Aging Promotes B-1b Cell Responses to Native, but Not Protein-Conjugated, Pneumococcal Polysaccharides: Implications for Vaccine Protection in Older Adults

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The efficacy of different vaccines in protecting elderly individuals against Streptococcus pneumoniae infections is not clear. In the current study, aged mice (22–25 months old) exhibited significantly increased susceptibility to respiratory infection with serotype 3 S. pneumoniae relative to younger adult mice, regardless of whether mice were naive or immunized with native pneumococcal polysaccharide (PPS; Pneumovax23) or protein-PPS conjugate (Prevnar-13) vaccines. Nonetheless, Pneumovax-immunized aged mice developed limited bacteremia following respiratory challenge and exhibited significantly increased survival following systemic challenge relative to Prevnar-immune aged mice and young mice that had received either vaccine. This was explained by >10-fold increases in PPS-specific immunoglobulin G (IgG) levels in Pneumovax-immunized aged mice relative to other groups. Remarkably, PPS3-specific B-cell expansion, IgG switching, plasmablast differentiation, and spleen and bone marrow antibody-secreting cell frequencies were 10-fold higher in aged mice following Pneumovax immunization relative to young mice, due to significantly increased B-1b cell participation. In summary, this study highlights (1) the need to devise strategies to enhance respiratory immunity in aged populations, (2) the diverse responses young and aged populations generate to Pneumovax vs Prevnar vaccines, and (3) the potential value of exploiting B-1b cell responses in aged individuals for increased vaccine efficacy.

Keywords. Pneumovax23; Prevnar-13; vaccines; B-1b cells; aging; Streptococcus pneumoniae; humoral immunity.
higher in elderly individuals vs young adults [10, 11], although the quality of Abs produced may differ in aged individuals [10, 12–14]. Studies suggest that conjugate vaccines may not yield titers that are superior to the native PPS vaccine in adults and do not boost as effectively as in young children [15–18]. This holds true for studies with elderly individuals [9, 15–17].

Alterations in the B-cell compartment are evident with advanced age [2, 19]. Nonetheless, the impact aging has on the frequency and functionality of distinct subsets, including Ag-specific B-cell responsiveness, is not clear. Understanding this, along with the respective contributions of follicular B cells and innate-like B cells (B-1 and MZB cells) to Ab responses against native PPS and PPS-protein conjugate vaccines, is of critical significance to devising age-appropriate vaccination strategies. In the current study, we investigated the effect extreme age has on protective Ab responses to currently used pneumococcal vaccines using a mouse immunization-challenge model. Our results reveal a remarkably enhanced capacity for PPS-specific B-1b cells in aged mice to expand, isotype switch, and produce Abs in response to the native, but not conjugate vaccine. Nonetheless, the impact aging has on the frequency and functionality of distinct subsets, including Ag-specific B-cell responsiveness, is not clear. Understanding this, along with the respective contributions of follicular B cells and innate-like B cells (B-1 and MZB cells) to Ab responses against native PPS and PPS-protein conjugate vaccines, is of critical significance to devising age-appropriate vaccination strategies. In the current study, we investigated the effect extreme age has on protective Ab responses to currently used pneumococcal vaccines using a mouse immunization-challenge model. Our results reveal a remarkably enhanced capacity for PPS-specific B-1b cells in aged mice to expand, isotype switch, and produce Abs in response to the native, but not conjugate vaccine. Overall, these results indicate that significant aging-induced alterations in B-1b cells and/or their responsiveness to PPS vaccine antigens may impact vaccine efficacy. Moreover, this study reveals that protein conjugation and/or the adjuvant used in conjugate vaccines may potentially limit the ability of B-1b cells and/or other B-cell subsets in aged individuals to optimally respond to PPS.

METHODS

Mice

Young adult (3–4 months) and aged (22–25 months) C57BL/6 male mice were obtained from Charles River Laboratories (through National Institute on Aging). Mice were housed under specific pathogen–free conditions, with the exception of infection experiments. All studies and procedures were approved by the Wake Forest School of Medicine Animal Care and Use Committee.

Immunizations and Infections

Mice were immunized intraperitoneally with 200 µL of diluted Pneumovax23 (Merck) containing 0.125 µg each of 23 PPS or Prevnar-13 (Wyeth Pharmaceuticals) containing approximately 0.125 µg each of 13 PPS. This mouse dose was determined using the Food and Drug Administration human dose equivalent surface area–based formula [20], whereby the mouse dose (mg/kg) = human dose (0.025 mg/70 kg) × (human K_m = 37)/ (mouse K_m = 3).

