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Immune response of neonates to pneumococcal polysaccharide-protein conjugate

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Summary. Immune responses were studied in adult and young mice exposed to pneumococcal 6A and 19F polysaccharides (PSs), as well as 19F PS conjugated to proteins, e.g. human immunoglobulin G (HIgG), pneumococcal R_{61} cell wall polypeptide, and bovine serum albumin (BSA). Significantly higher IgM and IgG2 antibody titres were induced in mice receiving 19F PS-protein conjugates than in the control group receiving 19F PS alone.

Maternal immunization with 19F PS-HIgG conjugate elicited a low immune response in the offspring. However, when young mice from immunized mothers were given an additional dose of polysaccharide-protein conjugate, they gave an antibody response greater than that of mice not given additional immunogen. Similarly, young mice exposed to 14-valent pneumococcal vaccine during gestation produced higher antibody response to 6A and 19F PSs.

Secondary immunization of 19F PS or PS-protein conjugate at 1 or 2 weeks after primary immunization did not enhance antibody formation but rather suppressed the immune response to that polysaccharide.

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INTRODUCTION

Some isolated bacterial capsular polysaccharides (PS) do not produce appreciable antibody responses, regardless of the dose and regimen employed. High titres of antisera can be prepared against capsular polysaccharides by the injection of the inactivated whole bacteria. Antibodies against polysaccharides or oligosaccharides can be elicited when these antigens are conjugated to a protein carrier molecule before immunization. Such antibodies are known to confer protective immunity. For example, mice immunized with cellobiuronic acid conjugated to bovine serum albumin were protected against a lethal dose of type 3 pneumococci (Goebel, 1939a, b). *Haemophilus influenzae* type b polysaccharide conjugated to protein was converted from a 'T-independent' to a 'T-dependent' immunogen; this resulted in a significant increase in immunogenicity (Schneerson, Barrera, Sutton & Robbins, 1980). Immunization with the oligosaccharide of *Klebsiella* K_2 (Geyer, Stirm & Himmelspach, 1979), or meningococcal polysaccharides (Jennings & Lugowski, 1981), conjugated to protein by reductive amination, produced a high level of specific antibody.

Maternal immunization with pneumococcal polysaccharide results in the transfer of polysaccharide to offspring via the placenta and milk (Lee, 1980). Offspring exposed to polysaccharides during gestation or lactation produce a higher antibody response upon subsequent immunization than mice not exposed previously to polysaccharide (Lee, 1980; Gill, Kunz &

Davis, 1971, 1977). Thus, maternal immunization with pneumococcal polysaccharide may not be immunologically harmful or suppressive; rather it may improve the capacity of neonates to respond to these antigens (Lee, Blair, Szu & Lin, 1981).

The current pneumococcal vaccine contains fourteen types of polysaccharides, including type 6A and 19F. However, type 6A and 19F polysaccharides have been reported to be poor immunogens in individuals less than 2 years of age (Cowan, Amman, Wara, Howie, Schultz, Doyle & Kaplan, 1978; Granoff, 1980; Borgono, McLean, Vella, Woodhour, Canepa, Davidson & Hilleman, 1978); the reasons for this are not clear. An examination of the immunopotentiation of polysaccharide antigens conjugate to carrier molecules, as well as better understanding of the immune response of neonates to capsular polysaccharides would help in the development of a more effective vaccine for prevention of pneumococcal disease in such individuals.

The present work analyses the immune response of pneumococcal polysaccharide conjugated to various proteins. The immune response of neonatal mice from mothers immunized with pneumococcal polysaccharides or polysaccharide-protein conjugates during gestation was also investigated.

MATERIALS AND METHODS

Materials

Pneumococcal polysaccharide type 19F and 6A as well as vaccine were obtained from Merck, Sharp & Dohme, West Point, Pa. The chemical composition and purity of these polysaccharides were reported previously (Krishnamurthy, Lee, Henrichsen, Carlo, Stondt & Robbins, 1978; Robbins, Lee, Rastogi, Schiffman & Henrichsen, 1979). Human immunoglobulin G (HIgG) was obtained from Miles Biochemicals, Inc., Elkhart, Ind. Pneumococcal R_{61} cell wall polypeptide was prepared as described and its composition reported previously (Lee & Liu, 1977). Sodium cyanoborohydride (sodium cyanotrihydride borate) was obtained from Alfa Products, Danvers, Mass. DEAE-cellulose was obtained from Whatman Biochemicals, Ltd, Maidstone, Kent. Polystyrene tubes (12 × 75 mm, number 2052) were obtained from Oxnard, Calif. Bovine serum albumin, Brij, alkaline phosphatase, p-nitrophenyl phosphate were purchased from Sigma Chemical Company, St. Louis, Mo. Lyophilized guinea-pig complement and rabbit anti-lymphocyte serum were purchased from Microbiological Associates, Bethesda, Md. BALB/c mice were obtained from the Small Animal Production Section of the National Institutes of Health, Bethesda, Md.

