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Streptococcus pneumoniae carriage and salivary antibodies induced by pneumococcus in Vilnius preschool children

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Background. *Streptococcus pneumoniae* (pneumococcus) is a potential respiratory pathogen causing high-risk diseases, particularly in young children. Otherwise, carriage of pneumococcus may be asymptomatic depending on the serotype and the immune system of the individual. The aim of this study was to determine nasopharyngeal carriage rates, serotype distribution and to evaluate the mucosal immune response to *S. pneumoniae* infection in healthy 2–7-year-old children attending day care centres in Vilnius, Lithuania.

Materials and methods. Nasopharyngeal (n = 601) and saliva samples were collected randomly. A pneumotest kit (Statens Serum Institute, Denmark) was used for serogrouping / serotyping; the sandwich ELISA with a double monoclonal system was used for immunoglobulin concentration measurement.

Results. Carriage rate in the children's population was 43%; from 33 serotypes found, the following six prevailed: 19F, 23F, 6B, 6A, 3 and 18C, which accounted for 58% of isolates. The carriage rates in children over four were found to be decreased. The concentrations of total and specific salivary antibodies of three isotypes (IgA, IgM and IgG) in carriers of six pneumococcal serotypes (3, 6B, 14, 18C, 19F and 23F) were measured.

Conclusions. The carriage rates and prevalent serotypes of *S. pneumoniae* in healthy preschool Lithuanian children were similar as in the neighbouring states. The response of total and specific salivary antibodies was found to be serotype-specific. The pneumococcal carriage stimulated the production of not only specific antibodies, but also of total immunoglobulins of S-IgA, IgM and IgG isotypes.

Key words: *Streptococcus pneumoniae*, serotypes, specific antibody, saliva, children

INTRODUCTION

Mucosal membranes are constantly exposed to antigens of different origin. The vast majority of pathogens penetrate the human body through mucosal membranes, and most of them are eliminated by the local immune system (1). Mucosal immunity forms the first line of defence against mucosally transmitted pathogens such as pneumococcis.

Streptococcus pneumoniae (pneumococcus) is a gram-positive, lancet-shaped encapsulated coccus, a frequent inhabitant of the respiratory tract, and it is able to cause both local diseases and life-threatening infections (2). Furthermore, the pneumococcus can be carried in the nasopharynx without any apparent symptoms, which helps it to persist in the human population and spread from person to person by aerosols. The most susceptible for this infection are children with their immature immune system. Moreover, the pathogenicity of pneumococcus depends on the serotype (3). Pneumococci are classified into 91 serotypes based on the antigenic variability of the capsular polysaccharides, which is an important virulence factor (4). Serotypes belonging to 6, 14, 18, 19, and 23 serogroups are prevalent and account for 60–80% of infections depending on the geographic area of the world. However, the prevalence of certain serotypes varies in different countries (5). Thus, it is reasonable to investigate the incidence of the serotypes in order to use or to develop the appropriate vaccination in a country. These microbes are able to stimulate immune responses. In addition to 'innate immunity', the specific, antibody-mediated defence takes action already on the mucosal surfaces. Relatively few data exist on the immune responses to pneumococcus after natural exposure. It has been demonstrated previously that contacts

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with *S. pneumoniae* induced natural salivary IgA responses against protein and polycaccharide antigens in children and adults (6). Mucosal IgA is predominant in all secretions and relatively important in host defence (1). In addition to IgA, pentameric IgM is likewise actively enriched in most exocrine fluids and is associated with the secretory component. In absence of IgA, higher concentrations of IgM can compensate for the IgA deficiency because it is the second possible antibody capable of binding the secretory component (1). IgG in mucosal secretions has traditionally been regarded as originating from serum by diffusion (7). In addition, IgG can be produced locally (8). It suggests that three isotypes (IgA, IgM and IgG) are important in mucosal immunity.

The objective of this study consisted of two related parts: the first task was to determine nasopharyngeal carriage rates and serotype distribution in Vilnius (Lithuania) preschool children population, and the second task was to evaluate the production and to compare the quantity of total and specific immunoglobulins in relation to serotype and pneumococcus carriage in children's saliva samples.

