

## Original article

## Remission of chronic fungal asthma in the absence of CCR8

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**Background:** Experimental studies have generated conflicting data regarding the role of CCR8 in antigen-driven allergic airway disease models, thereby dampening enthusiasm for further exploration of the targeting of CCR8 in asthma.

**Objective:** Recent data show that the absence of CCR8 leads to a marked amplification of the innate immune response, and these data provided impetus for the current study, which addressed the role of this chemokine receptor in a model of fungal asthma.

**Methods:** Wild-type (CCR8<sup>+/+</sup>) and CCR8-deficient (CCR8<sup>-/-</sup>) mice were sensitized to *Aspergillus fumigatus* antigens and challenged via intra-tracheal injection with live fungal conidia, and parameters of airway hyperresponsiveness, inflammation, and remodeling were examined.

**Results:** At day 7 after conidia challenge in wild-type (CCR8<sup>+/+</sup>) and CCR8-deficient (CCR8<sup>-/-</sup>) mice sensitized to *A. fumigatus* antigens, markedly less fungal material was present in the lungs of the CCR8<sup>-/-</sup> group compared with the CCR8<sup>+/+</sup> group. At day 14 after conidia challenge, all characteristic airway physiology, inflammatory, and remodeling parameters of fungal asthma were significantly decreased or abolished in the CCR8<sup>-/-</sup> group relative to the CCR8<sup>+/+</sup> group.

**Conclusion:** Together these data show that an enhanced innate immune response in the absence of CCR8 promotes the rapid clearance of fungal material from the lung, thereby facilitating the remission of fungal asthma.

**Clinical implications:** This study shows that the clearance of fungal material from the lung was enhanced in the absence of CCR8, which suggests that this receptor may be an attractive target in fungal-allergic asthma and other fungal-associated pulmonary diseases. (J Allergy Clin Immunol ■■■■;■■■: ■■■-■■■.)

**Key words:** *Aspergillus*, CCR8, CCL1, chemokine receptor, chemokines, fungal asthma, fungal allergy, and ABPA

## Abbreviations used

AHR: Airway hyperresponsiveness  
BAL: Bronchoalveolar lavage  
Con A: Concanavalin A  
DC: Dendritic cell  
NK: Natural killer cell  
mDC: Myeloid DC  
pDC: Plasmacytoid DC  
SPF: Specific-pathogen free

Among the many pathways that have been implicated during asthmatic airway disease, interest in the chemotactic cytokines or chemokines has grown because of the major role these factors exert in inflammatory and immune cell recruitment and activation during experimental allergic airway disease.<sup>1</sup> As most chemokines appear to bind and activate several different chemokine receptors, recent targeting efforts have been directed toward the G protein coupled receptors that bind chemokines.<sup>2</sup>

Unlike most chemokine receptors, the murine and human CCR8 genes share approximately 71% homology, thus encoding for a receptor that can be activated by either human or murine CCL1.<sup>3-5</sup> CCR8 also binds other mammalian-derived and viral-derived ligands, including liver expressed chemokine or CCL16 and viral chemokine macrophage inflammatory protein II.<sup>6-8</sup> CCR8 is expressed on activated T<sub>H</sub>2 cells and NK1.1<sup>+</sup>CD4<sup>+</sup> cells, and CCL1 is a potent chemoattractant for murine T<sub>H</sub>2 cells.<sup>9</sup> CCR8 is also selectively expressed on human T<sub>H</sub>2 but not on T<sub>H</sub>1 cells<sup>9</sup>, and its selective expression identified it as a potential therapeutic target for allergic disease and asthma.<sup>10</sup> In addition, CCR8 is expressed by a variety of cells that are also relevant to asthma, including monocytes, NK cells, stimulated eosinophils, neutrophils, and monocytes.<sup>5,9,11,12</sup>

Delineation of the role of CCR8 in asthma and allergy has been aided by the generation of CCR8-deficient (CCR8<sup>-/-</sup>) mice; however, the precise role of CCR8 remains unclear given that discordant results have been obtained from acute antigen-induced models of allergic airways disease.<sup>13-16</sup> The purpose of this study was to investigate the role of CCR8 in a model of chronic *Aspergillus*-induced asthma, which involves innate and adaptive

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Supported by National Institutes of Health Research Grant HL069865 (to C.M.H.).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication August 10, 2006; revised November 7, 2006; accepted for publication December 27, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.12.660

immune processes against this clinically relevant fungus. The absence of CCR8 promoted the effective and rapid clearance of *Aspergillus* conidia from the lungs of mice at day 7 after challenge. The effective clearance of fungal material in CCR8<sup>-/-</sup> mice facilitated airway disease remission at day 14 in CCR8<sup>-/-</sup> mice as evidenced by significant reductions in airway eosinophilia, airway hyper-responsiveness (AHR), and remodeling compared with wild-type CCR8<sup>+/+</sup> mice. Mechanistically, increased activation of macrophages, increased numbers of myeloid dendritic cells (DCs) in the lung, and a shift in the cytokine balance toward IFN- $\gamma$  and IL-10 production may explain the rapid clearance of fungal material from CCR8<sup>-/-</sup> mice. Thus, CCR8 exerts a prominent modulatory effect on the innate and adaptive immune responses during chronic fungal asthma.