WU2 strain S. pneumoniae was given intraperitoneally as previously described [4, 21, 22]. For intranasal inoculations, mice were anaesthetized using isoflurane and held in the supine position while 40 µL of bacterial suspension was slowly delivered into the nose (approximately 20 µL/nare). Mice were monitored every 12 hours and humanely euthanized upon demonstrating signs of morbidity. Lung colony-forming units (CFU) were determined by plating whole lung homogenate on 5% TSA-II sheep blood agar plates coated with 4 µg/mL gentamicin.

Enzyme-Linked Immunosorbent and Enzyme-Linked Immunospot Assays

Serum was diluted in tris-buffered saline containing 1% bovine serum albumin (TBS-BSA) and incubated with 10 µg/mL cell wall polysaccharide (CWPS; Statens Serum Institut) to adsorb noncapsular polysaccharide Abs. PPS3-specific Ab levels were determined by adding diluted sera to Maxisorp plates coated with 5 µg/mL PPS3 (ATCC) and blocked with TBS-BSA. Wake Forest School of Medicine-conjugated polyclonal goat antimouse immunoglobulin (Ig) Abs (Southern Biotechnology Associates) and pNPP (Sigma) were used to detect Abs. Endpoint titer PPS3-specific enzyme-linked immunosorbent assays (ELISAs) were performed as previously described [4], with the exception that CWPS-Multi (containing CWPS1 and CWPS2; Statens Serum Institut) and 2 structurally unrelated PPSs, PPS1 and PPS4 (ATCC), were preincubated (at 10 µg/mL each) with diluted sera to provide additional nonspecific polysaccharide blocking. Enzyme-linked immunospot assays (ELISpots) were performed as previously described [23] using ELISpot plates (MAHAS4510; Millipore) coated with 10 µg/mL PPS3.

PPS3 Biotinylation and Flow Cytometry

Biotinylated PPS3 (PPS3bio) was generated by combining 2 mL of 5 mg/mL PPS3 with 25 µL 100 mg/mL 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride, each in 0.1 M MES buffer, pH = 5.0. EZ-link biotin-CL hydrazide (Pierce) was added (1.25 mM final) and mixed for 2 hours at room temperature, followed by phosphate-buffered saline (PBS) dialysis. Single cell suspensions from spleen and peritoneal lavage (2 × 10^7/mL) were preblocked in PBS containing 10 µg/mL CWPS and 0.5 µg/mL Fc block (eBioscience) for 20 minutes, and then stained with 25 µg/mL PPS3bio for 45 minutes at room temperature. Cells were washed with PBS containing 2% newborn calf serum and stained with streptavidin-FITC and fluorochrome-conjugated Abs: CD5(53–7.3), B220(RA3-6B2), CD11b(M1/70), CD21/35(E9), and CD1d(1B1) (Biolegend); CD19 (1D3, eBio-science); CD138(281–2, BD Biosciences); and IgG3 (Southern Biotechnology). Cells were analyzed using a BD FACSCantoII cytometer with FSC-A/FSC-H doublet exclusion. Two million events were collected for Ag-specific B-cell analysis and 20,000 events collected for B-cell subset analysis. Positive and negative cell populations were determined using unreactive isotype-matched Abs. Data was analyzed using FlowJo software (Treestar).

Statistical Analysis

Data are shown as means ± SEM with differences assessed using Student t test. Differences in survival curves were assessed using the log-rank test.
RESULTS

Aged C57BL/6 Mice Are More Susceptible to Intranasal Infection With Invasive S. pneumoniae

Young (2-month-old and 3- to 4-month-old) and aged (22-month-old) C57BL/6 mice were challenged intranasally with 1 × 10^6 CFU WU2, an invasive serotype 3 pneumococcal strain. Aged mice rapidly succumbed to infection, with 80% mortality by day 4 (Figure 1). Younger mice had significantly delayed and reduced mortality, reaching only 50% in 2-month-old mice and 20% in 3- to 4-month-old mice. Interestingly, aged mice did not exhibit increased susceptibility to intratracheal infection with EF3030 (data not shown), a strain that readily colonizes the nasopharynx but does not cause bacteremia in young mice [24].

