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A thymus-independent (type 1) phosphorylcholine antigen isolated from *Trichinella spiralis* protects mice against pneumococcal infection

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SUMMARY

A phosphorylcholine (PC)-containing glycoprotein of 68,000 molecular weight (MW) was isolated from *Trichinella spiralis*. The potential of this antigen (Tsp) as a species-specific vaccine against *Streptococcus pneumoniae* was studied in both immunologically deficient (CBA/N) and normal (CFW) mice. Unlike the PC determinant found in *S. pneumoniae*, Tsp is a type 1 thymus-independent (TI-1) antigen, as it was able to stimulate PC-specific antibody production in CBA/N animals, though less well than in CFW mice. Immunological memory to this antigen was observed in both strains of mice, and the predominant class of antibodies formed was IgM. In further studies, Tsp-immunized CFW mice were protected against a fatal challenge of *S. pneumoniae* type 3. Protection in these animals is probably mediated by the PC-specific antibodies present, which comprised 87·9% of antibodies reactive to *S. pneumoniae*, or 58·7% of total antibodies formed.

INTRODUCTION

Streptococcus pneumoniae (pneumococcus) and other encapsulated bacteria are important causes of human infections, particularly in the developing world, and the development of effective vaccines against these organisms remains a challenge (Griffis *et al.*, 1987). Currently, the vaccines available are composed of capsular polysaccharides derived from the respective organisms. Although antibodies to these components are protective, the polysaccharides themselves do not stimulate good or lasting immunity or immunological memory, particularly in young infants for whom protection is most needed (Peltola *et al.*, 1977; Kayhty *et al.*, 1980; Sell *et al.*, 1981). A possible solution to this problem, as seen in both clinical (Zollinger *et al.*, 1979; Anderson *et al.*, 1981) and experimental (Snippe *et al.*, 1983; Moreno, Lifely & Esdaile, 1985) situations, is to use protein conjugates of the polysaccharides so that the antigens behave like proteins and thus become highly immunogenic in all age groups. In the pneumococcus, however, there are at least 83 capsular types and this presents problems to vaccine formulation. The vaccine presently available, known as Pneumovax 23 (PnX) (Merck Sharp & Dohme, West Point, PA), consists of polysaccharides derived from 23 serotypes.

Interest was recently focused on a hapten determinant present in the cell wall (and capsule) of all serotypes of *S. pneumoniae* for possible use as a species-specific vaccine against this bacterium. This is phosphorylcholine (PC). Anti-PC antibodies have been shown to be protective against infection caused by certain pneumococcal serotypes in mice (reviewed by Briles *et al.*, 1986), although such a role is less clear in man (Gray, Dillon & Briles, 1983; Musher *et al.*, 1986). This was demonstrated, for example, in mice passively immunized with PC-specific antibodies, in which it was found that antibodies of the IgG class and T15 idiotype (Briles *et al.*, 1981, 1982) were the most protective. The PC determinant present in the capsule and cell wall of *S. pneumoniae* is attached to polysaccharides (Bennett & Bishop, 1977; Jennings, Lugowski & Young, 1980) and, like the polysaccharides themselves, is not immunogenic in CBA/N mice (Scher, 1982). These animals, which carry an X-linked immunodeficiency presumably affecting the maturation (function) of their B cells, allow the differentiation of thymus-independent (TI) antigens into those (TI-1) to which they can respond to, e.g. lipoprotein-associated lipopolysaccharide, and those (TI-2) which they cannot respond to, e.g. PC polysaccharide (Scher, 1982). CBA/N animals are thus immunologically similar to the young infant (Ambrosino *et al.*, 1987). Consequently, the pneumococcal PC antigens may not be suitable as vaccine candidates unless they are chemically modified. Alternatively, since PC occurs widely in nature, namely, in numerous species of bacteria, nematodes and moulds (Potter, 1977), it is possible that this antigen derived from different sources may have different immunogenic potential. In this study, we examined the biological properties of the PC antigen isolated from *Trichinella spiralis* larvae and found this antigen to be different from the pneumococcal counterpart.

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MATERIALS AND METHODS

Mice

CBA/N mice were obtained from the Laboratory Animal Unit (LAU) in our University. CFW (Swiss) mice recently acquired

from Charles River Breeding Laboratories Inc., Wilmington, MA, were also bred at LAU. All mice used in the experiments were 3–4 weeks old.