## METHODS

### Mice

CCR8<sup>-/-</sup> mice were generated as described previously and bred under specific-pathogen free (SPF) conditions.<sup>14</sup> The lack of CCR8 transcripts in these mice was confirmed by real-time polymerase chain reaction (RT-PCR) (not shown) and quantitative PCR analysis (Fig 1, G). Age-matched and sex-matched, SPF C57BL/6 (CCR8<sup>+/+</sup>) mice were purchased from Taconic (Hudson, NY) and maintained in an SPF facility. Committee approval for this study was obtained from the University of Michigan Medical School.

### Chronic fungal asthma model and time point data collection

Sensitization and challenge of mice to *Aspergillus fumigatus* antigens and conidia was performed as described in detail previously.<sup>17</sup> After lung function assessment, bronchoalveolar lavage (BAL), serum, and whole lung samples were analyzed also as described previously.<sup>17</sup> mRNA expression was analyzed by TaqMan quantitative RT-PCR using appropriate primer and probe sets purchased from Applied Biosystems (Foster City, Calif) and performed as described in detail previously.<sup>18</sup>

### Flow cytometry

Single cell suspensions, derived from dissected lung tissue, were labeled with fluorescein isothiocyanate-conjugated, phycoerythrin-conjugated or CY5-conjugated antibodies to CD3, CD4, CD8, CD25, CD45RB, B220, CD11b, CD11c, NK1.1, TLR2, F4/80, or Gr-1 (BD Biosciences, Franklin Lakes, NJ) as described previously.<sup>19</sup> Data for 10,000 events were acquired by flow cytometry (Beckman Coulter, Fullerton, Calif) and analyzed using FlowJo (Tree Star Inc., Ashland, Ore).

### Splenocyte restimulation

Splenocytes were derived from pooled spleens of 5 mice per group by mechanical dissociation through a 40- $\mu$ m nylon cell strainer. Red blood cells were lysed, and splenocytes were resuspended in 10% FCS/RPMI containing penicillin, streptomycin, and L-glutamine in a 6-well tissue culture plate. A total of  $1 \times 10^7$  cells/well were stimulated with either Concanavalin A (Con A) or *A fumigatus* antigens and supernatants collected after 24 hours.

### Statistical analysis

All results are expressed as mean  $\pm$  SEM. Significant differences between the 2 groups were tested with a Student *t* test, with *P* values

are represented as follows: \* or #*P*  $\leq$  .05, \*\* or ##*P*  $\leq$  .01, and \*\*\**P*  $\leq$  .001.

## RESULTS

### Divergence of whole lung cytokine and chemokine levels in CCR8<sup>-/-</sup> mice at day 3

At day 3 after conidia challenge, histologic evidence of pulmonary inflammation was similar in both CCR8<sup>+/+</sup> and CCR8<sup>-/-</sup> mice (Fig 1, A and B, and data not shown). Systemic features of allergic disease were also similarly evident in CCR8<sup>+/+</sup> and CCR8<sup>-/-</sup> mice, including similarly elevated total serum IgE and IgG2A (Fig 1, C). Enumeration of the leukocytes retrieved from BAL did not reveal any significant differences in the cellularity of the alveolar compartment in these mice at day 3 (Fig 1, D). AHR to a systemic methacholine challenge showed that both *A fumigatus*-sensitized CCR8<sup>+/+</sup> and CCR8<sup>-/-</sup> mice exhibited a similar magnitude of airway hyperreactivity (Fig 1, E). Nonsensitized groups of wild-type and knockout mice showed similar baseline responses to methacholine challenge (not shown). Thus, many inflammatory and physiological features of allergic airway disease caused by *A fumigatus* conidia challenge in *A fumigatus*-sensitized mice were unaffected by the absence of CCR8 at day 3. Nevertheless, divergence between these 2 groups of mice at this time was observed after analysis of cytokine and chemokine levels in lung and BAL fluid. For example, CCR8<sup>-/-</sup> mice had significantly less IL-4 in whole lung tissue (Fig 1, F) and significantly increased levels of CCL2 in their BAL fluid compared with CCR8<sup>+/+</sup> mice at day 3 (Fig 1, F). Also, significantly greater mRNA transcript existed for CCL1, CCL17, and CCL24 in the lungs of the CCR8<sup>-/-</sup> mice compared with the CCR8<sup>+/+</sup> group at day 3 (Fig 1, G). Although these data suggested that many features of fungal asthma were similar in both the CCR8<sup>+/+</sup> and the CCR8<sup>-/-</sup> groups, the changes in IL-4 and CCL2 were impetus to examine later time points in this model.

