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Deficiencies in Opsonic Defense to Pneumococci in the Human Newborn Despite Adequate Levels of Complement and Specific IgG Antibodies

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ABSTRACT. We studied the major determinants of opsonophagocytosis against *Streptococcus pneumoniae* serotypes 14 and 19 in paired cord/maternal sera from 27 healthy term and 24 preterm infants in an attempt to gain more insight in the susceptibility of newborns to pneumococcal infection. For both pneumococcal serotypes studied, opsonic activity in neonatal sera varied greatly, but was moderately to profoundly deficient when compared to paired maternal sera, both in preterm (34.5 and 34.9% of the activity in maternal serum, for serotypes 14 and 19, respectively, $p < 0.001$ for both) and in term serum (43.5 and 52.7% of the activity in maternal serum, for serotypes 14 and 19, respectively, $p < 0.001$ for both). The opsonic deficiency in preterm sera could be ascribed to a diminished level of the major opsonins for pneumococci, *i.e.* complement factor C3 deposited on the bacterial surface (69.5 and 66.2% of C3 deposition in maternal serum on serotypes 14 and 19, respectively, $p < 0.01$ for both) and specific anticapsular IgG antibodies (48.5 and 14.1% of maternal levels for serotypes 14 and 19, respectively, $p < 0.001$ for both). However, the opsonic defect in serum from term infants could not be explained in a similar way, because C3 deposition and specific anticapsular IgG levels were equal to the values found in the paired maternal sera. Therefore, we conclude that the opsonic defect in newborn serum for pneumococci cannot be solely explained by a deficiency in the major opsonins for these bacteria. A dysfunction in these opsonins seems to be a more likely explanation for the observed opsonic defect in the neonate. (*Pediatr Res* 27: 514–518, 1990)

Abbreviations

CFU, colony forming units
C3, complement factor C3
GHBSS, Hanks' balanced salt solution containing 1% gelatin
NHS, normal human serum
PMN, polymorphonuclear leukocytes
PPS, pneumococcal capsular polysaccharides
VBS, veronal buffered saline
VBS^{2+}, 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+}

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Systemic infection due to *Streptococcus pneumoniae* is a serious disease in the neonatal period. In many infants the infection runs a fulminant course with a fatal outcome in 50%. The incidence of neonatal pneumococcal septicemia, however, is low. *S. pneumoniae* causes approximately 5% of all neonatal septicemias (1). Results of epidemiologic studies suggest that the majority of infants with neonatal pneumococcal septicemia acquire the microorganisms either by the ascending route or during passage through the birth canal. The rarity of neonatal pneumococcal disease is probably due to the low incidence of carriage of pneumococci in the genital tract (2–4).

To gain better insight in the apparent susceptibility of the newborn to pneumococcal septicemia, we determined the efficiency of opsonophagocytosis of pneumococci in paired cord/maternal sera of healthy term and premature neonates. This process is one of the major host-defense mechanisms against such bacteria in man.

MATERIALS AND METHODS

Sera. Serum was collected from the umbilical cord directly postnatally or by venipuncture within 48 h of birth from 27 healthy term neonates (mean gestational age 40 wk, range 37–42 wk; mean birth wt 3447 g, range 2750–4250 g), 20 preterm neonates (mean gestational age 33 wk, range 28–36 wk; mean birth wt 2064 g, range 1200–3400 g), and four immature neonates (mean gestational age 26 wk, range 25–26 wk; mean birth wt 1022 g, range 755–1330 g). These newborns were hospitalized in either the newborn nursery of the Department of Obstetrics in the University Hospital, Utrecht, or the neonatal intensive care unit of the Children's Hospital "Het Wilhelmina Kinderziekenhuis," Utrecht, and all were without signs of infection. Serum samples were also collected from their mothers within 1 wk of giving birth. Sera from 20 healthy adults were mixed, this pool was called NHS and used as the reference serum. All serum samples were stored in 1-mL aliquots at −70°C until use.