MATERIALS AND METHODS

Study subjects and ethical aspects

The subjects of our study were 2–7-year-old children attending day-care centres in different districts of Vilnius (Lithuania). Samples were collected by approval from the National Ethics Committee of Lithuania. Parents or legal guardians had received information about the study before the enrolment. An informed consent was obtained in each case. Data about the use of antibiotics and past diseases were collected with a special questionnaire. None of the subjects and controls had previous immunization with any pneumococcal vaccine.

Samples

Children were asked neither to eat nor drink one hour before sample collection. Nasopharyngeal swabs were obtained from a nostril with a "Mini-tip culturette" flexible swab (Becton Dickinson, Germany) and transported to the bacteriological laboratory of Vilnius University Children's Hospital to test for *S. pneumoniae* carriage. The nasopharyngeal swabs were cultured on chocolate agar and selective blood agar with 5% CO_2 according to the methods certified by the CLSI. Cell culture was used for identification by standard methods and for obtaining a pure culture for microtiter plate covering (9). Cell concentration was estimated by McFarland Turbidity Standards (10). Pneumococcal strains were serotyped / grouped with a Pneumotest kit from Statens Serum Institute (Copenhagen, Denmark) (11). Saliva samples were collected from every subject with a cotton swab placed under the cheek for 5 minutes, then centrifuged at 3000 rpm to collect the saliva, and stored at –70 °C until analyses. Saliva samples were thawed only once. Prior to analyzing, samples had been incubated at 56 °C for 15 min, centrifuged at 13000 g for 15 min, and the supernatant was used for the assay.

Enzyme immunoassay (EIA)

Measurement of anti-pneumococcal antibodies in saliva. Microtiter plates (Polysorp; Nunc, Roskilde, Denmark) were coated with a pure pneumococcal cell culture 2.4×10^8 cell/ml PBS (50 µl/well) and incubated without cover overnight at 37 °C to get it dried. The following pneumococcal serotypes were used: 3, 6B, 14, 18, 19B and 23. For measurements of antibodies specific to cell wall polysaccharides (CWPS), the plates were coated with 20 µg/ml of a pneumococcal cell wall polysaccharide mixture (CWPS Multi) from Statens Serum Institute (Copenhagen, Denmark) under the same conditions. The following steps were similar for detecting both types of pneumococcal antibodies. All plates were blocked with 2% BSA-PBS for 1 h at 37 °C. The human serum 89-SF from the U. S. Food and Drug Administration was used as a reference serum. Saliva samples were diluted 1 : 10 in BSA-PBS. Samples were assayed at a single dilution in duplicate and the reference serum at eight serial three-fold dilutions in duplicate. Samples were aliquoted and incubated for 1.5 h at 37 °C. Mouse monoclonal anti-human IgA, IgM and IgG biotin conjugated antibodies had been produced and characterized earlier (12). Biotinilated antibodies were incubated for 1 hour at 37 °C, followed by streptavidin-horseradish peroxidase conjugate incubation for 40 min at 37 °C. Bound conjugate was detected calorimetrically using the o-phenylenediamine / H_2O_2 substrate. The reaction was stopped by 2M H_2SO_4 solution. Between the steps, plates were washed four times with PBS containing 0.1% Tween 20 (Merck, UK). Absorbance was measured at 490 nm with an EL × 800 microtiter plate reader (BioTek, USA).

Measurement of total S-IgA, IgM and IgG quantities in saliva. The pears of capture and detection monoclonal antibodies were chosen for sandwich ELISA for the quantitative evaluation of appropriate immunoglobulin classes on the basis of developed test systems (12). Microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated with 10 µg/ml of capture monoclonal antibodies overnight at 4 °C. WHO International Standard Immunoglobulins G, A and M, Human Serum Code: 67/086 (NIBSC, UK) and purified human secretory IgA (Cat: 55905; MP Biomedicals, USA) were used as reference materials. Saliva samples were diluted in BSA-PBS 1 : 100 for IgM, 1 : 400 for S-IgA, 1 : 1000 for IgG. Detection antibodies, streptavidin-horseradish peroxidase conjugate and the substrate were the same as for measuring anti-pneumococcal antibodies.

