Airway hyperresponsiveness, but not airway remodeling, is attenuated during chronic pulmonary allergic responses to Aspergillus in CCR4−/− mice[1]

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SPECIFIC AIMS

In the present study, we explored the role of CC chemokine receptor 4 (CCR4) in innate and acquired immune responses using a model of chronic fungal asthma induced by the intrapulmonary introduction of live *Aspergillus fumigatus* conidia into mice previously sensitized to soluble Aspergillus antigens.

PRINCIPAL FINDINGS

1. Fungal elements from Aspergillus conidia were rapidly cleared from the lungs of *A. fumigatus*-sensitized CCR4−/− mice, coincident with the augmented recruitment of neutrophils and macrophages into the airways of these mice

At days 3 and 7 after conidia in *A. fumigatus*-sensitized CCR4 wild-type (+/+) mice, whole lung levels of the CCR4 ligands macrophage-derived chemokine (MDC/CCL22) and thymus- and activation-regulated chemokine (TARC/CCL17) were significantly elevated above baseline levels and levels measured in whole lung samples from CCR4 knockout (−/−) mice. However, at day 30 after conidia, whole lung expression of both CCR4 ligands had returned to baseline levels in CCR4+/+ mice. As indicated by histological staining (**Fig. 1**), fungal spores and other elements were absent from the lungs of *A. fumigatus*-sensitized CCR4−/− mice at day 7 after an intratracheal challenge of 5.0×10^6 live conidia, in contrast to *A. fumigatus*-sensitized mice CCR4+/+ mice challenged in the same manner whose lungs contained fungal material at all three times (days 3, 7, and 30) examined after the conidia challenge. Since macrophages and neutrophils both participate in host defense against Aspergillus, we next examined whether the numbers of these cells were altered in *A. fumigatus*-sensitized CCR4−/− mice after the conidia challenge. Significantly greater numbers of neutrophils were detected in bronchoalveolar lavage (BAL) samples removed from CCR4−/− mice compared with CCR4+/+ mice at days 3 and 7 after conidia. Even more dramatic was the increased level of MPO, a specific marker of neutrophil activation, in whole lung samples from CCR4−/− mice compared with CCR4+/+ mice at days 3 and 7 after conidia. Significant increases in macrophage numbers in BALs from CCR4−/− mice vs. similar samples from CCR4+/+ mice were observed at all three times examined after conidia challenge. Together, these data suggested that the increased recruitment and activation of inflammatory cells with potent anti-fungal properties enhanced clearance of conidia from the airways of *A. fumigatus*-sensitized CCR4−/− mice.

2. A significant Th2-type allergic response to *A. fumigatus* was present acutely at days 3 and 7, but not chronically at day 30, after conidia challenge in *A. fumigatus*-sensitized CCR4−/− mice

Several parameters of *A. fumigatus*-induced allergic airway disease, including total serum IgE and IgG1, airway hyper-responsiveness (**Fig. 2**), T cell accumulation in the BAL, and the major Th2 cytokines IL-4, IL-5, and IL-13, were all significantly elevated in CCR4−/− mice compared with the CCR4+/+ groups at days 3 and 7 after the conidia challenge. BAL and peribronchial eosinophilia were significantly lower in CCR4−/− mice compared with CCR4+/+ mice at early times after the conidia challenge. At day 30 after conidia challenge, the CCR4−/− group exhibited significantly lower total serum IgE, airway hyper-responsiveness, airway inflammation, and major Th2 cytokine levels compared with the CCR4+/+ group. Thus, these data suggested that CCR4 expression was not required for the initiation of Th2-dependent allergic responses to Aspergillus (except for pulmonary eosinophilia), but the maintenance of these responses was dependent on CCR4 in this model of fungal asthma.

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[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.02–0193fje; to cite this article, use *FASEB J.* (June 21, 2002) 10.1096/fj.02–0193fje

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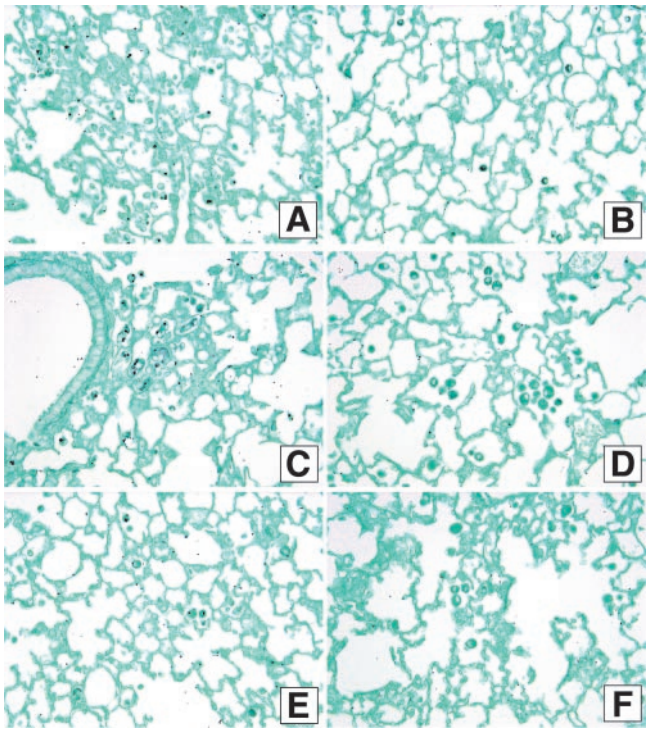

