Basophil Expansion Protects Against Invasive Pneumococcal Disease in Mice

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Background. Protein-based vaccination using pneumococcal proteins is a promising approach for efficient vaccines against Streptococcus pneumoniae. Basophils play an important role in enhancing memory immune responses to intact proteins. We examined the impact of increased basophil pool sizes on humoral memory responses to pneumococcal surface protein A (PspA).

Methods. Basophil pool sizes in blood, spleen, and bone marrow were increased by either interleukin 3 (IL-3) treatment or by adoptive basophil transfer before secondary PspA immunization. Subsequently, PspA-specific antibody titers and resistance of mice against invasive pneumococcal disease (IPD) was determined.

Results. Mice treated with IL-3, which increased basophil pool sizes, and mice receiving a single basophil transfusion responded with significantly higher PspA-specific antibody titers after immunization with PspA. Importantly, however, just a single transfusion of flow-sorted basophils into mice before secondary immunization with PspA significantly protected mice from lethal IPD. Moreover, concomitant blockade of inhibitory FcγRIIB on transfused basophils further substantially increased basophil-mediated protection against IPD in mice.

Conclusions. This is the first study to find that a single transfusion of basophils is sufficient to boost protein-based memory responses against pneumococcal protein antigens, thereby providing significant protection against IPD in mice.

Keywords. S. pneumoniae; pneumonia; vaccination; adaptive immunity; adjuvants; PspA; FcγRIIB; Fc-receptor.

Streptococcus pneumoniae is the most prevalent pathogen causing community-acquired pneumonia (CAP). CAP is known to frequently progress into invasive pneumococcal disease (IPD), which has a mortality rate of 10%–30% [1]. According to the World Health Organization, approximately 1.6 million people, particularly children <5 years old, die every year from pneumococcal pneumonia and IPD, mostly in developing countries [2, 3]. The worldwide spread of antibiotic-resistant clones of S. pneumoniae, together with the observation that, despite appropriate antibiotic therapy, mortality rates due to CAP remain high, particular interest has emerged for the development of broadly applicable and affordable preventive strategies. To date, 2 different pneumococcal vaccines have been approved: a 23-valent polysaccharide vaccine and a 13-valent conjugate vaccine [4, 5]. Although the use of conjugate vaccines has substantially contributed to reduced mortality rates among pediatric pneumococcal infections, clinical data demonstrate increased vaccine serotype replacement by nonvaccine serotypes, thus raising significant future problems for the use of conjugate vaccines [6]. In addition, since only a portion of the >90 different pneumococcal serotypes are covered by the currently available preventive strategies, there is urgent need for a vaccination approach that, optimally, providing protection against all pneumococcal serotypes. In particular, pneumococcal protein–based vaccines using pneumococcal antigens expressed by all known serotypes.

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14 • JID 2014:210 (1 July) • Bischof et al
of *S. pneumoniae* have emerged as reasonable alternatives for the currently available polysaccharide or conjugate vaccines. Pneumococcal surface protein A (PspA), which is expressed by virtually all clinical *S. pneumoniae* isolates, has been studied extensively for its protective properties against pneumococcal infections. PspA is immunogenic in mice, and PspA-specific antibodies have been shown to increase the deposition of complement on the bacterial cell surface, thus rendering them protective [7]. Although PspA is a heterologous protein, it shows cross-reactive and cross-protective properties [8, 9]. In fact, PspA immunization was protective against nasal *S. pneumoniae* carriage, focal pneumonia, and IPD in mice [10]. Additionally, in a phase 1 trial with recombinant PspA, the protein showed safety and immunogenicity in humans, and antiserum isolated from vaccinated individuals protected mice from pneumococcal infection [11]. Together, these findings make PspA a valuable candidate for future protein-based vaccination approaches.

Basophils are the least abundant granulocyte population, composing <1% of granulocytes in blood, spleen, and bone marrow. Their contribution to allergic reactions and helminth infections is mainly mediated by the high-affinity FcεRI receptor, which is activated when FceRI-bound immunoglobulin E (IgE) is cross-linked by antigen, resulting in the subsequent release of preformed proinflammatory mediators and interleukins from their granules [12, 13].

