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CXCR2 Is Necessary for the Development and Persistence of Chronic Fungal Asthma in Mice

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The role of CXCR during allergic airway and asthmatic diseases is yet to be fully characterized. Therefore, the present study addressed the role of CXCR2 during Aspergillus fumigatus-induced asthma. Mice deficient in CXCR2 (CXCR2<sup>-/-</sup>) and wild-type counterparts (CXCR2<sup>+/+</sup>) were sensitized to A. fumigatus Ags and challenged with A. fumigatus conidia, and the resulting allergic airway disease was monitored for up to 37 days. At days 3 and 7 after conidia, CXCR2<sup>-/-</sup> mice exhibited significantly greater methacholine-induced airway hyperreactivity than did CXCR2<sup>+/+</sup> mice. In contrast, CXCR2-deficient mice exhibited significantly less airway hyperresponsiveness than the wild-type control groups at days 14 and 37 after conidia. At all times after conidia, whole lung levels of IL-4, IL-5, and eotaxin/CC chemokine ligand 11 were significantly lower in CXCR2<sup>-/-</sup> mice than in the wild-type controls. Eosinophil and T cell, but not neutrophil, recruitment into the airways of A. fumigatus-sensitized CXCR2<sup>-/-</sup> mice was significantly impaired compared with wild-type controls at all times after the conidia challenge. Whole lung levels of IFN-γ, inflammatory protein-10/CXC chemokine ligand (CXCL) 10, and monokine induced by IFN-γ (MIG)/CXCL9 were significantly increased in CXCR2<sup>-/-</sup> mice compared with CXCR2<sup>+/+</sup> mice at various times after conidia. Interestingly, at day 3 after conidia, neutrophil recruitment and airway hyperresponsiveness in CXCR2<sup>-/-</sup> mice was mediated by inflammatory protein-10/CXCL10 and, to a lesser degree, MIG/CXCL9. Taken together, these data suggest that CXCR2 contributes to the persistence of asthmatic disease due to A. fumigatus.


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Materials and Methods

Murine model of chronic fungal asthma

Specific pathogen-free female CXCR2<sup>-/-</sup> and CXCR2<sup>+/+</sup> mice (6–8 wk of age) were purchased from The Jackson Laboratory (Bar Harbor, ME).
and were maintained in a specific pathogen-free facility for the duration of this study. All CXCR2−/− mice were genotyped before their use to confirm the homozygous deficiency of CXCR2. Prior approval for mouse usage was obtained from the University Laboratory of Animal Medicine facility at the University of Michigan Medical School (Ann Arbor, MI). Systemic sensitization of mice to a commercially available preparation of soluble A. fumigatus Ags was performed as described previously (20). Seven days after the third intranasal challenge, A. fumigatus-sensitized CXCR2+/− and CXCR2−/− mice received 5.0 × 10⁴ A. fumigatus conidia suspended in 30 μl 0.1% Tween 80 via the intratracheal route (20). In separate studies, A. fumigatus-sensitized CXCR2−/− mice received 10 μg purified rabbit IgG, purified rabbit anti-mouse IP-10/CXCL10, or purified rabbit anti-mouse MIG/CXCL9 via i.p. injection immediately before and at day 2 after the conidia challenge. Anti-IP-10/CXCL10 and anti-MIG/CXCL9 were generated in rabbits according to a previously published technique (21), and these Abs were extensively screened to ensure that each specifically targeted the appropriate chemokine.

**Measurement of bronchial hyperresponsiveness**

At days 3, 7, 14, and 37 after the A. fumigatus conidia challenge, bronchial hyperresponsiveness in A. fumigatus-sensitized CXCR2+/− and CXCR2−/− mice was measured in a Buxco plethysmograph (Buxco, Troy, NY) as previously described (20). Sodium pentobarbital (Butler, Columbus, OH; 0.04 mg/g of mouse body weight) was used to anesthetize each mouse before its intubation for ventilation with a Harvard pump ventilator (Harvard Apparatus, Reno, NV). The following ventilation parameters were used: tidal volume = 0.25 ml; breathing frequency = 120/min; and positive end-expiratory pressure = 3 cm H₂O. Within the sealed plethysmograph mouse chamber, transrespiratory pressure (i.e., Δ tracheal pressure = Δ mouse chamber pressure) and inspiratory volume or flow were continuously monitored online by an adjacent computer, and airway resistance was calculated by the division of the transpulmonary by the change in inspiratory volume. A dose response to methacholine was used to determine the optimal dose of this bronchoconstrictor for the study. Increasingly higher doses (0–420 μg/kg body weight) of methacholine were administered via tail vein injection to both CXCR2+/− and CXCR2−/− mice at 3 days postconidia. The 210-μg/kg dose resulted in airway hyperresponsiveness in the CXCR2−/− allergic mice, whereas the CXCR2−/− mice exhibited little response; as such, 200 μg/kg body weight was the dose used for each time point in the study. After a baseline period in the Buxco mouse chamber, each mouse received methacholine by tail vein injection. Airway responsiveness to this bronchoconstrictor was calculated online. At the conclusion of the assessment of airway responsiveness, a bronchoalveolar lavage (BAL) was performed with 1 ml normal saline. Approximately 500 μl of blood was then removed from each mouse and transferred to a microcentrifuge tube. Sera were obtained after the sample was centrifuged for 5 min. Whole lungs were then dissected from each mouse and snap frozen in liquid N₂ or prepared for histological analysis.