Preparation of pneumococcal polysaccharide-protein conjugate

Polysaccharide-protein conjugates were prepared by the reductive amination method (Gray, 1978, 1974). Briefly, protein (HIgG, pneumococcal R_{61} cell wall polypeptide, or bovine serum albumin) together with pneumococcal 19F polysaccharide and sodium cyanoborohydride were dissolved in 0·2 M dibasic potassium phosphate, pH 8·0 and the mixture was incubated at 37° for 8 hr. Then, the mixture was passed through a Sepharose 4B column (1·9 × 90 cm), and eluted with 0·2 M ammonium acetate solution. The fractions collected were assayed for both protein content by absorbance at 280 nm and for polysaccharide content by the anthrone reaction (Scott & Melvin, 1953). Fractions containing the conjugates were pooled, dialysed extensively against distilled water, and lyophilized.

In some cases, the polysaccharide-protein conjugate was passed through a DEAE-cellulose column (Lee & Lin, 1981), which was then washed with 0·2 M dibasic potassium phosphate, pH 8·0. The bound polysaccharide-protein conjugate was eluted with 2 M sodium chloride. The fractions collected were assayed for both protein and polysaccharide content and samples containing conjugates were pooled, dialysed against distilled water and lyophilized. The polysaccharide-protein conjugates, e.g. 19F PS-HIgG exhibited specific reaction in immunodiffusion with antisera against both polysaccharide and protein. The conjugate also co-chromatographed away from the PS and protein peaks of gel filtration columns (Lee & Lin, 1981).

Antibody determination by the method of passive immune haemolysis

Polysaccharide-coated sheep erythrocytes 0·2 ml, prepared by the method described previously (Lee & Koizumi, 1981), were placed in test tubes containing several two-fold dilutions of standard or test antisera in 0·2 ml barbital buffer; the tubes were kept at 25° for 10 min. Then, 0·1 ml of 10% guinea-pig complement in barbital buffer was added; the mixture was adjusted to 1·0 ml with barbital buffer, incubated at 37° for 30 min, and centrifuged at 700 *g* for 10 min. Suspensions containing PS-coated erythrocytes and complement, prepared in the same experimental conditions, were

used as controls. The optical density of the supernatant was measured at 540 nm to provide a measure of the amount of antibody-mediated haemolysis produced.

Enzyme-linked immunosorbent assay (ELISA)

A modified version of the enzyme-linked immunosorbent assay (Lee, 1980; Callahan, Woodhour, Meeker & Hilleman, 1979; Barrett, Ammann & Stenmark, 1980; Russell, Edward & Wortham, 1980) was used. Polystyrene tubes were coated with the immunoglobulin fraction of rabbit anti-pneumococcal type 19F serum, 0·1 μg in 1 ml of saline, stirred at 37° for 3 hr. The tubes were emptied, then filled with type 19F PS (1 μg in 1 ml of 0·1 M Tris buffer, pH 8·5), incubated at 37° for 3 hr and washed twice with detergent solution (0·1% Brij in saline). Mouse antiserum samples, 10 μl each in 1 ml of saline, were added to tubes, incubated on shaker at 37° for 3 hr and washed twice with detergent solution. A phosphate-buffered saline (PBS) solution containing 2·5% normal rabbit serum and 0·5% bovine serum albumin (BSA) was added to each tube which was incubated on a shaker at 37° for 30 min. The tubes were emptied and washed twice with detergent solution. One millilitre of dilute (1:300 in PBS) alkaline phosphatase conjugated to rabbit anti-mouse IgM or IgG2, was added to each tube which was incubated on a shaker at 37° for 3 hr. Excess conjugate was removed by washing twice with detergent solution. To each tube was added 1 mg of p-nitrophenyl phosphate in 1 ml of 1 M Tris buffer, pH 9·8, containing 0·3 mM magnesium chloride; the tubes were incubated at 37° for 90 min. The enzymatic reaction was stopped by the addition of 0·05 ml of 4 N sodium hydroxide. Yellow p-nitro-phenolate was measured spectrophotometrically at 400 nm. The antibody concentration of the standard mouse pneumococcal 19F antiserum was measured by radioimmunoassay (Schiffman, Douglas, Bonner, Robbins & Austrian, 1980).