### Fungal clearance at day 7 after conidia challenge was enhanced in the absence of CCR8

Fungal material was mainly internalized within mononuclear cells in whole lung sections from CCR8<sup>+/+</sup> mice at day 7 after conidia challenge (Fig 2, A), whereas few mononuclear cells contained fungal material in lung sections from CCR8<sup>-/-</sup> lungs at this time (Fig 2, B), which suggests that fungal clearance was accelerated in the absence of CCR8. Also a great abundance of highly activated macrophages or giant cells was observed in whole lung histologic sections from the CCR8<sup>-/-</sup> group (Fig 2, B). CCR8<sup>-/-</sup> mice had a significantly greater total serum IgE compared with their corresponding CCR8<sup>+/+</sup> group (Fig 2, C). A reduction in eosinophils was evident in the BAL fluid of the CCR8<sup>-/-</sup> mice compared with the wild-type group (Fig 2, D). AHR was significantly elevated above baseline in the CCR8<sup>+/+</sup> and CCR8<sup>-/-</sup> groups, but the increase was less in the CCR8<sup>-/-</sup> group

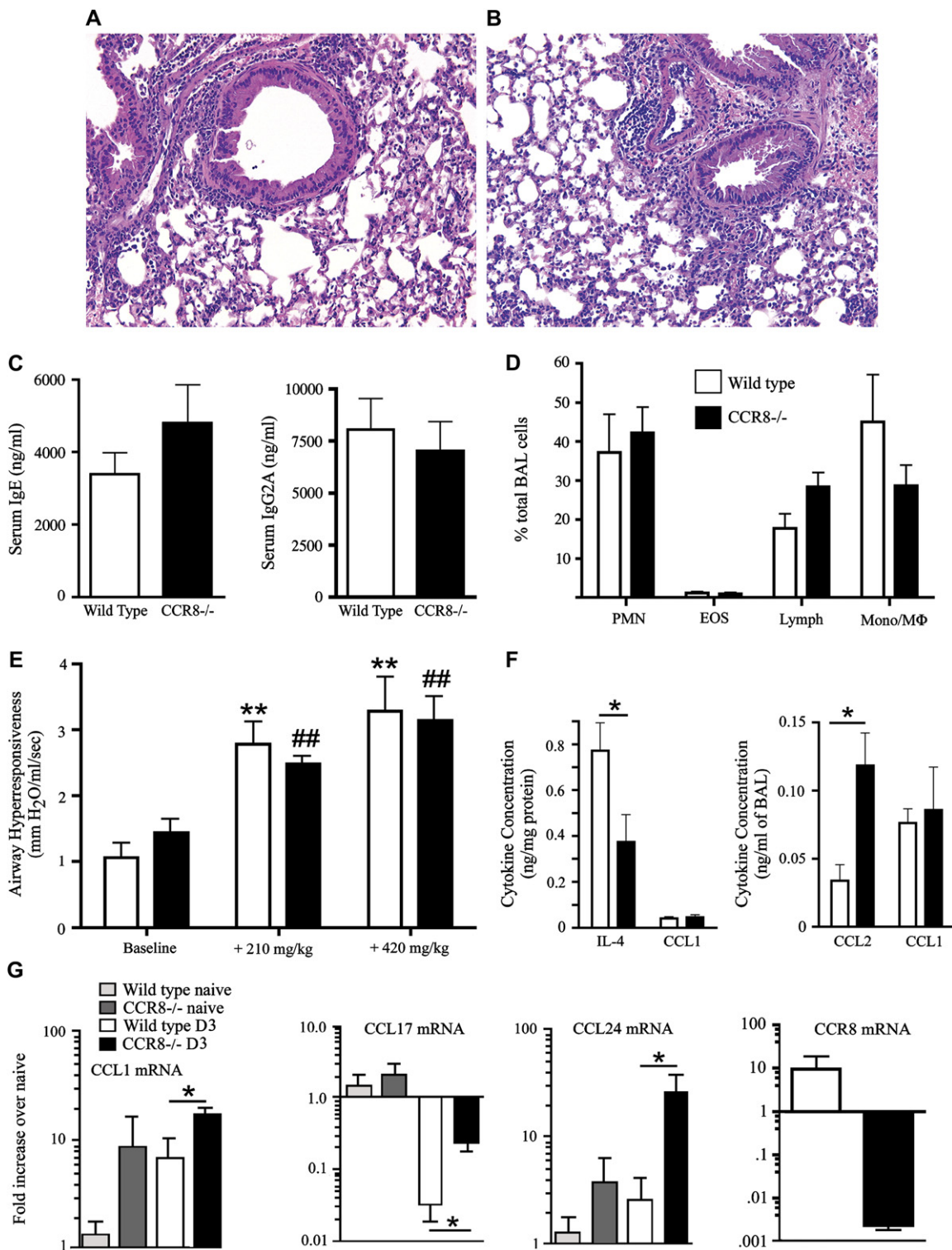


**FIG 1.** Detailed examination of the features of allergic airways disease at day 3 after conidia challenge. Representative photomicrographs of hematoxylin and eosin–stained histologic lung sections from CCR8<sup>+/+</sup> (A) and CCR8<sup>-/-</sup> (B) mice; original magnification  $\times 200$ . C, Serum IgE and IgG2A. D, Quantification of BAL leukocytes. E, AHR after methacholine provocation; statistical significance is compared with baseline values. F, Cytokine protein content in the lung tissue and BAL. G, Cytokine mRNA in the lung,  $n = 5$ . \* $P \leq .05$ ; \*\* $P \leq .01$  compared with baseline airway hyperresponsiveness in wild type; ### $P \leq .01$  compared with baseline airway hyperresponsiveness in CCR8<sup>-/-</sup>.

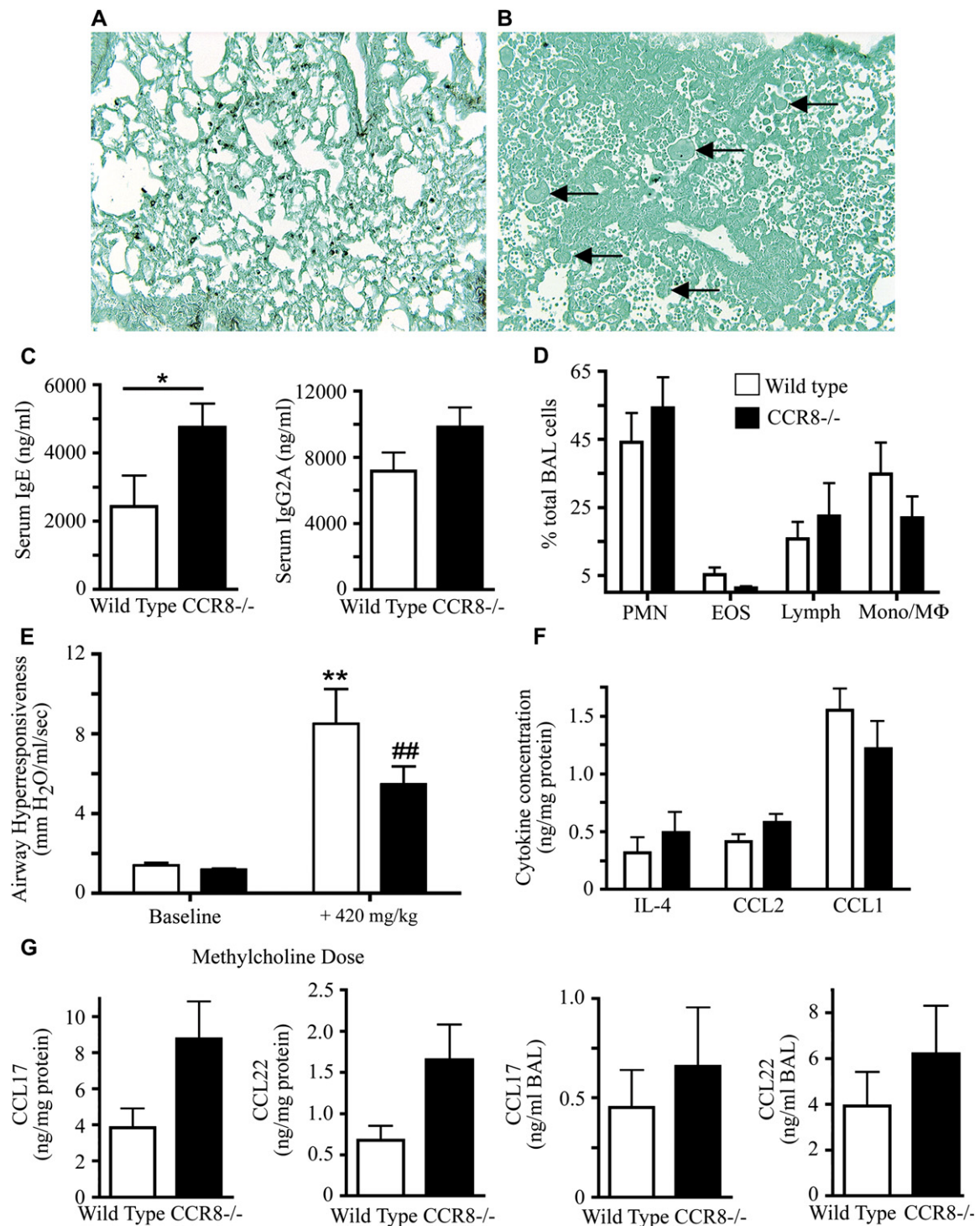


**FIG 2.** Examination of the features of allergic airways disease at day 7 after conidia challenge. Giant cells are depicted by arrows in photomicrographs of Gomori Methenamine Silver-stained histologic lung sections from CCR8<sup>+/+</sup> (**A**) and CCR8<sup>-/-</sup> (**B**) mice; original magnification  $\times 200$ , representative of  $n = 5$ . **C**, Serum IgE and IgG2A. **D**, Quantification of BAL leukocytes,  $n = 5$ . **E**, AHR after methacholine provocation; statistical significance is compared with baseline values,  $n = 3-4$ . **F**, Cytokine protein content in the lung tissue and BAL,  $n = 5$ . **G**, Cytokine protein content in lung tissue and BAL,  $n = 5$ . \* $P \leq .05$ ; \*\* $P \leq .01$  compared with baseline airway hyperresponsiveness in wild type; ## $P \leq .01$  compared with baseline airway hyperresponsiveness in CCR8<sup>-/-</sup>.