We have found previously that depletion of basophils before secondary PspA immunization led to significantly decreased PspA-specific antibody titers and diminished survival after lethal IPD in mice [14], thus supporting the view that basophils serve as a so-called cellular adjuvant in enhancing secondary memory immune responses to intact protein antigens. On the other hand, it has not been examined whether a transient expansion of basophil counts before secondary PspA immunization would further improve protective immunity against IPD in mice. Therefore, we determined whether experimentally increased basophil pool sizes induced by either interleukin 3 (IL-3)–complex pretreatment or by adoptive basophil transfusion would further improve PspA protein–dependent immunization efficacy in mice and protect mice from otherwise lethal IPD.

**METHODS**

**Mice**

Wild-type (WT) C57BL/6N (female, 8 weeks old) mice were purchased from Charles River. CD45.1-alloantigen expressing B6.SJL-Ptprca mice were purchased from Jackson. B6;129S4-Fcgr2b<sup>tm1(Tis)</sup>/J (FcyRIIB-KO) mice were kindly provided by Prof Gessner, Hannover School of Medicine. All animal experiments were approved by the local governmental authorities of the Hannover Medical School and the Lower Saxony State Office for Consumer Protection and Food Safety.

**Study Design**

Recombinant PspA production was generated as described previously [33]. On day 0, mice received a primary immunization with 10 µg PspA intraperitoneally in the absence of any adjuvant. Twenty-five days later, mice received a secondary immunization with 20 µg of PspA per mouse. Expansion of basophil pool sizes before secondary immunization was achieved by 2 different experimental approaches. In one approach, mice were treated by intraperitoneal injection of recombinant murine IL-3 or IL-3 complexed to anti-IL-3 antibodies (IL-3C), as described in the Supplementary Materials. Control mice received vehicle (phosphate-buffered saline [PBS]/0.1% human serum albumin). In the other approach, mice received intravenous transfusions of PspA-reactive basophils or FcγRIIB-blocked, PspA-reactive basophils collected from bone marrow and spleens of IL-3C–pretreated mice, as described in Supplementary methods. At indicated time points after secondary immunization PspA-specific antibody titers, cytokine levels and plasma cell numbers were determined as described in the Supplementary Materials. For infection experiments, mice were challenged orotracheally with serotype 3 *S. pneumoniae* (A66.1), which was prepared essentially as described previously [14]. Unless otherwise stated, 3×10<sup>6</sup>–6×10<sup>6</sup> colony-forming units (CFUs) of A66.1 were slowly instilled in a total volume of 50 µL of PBS, as described previously [34].

In selected experiments, mice were passively immunized by intraperitoneal injection of pooled antiserum collected from previously PspA-immunized mice. Since PspA-immunized mice were already protected to a high degree from IPD, we passively immunized mice by application of just 10 µL of pooled antiserum to allow valid monitoring of differences in survival rates between groups. Two hours after passive immunization, mice were challenged with *S. pneumoniae* at 3×10<sup>6</sup>–4×10<sup>6</sup> CFUs/mouse. After *S. pneumoniae* infection, mice were monitored daily for developing bacteremia and survival. Information about reagents, flow cytometric analysis, staining of cells, determination of CFUs, and in vitro stimulation of cells is given in the Supplementary Materials.

**Statistical Analysis**

The data are expressed as mean ± SD. Significant differences were calculated using SPSS software (IBM). For the comparison of ≥2 groups, the Kruskal-Wallis test, together with the Mann–Whitney *U* test, was used. Survival analysis was compared using the log-rank test. Significant differences between 2 groups were assumed with *P* values of <.05.

