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REVIEW ARTICLE

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Clinical bacteriology and immunology in acute otitis media in children

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Abstract Acute otitis media (AOM) is the most common disease seen in childhood. *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* (NTHi), and *Moraxella catarrhalis* are the most frequent pathogens of all AOM episodes. The high prevalence of drug-resistant pathogens such as penicillin-resistant *S. pneumoniae* (PRSP) and beta-lactamase producing or nonproducing ampicillin-resistant *H. influenzae* (BLPAR or BLNAR) is causing serious clinical problems worldwide. PRSP and BLNAR have become important risk factors for intractable clinical outcome of AOM. PRSP causes a three times higher incidence of intractable AOM than susceptible strains. BLNAR strains show penicillin-binding protein gene mutation and are not only resistant to ampicillin, but also have reduced susceptibility to cephalosporin. The resistant *H. influenzae* pathogen has shown clonal dissemination in Japan in ways different from those of penicillin-resistant *S. pneumoniae*. Protection against AOM due to these pathogens may depend on pathogen-specific antibodies. Pneumococcal capsular polysaccharides (PCPs) are type specific and poorly immunogenic in children younger than 2 years old. Approximately 50% of otitis-prone children showed subnormal levels of anti-PCP IgG2 antibody. In our immunological study in children with otitis media, however, otitis-prone children were not unusually vulnerable to infections except those resulting in otitis media. This fact seems to refute the presence of a broad immunological deficit in these children. Some pathogen-specific antibodies may be directed against protein immunogens such as pneumococcal surface protein A (PspA) of *S. pneumoniae*, P6 of NTHi, and UspA of *M. catarrhalis*. The levels of antibody to P6 of NTHi in healthy children were significantly higher than those in the otitis-prone children after the age of 18 months. In general, individual antibody levels in otitis-prone individuals did not have an age-dependent rise. The failure to develop a good antibody response to common antigens such as PspA and P6 may enable the pathogen to cause persistent or recurrent disease.

Key words Acute otitis media · penicillin-resistant *Streptococcus pneumoniae* (PRSP) · β-lactamase nonproducing ampicillin resistant (BLNAR) · Otitis prone · P6 · PspA

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Clinical bacteriology in acute otitis media

Bacteria are found in 50%–90% of cases of acute otitis media (AOM) with or without otorrhea.[1] *Streptococcus pneumoniae* (*S. pneumoniae*) and *Haemophilus influenzae (H. influenzae)* are the leading causative pathogens responsible for AOM, and they frequently colonize in the nasopharynx.[1,2] These two notorious pathogens have long been susceptible to β-lactams, and AOM caused by them had easily been improved by oral antimicrobial therapy. However, antimicrobial-resistant pathogens, especially penicillin-resistant *Streptococcus pneumoniae* (PRSP), has become the major cause of intractable otitis media.[2] Antimicrobial resistance in *H. influenzae* has also evolved significantly during the last 20 years, while ampicillin (AMP) had long been considered the drug of first choice for the treatment of infection due to *H. influenzae*.[3]

The mechanism of the resistance of *S. pneumoniae* to β-lactams is the stepwise alterations in the high molecular weight penicillin binding proteins (PBPs) and the reduction of the binding affinity of β-lactams to the PBPs. Among several PBPs, 1A, 2X, and 2B have transpeptidase activity and contain the conserved amino acid motif of SXXK, SXN, and KT(S) G in an active serine residue. *S. pneumoniae* acquires exogenous low affinity genes and causes genetic mutations that alter PBP affinity for β-lactams.[4–7]

Two well-known mechanisms of resistances to β-lactams in *H. influenzae* have been reported. One is the production

of either TEM-1 or ROB-1 type β-lactamase.[8,9] The other is designated as β-lactamase nonproducing ampicillin resistant (BLNAR), and involves a decreasing affinity of PBPs to β-lactams caused by conformational changes with genetic mutations.[10–12] Recent studies revealed that increasing resistances to β-lactams in BLNAR strains were closely related to mutations in the *ftsI* gene encoding PBP3, which mediates septum peptidoglycan formation.[13,14] The substitutions in the *ftsI* gene have been classified into the following three groups: group I, His is substituted for Arg-517 (Arg517His) near the KTG motif; group II, Lys is substituted for Asn-526 (Asn526Lys) near the KTG motif; group III, three residues (Met-377, Ser-385, and Leu-389) near the SSN motif are replaced by Ile, Thr, and/or Phe (Met377Ile, Ser385Thr,and/or Leu389Phe, respectively), in addition to the replacement of Asn526Lys. Isolates with susceptible or intermediate ampicillin resistance are commonly found in groups I and II, and isolates in group III are associated with a higher level of ampicillin resistance.[15]