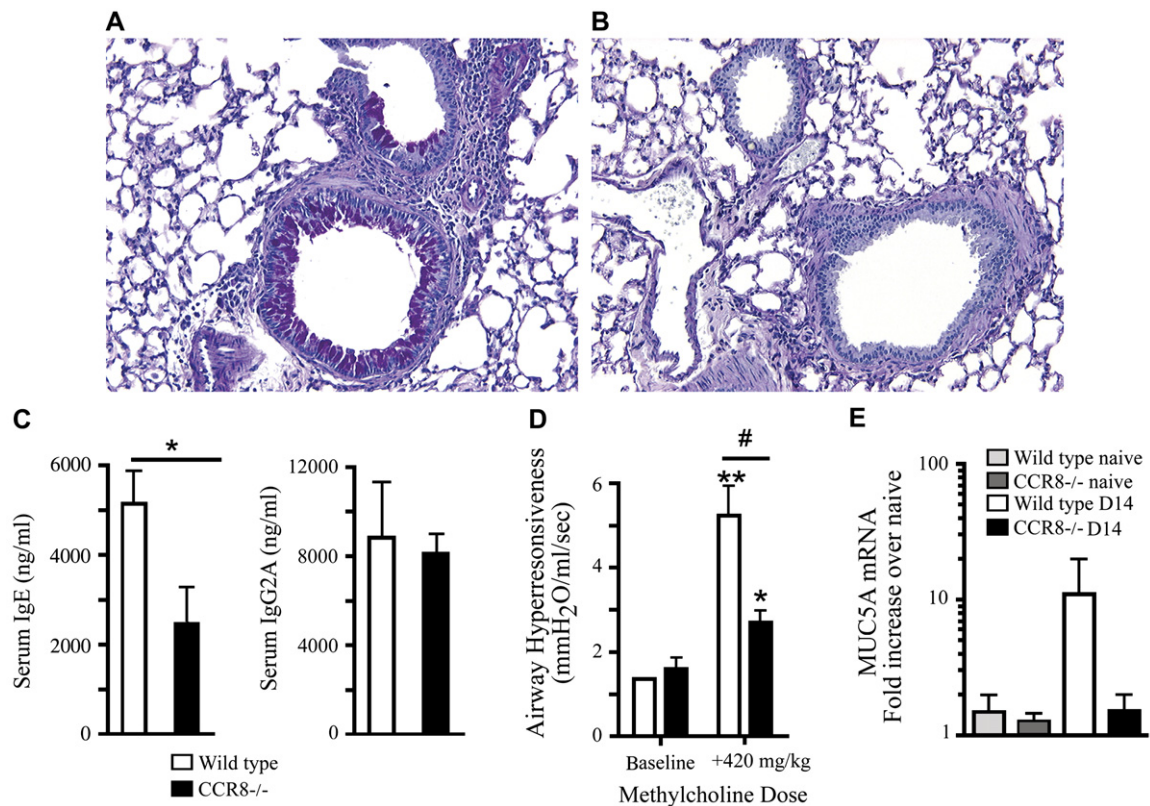


**FIG 3.** Reduced physiological and remodeling parameters of allergic airways disease at day 14 postconidial challenge. Representative photomicrographs of periodic acid-Schiff-stained histologic lung sections from CCR8<sup>+/+</sup> (A) and CCR8<sup>-/-</sup> (B) mice; original magnification  $\times 200$ . C, Serum IgE and IgG2A. D, Measurement of AHR after methacholine provocation; statistical significance is compared with baseline values. E, MUC5A mRNA detected in the lung,  $n = 5$ . \* $P \leq .05$ ; \*\* $P \leq .01$  compared with baseline airway hyperresponsiveness in wild type; # $P \leq .05$  compared with baseline airway hyperresponsiveness in CCR8<sup>-/-</sup>.

(Fig 2, E). Analysis of whole lung cytokine and chemokine levels at day 7 revealed few differences between these groups except that CCL17 and CCL22 were elevated in both the BAL and the whole lung samples taken from the CCR8<sup>-/-</sup> group (Fig 2, F and G). Together, these data suggested that the absence of CCR8 had a profound effect on the innate immune response to *A fumigatus* conidia.

