Immunosuppressive Effects of CCL17 on Pulmonary Antifungal Responses during Pulmonary Invasive Aspergillosis

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Aspergillus fumigatus-sensitized CCR4-deficient (CCR4−/−) mice exhibit an accelerated clearance of conidia during fungal asthma. In the present study, we examined the roles of CCL17 and CCL22, two CCR4 ligands, during pulmonary invasive aspergillosis in neutropenic mice. Kaplan-Meier survival curve analysis revealed that wild-type C57BL/6 (CCR4+/+) mice were significantly protected from the lethal effects of Aspergillus compared with their wild-type controls following systemic neutralization with anti-CCL17 but not anti-CCL22 antibodies. Systemic neutralization of CCL17 significantly increased whole-lung CCL2 levels. Mouse survival and histological analysis revealed that the receptor mediating the deleterious effects of CCL17 was CCR4 since mice genetically deficient in CCR4 (CCR4−/−) did not develop invasive aspergillosis. Enzyme-linked immunosorbent assay analysis of whole-lung samples at day 2 after conidial challenge in neutrophil-depleted CCR4−/− and CCR4+/+ mice revealed that whole-lung IL-12 levels were significantly increased in the CCR4−/− group compared with the wild-type group. Also at day 2 after conidial challenge, significantly greater numbers of CD11c+ F4/80+ and CD11c+/CD86+ but fewer CD3/NK1.1+ cells were present in the lungs of CCR4−/− mice compared with their wild-type counterparts. Thus, CCL17-CCR4 interactions dramatically impair the pulmonary antifungal response against A. fumigatus in neutropenic mice.

Aspergillus fumigatus is a major clinical menace in developed countries given its prevalence and ability to elicit a number of diseases, including asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis, aspergillosis, saprophytic involvement of infected tissue, chronic necrotizing pulmonary aspergillosis, and invasive aspergillosis (25). A number of factors participate in the development of Aspergillus-associated diseases, and these include the virulence of the fungal strain, the magnitude or duration of the exposure, and, probably most important, the status of host defense mechanisms and the type of exposure (25). All organs appear to be susceptible to infection, but pulmonary aspergillosis is the most common disease associated with this airborne saprophytic fungi (14). The spores or conidia from Aspergillus pose few problems for immunocompetent individuals; however, any perturbation to the systemic or pulmonary mucosal immune systems can lead to severe and usually fatal invasive aspergillosis (40), the incidence of which has increased alarmingly in this era of modern medicine (41).

Considerable research attention has been directed toward understanding the major immune factors that are required to contain the growth of A. fumigatus, particularly in the immunosuppressed host (56). Some very exciting antifungal strategies which work in the immunocompromised host have been elucidated, and these include manipulation of cytokine (16, 19, 47, 52) and chemokine levels (48, 51); anti-idiotypic monoclonal antibodies (18); they include manipulation of cytokine (16, 19, 47, 52) and chemokine levels (48, 51); anti-idiotypic monoclonal antibodies (18); and various vaccination strategies involving T cells (15), NK cells (51), and immunocompetent (11, 12, 54) or immature (6) dendritic cells. Although impressive progress has been made, further experimental work is required to fully elucidate the mechanisms responsible for protective pulmonary immunity against A. fumigatus.

Previous studies demonstrated that A. fumigatus-sensitized mice lacking CCR4, the chemokine receptor that binds thymus and activation-regulated chemokine (TARC/CCL17) (34) and macrophage-derived chemokine (MDC/CCL22) (35), due to gene deletion rapidly cleared A. fumigatus conidia from their lungs, whereas wild-type mice did not (57). This finding was surprising in light of other studies demonstrating the major enhancing effect of CCL22 on innate immune responses directed by monocytes, dendritic cells, and NK cells (29, 37, 44). However, CCL17, which is predominately generated by non-immune cells (27, 34), does not share CCL22’s effects on the innate immune response. In fact, enhanced production of this chemokine is driven by Th2 cytokines (26) and has a major enhancing role in highly skewed Th2-type immune responses, including asthma (7, 9, 39, 59), atopic dermatitis (61, 62), fulminant liver failure (63), and Schistosoma mansoni egg-induced pulmonary granulomatous responses (37). The differential roles of these two chemokines in immune responses may be due to the ability of CCL17 but not CCL22 to bind CCR8 (8). In addition, we recently observed that, unlike CCL22, CCL17 had a major immunosuppressive effect in a murine model of S. mansoni-induced pulmonary granuloma formation (37).