**Morphometric analysis of leucocyte accumulation in BAL samples**

Neutrophils, eosinophils, lymphocytes, and macrophages were enumerated in BAL samples cytospun (Shandon Scientific, Runcorn, UK) onto coded microscope slides. Each slide was stained with a Wright-Giemsa differential stain, and the average number of each cell type was determined after counting a total of 300 cells in 10–20 high power fields (×1000) per slide. A total of 1 × 10⁵ BAL cells were cytospun onto each slide to compensate for differences in cell retrieval.

**Whole lung histological analysis**

Whole lungs from both groups of mice at days 3, 7, 14, and 37 after A. fumigatus conidia challenge were fully inflated with 10% formalin, dissected, and placed in fresh formalin for 24 h. Routine histological techniques were used to paraffin-embed the entire lung, and 5-μm sections of whole lung were stained with H&E, Gomori methanamine silver (GMS), or periodic acid-Schiff (PAS). Inflammatory infiltrates and structural alterations were examined around small airways and adjacent blood vessels using light microscopy at a magnification of ×200.

**ELISA**

Murine IL-5, IL-4, IFN-γ, etoxin, and RANTES were measured in 50-μl samples from whole lung homogenates using a standardized sandwich ELISA previously described in detail (21). Each ELISA was screened to ensure Ab specificity and recombinant murine cytokines, and chemokines were used to generate the standard curves from which the concentrations present in the samples were derived. The limit of ELISA detection for each cytokine was consistently above 50 pg/ml. The cytokine and chemokine levels in each sample were normalized to total protein levels measured using the Bradford assay (Bio-Rad, Hercules, CA).

Serum levels of IgE and IgG1 were analyzed using complementary capture and detection Ab pairs for IgE and IgG1 (BD Pharmingen, San Diego, CA). Ig ELISAs were performed according to the manufacturer’s directions. Duplicate sera samples were diluted to 1/100 for IgE determination and 1/1000 for determination of IgG levels. Ig levels were then calculated from OD₄₉₂ readings, and Ig concentrations were calculated from a standard curve generated using recombinant IgE or IgG1 (both standard curves ranged from 5 to 2000 pg/ml).

**Measurement of myeloperoxidase (MPO)**

MPO is an enzyme found predominantly in azurophilic granules of neutrophils that catalyzes the formation of hypochlorous acid. MPO is commonly used as an index of the activation state or presence of neutrophils in a variety of tissues. A commercially available MPO-specific ELISA (Calbiochem, La Jolla, CA) was used to determine immunoreactive levels of MPO in BAL fluid per manufacturer’s instructions.

**Statistical analysis**

All results are expressed as mean ± SE. A one-way ANOVA and a Dunnett multiple comparisons test were used to reveal statistical differences between the CXCR2+/− and CXCR2−/− groups at days 3, 14, and 37 after the conidia challenge; p < 0.05 was considered statistically significant.

**Results**

A. fumigatus-sensitized CXCR2−/− mice were resistant to the lethal effects of an A. fumigatus conidia challenge

Previous studies by Mehrad et al. (19) demonstrated that immunocompetent mice in which CXCR2 was immunoneutralized developed lethal invasive aspergillosis (due to uncontrolled fungal growth) in contrast to a control group of immunocompetent mice that were resistant to Aspergillus infection. In the present study, we observed that 50% of nonsensitized CXCR2−/− mice that were challenged with 5 × 10⁴ conidia succumbed to this fungal challenge by day 3 after conidia (Table I). Conversely, no deaths were observed in a group of nonsensitized CXCR2+/− mice or in either A. fumigatus-sensitized group at any time after the conidia challenge (Table I). These data suggested that the allergic sensitization of CXCR2−/− mice to A. fumigatus conferred protection from the lethal effects of uncontrolled growth of A. fumigatus.