Complement assays. The classic and alternative pathways of complement activation were determined as previously described (5). Briefly, sera were diluted in either VBS containing VBS^{2+} to determine classic complement pathway activity or VBS containing 5 mM Mg^{2+} and 8 mM EGTA (EGTA-VB) to determine alternative complement pathway activity. The assays were performed in U-well microtiter plates (Greiner Labortechnik, Nurtingen, FRG). A total of 50 μL of either sensitized sheep erythrocytes (2×10^8/mL VBS^{2++}) or rabbit erythrocytes (1.5×10^8/mL EGTA-VB) were added to each well and mixed with 100 μL of various serum dilutions. Hemolysis was measured spectrophotometrically in a Titertek-Multiskan (Flow Laboratories, Helsinki, Finland) after incubating the mixtures for either 60 min at 37°C (classic pathway) or 30 min at 37°C (alternative pathway).

Complement activity was expressed at 50% hemolysis U/mL, as previously described (5).

Deposition of complement factor C3 on the surface of pneumococci was measured by ELISA. Wells of flat-bottom microtiter trays (Titertek Immunoassay-plate, Flow Laboratories, Amsterdam, The Netherlands) were coated with 100 μL of formaline-killed pneumococci of serotype 14 or 19. The trays were incubated overnight at 4°C and subsequently washed five times with tap water containing 0.05% Tween-20 (Merck, Schuchardt, Hohenbrunn, FRG). Subsequently 50 μL of a 4% dilution of Elk blocking agent (DMV-Campina BV, Eindhoven, The Netherlands) in PBS was added. After a period of 1 h serial dilutions of serum samples were pipetted into the antigen-containing wells and they were incubated again for 1 h at 37°C. Then the trays were washed and a horseradish-peroxidase-conjugated goat antiserum to human C3 (Cappel-Organon Teknika Corporation, West Chester, PA) was added to the wells. After incubation and washing a substrate solution containing tetra-methyl-benzidine (Sigma, St. Louis, MO) and urea peroxide (Organon Teknika, Oss, The Netherlands) dissolved in 1.1 M acetate buffer (pH 6.0) was added to the bound conjugate. The reaction was stopped after 10 min at 37°C by adding 50 μL of 2 NH_2SO_4 to the wells. The color reactions were measured by absorption at a wavelength of 450 nm in a Titertek Multiskan and expressed as OD units. The titer of the individual sera was defined as the reciprocal of OD_{50} = 1.0. Preliminary studies showed that this OD value resulted in samples being on the linear part of the ELISA curve. All values were related to the OD value of NHS.

Determination of antibodies. Determination of antibodies to either intact pneumococci or pneumococcal capsular polysaccharides serotypes 14 and 19 was performed using an ELISA as previously described (6).

To determine the antibodies to intact pneumococci, the initial steps were identical to the ones described above for determination of the C3 deposition, except for the blocking agent. After incubation of the antigen-containing wells with the different serum dilutions, the trays were washed and a horseradish-peroxidase-conjugated goat antiserum to human IgG (Nordic, Tilburg, The Netherlands) was added to the wells. The reference serum pool was arbitrarily set at 100 U/mL. The results obtained and the individual maternal and infant sera were expressed proportionally to this value. The coefficient of variation of the method determined by testing individual samples with the same internal control on different days was found to be <15%.

To determine the antibodies to PPS serotypes 14 and 19, microtiter trays were coated overnight at 37°C with 100 μL of 10 μg/mL purified polysaccharides (American Type Culture Collection, Rockville, MD) in 0.9% NaCl. Serial dilutions of serum samples and reference plasma pool were incubated for 4 h at room temperature. Subsequently, wells were incubated with peroxidase-conjugated goat anti-human IgG (Cappel-Organon). O-Phenylene-diamine (5.5 mM) was used as substrate, OD was measured at 450 nm. The reference serum pool contained 1778 (serotype 14) and 440 (serotype 19) ng of antibody nitrogen per mL (7). The results obtained with the individual maternal and infant sera were expressed proportionally to these values. The interassay variability of the procedure was assessed by incorporating next to the standard serum a normal human serum pool in every assay; the coefficient of variation of the method was found to be <15%.

Radioactive labeling of bacteria. Isolates of *S. pneumoniae* serotypes 14 and 19 from neonates with septicemia were maintained on blood-agar plates. Colonies were freshly inoculated into Mueller-Hinton broth (Difco Laboratories, Detroit, MI) supplemented with adenosine and either 2.5% FCS for serotype 14 or 2.5% ultroser G (Reactifs IBF, Villeneuve la Garenne, France) for type 19. A total of 10 μCi [^{3}H]glucosamine (sp act 22 Ci/mmol, Amersham Laboratories, Buckinhamshire, UK) was added for labeling serotype 14 and 20 μCi [^{3}H]glucosamine for labeling type 19. After incubation for 18 h at 37°C in a shaking water bath, bacteria were washed three times in isotonic PBS (pH 7.4) and subsequently resuspended in PBS at a concentration of 2.5 × 10^8 CFU/mL.

