Effect of Genetic Background on the Response to Bacterial Sinusitis in Mice

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Objective: To study the importance of ongoing allergen exposure and Th1/Th2 genetic background in augmented bacterial and inflammatory responses in allergic and infected mice.

Design: BALB/c and C57BL/6 mice were made allergic to ovalbumin. After 1 day of intranasal allergen exposure, they were inoculated intranasally with Streptococcus pneumoniae. The numbers of bacteria and inflammatory cells in the sinuses were determined, and nasal responsiveness to histamine was assessed.

Results: Infected BALB/c and C57BL/6 mice that received ongoing ovalbumin challenge following intraperitoneal sensitization showed significantly greater bacterial load and phagocyte level compared with the infected-only mice. Differences were diminished after the allergen challenge was stopped. Allergic and infected C57BL/6 mice showed fewer bacteria and phagocytes compared with the allergic and infected BALB/c mice. Surprisingly, in contrast to the nonallergenic C57BL/6 mice, the infected BALB/c mice showed a larger number of bacteria 28 days after infection.

Conclusions: Ongoing allergic reaction augments bacterial load in both BALB/c and C57BL/6 mice and induces nasal hyperreactivity to histamine. Allergic and infected C57BL/6 mice show less allergic inflammation and bacterial load compared with allergic and infected BALB/c mice. Stopping allergen exposure reduces the response. Infected BALB/c mice, which favor a Th2 response, were less able to clear infection than C57BL/6 mice, which favor a Th1 response. Inflammation and bacterial load are affected by genetic background of mice and ongoing allergen stimulation.

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Allergic rhinitis and rhinosinusitis are widespread diseases in man. Allergic rhinitis is considered a major predisposing factor for the development of acute bacterial and chronic rhinosinusitis. How allergic rhinitis predisposes to or affects the course of rhinosinusitis remains unclear.

We previously developed a mouse model of acute bacterial rhinosinusitis. In this model, an ongoing allergic reaction augmented the bacterial load in BALB/c mice 5 days after inoculation, and the infection was associated with an augmented inflammatory response. In that study, we only evaluated the response to Streptococcus pneumoniae at day 5 in BALB/c mice. Whether the augmented bacterial load persists after allergen exposure stops or whether the strain of mice is important are 2 important questions not answered by our prior studies.

CD4 T-cell subsets, type 1 helper T cells (Th1 cells), and type 2 helper T cells (Th2 cells) have a crucial role in balancing the immune response. Their cytokines act as an autocrine growth factor for further expansion of their cell type and as reciprocal inhibitory agents for the development of the opposite cell type. Th1 cells play a role in fighting primarily intracellular microbial infections. Th2 cells, besides playing a role in fighting extracellular pathogens, promote IgE production and eosinophil function, which are key players in the pathogenesis of allergic inflammation. Allergen-induced up-regulation of Th2 cytokines has been proved to play an important role in the pathogenesis of allergy. A shift from Th1 to Th2 response can enhance both infectious and allergic diseases.

In mice, several studies have shown that the genetic tendency of the animal to favor a Th1 or Th2 response plays a major role in determining the course and severity of infection with Leishmania major. C57BL/6 mice, with a Th1 tendency, show resistance to systemic infection and consistently produce high levels of interferon-γ and a localized inflammatory reaction. In contrast, BALB/c mice, with a
T_{H2} tendency, develop a disseminated and lethal infection. Other studies showed that the genetic background affects the induction of allergy. BALB/c mice respond well to allergen stimulation, whereas C57BL/6 mice respond less well.

In this study, we used a mouse model of acute bacterial rhinosinusitis, in which BALB/c and C57BL/6 mice were given ovalbumin (OVA) or phosphate-buffered saline (PBS) by intraperitoneal injection, followed by 28 days of intranasal OVA or PBS challenge, and were inoculated with S. pneumoniae 1 day after intranasal OVA exposure began. We evaluated and compared the effect of long-term intranasal OVA challenge on allergic inflammation, nasal responsiveness to histamine, and the augmented infectious effect between sensitized and nonsensitized mice and also the difference between BALB/c and C57BL/6 mice. We hypothesized that the augmented bacterial and inflammatory responses in mice could be influenced by the duration of allergen stimulation and the genetic background of the mice.