<table>
<thead>
<tr>
<th>Mouse Treatment</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
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<td>CXCR2−/− (A. fumigatus sensitized)</td>
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* Four groups of six mice were challenged with A. fumigatus conidia at day 0, and mouse survival was monitored up to day 3 after the challenge. Results are given as percent of animals surviving.

**Table I. Mouse survival after A. fumigatus conidia challenge**

* Both groups of mice either received saline or A. fumigatus Ag before an intratracheal challenge with 5.0 × 10⁴ A. fumigatus conidia.
A. fumigatus-sensitized CXCR2−/− mice exhibited significantly increased serum levels of IgE after a conidia challenge

It was recently shown that CXCR2−/− mice exhibit increased IgE levels in the context of allergic airway disease due to OVA sensitization and challenge (22). Therefore, our first objective was to determine whether CXCR2−/− mice exhibited enhanced IgE and IgG1 during the course of chronic fungal asthma. As shown in Fig. 1, major differences in Ig levels between CXCR2+/+ and CXCR2−/− mice were observed only at certain times after the conidia challenge. Total serum IgE levels were markedly increased in CXCR2−/− mice compared with their wild-type controls at days 7 and 37 after conidia, but not at any other times during the course of chronic fungal asthma. At all times after conidia, IgG1 levels were similar in both groups of mice. Taken together, these data suggested that major differences in Ig synthesis were not apparent or consistent between the CXCR2+/+ and CXCR2−/− groups during the course of chronic fungal asthma.

A. fumigatus-sensitized CXCR2−/− mice exhibited exaggerated but abbreviated methacholine-induced airway hyperresponsiveness after a conidia challenge

Given that OVA-sensitized and -challenged CXCR2−/− mice have been shown to exhibit decreased responsiveness to methacholine compared with CXCR2+/+ (22), we next determined whether airway hyperresponsiveness to methacholine was altered in CXCR2−/− mice during the course of chronic fungal asthma. At day 3, a methacholine dose-response curve was used to assess the working dose that would be used for this and each subsequent time point (Fig. 2A). A 200 μg methacholine per kg of body weight dose was used as the working dose, because this dose was the highest at which the CXCR2−/− mice were responsive to the drug, whereas the CXCR2+/+ mice were not. The full time course of airway hyperresponsiveness is summarized in Fig. 2B. It was apparent that the development of airway responsiveness differed greatly between the two groups of mice. First, CXCR2−/− mice exhibited a progressive increase in airway hyperresponsiveness such that the greatest airway responses in this group of mice were observed at day 37 after the conidia. Significantly higher airway responsiveness to methacholine was observed in the CXCR2−/− groups at days 3 and 7 after conidia compared with the CXCR2+/+ groups at the same times (Fig. 2B). In contrast, at days 14 and 37...
after conidia, airway hyperresponsiveness was significantly lower in the CXCR2−/− groups compared with the appropriate control group (Fig. 2B). Thus, there appeared to be an accelerated appearance of airway hyperresponsiveness in A. fumigatus-sensitized CXCR2−/− mice, but this response diminished with time during the course of chronic fungal asthma.

**Whole lung levels of IL-4, IL-5, eotaxin, and RANTES were significantly lower in CXCR2−/− mice than in CXCR2+/+ mice after conidia challenge**

The observation that the development of chronic fungal asthma was accelerated but not sustained in CXCR2−/− mice prompted us to examine the changes in whole lung levels of Th2 cytokines and proallergic chemokines such as eotaxin and RANTES (23, 24). Considering that both groups of mice developed airway hyperresponsiveness to methacholine, it was surprising to observe that whole lung levels of IL-5 (Fig. 3A), IL-4 (Fig. 3B), and eotaxin (Fig. 3C) were significantly lower in CXCR2−/− mice than in CXCR2+/+ mice. Whole lung RANTES levels did not differ between the two groups at days 3 and 7 after conidia, but whole lung levels of this CC chemokine were significantly lower at days 14 and 37 after conidia in CXCR2−/− mice than in CXCR2+/+ mice (Fig. 3D). The decrease in whole lung levels of RANTES correlated with the loss of airway hyperresponsiveness in CXCR2−/− mice, which is consistent with previous studies documenting the contribution of RANTES to the development of airway hyperresponsiveness during allergic airway diseases (25). Thus, CXCR2−/− mice did not exhibit increases in Th2 cytokines and eotaxin as similar to those of wild-type counterparts, suggesting that the development of Th2-mediated allergic airway events was partially inhibited in these mice.

