The Role of CC Chemokine Receptor 6 in Host Defense in a Model of Invasive Pulmonary Aspergillosis

Anagha P. Phadke[1], Gangaram Akangire[1], Stacy J. Park[1,2], Sergio A. Lira[3], and Borna Mehrad[1,2,4]

[1]Division of Pulmonary and Critical Care Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; [2]Department of Microbiology, University of Virginia, Charlottesville, Virginia; [3]Immunobiology Center, Mount Sinai School of Medicine, New York, New York; and [4]Division of Pulmonary and Critical Care Medicine, University of Virginia, Charlottesville, Virginia

***Rationale*: Invasive aspergillosis is a severe fungal infection afflicting immunocompromised patients, particularly patients with neutrophil defects. CCR6, a β-chemokine receptor, mediates migration of dendritic cells (DCs) and several lymphocyte subsets to sites of epithelial inflammation, but its role in infections has not been examined extensively.**

***Objectives*: To test the hypothesis that CCR6-mediated leukocyte recruitment is necessary for effective host defense in neutropenic hosts with invasive pulmonary aspergillosis.**

***Methods*: Neutropenic wild-type mice and mice with targeted deletion of CCR6 were infected with *Aspergillus fumigatus*. The host responses to the infection were compared *in vivo* and leukocyte responses to the fungus were examined *in vitro*.**

***Measurements and Main Results*: In the context of infection, immature myeloid DCs were the major population of CCR6-expressing cells in the lungs. As compared with wild-type animals, CCR6-deficient mice developed a more severe infection when challenged with *A. fumigatus* conidia, as documented by a higher mortality rate and greater lung fungal burden. This was associated with reduced accumulation of DCs in the lungs. CCR6-deficient and wild-type DCs did not differ in their phagocytosis of conidia, cytokine response, or maturation *in vitro*. In adoptive transfer experiments, however, DCs from CCR6-deficient donors showed lesser accumulation in the lungs of infected mice as compared with wild-type cells, and transfer of wild-type, but not CCR6-deficient, DCs resulted in attenuated severity of infection in CCR6-deficient recipients.**

***Conclusions*: Taken together, these results implicate CCR6-mediated DC influx into the lung in the initial host defense in invasive aspergillosis.**

Keywords: CCR6 protein, mouse; dendritic cells; fungi, filamentous; pneumonia

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Invasive aspergillosis is an infection of immunocompromised hosts, particularly those with neutrophil defects. CCR6, a chemokine receptor, mediates migration of several leukocyte subsets to epithelial surfaces, but its role in infections is not clear.

What This Study Adds to the Field

CCR6-mediated dendritic cell influx into the lung is critical to the initial host defense against invasive aspergillosis in neutropenic hosts.

Invasive aspergillosis is a severe infection afflicting immunocompromised patients and is caused by a filamentous fungus that is ubiquitously distributed in the environment. The illness characteristically begins in the respiratory tract, where inhaled spores germinate, penetrate the epithelium, and invade the surrounding tissues, leading to severe pneumonia. Despite recent progress in antimicrobial therapy, this infection is characterized by a steadily increasing incidence and a persistently poor outcome, as recently reviewed (1). The immune response of the host is critical to the pathogenesis of this infection—a point highlighted by the fact that, although all humans inhale hundreds of *Aspergillus* spores daily, the infection only develops in hosts with markedly impaired immunity. The best-characterized immune defects predisposing to invasive aspergillosis are qualitative or quantitative neutrophil defects, but increasing evidence points to the additional role of other immune cells in defense against this infection (2–4). A better understanding of the mechanisms involved in antimicrobial defense in this infection may identify opportunities for new therapeutic interventions.

CCR6 is a CC chemokine receptor for the chemokine ligand CCL20 and several members of the β-defensin family (5–8). CCR6 is expressed by immature myeloid dendritic cells (DCs), most mature B cells, and memory T cells, but not by plasmacytoid or lymphoid DC subsets nor by any mature DCs (7–14). CCR6 is important in regulating several aspects of mucosal immunity; this includes mediating the recruitment of immature DCs to sites of epithelial inflammation (15, 16), including the lung (17), and homing of both CD4 T cells and DCs to gut mucosal lymphoid tissue (18, 19). CCR6 has been implicated in the pathogenesis of a number of diseases, including inflammatory bowel disease (20), hypersensitivity skin diseases (21, 22), airway allergy (23, 24), and spread of cancer cells (25, 26).

Other groups have documented the importance of DCs and T-cell–mediated immunity to antimicrobial responses in invasive aspergillosis (2, 27–29). We therefore tested the hypothesis that CCR6-mediated leukocyte recruitment is necessary for effective host defense in neutropenic hosts with invasive aspergillosis.

Some of the results of these studies have been reported previously, in the form of an abstract (30).

