Genetic background impacts vaccine-induced reduction of pneumococcal colonization

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A B S T R A C T

Vaccination has been one of the most successful strategies to reduce morbidity and mortality caused by respiratory infections. Recent evidence suggests that differences in the host genetic background and environmental factors may contribute to heterogeneity in the immune response to vaccination. During preclinical testing, vaccines are often evaluated in a single mouse inbred strain, which may not translate well to the heterogeneous human population. Here, we examined the influence of host genetic background on vaccine-induced protection against pneumococcal colonization in two commonly used inbred mouse strains, i.e. C57BL/6 and BALB/c as well as the F1 cross of these two strains. Groups of mice were vaccinated intranasally with a vaccine formulation containing a model pneumococcal antigen, i.e. pneumococcal surface protein A (PspA), adjuvanted with cholera toxin subunit B (CTB). Even in the absence of vaccination, differences in colonization density were observed between mouse strains. Although vaccination significantly reduced pneumococcal density in all mouse strains, differences were observed in the magnitude of protection. We therefore examined immunological parameters known to be involved in vaccine-induced mucosal clearance of S. pneumoniae. We found that PspA-specific IgG levels in nasal tissue differed between mouse strains, but in all cases it correlated significantly with a reduction in colonization. Furthermore, increased mucosal IL17A, but not IFNγ, IL10, or IL4, was found to be mouse strain specific. This suggests that the reduction of bacterial load may be accompanied by a Th17 response in all genetic backgrounds, although the cytokine dynamics may differ. Increased insight into the different immune mechanisms that affect pneumococcal carriage will contribute to development of future vaccines against S. pneumoniae.

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1. Introduction

The human immune system is incredibly diverse and is capable of responding to many pathogens and environmental antigens [1,2]. From both animal studies as well as human studies it is known that the genetic background is an important determinant of pathogen susceptibility [3–9]. Host genetic components are also known to affect vaccine-induced responses [10–12]. While vaccines are designed to protect all individuals within the vaccinated population, differences in individual genetic makeup affecting the establishment of vaccine-induced protection could potentially lead to inadequate protection.

In this study, we focus on Streptococcus pneumoniae, a human-specific pathogen ranking among the top 10 of infection-related mortality and causing severe disease, including pneumonia and sepsis [13–15]. Since colonization is a prerequisite for both transmission and invasive disease, the impact of vaccination on pneumococcal carriage, likely via a Th2 mechanism, has led to a significant reduction of invasive pneumococcal disease [16]. Preclinical evaluation of vaccines is typically performed in inbred mouse strains, such as C57BL/6 and BALB/c. Considering that these vaccines are intended to work in a more heterogeneous setting, this may complicate successful translation of findings in the mouse model. C57BL/6 and BALB/c mice are known to induce Th1 and Th2-prone responses, respectively. Conversely, whole cell vaccine-induced reduction of pneumococcal colonization in experimental studies was previously shown to be dependent on a Th17 response [17,18].

In this study, we investigated the effect of mouse strain genetic background on the efficacy of mucosal vaccination against S. pneumoniae, using the model antigen pneumococcal surface protein A

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(PspA). Protection outcomes were then correlated to differences in cellular and humoral immune markers measured after bacterial challenge.

2. Materials and methods

2.1. Mouse vaccination and infection studies

Seven week-old female C57BL/6j (inbred), BALB/c (inbred), and CB6F1 (outcross) mice, a cross between BALB/c × C57BL/6j (Charles River Laboratories), received three intranasal (i.n.) immunizations with 10 μg purified recombinant PspA from TIGR4 (kindly provided by Mucosys B.V., Groningen, The Netherlands) with 4 μg CTB (Sigma) or 4 μg CTB alone (control) in a volume of 10 μL at two-week intervals, under anesthetics (2.5% v/v isoflurane, AU Veterinary Services) [19]. Three weeks after the final immunization mice were infected i.n. with 10⁶ CFU of *S. pneumoniae* TIGR4 [20]. Mice were euthanized five days after challenge, after which both nasal lavage was obtained and mucosal nasal tissue was harvested. Nasal lavage was performed using 1 mL PBS and serially diluted onto Columbia Agar with Gentamicin (Mediaproducts BV) to determine bacterial load (log CFU/organ). The lower limit of detection is 1.19 log CFU. Nasal tissue was homogenized in 1 mL PBS using an IKA T10 basic blender and stored for subsequent analyses. All animal experimentation was performed in accordance with and approved by the Radboud University Medical Center Committee for Animal Ethics (DEC2013-266).