**Temporal variations in the expression of IFN-γ and IL-12 in whole lung samples from CXCR2−/− mice were observed after a conidia challenge**

The observation that whole lung levels of IL-4 and IL-5 were significantly lower in whole lung samples from CXCR2−/− mice suggested that these mice did not develop the appropriate Th2 responsiveness to A. fumigatus. This observation also raised the possibility that the levels of Th1 cytokines such as IFN-γ and IL-12 may have also been affected by the CXCR2 deficiency during chronic fungal asthma. As shown in Fig. 4A, the whole lung levels of IFN-γ were significantly lower in whole lung samples from CXCR2−/− mice at days 3 and 7 after the conidia challenge. At the later time points, whole lung IFN-γ levels in this group were higher (significantly so at day 37) than those detected in whole lungs from CXCR2+/+ mice. Interestingly, the increases in whole lung IFN-γ levels in CXCR2−/− corresponded with the diminution of airway hyperresponsiveness, and these findings were in accordance with our previous findings from this model (26, 27). Temporal changes in whole lung levels of IL-12 are shown in Fig. 4B. It was apparent that whole lung levels of IL-12 were lower in the CXCR2−/− mice at all time points after the conidia challenge, but the differences between the two groups reached significance only at days 7 and 37 after conidia. Aside from the significant increase in whole lung levels of IFN-γ at day 37, these data suggested that the Th1 cytokine response was only modestly affected in A. fumigatus-sensitized CXCR2−/− mice after a conidia challenge.

**Eosinophil and T cell, but not neutrophil, recruitment into the airways of CXCR2−/− mice was markedly impaired at all times after conidia challenge**

The cytokine and chemokine profile in CXCR2−/− mice during the course of the allergic airway response to A. fumigatus conidia...
suggested that major defects in leukocyte recruitment to the airways were probably present in these mice. Examination of BAL levels of neutrophils, eosinophils, lymphocytes, and macrophages at days 3, 7, 14, and 37 after conidia confirmed recruitment differences were present between CXCR2\(^{+/+}\) and CXCR2\(^{-/-}\) mice (Fig. 5). At all time points after the conidia challenge, a markedly smaller percentage of the BAL cells were comprised of either eosinophils or lymphocytes, including T cells. However, an unexpected finding from the present study was that neutrophil counts in the BAL did not differ between the two groups at days 3 and 7 after the conidia challenge (Fig. 5). Clearly, this result differs from several other studies in these mice including the study that addressed the role of CXCR2 in allergen-induced pulmonary inflammation (22).

Overall, the movement of eosinophils and lymphocytes into the airways of CXCR2\(^{-/-}\) mice sensitized to and challenged with \textit{A. fumigatus} was markedly impaired during the course of this model. Airway remodeling features of chronic fungal asthma were absent from \textit{A. fumigatus}-sensitized CXCR2\(^{-/-}\) mice at day 37 after conidia challenge. Although histological analysis of lung sections from both CXCR2\(^{+/+}\) and CXCR2\(^{-/-}\) groups revealed no appreciable difference at early time points after conidia (days 3, 7, and 14), histology at day 37 was markedly dissimilar (summarized in Fig. 6). The peribronchial accumulation of inflammatory leukocytes was apparent in lung samples from CXCR2\(^{+/+}\) mice (Fig. 6A), whereas no evidence of peribronchial inflammation was apparent in CXCR2\(^{-/-}\) mice at this time after conidia (Fig. 6B). However, further histological assessment of lung samples from CXCR2\(^{-/-}\) mice revealed that these mice had major interstitial inflammation (shown in Fig. 6B, inset). A main feature of the remodeled airway during chronic fungal asthma is goblet cell hyperplasia (20), and this feature (magenta-stained cells in epithelium) was prominent in histological lung sections from CXCR2\(^{+/+}\) mice at day 37 after the conidia challenge (Fig. 6C). In contrast, goblet cell hyperplasia was not observed in the lungs of CXCR2\(^{-/-}\) mice at this time (Fig. 6D). The presence or absence of fungal elements in the lungs of both groups of mice was revealed using the GMS stain.
samples from CXCR2<sup>+/+</sup> (Fig. 6E), but not CXCR2<sup>−/−</sup> (Fig. 6F) mice, contained black-stained fungal elements. Taken together, these histological findings suggested that CXCR2<sup>−/−</sup> mice did not exhibit the peribronchial and airway changes typically associated with chronic fungal asthma in wild-type mice. Interestingly, CXCR2<sup>−/−</sup> mice exhibited much greater interstitial inflammation than the CXCR2<sup>+/+</sup> mice despite the observation that these mice did not appear to have a pulmonary burden of fungal material.

