The role of CC chemokine receptor 5 (CCR5) and RANTES/CCL5 during chronic fungal asthma in mice[1]

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SPECIFIC AIMS

In the present study, we explore the role of CC chemokine receptor 5 (CCR5) and RANTES/CCL5 in a murine model of chronic fungal asthma induced by an intrapulmonary challenge with *Aspergillus fumigatus* conidia.

PRINCIPAL FINDINGS

1. CCR5−/− mice limit the development of fungal asthma and only transiently express airway hyperresponsiveness (day 12) after fungal challenge

Airway hyperresponsiveness was significantly decreased after conidia challenge in CCR5−/− mice, except at day 12, at which time the response to methacholine was comparable to control mice (**Fig. 1**). A deficit in eosinophil chemoattractants, C10/CCL6 and eotaxin/CCL11, was noted in the CCR5 knockout strain at days 2 and 12, highlighting the importance of the CCR5 receptor in multiple facets of eosinophil recruitment. Control mice displayed obvious perivascular and peribronchial inflammation, while the inflammation in the CCR5-deficient mice was predominantly perivascular. The peribronchial inflammation that was noted in the CCR5−/− mice was nearly devoid of eosinophils.

2. RANTES mediates the appearance of fungal asthma in the absence of CCR5

RANTES/CCL5 levels in CCR5−/− mice were markedly higher than their wild-type counterparts, except on days 2 and 12 after conidia, at which time levels were similar. Immunoneutralization of RANTES/CCL5 during the first 12 days after conidia challenge effectively limited fungal asthma expression in CCR5−/− mice. Anti-RANTES/CCL5 curtailed leukocyte and eosinophil recruitment in CCR5−/− mice (**Fig. 2**) below that of controls treated with normal serum, ostensibly blocking the chemokine's binding to an alternative receptor, possibly CCR1, CCR3, and/or CCR4. Identification of this putative receptor is the aim of ongoing studies.

3. Airway remodeling is less severe in CCR5−/− mice than in controls

Goblet cell hyperplasia and peribronchial fibrosis, two hallmarks of airway remodeling in the fungal asthma model, were significantly reduced in CCR5−/− mice. Peribronchial fibrosis was observed through histological and collagen analysis to be significantly less prominent in the CCR5−/− mice when compared with controls. Anti-RANTES/CCL5 treatment reduced collagen deposition in both CCR5+/+ and CCR5−/− groups.

Goblet cell hyperplasia was prominent in the CCR5−/− mice at day 12 only, while CCR5+/+ mice harbored goblet cells through day 40. Goblet cell hyperplasia was still noted in CCR5−/− mice at day 12 even after neutralization of RANTES/CCL5, suggesting a limited role for RANTES/CCL5 in the overall aspects of airway remodeling.

CONCLUSIONS AND SIGNIFICANCE

CCR5 is a chemokine receptor for MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5. Lung T cells abundantly express CCR5. One of CCR5's major ligands, RANTES/CCL5, has been linked with both atopic and nonatopic asthma, making the study of RANTES/CCL5 germane to the treatment of this ubiquitous disease. A natural mutation in CCR5 exists, and individuals who are homozygous for a 32-base pair deletion in the coding sequence of CCR5 (CCR5Δ32) were found to be highly resistant to HIV infection and potentially other chronic diseases such as asthma. In the present study, we examined the contribution of CCR5 and RANTES/CCL5 to the development and maintenance of chronic fungal asthma induced by *A. fumigatus* conidia in mice sensitized to the fungus.