METHODS

MOUSE MODELS

BALB/c mice and C57BL/6 of either sex, aged 6 to 8 weeks, were obtained from Jackson Laboratory (Bar Harbor, Me). The animal safety committee of the University of Chicago, Chicago, Ill, approved all protocols.

The mouse models included the following 4 groups:

- **Allergic and infected mice:** Mice were OVA sensitized by intraperitoneal injection on days 0 and 8, followed by intranasal challenge with OVA starting on day 9, and were then infected by intranasal inoculation with S. pneumoniae on day 10.
- **Nonsensitized, OVA-challenged and infected mice:** Mice were given PBS by intraperitoneal injection on days 0 and 8, followed by intranasal challenge with OVA starting on day 9, and were then infected by intranasal inoculation with S. pneumoniae on day 10.
- **Infected-only mice:** Mice were given PBS by intraperitoneal injection on days 0 and 8, followed by intranasal challenge with PBS starting on day 9, and were then infected by intranasal inoculation with S. pneumoniae on day 10.
- **Control (untreated) mice:** Mice had no intervention before being killed and served as controls for flow cytometry.

In essence, we conducted 2 parallel studies, each of the 4 groups with BALB/c and C57BL/6 mice were run in parallel. The distinction between the 2 studies was the length of OVA exposure (5 vs 28 days). In each study, the intraperitoneal inoculation was started simultaneously in the mice, with the aim of having 8 mice per group, except for the untreated mice that served as controls for the flow cytometry. A few mice died prior to their designated time point, leaving some time points with fewer than 8 mice. Histamine challenges were performed 2 days prior to euthanasia. For clarity, the “Results” section presents the data in pieces to address specific questions.

Nasal challenge with 25 µL of serially diluted histamine solution (0.3mM, 3mM, and 30mM) was applied to each nostril. The number of nasal rubbings and sneezes was counted for a 10-minute interval after histamine challenge as index of the response. The sum of the 3 challenges is reported as the total number of nasal rubbings and sneezes.

NASAL LAVAGE CULTURE

At 5, 14, 21, and 28 days after infection, groups of mice were sedated with a respiratory-failure dose of 120 mg/kg of pentobarbital sodium (Nembutal; Abbott Laboratories, Abbott Park, Ill). While sedation ensued, nasal lavage with 200 µL of PBS was performed. The lavage fluid was serially diluted in 10-fold increments by PBS from 1:1 to 1:10^7. Ten microliters of each dilution were dropped and streaked on Columbia sheep blood agar plates. Plates were incubated for 24 hours. Bacterial colonies were counted and reported as colony-forming units per milliliter.

HARVESTING OF SINUS MUCOSA

After nasal lavage, the mice were decapitated. Skin, mandible, and tongue were removed, and then the skull was split in the midline to expose the sinuses. Sinus tissue was removed and placed in 2 mL of PBS and then mixed with 2 mL of 2 mg/mL collagenase P (Roche Diagnostics, Indianapolis, Ind) to give a final concentration of 1 mg/mL. The tissue was incubated at 37°C for 1 hour in a shaker for increasing the recovery of cells from sinus tissue. The material was passed through a Nytex filter (Genesee Scientific, San Diego, Calif), and cells were recovered in Dulbecco modified Eagle medium with 3% fetal calf serum. We counted cells by using 0.4% trypan blue at 1:10 dilution. The volume of solution that contained 3 × 10^6 cells was calculated, and this amount was placed in each flow cytometry tube. The tubes were filled with flow cytometry buffer, spun for 5 minutes at 1350 rpm. We discarded the supernatant and resuspended the pellet in 2 mL of Dulbecco modified Eagle medium with 3% fetal calf serum. We counted cells by using 0.4% trypan blue at 1:10 dilution. The volume of solution that contained 3 × 10^6 cells was calculated, and this amount was placed in each flow cytometry tube. The tubes were filled with flow cytometry buffer, spun for 5 minutes at 1350 rpm, and decanted. Twenty microliters of anti-FcγRII/III antibody (2.4G2) were added, and the tubes were incubated for 15 minutes at room temperature to stop nonspecific staining by fluorochrome-conjugated antibodies that are mediated by Fc receptors. Ten microliters of antibody was added, and the tubes were incubated for 60 minutes at 4°C. After incubation, the tubes were filled with flow cytometry buffer, spun, and decanted. We then added 300 µL of flow cytometry buffer.