**RESULTS**

**Effects of IL-3 and IL-3C Treatment on Basophil Counts in Blood, Spleen, and Bone Marrow of Mice**

In initial experiments, we analyzed the effect of IL-3 on basophil counts in blood, spleen, and bone marrow of mice treated with
IL-3 for 3, 5, or 7 consecutive days. Basophils were gated according to their forward- and side-scatter characteristics (P1, Supplementary Figure 1), followed by subgating on CD117<sup>neg</sup> cells to exclude mast cells (P2, Supplementary Figure 1). Subsequently, basophils were identified as a distinct, CD49b<sup>pos</sup>, FcεRI<sup>pos</sup> population (P3, Supplementary Figure 1). Under baseline conditions, basophils represented about 0.4%, 0.1%, and 0.6% of total leukocytes in blood, spleen, and bone marrow, respectively. Treatment with IL-3 for 3 days significantly increased basophil counts in spleen and bone marrow but not in blood (Figure 1A–C), with a further significant increase observed in all organ systems observed on days 5–7 after treatment. Based on this observation, when treating mice with IL-3 complexed with anti-IL-3 antibodies (IL-3C) for 5 days, we observed dramatically increased basophil counts in blood, spleen, and bone marrow, compared with the vehicle or IL-3 treatment regimen (Figure 1D). Fluorescence-activated cell sorting analysis revealed approximately 100 times higher basophil counts in blood and spleen and approximately 30 times higher basophil numbers in bone marrow of IL-3C treated mice, compared with baseline conditions (Figure 1E–G). Consistent with a previous report [15], slightly and nonsignificantly increased numbers of mast cells were noted in peritoneal lavages and bone marrow of mice after 5 days of IL-3C treatment (data not shown).

Effect of IL-3– or IL-3C–Elicited Increased Basophil Counts on PspA-Specific Antibody Titters

We next examined whether expanded basophil counts would result in enhanced adaptive immune responses to PspA. Therefore, mice were subjected to primary PspA immunization on day 0, followed by secondary immunization with PspA on day

![Figure 1](image_url)

**Figure 1.** Determination of basophil numbers in blood, spleen, and bone marrow of mice treated with interleukin 3 (IL-3) or IL-3 complexed to anti-IL-3 antibodies (IL-3C). A–C, Mice were treated for 3, 5, or 7 consecutive days with IL-3 (200 ng intraperitoneally/mouse 2 times daily). Control mice received vehicle (phosphate-buffered saline [PBS]/0.1% human serum albumin) for the respective periods. Subsequently, basophil pool sizes in blood (A), spleen (B), and bone marrow (C) were quantified using flow cytometric analysis. D–G, Comparative analysis of basophil numbers in mice after 5 days of IL-3 or IL-3C treatment. D, Representative dot plots of flow cytometric basophil analysis. Subgating according to CD49b and FcεRI cell surface expression is depicted for vehicle (PBS), IL-3, and IL-3C-treated mice. Total basophil numbers are shown for blood (E), spleen (F), and bone marrow (G). The data in A–C and E–G are shown as mean ± SD. Data in panels A–C are from 3–7 mice and are representative of 2 independent experiments. The experiment shown in panels E–G was performed 3 times, and data are for 9–12 mice per treatment group. *P < .05, **P < .01, and ***P < .001 denote significant increases, compared with PBS-treated mice. **P < .01 and ***P < .001 denote significant increases, compared with 3 days (B) or 5 days (E–G) of IL-3 treatment.
25. Basophil expansion was achieved in mice by IL-3 or IL-3C administration from days 19 to 23 after primary PspA immunization. Subsequently, mice were allowed to rest for 2 days before secondary immunization with PspA, as outlined in Figure 2A. As shown in Figure 2B–D, IL-3C treated mice responded with substantially elevated PspA-specific immunoglobulin G1 (IgG1) and IgG2a antibody titers in plasma on days 5 and 10 after restimulation with PspA, whereas IL-3–treated mice responded with significantly enhanced immunoglobulin M and nonsignificantly elevated PspA-specific IgG1 and IgG2a antibody titers to secondary PspA immunization. Without PspA immunization, all mice died after pneumococcal challenge within 72 hours. However, IL-3C treatment, although providing elevated anti-PspA antibody titers, failed to protect PspA-immunized mice against IPD (Figure 2E).

Effect of Passive Immunization With PspA-Specific Antiserum on Bacteremia and Survival of Mice Challenged With S. pneumoniae

In the next set of experiments, we aimed to determine whether passive immunization with antiserum from IL-3C–treated mice would protect naive mice against bacteremia and improve their survival after lethal challenge with invasive S. pneumoniae. As shown in Figure 3A and 3B, infection of naive mice with pneumococci resulted in 100% bacteremia by day 2 after infection and 100% mortality by day 3 after infection. In contrast, naive mice passively immunized with antiserum from PBS-treated, PspA-immunized mice demonstrated a nonsignificantly delayed bacteremia induction and mortality during an observation period of 10 days (Figure 3A). Importantly, however, passive immunization of naive mice with antiserum from

![Figure 2](image-url)
IL-3C-treated, PspA-immunized mice completely protected mice from lethal pneumococcal challenge during the first 4 days of infection (Figure 3A and 3B). In this experimental group, we observed a significantly decreased bacteremia induction of 50% and concomitantly improved survival of 40% during the 10-day observation period, compared with both control groups. Together, passive immunization of naive mice with antiserum from IL-3C–treated mice exerted significant protection against challenge with a lethal dose of *S. pneumoniae*.