METHODS

C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME), and mice with targeted deletion of CCR6 (18), on C57BL/6 background, were bred locally. (*See* the online supplement for additional details of methods used.) We used a previously characterized model

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Correspondence and requests for reprints should be addressed to Borna Mehrad, M.D., Box 800546, University of Virginia School of Medicine, Charlottesville, VA 22908. E-mail: mehrad@virginia.edu

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of invasive pulmonary aspergillosis in mice with transient antibody-mediated neutrophil depletion (4, 31–33), using *Aspergillus fumigatus* strain 13073 (American Type Culture Collection, Rockville, MD). In various experiments, neutralizing antibodies were administered intraperitoneally, and leukocytes were administered intravenously.

Whole-lung single-cell suspensions were processed for flow cytometry, cell sorting, and differential counts, as described (4, 31–33). The absolute number of each leukocyte subset was determined as the product of the percentage of the cell type and the number of cells in the sample, as determined under a hemacytometer. Molds, including *Aspergillus* species, grow as multicellular branching hyphae that do not form distinct reproductive structures in infected tissues. We therefore used a previously characterized assay for chitin, a carbohydrate component of hyphal wall absent from mammalian cells and conidia, to quantify the burden of hyphae in infected lungs, as detailed previously (4, 31–33). ELISA was performed on filtered supernatant of lung homogenates or cell culture supernatants using commercial kits. Lungs were fixed and processed for histology as described (4, 31–33), and stained with hematoxylin and eosin or Grocott's methenamine silver.

Immature bone marrow–derived myeloid DCs were prepared as a modification of a published protocol (34). Bone marrow cells were cultured in murine granulocyte macrophage–colony stimulating factor (mGM-CSF) and enriched using anti-CD11c magnetic microbeads according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA). Enrichment resulted in increased an proportion of $CD11b^+$ $CD11c^+$ cells, from approximately 55% to more than 95%. Recovered cells were routinely more than 98% viable by trypan blue exclusion and, consistent with immature DC phenotype, were approximately 10% $CD86^+$ and expressed low levels of major histocompatibility (MHC) class II molecules.

In adoptive transfer experiments, cells were administered intravenously via a lateral tail vein. In some experiments, transferred cells were first labeled with the vital fluorochromes PKH26 (Sigma-Aldrich, St. Louis, MO) or carboxyfluorescein diacetate succinimidyl ester (Invitrogen Life Technologies, Carlsbad, CA). In coculture experiments, immature DCs were incubated with freshly harvested conidia and were incubated in 96-well plates. To assess phagocytosis *in vitro*, fresh conidia were first conjugated to fluorescein isothiocyanate and washed, and uniform staining was verified by epifluorescent microscopy before coincubation. At designated times, phagocytosis was halted by addition of cytochalasin D and extracellular fluorescence quenched in trypan blue before flow cytometric analysis.

Data were analyzed on a Macintosh Powerbook G4 computer (Apple, Cupertino, CA) using the Prism statistical package (version 4.0a; Graphpad Software, San Diego, CA). Survival data were compared using Fischer's exact test. All other data were expressed as mean ± SEM. Values between two groups over multiple times were compared with two-way analysis of variance, and comparisons between two groups at a single time were performed with an unpaired, two-tailed Mann-Whitney (nonparametric) test. Probability values were considered statistically significant if they were less than 0.05.

RESULTS

Accumulation of CCR6-expressing Cells in the Lung in Invasive Aspergillosis

We began by examining the lungs of mice with invasive aspergillosis for leukocytes that express CCR6. In this context, we identified DCs in infected-lung single-cell suspensions using criteria described by other groups (17, 35–38). In the lungs of mice with invasive aspergillosis, DCs were predominantly CD11b-high and CD11c-positive ($CD11b^{hi}$ $CD11c^+$) (Figure 1A), thus identifying them as myeloid (rather than plasmacytoid or lymphoid) DCs. These cells displayed low autofluorescence, expressed low levels of MHC class II molecules and CD86, and did not express Gr-1 or B220 (data not shown). They had monocytic morphology when freshly sorted but acquired dendritic morphology after overnight culture in cytokine-free media (gate R1, Figure 1B). A separate and distinct CD11b-low and CD11c-high ($CD11b^{lo}$ $CD11c^{hi}$) cell population, previously identified as a subset of resident lung macrophages (36, 39, 40), retained macrophage morphology both when fresh and after overnight culture (gate R2, Figure 1B).