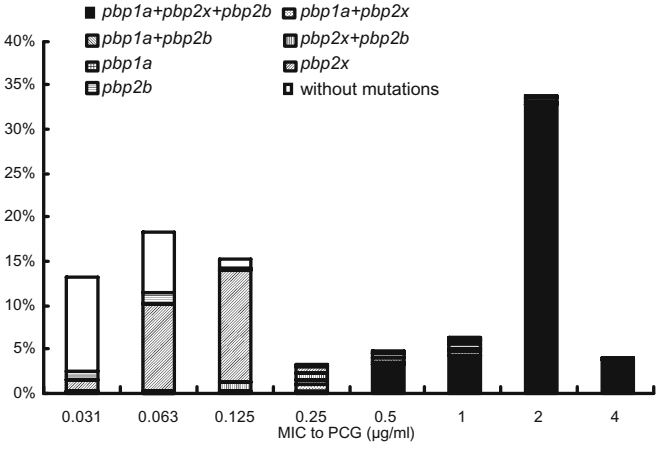

Fig. 1. Correlation between susceptibility to penicillin G (*PCG*) and mutations in penicillin binding protein (*pbp*) genes. *MIC*, minimal inhibitory concentration

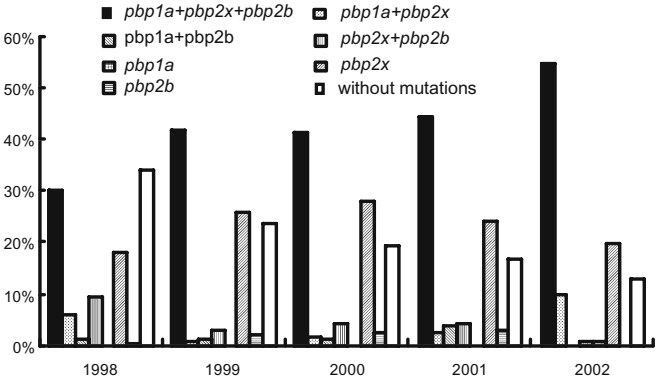

Fig. 2. Annual distributions of *pbp* gene mutations in *S. pneumoniae*

Antimicrobial resistance in *Streptococcus pneumoniae*

S. pneumoniae is the most important pathogen for AOM, and 20%–35% of AOM is caused by *S. pneumoniae*. The high incidence of PRSP strains has recently been a global issue. Resistance strains are appearing all over the world nowadays. Recent reports indicate that the rates of PRSP were 54.8% in Korea, 43.2% in Hong Kong, 38.6% in Taiwan, 71.4% in Vietnam, 29.3% in Japan, 12% in the United States of America, and 2% in Germany.[2,16] Almost 76% of strains had a mutation in their PBP 1a, 2b, and 2x in Japan. They were classified into seven genotypic classes after polymerase chain reaction (PCR) identification of abnormal *pbp1a*, *pbp2x*, and *pbp2b* genes: (i) penicillin-susceptible *S. pneumoniae* (PSSP) isolates with no abnormal *pbp* genes (24.2%); (ii) genotypic penicillin-intermediate *S. pneumoniae* (gPISP) isolates with only an abnormal *pbp2x* gene [gPISP (*2x*)] (26%); (iii) with only an abnormal *pbp1a* gene [gPISP (*1a*)] (0.1%); (iv) with only an abnormal *pbp2b* gene [gPISP (2b)] (2.2%); (v) gPISP isolates with abnormal *pbp1a* and *pbp2x* genes (2.8%); (vi) gPISP isolates with abnormal *pbp2x* and *pbp2b* genes (2.2%); (vii) genotypic penicillin-resistant *S. pneumoniae* (gPRSP) isolates with three abnormal *pbp* genes (38.5%). Almost 95% of strains had abnormal ppb 2x gene mutations (Fig. 1). The minimal inhibitory concentration (MIC) MIC_{50} and MIC_{90} of the strains with mutations in the three *pbp* genes to PCG were ≥2 μg/ml, whereas strains without mutations in either *pbp* genes were ≤0.03 μg/ml and 0.06 μg/ml, respectively. The MIC_{50} and MIC_{90} of the strains with mutations in *pbp2x* were 0.06 μg/ml and 0.125 μg/ml, respectively. On the other hand, the MIC_{50} and MIC_{90} of strains with mutations in two types of *pbp* genes (*pbp1a* and *pbp2x*, *pbp1a* and *pbp2b* or *pbp2x* and *pbp2b*) varied in the range between 0.125–0.5 μg/ml and 0.5–4 μg/ml, respectively. Annual changes of mutations in the three PBP genes were assessed for 5 years. Strains with mutations in three PBP genes gradually increased from 1998 to 1999. Then the increase of PBP-mutated strains became slower from 1999 to 2001. In 2002, the strains increased again. On the other hand, strains without mutations in PBP genes decreased in number. The strains with mutations in *pbp2x* gradually increased from 1998 to 2000, and then gradually decreased in numbers from 2001 to 2002 (Fig. 2). In the age distribution of mutations in PBP genes, strains with mutations in all three PBP genes were frequently identified among children younger than 2 years old (48.9% vs. 35.2%, $P < 0.01$) (Fig. 3). The other strains showed similar prevalences between the two age groups.