**PspA-Reactive Flow-Sorted Basophils Respond With T-Helper Type 2 (Th2) Cytokine Release to Stimulation With PspA-Specific Antibody Complexes**

Based on the finding that IL-3C treatment of PspA-immunized mice, although providing improved protection against *S. pneumoniae* in passive immunization experiments, was ineffective in a model of IPD, we next examined whether adoptive basophil transfer would confer improved protection in PspA-immunized mice against invasive *S. pneumoniae*. To prevent their activation, basophils were purified from spleens and bone marrow of IL-3C–treated mice by high-speed flow sorting. Gating of basophils was done according to their forward- and side-scatter characteristics (P1, Supplementary Figure 2), followed by hierarchical subgating of the CD49b\textsuperscript{high}, CD11b\textsuperscript{low} (P2), and CD49b\textsuperscript{high}, CD11b\textsuperscript{high} population (P3) [16]. Flow-sorted cells (purity, >96%) stained positive with anti-IgE (Figure 4A) and anti-Fc\varepsilonRI (data not shown) and were found to exhibit a typical basophil morphology [17] on Pappenheim-stained cytocentrifuge preparations (Figure 4B). Such flow-sorted basophils incubated with anti-PspA antiserum to generate PspA-reactive basophils strongly stained positive with PspA–fluorescein isothiocyanate (FITC), confirming that sorted cells represented basophils.

We next questioned whether flow-sorted basophils would respond to PspA antigen-antibody complex stimulation with Th2 cytokine release in vitro. As shown in Figure 4D and 4E, PspA-reactive basophils incubated for 24 hours with purified PspA

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**Figure 3.** Survival and bacteremia of mice passively immunized with antiserum collected from mice treated with interleukin 3 (IL-3) complexed to anti-IL-3 antibodies (IL-3C) or phosphate-buffered saline. Mice were passively immunized either with pneumococcal surface protein A (PspA)–specific antiserum from IL-3C–treated mice (dotted line) or with PspA–specific antiserum from PBS-treated mice (dashed line). Control mice received no passive immunization (black line). Subsequently, all mice were orotracheally infected with *Streptococcus pneumoniae*, and survival (A) and bacteremia (B) was monitored for 13 days. Survival and bacteremia analysis are presented as the percentage of mice surviving or developing bacteremia (n = 6–9 mice per treatment group). *P* < .01 indicates significant differences between mice that received antiserum from IL-3C–treated mice, compared with mice that received antiserum from PBS-treated mice.
demonstrated a significantly increased release of interleukin 4 (IL-4) and interleukin 6 (IL-6) into the cell culture supernatant, whereas flow-sorted basophils treated with PspA-specific antibody (α-PspA), and binding of PspA-specific antibodies to the basophil cell surface was further validated by staining of sorted cells with fluorescein isothiocyanate-conjugated PspA. D and E. Sorted basophils were left untreated or were incubated with PspA, α-PspA, or PspA together with α-PspA in vitro. After 24 hours, the cell-free supernatant was collected, and interleukin 4 (IL-4; D) and interleukin 6 (IL-6; E) levels were determined. As a positive control, basophils were incubated with α-FceRI antibody MAR-1. F, Basophils from CD45.1pos donor mice treated with interleukin 3 (IL-3) complexed to anti-IL-3 antibodies (IL-3C) were flow sorted and adoptively transferred via tail vein injection into CD45.2pos recipient mice. Twenty-four hours after transfusion, blood, spleen, and bone marrow basophils of recipient mice were analyzed for CD45.1pos basophils. Representative dot plots from recipient mice are shown. Gating according to FcεRI, CD49b, and CD117 was done as described in Methods. Positive staining of the gated cells with CD45.1 antibodies verified donor-type basophils. Cytokine levels in panels D and E are presented as mean ± SD of n = 5–6 determinations. Asterisks denote statistically significant differences from all other groups. Abbreviation: FITC, fluorescein isothiocyanate.

