-Emmons, J. E. Blalock, and H. Kohler. 1988. Inhibition of self-binding antibodies (autobodies) by a VH-derived peptide. *Science* **240**:1034–1036.
 25. Kang, C. Y., H. L. Cheng, S. Rudikoff, and H. Kohler. 1987. Idiotypic self binding of a dominant germline idiotype (T15). Antibody activity is affected by antibody valency. *J. Exp. Med.* **165**:1332–1343.
 26. Kenny, J. J., C. M. Moratz, G. Guelde, C. D. O'Connell, J. George, C. Dell, S. J. Penner, J. S. Weber, J. Berry, and J. L. Claffin. 1992. Antigen binding and idiotype analysis of antibodies obtained after electroporation of heavy and light chain genes encoding phosphocholine-specific antibodies: a model for T15-idiotype dominance. *J. Exp. Med.* **176**:1637–1643.
 27. Lai, E. H., E. A. Kabat, J. Meienhofer, E. P. Heimer, A. J. Olson, and R. Lerner. 1987. Inhibition of phosphorylcholine binding to antibodies using synthetic peptides. *Nature* **325**:168–171.
 28. Lee, W., H. Cosenza, and H. Kohler. 1974. Clonal restriction of the immune response to phosphorylcholine. *Nature* **247**:55–57.
 29. Limpanasithikul, W., S. Ray, and B. Diamond. 1995. Cross-reactive antibodies have both protective and pathogenic potential. *J. Immunol.* **155**:967–973.
 30. Lund, E., and J. Henrichsen. 1978. Laboratory diagnosis, serology and epidemiology of *Streptococcus pneumoniae*. *Methods Microbiol.* **12**:241–262.
 31. McNamara, M. K., R. E. Ward, and H. Kohler. 1984. Monoclonal idiotope vaccine against *Streptococcus pneumoniae* infection. *Science* **226**:1325–1326.
 32. Moe, G. R., S. Tan, and D. M. Granoff. 1999. Molecular mimetics of polysaccharide epitopes as vaccine candidates for prevention of *Neisseria meningitidis* serogroup B disease. *FEMS Immunol. Med. Microbiol.* **26**:209–226.
 33. Mond, J. J., R. Lieberman, J. K. Inman, D. E. Mosier, and W. E. Paul. 1977. Inability of mice with a defect in B-lymphocyte maturation to respond to phosphorylcholine on immunogenic carriers. *J. Exp. Med.* **146**:1138–1142.
 34. Morahan, G., C. Berek, and J. F. Miller. 1983. An idiotypic determinant formed by both immunoglobulin constant and variable regions. *Nature* **301**:720–722.
 35. Nicoletti, C., X. Yang, and J. Cerny. 1993. Repertoire diversity of antibody response to bacterial antigens in aged mice. III. Phosphorylcholine antibody from young and aged mice differ in structure and protective activity against infection with *Streptococcus pneumoniae*. *J. Immunol.* **150**:543–549.
 36. Nussbaum, G., W. Cleare, A. Casadevall, M. D. Scharff, and P. Valadon. 1997. Epitope location in the *Cryptococcus neoformans* capsule is a determinant of antibody efficacy. *J. Exp. Med.* **185**:685–694.
 37. Nussbaum, G., R. Yuan, A. Casadevall, and M. D. Scharff. 1996. Immunoglobulin G3 blocking antibodies to the fungal pathogen *Cryptococcus neoformans*. *J. Exp. Med.* **183**:1905–1909.
 38. Ortvist, A., J. Hedlund, L. A. Burman, E. Elbel, M. Hofer, M. Leinonen, I. Lindblad, B. Sundelof, and M. Kalin. 1998. Randomised trial of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. *Lancet* **351**:399–403.