### Demonstrably decreased allergic airways disease in CCR8<sup>-/-</sup> mice was apparent at day 14

At day 14 after conidia in this model of chronic fungal asthma, airways typically exhibit peak goblet cell metaplasia and mucus overproduction.<sup>17</sup> Representative histologic sections from the lungs of mice 14 days postconidia challenge revealed that the absence of CCR8 was associated with an absence of mucus staining (Fig 3, A and B). In accordance with the attenuation of histologically evident mucus cell metaplasia, whole lung transcript levels of the mucus gene MUC5A were decreased 10-fold in the CCR8<sup>-/-</sup> group compared with the wild-type group (Fig 3, E). Furthermore, the level of serum IgE in CCR8<sup>-/-</sup> mice was significantly lower than serum IgE

levels in the wild-type mice at day 14 after conidia, and levels of this Ig in the CCR8<sup>-/-</sup> group were 50% lower than those measured 7 days previously in this group (ie,  $2463.6 \pm 816.7$  ng/mL vs  $4747.1 \pm 705.9$  ng/mL, Fig 3, C). At day 14 after conidia challenge, AHR was significantly reduced in the CCR8<sup>-/-</sup> group compared with the CCR8<sup>+/+</sup> group (Fig 3, D). Thus, major inflammatory, physiological, and remodeling parameters of chronic fungal asthma were attenuated or abolished in the absence of CCR8.

### Flow cytometric analysis of whole lung immune cell populations during chronic fungal asthma in CCR8<sup>+/+</sup> and CCR8<sup>-/-</sup> mice

As CCR8 is upregulated on several cells relevant to asthma, we further examined the presence of T cells, granulocytes, monocytes, macrophages, and DCs into the lungs of both groups of mice. No differences in CD4<sup>+</sup> or CD8<sup>+</sup> cells in the lung were observed at either time point after conidia (Table I), nor did the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> cells differ between CCR8<sup>-/-</sup> and CCR8<sup>+/+</sup> mice (data not shown). No significant differences in the population size of activated and/or regulatory CD4<sup>+</sup> T lymphocytes were observed between CCR8<sup>-/-</sup> and wild-type groups,

**TABLE I.** Quantification of lung cell populations by flow cytometric identification of typical cell surface markers

| Cell population                                     | Day 7 (% gated) |                     | Day 14 (% gated) |                     | Day 21 (% gated) |                     |
|-----------------------------------------------------|-----------------|---------------------|------------------|---------------------|------------------|---------------------|
|                                                     | Wild-type       | CCR8 <sup>-/-</sup> | Wild-type        | CCR8 <sup>-/-</sup> | Wild-type        | CCR8 <sup>-/-</sup> |
| CD4 <sup>+</sup>                                    | 7.93 ± 1.34     | 7.73 ± 0.65         | 12.62 ± 0.73     | 12.58 ± 1.46        | 9.16 ± 0.92      | 9.09 ± 1.01         |
| CD8 <sup>+</sup>                                    | 4.23 ± 0.93     | 3.53 ± 0.88         | 5.41 ± 2.91      | 2.91 ± 0.47         | 4.81 ± 0.55      | 3.88 ± 0.59         |
| CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>+</sup> |                 |                     | 1.66 ± 0.61      | 6.92 ± 1.02         | 0.28 ± 0.14      | 0.04 ± 0.01         |
| CD3 <sup>+</sup> CD8 <sup>+</sup> CD25 <sup>+</sup> |                 |                     | 1.54 ± 0.49*     | 10.36 ± 2.65*       | 1.33 ± 0.30      | 1.10 ± 0.35         |
| CD11c <sup>+</sup>                                  | 46.94 ± 14.52   | 78.36 ± 25.34       |                  |                     | 4.59 ± 0.58      | 5.31 ± 0.59         |
| Gr <sup>-</sup> 1 <sup>+</sup>                      | 4.09 ± 0.53     | 3.95 ± 0.58         | 2.82 ± 0.16      | 2.61 ± 0.08         | 7.45 ± 0.67      | 8.36 ± 2.09         |
| F4/80 <sup>+</sup>                                  | 1.59 ± 0.35     | 1.65 ± 0.28         | 2.43 ± 0.18      | 2.23 ± 0.25         |                  |                     |

| DC population | Day 7 (% gated) |                     | Phenotype |
|---------------|-----------------|---------------------|-----------|
|               | Wild type       | CCR8 <sup>-/-</sup> |           |
| CD11c+CD11b+  | 3.82 ± 0.71†    | 9.97 ± 1.31†        | mDC       |
| CD11c+B220+   | 0.24 ± 0.03     | 0.15 ± 0.03         | pDC       |

\**P* < .05 compared with day 0.

