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Specificities and Opsonophagocytic Activities of Antibodies to Pneumococcal Capsular Polysaccharides in Sera of Unimmunized Young Children

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An enzyme immunoassay (EIA) for antibodies to pneumococcal capsular polysaccharides (Pnc PSs) detects in some cases antibodies that are cross-reactive within different Pnc PSs. Recently, it has been suggested that for detection of only serotype-specific antibodies, EIA can be modified by removing cross-reactive antibodies by absorption with an irrelevant PS, e.g., the type 22F PS. The opsonophagocytosis assay measures the functional activities of antibodies in vitro, and the results of that assay correlate with in vivo protection better than measurement of the antibody concentration by EIA. We compared these different methods for measuring antibodies to type 1, 6B, 11A, 14, 19F, and 23F Pnc PSs in the sera of unimmunized young children who had been monitored for pneumococcal carriage, acute otitis media, and acquisition of antibodies to Pnc PSs from 2 to 24 months of age. Serum samples with antibody increases after contact with a pneumococcus of a homologous serotype contained specific antibodies and often had opsonophagocytic activity (OPA) (20 of 46). In samples with antibody increases from children who had not had contact with a pneumococcus of a homologous serotype, the antibodies found to be type specific by conventional EIA were usually cross-reactive and infrequently had OPA (10 of 68). When type 22F PS absorption was used in the EIA, most of the false antibody increases were eliminated, but most of the true antibody increases were still detected and the association between the antibody concentration detected by EIA and OPA was improved. However, there were serotype-dependent differences in the frequency of OPA. Use of absorption with a heterologous PS in EIA should be encouraged, and both the specificity of EIA and the sensitivity of opsonophagocytic assays should be further evaluated and improved.

Immunity against *Streptococcus pneumoniae* (pneumococcus) is mediated by phagocytosis in the presence of complement and antibodies to pneumococcal capsular polysaccharides (Pnc PSs) (2). The in vitro opsonophagocytic activities (OPAs) of serum antibodies are believed to represent the functional activities of the antibodies in vivo and thus to correlate with protective immunity (7, 14).

Enzyme immunoassay (EIA) for the measurement of the concentrations of antibodies to Pnc PSs has been widely used to measure immunity to pneumococci and the immunogenicities of pneumococcal vaccines. However, for the estimation of immunity, a good correlation between the concentration of immunoglobulin G (IgG) measured by EIA and the OPAs of antibodies is needed. The correlation between the two methods has been reasonably good with postimmunization serum samples from infants and adults (1, 13, 21, 22). However, sera from unimmunized individuals may have lower OPAs than expected on the basis of the antibody concentration obtained by EIA (1, 11).

The Pnc PS preparations used in the present EIAs are contaminated with a common cell wall PS (CPS) (18), and antibodies to CPS should be absorbed to improve the specificity of the EIA (10). Recently, several investigators have reported that despite absorption with CPS, antibodies cross-reactive with several types of Pnc PSs are still measured by EIA (4, 15, 23). The reason for this cross-reactivity has not been confirmed. It has been suggested that the Pnc PS preparations used as EIA antigens contain impurities or cross-reactive epitopes common to many serotypes (15, 23). Removal of the cross-reactive antibodies by absorption with an irrelevant heterologous PS, e.g., the type 22F PS, improves the correlation between the antibody concentration obtained by EIA and the OPA (3). Thus, type 22F PS absorption has been suggested as an additional step in EIAs for antibodies to Pnc PSs. Cross-reactive antibodies are found more often in the sera of unimmunized infants and adults than in the sera of infants and adults immunized with pneumococcal vaccines, suggesting that the majority of the antibodies induced by vaccination are Pnc PS type or group specific (3, 15). The origin and development of the cross-reactive antibodies by age has not been studied.

We have previously described the natural development of antibodies to Pnc PSs, as detected by EIA, during the first 2 years of life (16), and antibody responses in children with pneumococcal acute otitis media (AOM) (17) in a Finnish Otitis Media (FinOM) Cohort Study (19). Pneumococcal carriage and AOM induced antibodies to the homologous Pnc PS, but there were serotype-specific differences (16, 17). Low concentrations of antibodies were also produced after contact with pneumococci with heterologous serotypes and even with no detectable contact with pneumococci (16, 17).