**BAL levels of MPO were significantly increased in A. fumigatus-challenged CXCR2<sup>−/−</sup> mice compared with CXCR2<sup>+/+</sup> mice at day 3 after conidia**

The finding that neutrophil numbers were similar in the BAL of CXCR2<sup>−/−</sup> and CXCR2<sup>+/+</sup> mice at days 3 and 7 after conidia led us to examine the activation state of the neutrophils recruited to the airways of CXCR2<sup>−/−</sup> mice. MPO is a marker of neutrophil activation in the context of a number of inflammatory diseases or disorders. MPO levels in BAL samples from CXCR2<sup>+/+</sup> and CXCR2<sup>−/−</sup> mice at days 3 and 7 after conidia are summarized in Fig. 7. MPO levels in BAL samples from CXCR2<sup>−/−</sup> mice were significantly higher at day 3 but significantly lower at day 7 after conidia challenge than were those of CXCR2<sup>+/+</sup> mice. These data suggested the activation of neutrophils in the BAL at day 3 after the conidia challenge was markedly enhanced in A. fumigatus-sensitized CXCR2<sup>−/−</sup> mice.

**Significantly increased whole lung levels of IP-10 and MIG were detected in A. fumigatus-sensitized CXCR2<sup>−/−</sup> mice at day 3 after conidia challenge**

The recruitment and increased activation of neutrophils in A. fumigatus-sensitized CXCR2<sup>−/−</sup> mice at day 3 after the conidia challenge were not readily explicable considering that this type of response had not been previously documented in these mice. Although it has been shown that MIP-2 induces chemokine production in astrocytes via an CXCR2-independent mechanism (28), we

**FIGURE 6.** Representative photomicrographs of H&E-, PAS- and GMS-stained whole lung sections at day 37 after A. fumigatus conidia challenge in A. fumigatus-sensitized CXCR2<sup>+/+</sup> and CXCR2<sup>−/−</sup> groups. A marked peribronchial accumulation of inflammatory leukocytes was apparent in histological sections from CXCR2<sup>+/+</sup> mice (A), whereas little peribronchial inflammation was apparent in CXCR2<sup>−/−</sup> mice at this time after conidia (B). However, as shown in B, inset, severe interstitial inflammation was apparent in CXCR2<sup>−/−</sup> mice at this time. PAS-stained goblet cells were prominent in the airways of CXCR2<sup>−/−</sup> mice (C), but were absent in CXCR2<sup>+/+</sup> mice at this time (D). The presence of fungal material in alveolar macrophages was revealed by GMS staining (black material highlighted by arrows) in CXCR2<sup>−/−</sup> mice (E), but GMS-stained alveolar macrophages were absent in CXCR2<sup>−/−</sup> mice (F). Original magnifications, ×200.

**FIGURE 7.** BAL levels of MPO at days 3 and 7 after A. fumigatus conidia challenge in A. fumigatus-sensitized CXCR2<sup>+/+</sup> (■) and CXCR2<sup>−/−</sup> (□) groups. Differential leukocyte counts in BAL samples at days 3, 7, 14, and 37 after A. fumigatus conidia challenge in A. fumigatus-sensitized CXCR2<sup>−/−</sup> and CXCR2<sup>+/−</sup> groups. BAL cells were dispersed onto microscope slides; neutrophils, eosinophils, lymphocytes, and macrophages were differentially stained with Wright-Giesma stain. A minimum of 15 high power fields or 300 cells was examined in each cytospin. A total of 1 × 10<sup>5</sup> BAL cells was cytospun onto each slide to compensate for differences in cell retrieval from each mouse. MPO levels were measured by specific ELISA according to the manufacturer’s directions. Values are expressed as mean ± SE; n = 5 mice/group. * p ≤ 0.05, compared with the MPO levels measured in CXCR2<sup>+/+</sup> control at the same day after the conidia challenge.
examined the possibility that non-ELR chemokines such as IP-10/ CXCL10 and MIG/CXCL9 were involved in the neutrophil response elicited by the introduction of conidia into A. fumigatus-sensitized mice. To date, there is no evidence that neutrophils can express CXCR3, but it is possible that other, as yet identified, CXCRs can bind non-ELR CXC chemokines (29). In addition, there is evidence that chemokines can influence the production and release of lipid-derived neutrophil chemoattractant factors including leukotrienes (30). ELISA analysis of whole lung levels of IP-10/CXCL10 and MIG/CXCL9 in both groups of mice revealed that both non-ELR CXC chemokines were significantly elevated in CXCR2−/− mice compared with those in CXCR2+/+ mice (Fig. 8). These data suggested that both IP-10/CXCL10 and MIG/CXCL9 were significantly elevated at the time (day 3 after conidia) of increased neutrophil recruitment and activation in CXCR2−/− mice.