2.2. Detection of nasal and serum antibody responses

Maxisorp high binding affinity plates (Nunc) were coated with 2 μg/mL of purified PspA in carbonate coating buffer (0.1 M carbonate/bicarbonate pH 9.6) at 4°C overnight [20]. The following day, wells were washed with PBS containing 0.05% Tween-20 (PBST; Merck), blocked with 1% BSA (Sigma) and incubated with individual mouse nasal and serum samples for 1 h at 37°C. Wells were washed with PBST and incubated with anti-mouse IgG-alkaline phosphatase (Sigma) for 1 h at 37°C. After washing, samples were developed using 1 mg/mL p-nitrophenylphosphate in substrate buffer, 1 M diethanolamine, 0.5 mM MgCl₂ pH 9.8, (Calbiochem, VWR) and optical density was measured at 405 nm (Calbiochem, VWR) and optical density was measured at 405 nm. Bacterial colonization was determined in the nasal cavity.

To determine the influence of the genetic background on pneumococcal colonization, we used three different mouse strains: C57BL/6 (inbred), BALB/c (inbred) and CB6F1 (outcross, BALB/c × C57BL/6). All groups received three intranasal immunizations with CTB only (mock) or PspA plus CTB five days after infection, bacterial colonization was determined in the nasal cavity.

We found clear mouse strain-dependent differences in pneumococcal density even in the mock-treated animals (Fig. 1). At day 5 post-infection, BALB/c mice showed significantly higher CFU titers than C57BL/6 or CB6F1 mice. Additionally, the variation in CFU counts was relatively small for BALB/c mice (Log CFU 1.56), while more variation was found in both C57BL/6 and CB6F1 mice (Log CFU 2.43 and 4.00, respectively).

3. Results

3.1. Pneumococcal colonization densities differ between genetically distinct mouse strains even in the absence of vaccine-induced immunity

We subsequently determined whether there was an association between genetic background and the reduction in *S. pneumoniae* colonization following vaccination with PspA. In all PspA-vaccinated mice, pneumococcal load was significantly reduced compared to mock-vaccinated mice at five days after infection (Fig. 1A), suggesting that the vaccines induce protection in all mouse strains. The largest fold reduction in pneumococcal load was observed in CB6F1 mice, which significantly differed from BALB/c (Fig. 1B). The degree of variation in colonization densities differed per mouse strain. At day 5 post-infection, BALB/c mice showed overall low variation in CFU counts (Log CFU 2.19) and thus an increased significant difference between CTB- and PspA-vaccinated animals as compared to C57BL/6 mice (Log CFU 4.60) (Fig. 1A). Similar in CB6F1 mice (Log CFU 2.79) in comparison to C57BL/6 mice, treatment groups showed a higher significant difference, primarily because almost all PspA-vaccinated animals had no detectable pneumococcal colonization (Fig. 1A). The results suggest that a higher level of colonization shows lower intra-group variation in PspA-vaccinated animals. Together this illustrates that PspA vaccine-induced reduction in pneumococcal colonization can be achieved in distinct genetic backgrounds, but vaccine efficacy varies per mouse genetic background.
Fig. 1. Baseline pneumococcal colonization and vaccine-induced protection against pneumococcal colonization in C57BL/6, BALB/c, and CB6F1 mice. Mice were 3× i.n. immunized with CTB only (control mice) and challenged i.n. at three weeks post-immunization with 10^5 S. pneumoniae. (A) Bacterial recovery at baseline and following vaccination and (B) fold reduction in bacterial load was determined in two independent studies at 5 days post-infection in C57BL/6, BALB/c, and CB6F1 mice. (A) Symbols represent individual mice receiving CTB (open circles) or PspA + CTB (black circles) and (B) Symbols represent the fold change in bacterial load by subtracting the mean CFU of the control mice (CTB) by CFU values of individual mice receiving PspA + CTB. Data show group mean ± SEM from n = 8–10 mice. *p < 0.05; **p < 0.01; ***p < 0.001.