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In this study, we evaluated the specificities and OPAs of antibodies in selected serum samples from our previous study (16). We used a set of samples that had twofold or greater increases in antibody concentrations compared to the concentration in a sample taken 6 months earlier and with approximately 1 μg or more of anti-Pnc PS antibodies per ml, as measured by the conventional EIA. Such samples were selected from children with and without a previous contact with pneumococci of the homologous serotype. The effect of the type 22F PS absorption step in the EIA was also evaluated.

MATERIALS AND METHODS

Study population and sera. Serum samples for this study were selected from among the samples from participants in the FinOM Cohort Study used in our previous study (16). The study population consisted of 329 healthy Finnish children who were monitored prospectively in a special study clinic from 2 to 24 months of age (19). During scheduled visits at 2, 3, 4, 5, 6, 9, 12, 15, 18, and 24 months of age, interview data and nasopharyngeal (NP) swab specimens for detection of pneumococcal carriage were obtained. In addition, NP aspirates (NPAs) and middle ear fluid (MEF) samples were obtained from patients with respiratory infection and from patients with a diagnosis of AOM, respectively. Serum samples (5 ml of venous blood) were obtained from the children at scheduled visits at 6 (±14 days), 12 (±14 days), 18 (±28 days), and 24 (±28 days) months of age. The sera were stored at −20°C. The NP swabs, NPAs, and MEF samples were collected and cultured as described previously (19) for detection of pneumococcal carriage or the etiology of AOM. Pneumococci were identified and serotyped by standard methods (9). The development of antibodies by age has been reported earlier (16, 17).

Serum samples used in this study. Selected serum samples taken at the scheduled visits at 18 or 24 months of age were used in this study. The following criteria were used for selection: a twofold or greater increase in the concentration of antibody to PS of type 1, 6B, 11A, 14, 19F, or 23F compared to the concentration in the sample taken during the previous scheduled visit (i.e., at 12 or 18 months, respectively) and the presence of an antibody concentration of approximately 1 μg/ml or greater. An antibody concentration of at least 1 μg/ml is needed to achieve detectable OPA in infant sera taken after vaccination (21). The serum samples were further grouped according to pneumococcal culture findings (Table 1). Sera in the Pnc PS contact-positive (Pnc 6B+, Pnc 11A+, Pnc 14+, Pnc 19F+, and Pnc 23F+) groups were from children who had had at least one culture-confirmed contact with a pneumococcus of the indicated serotype (pneumococci of that serotype were cultured from NP swabs, NPAs, and/or MEF samples) between the two scheduled visits. Accordingly, sera in the Pnc PS contact-negative (Pnc 1−, Pnc 6B−, Pnc 11A−, Pnc 14−, Pnc 19F−, and Pnc 23F−) groups were from children who had not had any detectable contact with the respective serotype by the indicated age, despite the increase in antibody concentrations. These children may have had contacts with other pneumococcal serotypes or no contacts with pneumococci at all. Data were derived from a total of 82 serum samples from 75 children. Some samples were included more than once.

PSs. Capsular PSs of *S. pneumoniae* serotypes 1 (lot 1211368), 11A (lot 963596), 14 (lot 2020510), 19F (lot 2033178), 22F (lots 1450702 and 2045906), and 23F (lot 1417200) were obtained from American Type Culture Collection (ATCC; Manassas, Va.). Capsular PS of serotype 6B was received via collaboration with the National Institute of Public Health and the Environment (Bilthoven, The Netherlands). The CPS used for absorption of anti-CPS antibodies from the sera was from the Statens Serum Institut (Copenhagen, Denmark).

EIA. The conventional EIA was performed as described earlier by Käyhty et al. (8). The results are expressed as micrograms per milliliter, calculated on the basis of the officially assigned IgG concentrations in reference serum sample 89-SF (12). In addition to the conventional EIA, antibody concentrations were determined by EIA with an absorption step with type 22F PS; the lowest serum dilution was incubated with 10 μg of CPS per ml and 30 μg of type 22F PS per ml for 1 h at room temperature before the EIA. The interassay coefficient of variance for both EIA methods was <15%.