Plaque-forming-cell assay

Mice were immunized intraperitoneally with various doses of pneumococcal polysaccharides in 0·2 ml saline and the immune response determined 5 days later. Splenic plaque-forming cells (PFC) were determined by the technique of localized haemolysis in gel (Lee, Malik & Robbins, 1978; Jerne & Henry, 1963; Dresser, 1978). Sheep erythrocytes coated with pneumococcal polysaccharide by chromium chloride coupling procedure were used as indicator target cells. The cell mixtures in the gel plates were incubated for 17 hr at 37° in 5% CO_2 and humid atmosphere. Complement was added and the plates again incubated for 1 hr at 37° after which total plaque-forming cells were counted.

Effect of maternal immunization on the immune response of neonates

Pregnant mice at 14 days of gestation were injected subcutaneously with doses of 19F PS-HIgG conjugate. The offspring from these mice were divided into two groups: one group was given an additional dose of 19F PS-HIgG conjugate subcutaneously, 0·1 μg in 0·1 ml of saline, at 2 weeks of age, whereas the other group was given 0·1 ml of saline without conjugate. The spleens and sera were collected for PFC assay and antibody determination 7 days later.

Maternal transfer of polysaccharides and immunoglobulins to offspring

The experimental scheme for maternal transfer of polysaccharides and immunoglobulins to offspring is shown in Fig. 1. Pregnant mice at 14 days of gestation were injected subcutaneously each with 0·5 μg/type of fourteen-valent pneumococcal vaccine in 0·2 ml saline without polysaccharide. Within 12 hr after birth, the

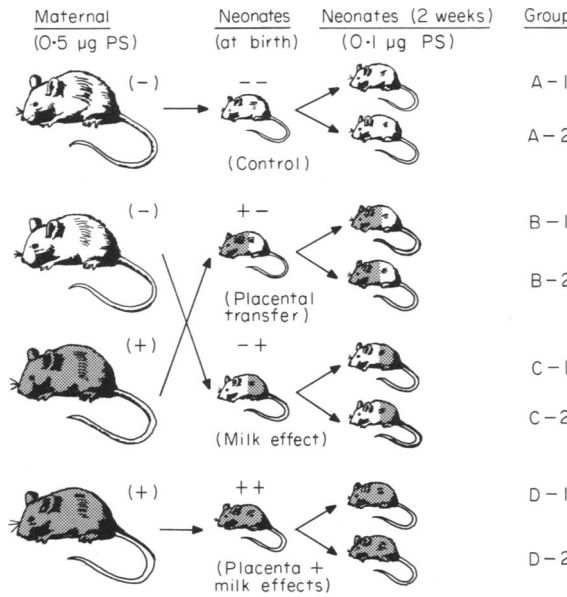

Figure 1. Experimental design on maternal transfer of polysaccharide to offspring through placenta and milk.

young from mothers immunized during gestation were nursed by the non-immunized foster mother; the young from non-immunized mothers were nursed by the mothers immunized during gestation. Some young mice from non-immunized mothers were nursed by the same mothers and served as control. In another group, the young mice were nursed by mothers immunized during gestation; they continued to be nursed by the same mothers during lactation. Thus, there are four groups of offspring: (A) control group, offspring from non-immunized mothers, nursed by the same mothers; (B) offspring exposed to maternal polysaccharides and immunoglobulins during gestation (placental transfer); (C) offspring exposed to maternal polysaccharides and immunoglobulins during lactation (milk effect); and (D) offspring exposed to maternal polysaccharides and immunoglobulins during both gestation and lactation. At 2 weeks of age, the young mice of each group were divided into two subgroups; one subgroup received an additional dose of pneumococcal vaccine, 0·1 μg/type in 0·1 ml of saline, whereas the other subgroup was given 0·1 ml of saline without polysaccharide. The immune responses to 6A and 19F polysaccharides were examined 7 days later.