S. pneumoniae resistance to macrolides has also been a big concern all over the world, and the rates of resistance were 70%–80% in Japan, 92.1% in Vietnam, 86% in Taiwan, 80.2% in Korea, 76.8% in Hong Kong, and 30% in the USA.[2,17,18] The majority of the strains had *mefA* (32.5%) or *ermB* (34%) and *mefA* and *ermB* (3.4%) gene-mediating macrolide resistance. Susceptibilities to clarithromycin of strains with *mefA* gene, *ermB* gene, and both were 1–4 μg/ml, >64 μg/ml, and >64 μg/ml, respectively. Macrolide-resistant genes were frequently identified among penicillin nonsusceptible strains (PISP + PRSP)[2,17] (Fig. 4).

PRSP causes a three times higher incidence of intractable AOM than PSSP. Serotypes or serogroup 19F, 23F, and

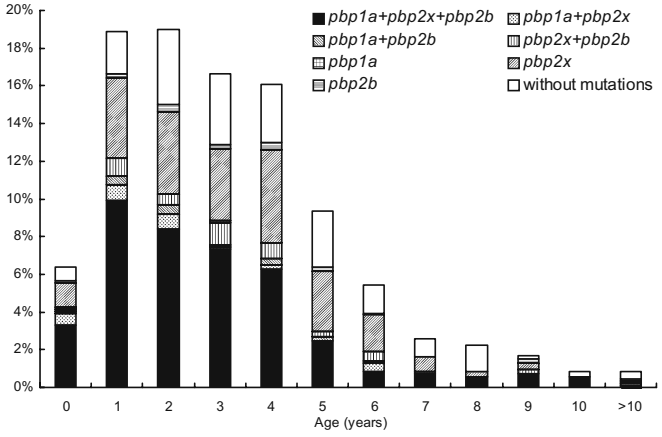

Fig. 3. Age distribution of mutations in *pbp* genes

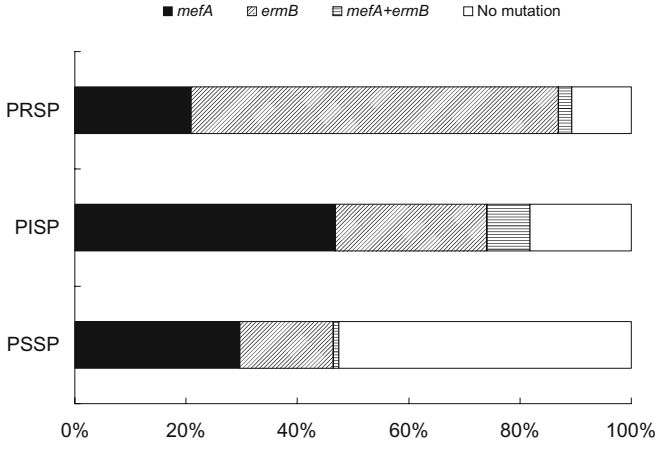

Fig. 4. Macrolide-resistant genes and susceptibility to PCG. *PRSP*, penicillin-resistant *Streptococcus pneumoniae*; *PISP*, penicillin-intermediate *Streptococcus pneumoniae*; *PSSP*, penicillin-susceptible *Streptococcus pneumoniae*

6 are the most prevalent serotypes, followed by serotype 3, serotype 9V, and serotype 7F all over the world. The increasing incidence of PRSP is nowadays a great concern all over the world, and most strains have also been getting multidrug resistant. Conjugated pneumococcal vaccine does reduce the colonization of *S. pneumoniae* in AOM patients, but the efficacy of vaccine in AOM patients still remains controversial.