Thus, the present study addressed the roles of CCL17, CCL22, and their common chemokine receptor in a well-characterized murine model of invasive aspergillosis. The neutralization of CCL17 or the lack of CCR4, but not CCR8, markedly enhanced mouse survival following the bolus administration of A. fumigatus conidia to neutrophil-depleted mice on a C57BL/6 background. This protective effect was associated with significant increases in the antifungal chemokine

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CCL2 (51) and interleukin-12 (IL-12). In addition, the numbers of activated CD11c+ F4/80+ and CD11c+ CD86+ cells were significantly higher in the lungs of neutrophil-depleted CCR4−/− mice at day 2 after conidial challenge compared with neutrophil-depleted CCR4−/− mice at the same time after conidial challenge. Thus, CCL17 via CCR4 activation markedly impaired the innate mononuclear cell response to Aspergillus conidia, thereby permitting the development of invasive pulmonary aspergillosis.

MATERIALS AND METHODS

Mice. Specific-pathogen-free female C57BL/6 (wild type, CCR4−/−, or CCR8−/−) mice (6 to 8 weeks of age) were purchased from Taconic (German-town, NY). CCR4 mice were generated as previously described in detail (22) and backcrossed 10 generations onto the C57BL/6 genetic background. Tassie Collins (Amgen Inc., South San Francisco, CA) kindly provided breeding pairs of and backcrossed 10 generations onto the C57BL/6 genetic background. Sergio Lira (Mount Sinai School of Medicine) kindly provided breeding pairs of the appropriately backcrossed CCR4−/− mice. CCR8−/− mice were generated at previously described in detail (21) and backcrossed 10 generations onto the C57BL/6 genetic background. Sergio Lira (Mount Sinai School of Medicine) kindly provided breeding pairs of the appropriately backcrossed CCR4−/− mice. Breeding colonies of CCR4−/− and CCR8−/− mice were maintained under specific-pathogen-free conditions in the University Laboratory of Animal Medicine (ULAM) facility. As previously described, CCR4−/− (22) and CCR8−/− (21) mice were born at the expected Mendelian ratios and showed no evidence of abnormal growth patterns. Prior approval for mouse usage in the present study was obtained from the ULAM.

Murine model of invasive aspergillosis. All mice used in the present study were depleted of neutrophils with an intraperitoneal injection of 100 μg of Rb6-C5 (anti-Ly6G) as previously described in detail (46). At 24 and 72 h after Rb6-C5 antibody injection, peripheral blood neutrophil count was reduced to less than 50 cells/μl (46). In the present study, all mice were anesthetized with a mixture of ketamine and xylazine (53) prior to receiving 5.0 mg/kg of A. fumigatus conidia suspended in 30 μl of 0.1% Tween 80 via an intratracheal (i.t.) injection (32) at 24 h after Rb6-C5 administration. A. fumigatus strain 13073 (American Type Culture Collection, Manassas, VA) was used since this is a highly virulent Aspergillus strain (49) which elicits a reproducible form of invasive aspergillosis in Rb6-C5-treated mice (46).

In the first series of experiments, neutrophil-depleted wild-type mice received goat immunoglobulin G (IgG), goat anti-mouse polyclonal anti-CCR4, or goat anti-mouse polyclonal anti-CCR2 (all at 20 μg/dose; R&D Systems, Minneapolis, MN) beginning 2 h prior to the i.t. conidial challenge and IgG or specific antibodies were readministered every 48 h thereafter. We have previously used anti-CCR4 and anti-CCR2 agonists for effective neutralization of chemokine activity in mice (37).

In the second series of experiments, groups of wild-type, CCR4−/−, and CCR8−/− mice were rendered neutropenic and challenged with 5 × 106 conidia. In both series of experiments, groups of seven mice were monitored for survival after the i.t. injection of conidia for 6 days. Additional groups of five mice were killed at designated time points after conidial challenge by anesthesia overdose and bled, and bronchoalveolar lavage (BAL) and whole-lung samples were processed as described below. The designated time points in this study were days 0 (i.e., 24 h after Rb6-C5 and immediately prior to conidial challenge), 2, and 6 after conidial challenge.