Figure 1. Gomori methenamine silver (GMS) -stained whole lung sections from CCR4+/+ (*A, C, E*) and CCR4−/− (*B, D, F*) mice at days 3 (*A, B*), 7 (*C, D*), and 30 (*E, F*). Fungal material appears black. Original magnification, 200×.

3. Chronic airway remodeling associated with fungal asthma persists in the absence of CCR4

Another chronic feature of the intrapulmonary conidia challenge in *A. fumigatus*-sensitized mice is an increase in goblet cell hyperplasia and peribronchial fibrosis. Specific staining highlighted the presence of goblet cells in the airways of CCR4+/+ mice at all three times after the conidia challenge and similar levels of goblet cell staining were detected in the airways of CCR4−/− mice at the same time points. Increased collagen deposition and peribronchial fibrosis were quantified by Sircol collagen assay, and no differences were noted in the level of collagen in whole lung samples from CCR4-deficient mice and controls at days 3, 7, or 30. This fact was reiterated via Masson trichrome staining, which revealed no significant differences in peribronchial fibrosis between knockout and wild-type mice (data not shown). Thus, airway remodeling during chronic fungal asthma was not inhibited by the lack of CCR4.

CONCLUSIONS AND SIGNIFICANCE

This murine model of chronic fungal asthma is characterized by an intricate interplay between the innate immune responses directed against the live Aspergillus conidia and the Th2-mediated events that modulate peribronchial eosinophilia, airway hyper-responsiveness, and airway remodeling. The present study demonstrated that CCR4−/− mice exhibited an aggressive anti-fungal response characterized by enhanced neutrophil function and increased macrophage recruitment into the airways. Defects in the ability of either neutrophils or macrophages to phagocytose and kill *A. fumigatus* conidia result in severe invasive lung disease and mortality. Whereas the increased prevalence and activation of neutrophils and macrophages in the lungs of CCR4−/− mice presumably account for the rapid clearance of conidia from the airways of these mice, the regulatory role that CCR4 exerts in the recruitment and/or activation of these inflammatory cells is not known.

The aggressive anti-fungal response in *A. fumigatus*-sensitized CCR4−/− mice occurred concomitantly with significantly abbreviated Th2-associated responses compared with the appropriate wild-type groups. Pulmonary eosinophilia was significantly decreased at all times examined after the conidia challenge in CCR4−/− mice. At day 3 after conidia, CCR4−/− mice had significantly higher levels of Th2 cytokines and T cell numbers were elevated in the BAL vs. their wild-type counterparts; however, Th2 cytokine levels of IL-4, IL-5, and IL-13 were significantly decreased in the lung of CCR4−/− mice at day 30. Th2 cells can develop normally in CCR4-deficient mice, but it was surprising that in vivo T cell recruitment did not seem to be affected in the CCR4−/− mice at any time after the conidia challenge in light of previous reports on the ability of CCR4 ligands to regulate the recruitment of Th2 cells. However, when allowing for the relatively small proportion of the total Th2 cell population

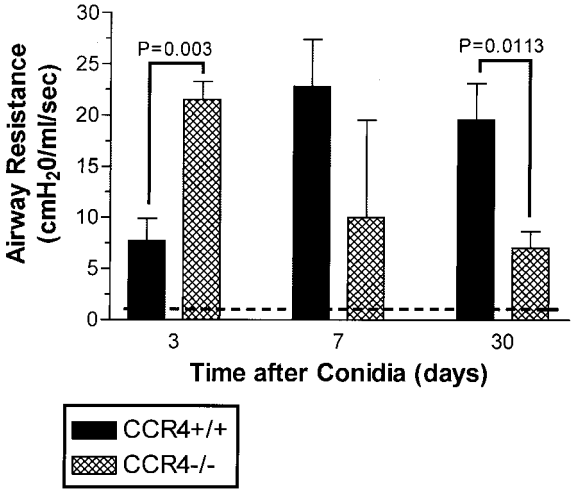

Figure 2. Airway hyper-responsiveness at days 3, 7, and 30 after *A. fumigatus* conidia challenge in *A. fumigatus*-sensitized CCR4+/+ and CCR4−/− mice. Baseline airway resistance in all groups was similar before the methacholine provocation and is indicated for both groups by the dashed line (1.6±0.5 cm H_2O/mL/s). Peak increases in airway resistance after i.v. injection of methacholine at 520 μg/kg are shown. This dose of methacholine caused maximal changes in airway hyperresponsiveness in both groups of mice. Values are expressed as mean ± SE; n = 5–7 mice/group.

comprised by any one Ag-specific clonal type, this result should be expected.