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[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.01–0528fje; to cite this article, use *FASEB J.* (December 14, 2001) 10.1096/fj.01–0528fje

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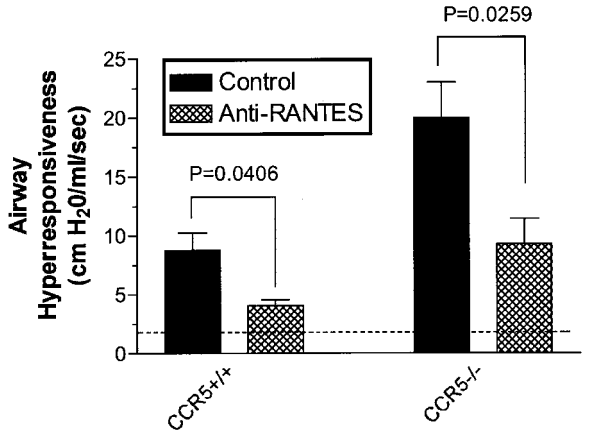

Figure 1. Airway hyperresponsiveness in *A. fumigatus*-sensitized CC chemokine receptor-5 (CCR5) wild-type (+/+) and CCR5 knockout (−/−) mice before and at various times after an intrapulmonary challenge with live *A. fumigatus* conidia. Peak increases in airway resistance or hyperresponsiveness (units=cm H_2O/ml/s) were determined at each time point after an intravenous (i.v.) injection of methacholine. The dashed line represents the baseline level of airway responsiveness. Values are expressed as mean ± SE; n = 4–7/group/time point.

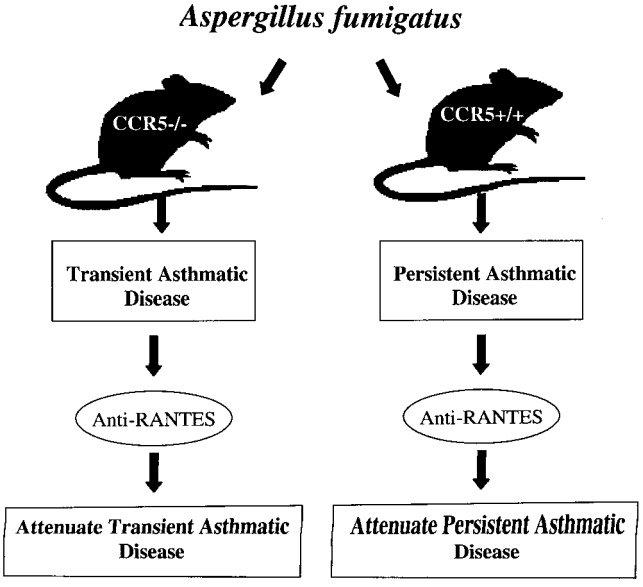

Figure 3.

Other experimental studies using acute models of allergic airway disease have corroborated these clinical findings by showing that RANTES/CCL5 is a major eosinophil and T cell chemoattractant that consequently modulates airway hyperresponsiveness. The role of RANTES/CCL5 in allergic wild-type mice has been examined using antibody immunoneutralization techniques, but a description of allergic airway disease in CCR5−/− mice has not been reported before.

The compilation of previous studies of immune responses by CCR5−/− mice results in a complex and multi-layered schema in which the involvement of CCR5 depends on the nature of the pathogen (i.e., bacterial, viral, or fungal), the site of infection, and the background strain of the CCR5−/− mouse. For example, CCR5−/− mice on an ICR background share similar developmental qualities with their wild-type counterparts, but exhibit a defect in the innate immune response necessary for clearing Listeria from the liver (though not lung and spleen) whereas T cell-dependent delayed-type hypersensitivity reactions and humoral responses were markedly enhanced in these mice. This finding agrees with data from the present study, which revealed that *A. fumigatus*-sensitized CCR5−/− mice had significantly higher serum levels of IgE before the conidia challenge compared with CCR5+/+ mice at the same time. However, serum IgE levels in these mice were comparable at all other times, except day 40 after conidia. Nonsensitized CCR5−/− mice (on a C57BL/6J×129/Ola background) effectively resist pulmonary infection with *Cryptococcus neoformans* but are susceptible to brain infection due to a lack of leukocyte recruitment into the brain. CCR5−/−