The recruitment of neutrophils into the airways of CXCR2−/− mice and development of airway hyperresponsiveness was mediated by IP-10/CXCL10 and MIG/CXCL9 at day 3 after the conidia challenge.

To examine the effects of IP-10/CXCL10 and MIG/CXCL9 on neutrophil recruitment and activation and airway hyperresponsiveness at day 3 after conidia, groups of A. fumigatus-sensitized CXCR2−/− mice received purified anti-IP-10/CXCL10 or polyclonal anti-MIG/CXCL9 before and at day 2 after conidia. These purified Abs effectively immunoneutralized the targeted chemokine (data not shown), and the effects of these treatments on neutrophil recruitment (Fig. 9A) and BAL myeloperoxidase (Fig. 9B) are summarized in Fig. 9. The immunoneutralization of IP-10/CXCL10 had a major inhibitory effect on the recruitment of neutrophils into the airways of A. fumigatus-sensitized CXCR2−/− mice at day 3 after conidia, whereas anti-MIG/CXCL9’s effect was minor. As shown in Fig. 9B, both Ab treatments significantly reduced BAL MPO levels compared with the control group that received IgG alone. These data suggested that the recruitment and activation of neutrophils in the context of this model was dependent on IP-10/CXCL10 and, to a lesser extent, MIG/CXCL9. The effects of anti-IP-10/ CXCL10 and anti-MIG/CXCL9 are summarized in Fig. 10. The immunoneutralization of IP-10/CXCL10 significantly reduced methacholine-induced airway hyperresponsiveness in A. fumigatus-sensitized and challenged CXCR2−/− mice at day 3 after conidia. Anti-MIG/CXCL9 treatment of CXCR2−/− mice reduced airway hyperresponsiveness by 50%, but this reduction did not reach statistical significance. These data showed that IP-10/CXCL10, and to a lesser extent MIG/CXCL9, modulated the airway hyperresponsiveness observed in CXCR2−/− mice at day 3 after conidia. In addition, the inhibitory effect of anti-IP-10/ CXCL10 and MIG/CXCL9 appeared to correlate with a decreased recruitment of neutrophils into the airways of CXCR2−/− mice.
The immunoneutralization of IL-18, another Th1 cytokine (35), induced allergic airway disease since we have recently shown that challenge of conidia (19). In the present study, CXCR2 expression is necessary for responses characterized by enhanced total serum IgE, Th2 cytokines, goblet cell hyperplasia, or fungal overgrowth (33, 34). These findings suggest that CXCR2 is required for the development and maintenance of the characteristic peribronchial features of chronic fungal asthma.

In the context of A. fumigatus exposure in immunocompromised mice, the presence of an intact and robust Th1 response mediated by IFN-γ and IL-12 is necessary for the containment of fungal growth and the prevention of invasive aspergillosis (31, 32). Accordingly, the increased expression of IL-4 or IL-10 appears to suppress the Th1-mediated antifungal response, consequently leading to invasive fungal disease in neutropenic mice (33, 34). These events appear to also hold true in the context of Aspergillus-induced allergic airway disease since we have recently shown that the immunoneutralization of IL-18, another Th1 cytokine (35), after a conidia challenge in A. fumigatus-sensitized mice leads to a markedly increased fungal burden and pronounced symptoms of fungal asthma (36). The pulmonary antifungal response depends on the activation of two leukocyte types, alveolar macrophages and neutrophils (37). The alveolar macrophage is the major cell type that phagocytoses and kills conidia introduced into the pulmonary environment (18), although neutrophils can also destroy conidia in this manner (38). The major role of neutrophils after a conidia challenge is to destroy conidia that germinate and form hyphal elements (37). In the present study, it was apparent that A. fumigatus-sensitized CXCR2−/− mice challenged with conidia did not retain fungal material, suggesting that fungal clearance was comparable with, or even more efficient than, the antifungal response in A. fumigatus-sensitized CXCR2+/+ mice. One explanation for this finding may be found in the fact that neutrophil activation (based on MPO levels) in A. fumigatus-sensitized mice was enhanced at day 3 after conidia. Considering previous findings (33), it is also conceivable that the presence of significantly lower whole lung levels of IL-4 and IL-5 and the significant increases in whole lung levels of IFN-γ, IP-10/CXCL10, and MIG/CXCL9 in A. fumigatus-sensitized CXCR2−/− mice facilitated the effective immune containment and/or elimination of the conidia by alveolar macrophages. Thus, the enhanced neutrophil activation and the whole lung cytokine profile in A. fumigatus-sensitized CXCR2−/− mice appeared to favor the containment and elimination of A. fumigatus conidia thereby facilitating the survival of A. fumigatus-sensitized CXCR2−/− mice.