Antimicrobial resistance in *Haemophilus influenzae*

H. influenzae is the second leading pathogen that causes AOM in children. Most AOM is caused by nontypeable *H. influenzae* (NTHi). Since the first reports of ampicillin-resistant strains of *H. influenzae* in 1974 from the USA, the major mechanism of the antimicrobial resistance of *H. influenzae* has been considered to be either TEM-1 or ROB-1 types of β-lactamase.[19–21] The prevalence of β-lactamase-producing strains increased markedly up to 15.2% in 1983–1984, 36.4% in 1994–1995, and 31.3% in 1997–1998 in the United States.[19,20] BLNAR strains had been isolated at low frequencies in the 1980s, but BLNAR strains have rapidly increased at the rate of 19.5% in the 1990s.[22] Recently, the rate of BLNAR was 58.1% in Korea, 37% in Japan, 0%–33% in Europe, and 4%–10.1% in the USA.[19–21,23,24]

In 2003, the Japan Society of Infectious Diseases in Otolaryngology conducted the 4th nationwide surveillance of causative pathogens responsible for upper respiratory tract infectious diseases in Japan in order to define the contemporary surveillance of antimicrobial-resistant pathogens. According to the criteria for the susceptibility of *H. influenzae* to AMP by the Clinical and Laboratory Standards Institute (CLSI), *H. influenzae* isolates were divided into 61.0% susceptible strains (MIC ≤ 1 μg/ml), 37 (14.0%) intermediately resistant strains (MIC = 2 μg/ml), and 66 (25.0%) resistant strains (MIC ≥ 4 μg/ml). Five strains produced TEM-type β-lactamase. These were divided into 3 (1.2%) strains with mutations in the *ftsI* gene (gBLPACR: genotypic β-lactamase-producing amoxicillin-clavulanate-resistant) and 2 (0.8%) strains without mutations in the *ftsI* gene gBLPAR (genotypic β-lactamase-producing ampicillin-resistant). According to PCR-based genotyping, 172 (65.1%) isolates had mutations in the *ftsI* gene without producing β-lactamase (gBLNAR: genotypic β-lactamase-nonproducing ampicillin-resistant). These were 98 (37.1%) strains with group I/II mutations in a variable mutated region (Group I/II gBLNAR) and 74 (28.0%) strains with group III mutations in a highly mutated region (Group III gBLNAR). The other 87 (33.0%) isolate were gBLNAS (genetically β-lactamase nonproducing ampicillin-susceptible) strains with mutations in neither the *ftsI* gene nor the *bla* gene (Table 1).[15] The Group III gBLNAR strains showed resistance to both penicillin and cephalosporin. Among the 61 gBLNAR strains with mutations in the *ftsI* gene, 6 clones were identified. As the MIC to AMP increased, the frequencies of clonal dissemination were getting higher. Six (25%) strains among 24 strains with MIC to AMP 4 μg/ml, 6 (23.0%) strains among 26 strains with MIC to AMP 8 μg/ml, and 7 (63.6%) strains among strains with MIC to AMP ≥ 16 μg/ml showed similar PFGE patterns (Fig. 5).[15] PBP gene-mutated *H. influenzae* is not only resistant to AMP, but also has reduced susceptibility to cephalosporin. Nowadays, the minimum inhibitory concentration to ampicillin is increasing rapidly, and the strains are becoming resistant to third-generation cephalosporin. Such a prevalence of BLNAR strains with mutations of the *ftsI* gene has been alarmingly high in Japan. The resistant *H. influenzae* pathogen will disseminate in different ways from penicillin-resistant *S. pneumoniae*. Consequently, we need to continue careful surveillance for BLNAR strains of *H. influenzae* in patient populations, and continue our efforts to understand why these antibiotic-resistant strains are becoming more prevalent. PCR-based genotyping and study of molecular characteristics bring us useful information to continue our surveillance of this resistant pathogen.

Table 1. Correlation between PCR-based genotyping and susceptibility to AMP

PCR-based genotype	No. of isolates	No. of isolates with MIC to AMP (μg/ml) of:									MIC (μg/ml)			Susceptibility (%)[a]		
		≤0.12	0.25	0.5	1	2	4	8	16	32	≥64	MIC_{50}	MIC_{90}	Range	S	I	R
gBLNAS	87	3	35	46	3							0.5	0.5	0.12–1	100	0	0
Group I/II gBLNAR	98	5	23	21	18	23	6	2				0.5	2	0.12–8	68.4	23.5	8.1
Group III gBLNAR	74				7	14	18	24	10	1		4	16	1–32	9.5	18.9	71.6
Group I/II gBLPACR	1										1	128	128	128	0	0	100
Group III gBLPACR	2										2	>128	>128	>128	0	0	100
gBLPAR	2										2	64	>128	64 ≥ 128	0	0	100
Total	264	8	58	67	28	37	24	26	10	1	5	0.5	8	0.12 ≥ 128			