Antigens

Crude *T. spiralis* antigen (Tsc) was obtained by sonication of larvae isolated from the muscle of infected CFW mice. This was followed by overnight extraction with 0·1% Triton X-100 and subsequent clarification at 11,000 ***g*** for 5 min (Choy *et al.*, 1988). Its concentration was determined from the protein content by the method of Lowry *et al.* (1951).

Purified *T. spiralis* antigen (Tsp) was obtained from Tsc by affinity chromatography (Lim, 1987). The reagent antibody, Ts2Ab (see below), used for this purpose was coupled to CNBr-activated Sepharose 4B (Sigma Chemical Co., St Louis, MO). Elution of the adsorbed antigen was achieved using 0·1 M glycine-NaOH buffer (pH 11·0) at room temperature. The eluted fractions, monitored by spectrophotometry at OD_{280}, were pooled and dialysed immediately against three changes of phosphate-buffered saline (PBS; pH 7·5) at 4°. The contents were concentrated in the same dialysis bag using aquacide (Calbiochem-Behring Corp., La Jolla, CA), and then dialysed against PBS before clarification in an Eppendorf centrifuge (Brinkman Instruments, Westbury, NY) for 5 min. Preparations from different runs were pooled together and then divided into small aliquots for storage at 4° until use. Estimation of the concentration of the purified material was based on its combined carbohydrate and protein content.

Pneumococcal C-polysaccharide (PnC) was prepared from cell lysates of *S. pneumoniae* type 3 (ATCC strain) according to the method of Sorensen *et al.* (1984). Briefly, the cells of an overnight culture were lysed with sodium deoxycholate, after which the polysaccharide was extracted with chloroform-butanol and subsequently precipitated with 4 vol of ethanol. The concentration of the antigen used in the study was based on its carbohydrate content.

Pneumovax 23 (lot no. 0676 N) (PnX) was obtained from Merck Sharp & Dohme, West Point, PA. Antigen concentration used was based on information supplied by the manufacturer. PC-conjugated human serum albumin (PC-HSA) was a kind gift of Dr U. Sundin, Karolinska Institute, Huddinge, Sweden. Ovalbumin (OVA) was purchased from Sigma.

Reagent antibodies

Ts2Ab was produced by somatic fusion of spleen cells derived from *T. spiralis*-infected BALB/c mice and NS1 myeloma cells (Lim & Choy, 1986). This PC-specific, $T15^+$ IgM protein was purified from mouse ascites fluid by protein A–chromatography (Lim & Choy, 1986) for use in Sepharose-coupling and as a standard in the immunoassays. The concentration of the purified antibody was determined by Lowry *et al.* (1951).

Purified mouse anti-OVA antibodies were obtained by affinity chromatography of sera derived from OVA-immunized CFW mice on an OVA-coupled Sepharose 4B column, using 0·1 M glycine-NaOH buffer (pH 11·0) as the eluent (Lim, 1987). The concentration of the purified antibody was determined as described by Lowry *et al.* (1951).

Immunization studies

In all cases, mice (five to 10 per group) were primed intraperitoneally (i.p.) with 0·2 ml of alum-precipitated antigen in complete Freund's adjuvant (CFA). Alum-precipitation was performed as described by Hudson & Hay (1980). Two weeks later, 0·2 ml (1/5th priming dose) of the alum-precipitated antigen in saline was administered intravenously by the femoral vein to each mouse. Blood was obtained from the retro-orbital plexus 13 days following the primary injection and 5 days following the booster immunization to determine the respective antibody responses.

Antibody assays

Enzyme-linked immunosorbent assays (ELISA) were performed according to previously described procedures (Lim & Ho, 1983) to determine the antibody activities in test sera. Briefly, Immunon-2 microplates (Dynatech Lab., Alexandria, VA) were coated with the antigen in bicarbonate buffer (pH 9·6) overnight at 4°. The antigens used were: Tsc (14 μg/ml), PnC (3 μg/ml), OVA (10 μg/ml) and PC-HSA (1:2000 stock dilution). Serial dilutions of the test serum were then added and incubated with the antigen for 2 hr at 37°. The plate was subsequently washed three times. In the determination of total antibody content, the assay was developed with peroxidase-labelled goat anti-mouse IgG (H + L) antibody (Tago, Burlingame, CA). For isotype determination, the assay was developed with class-specific reagents obtained from Sigma and subsequently with peroxidase-labelled goat anti-rabbit IgG (H + L) antibody (Sigma). Incubation in each step was carried out at 37° for 2 hr. In all cases, the assay was finally developed by addition of substrate (O-phenylenediamine). Results were read in a MR710 reader (Dynatech).