RESULTS

Immune response to 19F polysaccharide-protein conjugate

Figure 2 shows the standard curve for the assay of serum antibody specific for 19F polysaccharide, determined by the method of passive immune haemolysis. The curve was linear for mouse antibody concentrations of 3–10 ng antibody protein/ml. The complement control caused negligible or no haemolysis to PS-coated erythrocytes. Table 1 shows the antibody response of 19F polysaccharide-protein conjugate in young as well as adult mice. The antibody titre of 2 week old mice was 0·61 to 1·92 μg 19F antibody protein/ml serum for mice given 0·1 μg and 10 μg of 19F PS, respectively; 9 week old mice had 2·40 to 9·24 μg antibody protein/ml after immunization with 0·05 μg or 5 μg of 19F PS. Two week old mice given 0·1 to 10 μg 19F PS conjugated with HIgG, cell wall polysaccharide, or BSA, exhibited significantly ($P<0·05$) higher antibody levels than the control group, given the same dose of 19F PS alone. In young mice, given the 10 μg dose, antibody titres were 2 to 2·3 times higher ($P<0·01$) than the control group. Similarly, higher antibody responses ($P<0·05$) were

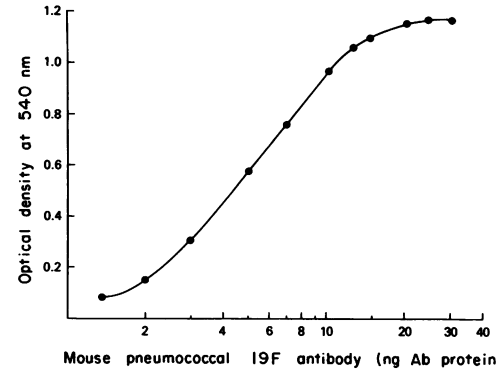

Figure 2. Standard curve of pneumococcal 19F antibody determined by the method of passive immune haemolysis. The 19F polysaccharide-coated sheep erythrocytes, 0·2 ml, were added with 0·2 ml of various concentration of mouse antisera of known antibody titres and 0·1 ml of 10% guinea-pig complement in barbital buffer, pH 7·4. The mixture was adjusted to 1·0 ml with barbital buffer, pH 7·4 and incubated at 37° for 30 min. The mixture was centrifuged and the optical density of the supernatant was measured at 540 nm.

observed in 9 week old mice. In adult mice, given 50 μg of polysaccharide conjugated with HIgG or BSA, antibody titres were 3·3 to 3·5 times higher ($P<0·01$) than the control group, given the same dose of 19F PS alone. This as well as the radioimmunoassay, measure total antibodies bound to polysaccharide antigen.

IgM and IgG2 antibody response to 19F polysaccharide-protein conjugate

Figure 3 shows the standard curves for the assay of

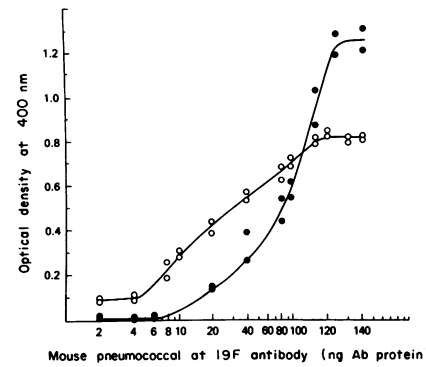

Figure 3. Standard curves for pneumococcal 19F IgM (●) and IgG2 (○) antibody determined by ELISA method.

