Vaccine 31 (2013) 4632–4640

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Pneumococcal polysaccharide vaccination induces polysaccharide-specific B cells in adult peripheral blood expressing $CD19^+CD20^+CD3^-CD70^-CD27^+IgM^+CD43^+CD5^{+/-}$

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ARTICLE INFO

Article history:
Received 31 January 2013
Received in revised form 20 June 2013
Accepted 16 July 2013
Available online 1 August 2013

Keywords:
Flow cytometry
Streptococcus pneumoniae
Polysaccharide
B cell
CD43
pneumococcus

ABSTRACT

Pneumococcal polysaccharide vaccines have been used to elicit a protective anti-pneumococcal polysaccharide antibody response against *Streptococcus pneumoniae* in healthy individuals. Identifying human B cells which respond to T-cell independent type-2 antigens, such as pneumococcal polysaccharides, has been challenging. We employed pneumococcal polysaccharides directly conjugated to fluorophores in conjunction with flow cytometry to identify the phenotype of B cells that respond to pneumococcal polysaccharide vaccination. We have previously identified that the majority of pneumococcal polysaccharide-selected cells responding to vaccination are $CD27^+IgM^+$ (IgM^+ memory) cells. In this study, we further characterized pneumococcal polysaccharide-selected cells in the peripheral blood to better identify how the various B cell phenotypes responded 7 and 30 days post-immunization. We show that 7 days post-immunization the majority of pneumococcal polysaccharide-selected IgM^+ memory cells ($PPS14^+$ 56.5%, $PPS23F^+$ 63.8%) were $CD19^+CD20^+CD27^+IgM^+CD43^+CD5^{+/-}CD70^-$, which was significantly increased compared to pre-immunization levels. This phenotype is in alignment with recent publications describing human B-1 cells. PPS-responsive B cells receded to pre-immunization levels by day-30. These findings suggest that this B-1 like cell population plays an important role in early responses to *S. pneumoniae* infection and possibly other T-cell independent type-2 antigens in humans

1. Introduction

Increased antibiotic resistance among many *Streptococcus pneumoniae* serotypes associated with disease, including pneumococcal polysaccharides 14 and 23F (PPS14, PPS23F), emphasizes the need for improved vaccine strategies, especially for those at highest risk for invasive disease including elderly and immunocompromised individuals [1–5]. Vaccination results in PPS-specific IgM and IgG opsonophagocytic antibodies (Ab) which are critical for bacterial clearance [6–10].

The nature of the immune cells involved in the production of Ab against these T-independent Type II (TI-2) polysaccharide antigens is controversial. Splenic marginal zone B cells (MZB) produce recirculating plasmacytes and memory B cells, capable of rapidly producing opsonizing IgM and IgG Abs against TI-2 antigens [7,11–16]. The role of MZB in response to TI-2 antigens is also supported by the finding that individuals who respond poorly to pneumococcal vaccinations tend to lack IgM^+ memory B cells. This includes patients with congenital neutropenia, common variable immunodeficiency, HIV infection, have been splenectomized, infants <2 years old with an underdeveloped marginal zone, and elderly populations [11,14,15,17–20]. Alternatively, B-1 cells have also been implicated in the production of plasmacytes and memory B cells capable of rapidly producing IgM and IgG Abs against TI-2 antigens [7,12,21–25].

Previous studies demonstrate mouse B-1 cells transferred into $RAG^{-/-}$ mice produce PPS-specific Abs and provide protection against lethal challenge [21,22]. While it is thought that B-1 cells contribute to the immune response against pathogens expressing TI-2 antigens in humans, the direct relevance of B-1 cells has been

Abbreviations: Ab, antibodies; PPS, pneumococcal polysaccharide; TI-2, T-independent type II; MZB, splenic marginal zone B cell; PPV, pneumococcal polysaccharide vaccination; OPT, opsonophagocytic titer; FMO, Fluorescence Minus One.

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http://dx.doi.org/10.1016/j.vaccine.2013.07.030

unclear due to the difficulty in identifying human B-1 cell equivalents. In mice, B-1 cells can be divided into two subtypes, B-1a and B-1b cells. B-1b cells have the ability to produce Abs that can provide a long-term adaptive immune response to TI-2 antigens like polysaccharides [21–23,26,27]. Human B-1 cells on the other hand are controversial themselves. It is unclear if the same division of B-1 cells that exist in mice is recapitulated in humans. Previous studies demonstrate that CD5 expression on human B cells is insufficient to characterize B-1 cells as it is used in mice [27–30]. Recent publications have described a mouse B-1a like subset in humans [27,30]. It is currently unclear if there is a mouse B-1b like equivalent in humans capable of responding to TI-2 antigens such as those used for pneumococcal polysaccharide vaccination (PPV).

We have previously shown, using fluorescently labeled PPS14 and PPS23F, the majority of PPS-specific B cells responding to vaccination are IgM^+ memory cells ($CD27^+IgM^+$) [31]. The goal of the present study was to further characterize PPS-specific PPV responding cells with respect to expression of CD43 and CD5 used to characterize this putative B-1 cell population. Our results identify PPS14- and PPS23F-reactive B cell populations that circulate in the peripheral blood 7- and 30-days post-immunization in response to PPV. We show the majority of PPS-specific B cells on day-7 are phenotypically characterized as $CD19^+CD20^+CD3^-CD70^-CD27^+IgM^+CD43^+CD5^{+/-}$. This population is in alignment with recent reports of human B-1 cells [30,32–34]. We also show that 30 days post-immunization, this population recedes toward pre-immunization levels.