In some instances, the total antibody binding assay was performed in the presence of 10^{-3} M PC (phosphorylcholine chloride; Sigma) (final) included in the test sample.

All microplates used contained serial dilutions of the appropriate standard (Ts2Ab or anti-OVA antibody) as well as relevant controls.

Protection studies

The method of Wallick, Claflin & Briles (1983) was followed. Briefly, *S. pneumoniae* type 3 (ATCC strain) bacteria were grown for 6 hr at 37° to log phase in nutrient broth (Oxford Ltd, Basingstoke, Hampshire, U.K.) supplemented with 5% horse serum (Oxford). The cells were obtained by centrifugation at 4°, washed and resuspended in the serum broth, and kept on ice until use. The concentration of bacteria was determined by spectrophotometry at OD_{420}, based on previous calibration against viable count (1 OD_{420} unit $= 3 \times 10^8$ bacteria/ml). $7{\cdot}5 \times 10^4$ organisms in 0·2 ml serum broth were injected intravenously by the femoral vein into each mouse for the protection test. This was the actual number of bacteria administered, as determined by viable count of the inoculum just before and after challenge.

Miscellaneous tests

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously (Lim, 1987). Staining was done using the modified silver stain of Oakley, Kirsche & Morris (1980). Protein estimation was performed according to Lowry *et al.* (1951), and carbohydrate

determination by the phenol-sulphuric acid method (Williams & Chase, 1968).

Comparison of data was performed by the Wilcoxon two-sample test.

RESULTS

Isolation of PC antigen from *T. spiralis*

Affinity chromatography was used to isolate the PC antigen of *T. spiralis* from crude extracts of the larvae. The reagent antibody used for this purpose was an IgM PC-specific hybridoma antibody raised against the nematode previously. In a typical experiment, 7 mg (protein) of crude *T. spiralis* antigen in 0·5 ml were loaded into the 4-ml column, and 20 μg (protein) were specifically recovered. Chemical analysis of the purified antigen (Tsp) pooled from different runs indicated a carbohydrate: protein content of 11:1 (lipid determination not done). On SDS–PAGE analysis, Tsp showed a single band at 68,000 MW (Fig. 1). [Reagent antibody, if present as a contaminant from the column, would show two bands: one (μ-chain) at 77,000 MW and the other (L-chain) at 23,000 MW (Lim & Choy, 1986)].

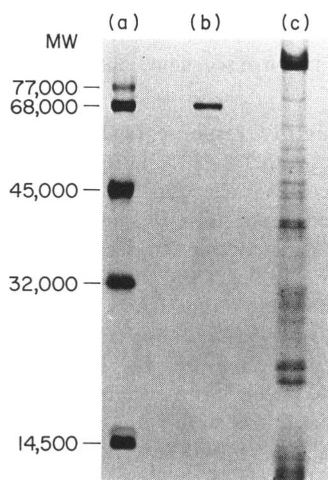

Figure 1. Characterization of Tsp (b, 2 μg) purified from crude lysates (Tsc) of *T. spiralis* (c, 5 μg) on 10% SDS–PAGE, stained with silver nitrate. (a) Molecular weight markers.

Immunogenicity studies in CBA/N mice

The immunogenicity of Tsp and pneumococcal PC-associated antigens (PnC and PnX) in CBA/N mice was studied. For comparison, a protein antigen (OVA) was included. Immune response was measured in terms of antibodies produced which were reactive to the *Trichinella* crude antigen (Tsc), PnC or OVA.

As shown in Table 1, mice immunized intraperitoneally with 80 μg Tsp in CFA developed, 13 days later, low levels of antibodies to Tsc and also, in some cases, to PnC. When given an intravenous booster (8 μg) 3 weeks following the primer, these mice developed high levels (261 μg/ml) of antibodies to the homologous antigen, Tsc, 3·0% (7·7 μg/ml) of which also bound to the heterologous antigen, PnC (Table 1, Fig. 2). Only 5·9% of the antibodies to Tsc were PC-inhibitable whereas more than 96·1% of those reactive to PnC could be inhibited. Thus, the increase in anti-PC (PnC) antibody content from the primary to the secondary response was about 22-fold. Smaller quantities (e.g. 10 μg, primary dose) of Tsp were also immunogenic.