Impact of Adoptive Transfer of PspA-Reactive Basophils on Secondary Adaptive Immune Responses to PspA

We next questioned whether increased basophil pool sizes achieved by adoptive transfer of PspA-reactive basophils would improve humoral immune responses and survival in recipient mice after challenge with invasive S. pneumoniae. Therefore, flow-sorted PspA-reactive basophils were transfused into mice immediately before PspA restimulation (Figure 5A). Importantly, transfusion of 3 × 10⁷ basophils significantly increased IgG1 antibody titers in plasma of recipient mice.
compared with control mice, 7 and 10 days after secondary PspA immunization, which was further accentuated by transfusion of $1 \times 10^6$ PspA-reactive basophils (Figure 5B). In contrast, no differences in IgG2a antibody titers were observed between experimental groups (data not shown).

Since splenic basophils are known to be a major source of IL-6 after rechallenge with antigen [14], we next analyzed whether adoptive transfer of PspA-reactive basophils would also trigger increased cytokine responses in immunized mice. Indeed, cell-free supernatants from spleen homogenates of basophil-transfused, PspA-immunized mice collected 12 hours after restimulation demonstrated significantly higher IL-6 concentrations than non-basophil-transfused, PspA-immunized mice (Figure 5C). Moreover, mice receiving transfusions of $3 \times 10^5$ or $1 \times 10^6$ basophils exhibited about 2–3-fold greater number of PspA-specific plasma cells than non-basophil-transfused, PspA-immunized control mice, as judged by intracellular staining of bone marrow cells with PspA-FITC (Figure 5D). Additionally, we found that bone marrow basophils analyzed on day 14 after restimulation were still able to bind PspA-FITC (Figure 5E). Collectively, these data demonstrate that adoptive basophil transfer results in substantially increased secondary humoral memory responses to intact pneumococcal protein antigen in mice.

**Effect of Basophil Transfusion on Survival of Mice Challenged With Invasive *S. pneumoniae***

Having shown that adoptive transfer of PspA-reactive basophils triggered increased humoral immune responses against PspA in mice, we next examined the efficacy of basophil transfusion in protecting mice against invasive pneumococcal infections. Groups of mice that did or did not receive PspA-reactive basophil transfusion before secondary PspA immunization were infected 10 days after restimulation with PspA with invasive *S. pneumoniae*. As shown in Figure 6A, PspA-immunized mice with normal basophil counts showed a survival of approximately 40%–50% during an observation period of 12 days, whereas mice transfused with PspA-reactive basophils demonstrated a significantly improved survival of 80% after challenge with pneumococci (Figure 6A). Importantly, just 12% of basophil-transfused mice developed bacteremia during the observation period, compared with bacteremia induction in approximately 40% of PspA-only immunized mice exhibiting normal basophil counts (Figure 6B). Basophil-transfused mice also demonstrated significantly lower bacterial CFUs in their bronchoalveolar lavage fluids relative to non-basophil-transfused, PspA-immunized mice (Figure 6C). To verify that the observed improved survival was indeed basophil dependent, in control
transfusion experiments, identically prepared flow-sorted eosinophils were transfused into PspA-immunized mice 1 hour before secondary PspA immunization as shown in Figure 5A. Control mice were immunized with PspA but did not receive an adoptive cell transfer. All mice were intratracheally infected with $4 \times 10^5$–$6 \times 10^5$ colony-forming units (CFUs) of S. pneumoniae on day 10 after restimulation (day 35), and survival (A and D) and bacteremia (B and E) were monitored during an observation period of 14 days. CFUs in bronchoalveolar lavage (BAL) were determined on days 1 and 2 after infection (C). Bacterial CFUs are presented as mean ± SD for 12–13 mice per time point and treatment group from 3 independently performed experiments, whereas survival and bacteremia in experiments in panels A and B was monitored in 16 mice per treatment group in 2 independently performed experiments. Survival and bacteremia in panels D and E represent 9 mice per treatment group. The experiment was repeated 2 times with similar results. *$P<.05$, compared with the control group.