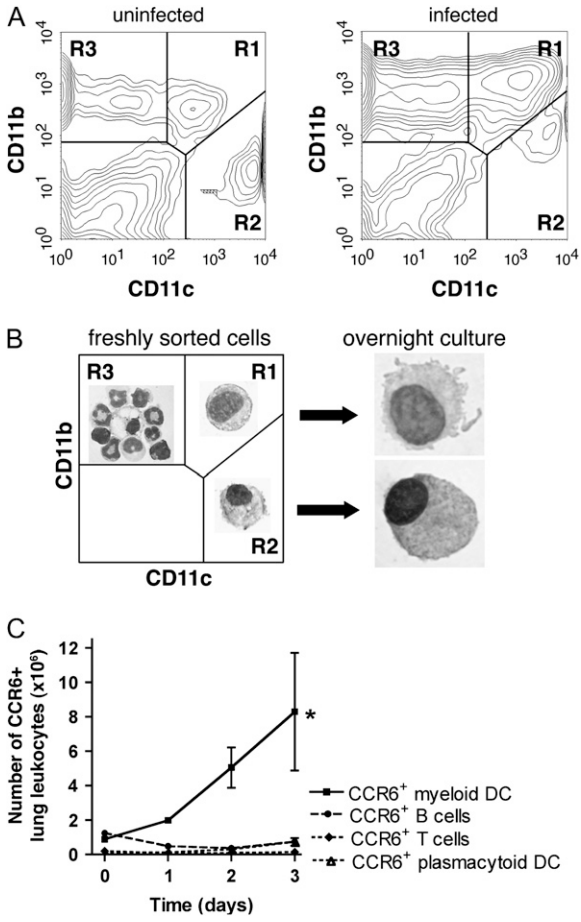

Figure 1. Identification of lung dendritic cells (DCs) in mice with invasive aspergillosis. (*A*) Representative contour plots of lung single-cell suspension from an uninfected animal on Day 2 of infection. *Panels* are gated on $CD45^+$ cells. (*B*) Microscopic appearance of cells in each gate, sorted from a mouse on Day 2 of infection. When fresh, R1 cells were monocytic, R2 cells had macrophage morphology, and R3 cells consisted of a mixed population of neutrophils, band forms, monocytes, and macrophages. After overnight culture in cytokine-free media, most R1 cells assumed DC morphology, whereas R2 cells retained macrophage morphology. (*C*) Lung single-cell suspensions were examined by flow cytometry for CCR6 expression on various days after onset of infection. The following markers were used to identify cell types: myeloid DCs, gate R1 in *A*; plasmacytoid DCs, $CD11c^+$ $B220^+$; B cells, $CD11c^-$ $B220^+$; T cells, $CD3^+$. Day 0 represents uninfected mice. Data shown are mean ± SEM; n = 4 for each group at each time point. *$p < 0.05$ compared with each of the other groups over time.

Because CCR6 expression has been reported on DCs, mature B cells, and memory T-cell subsets (9–11), we examined the lungs of infected animals for CCR6 expression on these leukocyte subsets at various points after the onset of infection. In uninfected animals, the lungs contained similar numbers of CCR6-expressing B cells and myeloid DCs, but myeloid DCs became the predominant CCR6-expressing cell in the lung at all examined times after onset of infection, constituting 11 to 17% of leukocyte-enriched lung cell suspensions. As expected (12, 13), we found very few plasmacytoid DCs expressing CCR6 in the lungs of infected mice at any time; similarly, CCR6-expressing T cells were not detectable in the lungs of these animals.

Outcome of Invasive Aspergillosis in CCR6-deficient Mice

To assess the role of CCR6 in host defense against this infection, we next compared the outcome of the infection in wild-type and CCR6-deficient mice. Using small inocula of *A. fumigatus* to produce low mortality in wild-type animals, we found CCR6-deficient mice to be more susceptible to death from invasive aspergillosis than their wild-type counterparts (Figure 2A). This increased mortality from the infection was associated with a sixfold increase in the lung fungal content on the third day of infection, again using small fungal inocula (Figure 2B). At this time point, histology of the lungs showed lung consolidation in association with areas where fungal hyphae invaded from alveoli and airways into the lung parenchyma in both wild-type and CCR6-deficient mice. On high-power examination, many of the infiltrating leukocytes had mononuclear morphology (Figure 3). In the absence of neutrophil depletion, on the other hand, CCR6-deficient animals challenged with large inocula of conidia had undetectable lung chitin content, demonstrating a high degree of resistance to infection, similar to what is observed in immunocompetent wild-type animals (31).

Comparison of Lung Leukocytes in CCR6-deficient and Wild-Type Animals with Invasive Aspergillosis

To assess the mechanism of increased susceptibility of CCR6-deficient mice to invasive aspergillosis, we compared the number of various leukocyte subsets in the lungs between wild-type and CCR6-deficient mice at various times after onset of infection (Figure 4). In these experiments, myeloid DCs, defined as cells in gate R1 in Figure 1, were fewer in the lungs of CCR6-deficient, as compared with wild-type, mice over the examined times. To quantify resident macrophages and recruited monocytes/macrophages, we used criteria published by other groups (17, 39, 41). Accordingly, the number of recruited monocyte/macrophages (cells with midlevel expression of F4/80 and CD11b, but not CD11c [$F4/80^{mid}$ $CD11b^{+}$ $CD11c^{-}$]) was also decreased in the lungs of CCR6-deficient, as compared with wild-type, mice over the time course. Resident lung macrophages were defined as $F4/80^{hi}$ cells with high autofluorescence; most, but not all, of these cells corresponded to the $CD11b^{lo}$ $CD11c^{hi}$ population (gate R2 in Figure 1). The number of these cells did not change significantly in either group during the course of infection, and also did not differ between wild-type and CCR6-deficient animals. As noted previously, the number of lung plasmacytoid DCs was small during this infection, and did not differ between the two groups. Because mature B cells and a subset of T cells are known to express CCR6, we also enumerated these cells in the infected lungs. The numbers of lung B cells and CD4 and CD8 T cells all decreased in both groups after intratracheal inoculation, a phenomenon previously described with intrapulmonary inoculation of particulate antigens (42, 43). In addition, the numbers of lung natural killer cells and neutrophils (which begin to recover and appear in the lung on Day 2 of infection [31–33]) did not differ between wild-type and CCR6-deficient mice over the course of infection (data not shown).