3.2. Levels of PspA-specific IgG in all mouse strains

It has previously been described that antibodies contribute to prevention or clearance of pneumococcal colonization [22–26]. To examine the antibody response in these mice, we measured PspA-specific IgG concentrations in nasal washes at five days post-infection. Although all mouse strains showed significantly increased nasal PspA-specific IgG levels (Fig. 2A–C) compared to the CTB-treated control mice, the total level of PspA-specific IgG differed between mouse strains. High PspA-specific IgG concentrations were measured in both C57BL/6 and CB6F1 mice, i.e. mean levels of 181 ng/mL and 325 ng/mL respectively, while in BALB/c mice mean levels of 45 ng/mL were measured. The fold change in nasal IgG was significantly increased in CB6F1 as compared to BALB/c mice (Fig. S1A), but was not observed for C57BL/6 animals. In all mouse backgrounds, there is a trend for nasal IgG correlating with a reduction in colonization as determined by the non-parametric Spearman-rank test (Fig. 2D–F). Furthermore, sera from the different mouse strains contained similarly high PspA-specific IgG concentrations (Fig. 2G–I) and had slightly different agglutinating capacity (Fig. 2J–L). Both C57BL/6 and CB6F1 mice showed increased fold change in serum IgG as compared to BALB/c mice, but was only significant for C57BL/6 animals (Fig. S1B). However, for unknown reasons a few sera from C57BL/6 and CB6F1 mice did not show any agglutinating activity and generally levels of pneumococcal agglutination differed per mouse strain, with C57BL/6 and CB6F1 mice showing enhanced agglutination as compared to BALB/c mice.

3.3. Nasal T helper signature cytokine levels in distinct mouse strains

Cellular responses have also been associated with clearance of pneumococcal colonization [17,18]. To investigate the contribution of cellular immunity, hallmark T helper cytokines IFNγ (Th1), IL17A (Th17), IL4 (Th2), and IL10 (Treg) were measured in nasal tissue at five days post-infection. In none of the samples IL4 was detected. IFNγ concentrations were very low to undetectable. In mice with detectable IFNγ, no differences were observed between C57BL/6, BALB/c and CB6F1 mice. Following challenge, all three mouse strains showed increased IL17A levels after vaccination with PspA, compared to mock-vaccinated animals (Fig. 3A–C). Of note, a slight trend of increased IL17A was observed in C57BL/6 and CB6F1 mice, while this increase was highly significant in BALB/c mice. Although IL10 was detectable in the nasal tissue of all mice, no significant differences were observed between mice immunized with PspA as compared to their respective mock-treated controls (Fig. 3D–F). Taken together, increased concentrations of nasal IL17A was measured in all three mouse genetic backgrounds following PspA vaccination, albeit in varying levels.

To identify relations between T helper cytokines and vaccine-induced reduction of colonization of S. pneumoniae, we correlated the nasal cytokine concentrations of individual mice with their respective pneumococcal load. We observed a trend of increased nasal IL17A that correlated with reduced colonization in C57BL/6, BALB/c and CB6F1 mice (Fig. 4A–C), but significance was not reached for C57BL/6 animals. A negative correlation was observed between nasal IL17A and bacterial load in the noses of BALB/c mice (Fig. 4B), with a p-value of >0.01. Nasal IL10 did not correlate with bacterial load in any of the three mouse strains (Fig. 4D–F).

4. Discussion

It is widely accepted that the genetic makeup of the host is a critical determinant for susceptibility to infection [1,2,8]. Although it is known that genetic variation affects the quantity, persistence and type of immune responses [10], it is still unclear how genetic factors affect vaccine efficacy. Here, we studied whether the mouse genetic background influences vaccine-induced reduction of pneumococcal colonization. Immunization with PspA in combination with an adjuvant has previously been shown to confer protection against pneumococcal colonization in a range of different mouse strains [20,27]. In this study, we found significant differences in pneumococcal density between C57BL/6, BALB/c and CB6F1, following mock-vaccination and vaccination with PspA. We have previously described that the CTB adjuvant modulates the response to pneumococcal infection independently of specific antigenic stimulation [28]. Despite the difference in genetic background, all three mouse strains showed increased nasal IL17A and IgG levels, however no significant differences in IL17A responses were observed for C57BL/6 mice. In addition, clear differences in levels of nasal IgG and IL17A were measured between the different strains.

The lower pneumococcal densities in C57BL/6 and CB6F1 suggest that these strains are intrinsically more resistant to pneumococcal colonization than BALB/c mice. CB6F1 mice are an outcross of C57BL/6 × BALB/c and the immunological characteristics of both strains, leading to a Th1 or Th2 dominated response, respectively, are combined in CB6F1 mice. In our studies, CB6F1 mice showed the highest resistance to S. pneumoniae colonization, suggesting that a combination of cellular and humoral responses has
Fig. 2. Nasal antigen-specific IgG significantly correlates with protection. Mice received three i.n. immunizations with PspA + CTB, or CTB only (control mice) and i.n. infection with $10^6$ S. pneumoniae three weeks post-immunization. (A–C) Nasal lavages and (G–I) serum obtained five days post-infection from two independent studies were assessed for PspA-specific IgG in C57BL/6, BALB/c, and CB6F1 mice. Antigen-specific IgG concentrations were correlated with bacterial density recovered from the nasal lavage of (D) C57BL/6, (E) BALB/c, and (F) CB6F1 mice using Spearman rank test. Spearman’s correlation coefficient (r) and p-value are indicated. (J–L) Pneumococcal agglutination by serum was measured using flow cytometry in C57BL/6, BALB/c, and CB6F1 mice. Symbols represent individual mice receiving CTB (open circles) or PspA + CTB (black circles) and show group mean ± SEM from n = 8–10 mice from two independent experiments. Significance is indicated with *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001.
Fig. 3. Increase in nasal IL17A, but not IL10, in C57BL/6, BALB/c, and CB6F1 mice. T helper cytokines were assessed in nasal tissue, from i.n. immunized mice, at five days after homologous pneumococcal infection. Concentrations (A–C) IL17A and (D–F) IL10 were measured using flow cytometry in C57BL/6, BALB/c, and CB6F1 mice respectively. Data show individual mice with group mean ± SEM from n = 8–10 mice and significance was accepted at p-value 0.05 with *p < 0.05.