In contrast to Tsp, as much as 100 μg PnX stimulated virtually no antibody in the CBA/N animals (Table 1, Fig. 2). PnC (1 μg), on the other hand, elicited small amounts of antibodies to the immunizing antigen, none of which appeared to be inhibitable by 10^{-3} M PC.

OVA-immunized mice, which had no detectable level (< 0·3 μg/ml) of antibodies to Tsc or PnC, developed high titres of anti-OVA antibodies in the primary response, which increased five-fold in the secondary response (Table 1).

Table 1. Immunogenicity of Tsp and other PC-associated antigens

Immunogen (primary dose, μg)	Primary antibody response to (μg/ml)*			Secondary antibody response to (μg/ml)*		
	Tsc	PnC	OVA	Tsc	PnC	OVA
CBA/N mice						
Tsp (80)	5·44±0·35	<0·34±0·04	—	261±20	7·70±0·68	—
Tsp (10)†	—	—	—	10·5±1·2	1·40±0·25	—
PnC (1)	≤0·13±0·01	≤0·14±0·02	—	≤0·40±0·03	≤1·22±0·21	—
PnX (100)†	—	—	—	≤0·58±0·08	<0·30	—
PnX (1)	≤0·10±0·0	≤0·05	—	≤0·20±0·01	<0·15	—
OVA (10)	≤0·10±0·01	≤0·05	377±50	≤0·27±0·02	≤0·19±0·01	1869±123
CFW mice						
Tsp (80)	51·3±5·8	7·30±0·84	—	1486±139	71·6±6·3	—
PnC (1)	6·19±0·26	19·7±2·1	—	6·78±0·20	48·9±5·4	—
PnX (1)	3·65±0·19	7·83±0·53	—	4·47±1·88	24·1±2·0	—
OVA (10)	0·73±0·04	≤0·52±0·03	97·0±9·4	≤1·02±0·16	≤0·82±0·08	646±111

* Based on total binding to Tsc, PnC or OVA (mean±SEM). Purified Ts2Ab and purified mouse anti-OVA antibodies were used as standards for the respective assays; the limits of detection of these antibodies were 2–4 ng/ml. —, test not performed.

† Five mice per group. Other groups had 10 mice.

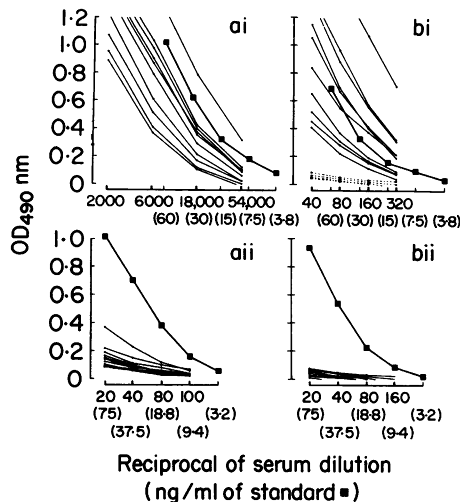

Figure 2. Titration of individual sera obtained from CBA/N mice immunized with Tsp (80 μg) (i) or PnX (1 μg) (ii) for ELISA activity to Tsc (a) or PnC (b). Determination in some cases was made in the presence of 10^{-3} M PC (----). This is shown for representative cases only in (bi) and not at all for (ai). In the latter, there was virtually no difference whether the inhibitor was present or not in all cases.

Immunogenicity studies in CFW mice

The antibody response experiment were repeated in immunologically normal (CFW) mice (Table 1).

Tsp (80 μg) stimulated higher antibody production in these animals than in CBA/N mice in both the primary and secondary responses. Thus, in the secondary response, 1486 μg/ml of anti-Tsc antibodies were produced, which is 5·7-fold more than that observed in CBA/N animals. Furthermore, 50% (compared to 5·9% in CBA/N mice) of these antibodies were inhibitable by 10^{-3} M PC, while 4·8% (71·6 μg/ml) also bound to the heterologous antigen, PnC, which were mostly (87·9%) PC-inhibitable. Compared to the corresponding response in CBA/N mice, the secondary anti-PC (PnC) response in CFW animals was 9·3-fold higher. This particular antibody activity in the CFW mice was also singificantly higher (9·8-fold) than that observed in the primary response of these animals.