Pneumovax23 and Prevnar-13 Do Not Increase Survival Against Pneumococcal Respiratory Infection in Aged Mice

We next determined whether current pneumococcal vaccines would provide protection against intranasal infection. Young and aged mice were immunized with an equivalent 0.125 μg dose of PPS3 contained in Pneumovax23 and Prevnar-13 vaccines. Mice were then challenged with a lethal intranasal dose (1 × 10^7 CFU WU2) that yields approximately 90% mortality in young adult naive C57BL/6 mice. Although immunization yielded significant survival in young mice (55%–75%), only 10%–20% of aged mice survived, regardless of whether Pneumovax or Prevnar was used (Figure 2A). CFU lung burden was also significantly higher in morbid immune aged mice relative to morbid immune young mice on day 8 (Figure 2B). Proinflammatory cytokine levels (interleukin 6, tumor necrosis factor α, and interleukin 17) were higher in lung homogenates of d8 morbid aged mice compared to morbid young mice (Figure 2C), perhaps due to higher bacterial loads. Notably, interleukin 10 (IL-10) levels (known to suppress pneumococcal clearance [25, 26]) were significantly higher in both naive aged mice and morbid aged mice relative to their younger counterparts (Figure 2C). Thus, immunization of aged mice with either PPS vaccine did not promote increased survival following a lethal S. pneumoniae respiratory challenge.

Pneumovax, but Not Prevnar, Limits Bacteremia Resulting From Respiratory Infection and Confers Significant Protection Against Systemic Challenge in Aged Mice

Bacteremia is commonly associated with invasive pneumococcal pneumonia. Eight days following intranasal challenge, the majority of young immune morbid mice were bacteremic, regardless of whether they had been immunized with Pneumovax or Prevnar (Figure 2D). Prevnar-immunized aged morbid mice were also bacteremic. However, Pneumovax-immune morbid aged mice exhibited significantly less bacteremia relative to all other groups (Figure 2D), despite high lung CFU counts and the lowest survival rates (Figure 2A and 2B).

Remarkably, aged mice immunized with Pneumovax were afforded the highest level of protection (approximately 60% survival) against a lethal systemic WU2 challenge delivered intraperitoneally (Figure 3). Survival in this group was significantly higher than in aged mice immunized with Prevnar and both groups of immunized young mice. Prevnar nonetheless elicited partial protection in young mice, in contrast to aged mice (Figure 3). Thus, although neither pneumococcal vaccine afforded aged mice with a significant survival advantage following respiratory challenge, Pneumovax specifically and selectively limited bacteremia stemming from WU2 respiratory challenge and provided optimal protection against systemic challenge in aged mice.

Aged Mice Generate Significantly Increased PPS-Specific Ab Levels to Pneumovax but Impaired Levels to Prevnar

Aged mice immunized with Prevnar generated similar to higher PPS3-reactive IgM and IgG responses 7 days postimmunization, but by day 28, levels in Prevnar-immune young mice were significantly higher (Figure 4A). Mean day 7 IgM titers were 2-fold higher in Prevnar-immunized aged mice relative to young mice, whereas day 28 IgG titers were approximately 40% lower in aged mice (Figure 4B). In contrast to young mice, which produced high levels of PPS3-specific IgG1 following Prevnar immunization (Figure 4C), aged mice produced low amounts of IgG1 and had moderately increased IgG3.

In contrast to results with Prevnar, aged animals immunized with Pneumovax produced significantly higher PPS3-specific IgM and IgG levels than aged mice immunized with Prevnar and young mice immunized with either vaccine (Figure 4A). IgM (day 7) titers for Pneumovax-immunized aged mice were 9-fold higher than Prevnar-immunized aged mice and 6-fold higher than young mice, and IgG (day 28) titers were 14-fold higher than Pneumovax-immunized young mice and

![Figure 1](https://academic.oup.com/jid/article-abstract/209/1/87/2193108/Downloaded_from_https://academic.oup.com/jid/article-abstract/209/1/87/2193108)
7.5-fold higher than Prevnar-immunized aged mice (Figure 4B). Importantly, increased PPS3-specific IgG levels in Pneumovax-immune aged mice were largely due to significantly increased IgG3 and IgG2b (Figure 4C). Pneumovax-specific ELISAs yielded similar results (Supplementary Figure 1A), suggesting that the effect was not restricted to PPS3. No age-specific differences were detected in the levels of IgM and IgG reactive against the CWPS vaccine contaminant (Supplementary Figure 1B).