Data analysis

The concentration of anti-pneumococcal IgA, IgM and IgG antibodies was calculated on the basis of the OD value obtained by subtracting the OD value of a saliva sample obtained on a plate covered with CWPS Multi from a sample obtained on a plate covered with the pneumococcal culture. The concentrations were determined by using a four-parameter logistic-log curve fitting model (Gen5 software) and expressed in nanograms per millilitre (ng/ml) of saliva. The lowest detected concentration was 0.4 ng/ml for IgA, IgM and IgG.

For quantitative measurement of antibodies to CWPS, a cut off value was established. A sample with the mean OD value obtained on a plate covered with CWPS Multi ≥ 2 SD of the blank was regarded as positive (cutoff value).

Non-parametric statistical methods were used because of the non-normality of antibody concentration data. Antibody concentrations in two groups (age, gender and carriers / non-carriers) were compared using the Mann–Whitney rank sum test. Differences in antibody concentrations between the serotype groups were analysed by the Kruskal–Wallis test. The significance of the correlation between the total and specific antibody concentrations was estimated by Spearman's correlation analysis. Differences were considered statistically significant when the p value was <0.05.

RESULTS

Microbiological studies

Nasopharyngeal swabs were collected from 601 healthy children 1–7 years old (median age 5.1) attending day care centres in Vilnius, Lithuania. The children's population consisted of 297 (49%) girls and 304 (51%) boys. *S. pneumoniae* was found in 257 children (43%). Twenty-two serogroups consisting of 33 different serotypes were identified. As presented in Table 1, the most frequent were serotypes 19F and 23F (approx. 14% each), serogroup 6 (16.8%) consisting of two serotypes, serotypes 3 and 18C formed more than 5% each, and the other *S. pneumoniae* serotypes made less than 3.1%.

The children (n = 601) were divided into two clusters according to age (2–4 and 4–7 years) consisting of 266 (44%) and 335 (56%) individuals. The nonparametric Mann–Whitney rank sum test showed a statistically significant difference ($p < 0.001$) in pneumococcal carriage between these clusters: the carriage rate was higher in 2–4-year-old children than in those aged 4–7 years. No statistically significant difference was found in pneumococcal carriage between males and females ($p = 0.459$).

Serotype-specific salivary antibody response

Total (S-IgA, IgM, IgG), specific to cell wall polysaccharide (IgA CWPS, IgM CWPS, IgG CWPS) and anti-pneumococcal (IgA Pn, IgM Pn, IgG Pn) antibodies were assessed in saliva samples of 129 non-carriers (pneumococcus-free) and in 107 carriers of *S. pneumoniae*. Anti-pneumococcal antibodies were measured by ELISA; test plates were coated with suspensions of a pure pneumococcal cell culture of appropriate serotype. The data obtained were not normally distributed; the normality was not achieved by log-transformation in some data sets. Therefore, nonparametric statistics was used to compare the distribution of the concentration of different isotypes and the specificity of antibodies to six most abundant serotypes. The distribution of the concentrations of three isotypes of total immunoglobulins and pneumococcal antibodies (IgA Pn, IgM Pn, IgG Pn) by six serotypes was statistically significant ($p < 0.01$). Data on the distribution of immunoglobulin isotypes according to serotypes are presented in Table 2. The numerals in the table show the percentage of children whose antibody concentration of separate isotypes exceeded the median. The data were expressed in percentage because of a high variation in the count of serotype representatives. The highest percentage of children with the total immunoglobulin production exceeding the median was related to serotypes 19F and 18C and the lowest one to serotype 23F. Similar results were obtained for anti-pneumococcal IgA Pn. In the case of anti-pneumococcal IgM Pn, 91% of children possessed an enhanced concentration of antibodies to serotype 19F.