Phagocytosis assay. A total of 0.1 mL of [^{3}H] labeled *S. pneumoniae* were mixed with 0.4 mL of a serum diluted to a final concentration of 30% in GHBSS and incubated for 30 min at 37°C in a slowly rotating water bath. The bacteria were then washed once in ice-cold PBS and centrifuged at 1500 × *g* at 4°C for 15 min to separate the opsonized bacteria. The pellet was resuspended in GHBSS at a concentration of 5 × 10^7 CFU/mL. In two biovials (Bio-Vial, Beckman Instruments, Chicago, IL) 0.1 mL (5 × 10^7 CFU/mL) of opsonized labeled bacteria were

Table 1. *Complement activity in sera from term and preterm newborns*

Complement activation pathway	Complement activity mean ± SD (range)	% of NHS* mean ± SD (range)
Classic		
Term (*n* = 26)	1129 ± 764 (365–3577)†	52 ± 36 (15–171)
Preterm (*n* = 23)	900 ± 670 (76–2933)†	41 ± 29 (6–85)
Alternative		
Term (*n* = 17)	36 ± 12 (12–67)‡	59 ± 25 (13–119)
Preterm (*n* = 17)	33 ± 8 (19–47)‡	49 ± 12 (21–75)

* NHS (pooled serum from 20 normal healthy adults) with a CH50 value of 2435 U/mL and a AP50 value of 68 U/mL.

† Measured as hemolytic units of complement that lysed 50% of sensitized sheep red blood cells (CH50)/mL.

‡ Measured as hemolytic units of complement that lysed 50% of unsensitized rabbit red blood cells (AP50)/mL.

Table 2. *C3 deposition on surface of S. pneumoniae serotypes 14 and 19 in sera of term and preterm newborns and in paired maternal serum*

C3 deposition*	Serotype 14	Serotype 19
Term (*n* = 20)	77 ± 49† (22–197)	77 ± 49† (22–176)
Mother (*n* = 20)	86 ± 40 (39–181)	89 ± 40 (33–176)
Preterm (*n* = 23)	57 ± 24‡ (26–124)	45 ± 19‡ (20–93)
Mother (*n* = 23)	82 ± 34 (41–180)	68 ± 29 (32–147)

* Expressed as percent of NHS values, mean ± SD (range).

† Value not significantly different from maternal serum.

‡ Value significantly different from maternal serum, $p < 0.001$.

Table 3. *IgG antibody concentration to intact S. pneumoniae serotypes 14 and 19 in sera of term and preterm newborns and in paired maternal serum*

IgG antibody concentration (U/mL)*	Mean ± SD	Range	*p* value†
Intact serotype 14			
Term (*n* = 20)	86 ± 76	14–292	NS
Mother (*n* = 20)	71 ± 72	15–312	
Preterm (*n* = 23)	47 ± 38	8–143	NS
Mother (*n* = 23)	57 ± 67	14–356	
Intact serotype 19			
Term (*n* = 20)	77 ± 67	5–294	<0.02
Mother (*n* = 20)	64 ± 62	17–285	
Preterm (*n* = 23)	48 ± 34	9–138	NS
Mother (*n* = 23)	55 ± 53	16–279	

* Data expressed proportionally to the reference serum pool (NHS), which was arbitrarily assigned a value of 100 U/mL.

† *p* value of difference between infant sera and paired maternal sera (assessed by *t* test for paired observations).

Table 4. *IgG antibody concentration to PPS serotypes 14 and 19 in sera of term and preterm newborns and in paired maternal serum*

IgG antibody concentration (ng/mL)*	Mean ± SD	Range	*p* value†
PPS serotype 14			
Term (n = 19)	838 ± 622	182–2260	NS
Mother (n = 19)	1093 ± 950	166–3768	
Preterm (n = 24)	356 ± 362	15–1658	<0.001
Mother (n = 24)	734 ± 592	151–2713	
PPS serotype 19			
Term (n = 19)	178 ± 240	7–910	<0.001
Mother (n = 19)	261 ± 266	18–1019	
Preterm (n = 24)	26 ± 34	4–146	<0.001
Mother (n = 24)	185 ± 186	18–874	

* Data expressed proportionally to the values of the reference serum pool, which were 1778 ng/mL for serotype 14 and 440 ng/mL for serotype 19.

