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Genetic background impacts vaccine-induced reduction of pneumococcal colonization

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ABSTRACT

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*

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 heterogenic 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 Mucosis 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 10^6 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 Colombia 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*-nitrophenylposphate in substrate buffer, 1 M diethanolamine, 0.5 mM $MgCl_2$ pH 9.8, (Calbiochem, VWR) and optical density was measured at 405 nm 10 and 30 min after substrate addition. The lower limit of detection for nasal and serum IgG was 2 ng/mL and 103 ng/mL respectively.

2.3. Agglutination assay

Unencapsulated pneumococci (TIGR4) were incubated with 5 μL serum dilutions in a volume of 10 μL at 37 °C for 90 min. Bacteria were fixed with 1% paraformaldehyde and measured using a BD LSRII Flow Cytometer (BD Biosciences) as previously described [21].

2.4. Measurement of nasal IFNγ, IL4, IL17A and IL10

IFNγ, IL4, IL17A and IL10 concentrations in nasal tissue were determined using the Cytometric Bead Array kit. The Mouse Enhanced Sensitivity buffer kit (Becton Dickinson) in combination with the IFNγ, IL4, IL17A and IL10 Enhanced Sensitivity Flex sets (Becton Dickinson) was used according to manufacturer's instructions. Concentrations were calculated using Soft Flow FCAP Array v1.0 (Becton Dickinson) with 274 fg/mL as the lower limit of detection.

2.5. Statistical analyses

The nonparametric Kruskall Wallis and Mann-Whitney *t* test was applied to CFU numbers, antibody and cytokine levels for comparison between vaccinated and control mice and between different mouse backgrounds. Correlations between cytokine and antibody concentrations with bacterial load were analyzed using Spearman rank test. Fold changes in CFU numbers and antibody levels between mouse backgrounds were analyzed using one-Way ANOVA. To generate outcomes that are not affected by multicollinearity, we calculated adjusted R squares and residual standard deviations by means of univariate and multivariable linear regression for the effect of cytokine level(s) on pneumococcal colonization density.

All statistical analyses were performed using GraphPad Prism version 5.0 (Graphpad Software) and SPSS (IBM SPSS Statistics 22). Differences were considered significant at p-values <0.05 and were indicated as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$; ns, not significant.

3. Results

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

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 then 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).

3.2. Vaccine-induced reduction of S. pneumoniae colonization in genetically distinct mouse strains

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.

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