Inhibition EIA. Serum samples were diluted 1:100 in 10% fetal bovine serum (Life Technologies, Ltd., Paisley, Scotland) in phosphate-buffered saline containing 10 μg of CPS per ml and aliquoted and placed into three separate tubes. Either homologous (type 1, 6B, 11A, 14, 19F, or 23F) or heterologous (type 22F) Pnc PS (30 μg/ml) was added, and the tubes were incubated for 1 h at room temperature. A control tube containing only CPS was included. The concentration of 30 μg/ml was found to be optimal for absorption for the PS types studied (15). After absorption, EIA was performed as described above. The percent inhibition was calculated from the optical density values. Inhibition of antibody binding by ≤20% was considered low, and inhibition of antibody binding by ≥80% was considered effective.

TABLE 1. Selection of serum samples from among the FinOM Cohort Study samples for the different groups

Group	No. (%) of serum samples			
	From children with no contact with the indicated serotype by that age[a]	From children with contact with the indicated serotype between 12 and 18 mo or 18 and 24 mo, respectively[a]	With twofold or greater increase in concn of IgG to the indicated Pnc PS and with approximately 1 μg of IgG per ml	Available for the analyses
Pnc 1−	264	0	13 (5)	12
Pnc 6B+		36	4 (11)	4
Pnc 6B−	201		8 (4)	8
Pnc 11A+		27	19 (70)	16
Pnc 11A−	225		20 (9)	17
Pnc 14+		19	7 (37)	6
Pnc 14−	236		10 (4)	10
Pnc 19F+		56	16 (29)	13
Pnc 19F−	173		34 (20)	13
Pnc 23F+		46	7 (15)	7
Pnc 23F−	189		12 (6)	8

[a] Number of serum samples at 18 and 24 months of age from children without (Pnc 1−, 6B−, 11A−, 14−, 19F−, and 23F− groups) or with (Pnc 6B+, 11A+, 14+, 19F+, and 23F+ groups) previous contact with the indicated serotype.

Opsonophagocytosis assay. The OPAs of the antibodies were analyzed by measuring the killing of live pneumococci by differentiated HL-60 cells in the presence of serum antibody and complement. A modification (1) of the method described by Romero-Steiner et al. (13) was used. *S. pneumoniae* serotypes 1, 6B, 14, 19F, and 23F (reference strains received from the Centers for Disease Control and Prevention, Atlanta, Ga.) and serotype 11A (reference strain obtained from the World Health Organization Collaborating Center for Reference and Research on Streptococci, Prague, Czech Republic) were grown in Todd-Hewitt broth supplemented with 0.5% yeast extract and kept frozen (−70°C) in aliquots in Todd-Hewitt broth with 15% glycerol. OPA is expressed as a titer that is the reciprocal of the serum dilution with 50% killing compared with the bacterial growth in the controls without serum. A titer of 4 was given to sera with undetectable OPAs. To demonstrate the specificities of opsonic antibodies, serum samples were preincubated with either CPS or homologous or heterologous Pnc PS (30 min at room temperature with 1 mg of PS or CPS in 1 ml of undiluted sera) prior to the assay for OPA. OPA was totally removed only from samples preincubated with the homologous Pnc PS.

Statistical analyses. The average antibody concentrations were expressed as geometric mean concentrations (GMCs) and the average OPAs were expressed as geometric mean OPAs (GMOPAs).

RESULTS

In the whole FinOM Cohort Study population, the number of serum samples with twofold or greater increases in antibody concentrations that yielded an approximately 1 μg/ml or higher concentration in the sample obtained at 18 or 24 months of age depended on the serotype (Table 1). Among the serum samples from children without a previous contact with the homologous serotype, increases in the concentrations of antibodies to type 1, 6B, 11A, 14, and 23F PSs were detected in 4 to 9% of

TABLE 2. Number of serum samples with levels of inhibition of <20% and >80% with the indicated homologous and heterologous (type 22F) Pnc PSs, GMCs of antibody measured by conventional EIA or EIA with type 22F PS absorption, and GMOPAs of antibodies to Pnc PSs 1, 6B, 11A,14, 19F, and 23F in sera of unimmunized children in the different groups