† p value of difference between infant sera and paired maternal sera (assessed by t test for paired observations).

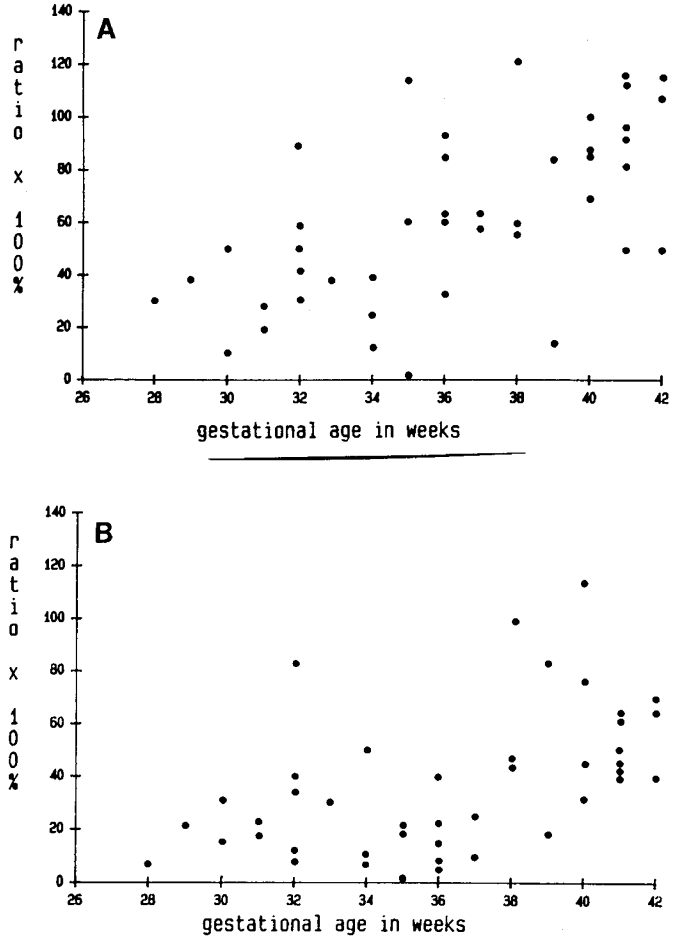

Fig. 1. *A*, Neonatal/maternal ratio of IgG antibodies to capsular polysaccharides of *S. pneumoniae* serotype 14 in 43 paired serum samples related to gestational age (wk). Correlation coefficient $r = 0.596$ ($p < 0.001$). *B*, Neonatal/maternal ratio of IgG antibodies to capsular polysaccharides of *S. pneumoniae* serotype 19 in 43 paired serum samples related to gestational age (wk). Correlation coefficient $r = 0.561$ ($p < 0.001$).

Table 5. *Uptake of S. pneumoniae after incubation in sera of term and preterm newborns and in maternal serum*

	% uptake*	*p* value†
Serotype 14		
Term (n = 27)	47 ± 42 (5–143)	$0.01 < p < 0.001$
Mother (n = 27)	108 ± 57 (11–250)	
Preterm (n = 24)	38 ± 29 (6–100)	<0.001
Mother (n = 24)	110 ± 53 (26–250)	
Serotype 19		
Term (n = 27)	39 ± 36 (2–152)	<0.001
Mother (n = 27)	74 ± 57 (15–263)	
Preterm (n = 22)	29 ± 29 (6–102)	<0.001
Mother (n = 22)	83 ± 54 (11–205)	

* Expressed as percent of NHS value, mean ± SD (range).

† p value of difference between infant sera and paired maternal sera (assessed by t test for paired observations).