ELISA analysis. Murine CCL1, CCL17, CCL22, CCL2, CCL3, tumor necrosis factor alpha (TNF-α), gamma interferon (IFN-γ), IL-10, and IL-12 levels were determined in 50-μl samples from whole-lung homogenates by a standardized sandwich enzyme-linked immunosorbent assay (ELISA) technique provided by the supplier (R&D Systems, Minneapolis, MN). Each ELISA was screened to ensure the specificity of each antibody used. Recombinant murine cytokines or chemokines (R&D Systems, Minneapolis, MN) 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. Cytokine and chemokine levels were normalized to total protein levels with the Bradford assay.

Chitin analysis. Chitin is a major component of the hyphal wall and is not present in conidia, nor is it present in mammalian tissues. This hyphal wall component is often quantified as a measure of the fungal load in the lung, rather than relying on the dilution and culture of lung homogenates, which may underestimate the number of hyphae present in these samples. Whole-lung samples were processed as described in detail elsewhere (51). The chitin content in each sample was expressed as μg of glucosamine per lung.

RESULTS

Bronchoalveolar lavage levels of the CCR4 agonists CCL22 and CCL17 were significantly elevated at day 2 after conidial challenge in neutrophil-depleted CCR4−/− mice. Prior to the $A. fumigatus$ conidial challenge, baseline whole-lung levels of CCL22 and CCL17 in CCR4−/− mice were similarly below 250 pg/ml, whereas immunoreactive CCL1 levels were below the level of ELISA detection (Fig. 1A). Both CCL22 and CCL17 were elevated at days 2 and 6 after conidial challenge in neutrophil-depleted CCR4−/− mice. Elevations in whole-lung levels of CCL22 and CCL17 at days 2 and 6 after conidial challenge reached statistical significance compared with immunoreactive levels of both chemokines at day 0. At all three time points, CCL1 levels were consistently below the limits of ELISA detection in whole-lung samples.

In BAL samples, CCL22, CCL17, and CCL1 were not detected immediately prior to $A. fumigatus$ conidial challenge in CCR4−/− mice (Fig. 1B). However, at day 2 after conidial challenge, both CCL22 and CCL17 were significantly increased compared with day 0 levels. At this time, BAL CCL17 levels were approximately threefold greater than BAL CCL22 levels (Fig. 1B). At day 6 after conidial challenge, BAL levels of both CCR4 agonists were again below the limits of ELISA detection. Again, ELISA did not detect CCL1 in BAL samples either before or after conidial challenge.

Neutrophil-depleted C57BL/6 mice were highly resistant to invasive pulmonary aspergillosis following neutralization of CCL17. The roles of CCL22 and CCL17 during invasive aspergillosis in CCR4−/− mice were examined next. As shown in Fig. 2 (top panel), the neutralization of CCL17 significantly ($P \leq 0.05$; log rank test) improved survival of neutrophil-depleted CCR4−/− mice compared with neutrophil-depleted
Neutrophil-depleted CCR4<sup>−/−</sup> mice that received IgG at the same time points. At day 6 after conidial challenge, approximately 85% of CCR4<sup>−/−</sup> mice receiving anti-CCL17 were alive. In contrast, 57% and 43% of mice that received anti-CCL2 antibody and IgG, respectively, were alive at the same time point (Fig. 2, top panel). Thus these data suggested that CCL17 exerted an inhibitory effect on the innate antifungal response.

**Significantly elevated whole-lung CCL2 levels were present in anti-CCL17 antibody-treated mice.** Whole-lung ELISA analyses for TNF-α, IL-12, CCL2, and CCL3 are shown in Fig. 2, bottom panel. At day 6 after conidial challenge in neutropenic wild-type mice, whole-lung levels of TNF-α, IL-12, and CCL3 did not differ among the treatment groups examined. These groups included mice that received 20 μg of IgG, anti-CCL17, or anti-CCL22 at 2 h prior to and at days 2, 4, and 6 after conidial challenge. Whole-lung levels of CCL2 were significantly greater in the anti-CCL17-treated group than in the other two groups of treated mice at day 6 after conidial challenge (Fig. 2, bottom panel). Thus, the neutralization of CCL17 appeared to promote the generation of CCL2 during pulmonary invasive aspergillosis.