Unlike the lack of response seen in CBA/N animals, PnX stimulated good primary and secondary responses in CFW animals even with a primary dose as low as 1 μg. The antibodies produced to Tsc were predominantly PC-specific. However, there was little (1·2-fold) increase in the production of anti-PC (Tsc) antibody from the primary to the secondary response in these animals.

PnC was also highly immunogenic in CFW mice. Thus, the secondary anti-PC (Tsc) response was at least 17-fold higher than the corresponding response seen in CBA/N mice. There was, however, no increase in magnitude in this acivity over the primary response observed in the same (CFW) animals.

Control OVA-immunized mice showed low levels (< 1·0 μg/ml) of activities to Tsc and PnC, but high concentrations of antibodies of OVA in both the primary and secondary responses. Thus, there was a 6·6-fold increase in the anti-OVA antibody concentration following antigen booster.

Isotype determination of secondary antibody responses in CBA/N and CFW mice

The secondary anti-PC responses (Table 1) of CBA/N and CFW mice immunized with Tsp (80 μg, primary dose), PnX (1 μg) or PnC (1 μg) were characterized further according to isotype distribution. For comparison, the anti-OVA responses in these mice were also similarly characterized.

As shown in Table 2, the predominant class of anti-PC antibodies produced in both CBA/N and CFW mice was IgM. This isotype was 4·6–5·7-fold more than IgG, and 2·4–4·3-fold more than the IgA responses. (The IgA content may be over-estimated due to possible presence of contaminating anti-T15 antibodies in the anti-α serum used; Lim & Choy, 1986.) In marked contrast, the anti-OVA responses were predominantly IgG in both animals; significantly lower (12·5–14·3-fold) amounts of IgM antibodies were produced.

Protection studies in CFW mice

CFW mice immunized with Tsp (80 μg), PnC (1 μg), PnX (1 μg) or OVA (10 μg) in the previous experiment (Table 1) were challenged intravenously with $7·5 \times 10^4$ live type 3 pneumococci. The survival of these animals was observed daily for 10 days. As shown in Table 3, all OVA-immunized (control) mice died

Table 2. Characterization of secondary antibody responses to PC and OVA according to isotype

Mouse strain	Immunogen	Antibody specificity*	IgM†	IgM/IgA‡	IgM/IgA‡
CBA/N	Tsp	PC	3·39±0·25	5·14±0·25	4·34±0·14
	OVA	OVA	ND	0·07±0·00	—§
CFW	Tsp	PC	39·78±2·89	4·56±0·12	3·87±0·10
	PnC	PC	6·71±0·40	5·72±0·23	2·64±0·05
	PnX	PC	3·57±0·10	4·62±0·08	2·40±0·03
	OVA	OVA	ND	0·08±0·01	—

* PC-specific responses were based on total binding to the heterologous immunoadsorbent (PnC or Tsc) rather than on inhibition of binding by 10^{-3} M PC.

† IgM conc. based on Ts2Ab standard (mean±SEM). ND, not done.

‡ Quotient (mean±SEM) of antibody titres determined at OD_{490} 0·2–0·4.

§ Results not readable due to high non-specific binding of reagent anti-α serum to OVA.

Table 3. Effect of immunization on survival of CFW mice to pneumococcal challenge

Immunogen (dose, μg)	No. mice alive on day 0	1	2	3	4	5	6	7	8	9	10	Median days alive
Tsp (80)	10	10	10	10	10	10	10	10	10	10	9	> 10*
PnC (1)	10	10	10	10	10	10	10	10	10	10	10	> 10*
PX (1)	10	10	10	10	10	10	10	10	10	10	8	> 10*
OVA (10)	10	10	5	1	1	1	0	0	0	0	0	2·5†

* Statistically different from OVA-immunized (control) group; $P < 0{\cdot}005$ (Wilcoxon's rank test).

† Calculated from the formula of Briles *et al.* (1982): median days alive = $n/\left(\sum_{i \leq l}^{n} \frac{1}{Xi}\right)$,

where X is the number of days that mouse i lived and n is the number of mice in the group.

within 5 days after challenge (median days alive = 2·5), while mice from the other groups surviuved significantly ($P < 0{\cdot}005$) longer (median days alive > 10).