Table 1. Immune response to 19F polysaccharide-protein conjugate

Age (weeks)	Immunizing dose (μg)		Immunogen: 19F PS	PS-HIgG	PS-wall polypeptide	PS-BSA
2	0·01		0·95±0·10* (3)†	0·80±0·03 (7)	0·84±0·07 (6)	0·82±0·02 (6)
	0·1		0·61±0·05 (3)	0·76±0·04‡ (5)	1·06±0·13‡ (5)	0·81±0·01§ (6)
	1·0		1·80±0·24 (6)	3·17±0·56‡ (6)	3·70±0·36§ (6)	1·84±0·18 (6)
	10·0		1·92±0·33 (6)	4·44±0·76§ (8)	3·96±0·29§ (6)	3·89±0·47§ (6)
9	0·05		2·40±0·15 (5)	2·08±0·05 (6)	3·65±0·62† (4)	2·45±0·12 (6)
	0·5	(I)	8·95±0·53 (6)	ND¶	11·7±0·87† (6)	ND
		(II)	7·70±0·54 (6)	12·8±0·73§ (6)	12·1±0·54§ (6)	ND
	5·0	(I)	9·24±0·50 (5)	14·8±1·74‡ (6)	10·6±1·89 (4)	10·2±0·54 (4)
		(II)	7·27±1·14 (4)	14·5±1·35§ (5)	10·2±1·33 (4)	17·3±1·71§ (4)
	50·0		4·43±1·39 (6)	15·8±1·80§ (6)	ND	14·6±1·81§ (6)

*Mean ± standard error, μg anti 19F antibody protein/ml serum by the method of passive immune haemolysis, 5 days after immunization.

†Number of animals.

‡$P < 0·05$, §$P < 0·01$, when the immune response of mice given 19F PS-protein conjugate was compared with the response of mice given the same dose of unconjugated 19F PS.

¶ND, not determined. I, II, Two separate experiments were performed with the same dose of immunogens.

IgM and IgG2 antibody specific for 19F PS, by a modified ELISA method. Optical density was directly proportional to IgM antibody concentration from 10 to 120 ng antibody protein/ml, whereas the curve was linear for mouse IgG2 antibody at concentrations of 4–120 ng antibody protein/ml. Table 2 shows the response of IgM and IgG2 antibody in mice given 19F PS-protein conjugate. The antibody levels of adult mice were 3·57–5·88 μg IgM antibody protein/ml serum and 1·70–5·52 μg IgG2 antibody protein/ml. In many cases, the mice immunized with 19F PS-protein conjugate induced IgM and IgG2 antibodies significantly ($P < 0·01$) higher than the control group given the same dose of 19F PS alone.

Table 2. IgM and IgG2 antibody response to 19F polysaccharide-protein conjugate

Antibody response	Immunizing dose (μg)	Immunogen: 19F PS	PS-HIgG	PS-wall polypeptide	PS-BSA
IgM	0·05	3·57±0·35* (6)†	6·68±0·79§ (6)	9·80±1·44§ (5)	9·39±1·94§ (7)
	0·5	5·78±0·48 (5)	8·68±1·37† (6)	8·56±0·59§ (7)	8·80±0·76§ (6)
	5·0	5·88±0·88 (6)	7·70±0·66 (6)	8·87±1·08‡ (6)	9·71±0·59‡ (6)
IgG2	0·05	1·70±0·35 (5)	1·79±0·19 (6)	2·70±0·66 (5)	2·46±0·69 (5)
	0·5	3·74±0·69 (6)	3·49±0·10 (5)	5·93±0·60‡ (7)	3·02±0·20 (5)
	5·0	5·52±0·52 (6)	9·95±2·07§ (6)	ND	11·7±3·00§ (8)

*Mean ± standard error, μg antibody protein/ml serum by the ELISA method, 5 days after immunization. Ten microlitres of sample sera were diluted to 1 ml of saline, so that the optical density values fell within the linear part of the standard curve.

†Number of animals.

‡$P < 0·05$, §$P < 0·01$, when the immune response of mice given 19F PS-protein conjugate was compared with the response of mice given the same dose of unconjugated 19F PS.

¶ND, not determined.

Immune response of neonates to 19F PS-HIgG conjugate

Table 3 shows the effect of maternal immunization with 19F PS-HIgG conjugate on the direct IgM PFC response of neonates. Maternal immunization with

Table 3. Immune response of neonates to pneumococcal 19F PS-HIgG conjugate

Maternal immunization (μg)	Additional immunogen given to young (0·1 μg)	
	−	+
0·05	98·0 ± 10·5* (5)	6254 ± 321‡§ (7)
0·5	132 ± 30·1 (5)	4620 ± 671‡§ (5)
5·0	198 ± 23·2 (6)	3787 ± 239‡§ (7)
50·0	139 ± 14·4 (7)	2057 ± 293‡ (7)
Immunization to neonates (0·1 μg)		
19F polysaccharide		1015 ± 33·0 (9)
19F PS-HIgG		1919 ± 32·7¶ (10)

*Mean ± standard error, direct IgM PFC/spleen by the method of PFC.