FLOW CYTOMETRIC ANALYSIS

Cells were stained and then analyzed on a CyAn flow cytometer (Dako-Cytomation, Ft Collins, Colo) with Summit software (Summit Software Inc, Fort Wayne, Ind). For staining, all conjugated antibodies were purchased from BD Biosciences, Pharmingen, Calif, except anti-CCR3 fluorescein isothiocyanate (FITC), which was purchased from R&D Systems Inc, Minneapolis, Minn (anti-CD11b FITC for CD11b+ myeloid cells [mainly macrophages and neutrophils] and double-stained anti-GR1-PE and CCR3 FITC for GR1hi+ CCR3- neutrophils and GR1lo-mihi+ CCR3+ eosinophils, respectively).

STATISTICAL ANALYSIS

Parametric statistics were used for analysis of the data because the data were normally distributed. Nasal responsiveness was presented in total number of sneezes or rubbing for the 3 histamine concentrations. The number of bacterial colonies grown in nasal lavage culture and positively stained cells in flow cytometry was normalized by conversion onto logarithm base 10 before analysis. Because the lower limits of detection for the bacteria is 10^2, we plotted the lowest number as 50. Statistical
sensitized intraperitoneally and exposed intraperitoneally to OVA for 28 days but not infected with S pneumoniae (S pneumoniae) represents mice that were not sensitized intraperitoneally but exposed intranasally to OVA and infected with S pneumoniae.

assessed by flow cytometry; untreated [UT] mice served as a control). OVA (ovalbumin) infection (∗) statistically significantly elevated from 5 to 28 days after infection (∗). Bars indicate mean. In all tests, 2-tailed alternatives were used. ∗P<.05. Analyses were performed by use of analysis of variance, followed by a Tukey HSD (Honestly Significant Difference) if analysis of variance showed a significant difference between groups. In all tests, 2-tailed alternatives were used. P<.05 was considered statistically significant.

**RESULTS**

**AUGMENTED BACTERIAL AND INFLAMMATORY RESPONSES DURING LONG-TERM ALLERGIC INFLAMMATION**

We compared the augmented infectious response between allergic and infected BALB/c mice and nonsensitized, OVA-exposed, and infected BALB/c mice by measuring the number of bacterial colonies grown and the inflammatory cell influx into sinuses. At 5 days after inoculation, allergic and infected BALB/c mice showed significantly more bacteria, CD11b⁺ (macrophages), GR1⁺⁺⁺ CCR3⁺ (neutrophils), and GR1⁺⁻⁻ CCR3⁺ (eosinophils) cells compared with the nonsensitized, OVA-exposed, and infected BALB/c mice. The number of bacteria and inflammatory cells between groups remained statistically significantly elevated from 5 to 28 days after infection (**Figure 1**).

No bacterial colonies were grown in the allergic-only mice. The number of GR1⁺⁻⁻ CCR3⁺ cells in allergic-only BALB/c mice was significantly higher than that in nonsensitized, OVA-challenged, and infected BALB/c mice but was not different from that in allergic and infected BALB/c mice.