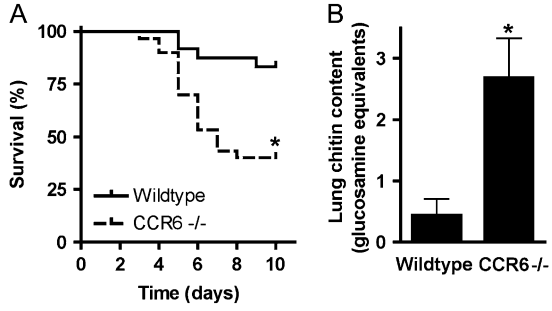

Figure 2. Outcome of infection in CCR6-deficient mice with invasive aspergillosis. (*A*) Survival study; n = 26–30 for each group, pooled from two experiments; *p < 0.05. (*B*) Lung chitin content on Day 3 of infection. Data represent mean ± SEM; n = 6 for each group; *p < 0.05.

Role of CCR6 in Recruitment of Myeloid DCs to the Lung in Invasive Aspergillosis

Given our observations that lung myeloid DCs were the major cell population expressing CCR6 in the context of invasive aspergillosis, and that CCR6-deficient mice had fewer lung DCs, leads us to hypothesize that CCR6 expression on myeloid DCs was important for their influx into the lung in this infection. The number of lung DCs at any time represents the sum of cellular influx or local differentiation, on the one hand, and efflux or apoptosis, on the other; we therefore sought to examine the contribution of CCR6 expression on DC influx into the lungs more directly by tracking the arrival of labeled DCs to the infected lungs. To this end, we cultured immature myeloid DCs from bone marrow of wild-type and $CCR6^{-/-}$ donors, labeled the cells with a vital fluorochrome, and transferred them intravenously into wild-type neutropenic recipient mice before the intratracheal challenge with *Aspergillus* conidia (Figure 5A). After 18 hours, we found 68% fewer transferred DCs in the lungs of mice given CCR6-deficient, as compared with wild-type, DCs. Although CCR6-deficient DCs do demonstrate some degree of influx to the infected lung, suggesting a secondary CCR6-independent mechanism for this process, these results indicate that optimal influx of DCs into the lungs in the setting of invasive aspergillosis requires their expression of CCR6.

To assess whether the observed CCR6-dependent recruitment of DCs to the lungs in this infection might explain the increased susceptibility of CCR6-deficient animals, we compared the severity of infection in animals after the transfer of DCs from various donors (Figure 5B). Transfer of wild-type DCs to CCR6-deficient mice resulted in an approximately 60% reduction in lung fungal burden on Day 3 of infection, but similar transfer of $CCR6^{-/-}$ DCs did not result in a statistically significant effect on lung fungal content.

In addition to DCs, CCR6-deficient mice with invasive aspergillosis had reduced numbers of lung monocytes/macrophages (Figure 4B). We therefore examined the effect of depletion of these cells with intrapulmonary clodronate liposomes on the outcome of the infection. As expected (44), intrapulmonary administration of clodronate liposomes did not affect the number of lung DCs. When both neutrophils and monocytes/macrophages were depleted from wild-type and CCR6-deficient mice and infection was established with a small inoculum of *Aspergillus* conidia, lung chitin content did not differ significantly between the two groups (1.68 ± 0.56 vs. 1.31 ± 0.55 glucosamine equivalents, respectively; p = 0.66). This indicates that protective effects of CCR6 are not apparent in mice with depletion of both neutrophils and monocytes/macrophages, which is likely due to the profound impairment of host defenses in this setting.

Characterization of CCR6-deficient Myeloid DCs

Given that DC expression of CCR6 appeared to be required for DC influx into the lungs in invasive aspergillosis and for enhanced host defense against the infection, we hypothesized that the impaired defense of CCR6-deficient mice is solely due to impaired influx of DCs into their lungs. The competing hypothesis is that CCR6-deficient DCs have abnormalities beyond their chemotactic properties, which contribute to its role in host