†Number of animals.

‡$P < 0·01$, when the immune response of neonates to maternal immunization is compared with young mice given an additional immunogen.

§$P < 0·01$, when the immune response of neonates to maternal immunization followed by an additional dose of immunogen given to young mice is compared with the mice given conjugate immunogen alone at 2 weeks of age.

¶$P < 0·01$, when the immune response of neonates given a conjugate immunogen is compared with the mice given an unconjugated 19F PS alone.

19F PS-HIgG conjugate elicited a low immune response in the offspring. However, when young mice of immunized mothers were given an additional dose of PS-protein conjugate, they gave a PFC response greater than that of mice not given additional immunogen. These mice exposed to PS-protein conjugate during gestation and lactation, also exhibited higher PFC response than those given 19F PS-protein conjugate alone at 2 weeks of age.

Effect of maternal transfer of polysaccharide and immunoglobulin to offspring

Table 4 shows the effect of maternal transfer of polysaccharides and immunoglobulins to offspring on the immune response of neonates to 6A or 19F PS. Maternal immunization with fourteen-valent polysaccharide vaccine induced a low antibody response to 19F PS in the offspring. However, as observed previously, when young mice were given an additional dose of vaccine at 2 weeks of age, they gave a significantly higher antibody response. Maternal immunization with pneumococcal vaccine during gestation or/and lactation, induced a significantly higher PFC response to 19F PS. The relative effect or importance of maternal immunization during gestation or lactation on immune response of neonates was not clear. Immunization during both gestation and lactation appears to produce a synergistic effect which gave the neonates a greater immune response specific for 19F PS. The PFC responses to 6A PS were very low in young mice given 6A vaccine, either monovalent or as a component of fourteen-valent vaccine. However, when young mice were exposed to polysaccharide during gestation or/and lactation, they induced a high antibody response to 6A PS.

Effect of secondary immunization of 19F PS or PS-protein conjugate

Table 5 shows the effect of secondary immunization with 19F PS or PS-protein conjugate on antibody response. Secondary immunization with 19F PS or PS-protein conjugate at 1 or 2 weeks after primary immunization did not enhance the PFC response or serum antibody level, but rather suppressed the antibody formation to that polysaccharide. Treatment by 0·3 ml anti-lymphocyte serum (ALS) at the time of secondary immunization of 19F PS did not restore the decreased antibody response to 19F PS [direct IgM PFC/spleen; 3633 ± 560 (6) in 19F PS group against 4450 ± 886 (6) in 19F PS + ALS treated group]. In contrast, ALS treatment in mice given secondary immunization with 19F PS-HIgG conjugate restored partially the decreased antibody response [direct IgM PFC/spleen; 4433 ± 589 (6) in 19F PS-HIgG group against 7580 ± 940* (5) in conjugate + ALS treated group, $P < 0·05$].

DISCUSSION

Pneumococcal infection remains the primary cause of pneumonia, otitis media and meningitis in infants and children (Loda, Collier, Glezen, Strangert, Clyde & Denny, 1975; Baird, Whittle & Greenwood, 1976;

Table 4. Effect of maternal transfer of polysaccharides and immunoglobulins to offspring on immune response of neonates

Group	Maternal transfer of PS and Ig during: Gestation	Lactation	Additional PS given to neonates (0·1 μg/type)	Immunogen: 6A, Immunization with: Monovalent	6A, 14-valent	19F, 14-valent
A-1	—	—	—	61·1 ± 6·8 (7)†	72·9 ± 6·6 (6)	123 ± 12·0 (6)
A-2	—	—	+	77·9 ± 7·0 (7)	88·6 ± 9·1 (7)	1696 ± 119 (10)
	(Control)					
B-1	+	—	—	158 ± 17·3§ (7)	159 ± 21·6§ (5)	173 ± 15·4‡ (6)
B-2	+	—	+	168 ± 20·9§ (8)	184 ± 19·0§ (9)	3230 ± 289§ (8)
	(Placental transfer)					
C-1	—	+	—	132 ± 11·4§ (6)	125 ± 7·0§ (6)	210 ± 27·6‡ (6)
C-2	—	+	+	124 ± 14·9‡ (7)	127 ± 5·5‡ (8)	2597 ± 375§ (7)
	(Milk effect)					
D-1	+	+	—	162 ± 15·6§ (7)	138 ± 10·4§ (5)	288 ± 47·5§ (6)
D-2	+	+	+	178 ± 9·6§ (8)	159 ± 15·8§ (5)	5219 ± 746§ (9)
	(Placental and milk effect)					

*Mean ± standard error, direct IgM PFC/spleen by the method of localized haemolysis in gel. Maternal group was injected subcutaneously each with pneumococcal vaccine, 0·5 μg/type in 0·2 ml of saline, or 0·2 ml of saline without polysaccharide.