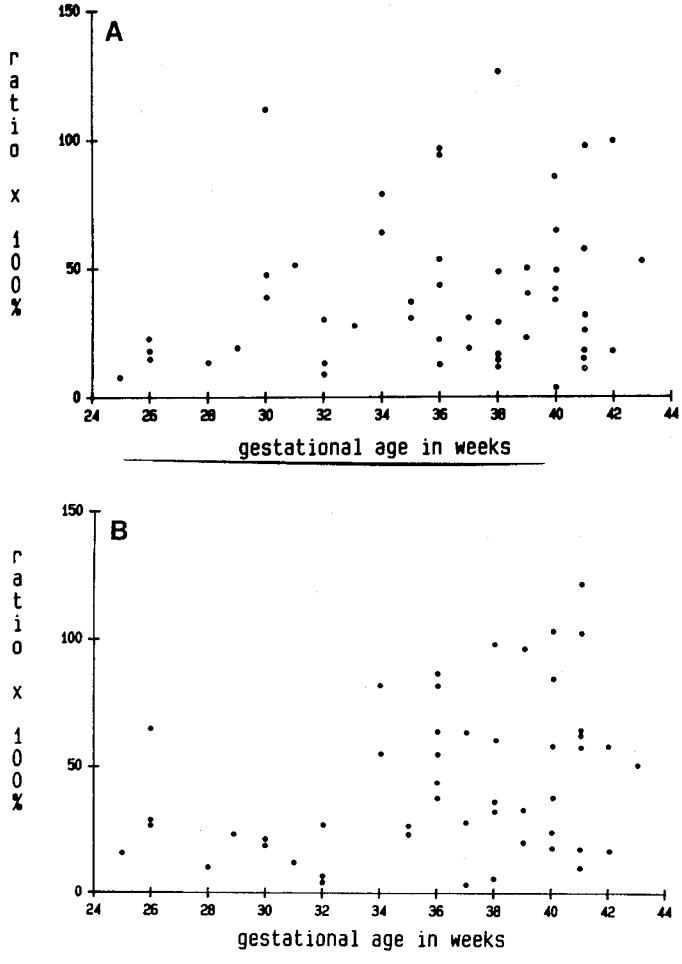

Fig. 2. *A*, Neonatal/maternal uptake ratio of ^{3}H-labeled *S. pneumoniae* serotype 14 in 51 serum samples related to gestational age (wk). The uptake ratio was not significantly related to gestational age ($r = 0.264$, $p > 0.05$). *B*, Neonatal/maternal uptake ratio of ^{3}H-labeled *S. pneumoniae* serotype 19 in 49 serum samples related to gestational age (wk). The uptake ratio was only weakly significantly related to gestational age ($r = 0.342$, $0.02 < p < 0.01$).

mixed with 0.1 mL (5×10^6 CFU/mL) of PMN, isolated from the blood of normal humans as previously described (8). To measure the total amount of radioactivity 2.5 mL of scintillation fluid (Aqualuma Plus, Lumac/3M, Schaesberg, The Netherlands)

was added to a third biovial containing 0.1 mL of opsonized labeled bacteria. The mixtures were incubated at 37°C in a shaking water bath. After 12 min the reaction was stopped by adding 2.5 mL of ice-cold PBS to the biovials. PMN and associated pneumococci were separated from free bacteria by centrifugation at 160 × *g* at 4°C for 5 min. This procedure was repeated three times. The pellets were then resuspended in 2.5 mL of scintillation fluid. Radioactivity was counted in a liquid scintillation counter (Philips PW 4700, Almelo, The Netherlands) and uptake of radiolabeled pneumococci by PMN after 12 min was expressed as a percentage of the total amount of radioactivity added, which was determined in a separate biovial. The percent uptake at 12 min was considered the measure of bacterial opsonization. The uptake percent achieved with NHS was arbitrarily set at 100% and the data of the individual sera were expressed proportionally to this value. The coefficient of variation of the method, determined by testing individual samples with the same internal control on different days was found to be <10%.

Statistical analysis. The significance of differences between means of paired data was assessed by two-tailed *t* test for paired observations. Correlation coefficients were calculated where appropriate.

RESULTS

Complement activity in serum from term and prematurely born neonates. Complement activity in serum from neonates was lower than that found in NHS (Table 1). The classic complement activity in the sera of term and premature newborns was 52% ± 36% and 41% ± 29% (mean level ± SD), respectively, of the activity present in NHS. The alternative complement activity in the sera of term and premature newborns was 59% ± 25% and 49% ± 12% (mean level ± SD), respectively of the activity present in NHS. Complement activity showed no significant correlation with either gestational age or birth wt ($p > 0.1$).