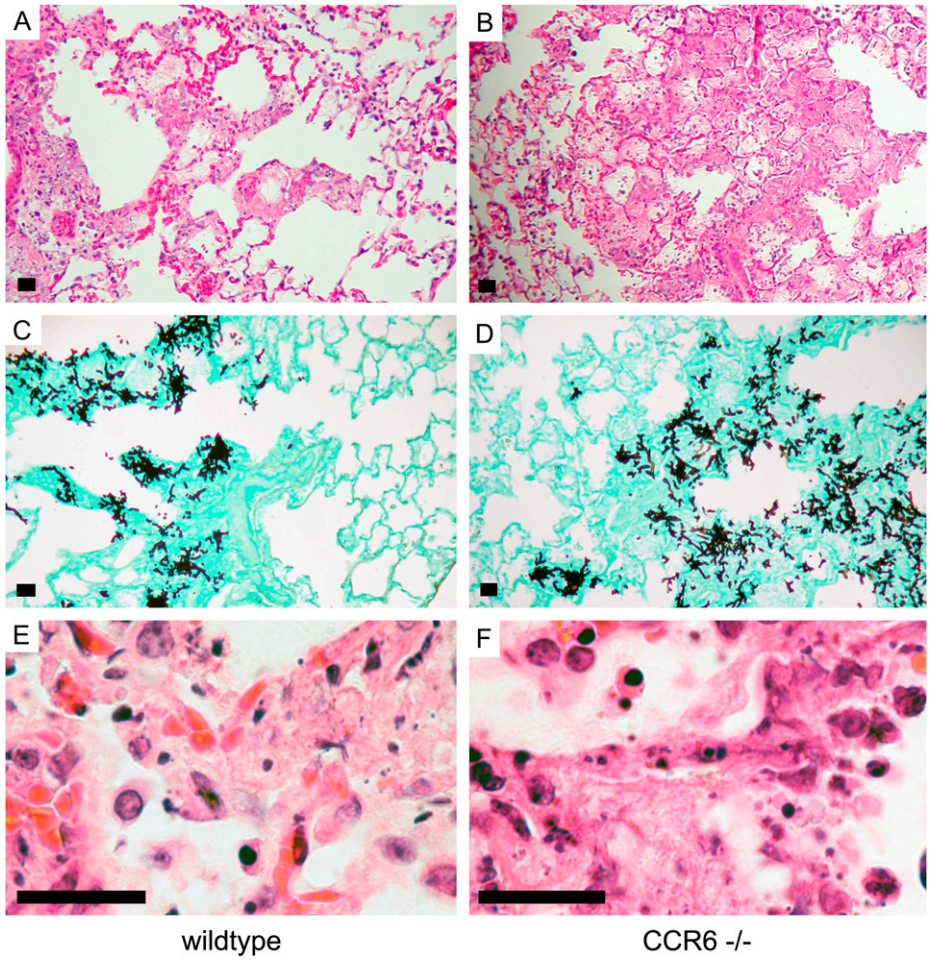

Figure 3. Effect of CCR6 deficiency on lung histology in mice with invasive aspergillosis. Representative lung hematoxylin and eosin (*A–B* and *E–F*) and Grocott's methenamine silver (to demonstrate fungal elements; *C–D*) stains 3 days after onset of infection are shown. *A–D* show consolidation of lung parenchyma in association with areas of hyphal invasion, and *E–F* show leukocytes in the lung parenchyma. Many of the leukocytes have mononuclear morphology; some polymorphonuclear cells are also visible. All *scale bars* are 20 μm; original magnifications were ×100 for *top* and *middle panels* and ×1,000 for *lower panels*.

defense against invasive aspergillosis. To address this possibility, we compared the *in vitro* interaction of purified immature myeloid DCs from wild-type and CCR6-deficient mice with *A. fumigatus* (Figure 6). As expected, immature DCs displayed rapid and efficient phagocytosis of *A. fumigatus* conidia, but there was no difference in the phagocytic rate of conidia between wild-type and CCR6-deficient DCs (Figure 6A). Similarly, cells displayed no difference in production of tumor necrosis factor, a cytokine prominently produced by DCs exposed to the fungus and critical to host defense in this infection (2, 45), when coincubated with live *A. fumigatus* spores (Figure 6B). In addition, the rate of maturation of DCs induced by exposure to *A. fumigatus*, as determined by surface expression of CD86 and up-regulation of MHC class II molecules, did not differ between CCR6-deficient and wild-type cells (Figures 6C and 6D). Although limited by the *in vitro* approach and nonpulmonary origin of the leukocytes, these data suggest that the absence of CCR6 does not, *per se*, influence the interaction of DCs with *Aspergillus* beyond its effects on chemotaxis.

Effect of CCL20 Neutralization on Recruitment of Myeloid DCs to the Lung in Invasive Aspergillosis

Our data indicate that CCR6 expression by DCs is important in their recruitment to the lung in invasive aspergillosis. Because the ligand CCL20 signals solely via CCR6, we sought to provide further support for this notion by examining this ligand in lungs of mice with invasive aspergillosis. We found detectable levels of CCL20 in the lungs of uninfected subjects, consistent with previous reports in mice (46) and humans (47, 48). There was a marked induction of CCL20 in the lungs in the context of invasive aspergillosis in wild-type animals (Figure 7A). To determine the role of CCL20 in influx of DCs into infected lungs, we next transferred live, fluorochrome-labeled, immature DCs to mice intravenously. In the context of infection, accumulation of transferred DCs in the lungs increased approximately fivefold (Figure 7B). Neutralization of CCL20 using polyclonal antibodies resulted in a 55% decrease in accumulation of transferred DCs in the lungs of infected mice, providing evidence for a role for CCR6 ligands in this infection. Finally, neutralization of CCL20 in the context of infection resulted in a threefold increase in lung chitin content in neutropenic mice (Figure 7C), providing further evidence of the importance of the CCL20–CCR6 axis in defense against invasive aspergillosis.