A surprising finding from the present study was that neutrophil recruitment into the airways of A. fumigatus-sensitized CXCR2+/+ mice was comparable with that observed in A. fumigatus-sensitized CXCR2−/− mice. This finding is in contrast to a number of previous reports that showed that neutrophil recruitment was markedly impaired in nonallergic, immunocompetent mice to survive an intrapulmonary challenge of conidia (19). In the present study, CXCR2−/− mice sensitized to soluble A. fumigatus Ags were not susceptible to the lethal effects of a conidia challenge. This resistance appeared to be a consequence of the fact that neutrophil recruitment in A. fumigatus-sensitized CXCR2+/+ mice was comparable with that measured in A. fumigatus-sensitized CXCR2+/+ mice. However, the recruitment of eosinophils and T cells and the concomitant Th2 response that was prominently expressed in A. fumigatus-sensitized CXCR2+/+ mice were not observed in A. fumigatus-sensitized CXCR2−/− mice after the conidia challenge. Instead, various Th1-associated chemokines and cytokines such as IP-10/CXCL10, MIG/CXCL9, and IFN-γ were significantly increased in CXCR2−/− mice compared with the wild-type controls at various times after the conidia challenge. The histological features of A. fumigatus-induced lung disease differed greatly between the two groups of mice examined. For example, in contrast to CXCR2+/+ mice, CXCR2−/− mice exhibited a marked pan-lung inflammatory response that was not localized around the airways. Furthermore, A. fumigatus-sensitized CXCR2−/− mice did not exhibit peribronchial inflammation, goblet cell hyperplasia, or fungal overgrowth after the conidia challenge. Thus, these findings suggest that CXCR2 is required for the development and maintenance of the characteristic peribronchial features of chronic fungal asthma.

In the context of A. fumigatus exposure in immunocompromised mice, the presence of an intact and robust Th1 response mediated by IFN-γ and IL-12 is necessary for the containment of fungal growth and the prevention of invasive aspergillosis (31, 32). Accordingly, the increased expression of IL-4 or IL-10 appears to suppress the Th1-mediated antifungal response, consequently leading to invasive fungal disease in neutropenic mice (33, 34). These events appear to also hold true in the context of Aspergillus-induced allergic airway disease since we have recently shown that the immunoneutralization of IL-18, another Th1 cytokine (35), after a conidia challenge in A. fumigatus-sensitized mice leads to a markedly increased fungal burden and pronounced symptoms of fungal asthma (36). The pulmonary antifungal response depends on the activation of two leukocyte types, alveolar macrophages and neutrophils (37). The alveolar macrophage is the major cell type that phagocytoses and kills conidia introduced into the pulmonary environment (18), although neutrophils can also destroy conidia in this manner (38). The major role of neutrophils after a conidia challenge is to destroy conidia that germinate and form hyphal elements (37). In the present study, it was apparent that A. fumigatus-sensitized CXCR2−/− mice challenged with conidia did not retain fungal material, suggesting that fungal clearance was comparable with, or even more efficient than, the antifungal response in A. fumigatus-sensitized CXCR2+/+ mice. One explanation for this finding may be found in the fact that neutrophil activation (based on MPO levels) in A. fumigatus-sensitized mice was enhanced at day 3 after conidia. Considering previous findings (33), it is also conceivable that the presence of significantly lower whole lung levels of IL-4 and IL-5 and the significant increases in whole lung levels of IFN-γ, IP-10/CXCL10, and MIG/CXCL9 in A. fumigatus-sensitized CXCR2−/− mice facilitated the effective immune containment and/or elimination of the conidia by alveolar macrophages. Thus, the enhanced neutrophil activation and the whole lung cytokine profile in A. fumigatus-sensitized CXCR2−/− mice appeared to favor the containment and elimination of A. fumigatus conidia thereby facilitating the survival of A. fumigatus-sensitized CXCR2−/− mice.
the present study, A. fumigatus-sensitized CXCR2−/− mice exhibited airway hyperresponsiveness that appeared to be dependent on the presence of recruited neutrophils. The strongest evidence for this came from the immunoneutralization studies targeting IP-10/CXCL10 and MIG/CXCL9 that revealed the importance of these factors in the recruitment of neutrophils and the subsequent development of airway hyperresponsiveness. These findings concur with other experimental studies that demonstrated that neutrophils, in the absence of eosinophils, could elicit airway hyperresponsiveness in allergic (9) and nonallergic (49) settings. It was also interesting in this study that changes in airway hyperresponsiveness in A. fumigatus-sensitized CXCR2−/− mice closely correlated with the presence of neutrophils around the airways of these mice at days 3 and 7 after conidia.