Nonparametric statistics was also used to compare the distribution of different antibody concentrations by age. The distribution of total S-IgA between the two age groups (2–4 and 4–7 years) was statistically significant ($p < 0.01$). The amount of S-IgA (mean $\text{rank}_{2-4\ \text{yrs}}$ 46, mean $\text{rank}_{4-7\ \text{yrs}}$ 75) was higher in older children.

Table 1. **Data on serotype-specific carriage of *Streptococcus pneumoniae* in nasopharynx of healthy 2–7-year-old children (n = 257)**

Serotype	Size of group	%
19F	37	14.4
23F	36	14.0
6B	22	8.6
6A	21	8.2
3	19	7.4
18C	14	5.4
9V	10	3.9
10	10	3.9
14	9	3.5
37	9	3.5
Others	70	<3.1

Table 2. **Median immunoglobulin concentrations and distribution of total and anti-pneumococcal antibodies by serotypes**

Ig class	Median, µg/ml	Pneumococcal serotype						p-value*
		3	6B	14	18C	19F	23F	
S-IgA	134	62	60	20	78	70	4	0.000
IgM	2.3	54	53	40	56	57	13	0.022
IgG	6	62	63	60	33	61	13	0.002
IgA Pn	0.02	46	50	20	67	74	29	0.031
IgM Pn	0.003	23	77	60	11	91	4	0.000
IgG Pn	0.002	54	77	40	56	48	17	0.001

* Kruskal–Wallis test.

Total antibody of S-IgA, IgM, IgG isotypes in saliva

Sandwich ELISA with a double monoclonal system was used to measure immunoglobulin concentration. Corresponding pairs of monoclonal antibodies demonstrated a strict specificity to human S-IgA, IgM, IgG and did not show any cross-reactions with other immunoglobulin isotypes (12).

Statistical data on separate immunoglobulin isotype concentrations in saliva samples of healthy preschool children are presented in Table 2 (the exceptional cases with supposed IgA defficiency were excluded). Three children (n = 236) were identified as exceptional with a zero level of both total and specific imunoglobulins of S-IgA and IgA isotypes. Three exceptional cases are presented in Table 3. The concentration of total IgM was 3.3-, 25-, 18- fold higher in comparison with the group median (Table 2) for three cases. The concentration of total IgG was 3-, 6.6-, 13.2- fold higher, respectively. The concentrations of the specific antibody of IgM and IgG isotypes were also higher than the median. The parents of these children did not declare any complaints during the last six months.

The nonparametric Mann–Whitney test revealed a statistically significant difference in the distribution of total S-IgA, IgM ($p < 0.001$) and IgG ($p = 0.005$) concentrations between pneumococcal carriers and non-carriers: the carriers had significantly higher immunoglobulin concentrations in saliva in comparison with pneumococcus-free children (Figure, A and B).

Anti-pneumococcal antibody of IgA, IgM, IgG isotypes in saliva

Sandwich ELISA with the same biotinilated monoclonals as for total Ig detection was used to assess the concentration of a specific antibody of three isotypes. Plates were covered with a pure pneumococcal culture of 3, 6B, 14, 18C, 19F and 23F serotypes. Thus, the antibodies detected were polyspecific to the structural components of the bacteria. The concentration of anti-pneumococcal IgA Pn, IgM Pn, IgG Pn (Table 2) was calculated from the OD of a sample after subtraction of the OD obtained in saliva reaction with cell wall polysaccharides. Specific IgA Pn antibodies were not detected in the saliva