Figure 6. Effect of adoptive basophil transfer as opposed to eosinophil transfer on survival, bacteremia, and bacterial loads in mice challenged with Streptococcus pneumoniae. A total of $2 \times 10^5$–$3 \times 10^5$ flow-sorted, pneumococcal surface protein A (PspA)–reactive basophils or equally treated eosinophils were transfused into PspA-immunized mice 1 hour before secondary PspA immunization as shown in Figure 5A. Control mice were immunized with PspA but did not receive an adoptive cell transfer. All mice were intratracheally infected with $4 \times 10^5$–$6 \times 10^5$ colony-forming units (CFUs) of S. pneumoniae on day 10 after restimulation (day 35), and survival (A and D) and bacteremia (B and E) were monitored during an observation period of 14 days. CFUs in bronchoalveolar lavage (BAL) were determined on days 1 and 2 after infection (C). Bacterial CFUs are presented as mean ± SD for 12–13 mice per time point and treatment group from 3 independently performed experiments, whereas survival and bacteremia in experiments in panels A and B was monitored in 16 mice per treatment group in 2 independently performed experiments. Survival and bacteremia in panels D and E represent 9 mice per treatment group. The experiment was repeated 2 times with similar results. *$P<.05$, compared with the control group.

Effect of Basophil-Directed FcγRIIB Blockade on Basophil-Mediated PspA-Specific Antibody Titers and Survival of Mice After Passive Immunization and S. pneumoniae Infection

Recent reports by Cassard et al demonstrated that both mouse and human basophils exhibit inhibitory FcγRIIB expression on their cell surface [18], the engagement of which resulted in attenuated IgG- and IgE-induced activating responses by basophils. Similarly, FcγRIIB-KO mice were shown to exhibit higher immunoglobulin levels after immunization [19]. Therefore, we examined what effect FcγRIIB deficiency or blockade would have on PspA immunization efficacy. FcγRIIB-KO mice responded to secondary PspA immunization with elevated PspA-specific IgG1 and IgG2a antibody titers, compared with WT mice (Figure 7A and 7B). On the basis of these findings, we next examined whether blockade of FcγRIIB on basophils before their transfusion into recipient mice would additionally enhance basophil-mediated secondary immune responses. Indeed, anti-FcγRIIB-F(ab’)2-pretreated basophils incubated with anti-PspA antiserum responded with significantly increased IL-4 and IL-6 release in vitro, compared with normal PspA-reactive basophils (Figure 7C and 7D). Along this line, adoptive transfer of anti-FcγRIIB-F(ab’)2-pretreated basophils into PspA-immunized mice significantly increased PspA-specific IgG1 and IgG2a antibody titers in recipient mice, compared with mice receiving transfusions with PspA-reactive basophils only (ie, without previous anti-FcγRIIB-F(ab’)2 incubation; Figure 7E and 7F). Importantly, passive immunization of naive mice with antiserum from PspA-immunized mice that received a single transfusion of FcγRIIB-blocked, PspA-reactive basophils demonstrated a significantly reduced bacteremia and, thereby, improved survival after challenge with invasive pneumococci, relative to mice receiving a
single transfusion of PspA-reactive basophils without previous FcγRIIB blockade (Figure 7E and 7F). Collectively, these data show that blockade of FcγRIIB on the cell surface of basophils further accentuated their role as enhancers of humoral immune responses against PspA, thereby additionally improving antibacterial resistance of mice against IPD.

**DISCUSSION**

Previous reports from our group and others have unraveled a role for basophils to serve as so-called cellular adjuvants during secondary humoral immune responses against intact protein antigens [14]. Using 2 different experimental approaches, we showed that expanded pool sizes of antigen-specific basophils substantially enhanced secondary humoral memory responses to a virulence-associated pneumococcal protein. In particular, adoptive basophil transfer but not growth factor–dependent expansion of basophil counts was found to rescue mice from lethal IPD after challenge with highly virulent *S. pneumoniae*. These protective effects were additionally increased by blockade of inhibitory FcγRIIB on basophils before their transfusion into recipient mice. The current study thus establishes a novel perspective to enhance future protein-based vaccination efficacies for the prevention of IPD in humans. We previously showed that depletion of basophils before PspA restimulation led to significantly decreased antigen-
specific antibody titers and that adoptive transfer of antigen-reactive basophils into naive mice was found to enhance memory responses to the infection-unrelated allophycocyanin protein used in that study [14]. However, that report did not address the functional consequence that experimentally expanded basophil pool sizes would have on outcomes in lethal bacterial lung infection models in mice. The current study shows that IL-3– or IL-3C–elicited elevated basophil counts triggered significantly higher PspA-specific antibody titers in mice immunized with pneumococcal virulence-associated PspA. These data are the first to show that increased numbers of basophils achieved through growth factor treatment of mice substantially increased memory responses to pneumococcal proteins. Additionally, mice passively immunized with antisera from PspA-immunized, IL-3C–treated mice accordingly demonstrated substantially improved survival, compared with mice receiving antisera from PspA-immunized, vehicle-treated mice. Surprisingly, IL-3C–treated, PspA-immunized mice were not protected from lethal pneumococcal challenge, which we believe is most likely due to the prolonged IL-3 treatment regimen, which to some extent also increased numbers of other granulocyte subsets (data not shown) known to respond with activation and enhanced degranulation to IL-3 treatment [20, 21].