C3 deposition. The amount of C3 deposited on the surface of the *S. pneumoniae* serotypes 14 and 19 after incubation in sera of term and preterm newborns varied considerably (Table 2). Compared to paired maternal serum, term neonates showed no significant difference ($p > 0.5$) in the amount of C3 deposited on the bacterial surface, whereas C3 deposition after incubation in sera of premature neonates was significantly less ($p < 0.01$).

Antibodies to intact pneumococci and capsular polysaccharides. IgG antibody titers to intact *S. pneumoniae* serotypes 14 and 19 and to PPS showed a wide range in the sera of both mothers and neonates, as demonstrated in Tables 3 and 4. The IgG titer against intact pneumococci in 20 serum samples of term neonates was higher than in paired maternal serum. This difference was significant ($p < 0.02$) for serotype 19, but not for serotype 14 ($p > 0.5$). The concentrations in 23 preterm sera did not differ significantly from paired maternal sera ($p > 0.1$).

The IgG titers against PPS in neonatal sera of both term and preterm neonates were significantly lower for serotype 19 than those found in paired maternal sera ($p < 0.001$). This was also found in sera from preterm neonates for serotype 14. Titers for this serotype in sera from term neonates were not, however, significantly different from maternal serum ($p > 0.05$).

A significant correlation was found between the neonatal/maternal ratio of IgG against PPS and the gestational age (Fig. 1 *A* and *B*). For serotype 14 the correlation coefficient was 0.596 ($p < 0.001$), whereas for serotype 19 it was 0.561 ($p < 0.001$). IgM antibodies against intact pneumococci and PPS in neonatal sera were either absent or very low in titer.

Phagocytosis. The opsonic activity of both maternal and neonatal sera for *S. pneumoniae* serotypes 14 and 19 varied considerably as can be seen in Table 5. This table, however, also shows that both serotypes were opsonized significantly less well in serum of newborns than in paired maternal serum. This applies to opsonic activity in sera from both preterm (serotype 14: 34.5% of maternal serum, $p < 0.001$; serotype 19: 34.9% of maternal serum, $p < 0.001$) and term neonates (serotype 14: 43.5% of maternal serum, $0.01 < p < 0.001$; serotype 19: 52.7% of maternal serum, $p < 0.001$). Figures 2 *A* and *B* demonstrate that there was no or only a very weak correlation between the ratio of neonatal/maternal uptake and gestational age (serotype 14: $r = 0.264$, $p > 0.05$; serotype 19: $r = 0.342$, $0.02 < p < 0.01$). Moreover, these figures emphasize that opsonic activity in more than half of the neonatal sera was less than 50% that in paired maternal serum. In addition, in neither maternal nor in the paired neonatal sera was a correlation detectable between uptake and antibody titers to capsular polysaccharides or whole bacterial cells. Similarly, no correlation was found between uptake and complement activity (for all correlation coefficients: $p > 0.1$). In both maternal and cord serum opsonization of *S. pneumoniae* serotypes 14 and 19 was almost abolished by heat treatment (30 min, 56°C). The amount of opsonic activity (mean ± SD, range) remaining in heated maternal, term and preterm neonatal serum was: for serotype 14: 8 ± 4% (range 2–18%), 7 ± 3% (range 2–14%), 5 ± 2% (range 0–10%), respectively; and for serotype 19: 7 ± 3% (range 2–19%), 6 ± 2% (range 0–13%), 3 ± 2% (range 0–7%), respectively.

DISCUSSION

The very low rate of urogenital pneumococcal colonization in pregnant women (2–4) and the severity of neonatal pneumococcal sepsis (1, 9, 10) suggest that the likelihood of a confrontation between the human newborn and pneumococci in the immediate postnatal period is indeed a rare occurrence and that neonatal host defense to this agent is rather insufficient.

Our studies demonstrated that opsonic acitivity for *S. pneumoniae* serotypes 14 and 19 in sera from healthy neonates is clearly diminished when compared to the paired maternal serum or pooled serum from normal human adults. More than half of the neonatal sera showed an opsonic activity of less than 50% that found in the paired maternal serum, which is in agreement with data reported by Chudwin *et al.* (11).

Brown *et al.* (12) showed earlier that optimal phagocytosis of pneumococci is dependent on specific anticapsular antibodies and complement activation. The crucial role of complement was again documented in our study because opsonic activity was almost completely abolished by heat treatment of the sera.