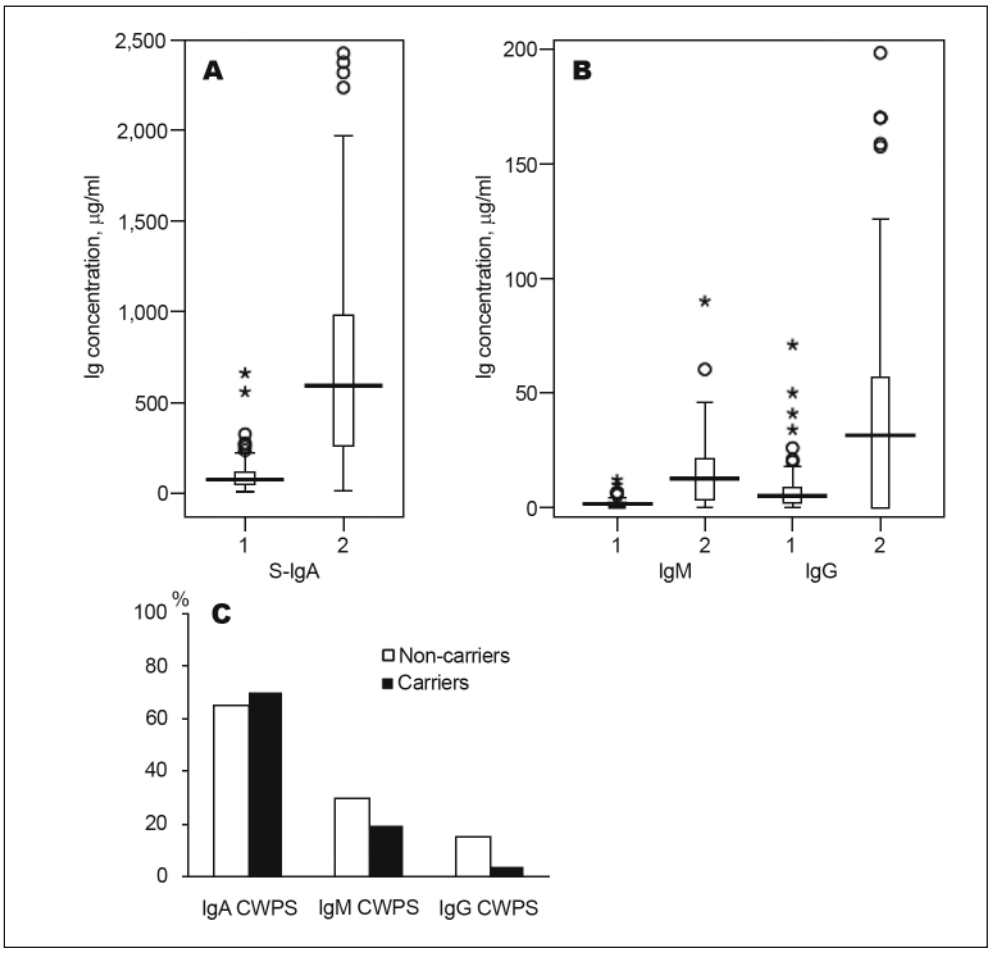

Figure. Distribution of immunoglobulin concentrations between non-carriers (1) and *S. pneumoniae* carriers (2) in saliva of 2–7-year-old healthy children: A – total S-IgA; B – total IgM and IgG; C – percentage of children possessing specific antibodies of IgA, IgM and IgG isotypes against cell wall polysaccharides (CWPS). Whiskers extend from box to highest and lowest values, excluding outliers; horizontal lines – medians; box – interquartile range that contains 50% of values; white square – outliers; asterisk – extreme values in box plots A and B

Table 3. **Exceptional cases as having no salivary IgA**

	No. 78	No. 303	No. 156
S-IgA, µg/ml	0	0	0
IgA, µg/ml	0	0	0
IgM, µg/ml	7.6	57.6	41.4
IgG, µg/ml	2.0	39.7	79.4
IgA Pn, ng/ml	0	0	0
IgM Pn, ng/ml	0	22	46
IgG Pn, ng/ml	0	10	26
Serotype	–	18C and 3	6A and 14
Growth	–	+++	+
Complaints	no	no	no
Age	4.8	5.9	3.9

of only one child, IgM Pn antibodies were not found in 34 children and IgG Pn in 30 children. This implies that 99% of pneumococcal carriers had specific IgA Pn, 68% of IgM Pn and 70% of IgG Pn class antibodies. The most abundant class of specific anti- pneumococcal antibodies was IgA Pn (median 40 ng/ml).

The nonparametric Spearman's rank correlation analysis indicated a moderate positive correlation between the total and specific immunoglobulins of appropriate isotypes in carriers (n = 105): the correlation coefficients for three isotypes of total and anti-pneumococcal immunoglobulins were 0.60 ± 0.02 (p = 0.01). The weakest significant correlation was assessed for the IgG–IgG CWPS pair (r = 0.27, p = 0.05). These correlations showed that a rising concentration of total immunoglobulin matched moderately the rising concentration of a specific one.