Consistent with the above results, PPS3-specific IgM antibody secreting cells (ASCs) were increased 10- to 20-fold and IgG ASCs were increased 5- to 10-fold in spleens and bone marrow of aged mice relative to young mice (Figure 4D). Thus, aged mice were hyperresponsive to the native PPS vaccine but hyporesponsive to the protein-conjugated PPS vaccine, thereby explaining the differing efficacies these vaccines had against systemic infection and bacteremia in aged mice (Figures 2 and 3).

**B-1b Cells Are Increased in Aged C57BL/6 Mice**

B-1b cells play a key role in generating PPS3-specific IgM and IgG3 [4]. MZ and B-1a B cells also produce TI Ab responses [4–6, 23, 27–32]. We therefore assessed these subsets in aged mice. Total splenic IgM+B-cell frequencies and numbers did not differ between young and aged mice and therefore results were pooled. Asterisks (*) indicate significant differences between means indicated by horizontal bars. Tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) kits and interleukin 10 (IL-10) (JES5-2A5, biotinylated JES5–16E3) and interleukin 17 (IL-17) (TC11-18H10.1, biotinylated TC11-8H4) monoclonal antibodies and cytokines were used (Biologend). Cytokine concentrations were determined by interpolation of optical density values into the appropriate reference standard regression curve obtained with recombinant mouse cytokines and corrected for any dilution factor. D. Bacteremia in morbid mice 8 days postinfection. Blood (10 µL) was streaked on gentamicin-coated blood agar plates with α-hemolytic colony growth assessed the following day. Asterisks (*) indicate significant differences between means of young and aged mice, and pound signs (#) indicate differences in values obtained for age-matched mice immunized with Pneumovax vs Prevnar as determined by Fisher exact test (P < .05).
B cells are detected in young mice (Figure 5C). Interestingly, splenic CD11b+ B cells were significantly increased in aged mice, and this was largely due to an increased CD5−CD11b+CD21/35lo B-cell population, phenotypically representative of the B-1b subset (Figure 5D). Splenic CD5−CD11b+ B-cell numbers did not differ between young and aged animals (Figure 5D), although total splenic CD5− B-cell numbers were reduced in aged mice (data not shown). Finally, although peritoneal cavity B-1a, B-1b, and B-2 subset frequencies did not differ between age groups (Figure 5E), peritoneal B-2 and B-1b cell numbers were significantly increased in aged animals. Thus, B-1b cells were increased in aged mice.

PPS3-Specific B-1b Cell Responses Are Significantly Increased in Aged Mice

We next assessed PPS3-specific B-cell responses following Pneumovax immunization. PPS3-specific splenic and peritoneal B-cell frequencies and numbers were similar or lower in naïve aged mice relative to naïve young mice (Figure 6A and 6B). However, 5 days postimmunization, PPS3-specific splenic B-cell frequencies were increased 6- to 9-fold in aged mice in contrast to the approximately 1.5-fold increase in young mice (Figure 6A and 6B). Peritoneal PPS3-specific B-cell frequencies and numbers were also significantly increased (2- to 3-fold) in aged mice, unlike young mice (Figure 6B). Whereas 62% ± 2% of PPS3-specific B cells in aged mice were CD138⁺ (plasmablasts; 0.34% of splenocytes), only 16% ± 6% of PPS3-specific B cells expressed CD138 in young mice (0.03% of splenocytes; Figure 6C). Thus, PPS3-specific B-cell plasmablasts in aged mice were increased 10-fold over young mice. By day 5, total IgG3⁺ PPS3-specific B-cell frequencies and numbers in aged mice were already 6-fold higher than those found for young mice (Figure 6D). Thus, Pneumovax-induced PPS3-specific B-cell expansion, isotype switching, and plasmablast differentiation was significantly increased in aged mice.

Consistent with B-1b cell participation in the response to PPS3 [4] and other T1-2 Ags [6, 23], PPS-3-specific splenic B cells expressing CD11b and very low to undetectable levels of CD5 were present in both young and aged animals following immunization (Figure 6E). Notably, PPS-3-specific splenic CD11b⁺ (B-1b) cell frequencies and numbers were significantly higher in aged mice (>4-fold) relative to young mice (Figure 6E). Moreover, Pneumovax-immunized aged mice had significantly higher frequencies and numbers of PPS3-specific splenic B-1b cells that were plasmablasts (3- to 5-fold higher; Figure 6F) and IgG3⁺ (8-fold higher; Figure 6G). PPS3-specific IgG3⁺ B-1b cells coexpressed CD138 (Figure 6H), demonstrating the key role B-1b cells play in the increased levels of PPS3-specific serum IgG observed following Pneumovax immunization in aged mice.