**ALLERGIC INFLAMMATION AND AUGMENTED BACTERIAL AND INFLAMMATORY RESPONSES AFTER STOPPING ALLERGEN CHALLENGE**

There were significantly more bacteria, CD11b⁺ (macrophages), GR1⁺⁺⁺ CCR3⁺ (neutrophils), and GR1⁺⁻⁻ CCR3⁺ (eosinophils) cells in allergic and infected BALB/c mice than in nonsensitized, OVA-challenged, and infected BALB/c mice at 5 days after infection, as seen in the first experiment. After we stopped the exposure to allergen, the number of bacteria and inflammatory cells was reduced and comparable to that in nonsensitized, OVA-challenged, and infected BALB/c mice at 21 and 28 days after infection (**Figure 2**). There were 6 mice for each time point.

**LONG-TERM INTANASAL ALLERGEN CHALLENGE**

Long-term intranasal OVA challenge slowly induced an allergic inflammation in BALB/c mice, with a significantly higher number of CD11b⁺ (macrophages) and GR1⁺⁻⁻ CCR3⁺ (eosinophils) cells in sinuses at 28 days (**Figure 3**), and these mice were more nasally responsive to histamine.
OVA exposure. Mice were followed for up to 28 days after infection. PBS (phosphate-buffered saline) represents mice that were sensitized intraperitoneally to OVA, exposed intranasally to OVA for 5 days, and infected with *Strep*, which was considered a major risk factor for rhinosinusitis. We previously showed that mice with 5 days of on-going allergic reaction had augmented bacterial and inflammatory responses.

ALLERGIC INFLAMMATION AND AUGMENTED INFECTIOUS RESPONSE DURING ALLERGEN EXPOSURE

Allergic-only C57BL/6 mice showed typical allergic inflammation, as indicated by a significantly higher number of *GR1^hi^CCR3^+^ eosinophils (eosinophils) cells in the sinuses (Figure 5) and nasal responsiveness to histamine (Figure 6). The allergic and infected C57BL/6 mice had a significantly higher number of bacteria, CD11b^+^ (macrophages), *GR1^hi^CCR3^+^ (eosinophils) cells (Figure 5) and more nasal responsiveness to histamine (Figure 6) compared with nonsensitized, OVA-challenged, and infected C57BL/6 mice. These differences were also seen between allergic and infected BALB/c mice and nonsensitized, OVA-challenged, and infected C57BL/6 mice.

Long-term (28 days) intranasal OVA challenge in nonsensitized and infected C57BL/6 mice did not increase the number of *GR1^lo-int^CCR3^+^ cells (eosinophils) (Figure 5) or nasal responsiveness (Figure 6). They also cleared bacteria in sinuses at day 28 (Figure 5).

Comparing these 2 mouse strains, allergic and infected BALB/c mice showed more bacteria, CD11b^+^ (macrophages), *GR1^lo-int^CCR3^+^ (eosinophils) cells, and nasal responsiveness to histamine than did allergic and infected C57BL/6 mice. They also showed these differences when nonsensitized, OVA-challenged, and infected BALB/c mice and nonsensitized, OVA-challenged, and infected C57BL/6 mice were compared.

ABILITY TO CLEAR INFECTION

We have shown that nonsensitized and infected C57BL/6 mice did not become allergic after 28 days of intranasal OVA exposure and were able to clear infection (Figure 5), in contrast to nonsensitized, PBS-challenged, and infected BALB/c mice, which showed a significant number of bacteria at 28 days after infection (Figure 3).

Allergic rhinitis is an inflammatory disease of the nasal mucosa that is mediated by IgE and Th2 lymphocytes. It is considered a major factor risk factor for rhinosinusitis. We previously showed that mice with 5 days of on-going allergic reaction had augmented bacterial and inflammatory responses.
In the present study, we established mouse models in which mice (BALB/c and C57BL/6) were made allergic and infected, and we showed that the induction and severity of allergic inflammation and the augmented infectious response were affected by the duration of allergen stimulation and the Th1/Th2 genetic background of the mice.