[a]S, susceptible; I, intermediate; R, resistant
AMP: ampicillin

Clinical immunology in acute otitis media

Otitis media is the most common disease seen in childhood. *S. pneumoniae*, NTHi, and *Moraxella catarrhalis (M. catarrhalis)* are the most frequent pathogens in about 35%–40%, 30%–35%, and 10%–15%, respectively, of all episodes. Protection against the disease due to these pathogens may depend on pathogen-specific antibodies. In the case of *S. pneumoniae*, the protective antibody has been thought to be directed mainly toward capsular polysaccharide antigens.[25] Capsular polysaccharides of *S. pneumoniae* are type-specific and poorly immunogenic in children younger than 2 years old.[26,27] Some pathogen-specific antibodies may be directed against protein immunogens such as pneumococcal surface protein A (PspA) of *S. pneumoniae*, P6 of NTHi, and UspA of *M. catarrhalis*.

In our immunological study in children with otitis media,[28–31] otitis-prone children were not unusually vulnerable to infections except those resulting in otitis media. This fact seems to refute the presence of a broad immunological deficit in the children. However, children who had recurrent episodes of otitis media caused by *S. pneumoniae* or nontypeable *H. influenzae* did not mount a normal response to PspA, PCP-IgG_2, and P6 during the episodes, and failed to have a secondary immune response on repeated challenge. It is likely, therefore, that these children will not respond adequately to immunization with PspA or P6 vaccines. Otitis-prone children also fail to respond appropriately to pneumococcal antigens, and thus may not be immunized effectively with a vaccine for otitis media that contains pneumococcal polysaccharides. Selective immunological derangements in otitis-prone children may therefore be more widespread than previously believed. Effective active immunoprophylaxis against otitis media will be possible only when the mechanism of the immunological defect in otitis-prone children is understood.

Immune response to PspA of *S. pneumoniae* in children with acute otitis media

A number of recent publications have described the importance of PspA in both disease production and immunity. PspA is attached to the surface of the pneumococcus by the C-terminal end of the molecule, and much of the immune response elicited by immunization in animals is directed against the N-terminal α-helical portion of the molecule.[32] The PspA gene is expressed in all strains of pneumococci, regardless of their capsular serotype.[33] Antibody responses to PspA in animals protect against sepsis and nasopharyngeal colonization.[33] Although PspA is a heterologous protein, there is a high degree of serological cross-reactivity among different PspA molecules from the two major families of PspA.[34] A single recombinant PspA protein is capable of inducing protection against pneumococcal strains of diverse capsular serotypes and different PspA serotypes in animal models. Thus, it is hypothesized that a single PspA