**Significantly elevated whole-lung TNF-α, IL-12, CCL2, and CCL3 levels in CCR4<sup>−/−</sup> mice.** TNF-α (47, 52, 55) and IL-12 (54, 60) have been shown to have protective effects in murine models of invasive aspergillosis. In the present study, ELISA analysis of whole-lung samples at days 2 and 6 after conidial challenge revealed marked differences in the temporal changes of these two important innate immune effector cytokines. As shown in Fig. 6, TNF-α levels were increased at day 2 after conidial challenge in both the CCR4<sup>−/−</sup> and CCR4<sup>−/−</sup> groups,
but the increase in TNF-α in CCR4−/− mice reached statistical significance (compared with TNF-α levels in the group immediately prior to conidial challenge; i.e., day 0) (Fig. 6). The temporal patterns of expression of whole-lung IL-12 also differed between the two groups of mice. Specifically, IL-12 levels dropped significantly at day 2 after conidial challenge in the CCR4−/− group. Conversely, whole-lung IL-12 levels in the CCR4−/− group at days 2 and 6 after conidial challenge did not differ from levels detected in whole-lung samples removed immediately prior to conidial challenge. However, a comparison between the CCR4+/+ and CCR4−/− groups at day 2 after exposure to conidia revealed that whole-lung IL-12 levels were significantly increased in the knockout group relative to the wild-type group (Fig. 6).
CCL2 (51) and CCL3 (45) exert prominent protective roles in the lung during invasive aspergillosis. Major differences in the expression of both CC chemokines were observed in CCR4+/+ and CCR4−/− mice, particularly at day 2 after conidial challenge. Whole-lung CCL2 levels were significantly increased at day 2 after conidial challenge in CCR4+/+ mice but not CCR4−/− mice compared with whole-lung CCL2 levels measured at the day zero time point in these groups of mice (Fig. 6). Whole-lung CCL3 was significantly increased in both groups at day 2 after conidial challenge, but markedly less variability in whole-lung CCL3 levels was observed in the CCR4+/+ group relative to the CCR4−/− group. Accordingly, CCL3 levels were significantly higher in the CCR4−/− group at the day 2 time point compared with the other two time points analyzed in this group. Thus, together the whole-lung ELISA results demonstrated that protective cytokines such as TNF-α, CCL2, and CCL3 were significantly induced in the lungs of CCR4−/− mice at day 2 of invasive aspergillosis compared to whole-lung levels in neutropenic CCR4+/+ mice before the conidial challenge. CCR4+/+ mice only exhibited a significant increase in whole-lung levels of CCL3 at day 2 after the conid-

FIG. 3. (Top) GMS staining of whole-lung tissue sections from neutropenic wild-type (A and D), CCR4−/− (B and E), and CCR8−/− (C and F) mice at days 2 (A, B, and C) and 6 (D, E, and F) after an i.t. challenge with 5 × 10⁶ conidia. To induce neutropenia, all mice were injected with 100 μg of RB6-8C5 antibody 24 h prior to the conidial challenge. *Aspergillus conidia and hyphae stained black with this staining procedure. The original magnification was ×200. (Bottom) Whole-lung chitin analysis in neutropenic wild-type (C57BL/6), CCR4−/−, and CCR8−/− mice at day 2 after an i.t. challenge with 5 × 10⁶ conidia. To induce neutropenia, all mice were injected with 100 μg of RB6-8C5 antibody 24 h prior to the conidial challenge. Data shown are means ± SEM (n = 5/group). *, P ≤ 0.05 compared with the C57BL/6 group; †, P ≤ 0.05 compared with the CCR8−/− group.

FIG. 4. Kaplan-Meier survival analysis of neutropenic wild-type (C57BL/6) and CCR4−/− mice after an i.t. challenge with 5 × 10⁶ conidia. To induce neutropenia, all mice were injected with 100 μg of RB6-8C5 antibody 24 h prior to the conidial challenge. Groups of seven mice were monitored for 6 days after conidial challenge. CCR4−/− mouse survival was significantly (P ≤ 0.05) greater than that of the wild-type group (C57BL/6). Data are representative of two separate survival experiments.
Most importantly, whole-lung IL-12 levels were significantly greater in the knockout group at day 2 of invasive aspergillosis than those in the wild-type group at the same time in this lung infection.

Enhanced mononuclear but not lymphocytic cell recruitment into the lungs of CCR4−/− mice during invasive pulmonary aspergillosis. Previous studies have highlighted the importance of mononuclear (45, 48), dendritic (4, 12, 54), and NK (51) cells in the protective antifungal response against *A. fumigatus* conidial challenge in neutropenic or immunocompromised mice. Prior to the conidial challenge, whole-lung samples from neutropenic CCR4+/+ and CCR4−/− mice contained similar numbers of dendritic cells (CD11c+ cells expressing CD86) and pulmonary macrophages (CD11c+ cells expressing F4/80) were similar in the CCR4+/+ and CCR4−/− groups (Fig. 7). Conversely, both cell types were significantly increased in whole-lung samples from the CCR4−/− group at day 2 after conidial challenge compared with the CCR4+/+ group at the same time after conidial challenge (Fig. 7).