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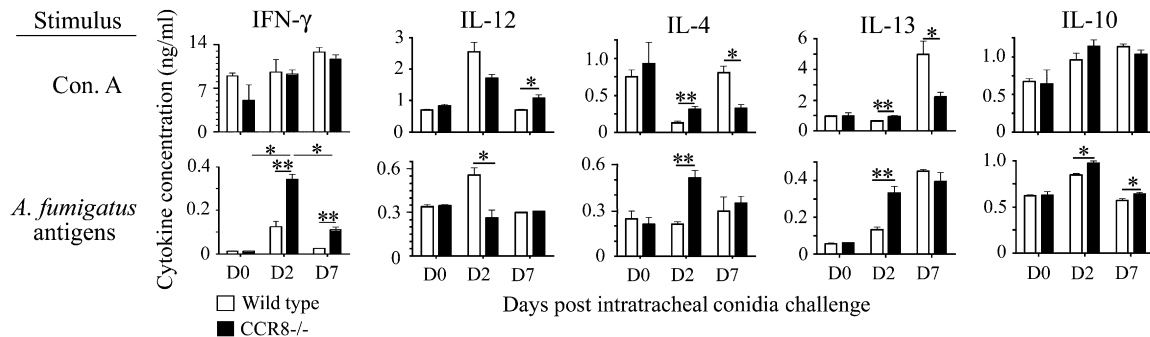


**FIG 4.** Cytokine production by antigen-restimulated splenocytes. Cytokine production by whole spleen cell populations from *A fumigatus*-sensitized CCR8<sup>-/-</sup> (open bars) and CCR8<sup>+/+</sup> (closed bars) mice before (ie, day 0) and at days 2 or 7 after conidia cultured for 24 hours with Con A (10 µg/mL) or *A fumigatus* antigens (1 µg/mL); data shown are means ± SEMs from 3 replicate wells of cells pooled from 5 mice. \**P* ≤ .05; \*\**P* ≤ .01.

but a significantly increased percentage of CD3<sup>+</sup>CD8<sup>+</sup>CD25<sup>+</sup> lymphocytes in the CCR8<sup>-/-</sup> group was found (Table I). Using selective markers to identify plasmacytoid DCs (pDCs), and myeloid DCs (mDCs), a significantly increased proportion of mDCs were present in the CCR8<sup>-/-</sup> group compared with the CCR8<sup>+/+</sup> group (Table I). Thus, although T-cell, granulocyte, monocyte, and macrophage recruitment was not altered in the absence of CCR8, a major increase in mDCs was present in CCR8<sup>-/-</sup> mice compared with wild-type mice.

### The cytokine response by *A fumigatus* antigen restimulated CCR8<sup>-/-</sup> splenocytes is dominated by IFN-γ and IL-10

Given that T-cell recruitment was not altered in the absence of CCR8, more examination of the cytokine responses by antigen rechallenged mixed splenocyte populations was examined. At day 2 after conidia challenge, Con A stimulated greater production of T<sub>H</sub>2-type cytokines IL-4 and IL-13 in CCR8<sup>-/-</sup> compared with

CCR8<sup>+/+</sup> splenocyte cultures, whereas at day 7 this was reversed (Fig 4). Less IL-12 was produced by Con A-activated CCR8<sup>-/-</sup> splenocytes isolated at day 2 and more IL-12 in the CCR8<sup>-/-</sup> cells from day 7. IFN-γ and IL-10 levels promoted by Con A did not differ between the 2 groups. Compared with cytokine responses generated in the presence of Con A, splenocytes stimulated with *A fumigatus* antigens released less IFN-γ, IL-4, IL-12, and IL-13, whereas IL-10 was similar. At day 2 after conidia challenge, CCR8<sup>-/-</sup> splenocytes respond to *A fumigatus* antigens with a substantial amount of IFN-γ, which was significantly greater than that produced by CCR8<sup>+/+</sup> splenocytes and a 10-fold increase over the IFN-γ produced at day 0. The enhanced IFN-γ production in the CCR8<sup>-/-</sup> group versus the CCR8<sup>+/+</sup> group was also observed in cultures from day 7 mice. IL-10 production was significantly greater in the CCR8<sup>-/-</sup> cells compared with the CCR8<sup>+/+</sup> splenocytes from days 2 and 7 splenocyte cultures challenged with *A fumigatus* antigens (Fig 4). The enhanced IL-10 production was only observed with

*A. fumigatus* and not with Con A stimulation and, therefore, was antigen-specific. Thus, these data indicate that the activation of splenocytes differed substantially depending on the presence or absence of CCR8.

## DISCUSSION

Asthma is characterized by AHR, chronic inflammation of the airways, reversible airways obstruction, and airways remodeling.<sup>20</sup> The chronicity of this lung disease seems to be, in part, caused by repeated exposure to specific environmental allergens such as insect byproducts, animal danders, and fungi such as *A. fumigatus*.<sup>21-23</sup> In the current study, we employed a model of fungal asthma initiated by a live conidial challenge in *A. fumigatus*-sensitized mice, to examine the immunologic contribution of CCR8 to the pathology of this condition.<sup>17</sup> The use of conidia to model chronic fungal asthma permitted the examination of CCR8's role in the link between innate and adaptive immunity in the context of an allergic condition. *A. fumigatus*-sensitized and challenged CCR8<sup>-/-</sup> mice exhibited an enhanced anti-fungal response at day 7 and a marked remission of many features of allergic airway disease at day 14 after conidia challenge. The remission of allergic airway disease in CCR8<sup>-/-</sup> mice seemed to be associated with enhanced macrophage activation, increased mDC numbers in the lung, and enhanced *A. fumigatus*-antigen driven IFN- $\gamma$  and IL-10 in the spleen. Thus, CCR8 is required for the maintenance of chronic fungal asthma.