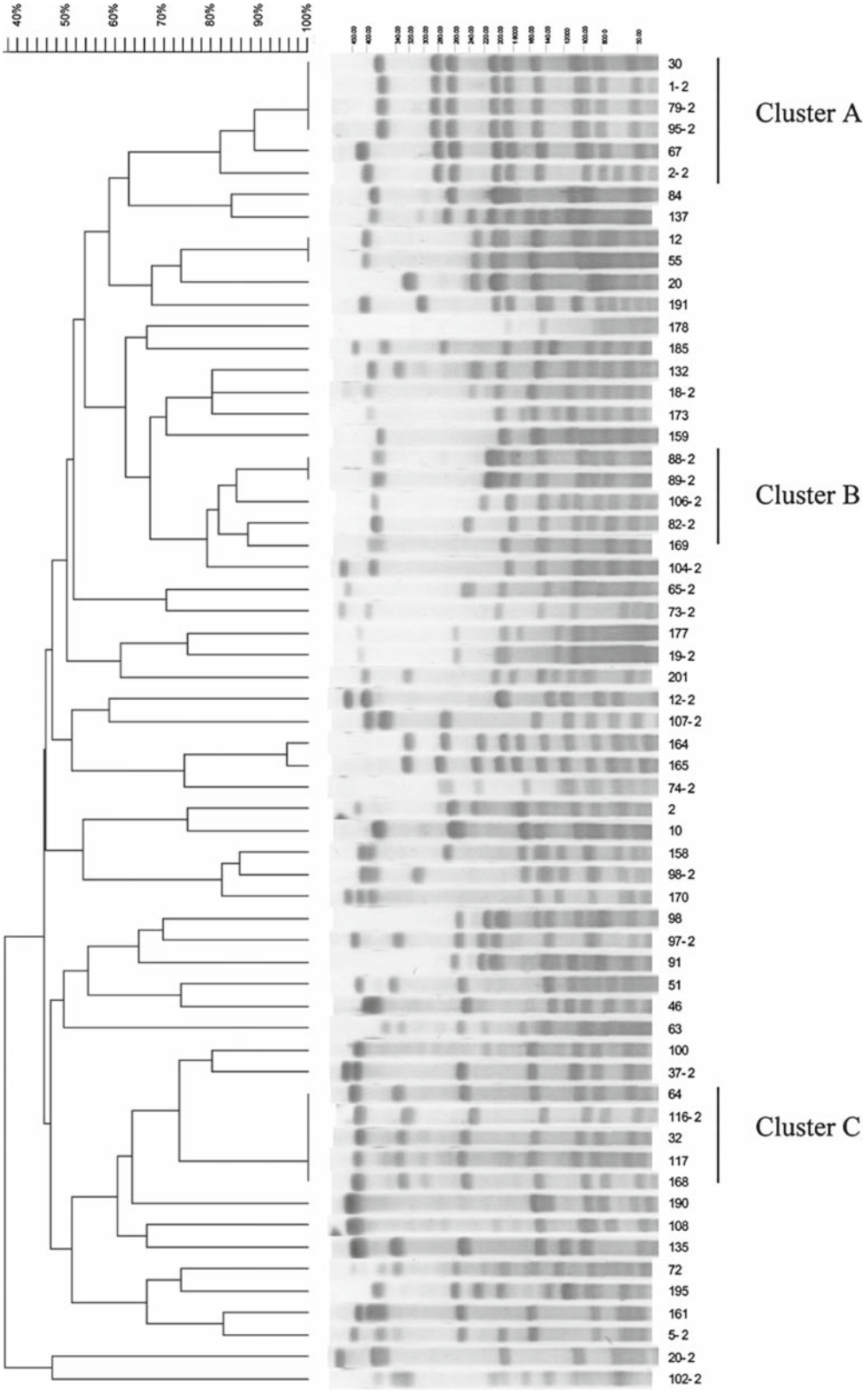

Fig. 5. Genetic identity and/or high relatedness among β-lactamase nonproducing ampicillin-resistant (BLNAR) isolates. Pulsed field gel electrophoresis (PFGE) dendrogram (unweighted pair group method with arithmetic means) of 61 BLNAR strains (MIC 4 μg/ml). Dice coefficients are shown above the dendrogram. Isolates with 80% relatedness on the dendrogram are considered to be strongly genetically related

protein may be able to provide protection against multiple diverse strains of *S. pneumoniae*.[35]

Immune responses to PspA in the sera of various age groups in the general population and in the nasopharynx of 30 children monitored from birth until 1 year of age were evaluated[29] (Fig. 6). IgG was the dominant serum antibody to PspA. In the first 2 years of life, comparable amounts of IgM and IgG antibodies were observed. In older people, IgG antibodies to PspA predominated over IgM antibodies. The level of IgA antibodies to PspA in serum remained low

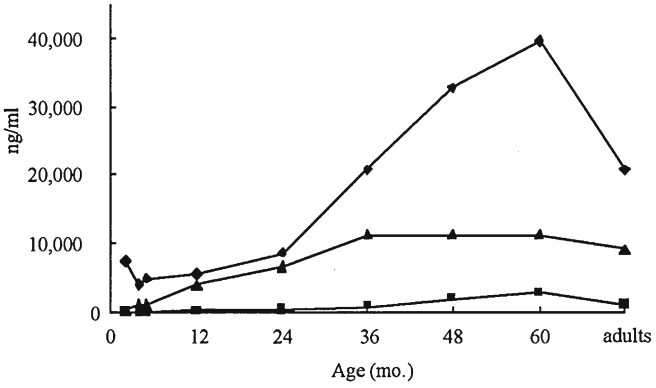

Fig. 6. Antibody to pneumococcal surface protein A (PspA) of *S. pneumoniae* in serum according to age