Analysis of CD3+ and NK1.1+ cell populations revealed that both cell types were detected in similar numbers immediately prior to conidial challenge in neutropenic CCR4+/+ and CCR4−/− mice (data not shown). A day 2 after saline challenge alone, similar numbers of CD3+ and NK1.1+ cells were observed in the lungs of CCR4+/+ and CCR4−/− mice (Fig. 7). However, both cell types were significantly decreased in whole-lung samples from the CCR4−/− group at day 2 after conidial challenge compared with the CCR4+/+ group at the same time after conidial challenge (Fig. 7). Thus, the absence of CCR4...
during invasive pulmonary aspergillosis was associated with increased dendritic cells and macrophages and decreased T-cell or NK cell recruitment into the lung.

**DISCUSSION**

The immunopathogenesis of invasive pulmonary aspergillosis remains enigmatic, although considerable progress has been made examining the soluble and or soluble factors that inhibit or enhance immune activation. Inflammatory cytokines such as TNF-α (47, 52, 55), IL-12 (54, 60), and IL-6 (16) protect, whereas IL-4 (19) predisposes immunocompromised mice to the pulmonary ravages of invasive growth by *A. fumigatus*. Chemokines such as CCL2 (through its effects on NK cell recruitment (51) and CCL3 and CXCL1 (through their effects on monocyte/macrophage recruitment) (45, 48) exert prominent protective effects in the context of invasive pulmonary aspergillosis. In the present study, we demonstrate that the de novo generation of CCL17 in the lung has deleterious effects on the innate immune response during experimental aspergillosis in neutropenic mice. The effects of CCL17 appeared to be mediated through CCR4 and not CCR8. Finally, the immunosuppressive effects of CCL17 appeared to be directed, in part, toward the modulation of lung mononuclear cell recruitment into the Aspergillus-infected lung.

Despite the presence and upregulation of both CCR4 ligands in whole-lung and BAL samples from wild-type mice with invasive pulmonary aspergillosis, it was clear from the present study that CCL17 and CCL22 had differential roles in the context of this model. Previous studies have demonstrated that although often concomitantly expressed in a variety of noninflamed and inflamed tissues, the regulation and action of these CCR4 ligands may differ markedly. For example, CCL17 and CCL22 are produced by a variety of immune cells, including dendritic cells, B cells, macrophages, NK cells, monocytes, and CD4 T cells (1, 2), but the expression of CCL17 alone has been documented in non-immune cells, including bronchial epithelial cells (58), keratinocytes (61), airway smooth muscle cells (26), and fibroblasts (5, 27, 37). In isolated monocytes, CCL22, but not CCL17, expression is inhibited by IL-10 (2) while corticosteroids appear to inhibit both chemokines regardless of the cell of origin (58). Differential roles for CCL17 and CCL22 have been reported in a number of experimental models of disease, including bacterium-induced fulminant hepatic failure (CCL17 alone) (63), cardiac allograft rejection and contact hypersensitivity (both CCL17 alone (1), pulmonary fibrosis (CCL17 but not CCL22) (5), pulmonary granulomatous inflammation (CCL22 > CCL17) (37), and allograft transplant rejection (CCL22 > CCL17) (42). However, it has been uniformly reported that both chemokines contribute to Th2-dominated inflammatory responses such as atopic dermatitis (62) and allergic airway disease or asthma (30, 39, 43). In the present study, we noted a clear demarcation between the roles of CCL17 and CCL22 during invasive pulmonary aspergillosis that the neutralization of CCL17 but not CCL22 improved mouse survival during the course of invasive pulmonary aspergillosis.

Part of the impetus to pursue the role of CCL17, CCL22, and CCR4 in invasive pulmonary aspergillosis stemmed from our previous study on the role of CCR4 during chronic fungal
Asthma induced by *A. fumigatus* (57). We previously noted that the elimination of conidia and fungal material proceeded at a much faster rate in the CCR4/H11002/H11002 mice than in the CCR4/H11001/H11001 mice. The augmented clearance was associated with an increase in a number of cytokines and chemokines normally associated with these cells. Studies previous to ours suggested that the innate immune response in CCR4/H11002/H11002 mice was altered in that these mice were dramatically less susceptible to the development of lipopolysaccharide-induced endotoxic shock (22). More recently, CCR4/H11002/H11002 mice showed prolonged cardiac allograft survival compared with their wild-type counterparts (33). Given that previous reports have suggested that CCL17 can bind and signal through CCR8 (8, 36), the development of invasive pulmonary aspergillosis was also examined in CCR8+/− mice.