DISCUSSION

Given that invasive pulmonary aspergillosis occurs almost exclusively in patients with marked immunodeficiency, the pathogenesis of the infection is evidently closely linked to aberrations in the host's antimicrobial responses. Although the importance of neutrophils in host defense against this infection is well documented, the current understanding of other effector mechanisms operating in immunocompromised hosts with invasive aspergillosis is limited (49). In this article, we provide data that link CCR6-mediated DC influx into the lung as a necessary component of host defense in neutropenic mice with invasive aspergillosis.

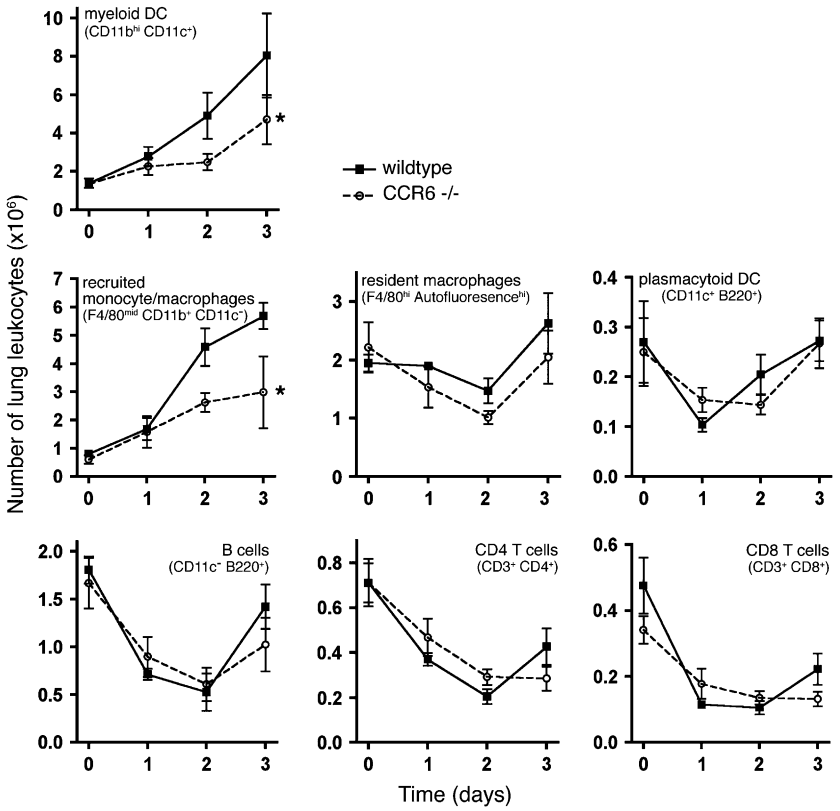

Figure 4. Comparison of lung leukocyte influx between wild-type and CCR6-deficient mice with invasive aspergillosis. Cells were enumerated in whole-lung single-cell suspensions, and populations were identified as $CD45^+$ cells expressing the markers in *parentheses*. Day 0 represents uninfected mice. *$p < 0.05$ comparing trend between the two groups over time. Data shown represent mean ± SEM of pooled data from two experiments; n = 4–8 for each group at each time point.

Several studies have examined the role of DCs in the immune response to *Aspergillus* species. Both human and mouse DCs efficiently phagocytose *Aspergillus* fungal elements *in vitro* (2, 50). Myeloid DCs bind conidia via the C-type lectin dendritic cell–specific intercellular adhesion molecule-3–grabbing nonintegrin (DC-SIGN) (50), and possibly dectin-1 (51, 52), and are activated via Toll-like receptor (TLR)–2 and TLR-4 and MyD-88–dependent signaling (53, 54). *In vivo*, myeloid DCs transport conidia and hyphae from the lung to lymph nodes and spleen, where they initiate a Th-1 acquired immunity to conidia but a Th-2 response to hyphae (2). When DCs are exposed to *Aspergillus* antigens *in vitro* and then transferred into naive mice, the recipients develop protective T-cell–mediated immunity to subsequent infection, an effect comparable to the transfer of Th-1 T cells from immunized to naive animals (3, 27, 28). In the context of this literature, our study found the phenotype of lung DCs in the infected animals to be that of immature myeloid DCs, consistent with prior reports with this organism (2), and similar to the DC phenotype reported in several other lung inflammation models (17, 55). In contrast to models of airway allergy (56, 57) and viral infection (58–60), we found few of the plasmacytoid or more mature myeloid DC subsets in mice with invasive aspergillosis. In addition, our data suggest that CCR6-deficient DCs have an isolated defect in homing to the infected lung but have normal interaction with the pathogen *in vitro*.