When mice were infected during an ongoing allergic reaction, they showed significantly higher bacterial recovery and phagocytic cell influx into the sinus mucosa compared with nonsensitized, OVA-challenged, and infected mice. The augmented infectious response persisted during long-term allergen challenge and was diminished when the allergen was removed. The experiments mimic epidemiologic data in human beings that show worsening of rhinosinusitis in allergic patients and an increased incidence of rhinosinusitis during the allergy season, suggesting that control of allergic inflammation might be helpful in reducing infections.

In lower-airway allergy, long-term (25 days) allergen challenge reduced allergic airway hyperreactivity. We obtained a different result in our mouse model. We found that allergic inflammation, nasal hyperreactivity, and the augmented infectious response persisted during 28 days of allergen challenge. This may be explained by the differences in anatomy and physiologic response between the upper and lower airways.

We also demonstrated that long-term intranasal allergen challenge without intraperitoneal sensitization in BALB/c mice can induce allergic inflammation in 21 days, as indicated by increased eosinophil influx and nasal hyperreactivity, and a tendency toward augmented infection. The changes, however, occurred more slowly and were less severe than following intraperitoneal sensitization. It is unknown whether additional continuous allergen exposure would lead to further increases in allergic stimulation, as suggested by our data, and approaches the allergic simulation following intraperitoneal sensitization. Mucosal sensitization has been shown to induce both antigen-specific IgE and nasal eosinophilia in BALB/c mice. In contrast to BALB/c mice, C57BL/6 mice, with a Th1 tendency, did not develop mucosal sensitization to OVA following 28 days of intranasal allergen challenge.

We focused on how Th1 and Th2 genetic backgrounds augment bacterial and inflammation response.
We demonstrated that C57BL/6 mice, with a Th1 tendency, can be made allergic, as indicated by increases in eosinophil influx, nasal hyperreactivity, and infection. The intraperitoneally exposed C57BL/6 mice had significantly more bacteria recovered and more phagocytic cells in sinuses compared with nonsensitized, OVA-exposed, and infected C57BL/6 mice. However, allergic and infected BALB/c mice, with a Th2 tendency, showed more...
allergic inflammation and a greater infectious response than did allergic and infected C57BL/6 mice.

Interestingly, 28 days after infection, nonsensitized, OVA-challenged, and infected BALB/c mice had a large number of bacteria in the sinuses in contrast to nonsensitized, OVA-challenged, and infected C57BL/6 mice, which essentially resolved the infection. The earlier time points (up to 14 days) did not show a significant difference, as we had previously reported.16 Infected BALB/c mice without allergy, in contrast to C57BL/6 mice, had a significant number of bacteria at our last time point. These results demonstrated that BALB/c mice were less able to clear infection than were C57BL/6 mice and emphasized the importance of the T91 and T92 genetic background, which played a role in the severity and course of infection. These findings might explain the high incidence of chronic rhinosinusitis in patients with allergic rhinitis.11-17-20

Our data suggest that the T91 genetic background plays an important role in allergy induction, an augmented infectious process, and infection control. A T92 genetic tendency leads to an exaggerated allergic response. The T cells respond to allergens by inducing cytokines produced by T912 cells (ie, interleukin 4, 5, and 13) rather than cytokines produced by T911 cells (ie, interferon-γ) and inhibiting T911 cell function.21 These might explain the augmented bacterial and inflammatory reaction when these 2 diseases interact.

In conclusion, topical nasal allergen challenge in BALB/c mice, but not C57BL/6 mice, can slowly induce an allergic reaction and shows a tendency to augment infection. C57BL/6 mice, with a T911 tendency, can be made allergic by systemic sensitization and augmented infection but with less severity than in BALB/c mice, with a T912 tendency. BALB/c mice without allergy were less able to clear infection than were C57BL/6 mice. The data suggest that the allergic inflammation and augmented infectious response are regulated and influenced by genetics and allergen stimulation.

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