 39. Phalipon, A., A. Folgori, J. Arondel, G. Sgaramella, P. Fortugno, R. Cortese, P. J. Sansonetti, and F. Felici. 1997. Induction of anti-carbohydrate antibodies by phage library-selected peptide mimics. *Eur. J. Immunol.* **27**:2620–2625.
 40. Pincus, S. H., M. J. Smith, H. J. Jennings, J. B. Burritt, and P. M. Glee. 1998. Peptides that mimic the group B streptococcal type III capsular polysaccharide antigen. *J. Immunol.* **160**:293–298.
 41. Quintans, J. 1977. The "patchy" immunodeficiency of CBA/N mice. *Eur. J. Immunol.* **7**:749–751.
 42. Robbins, J. B., R. Austrian, C. J. Lee, S. C. Rastogi, G. Schiffman, J. Henrichsen, P. H. Makela, C. V. Broome, R. R. Facklam, and R. H. Tiesjema. 1983. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. *J. Infect. Dis.* **148**:1136–1159.
 43. Sorensen, U. B., and J. Henrichsen. 1987. Cross-reactions between pneumococci and other streptococci due to C polysaccharide and F antigen. *J. Clin. Microbiol.* **25**:1854–1859.
 44. Spira, G., H. L. Aguila, and M. D. Scharff. 1988. T15 PC-binding monoclonal antibodies retain specificity when they switch from IgM to IgG. *J. Immunol.* **140**:2675–2680.
 45. Valadon, P., G. Nussbaum, L. F. Boyd, D. H. Margulies, and M. D. Scharff. 1996. Peptide libraries define the fine specificity of anti-polysaccharide antibodies to *Cryptococcus neoformans*. *J. Mol. Biol.* **261**:11–22.
 46. Valadon, P., G. Nussbaum, J. Oh, and M. D. Scharff. 1998. Aspects of antigen mimicry revealed by immunization with a peptide mimetic of *Cryptococcus neoformans* polysaccharide. *J. Immunol.* **161**:1829–1836.
 47. Wallick, S., J. L. Claffin, and D. E. Briles. 1983. Resistance to *Streptococcus pneumoniae* is induced by a phosphocholine-protein conjugate. *J. Immunol.* **130**:2871–2875.
 48. Westerink, M. A., P. C. Giardina, M. A. Apicella, and T. Kieber-Emmons. 1995. Peptide mimicry of the meningococcal group C capsular polysaccharide. *Proc. Natl. Acad. Sci. USA* **92**:4021–4025.
 49. Young, A. C., P. Valadon, A. Casadevall, M. D. Scharff, and J. C. Sacchettini. 1997. The three-dimensional structures of a polysaccharide binding antibody to *Cryptococcus neoformans* and its complex with a peptide from a phage display library: implications for the identification of peptide mimotopes. *J. Mol. Biol.* **274**:622–634.
 50. Young, N. M., M. A. Gidney, B. M. Gudmundsson, C. R. MacKenzie, R. To, D. C. Watson, and D. R. Bundle. 1999. Molecular basis for the lack of mimicry of *Brucella* polysaccharide antigens by Ab2γ antibodies. *Mol. Immunol.* **36**:339–347.
 51. Yuan, R., A. Casadevall, G. Spira, and M. D. Scharff. 1995. Isotype switching from IgG3 to IgG1 converts a nonprotective murine antibody to *Cryptococcus neoformans* into a protective antibody. *J. Immunol.* **154**:1810–1816.
 52. Yuan, R., R. Clynes, J. Oh, J. V. Ravetch, and M. D. Scharff. 1998. Antibody-mediated modulation of *Cryptococcus neoformans* infection is dependent on distinct Fc receptor functions and IgG subclasses. *J. Exp. Med.* **187**:641–648.
 53. Yuan, R. R., G. Spira, J. Oh, M. Paizi, A. Casadevall, and M. D. Scharff. 1998. Isotype switching increases efficacy of antibody protection against *Cryptococcus neoformans* infection in mice. *Infect. Immun.* **66**:1057–1062.