The lack of reference material prevented the quantitative measurements of the level of specific antibodies to CWPS. The cutoff value allowed to classify children to CWPS-positive (OD ≥ cutoff) and CWPS-negative (OD < cutoff). The level of antibodies to CWPS was expressed by the percentage of CWPS-positive children. The full amount of the anti-CWPS antibody (three isotypes as one) posessed 45% of children from the group of non-carriers and 61% from carriers. The distribution percentage among three immunoglobuline isotypes is presented in Figure (C).

DISCUSSION

This study determined nasopharyngeal carriage rates of *Streptococcus pneumoniae* among healthy children aged 2–7 years in Vilnius, Lithuania. Transmission and nasopharyngeal colonization are the main factors for the development of an invasive pneumococcal disease, but their patterns vary geographically. Pneumococcus was identified in 43% of children attending day care centres in Vilnius. This carriage rate was similar to that in geographically close regions: 44% in Estonia (13), 47% in St. Petersburg, Russia (14) and 38% in Czech Republic (15). Six serotypes (19F, 23F, 6B, 6A, 3 and 18C) from the 33 identified were predominant and comprised almost 60% of all isolates. The prevalent serotypes represented a similar distribution in the neighbouring states (13–15).

Studies have shown that colonization of the nasopharynx by the potential respiratory pathogen *S. pneumoniae* is established in early childhood, although the rates vary greatly not only depending on locality, but also on individual factors. We found higher carriage rates in younger (2–4 years old) than in older (4–7 years) children. These findings were in agreement with the information concerning the maturation of children's immune system. Gleeson et al. determined the dynamics developing IgA-specific antibodies to *Escherichia coli* O antigen in children's saliva. The total IgA levels were low from birth to 4 years of age; a gradual increase in specific antibodies to the levels found in adults was detected in 5–9-year-old children (16). Our findings showed significant differences in the total S-IgA level between the two age groups both in pneumococcal carriers and in pneumococcus-free children. This is in agreement with the data mentioned above, especially as S-IgA represents one of the main immunoglobulins in mucosal immunity.

The previous findings implied that at least some serotype-generated anti-capsular antibodies could reduce the risk of carriage in unimmunized toddlers. It was reported that, for serotypes 14 and 23F, specific protection correlated with an increased concentration of the serotype-specific antibody (17). For our analysis, we have chosen the most often carried serotypes (3, 6B, 18C 19F and 23F) and additionally included the invasive serotype 14. Our findings revealed significant differences in the distribution of the total and anti-pneumococcal S-IgA, IgM, IgG concentration in saliva by six serotypes. Serotypes 18C and 19F most intensively stimulated the local production of total and anti-pneumococcal antibody, especially that of IgA isotype. The weakest mucosal immune response was related to serotype 23F and to a less extent to serotype 14 (Table 2). The obtained data showed that protection against carriage is serotype-specific.

There are plenty of investigations on immunoglobulin production in the saliva of children of different age infected by various microorganisms. The factors influencing colonization and elimination of *S. pneumoniae* are not yet fully understood, however, immune responses are implicated in addition to bacterial properties. Our results demonstrated a considerable distribution of the total and anti-pneumococcal antibody concentration in saliva samples of separate individuals, possibly due to the turning-point in mucosal immune response at the age of about 4 years (16). Highly significant differences ($p < 0.005$) in the production of total S-IgA, IgM and IgG were determined between the two groups of children – pneumococcal carriers and non-carriers. The pneumococcal presence obviously stimulated the total antibody production of all three isotypes. This increase could not be assigned to the development of specific mucosal immunity because of the low specific antibody concentrations in comparison to the total immunoglobulin levels. It looks like these bacteria invading the human body through mucosal

membranes prompt their response, and local antibodies most probably influence the defence against them. Otherwise, it should be always taken into account that an increase in total immunoglobulin concentration may be due to elevated levels of specific immunoglobulins to other antigens.