Group[a]	No. of serum samples	No. of serum samples with the following % inhibition[b]:				GMCs of IgG (95% CI[c])		GMOPA (no. of serum samples with detectable OPA)
		Homologous PS		Heterologous (type 22F) PS				
		<20%	>80%	<20%	>80%	EIA	EIA with type 22F PS absorption	
Pnc 1−	12	0	12	0	12	1.57 (1.20–2.07)	0.05 (0.05–0.05)	4 (0)[d]
Pnc 6B+	4	0	3	4	0	1.19 (0.52–2.71)	1.10 (0.47–2.59)	23 (2)
Pnc 6B−	8	0	3	6	0	1.16 (0.44–3.04)	0.77 (0.22–2.71)	7 (1)
Pnc 11A+	16	0	15	16	0	3.06 (2.11–4.44)	2.95 (2.06–4.23)	18 (5)
Pnc 11A−	17	0	17	8	8	1.86 (1.27–2.74)	0.41 (0.15–1.10)	5 (2)
Pnc 14+	6	0	5	5	0	2.87 (1.29–6.40)	2.90 (1.34–6.31)	1,024 (6)
Pnc 14−	10	2	3	10	0	2.61 (1.48–4.58)	2.80 (1.62–4.83)	24 (3)
Pnc 19F+	13	2	4	7	2	2.09 (1.61–2.71)	1.59 (0.91–2.79)	4 (0)
Pnc 19F−	13	1	6	4	5	3.89 (2.58–5.88)	1.04 (0.46–2.33)	4 (0)
Pnc 23F+	7	1	5	7	0	2.27 (1.24–4.15)	1.96 (1.14–3.39)	624 (7)
Pnc 23F−	8	0	8	2	5	1.79 (0.86–3.75)	0.19 (0.04–0.92)	54 (4)

[a] Serum samples in the Pnc 1−, Pnc 6B−, Pnc 11A−, Pnc 14−, Pnc 19F−, and Pnc 23F− groups were obtained from children with no contacts with the indicated serotype; serum samples in the Pnc 6B+, Pnc 11A+, Pnc 14+, Pnc 19F+, and Pnc 23F+ groups were from children with contact with the indicated serotype.
[b] Percent inhibition was calculated as optical density by EIA of a serum sample absorped with either the homologous or heterologous PS compared to that for the sample absorped with CPS only.
[c] CI, confidence interval.
[d] Values in parentheses represent the number of serum samples with titers ≥8.

serum samples and increases in the concentrations of antibodies to type 19F PS were detected in 20% of the serum samples (Table 1). Among the children with antibody increases but without contact with homologous type 1, 6B, 11A, 14, 19F, or 23F pneumococci, 8 of 13, 7 of 8, 16 of 20, 7 of 10, 20 of 34, and 5 of 12, respectively, had had contact with other serotypes of pneumococci. Antibody increases occurred more frequently (11 to 70%) in children with contact with a homologous serotype (Table 1).

Specificities of antibodies and effect of type 22F PS absorption on the antibody concentration measured by EIA. The specificities of antibodies to serotypes 1, 6B, 11A, 14, 19F, and 23F measured by EIA were analyzed by inhibition of antibody binding with the homologous PS (type 1, 6B, 11A, 14, 19F, or 23F) or a heterologous PS (type 22F). Serotype-specific antibodies were considered those that were not inhibited (<20%) by the heterologous PS but that were effectively inhibited (>80%) by the homologous PS. Cross-reactive, nonspecific antibodies were inhibited (>20%) by heterologous PS.

The antibodies to serotypes 6B and 14 in the serum samples from the Pnc 6B+ and Pnc 14+ groups were highly specific. In most cases the level of inhibition by heterologous PSs was <20%, and it was never >80% (Table 2). Furthermore, they were mainly inhibited only by the homologous PSs: >80% inhibition by homologous PSs was seen in three of four and five of six serum samples, respectively (Table 2). However, poorer inhibition by homologous PSs was found in the serum samples from the Pnc 6B− and Pnc 14− groups. It should be noted that three of the eight serum samples in the Pnc 6B− group were from children who had had contact with serotype 6A, and in two of the three serum samples the antibodies to PS type 6B were specific. Consequently, in all groups the concentrations of antibodies to type 6B and type 14 PSs obtained either by the conventional EIA or by EIA with the type 22F PS absorption step were equal; in addition, neither the GMCs (Table 2) nor the antibody concentrations in the individual serum samples (data not shown) were affected by the use of type 22F PS absorption.