DISCUSSION

We have isolated the PC-associated antigen (Tsp) from *T. spiralis* and biologically characterized it. Based on immunogenicity studies in CBA/N (Scher, 1982) and immunologically normal (CFW) mice, we conclude that the PC determinant of this antigen is TI-1 in nature. Accordingly, it stimulated anti-PC antibody production in CBA/N animals, although this was 9·3-fold lower than the corresponding (secondary) response in CFW mice (Table 1). In both animals, an anamnestic response to the antigen was evident: the secondary anti-PC responses were 22- and 9·8-fold higher than the corresponding primary responses in CBA/N and CFW mice, respectively. In contrast, the PC-associated antigens of *S. pneumoniae* (PnC and PnX) elicited little or no anti-PC antibodies in CBA/N animals, whereas the same antigens stimulated vast amounts of these antbodies in CFW mice (Table 1). This is consistent with previous observations that PC is an important component of these antigens (Sorensen *et al.*, 1984; Fairchild *et al.*, 1986), and that, like the polysaccharides, the pneumococcal PC determinants are TI-2 in nature (Scher, 1982). Moreover, like polysaccharides (Snippe *et al.*, 1983), these antigens induced poor immunological memory (< 1·2-fold enhancement only). In comparison, a thymus-dependent (TD) antigen (OVA) included in the study, induced, expectedly (Scher, 1982; Snippe *et al.*, 1983), excellent anti-OVA responses in both CBA/N and CFW mice, including memory in these animals, as shown by the 5–7-fold enhancement of response (Table 1).

Tsp resembles a synthetic antigen made by coupling PC groups to lipopolysaccharide via tripeptide linkage (Kohler, Smyk & Fung, 1981). Such a conjugate was found to be TI-1 in nature, and the magnitude of the anti-PC response stimulated by it in CBA/N mice was very similar to that observed with Tsp (Kohler *et al.*, 1981). Moreover, the predominant class of PC-specific antibodies induced by both the antigens in these animals was IgM, characteristic of TI responses (Snippe *et al.*, 1983). IgM was also the predominant isotype formed to PC in CFW mice, whether Tsp, PnC or PnX was used as the immunogen (Table 2). This is in sharp contrast to the anti-PC response induced with a protein (haemocyanin) conjugate of PC, in which IgG but no IgM antibodies were found (Kenny *et al.*, 1981). This typically TD response (Snippe *et al.*, 1983) was also seen with OVA in our studies, in which 12–14-fold more IgG than IgM anti-OVA antibodies were produced in both CBA/N and CFW mice (Table 2). Thus, it seems the type of carrier (polysaccharide or protein) to which PC is attached determines the isotype distribution of the anti-PC response in the host.

Ubeira *et al.* (1987) recently described a PC-rich fraction isolated from *T. spiralis* larvae. This crude preparation readily induced anti-PC plaque-forming cell responses in (BALB/c × CBA/j)F_1 mice, but was TD, rather than TI, in nature. We do not understand the discrepancy between this finding and ours, presuming that the same PC determinant is shared by Tsp and the crude fraction. In our hands, however, immunogenicity tests performed in *nu/nu* BALB/c mice confirmed the TI characteristic of Tsp. Thus, mice immunized i.p. with 40 μg Tsp in CFA and subsequently boostered developed $11{\cdot}4 \pm 0{\cdot}3$ (mean ± SE of three mice) μg/ml of antibodies to PnC, whereas control animals (given bovine serum albumin) had only $3{\cdot}9 \pm 0{\cdot}2$ μg/ml of these antibodies. Tsp itself contained other determinants besides PC. Based on the immune response data in CFW mice, PC constitutes about half the antigen moiety, whereas in CBA/N animals only 6% of the antibodies produced to Tsp were PC-specific. Thus, it seems the non-PC half of Tsp is different from the PC determinant in being, possibly, TD in nature.