Activation of complement by either antibodies or bacterial cell wall and capsular components results in the deposition of C3b on the pneumococcal capsule and the degradation of this complement factor into C3d and iC3b (13). Brown *et al.* (12) showed that in the absence of anticapsular antibodies no opsonically active C3b is deposited on the pneumococcal surface. Recently, it was suggested that differences in C3 degradation patterns may in part explain some of the antiphagocytic properties of different pneumococcal serotypes (13). Significantly less C3 was deposited on the pneumococcal surface in preterm sera than after incubation in the paired maternal serum. This is possibly due to the diminished activity of complement in preterm sera. However, the reduced opsonic activity for pneumococci in sera from term newborns could not be explained by a deficiency of the major opsonins for pneumococci, *i.e.* C3 and specific antipneumococcal antibodies. In term infants C3 deposition on the pneumococcal surface as well as the titer of IgG antibodies to either whole pneumococci or capsular polysaccharides were at least equal to the values obtained with paired maternal sera. Moreover, although CH50 and AP50 values in neonatal sera were less than in the paired maternal sera, we failed to demonstrate a relationship between either classical or alternative complement activity and uptake of bacteria. This raises the possibility that complement deposited on the pneumococcal surface after incubation in some neonatal sera is less opsonically active either because of the C3 degradation pattern or because of dysfunction of transplacentally transferred IgG. The presumed dysfunction of trans-

ferred IgG apparently manifests itself both in an inefficient activation of complement and in a diminished capacity to stimulate uptake through the PMN receptor once this process has been initiated by C3.

Sera of term neonates had higher IgG concentrations against intact pneumococci than did the paired maternal serum, whereas the concentrations in preterm sera did not differ significantly. Specific IgG antibody titers against capsular polysaccharides of serotype 19 were significantly lower in sera from both term and preterm newborns. These findings are in agreement with those of Chudwin *et al.* (11) who found lower levels of anti-PPS antibodies against *S. pneumoniae* serotype 7F in cord sera of both term and preterm neonates. Preterm sera also had significantly lower concentrations of anti-PPS antibodies against serotype 14. In contrast we did not find a significant difference between anti-PPS antibodies against serotype 14 in sera of term newborns and paired maternal serum. This finding supports the possibility of a differential transport of specific anticapsular pneumococcal antibodies as compared to antibodies against other cell wall constituents, such as surface proteins or the peptidoglycan moiety (11).

No significant relationship between specific IgG capsular antibodies and bacterial uptake could be demonstrated in our experiments. Because IgM antibodies also play a major role in host defense against pneumococcal infections (14), it is possible that the higher opsonic activity in maternal sera is explained by the presence of IgM anticapsular antibodies in these sera, which were either absent or present in a only very low concentration in neonatal serum. However, this is not a very likely explanation, because IgM is not opsonically itself, but instead functions through the deposition of C3 on the bacterial surface. As already mentioned above, the amount of C3 deposited on the pneumococcal surface after incubation in newborn serum, at least in term serum, was equal to that deposited by incubation in maternal serum.

In conclusion, it is clear that although *S. pneumoniae* is not a common pathogen for the newborn infant, the almost universally present deficiency of opsonic defense as detected in the sera from healthy newborns may contribute to the high mortality rate of neonatal pneumococcal disease. Opsonic activity in neonatal sera varied widely, but was moderately to profoundly deficient when compared to paired maternal serum. This is even true for healthy term infants, in whom the major opsonins for pneumococci, *i.e.* C3 and specific anticapsular IgG antibodies, did not appear to be deficient. The opsonic defect cannot be solely explained by a diminished activity of complement or lower antipneumococcal antibody titers to either intact pneumococci or capsular polysaccharides. Rather, a dysfunction of either one or both of the major opsonins for pneumococci seems to offer a more likely explanation for this opsonic defect in the neonate. Thus, the exact role of factors such as C3-degradation patterns and the functional integrity of IgG subclasses after placental transfer in the process of opsonization of pneumococci in neonatal serum remains to be elucidated. Finally, because opsonic deficiency is more the rule than the exception even in healthy term infants, it is likely that the rarity of neonatal pneumococcal disease is due to the low incidence of genital carriage of pneumococci by pregnant women.

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