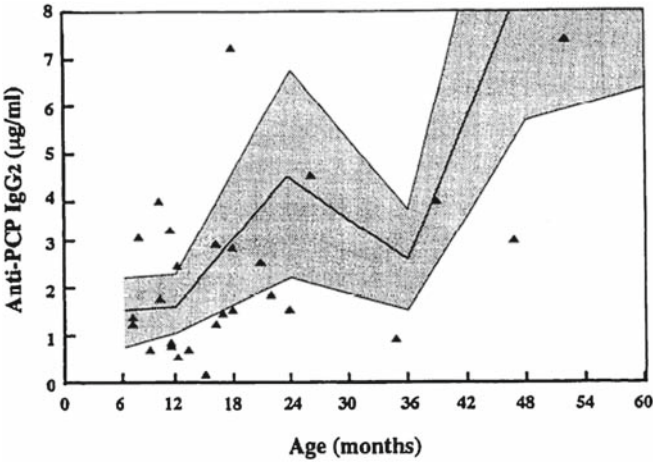

Fig. 7. Anti-PCP IgG2 in otitis-prone children. Normal values are plotted as a shaded area encompassing 2 SD around the mean. Otitis-prone children are represented as individual points (*small black triangles*). *PCP*, polysaccharide capsular protein

during the first 2 years of life. Although IgA was the dominant antibody to PspA in airway secretions, it was detected in a minority of children. Even the majority of children previously colonized with *S. pneumoniae* lacked antibody to it in their secretions. A decline in PspA IgG antibody concentrations was noted in sera from adults, and this was reflected in a similar decline in the proportion of total IgG represented by PspA-specific IgG. Epidemiological studies with *S. pneumoniae* indicate that acquisition of the strain and length of colonization decreases with increasing age, suggesting that maturation of the immune system in some way plays a role in controlling colonization patterns.[36]

The antibody response was evaluated in children with acute otitis media due to *S. pneumoniae*.[30] The age of the children had a range of 4–32 months. The mean IgG, IgM, and IgA antibody responses to PspA in sera from children at the acute and convalescent stages were 4864 vs 5831 ng/ml, $P < 0.05$, 1075 vs 3752 ng/ml, $P < 0.05$, and 67 vs 93 ng/ml, nonsignificant, respectively.

Studies of natural immunity to pneumococcal infections have focused almost exclusively on antibodies directed against the capsular polysaccharides. Although the introduction of conjugate vaccine has given satisfactory protective responses to polysaccharides in young children and raised expectations regarding a capsular-based vaccine, there are still more than 90 individual types of capsule in the pneumococcus. A single protein immunogen capable of eliciting protective antibodies would be attractive when compared with the need to include multiple polysaccharides in a vaccine for young children. This study showed that the majority of children responded to an infection by *S. pneumoniae* by making antibody to PspA. Nevertheless, the specific antibody to PspA may not always be protective to middle-ear infections of *S. pneumoniae* owing to several factors. Mucosal antibodies, which are expected to be those most crucial for protection at the respiratory surface, might not be parallel to serum antibodies. Moreover, it is quite possible that anti-PspA antibodies elicited during otitis media may be protective against invasive disease even if protection against otitis media is not always achieved.

Antibody response to pneumococcal capsular polysaccharides (PCP) in normal and otitis-prone children[31]

Antipneumococcal capsular polysaccharide (PCP) IgG_2 antibodies were measured by the quantitative enzyme-linked immunosorbent assay (ELISA). A polyvalent pneumococcal vaccine (Pneumovax; Merck Sharp & Dohme, West Point, PA, USA) containing 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F) was used as a coating antigen. In healthy children, the total IgG2 level was lowest at 6 months of age. The level increased until 2 years of age, and then gradually decreased until 4 years of age. Thereafter, at 4–5 years, it increased again. Anti-PCP IgG2 was lowest at 6 months of age. The level increased until 2 years of age and decreased at 3 years of age. Thereafter, at 4–5 years, it increased again. In otitis-prone children, subnormal levels of total IgG, and anti-PCP IgG were defined if the concentration was lower than 2 SD below the mean for that age in normal children. Five of 36 otitis-prone children (13.9%) showed subnormal levels of total IgG. Thirteen of 27 otitis-prone children (48.1%) showed subnormal levels of anti-PCP IgG2 antibody (Fig. 7). The number of children with subnormal levels of total IgG2 was not higher in the otitis-prone group than in the normal group ($P = 0.1484$). However, the number of children with subnormal levels of anti-PCP IgG antibody was significantly higher in the otitis-prone group than in the normal group (anti-PCP IgG2, $P < 0.01$).

Immune responses to P6 of NTHi in children with acute otitis media

NTHi is frequently associated with recurrent and chronic episodes of middle ear disease.[37] One of the major outer membrane proteins of NTHi, P6, is highly conserved among

strains, is antigenically stable, serves as a target for bactericidal antibody, and has been proposed as a possible candidate for vaccine formulation.[38–40]

The serum antibody response to P6 was studied in otitis-prone and normal children by ELISA.[28] The study group consisted of 43 children, who ranged in age from 1 to 92 months and were included in a prospective study of otitis media. Thirty of the subjects were classified as otitis-prone because they had had four or more episodes of otitis media in the first year of life, or six or more episodes of otitis media by the second year, or needed placement of tympanostomy tubes. The other 13 children were considered to be healthy and had had two or fewer episodes in the first year of life, or three or fewer by the end of the second year.