Our study confirms previous observations (Briles *et al.*, 1986) that PC-specific antibodies are protective against pneumococcal infection in mice. CBA/N animals were not used for this experiment since they do not make PC-specific antibodies of the protective (T15) idiotype (Kohler *et al.*, 1981; Kenny *et al.*, 1981; Briles *et al.*, 1982). Protection seen in the Tsp-immunized CFW mice is most probably mediated by the PnC-reactive antibodies (71·6 μg per ml) present in these animals, and specifically, by the anti-PC antibodies which comprised 88% of these antibodies (Table 1). However, direct proof of this requires passive transfer experiments, which we have not performed (due to lack of sufficient material). It remains to be determined whether Tsp is indeed more immunogenic in young infants than PnX and hence more suitable as a pneumococcal vaccine. On the other hand, there may not be any real advantage in using Tsp over the more immunogenic and more easily obtainable PC-protein conjugates (Kenny *et al.*, 1981; Wallick *et al.*, 1983) for this purpose. Protection in the CFW animals was also achieved with small amounts (1 μg) of PnC or PnX (Table 1). The

contribution of anti-PC antibodies to protection in these animals, however, is less clear since these comprised less than 40% (data not shown) of the total (24–49 μg per ml) PnC-binding antibodies. Moreover, antibodies to other cell envelope determinants, such as capsule (Briles *et al.*, 1986), which were not measured in our studies, are undoubtedly important in this regard.

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REFERENCES

AMBROSINO D.M., SIBER G.R., CHILMONCZYK B.A., JERNBERG J. & FINBERG R.W. (1987) An immunodeficiency characterized by impaired antibody responses to polysaccharides. *N. Engl. J. Med.* **316,** 790.

ANDERSON P., INSEL R.A., SMITH D.H., CATE T.R., COUCH R.B. & GLEZEN W.P. (1981) A polysaccharide-protein complex from *Haemophilus influenzae* type b. III. Vaccine trial in human adults. *J. Infect. Dis.* **144,** 530.

BENNETT L.G. & BISHOP C.T. (1977) Structure of type 27 *Streptococcus pneumoniae* capsular polysaccharide. *Can. J. Chem.* **55,** 8.

BRILES D.E., CLAFLIN J.L., SCHROER K. & FORMAN C. (1981) Mouse IgG3 antibodies are highly protective against infection with *Streptococcus pneumoniae. Nature (Lond.)*, **294,** 88.

BRILES D.E., FORMAN C., HUDAK S. & CLAFLIN J.L. (1982) Anti-phosporylcholine antibodies of the T15 idiotype are optimally protective against *Streptococcus pneumoniae. J. exp. Med.* **156,** 1177.

BRILES D.E., HOROWITZ J., MCDANIEL L.S., BENJAMIN W.H., JR., CLAFLIN J.L., BOOKER C.L., SCOTT G. & FORMAN C. (1986) Genetic control of the susceptibility to pneumococcal infection. In: *Current Topics in Microbiology and Immunology: Genetic Control of the Susceptibility to Bacterial Infection* (eds. D. E. Briles and M. D. Cooper), Vol. 124, pp. 103–120. Springer-Verlag, Berlin and New York.

CHOY W.F., LIM P.L. & NG M.H. (1988) A comparison of immunological methods for the detection of *Trichinella spiralis* antigen. *J. immunol. Meth.* **113,** 17.

FAIRCHILD R.L., STERNER K.E. & BRALEY-MULLEN H. (1986) Primary murine immunoglobulin M responses to certain pneumococcal capsular polysaccharides consist primarily of anti-pneumococcal cell wall carboydrate antibodies. *Infect. Immun.* **52,** 867.

GARY B.M., DILLON H.C. & BRILES D.E. (1983) Epidemiological studies of *Streptococcus pneumoniae* in infants: development of antibody to phosphocholine. *J. clin. Microbiol.* **18,** 1102.

GRIFFIS J.M., APICELLA M.A., GREENWOOD B. & MAKELA P.H. (1987) Vaccines against encapsulated bacteria: A global agenda. *J. Infect. Dis.* **9,** 176.

HUDSON L. & HAY F.C. (1980) *Practical Immunology*. Blackwell Scientific Publications, Oxford.

JENNINGS H.J., LUGOWSKI C.A., YOUNG N.M. (1980) Structure of the complex polysaccharide C-substance from *Streptococcus pneumoniae* type 1. *Biochemistry*, **19,** 4712.

KAYHTY H., KARANKO V., PELTOLA H.,SARNA S. & MAKELA P.H. (1980) Serum antibodies to capsular polysaccharide vaccine of group A *Neisseria meningitidis* followed for three years in infants and children. *J. Infect. Dis.* **142,** 861.