Antibodies to serotype 11A were highly specific in the Pnc 11A+ group: heterologous inhibition of <20% was detected in all 16 serum samples and homologous inhibition of >80% was detected in 15 of the 16 serum samples (Table 2). In the Pnc 11A− group, however, heterologous inhibition of >20% was detected in 9 of 17 serum samples. Consequently, the EIA with type 22F PS absorption gave lower antibody concentrations than the conventional EIA for the Pnc 11A− group but not the Pnc 11A+ group (Table 2). Very similar observations were made for serotype 23F: antibodies were specific in the Pnc 23F+ group but mostly cross-reactive in the Pnc 23F− group. The type 22F PS absorption step in EIA markedly reduced the concentrations of antibodies to type 23F PS in the Pnc 23F− group but not the Pnc 23F+ group (Table 2).

Antibodies to type 19F PS had a complex pattern. The specificities of the antibodies were somewhat better in the Pnc 19F+ group than in the Pnc 19F− group; 7 of 13 and 4 of 13 serum samples, respectively, were specific and were not inhibited (>20%) by the heterologous PS (Table 2). However, many serum samples showed antibody binding that could not be effectively (>80%) inhibited by either type 19F or type 22F PS. All the serum samples from the Pnc 19F− group had antibod-

ies with poor specificities; 5 were effectively (>80%) inhibited by heterologous PS, in addition to the type 19F PS, and in 5 additional serum samples the level of inhibition by either the homologous PS or the heterologous PS was low. The EIA with the type 22F PS absorption step gave lower concentrations than the conventional EIA for both the Pnc 19F− and Pnc 19F+ groups. However, the difference was more remarkable in the Pnc 19F− group (Table 2).

Serotype 1 was not cultured from any of the samples during the study. In spite of this, 13 children had increased levels of antibodies to the type 1 Pnc PS, as measured by conventional EIA (Table 1). The antibodies to serotype 1 were not specific: inhibition by the homologous serotype as well as inhibition by the heterologous serotype was >80% for all serum samples (Table 2). The EIA with type 22F PS absorption detected practically no antibodies to the type 1 Pnc PS (Table 2).

OPAs of antibodies. Higher OPAs against pneumococci of serotypes 6B, 11A, 14, and 23F were measured in the sera of children taken after contact with a homologous serotype than in the sera of children with no contact with a homologous serotype (Table 2). Even though the concentrations of specific antibodies (determined by EIA with type 22F PS absorption) to type 6B and type 14 PSs were equal, the OPAs were higher and were found more frequently in the Pnc 6B+ and Pnc 14+ groups than in the Pnc 6B− and Pnc 14− groups (Table 2). Although most (16 of 26) of the serum samples in the Pnc 19F+ and 19F− groups had concentrations of antibodies to type 19F PS of ≥1 μg/ml, as measured by EIA with type 22F absorption, none of the samples had OPAs against the type 19F strain (Table 2). Antibodies to the type 1 PS were removed from all serum samples by type 22F PS absorption. Accordingly, none of the serum samples had OPAs against the type 1 strain (Table 2). Overall, the sera of children with contact with pneumococci of a homologous serotype had OPA (titers, ≥8) more often than the sera of children without homologous contact (20 of 46 versus 10 of 68 for the pneumococcal serotype contact-positive versus the pneumococcal serotype contact-negative groups combined; Table 2). Furthermore, the antibodies were more often specific in the samples of children with contact with pneumococci of the homologous serotype than in the samples of children without contact with pneumococci of the homologous serotype (Table 3). Only 3 of the 45 samples with cross-reactive antibodies had OPAs, whereas 27 of the 69 samples with specific antibodies had OPAs (Table 3).

Association between antibody concentration and OPA. The concentrations of antibodies to type 6B, 11A, 14, and 23F PSs in serum measured by the conventional EIA and the EIA with type 22F PS absorption were compared to the OPAs (Fig. 1). For these analyses, serum samples in the pneumococcal serotype contact-positive group and the pneumococcal serotype contact-negative group were combined. Serotypes 1 and 19F were not included because no OPAs against these serotypes were detected (Table 2). For serotypes 6B and 14, the two EIAs gave very similar antibody concentrations (Table 2), and thus, the type of EIA did not influence the association of concentration and OPA (Fig. 1). For serotypes 11A and 23F the cross-reactive nonfunctional antibodies (Table 3) were markedly inhibited by type 22F PS (Table 2) and the association between the concentrations and the OPAs of antibodies was improved when EIA with the type 22F PS absorption step was used to determine the antibody concentration (Fig. 1).