†Number of animals.

‡$P < 0{\cdot}05$, §$P < 0{\cdot}01$, when immune response of neonates exposed to maternal treatment was compared with the response of mice that were not exposed to maternal treatment.

Table 5. Effect of secondary immunization on pneumococcal 19F antibody response

Age (weeks)	Dose (μg)	Secondary immunization (weeks)	Immunogen: 19F PS	PS-HIgG	PS-wall polypeptide
			(Direct IgM PFC/spleen)		
8	5·0	(Primary)	5400 ± 623* (6)†	14205 ± 1845 (6)	12650 ± 940 (6)
		1	3575 ± 380‡ (6)	5025 ± 589‡ (6)	3413 ± 410‡ (6)
		2	2825 ± 385‡ (6)	2025 ± 247‡ (6)	1633 ± 82·9‡ (6)
			(μg Ab protein/ml serum)		
9	5·0	(Primary)	9·92 ± 1·44 (6)	12·9 ± 1·41 (6)	10·6 ± 1·89 (4)
		1	4·42 ± 0·78‡ (6)	5·61 ± 0·96‡ (6)	4·04 ± 0·43‡ (6)

*Mean ± standard error, determined by the method of localized haemolysis in gel, or passive immune haemolysis.

†Number of animals.

‡$P < 0{\cdot}01$, when immune response of mice given secondary immunization is compared with the response of mice given immunization alone.

Brownless, Deloache, Cowan & Jackson, 1969). In the high risk population, including sickle cell disease, chronic liver disease, nephrotic syndrome, or immunodifficiency, pneumococcal infection is an even more serious problem. For pneumococcal type 6A, 14, 19F and 23, the highest incidence rate occurs in infants and children (Austrian, Howie & Ploussard, 1977; Austrian, 1977). However, a difficult problem confronting the development of pneumococcal vaccine is the low immune response of infants and children to the purified polysaccharides, particularly to types 6A and 19F (Cowan *et al.*, 1978; Granoff, 1980; Borgono *et al.*, 1978). Multiple or 'booster' immunizations of infants with these immunogens do not elicit sufficient antibody formation for protection against pneumococcal diseases.

The transfer of immunity from mother to young has been reported to occur prenatally or shortly after birth (Sterzl & Silverstein, 1967; Martensson & Fudenberg, 1965; Fudenberg & Fudenberg, 1964). In the rat, mouse, rabbit, and human, such transfer appears to be restricted to IgG class. The time when immunoglobulin is transferred from mother to young varies in different animal species. In humans and rabbits, passive immunity is acquired before birth, whereas in mice and rats it is acquired during pre- and postnatal periods (Schlamowitz, 1976). In the present study, pneumococcal type 19F polysaccharide was conjugated to various proteins, including human immunoglobulin G (HIgG), pneumococcal R_{61} cell wall polypeptide and bovine serum albumin. The polysaccharide-protein conjugate, e.g. 19F PS-HIgG exhibited specific reaction in immunodiffusion with antisera against both polysaccharide and protein. The conjugate co-chromatographed away from the PS and protein peaks of a gel filtration column. A mixture of 19F PS and HIgG did not enhance the antibody response indicating that covalent linkage or a strong bond between PS and protein is necessary for immunopotentiation (Lee & Lin, 1981).