DISCUSSION

Age-related alterations in the immune system are typically associated with decreased function and declining protection. In the current study, we report a remarkable increase in the quantity and quality of PPS3-specific B-1b cell responses to Pneumovax in extremely aged mice. Equally remarkable was the finding that the PPS-protein conjugate vaccine rendered aged mice unable to generate enhanced PPS-specific Ab responses. Pneumovax-induced Ab responses enhanced protection against systemic serotype 3 pneumococcal challenge, but did not attenuate age-related increases in mortality after intranasal challenge despite reducing bacteremia more effectively in old than young mice. Collectively, these novel findings reveal the differential responsiveness of aged PPS3-specific B-1b cells to native PPS vs PPS-conjugate vaccines, and suggest age-distinct mechanisms of pathogenesis and protection from pneumococcal respiratory infection. Our findings are particularly relevant for understanding aging-dependent changes in immune responses to carbohydrate vs protein-based vaccines.

Figure 3. Pneumovax immunization protects against systemic Streptococcus pneumoniae infection in aged mice. Young (4 months) and aged (22–24 months) mice were immunized intraperitoneally with Pneumovax and Prevnar vaccines. Twenty-eight days later, mice were challenged with a lethal systemic (intraperitoneal) dose (1.5 × 10⁵ colony-forming units) of WU2. Mice were monitored every 12 hours for morbidity. Percentage of survival in young (n = 6 mice/group) and aged (n = 7 mice/group) mice is shown. P values indicate significant differences in survival curves as determined by log-rank analysis.
Our data demonstrate that Ag-specific B-cell responses to carbohydrate Ags are significantly different between aged and young adult animals. Following Pneumovax immunization, PPS3-specific B-cell numbers were 5- to 10-fold higher in aged mice, and a much higher frequency of these B cells underwent IgG3 switching and ASC differentiation compared to young

Figure 4. Aged mice generate significantly increased pneumococcal polysaccharide (PPS)–specific antibody levels in response to Pneumovax but impaired levels in response to Prevnar. Young (4 months) and aged (22–24 months) mice were immunized intraperitoneally with Pneumovax or Prevnar vaccines. Enzyme-linked immunosorbent assays were performed to detect PPS3-specific mean (±SEM) immunoglobulin (Ig) M (1:800 serum dilution) and IgG (1:400 dilution) optical density (OD) levels for days 0, 7, 14, and 28 postimmunization (A) and IgM (day 7) and IgG (day 28) endpoint dilution titers (B). C, PPS3-specific IgG1, IgG2c, IgG2b, IgG3, and IgA levels in aged and young mice on day 0 and day 28 postimmunization. D, PPS3-specific IgM and IgG antibody secreting cell numbers in spleen and bone marrow 5 days post immunization. A–D, Asterisks (*) indicate significant (P<.05) differences between aged and young mice immunized with the same vaccine, and pound signs (#) indicate significant (P<.05) differences between age-matched mice immunized with Pneumovax vs Prevnar (n = 12–15 mice/group). Results are representative of 2 independent experiments.
mice. This is consistent with the 6- to 14-fold higher PPS-specific IgM and IgG titers and >10-fold higher ASC numbers in aged mice. Whether these outcomes are influenced by dose will be determined in future experiments. Importantly, B-1b cells contributed to PPS-specific B cell responses in both young and aged animals, consistent with our previous results showing that these cells selectively reconstitute PPS-specific IgM and IgG3 responses in immunodeficient mice [4]. Nonetheless, B-1b cells made much more substantial PPS-specific responses in aged mice. That PPS-specific B-1b cells in aged mice coexpressed IgG3 and CD138 at much higher frequencies indicates the key role they play in the increased PPS-specific IgG3 levels observed.