The persistence of fungal material in the lungs of allergic mice after conidial exposure has been shown to explain the chronicity of the allergic airway response in this model, as the fungal material supplies a continual allergen exposure.<sup>19,24</sup> From the current study, histologic sections showed that fungal material was cleared from the lungs of CCR8<sup>-/-</sup> mice, whereas considerable fungal material was present in the lungs of the wild-type mice at days 7 and 14 postconidia challenge. One explanation for the rapid clearance of the fungus from the lungs of CCR8<sup>-/-</sup> mice may lie in enhanced macrophage activation in the lung, which was apparent histologically by the presence of giant cells or multinucleated macrophages in the lungs of these mice.<sup>25</sup> Isolated CCR8<sup>-/-</sup> macrophages have previously been shown to exhibit augmented microbicidal activities and when stimulated with LPS produce higher levels of superoxide anion,  $\beta$ -glucuronidase, and nitric oxide compared with CCR8<sup>+/+</sup> macrophages.<sup>26</sup> Likewise the enhanced phagocytic activity of macrophages in *A. fumigatus*-sensitized CCR8<sup>-/-</sup> mice may explain the rapidity of fungal clearance. Alternatively, the increased clearance of fungal material in the CCR8<sup>-/-</sup> group maybe explained by elevated CCL2 levels. Both CCL2 and its receptor have been shown to have an important role in the immune response to *A. fumigatus*, limiting the allergic remodeling responses during chronic fungal asthma.<sup>27-29</sup> Finally, the increased presence of mDCs in the CCR8<sup>-/-</sup> group may also enhance the clearance of fungus as this cell population is critical in the antifungal response.<sup>18,30,31</sup>

Thus, several immune-related protein and cellular elements seemed to combine to enhance the clearance of fungal material from the lungs of mice lacking CCR8, and future studies in this model will address the relative importance of these elements during the progression of fungal asthma in these mice.

The restricted expression of CCR8 suggested the CCR8-CCL1 axis as a mechanism for the selective induction of migration and activation of T<sub>H</sub>2-type cells during inflammation and perhaps also in normal immune homeostasis.<sup>32-34</sup> Chensue et al<sup>14</sup> originally reported that CCR8<sup>-/-</sup> mice had a reduced T<sub>H</sub>2 cell response and a specific reduction in eosinophil recruitment in 2 acute models of allergic pulmonary inflammation. However, these findings were not substantiated in subsequent studies in which acute allergic airway diseases to similar soluble allergens were examined.<sup>15,16</sup> Coinciding with the latter studies, we noted minor effects on T-cell and eosinophil recruitment into the lungs of CCR8<sup>-/-</sup> mice. Nevertheless, our findings support the former study that, in the absence of CCR8, whole lung IL-4 levels were significantly lower at day 3 after conidia challenge, and AHR and mucus cell metaplasia eventually subsided. Another explanation for the remission of fungal asthma in CCR8<sup>-/-</sup> mice may relate to the changes in adaptive immune responses in these mice. Specifically, *A. fumigatus* antigens significantly promoted the generation of IFN- $\gamma$  and IL-10 in splenocyte cultures from CCR8<sup>-/-</sup> mice. The precise source of these cytokines is unknown, but Th1 and T regulatory cells, respectively, are candidates. IFN- $\gamma$  has a major inhibitory effect on the maintenance of chronic fungal asthma.<sup>35</sup> IL-10-producing CD4<sup>+</sup>CD25<sup>+</sup> T regulatory cells respond to CCR8 ligands in chemotaxis assays.<sup>36</sup> Both IFN- $\gamma$  and IL-10 appropriately modulate the innate immune response to *A. fumigatus* to promote fungus clearance without excessive tissue injury.<sup>37,38</sup> Thus, the absence of CCR8 seems to favor the generation of cytokines that modulate innate and adaptive features of this model.

In summary, CCR8 alters the innate and adaptive immune responses during experimental fungal asthma. Deletion of CCR8 enhanced several features of the anti-fungal response, thereby leading to the remission of allergic airway disease caused by *A. fumigatus* conidia. Thus, these studies suggest that CCR8 has a pivotal role in the progression of fungal asthma and further suggest that this receptor may be an attractive target in diseases mediated by this pathologic fungus.

We thank Ms Robin Kunkel for her assistance and Drs Joost Smit and Mathew Schaller for helpful discussions.

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