As a second approach to increase basophil counts in mice, we adoptively transferred PspA-reactive basophils before secondary PspA immunization. Importantly, PspA-reactive basophils not only released substantial amounts of IL-4 and IL-6 in vitro, mostly likely in an Fc-receptor–dependent manner [14], but their transfer also elicited significantly higher IL-6 levels in spleens of recipient mice, along with significantly increased PspA-specific IgG1 titers. These data show that PspA is able to trigger increased Th2 cytokine (ie, IL-4 and IL-6) release by antigen-reactive basophils and that increased numbers of antigen-specific basophils further increase such cytokine responses, which are essential for B-cell–dependent antibody responses [14], which we believe is the primary role of increased IL-4 and IL-6 release in the current model system. The role of Th1 versus Th2 responses in protective immunity against S. pneumoniae is controversial. IgG2a, which is mainly associated with Th1 responses, is considered to have the highest capacity to induce complement deposition on the pneumococcal surface, suggesting that Th1-biased immune responses are more effective against pneumococci [22–25]. On the contrary, IgG1 antibodies mediated by Th2 cells and IL-4 cytokine responses were shown to be as protective as IgG2a antibodies in various PspA immunization models [26, 27]. Our report demonstrates that Th2-biased responses mediated through IgG1 were sufficient to protect mice from IPD, as judged by significantly reduced bacteremia and mortality after pneumococcal challenge.

These data support the view that basophils respond to intact protein antigen by providing an early source of Th2 cytokines to facilitate accentuated Th2 cell responses with subsequently increased plasma cell numbers and enhanced antibody production in mice. However, we did not address whether basophils may serve as the principal antigen-presenting cell to initiate a Th2 response [17, 28–31]. A recent study using a house dust mite (HDM) allergy model favored a mechanism by which dendritic cells were the principal antigen-presenting cell to take up HDM antigen, whereas basophils amplified Th2 immunity to HDM [28]. Additionally, a more recent report demonstrated that basophils were capable of taking up haptons or peptides but not proteins for induction of Th2 responses, for which the presence of dendritic cells was needed [32]. Addressing this aspect of basophil-dependent immunity against IPD awaits future investigation.

A novel aspect of the current report is that a single transfer of FcγRIIB-blocked PspA-reactive basophils additionally increased PspA-specific antibody titers and further improved their protection against IPD. These data illustrate that not only numbers of basophils, but also their specific Fc-receptor–dependent activator/inhibitor balance shape the overall response to intact pneumococcal vaccination proteins and therefore should be considered as additional means by which to enhance future basophil-dependent pneumococcal vaccination strategies in humans.

An important aspect of future basophil-dependent pneumococcal vaccination approaches in humans will be the sufficient availability of nonactivated autologous basophils for transfusion purposes. Principally, autologous basophils could be generated starting with a patient’s bone marrow biopsy in vitro, again making use of IL-3 as a growth factor to expand basophils from bone marrow progenitors in vitro. However, this aspect of basophil biology awaits future investigation.

Together, the data of the current study show that adoptive transfer of PspA-reactive basophils is a powerful tool to increase Th2-dominated PspA-specific IgG1 antibody responses in mice, thereby substantially improving the host defense capacity of mice to withstand a lethal challenge with a bacteremia-inducing strain of S. pneumoniae.

**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 copyrighted. 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**

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