TABLE 3. Numbers of serum samples taken at 18 and 24 months of age from unimmunized children in the different groups containing either specific or cross-reactive antibodies to type 1, 6B, 11A, 14, 19F, and 23F Pnc PSs and OPAs of the samples

Group[a]	Antibody specificity[b]	No. (%) of serum samples		
		Total	With OPA[c]	Without OPA[d]
Pnc 1−	Specific	0	0	0
	Cross-reactive	12	0	12
Pnc 6B+	Specific	4	2	2
	Cross-reactive	0	0	0
Pnc 6B−	Specific	6	1	5
	Cross-reactive	2	0	2
Pnc 11A+	Specific	16	5	11
	Cross-reactive	0	0	0
Pnc 11A−	Specific	8	2	6
	Cross-reactive	9	0	9
Pnc 14+	Specific	5	5	0
	Cross-reactive	1	1	0
Pnc 14−	Specific	10	3	7
	Cross-reactive	0	0	0
Pnc 19F+	Specific	7	0	7
	Cross-reactive	9	0	9
Pnc 19F−	Specific	4	0	4
	Cross-reactive	6	0	6
Pnc 23F+	Specific	7	7	0
	Cross-reactive	0	0	0
Pnc 23F−	Specific	2	2	0
	Cross-reactive	6	2	4
All groups	Specific	69	27 (39)	42 (61)
	Cross-reactive	45	3 (7)	42 (93)

[a] Serum samples in the Pnc 1−, Pnc 6B−, Pnc 11A−, Pnc 14−, Pnc 19F−, and Pnc 23F− groups were obtained from children with no contacts with the indicated serotype; serum samples in the Pnc 6B+, Pnc 11A+, Pnc 14+, Pnc 19F+, and Pnc 23F+ groups were from children with contact with the indicated serotype.

[b] For this table, antibodies were considered specific if there was <20% inhibition with the heterologous Pnc PS (type 22F) and cross-reactive if there was >20% inhibition with the heterologous Pnc PS.

[c] Titer of ≥8.

[d] Titer of <8.

DISCUSSION

We have previously studied the natural development of antibodies to Pnc PSs and its association with previous contacts with pneumococci, nasopharyngeal carriage, or AOM in the FinOM Cohort Study population (16). Previous contacts were associated with increased concentrations of antibodies to the