Numerous studies have noted the effect of CCR4 ligands on the recruitment of CD4+ T cells (3, 20, 24) and NK cells (36). Most recently, CCL22 (via CCR4) has been shown to regulate the recruitment of Foxp3+ T regulatory cells to cardiac allografts (42). In the present study, we examined the impact of CCR4 deficiency on the recruitment of T and NK cells into the lungs during invasive aspergillosis because of previous reports highlighting the importance of these two cell populations in mediating protective immune responses against *Aspergillus* (15, 17, 51). We observed that the presence of both cell types was significantly lower in the lungs of CCR4−/− mice than in those of CCR4+/+ mice at day 2 after conidial challenge. The diminution in NK cell numbers in the lungs of CCR4−/− mice occurred despite the significantly increased levels of CCL2 in the lungs of these mice. Morrison and colleagues (51) previously reported that CCL2 mediated the recruitment of NK cells to the lungs as a critical early host defense mechanism during invasive pulmonary aspergillosis, but data from the present study suggest that the recruitment signal mediated through CCR4 supersedes that provided by CCL2/CCR2 for the recruitment of NK cells. Thus, these data suggested although CCR4 deficiency impaired the recruitment of T and NK cells into the lung, these cells did not appear to contribute to the protective immune response present in CCR4+/+ mice.

At day 2 after conidial challenge, pro-innate immune mediators including TNF-α, CCL2, and CCL3 were significantly increased or altered in the lungs of neutropenic CCR4−/− mice relative to levels of these factors in this group of mice immediately prior to the conidial challenge. In the CCR4+/− group, similar significant increases in these cytokines and chemokines above baseline levels (prior to conidial challenge) were only observed following statistical analysis of whole-lung levels of **FIG. 7. Flow cytometric analysis of CD11c+ F4/80−, CD11c+ CD86+, CD3+, and NK1.1+ cells in whole-lung samples from neutropenic wild-type (CCR4+/+) and CCR4−/− mice at day 2 after an i.t. challenge with 5 × 106 conidia. To induce neutropenia, all mice were injected with 100 μg of RB6-8C5 antibody 24 h prior to the conidial challenge. Data shown are means ± SEM (n = 5 mice/group). ***P ≤ 0.001 compared with the CCR4+/+ group that received conidia; τ, P ≤ 0.05 compared with the CCR4+/+ group that received conidia.**
CCL3. At day 2 after conidial challenge, whole-lung IL-12 levels were significantly decreased in the CCR4+/− group but unchanged in the CCR4+/- group compared with levels of this cytokine measured at day 0. Most importantly, levels of this cytokine were significantly increased in the CCR4+/- group relative to the CCR4+/- group at day 2 after conidial challenge. Shao and colleagues (60) recently demonstrated that the transfection of dendritic cells with adenovirus encoding IL-12 and the adoptive transfer of these cells pulsed with heat-inactivated *Aspergillus fumigatus* protected naive mice from invasive aspergillosis. Further studies will address the relative importance of IL-12 in the innate immune response present in CCR4−/− mice during invasive aspergillosis.

The source of protective cytokines and chemokines has yet to be determined, but their presence and/or actions may have contributed to the increased recruitment of dendritic (CD11c+ CD86+) and macrophages (CD11c+ F4/80−) into the lungs of CCR4−/− mice during invasive pulmonary aspergillosis. Both cell types have been previously shown to play key roles during host resistance to fungi (4, 13, 23, 28). While less is known about the effects of CCL17 on the activation of dendritic cells, these cell types have been previously shown to play key roles during the innate immune response directed against this fungal pathogen. Various vaccine- and drug-based therapies have been proposed, but the present study suggests that appropriate protection from invasive pulmonary aspergillosis may be achieved through the selective targeting of a specific chemokine, namely CCL17. Additional experimental studies are required to fully elucidate the effects of CCL17 on the innate immune response directed against this fungal pathogen, and clinical studies are required to determine whether this chemokine is elevated in patients experiencing invasive aspergillosis. Thus, the present studies demonstrate that CCL17 is a critical regulator of host defense against *A. fumigatus*, and this chemokine may be an important therapeutic target during invasive aspergillosis.

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