The reasons for significantly increased B-1b cell responses to native PPS in aged mice are not presently clear. Young B cell-deficient mice reconstituted with young or aged peritoneal B-1 cells or splenic B cells produce similar PPS-specific responses (Supplementary Figure 2), suggesting that B-cell extrinsic differences may be involved. The increase in total B-1b cells in aged mice may partly explain the increased responsiveness to PPS. However, additional factors likely contribute as naive PPS-specific B-cell numbers were similar in aged and young mice and the differences in PPS-specific B-cell expansion, isotype switching, and plasmablast differentiation following immunization between aged and young mice far exceeded the 2-fold increase in total B-1b cells. For example, alterations in the balance between the T-cell amplifier and suppressor populations described >40 years ago to regulate PPS-specific Ab responses may become altered with age [35]. Factors such as these that may regulate B-1b cell responses to TI-2 Ags and/or B-1b cell development are not well understood, although it is

Figure 5. B-1b cells are increased and marginal zone (MZ) B cells are decreased in aged mice. B-cell subsets were analyzed in young (4 months; white bars) and aged (24 months; black bars) C57BL/6 mice by flow cytometry. A–E, Mean (±SEM) total IgM⁺CD19⁺ B cell (A), IgM⁺CD21/35⁺CD1d⁺CD19⁺ (MZ) B cell (B), CD11b⁺CD19⁺ (B-1) (C), CD11b⁺CD19⁺CD65⁺ (B-1a) and CD11b⁺CD19⁺CD5⁺CD21/35⁻ⁿ/ᵐ (B-1b) (D) cell frequencies and numbers in spleen. E, Mean (±SEM) total peritoneal B-1a (CD19⁺CD11b⁺CD65⁺), B-1b (CD19⁺CD11b⁺CD5⁻), and B-2 (CD19⁺CD11b⁺CD5⁻) frequencies and numbers in young and aged mice. Asterisks (*) indicate significant (P<.05) differences between values for aged (black bars) and young mice (white bars).
Figure 6. B-1b cells contribute to hyperresponsive PPS3-specific B-cell responses in aged mice. A–H, Flow cytometric analysis and enumeration of PPS3-binding (antigen-specific) spleen cells from naive and immune (day 5; Pneumovax) young (white bars) and aged (black bars) mice. A, Representative flow cytometric analysis of PPS3-specific splenic CD19+ B cells in naive (left plots) and immune (right plots) mice. Frequencies of gated cells are indicated. B, Mean (±SEM) frequencies and numbers of splenic and peritoneal PPS3-specific CD19+ B cells in naive and immune mice. C, CD138 expression by PPS3-specific splenic CD19+ B cells in immune mice (thick solid line). Mean frequencies (±SEM) of CD138+ cells among PPS3-specific B cells are indicated in the histograms. Graphs indicate total mean (±SEM) frequencies and numbers of PPS3-specific splenic CD19+CD138+ B cells in young (white bars) and aged (filled bars) mice. D, IgG3 expression by PPS3-specific splenic CD19+ B cells in immune mice (thick solid line). Mean frequencies (±SEM) of IgG3+ cells among PPS3-specific B cells are indicated in the histograms. Young and aged PPS3-specific splenic CD19+ B cell IgG3 staining for naive mice is indicated by gray shaded histograms. Graphs indicate total mean (±SEM) frequencies and numbers of PPS3-specific IgG3+CD19+ B cells. E, CD11b and CD5 expression by PPS3-specific splenic CD19+ B cells in immune animals are indicated in the histogram. Graphs indicate total mean (±SEM) frequencies and numbers of PPS3-specific CD11b+ B cells. F–G, Mean (±SEM) frequencies and numbers of PPS3-specific CD19+CD11b+CD138+ (F) and CD19+CD11b+IgG3+ (G) B cells in young (white bars) and aged (filled bars) mice 5 days postimmunization. H, Representative CD138 expression on IgG3+CD11b+ PPS3-specific CD19+ cells. In C, E, and H, gray shading indicates monoclonal antibody isotype control staining for PPS3-specific B cells from day 5 immune mice. Asterisks (B–G) indicate significant differences between values for aged (black bars) and young (white bars) mice (*P < .05; n ≥ 4 mice/group).
clear that their regulation differs from that of B-2 cells [4, 21, 23, 32]. Future studies are required to determine the extent to which aging-associated alterations in B-1b cell development, repertoire specificity, and intrinsic and extrinsic regulation influence the magnitude of PPS-specific B-cell responses.