Three children were identified as having no salivary IgA during this study: the levels of both IgA and S-IgA were below the limit of detection (0.4 ng/ml). IgA deficiency is the most common immunodeficiency described (18). Usually, the serum IgA level in IgA-deficient patients is <0.05 mg/ml. This genetic defect of isotype switching had an influence on mucosal and systemic plasma cells; however, quantitative aspects remained controversial (19). The design of our experiment determined the random one-time selection of children; consequently, the serum immunoglobulin level was not assessed. However, the frequency 1 to 80 of IgA deficiency determined in this study for healthy children was considerably higher than that declared in adult people (1), but lower than in diseased hospitalized children (18). Patients with IgA deficiency were found to be at an increased risk of upper respiratory tract infections, allergies, coeliac-like enteropathies and autoimmune disorders. Nevertheless, many patients are asymptomatic. The children in our study had no complaints during the last months, although two of them were double and invasive serotype carriers. The reason for such asymptomatic carriage in detected cases of IgA deficiency could possibly be the mechanisms of compensation and cooperative defence with the participation of increased levels of salivary IgM and IgG. It is known that systemically administered IgG protects against mucosal infection by the respiratory syncytial virus in human lungs (20). The increased level of IgM can compensate for the absence of IgA in secretions, as IgM is the only antibody capable of binding the secretory component (1).

Pneumococcal antigens are so prevalent in our environment that most normal adults have significant titers of anti-pneumococcal antibodies (6). Obviously, an increase in antibody concentrations in nonvaccinated children is a consequence of natural contacts with pneumococci, the first contact occurring through mucosal surfaces. IgA is the prevalent isotype on mucosal surface; therefore, salivary anti-pneumococcal IgA in children was detected more often than IgM and IgG; it is in full agreement with other reports (8, 21, 22). Furthermore, only one child from the group of pneumococcal carriers (n = 105, excluding two cases having no IgA) did not possess anti-pneumococcal IgA. In order to evaluate mucosal response to *S. pneumoniae*, a more precise study of the relation between total and anti-pneumococcal antibody levels was carried out. The correlation between total and specific antibodies in most cases was moderate or even lower. Similar correlations between total IgA and anti-pneumococcal salivary antibodies for 7–16-month-old children had been found earlier by Nurkka et al. (21). A low correlation implies the other factors, including different individual mucosal response to the antigens of different origin.

Simell et al. found that pneumococcal carriage could induce both antibodies specific to pneumococcus protein and to polysaccharide in saliva of infants. The immune response was different depending on the origin of the antigen (22). We used two types of antigens for plate covering in our study. The first was a pure pneumococcus culture of various serotypes representing a mixture of bacterial antigens of different kind. The second was a mixture of purified pneumococcal cell wall polysaccharide antigens (CWPS) that are common to all pneumococcal serotypes (23). The final anti-pneumococcal antibody concentration was obtained after subtraction of OD values on pure culture plates from CWPS plates. Such separation of serotype-specific and nonspecific anti-pneumococcal antibodies revealed some differences in response to *S. pneumoniae* carriage. Naturally, the main response to CWPS was that of IgA class in both groups of children. However, the amount of IgG CWPS was four-fold higher in the group of pneumococcus-free children than in carriers. The results obtained by Nurkka et al. showed that even after three to four years following the series of four doses of a pneumococcal conjugate vaccine, the detection rate of salivary anti-pneumococcal IgG polysaccharide remained approximately at the same level as one month after the booster (21). Evidently, an increase in antibody concentrations in these unvaccinated and pneumococcus-free children is a consequence of natural permanent contacts with the bacteria in day care centres. The present study demonstrated that response to capsular components and CWPS differ. Furthermore, it appeared that the response to CWPS (as anti-pneumococcal IgG CWPS) persisted for a longer time even when microorganisms were not found in the nasopharynx. Otherwise it is known that specific antibodies generated in response to *S. pneumoniae* provide protection to the host (17). Despite the fact that the secretory IgA is the predominant isotype of mucosal surfaces, its role in protection from colonization is not proportional to the amount, possibly by reason of IgA but not IgG cleavage by specific proteases. The inhibition of colonization was found strongly to depend on serum IgG concentration which is related to the concentration in saliva due to diffusion (24). Probably, the enhanced amount of IgG CWPS in the saliva of non-carriers reduced pneumococcal carriage.