The number of DCs in the lungs at a given time point represents the sum of cellular influx or local differentiation, on the one hand, and their efflux or apoptosis, on the other. CCR6 is necessary for influx of immature DCs to sites of mucosal inflammation, including the lung, skin, and small intestine (17, 18, 61). The efflux of DCs from the inflamed lung is a result of down-regulation of CCR6 and up-regulation of CCR7 as part of DC maturation, mediating homing of DCs to secondary lymphoid tissues (62, 63). Our data indicate that CCR6-mediated recruitment is involved in the marked accumulation of immature DCs in the lungs noted in our model. In addition, however, reduced maturation and efflux of lung DCs or alteration in their

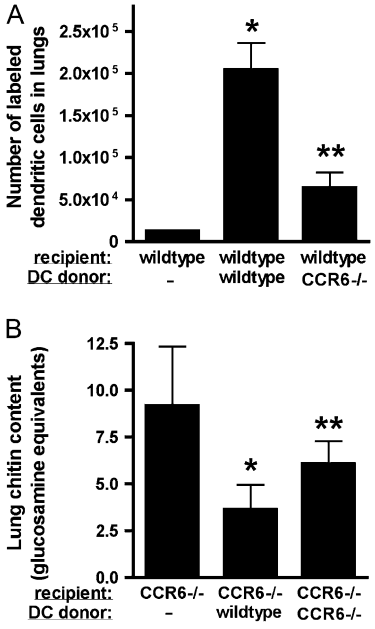

Figure 5. Role of CCR6 in dendritic cell (DC) influx to the lungs in invasive aspergillosis. (*A*) Cultured immature DCs from wild-type and CCR6-deficient donors were labeled with a vital fluorochrome, transferred intravenously to neutrophil-depleted wild-type animals before inoculation with *Aspergillus fumigatus* conidia, and fluorochrome-labeled cells in the lungs were enumerated after 1 day. Data shown are mean ± SEM; n = 6 for each group; *$p < 0.05$ compared with mice not receiving transferred cells; **$p < 0.05$ compared with mice receiving cells from wild-type donors. Representative of two experiments. (*B*) Lung chitin content in CCR6-deficient mice receiving intravenous phosphate-buffered saline or cultured immature DCs from various donors. Data shown are mean ± SEM of pooled data from two experiments; n = 11–13 mice per group; *$p < 0.05$ compared with mice not receiving transferred cells; **$p < 0.05$ compared with mice receiving cells from wild-type donors.

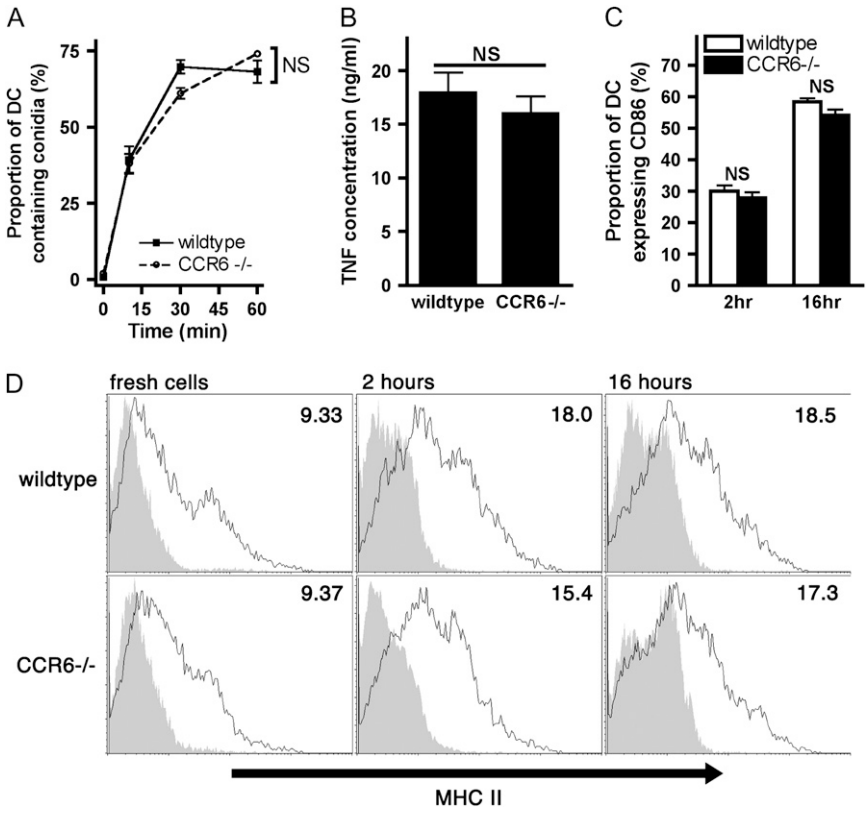

Figure 6. Comparison of immature myeloid dendritic cells (DCs) from wild-type and CCR6-deficient mice. (*A*) Phagocytosis of *Aspergillus fumigatus* conidia *in vitro*. (*B*) Supernatant tumor necrosis factor (TNF) concentration when DCs and live conidia were coincubated overnight. (*C-D*) CD86 and major histocompatibility (MHC) class II expression in DCs coincubated with live conidia. In *A–C,* data points denote mean ± SEM of 2–4 replicates and are representative of two experiments. NS = no statistically significant difference. In *D,* data are representative of 4–8 replicates in two experiments. *Shaded histograms* denote autofluorescence and *numbers* denote geometric mean fluorescence intensity of open histograms.