The initiation and maintenance of chronic fungal asthma were severely compromised in A. fumigatus-sensitized CXCR2−/− mice compared with A. fumigatus-sensitized CXCR2+/+ mice. The abbreviuated duration of allergic airway disease in CXCR2−/− mice may have been related to the suppressed whole lung levels of IL-4, IL-5, eotaxin/CCL11, and RANTES/CCL5. These cytokines and chemokines have been shown to be necessary at various levels, including eosinophil and T cell recruitment, for the development and maintenance of allergic airway disease in the context of a number of experimental settings (50). The suppressed levels of Th2 cytokines and chemokines did not appear to be a consequence of the failure of CXCR2−/− mice to develop allergic responsiveness to A. fumigatus because CXCR2-deficient mice had serum levels of IgE and IgG1 that were equivalent to or exceeded Ig levels in CXCR2-wild-type mice. One explanation for the defect in Th2 cytokine and chemokine generation may have been related to the increased presence of Th1-associated chemokines and cytokines. The adeno-virus-induced overexpression of IP-10/CXCL10 in the context of experimental allergic airway disease was recently shown to a profoundly inhibit the migration of eosinophils and T cells into the airways and ablate IL-4 levels in the BAL (51). A. fumigatus-sensitized CXCR2−/− mice exhibited significantly increased levels of IP-10/CXCL10 and MIG/CXCL9 at day 3 after the conidia challenge, and it is conceivable that the increased levels of these IFN-γ-inducible chemokines similarly down-regulate the Th2 responses to A. fumigatus conidia in these mice.

Although A. fumigatus-sensitized CXCR2−/− mice did not develop the characteristic allergic airway disease after the conidia challenge, the lungs of these mice were clearly inflamed. In particular, at day 37 after the conidia challenge, the pulmonary inflammatory infiltrate in these mice at this time was mainly comprised of monocytes and macrophages, but this infiltrate was not localized around the airways. It was not apparent from this study why A. fumigatus-sensitized CXCR2−/− mice displayed this type of inflammatory response, given that there was no evidence that excessive amounts of fungal material was present in the lungs of these mice. This finding contrasts with our previous studies of chronic fungal asthma in A. fumigatus-sensitized CCR2−/− mice, which failed to eliminate A. fumigatus conidia and as a consequence developed severe allergic disease (52). Further studies are necessary to determine what factors are responsible for the increased interstitial pulmonary inflammation in A. fumigatus-sensitized CXCR2−/− after a conidia challenge.

In conclusion, it was shown that A. fumigatus-sensitized mice were resistant to the lethal effects of an A. fumigatus conidia challenge in contrast to nonsensitized CXCR2−/− mice. The ability of A. fumigatus-sensitized CXCR2−/− mice to survive a conidia challenge is probably multifactorial, but it was observed that the generation of Th2 cytokines and chemokines was significantly lower whereas Th1 cytokine and chemokine levels were significantly enhanced in these mice at various times after the conidia challenge. A. fumigatus-sensitized CXCR2−/− mice failed to develop many of the characteristic features of chronic fungal asthma including persistent peribronchial accumulation of eosinophils and T cells, goblet cell hyperplasia, and persistent airway hyperresponsiveness. Thus, these findings demonstrate that CXCR2 expression is necessary for the development and maintenance of chronic fungal asthma in mice.

References