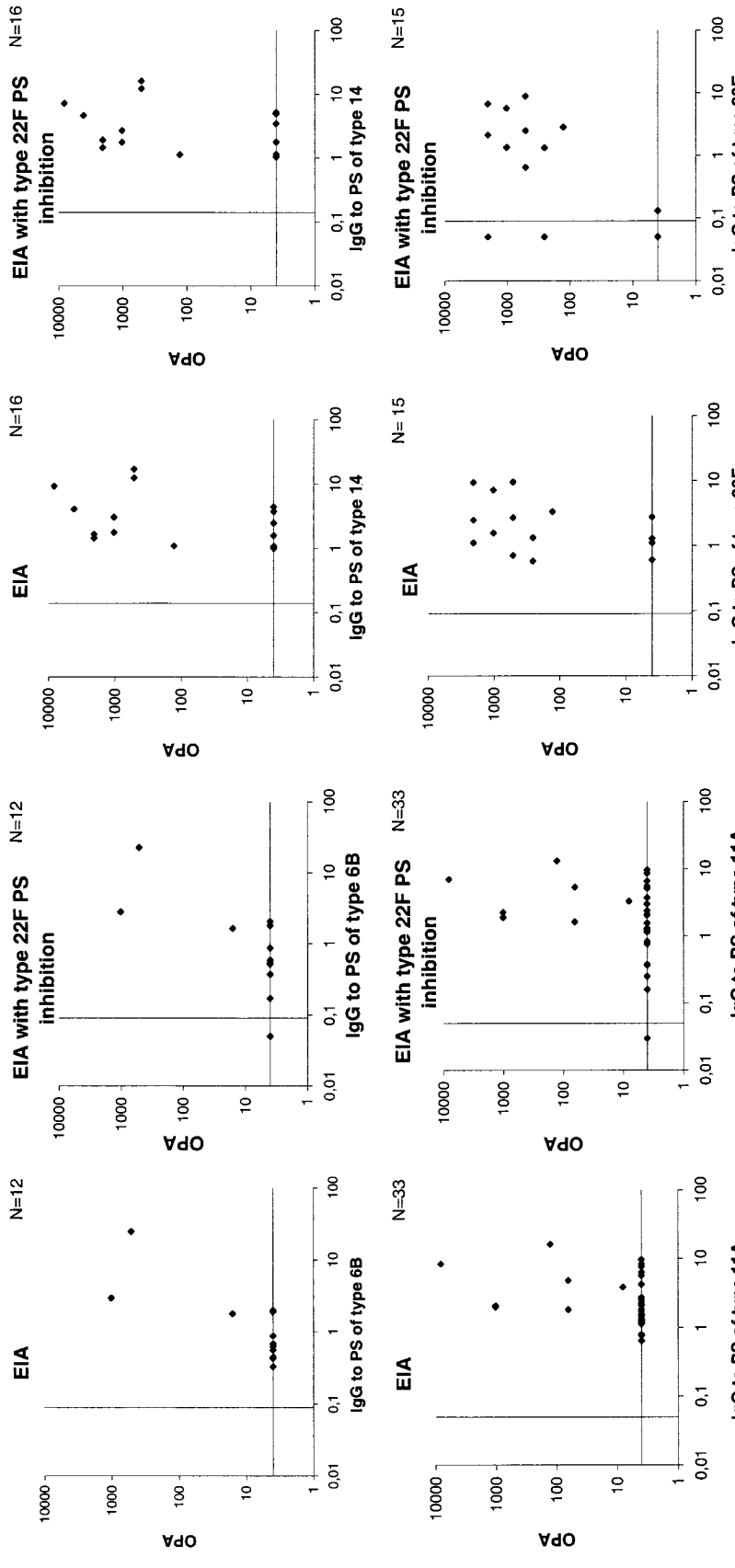

FIG. 1. Association between IgG concentration (in micrograms per milliliter), as measured by conventional EIA or by EIA with type 22F PS absorption, and OPAs (as titers) of antibodies to type 6B, 11A, 14, and 23F Pnc PSs. Serum samples were taken from unimmunized children at 18 or 24 months of age. Horizontal lines, a titer of 4 was given to sera with undetectable OPA; vertical lines, a concentration of 1.0 μg/ml was used as a cutoff for the EIAs.

homologous serotype, but there were serotype-specific differences. However, the children developed low concentrations of antibodies to all the Pnc PS types studied even without contact with the homologous serotype (16, 17). The reason seems to be the fact that the detection of antibodies to Pnc PSs is affected by the cross-reactivity of the Pnc PS antigens used in the present EIA (15, 23). We studied here the specificities and functional activities, as measured by opsonophagocytosis assay, of these antibodies and correlated these characteristics with culture-confirmed contacts with pneumococci. We also evaluated the possibility of using the type 22F PS absorption step in EIA (3) to improve the specificity.

For the whole study population, antibody increases yielding $\geq$1 μg of antibodies per ml in the children without contact with the homologous serotype were rare compared to the antibody increases in children with contact with the homologous serotype: 20 versus 29% for serotype 19F and 4 to 9% versus 11 to 70% for the other five serotypes studied. We included in this study serum samples taken at either 18 or 24 months of age; these ages of sample collection were chosen to exclude maternal antibodies. Sera were grouped according to the pneumococcal contacts that the children had had by the indicated age. In general, serum samples from children with contact with a homologous serotype contained serotype-specific antibodies. Moreover, these samples often had OPAs, although there were clear serotype-specific differences. In contrast, antibodies in serum samples from children without contacts with a homologous serotype were mostly cross-reactive and infrequently had OPAs.

Previous studies by us and other investigators with sera from adults have shown that detection of antibody to serotype 14 by EIA is serotype specific, whereas detection of antibody to the other serotypes is hampered by cross-reactivity (4, 15, 23). The present data with sera from a pediatric population confirm that antibodies to type 14 PS are highly specific. All serum samples in the Pnc 14+ group also had OPA against type 14. In contrast, only a few serum samples in the Pnc 14− group had OPA against type 14. This is interesting since this difference in OPA between the Pnc 14+ and the Pnc 14− groups cannot be explained by differences in antibody concentrations or antibody specificity. The origins of the antibodies in samples in the Pnc 14− group are not known; the antibodies were not absorbed by type 22F PS, and in a few cases they also had OPA. It is possible that some contacts with pneumococci remained undetected or that the children had had contacts with bacteria with PSs similar to type 14, e.g., the type III PS from group B streptococci (6). In the case of serotype 14, the increase in antibody levels or the concentration alone was not as good a marker of previous contact with type 14 as OPA was.