Given that our previous studies have demonstrated that eosinophils and T cells are major contributors to the airway hyper-responsiveness associated with acute Aspergillus-induced allergic airway disease and chronic fungal asthma, it is conceivable that the paucity of eosinophils and T cells around the airway contributed in part to the changes in airway hyper-responsiveness in CCR4−/− mice. However, we had observed that recruited neutrophils also appear to modulate the airway hyper-responsiveness during the first week after the installation of conidia into *A. fumigatus*-sensitized mice. Because CCR4−/− mice exhibit markedly higher numbers of activated neutrophils in their airways than their wild-type counterparts, it is possible these cells contributed to the airway hyper-responsiveness observed in these mice at days 3 and 7 after the conidia challenge. However, in the present study it was apparent that the major deficit in airway hyper-reactivity in CCR4−/− mice was observed only at day 30 after conidia when the whole lung levels of Th2 cytokines were significantly lower in the knockout group vs. the control group, illustrating that the presence of CCR4 is necessary for maintenance of the Th2 response associated with Aspergillus-induced lung disease. Studies are ongoing to more fully characterize the mechanism through which CCR4 maintains allergic airway disease.

Goblet cell hyperplasia and mucus hypersecretion are common characteristics of asthma. Goblet cell hyperplasia was not attenuated in CCR4−/− mice. As most of the other disease features in this model were attenuated or abolished, this was unexpected. A possible explanation for the maintenance of goblet cell hyperplasia in the absence of CCR4 could stem from the augmented lung neutrophil response observed in these mice during the course of the model. Previous studies have proposed that neutrophilia contributes to increased mucin expression and goblet cell degranulation. Others have suggested that enhanced numbers of neutrophils in the airways contribute to goblet cell activation in patients with chronic obstructive pulmonary disease apparently via the action of neutrophil elastase. Neutrophils have been implicated in goblet cell hyperplasia during OVA-induced allergic airway disease in guinea pigs.

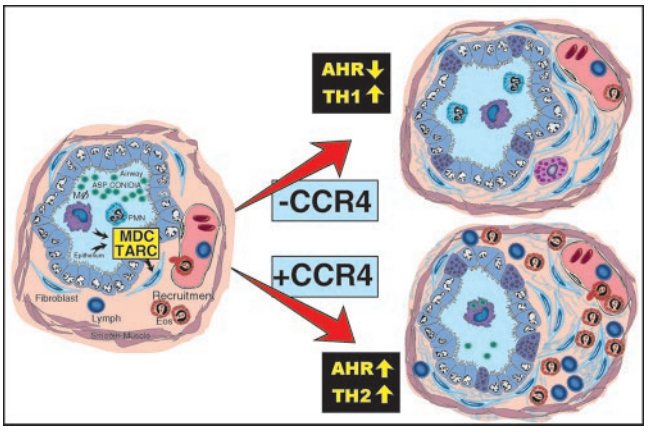

Figure 3. Summary of Aspergillus-induced airway changes in the presence and absence of CC chemokine receptor 4 (CCR4). The instillation of live Aspergillus conidia into the airways of mice previously sensitized to *A. fumigatus* evokes some immune events, including the elaboration of CCR4-specific ligands MDC/CCL22 and TARC/CCL17. In CCR4+/+ mice, chronic fungal asthma develops that at day 30 is characterized by retained fungal material, peribronchial inflammation, airway hyper-responsiveness, and a predominant Th2 cytokine response. In contrast, at the same time after conidia, CCR4−/− mice did not exhibit retention of fungal material, airway hyper-responsiveness was markedly diminished, and the systemic cytokine profile in these mice favored the generation of Th1 cytokines. However, airway remodeling characterized by goblet cell hyperplasia and peribronchial fibrosis was present to the same degree in both groups at day 30 after the conidia challenge.

In summary, we demonstrate here that the lack of CCR4 markedly alters the innate and acquired immune features of a chronic fungal allergic disease model precipitated by live fungal spores (see **Fig. 3** for summary). Although attenuating or reversing chronic remodeling features of fungal asthma may require another therapeutic strategy, CCR4 appears overall to be an attractive target during the course of chronic fungal asthma given that its absence leads to rapid clearance of fungal elements and the attenuation of many of the Th2-cytokine associated features of this disease. FJ