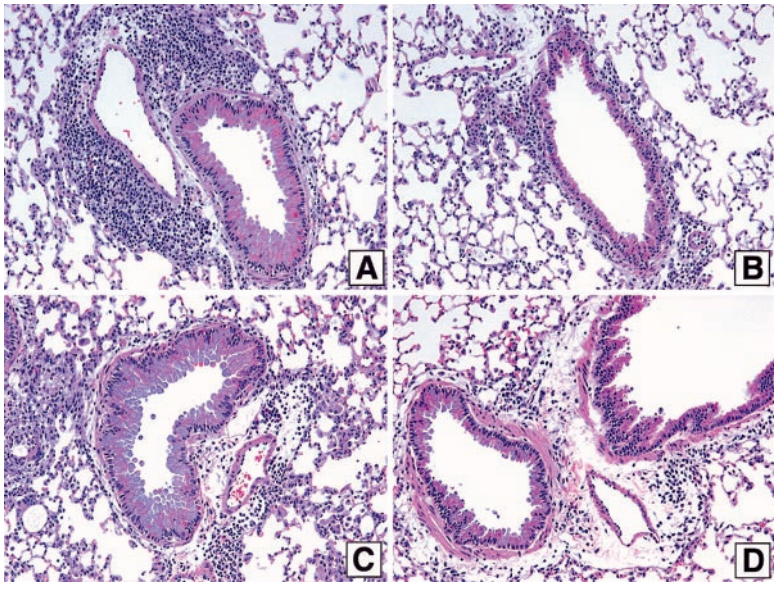

Figure 2. Representative photomicrographs of hematoxylin and eosin (H&E)-stained whole lung sections from *A. fumigatus*-sensitized CC chemokine receptor-5 (CCR5) wild-type (+/+) and CCR5 knockout (−/−) mice treated with intraperitoneal injections of normal goat serum or anti-RANTES antibody through day 12 after a live *A. fumigatus* conidia challenge. Both perivascular and peribronchial inflammation is apparent in the CCR5+/+ mice treated with NGS (*A*), whereas inflammation was predominantly observed around the vessels of CCR5−/− mice (*B*). Although infiltration around the airways and blood vessels was only moderate, interstitial inflammation was apparent in CCR5+/+ mice treated with anti-RANTES (*C*). CCR5−/− mice showed clear reduction of interstitial, perivascular, and peribronchial cell infiltrates (*D*). Original magnification was 200 53 for each photomicrograph.

mice on the same genetic background appear to be susceptible to the lethal effects of a primary pulmonary influenza infection; this response appears to be related to a massive inflammatory response rather than the cytopathic effects of an increased viral burden. However, other studies suggest that CCR5−/− mice (on a C57BL/6J×129/Ola background) effectively clear or contain systemic and pulmonary pathogens such as *Leishmania donovani* without apparent or persistent immune abnormality.

In summary, we observed that the appearance of fungal asthma in *A. fumigatus*-sensitized CCR5−/− mice was transient; allergic airway disease was only observed at one of the four time points examined after the introduction of conidia. RANTES/CCL5 appeared to mediate the transient appearance of fungal asthma in *A. fumigatus*-sensitized CCR5−/− mice through its binding to a putative alternative receptor. Finally, airway remodeling, demonstrated by goblet cell hyperplasia and peribronchial fibrosis, was attenuated in CCR5−/− mice. Although immunoneutralization of RANTES/CCL5 did not have a significant effect on goblet cell hyperplasia at the day-12 time point, the treatment did eliminate the fibrosis observed at this stage.

To conclude, while the lack of CCR5 markedly restricted the development of fungal asthma, it was apparent that its major ligand, RANTES/CCL5, could function in its absence, and neutralization of RANTES/CCL5 further reduced the hallmarks of allergic asthma (**Fig. 3**). Taken as a whole, these data suggest that CCR5 and RANTES/CCL5 are key contributors to the development and maintenance of chronic fungal asthma in the mouse model. FJ