In the general population, anti-P6 IgG antibody at birth was found at almost the same level as in adults, whereas no IgM or IgA antibodies specific for P6 were detected. Anti-P6 antibody levels in the three isotypes studied were lowest at 6 months of age and rose significantly after 2 years; IgG levels peaked at 10 years, whereas IgM and IgA peaked at 6 years. In every age group, IgG antibody specific for P6 was in the highest concentration among the three isotypes. Anti-P6 IgG antibody was detected in all individuals in each age group; however, IgM antibody specific for P6 was detected in all individuals older than 6 years of age, and IgA antibody specific for P6 was detected in all individuals only after 10 years of age. During the episode of otitis media, antibody levels in convalescent-phase sera exceeded those in acute-phase sera in 60% of cases. Sera obtained during acute and convalescent periods were screened for bactericidal antibody. Ten acute-phase sera possessed bactericidal antibody and 10 did not; all convalescent-phase sera had bactericidal antibody. When the paired sera were divided into two groups depending on the presence or absence of bactericidal antibody in the acute period and then analyzed for antibody to P6, a significant rise in anti-P6 antibody was detected in the group initially lacking bactericidal antibody.

In order to evaluate the immunological derangement in otitis-prone children, anti-P6 antibody levels were measured longitudinally in 30 otitis-prone and 13 healthy children on 93 and 32 occasions, respectively. The age at time of sampling varied between 1 and 92 months. Antibody levels increased seven-fold in the normal group for 36 months in comparison with less than three-fold in the otitis-prone group for 48 months. The levels of antibody in the normal group were significantly higher than those in the otitis-prone group after the age of 18 months. In general, individual antibody levels in otitis-prone individuals did not have an age-dependent rise. Furthermore, children who experienced two or more episodes of otitis media caused by nontypeable *H. influenzae* had no anamnestic antibody response to P6. Immunoglobulin IgM and IgA antibody responses to P6 in otitis-prone children reached a plateau after 18 months of age, and the anti-P6 IgM antibody level remained below the adult serum level even after 4 years of age. Differences between otitis-prone and normal children were not statistically significant. Antibody levels to P6 in otitis-prone children were measured, and anti-P6 IgG was defined if the concentration was lower than 2 SD below the mean for that age in normal children. As shown in Fig. 8, 11 of 20 otitis-prone children (55%) showed subnormal levels of anti-P6 IgG.[31]

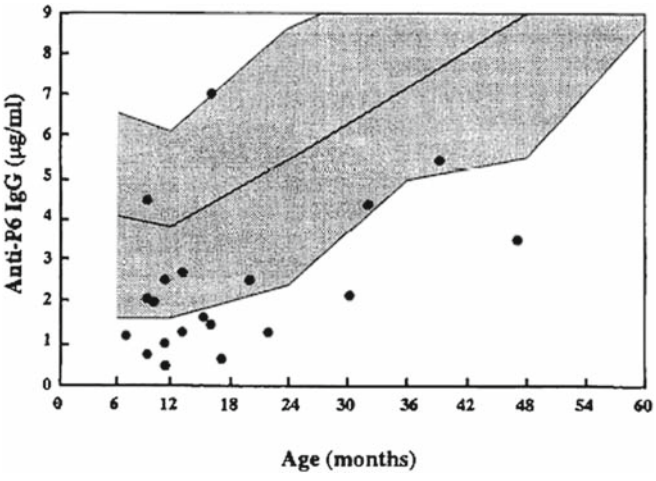

Fig. 8. Anti-P6 IgG in otitis-prone children. Normal values are plotted as a shaded area encompassing 2 SD around the mean. Otitis-prone children are represented as individual points (*small black circles*)

The failure to develop a good antibody response to common antigens may enable the pathogen to cause persistent or recurrent disease. As demonstrated by Harabuchi et al.[40] the level of anti-P6 antibody correlated to the severity of otitis media with effusion. The basis for these observed immunological abnormalities remains obscure. However, Kodama and Faden[41] suggested that in the case of NTHi, a lack of memory T lymphocytes might contribute to the poor antibody response despite repeated exposure to the pathogen. These results provide further information on the immunological aspects of otitis proneness.

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