Antigen-Specific B-Cell Response to 13-Valent Pneumococcal Conjugate Vaccine in Asplenic Individuals With β-Thalassemia Previously Immunized With 23-Valent Pneumococcal Polysaccharide Vaccine

Ioanna Papadatou,1 Cristina Piperi,2 Krystallenia Alexandraki,2 Antonis Kattamis,3 Maria Theodoridou,1 and Vana Spoulou1

1Infectious Diseases Unit, First Department of Paediatrics, Aghia Sophia Children’s Hospital, 2Department of Biological Chemistry, Medical School, and 3Thalassemia Unit, Aghia Sophia Children’s Hospital, University of Athens, Greece

Current guidelines recommend a combined schedule of a 13-valent pneumococcal conjugate vaccine (PCV13) and PPSV23 (23-valent polysaccharide vaccine) for asplenic individuals. We show that PCV13 induces a T-dependent immune response in asplenic individuals with β-thalassemia, but previous PPSV23s affect the memory B-cell response in a dose- and time-dependent manner.

Methods

Thirty-nine adults (21 male) with β-thalassemia and asplenia, aged 19–48 years (mean, 36.5 years), were recruited. All patients had received 1 dose of PCV7 seven years earlier and 1–4 doses of PPSV23s in the past. Time from their last PPSV23 ranged from 1 to 11 years with mean value of 4.7 years (SD, 3.0 years). All patients were vaccinated with 1 dose of PCV13. Blood samples were obtained immediately before (day 0), on day 7, and on day 28 after vaccination for polysaccharide-specific antibody and MBC analysis.

Total and polysaccharide-specific immunoglobulin G (IgG) and immunoglobulin M (IgM) MBCs were quantified using enzyme-linked immunospot assay (ELISpot) assay, as previously described [9]. In brief, washed peripheral blood mononuclear cells (PBMCs) were seeded at 10^5 cells/well on ELISpot plates previously coated with monoclonal IgG or IgM, or pneumococcal polysaccharides (3, 9V, 19A, 19F, and 23F). After 18 hours of incubation, cells were washed and plates were incubated with horseradish peroxidase (HRP) IgG or HRP IgM; the reaction was developed using TrueBlue (KLP, Insight Biotech). Plate drying revealed spot formation, enumerated automatically in AID ELISpot reader. MBCs were defined as the number of MBCs/10^5 cultured PBMCs.

The World Health Organization (WHO) enzyme-linked immunosorbent assay protocol was used for the detection of IgG

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antibodies in serum samples against pneumococcal serotypes. The trial was registered at ClinicalTrials.gov (NCT01846923).

**Statistical Analysis**

Continuous variables are presented as mean and standard deviation (SD) or median and interquartile range (IQR). Correlation coefficients were calculated to relate baseline values with those at day 7 and day 28. To longitudinally assess changes in IgG and IgM MBCs and IgG antibodies, mixed linear regression models with time were fitted. The independent covariates were PPSV23 doses, time since the last PPSV23, timing of PCV7 in relation to PPSV23 doses, age, and sex. Possible interactions of variables tested via regression models and none was significant. IgG antibody levels were log-transformed, whereas IgG and IgM MBCs were normally distributed and analyzed as raw data. Statistical significance was set at $P = .05$, and analyses were conducted using SPSS software (version 18.0).

**RESULTS**

Prior to vaccination, all study participants had detectable IgM and IgG MBCs against at least 1 serotype tested. On day 7, IgG MBCs increased significantly for serotypes 9V ($P < .001$), 19F ($P = .021$), 23F ($P = .001$), and 3 ($P < .001$) and for all serotypes on day 28: $P < .001$ for 9V, 19F, 23F, and 3 and $P = .17$ for 19A. In contrast, IgM MBCs did not rise post-PCV13 on day 7 or day 28 for any serotype tested, remaining at prevaccination levels. Polysaccharide-specific IgG antibody geometric mean concentrations above the threshold of 0.35 µg/mL were detected in all patients before vaccination and increased significantly on day 7 ($P \leq .001$ for 9V, 19F, 23F, and 19A and $P = .026$ for 3) and day 28 ($P < .001$) for all serotypes tested. Response to PCV13 was similar after adjusting for age, sex, and timing of PCV7 in relation to PPSV23s.

The investigation of the impact of previous PPSV23s on the B-memory and humoral response to PCV13 in a dose and time-dependent manner revealed consistent trends for all studied serotypes with IgG and IgM MBCs, as well as IgG antibody levels on day 28 being negatively correlated with number of previous PPSV23 doses and positively correlated with time elapsed since last PPSV23 vaccination (Figure 1).

More specifically, IgG MBCs were significantly ($P < .05$) negatively correlated with the number of previous PPSV23s for serotype 23F ($r = -0.36$) and IgM MBCs for serotypes 19F ($r = -0.47$), 23F ($r = -0.45$), and 3 ($r = -0.47$). The fold increase of IgG antibodies from baseline to day 28 was negatively correlated with the number of PPSV23s for serotypes 19F ($r = -0.40$) and 23F ($r = -0.38$) and positively correlated with time since last PPSV23 for serotype 19F ($r = 0.33$, Spearman correlation coefficient). Similar trends were observed for the IgG and IgM MBCs and IgG antibodies for the rest of the serotypes tested that did not reach statistical significance (Figure 1).