KENNY J.J., GUELDE G., CLAFLIN J.L. & SCHER I (1981) Altered idiotype response to phosphocholine in mice bearing an X-linked immune defect. *J. Immunol.* **127,** 1269.

KOHLER H., SMYK S. & FUNG J. (1981) Immune response to phosphorylcholine. VIII. The response of CBA/N mice to PC-LPS. *J. Immunol.* **126,** 1790.

LIM P.L. (1987) Isolation of specific IgM monoclonal antibodies by affinity chromatography using alkaline buffers. *Mol. Immunol.* **24,** 11.

LIM P.L. & CHOY W.F. (1986) Monoclonal IgM/A hybrid antibodies: artifacts due to anti-idiotype (T15) antibodies in commercial anti-alpha sera. *Molec. Immunol.* **23,** 909.

LIM P.L. & HO M.Y. (1983) Diagnosis of enteric fever by inhibition assay using peroxidase-labelled monoclonal antibody and *Salmonella typhi* lipopolysaccharide. *Aust. J. exp. Biol. Med. Sci.* **61,** 687.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L. & RANDALL R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

MORENO C., LIFELY M.R. ESDAILE J. (1985) Immunity and protection of mice against *Neisseria meningitidis* group B by vaccination, using polysaccharide complexed with outer membrane proteins: a comparison with purified B polysaccharide. *Infect. Immun.* **47,** 527.

MUSHER D.M., CHAPMAN A.J., GOREE A., JONSSON S., BRILES D. & BAUGHN R.E. (1986) Natural and vacine-related immunity to *Streptococcus pneumoniae. J. Infect. Dis.* **154,** 245.

OKALEY B.R., KIRSCHE D.R. & MORRIS N.R. (1980) A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Analyt. Biochem.* **105,** 361.

PELTOLA H., MAKELA P.H., KAYHTY H., JOUSIMIES H., HERVA E., HALLSTROM K., SIVONEN A., RENKONEN O.-V., PETTAY O., KARANKO V., AHVONEN P. & SARNA S. (1977) Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. *N. Engl. J. Med.* **297,** 686.

POTTER M. (1977) Antigen-binding myeloma proteins of mice. *Adv. Immunol.* **25,** 141.

SCHER I. (1982) The CBA/N mouse strain: an experimental model illustrating influences of the X-chromosome on immunity. *Adv. Immunol.* **33,** 1.

SELL S.H., WRIGHT P.F., VAUGHN W.K., THOMPSON J. & SCHIFFMAN G. (1981) Clinical studies of pneumococcal vaccine in infants. I. Reactogenicity and immunogenicity of two polyvalent polysaccharide vaccines. *Rev. Infect. Dis.* **3** (Suppl.), 597.

SNIPPE H., VAN HOUTE A.-J., VAN DAM J.E.G., DE REUVER M.J., JANSZE M. & WILLERS J.M.N. (1983) Immunogenic properties in mice of hexasaccharide from the capsular polysaccharide of *Streptococcus pneumoniae* type 3. *Infect. Immun.* **40,** 856.

SORENSEN U.B.S., AGGER R., BENNEDSEN J. & HENRICHSEN J. (1984) Phosphorylcholine determinants in six pneumococcal capsular polysaccharides detected by monoclonal antibody. *Infect Immun.* **43,** 876.

UBEIRA F.M., LEIRD J., SANTAMARINA M.T., VILLA T.G. & SANMARTIN-DURAN M.L. (1987) Immune response to *Trichinella* epitopes: the antiphosphorylcholine plaque-forming cell response during the biological cycle. *Parasitology*, **94,** 543.

WALLICK S., CLAFLIN J.L. & BRILES D.E. (1983) Resistance to *Streptococcus pneumoniae* is induced by a phosphocholine-protein conjugate. *J. Immunol.* **130,** 2871.

WILLIAMS C.A. & CHASE M.W. (1968) *Methods in Immunology and Immunochemistry*, Vol. II. Academic Press, New York.

ZOLLINGER W.D., MANDRELL R.E., GRIFFIS J. M., ALTIERI P. & BERMAN S. (1979) Complex of meningococcal group B polysaccharide and type 2 outer membrane protein immunogenic in man. *J. clin. Invest.* **64,** 836.