The results of the present study confirm that the Pnc PS type 6B preparation used here and in our previous studies (15–17) is specific. However, although the specificities of antibodies to PS 6B were good in both the Pnc 6B+ and the Pnc 6B− groups, only a few serum samples had OPA. This may be due to the overall low antibody concentrations that were insufficient for detection of OPA by the present opsonophagocytosis assay. A previous contact with serotype 6A, which is cross-reactive with 6B, explained the increases in the levels of antibodies to type 6B PS in three of the eight serum samples in the Pnc 6B− group, and in two of the three samples the antibodies specificities were high. However, the antibodies in these three samples had no OPA against type 6B, despite the presence of anti-type 6B antibody concentrations equal to those in the samples from the Pnc 6B+ group showing OPA. This is in accordance with data showing that antibodies to type 6A elicited by vaccines containing type 6B may not be functional against type 6A strains (11, 20) or that more antibodies to type 6B PS are needed for killing of a type 6A strain than for killing of a type 6B strain (20).

Altogether 26 serum samples with $\geq$1 μg of IgG against type 19F PS per ml, as detected by the conventional EIA, were analyzed, and none of these serum samples had OPA against type 19F. The specificities of the antibodies in samples from children without previous contact with type 19F were poor. However, despite the higher concentrations and better specificities of antibodies in sera from children with cultures positive for type 19F, the sera showed no OPA. The specificities of antibodies to type 19F, as determined by EIA, in general, were confusing; in some cases antibody binding could not be inhibited by either the homologous PS or the heterologous PS. Furthermore, high concentrations of antibodies to type 19F PS even in the sera of vaccinated children do not seem to offer protection against AOM (5). Also, higher anti-type 19F antibody concentrations than anti-type 6B antibody concentrations are needed in the sera of vaccinated children for OPA (1).

We have previously described that antibodies to type 1 PS can be detected by conventional EIA in serum samples of children participating in the FinOM Cohort Study, even though type 1 pneumococcus was not detected in any of the NP, NPA, or MEF samples during the study (16). We have now shown that these antibodies are not specific for type 1 PS and therefore are probably produced by a non-type-specific antigenic stimulus. These antibodies possessed no OPA against type 1 pneumococci. Use of the type 22F PS absorption step in the EIA removed all antibodies to type 1 PS detected by the conventional EIA in all samples.

The data obtained in this study highlight the need for heterologous type PS absorption in EIA, especially when samples from an unimmunized population are studied. Type 22F PS absorption did not have a notable effect on the concentrations of antibodies to type 6B and type 14 Pnc PSs. However, the type 6B antigen was especially chosen for this study (15, 16), and the commercially available preparations seem to be more cross-reactive (15, 23), indicating that, in general, the type 22F PS absorption step is also needed for the anti-type 6B EIA. Because the nature of the cross-reactivity is unknown (23), the specificity and amount of antibodies inhibited by type 22F PS cannot be determined. It is, however, probable that the antibodies removed are not serotype specific and, in principle, should not be detected. Data from us and others (3) show that the type 22F PS absorption step increases the association between the antibody concentration obtained by EIA and OPA. Furthermore, the results of the present study suggest that if antibody detection by EIA is used to study the seroepidemiology of pneumococcal infections, an EIA with type 22F PS absorption instead of the conventional EIA is highly recommended.

The present opsonophagocytosis assay requires approximately 1 μg of antibody per ml for detection of OPA in the sera of immunized infants (21). As shown here, the concentra-

tion of specific antibodies needed may be even higher in sera of unimmunized children and may be different for the different serotypes. In the present study, high concentrations of specific antibodies to type 11A and 19F PSs were insufficient for OPA, while lower concentrations were associated with OPA against type 14 and 23F strains. It is possible that in addition to the antibody concentration and specificity, other qualitative characteristics of the antibodies, e.g., antibody avidity, affect OPA (1). Contacts with pneumococci seem to elicit the development of specific and functional antibodies, and thus, detection of OPA in sera could be an indicator of a serotype-specific pneumococcal contact. However, the sensitivity of the assay for OPA described here should be improved to detect activity in the sera of unimmunized individuals.

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