CONCLUSIONS

Primarily, this study provides data on the local carriage rate and serotype distribution of *S. pneumoniae* strains in Vilnius (Lithuania) children, which is important in predicting the possible effects of different valence pneumococcal conjugate vaccines in this population.

In the light of the fact that the pneumococcus invade the human body through mucosal membranes, local antibodies most probably play a defencive role against them. Our study showed that the production of three main isotypes of both total and specific antibodies in the saliva of infected children

depended on the serotype carried. The pneumococcal carriage stimulated the production not only of specific antibodies, but also of total immunoglobulins. The response to capsular components and cell wall polysaccharides was found to be different. During this study, the frequency 1 to 80 of IgA deficiency was determined, and it was higher than in adult population.

However, the importance of salivary antibodies is not fully elucidated; for example, the concentrations of antibodies needed for protection are unknown. Despite the numerous studies in the field of mucosal immune response, the protective role and importance of salivary naturally induced antibodies require further investigations.

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VILNIAUS IKIMOKYKLINIO AMŽIAUS VAIKŲ *STREPTOCOCCUS PNEUMONIAE* NEŠIOJIMO DAŽNIS BEI PNEUMOKOKŲ PASKATINTA ANTIKŪNŲ GAMYBA SEILĖSE

S a n t r a u k a

Prielaidos ir tikslas. *Streptococcus pneumoniae* (pneumokokai) yra kvėpavimo takų patogenas, galintis sukelti pavojingas ligas, kurioms ypač neatsparūs maži vaikai. Antra vertus, pneumokokų nešiojimas priklausomai nuo nešiojamo serotipo ir individo imuninės sistemos savybių gali būti ir besimptomis. Šio darbo tikslai – pneumokokų nešiojimo nosiaryklėje ir serotipų paplitimo dažnio nustatymas, taip pat gleivinių imuninio atsako *S. pneumoniae* infekcijai įvertinimas sveikų 2–7 metų vaikų, lankančių ikimokyklinio ugdymo centrus Vilniuje (Lietuva), populiacijoje.

Medžiagos ir metodai. Atsitiktinės atrankos būdu buvo surinktas 601 nosiaryklės tepinėlis ir seilių pavyzdžiai. Darbe naudotas pneumokokų serotipavimo antikūnų rinkinys (Statens Serum Institute, Danija); imunoglobulinų koncentracija buvo nustatoma netiesioginės ELISA metodu taikant dvigubą monokloninių antikūnų sistemą.

Rezultatai. Tirtoje vaikų populiacijoje pneumokokų nešiojimo dažnis – 43 %; iš 33 nustatytų serotipų dažniausi 19F, 23F, 6B, 6A, 3 ir 18C serotipai sudarė 58 %. Vyresnių nei ketverių metų vaikų nosiaryklėje, palyginus su jaunesniais, pneumokokų nešiojimo dažnis buvo mažesnis. Nustatytos trijų izotipų (IgA, IgM ir IgG) bendrų ir specifinių antikūnų koncentracijos šešių serotipų (3, 6B, 14, 18C, 19F ir 23F) nešiotojų seilėse.

Išvados. *S. pneumoniae* nešiojimo dažnis ir vyraujantys serotipai ikimokyklinio amžiaus Vilniaus (Lietuva) vaikų populiacijoje panašūs į kaimyninių valstybių. Bendrų ir specifinių antikūnų atsakas į pneumokokinę infekciją priklauso nuo bakterijų serotipo. Pneumokokų nešiojimas skatina ne tik specifinių antikūnų gamybą, bet ir bendrų S-IgA, IgM ir IgG izotipų imunoglobulinų produkciją.

Raktažodžiai: *Streptococcus pneumoniae*, serotipai, specifiniai antikūnai, seilės, vaikai