Fig. 4. IL17A correlates with pneumococcal density in the nasopharynx. T helper cytokines were correlated with bacterial recovery derived from the nasal tissue of mice that received three i.n. immunizations, five days after homologous pneumococcal infection. Single cytokines concentrations of IL17A (A–C), and IL10 (D–F) in C57BL/6, BALB/c and CB6F1 mice respectively were correlated with pneumococcal load illustrated as CFU. Spearman rank test was applied for analysis from n = 8–10 mice and Spearman’s correlation coefficient (r) and p-value are indicated. Significance was accepted at a p-value of 0.05.
the largest impact on a reduction in pneumococcal load. This is in agreement with existing literature describing that both cellular and humoral immunity reduce pneumococcal colonization [22,29] Notably, the observations in CB6F1 mice are comparable to the results in C57BL/6 mice rather than BALB/c, suggesting that cellular immunity has a larger impact on pneumococcal colonization as compared to humoral immunity, this is supported by the literature [5,17]

Murine studies of pneumococcal carriage have demonstrated that protection against colonization is critically dependent on a Th17 response. This response is induced by colonization, but also by intranasal vaccination with e.g. inactivated whole pneumococci [17,18,20,29]. Notably, these studies were performed only in the C57BL/6 background. In the current study, a trend of increased IL17A response was observed in C57BL/6, BALB/c, CB6F1 mice vaccinated with PspA. However, it should be noted that nasal IL17A may also be a reflection of the bacterial load in the nasal cavity. Strikingly, BALB/c mice showed the highest increase in nasal IL17A (Fig. 3B), while BALB/c mice also carried higher loads of pneumococci after challenge than C57BL/6 and CB6F1. We hypothesize that IL17A is of great importance for reduction of pneumococcal colonization, but that levels and kinetics are determined by the mouse genetic background. In addition, differences observed between genetic backgrounds in colonization density and mucosal immune responses could also be influenced by the type of microbiota present in the different mouse strains [30].

The pneumococcal conjugate vaccine induces strong antibody responses which correlated with protection against pneumococcal acquisition, a prerequisite for colonization [31]. In this process, antibodies agglutinate bacterial cells and accelerate clearance from the nasopharyngeal mucosa [22,32]. Recently, our group observed that incubation of PspA-specific antibodies with pneumococci led to agglutination [21]. In this study, we observed that all three mouse strains vaccinated with PspA showed strong specific antibody responses, as measured in both nasal mucosa and sera, with significant agglutinating capacity (Fig. 2). Our findings further illustrate a marked contrast between nasal and serum IgG levels in BALB/c mice, which was not observed for C57BL/6 and CB6F1 mice. Possible explanations for this observation include differences between mouse strains (1) in the number and functionality of tissue resident B cells in the nasopharyngeal mucosa, or (2) in the efficiency of IgG translocation by the FcRn receptor, as FcRn was shown to maintain both quantity and quality of IgG in the mouse lung during infection [33]. Whether these differences exist between these mouse strains is currently unknown. The vaccine-induced antibodies, together with nasal IL17A responses may work synergistically, at different stages of colonization, leading to a reduction in pneumococcal load.

In conclusion, the current study demonstrates that vaccine-induced reduction of S. pneumoniae colonization is independent of the genetic background. However, the level of reduction strongly varied between the different mouse strains. IL17A and IgG responses measured post-challenge infection suggest that similar immune mechanisms may lead to reduction of pneumococcal load. Altogether, improved understanding of the immune mechanisms underlying vaccine-induced protection against pneumococcal colonization will contribute to the development of future pneumococcal vaccines.

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Conflict of interest

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.08.023.

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