rate of apoptosis, as has been noted in some DC–pathogen interactions (64, 65), may certainly contribute to this accumulation.

Another interesting feature of our results was that absence of CCR6 resulted not only in reduced numbers of DCs but also of monocytic cells, expressing CD11b and moderate levels of F4/80 but not CD11c, in the infected lungs. Cells with similar surface phenotype and morphology have been described as "monocytes and small macrophages" and "macrophage-like cells" in other models of pneumonia (39, 41). It has recently become clear that recruited inflammatory DCs and macrophages share a common clonogenic bone marrow precursor, which is distinct from the bone marrow precursor of resident tissue macrophages (66). Furthermore, peripheral blood monocytes have been recognized as a heterogonous population, a subset of which are recruited to tissue in the context of inflammation and are the precursors of both recruited immature DCs and recruited macrophages (67–69). Several lines of evidence indicate that the original recruitment of these circulating monocytes to the sites of inflammation requires CCR2 (17, 61, 68), but the sequence of phenotypic changes that occur after these cells arrive in the tissue has not been fully elucidated. Nevertheless, depletion of these cells abrogated the protective effect of CCR6 in our model, indicating their importance in defense against invasive aspergillosis. We speculate that this population of cells includes inflammatory macrophages and cells at an intermediate stage of maturation between circulating blood monocytes and immature tissue DCs, but recognize that the origin and fate of this population in this infection requires further study.

The findings of this study have a number of implications. First, we document the importance of DC influx into the lung

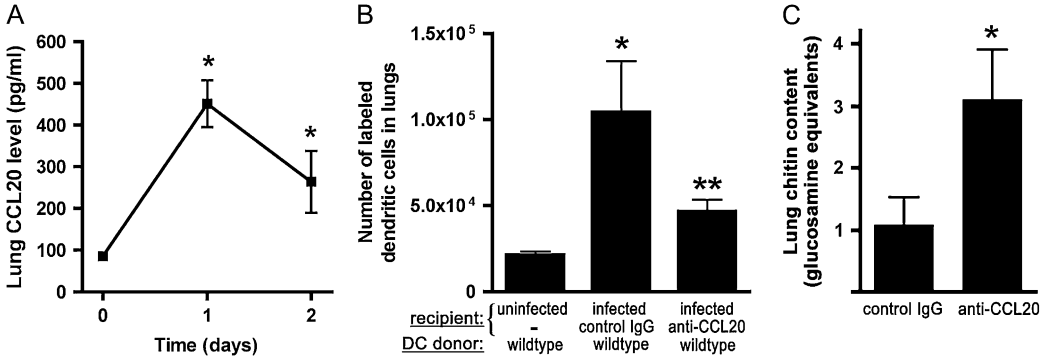

Figure 7. Role of CCL20 in invasive aspergillosis. (*A*) Lung CCL20 protein concentration, as measured in lung homogenates, at various time points after onset of infection. Day 0 represents uninfected animals. Data represent mean ± SEM; n = 4–6 mice per time point; *$p < 0.05$ compared with uninfected mice. (*B*) Fluorochrome-labeled cultured immature dendritic cells (DCs) from wild-type donors were administered intravenously to wild-type animals and fluorochrome-labeled cells in the lungs were enumerated after 1 day. Data shown are mean ± SEM; n = 4 for each group; *$p < 0.05$ compared with uninfected mice; **$p < 0.05$ compared with mice receiving control IgG. (*C*) Lung chitin content on Day 3 of infection in neutropenic mice treated with neutralizing anti-CCL20 antibody or control IgG. Data shown are mean ± SEM of pooled data from two experiments; n = 10–12 mice per group; *$p < 0.05$.

in invasive aspergillosis to the outcome of the infection. Interestingly, DC homing to the lung was important to survival and clearance of the infection despite the relatively brief duration of the infection in this model. This observation suggests that the beneficial effects of DC recruitment to the site of infection involve either very rapid development of T-cell–mediated acquired responses, or a T-cell–independent, DC-mediated innate immune mechanism. Second, our data underscore the relevance of CCR6 in an infection model, an area that has not been investigated in detail. Last, this study provides a rationale for studying mechanisms of DC influx in patients with invasive aspergillosis, and for examining strategies to enhance DC influx to the site of infection as potential therapeutic modalities.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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