2. Materials and methods

2.1. Human volunteers

Seventeen healthy volunteers participated in the University of Toledo IRB committee approved study (IRB #105137). Volunteers were 24–30 years old (mean = 26.6) and pneumococcal polysaccharide vaccine naive. Volunteers were questioned about medications, previous illness, and present health before immunization with PPV, Merck (23-valent pneumococcal polysaccharide vaccine).

2.2. Labeling of polysaccharide 14 and 23F with fluorescent dye

Conjugation of PPS14 to cascade blue (CB) ethylenediamine (Invitrogen catalog C-621) or PPS23F to 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF; Sigma–Aldrich Fluka catalog 36565) was carried out as previously described [31].

2.3. Flow cytometry

Peripheral blood was collected from volunteers pre- and post-immunization at days 0, 7, and 30. After Ficoll-gradient centrifugation and washing, cells were resuspended in FACS buffer (PBS, 0.1% FCS, 2 mM EDTA). Before staining, cells were absorbed with 10 μg/ml cell wall polysaccharide (Statens Serum Institut, MiraVista Diagnostics, Indianapolis, IN) and PPS22F (American Type Culture Collection) to reduce nonspecific binding [35]. B cells were labeled with 10 μg/ml, either PPS14-CB or PPS23F-DTAF (BioCentra, Sugar Land, TX). Fluorochrome-conjugated mAbs (BD Bioscience or eBioscience) to the following anti-human Ags were used: CD19 (APC-Cy7), CD27 (PerCP-Cy5.5), IgM (allophycocyanin), IgD (Alexa Fluor 700), CD5 (PE-Cy7), CD43 (PE), CD20 (Alexa Fluor 700), CD70 (CB and FITC). Cells were washed and suspended in FACS buffer and analyzed with FACSAria using FACSDiva software (BD Biosciences). FCS files were further analyzed using FlowJo software (Tree Star, Ashland, OR).

2.4. Pneumococcal polysaccharide ELISA

ELISA was performed to examine anti-PPS-specific human Abs in all volunteers. The PPS-ELISA is modification from the World Health Organization assay [36,37]. All steps were performed as reported previously [31].

2.5. Opsonophagocytic assay

Opsonophagocytic assay was performed as previously described [38,39]. Briefly, *S. pneumoniae*, serotypes 14 and 23F, were incubated with serial diluted heat-inactivated sera. Newborn rabbit serum (Pel-Freez, Brown Deer, WI) was added as source of complement. Differentiated HL-60 cells were added at an E:T ratio of 400:1. Sera were tested in duplicate. Results were obtained using Opsotiter1 software program (University of Alabama at Birmingham).

2.6. Statistical analysis

Geometric mean concentration of IgG, IgM, and IgA, and flow cell numbers, specific to PPS14 and PPS23F, were calculated for each group. Correlation between groups was examined using Pearson's correlation coefficient. Comparison between two group values was performed using unpaired *t* test. The *p*-values < 0.05 were considered to be significant.

3. Results

3.1. Ab titers increase significantly post-immunization with PPV

To show that our young healthy donor population ($n = 17$) responded normally to immunization with PPV, we tested sera collected on day-0, day-7, and day-30 for PPS14 and PPS23F Ab responsiveness. Following the World Health Organization recommendations, sera were first absorbed with PPS22F and cell wall polysaccharide to prevent overestimation of PPS-specific Ab concentrations [35,39,40]. Day-30 post-immunization, donors showed a significant increase in PPS14-specific IgM from 2.4 ± 2.4 μg/ml to 32.7 ± 18.0 μg/ml ($p < 0.001$), IgG from 3.3 ± 2.5 μg/ml to 47.1 ± 24.0 μg/ml ($p = 0.0023$), and IgA from 0.3 ± 0.2 μg/ml to 2.7 ± 2.9 μg/ml ($p = 0.0056$) (Fig. 1A). Similarly, post-immunization PPS23F-specific Ab levels were significantly increased for IgM from 1.2 ± 0.8 μg/ml to 22.3 ± 7.9 μg/ml ($p < 0.0001$), IgG from 2.8 ± 2.3 μg/ml to 31.9 ± 18.9 μg/ml ($p < 0.0001$), and IgA from 0.2 ± 0.1 μg/ml to 0.9 ± 1.1 μg/ml (Fig. 1B). IgG concentrations showed a greater increase post-immunization compared to IgM concentrations for both PPS. These ELISA data confirm that our donor population responded to PPV immunization resulting in a minimal two-fold increase in serotype-specific Ab.

3.2. OPT increases significantly 30 days post-immunization with PPV

Donor sera collected on day 0 and day 30 were tested for functional opsonophagocytic response using both PPS14 and PPS23F expressing *S. pneumoniae*. The reciprocal of the Ab dilution required to obtain 50% opsonophagocytic killing by differentiated HL-60 cells (opsonophagocytic titer-OPT) was calculated. There was a significant increase in the OPT post-immunization compared to pre-immunization for both PPS14 ($p = 0.0001$) and PPS23F ($p < 0.0001$) (Fig. 1C). These results confirmed that vaccination of our sample population with PPV elicited a functional immune response against serotype-specific PPS [41].