19F PS-protein conjugates induced higher IgM and IgG2 antibodies in young and adult mice than the control groups given the same dose of 19F PS alone. Furthermore, offspring exposed to PS-protein conjugate during gestation and lactation also exhibited an antibody response greater than that of mice given 19F PS or PS-protein conjugate alone at 2 weeks of age. Mice pretreated with a carrier known to activate helper T cells have been reported to produce a high antibody response to weakly immunogenic lipoteichoic acid, when immunized with lipoteichoic acid bound to the same carrier (Beining, Flannery, Prescott & Baker, 1980). In the present study, it was considered that the enhanced immunogenicity of PS-protein conjugate was likely to be due to the participation of carrier-specific helper T cells; the protein molecule in the PS-protein conjugate may stimulate helper T-cell activity to achieve an enhanced antibody response to the polysaccharide. Similar to previous observations on pneumococcal polysaccharide, maternal immunization with 19F PS-protein conjugate did not appear immunologically harmful or suppressive; rather it improved the capacity of neonates to respond to these antigens.

Treatment of mice with ALS has been found to induce an increase in the antibody response to pneumococcal polysaccharide (Barthold, Prescott, Stashak, Amsbaugh & Baker, 1974). The stimulating effect following ALS treatment was apparently due to the inactivation of suppressor T cells. These cells act primarily by limiting the extent to which B cells proliferate after immunization (Baker, Stashak, Amsbaugh & Prescott, 1974). The magnitude of the antibody response to pneumococcal type 3 polysaccharide is greatly influenced by the activities of two types of T cells: suppressor and amplifier T cells. Suppressor T-cell activity appears to be fully developed as early as 2 weeks of age; in contrast, amplifier T-cell activity does not reach maturity until 8–10 weeks of age (Morse, Prescott, Cross, Stashak & Baker, 1976). The inhibitory effects of suppressor T cells are predominant in young mice and may play an important role in the low immune response to polysaccharide in neonates (Lee *et al.*, 1978; Morse *et al.*, 1976). The PS-protein conjugate appears to interact with these regulatory T cells in such a way that it decreases or modifies the inhibitory effects of suppressor T cells; it is not known whether this enhanced antibody response is due to the ability of carrier-specific helper T cells to overcome the effects that would normally be induced by suppressor T cells in mice immunized with unconjugated PS.

A study on maternal immunization with meningococcal polysaccharides, group A and C during pregnancy revealed increased antibody titres in mothers as well as the placental transfer of antibodies to the newborn, especially with regard to group C polysaccharide (Carvalho, Giampaglia, Kimara, Pereiea, Farhat, Neves, Prandini, Carvalho & Zarvos, 1977). However, the relation of high levels of maternal antibodies to protection of the newborn from the infection was not established. It has been observed

that a steady progression in development of B cells in mice proceeds between 13 days of gestation and birth. The first surface immunoglobulin-bearing cells occur at 16 to 17 days of gestation. B cells do not become fully developed upon first expression of surface immunoglobulin (Rosenberg & Cunningham, 1976). In the present study, the polyvalent pneumococcal polysaccharide vaccine was given to pregnant mice at 14 days of gestation and the effects of maternal transfer of polysaccharide and immunoglobulin to offspring during gestation and/or lactation were studied. Young mice exposed to polysaccharides during gestation and/or lactation appear to produce a higher antibody response to 19F and 6A polysaccharides than unexposed mice. It is plausible that maternal immunization with excessive dose of polysaccharide antigens leads to tolerance or paralysis of neonatal immune responses. However, the results of present study suggest that maternal immunization under certain conditions, e.g. with an optimum dose of polysaccharide vaccines at a critical period of perinatal development, could provide the offspring with an enhanced immune response in postnatal life.

An increased antibody response has been observed in young children that received a booster immunization with meningococcal group A polysaccharide (Gold, Lepow, Goldschneider, Draper & Gotschlich, 1975). It has been recommended that immunization with pneumococcal polysaccharide vaccine be given to children at 6 months of age, with a repeat or booster immunization at 2 years of age (Cowan *et al.*, 1978). Contrary to these observations or suggestions, the present work indicates that secondary or 'booster' immunization of 19F PS or PS-protein conjugate does not enhance the immune response of neonates, rather, their antibody formation is suppressed. This inhibitory effect of secondary immunization may not be directly related to the activation of suppressor T cells, since the ALS treatment could not restore to normal the decreased antibody response. Priming with a subimmunogenic dose followed by an optimum dose of pneumococcal type 3 polysaccharide results in the development of a state of immunological unresponsiveness; this low-dose paralysis was an antigen-specific T-cell-dependent phenomenon (Baker *et al.*, 1974). Booster immunization children with pneumococcal vaccine appears to induce impairment of antibody response (Borgono *et al.*, 1978).

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