**DISCUSSION**

This is the first study to our knowledge to investigate the immunological memory induced by PCV13 in asplenic patients with β-thalassemia and the effect of previous immunizations with PPSV23 on antibody and MBC responses to PCV13. Despite the relatively small study size, we demonstrated that PCV13 establishes serotype-specific immune memory in this group in a real-life clinical setting. Moreover, we demonstrated consistent trends on the negative effect of previous PPSV23s to PCV13 immunogenicity and immunological memory in a dose- and time-dependent manner.

The increase of IgG MBCs on day 28 after vaccination provides evidence for the T-dependent nature of the immune response to PCV13, which has been associated with the production of new MBCs and replenishment of the MBC pool. Increased IgG MBCs following PCV7 have been previously reported by Clutterbuck et al in a cohort of healthy older adults and are in contrast to the significant reduction of IgG MBCs observed in the same setting following PPSV23 vaccination [10].

Our findings that IgM MBCs remained stable postvaccination provide further evidence that PCV13 does not deplete the MBC pool. It has been suggested that IgM MBCs generate IgM plasmablasts [11] and new IgM MBCs upon stimulation with pneumococcal antigens. Our findings that IgM MBCs had been maintained to baseline levels 1 month after PCV13 despite antigen stimulation suggest that PCV13 induces the production of new IgM MBCs. However, it should be noted that the kinetics of IgM MBCs in our patients could be affected by the lack of splenic marginal zone, which has been proposed as the predominant source of circulating IgM memory cells [12].

The impact of previous PPSV23s on MBC and antibody response to PCV13 was evaluated according to patients’ vaccination history. Consistent trends for dose- and time-dependency of hyporesponsiveness were demonstrated, and reached statistical significance for some of the studied serotypes. Patients with a history of more and recent PPSV23s immunizations had consistently inferior MBC and antibody counts post-PCV13 in comparison with those with fewer and earlier immunizations with PPSV23s in the past.

Our finding that multiple PPSV23s result in lower numbers of polysaccharide-specific MBCs is in accordance with the hypothesis that the polysaccharide antigens drive preexisting switched MBCs into terminal differentiation without replenishing the memory cell pool with new polysaccharide-specific MBCs [5, 10]. Hyporesponsiveness driven by polysaccharide
Vaccines was first described with meningococcal vaccines [13] and later with PPSV23. The PPSV23-induced depletion of MBCs is one of the proposed underlying mechanisms of hyporesponsiveness, alone or in association with other mechanisms under investigation, including the involvement of neutralizing circulating polysaccharide antigens, the depletion of B1b cells, or a dendritic cell–driven immunosuppressive mechanism [10, 14, 15]. The generation of MBCs by PCV13 could in part overcome the state of hyporesponsiveness in patients with previous PPSV23s. However, it is possible that 1 PCV is not enough to overcome established hyporesponsiveness [5, 15].

Long intervals since PPSV23 vaccination led to improved cellular and humoral immune response to PCV13, possibly due to waning of circulating antigens with time and the recharge of the B-cell pool by natural immunity, cross-reacting antigens, or homeostatically proliferating B-cell clones [16]. Time-dependency could explain the controversy on hyporesponsiveness among groups investigating the effect of PPSV23 in various intervals [5].

This study is subject to several limitations, the most important one being the relatively small study sample and the non-randomization of vaccine schedules. The total number of β-thalassemia major cases is low and there is a high incidence of significant comorbidities, which made patients ineligible for enrollment. Randomization of patients to receive various numbers of PPSV23 before PCV13 was impossible under the effect current guidelines [6]. The study also lacks a PPSV23-naive group, as all patients had received at least 1 dose of PPSV23 before they underwent splenectomy [3]. However, the study design attempts to address a real-life clinical problem for clinicians treating asplenic patients already vaccinated with PPSV23s.

Our finding for PPSV23-driven hyporesponsiveness provides further evidence that PCV13/PPSV23 combined schedules, currently recommended for at-risk individuals, could be justified if epidemiology of invasive pneumococcal disease following the extensive use of PCV13 indicates the prevalence of non-PCV13 serotypes.
Future multivalent conjugate vaccines or innovative peptide vaccines will, we hope, meet the dual need for maximum serotype coverage and optimal immunological characteristics for at-risk individuals.

Notes

Author contributions. All authors have substantially contributed to the submitted work.
I. P. co-designed and performed the research; collected, analyzed, and interpreted the data; and wrote the manuscript. C. P. and M. T. provided expertise and supervision in the performance of the study and edited the manuscript. A. K. co-designed the study, collected data, and followed up the study participants. V. S. co-designed the research, analyzed and interpreted the data, and edited the manuscript.

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