Remarkably, aged mice generated suboptimal responses to Prevnar-13. This indicates that the Ag-specific B cells that hyperrespond to native PPS in aged mice are rendered hyporesponsive to PPS that is either protein conjugated and/or alum associated. This may be due to altered Ag epitope accessibility/density upon protein conjugation, adjuvant suppression, and/or suppression by cells recruited into the T cell–dependent PPS-protein response. If B-2 cells are responsible for Ab production against PPS conjugates, this would also suggest that PPS-specific B-2 cell responses are inferior in aged mice, as aged mice produced significantly less PPS-specific Ab in response to Prevnar. Indeed, a previous study demonstrated that although 16- to 18-month-old and 2-month-old mice produced similar Ab responses to native PPS14, aged mice made significantly less Ab to a PsP3 protein-PPS14 conjugate when a CpG-based adjuvant was omitted [36]. This study demonstrated that T cell–associated aging defects play an important role in diminished humoral responses to PPS-protein conjugate vaccines, but that alternative adjuvants, as suggested by others [37], may overcome this. That native and conjugate PPS vaccines elicit differential B subset and T-cell involvement as well as altered idiotype/variable gene utilization [38, 39] may ultimately explain the dichotomous responses elicited to native or protein-conjugated pneumococcal vaccines by young vs aged mice.

Although further work is necessary to understand the implications our results have for elderly humans, several similarities between aged mice and humans are evident. First, as shown in our study and that from another group [40], aged C57BL/6 and BALB/c mice exhibit significantly increased susceptibility to respiratory challenge with invasive S. pneumoniae strains. This may be due to Ab-independent factors, including aging-associated Toll-like receptor desensitization and altered cytokine production [40, 41] (such as increased IL-10 [25, 26] as shown herein), decreased mucosal clearance, and increased pneumococcal adherence [42, 43]. Second, as we show for aged mice, in elderly humans Pneumovax demonstrates efficacy against systemic infection (bacteremia) but not pneumococcal pneumonia [10]. That Pneumovax-immune aged mice (producing 10-fold higher PPS3-specific IgG) were significantly more protected against systemic serotype 3 infection, but significantly less protected against respiratory infection, indicates that elucidating age-associated changes in mucosal defense may be key to reducing the burden and severity of pneumococcal pneumonia in aged individuals [41]. Third, multiple reports indicate that Prevnar does not elicit superior titers to Pneumovax in adults, including the elderly [9, 15–18]. Fourth, a recent study found that Pneumovax elicited increased IgG to some PPS in the elderly relative to young adults [11]. PPS-specific IgM levels and/or opsonophagocytic Ab capacity may nonetheless be decreased in the elderly [10, 12–14], and some studies do not show increased IgG [10]. The reasons for these differences and the extent to which mice model these findings are not yet clear. Importantly, young mice and humans are both deficient in Ab responses to polysaccharides. The similar ontogeny of polysaccharide-specific responsiveness may point to shared mechanisms of B-cell regulation and/or development at both extremes of the aging spectrum.

Despite the above similarities between mouse and human responses to PPS vaccines and pneumococcal infections, the extent to which our results may translate to humans is not yet clear. In contrast to laboratory mice, humans encounter multiple encapsulated pneumococcal strains by the time they reach an advanced age. Hence, there may be a degree of PPS refractivity in elderly individuals. This could explain why Pneumovax responses in the elderly are sometimes similar (instead of increased [11]) relative to younger adults [10]. Moreover, whether humans have a B-1b cell counterpart remains to be established, although our work in nonhuman primates strongly supports this possibility [6]. Finally, given the large variation in aging and immune response phenotypes among different mouse strains [44, 45], it is possible that our findings with aged C57BL/6 male mice may not translate to all strains/sex of aged mice, let alone all elderly humans, especially given differences between mouse and human aging [46]. Nonetheless, aged B6 mice could have a translatable counterpart within the human population. Further work is necessary to determine whether this is the case and whether pathways controlling B-1b cell (or likened subset) hyperresponsiveness to PPS can be identified and manipulated in elderly humans to improve vaccine efficacy.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. The authors thank Dr Christopher Jones, National Institute of Biological Standards and Control, Potters Bar, UK, for advice regarding biotin conjugation of PPS3.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant number R21AI095800) and the Wake Forest Translational Sciences Institute. Shared resources support was provided by a support grant from the National Cancer Institute Cancer Center (P30CA012197).

Potential conflicts of interest. All authors: No reported conflicts.
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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