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Immunomodulatory Role of C10 Chemokine in a Murine Model of Allergic Bronchopulmonary Aspergillosis

Cory M. Hogaboam,* Chad S. Gallinat,* Dennis D. Taub,‡ Robert M. Strieter,† Steven L. Kunkel,* and Nicholas W. Lukacs*†

The immunomodulatory role of the chemokine C10 was explored in allergic airway responses during experimental allergic bronchopulmonary aspergillosis (ABPA). The intratracheal delivery of Aspergillus fumigatus Ag into A. fumigatus-sensitized mice resulted in significantly increased levels of C10 within the bronchoalveolar lavage, and these levels peaked at 48 h after A. fumigatus challenge. In addition, C10 levels in BAL samples were greater than 5-fold higher than levels of other chemokines such as monocyte-chemoattractant protein-1, eotaxin, and macrophage-inflammatory protein-1α. From in vitro studies, it was evident that major pulmonary sources of C10 may have included alveolar macrophages, lung fibroblasts, and vascular smooth muscle cells. Experimental ABPA was associated with severe peribronchial eosinophilia, bronchial hyperresponsiveness, and augmented IL-13 and IgE levels. The immunoneutralization of C10 with polyclonal anti-C10 antiserum 2 h before the intratracheal A. fumigatus challenge significantly reduced the airway inflammation and hyperresponsiveness in this model of ABPA, but had no effect on IL-10 nor IgE levels. Taken together, these data suggest that C10 has a unique role in the progression of experimental ABPA. The Journal of Immunology, 1999, 162: 6071–6079.

Airway allergic responses to the ubiquitous fungal organism Aspergillus fumigatus can complicate asthma and are often characterized by a Th2-type cytokine response with varying severity of bronchial hyperresponsiveness, eosinophilia, and elevations in IgE (1, 2). These symptoms follow the development of hypersensitivity to A. fumigatus Ag (3), and collectively describe a disease known as allergic bronchopulmonary aspergillosis (ABPA) (4, 5). While the pathogenesis of ABPA still remains speculative, murine models of ABPA have provided clues regarding the immune events that precipitate this form of allergic airway disease (6). For example, both IL-4 and IL-5 contribute to the elevations in IgE, eosinophilia, and bronchial hyperresponsiveness associated with experimental ABPA (7–9, 10). Interestingly, lung injury in A. fumigatus Ag-challenged mice appears to require eosinophils and CD4+ T cells (10) in the absence of IgE-mediated events. This latter observation is supported by mutual observations that A. fumigatus Ag exposure induces eosinophilia in mice before the elevation of serum IgE levels (11, 8) and that ABPA proceeds normally in IgE knockout mice (12, 9). Other recent studies have revealed that this allergic disease is not necessarily a Th2-polarized event either, rather a combined Th1- and Th2-type cytokine response is present and these cytokine responses are regulated by endogenous IL-10 (13).

Despite advances in the characterization of immune events in experimental ABPA, a major deficit in knowledge persists regarding the role of chemotactic cytokines in airway allergic responses to Aspergillus fungus. Clinical observations in asthmatic patients show that C-C chemokines such as monocyte-chemoattractant protein-1 (MCP-1), RANTES, eotaxin, and macrophage-inflammatory protein-1α (MIP-1α) are elevated in allergic asthmatic patients before and following Ag challenge (14–16, 17). These findings have supplied the impetus for the exploration of the role of these and other chemokines in experimental allergic airway responses to a number of small protein allergens (18–20). Surprisingly, functional redundancy among the chemokines involved in the allergic airway response appears to be minimal due in part to the orchestrated timing and the tissue-specific localization of chemokine production (18, 21).

Growing evidence also suggests that certain chemokines possess shadow or modulating roles within immune responses (22). Originally identified in GM-CSF-activated bone marrow cells (23), MIP-related protein 1 or C10 is a chemokine that is postulated to fit this role. C10 has a genomic structure that includes an additional exon that delays its appearance for at least 24 h after cell activation (26). Thus, the present study was directed at elucidating the role of C10 in the development of allergic airway inflammation and hyperresponsiveness to A. fumigatus challenge.

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Abbreviations used in this paper: ABPA, allergic bronchopulmonary aspergillosis; BAL, bronchoalveolar lavage; MCP, monocyte-chemoattractant protein; MIP, macrophage-inflammatory protein; VSMC, vascular smooth muscle cell.

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Materials and Methods

Marine model of allergic bronchopulmonary aspergillosis

Specific pathogen-free, female CBA/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were maintained under specific pathogen-free conditions before and during experiments. Sensitization of mice to soluble A. fumigatus Ags was achieved using a previously described procedure (30, 31). Briefly, all mice received a total of 10 µg of A. fumigatus crude Ag (Greer Laboratories, Lenoir, NC) dissolved in 0.2 ml of IFA (Sigma, St. Louis, MO). One-half of this preparation was then deposited in the peritoneal cavity, and the remainder was delivered s.c. Two weeks later, mice received a total of 20 µg of A. fumigatus Ags dissolved in normal saline via the intratracheal route. Four days after the intranasal challenge, mice received 20 µg of A. fumigatus Ags dissolved in normal saline via the intratracheal route. In additional groups of A. fumigatus-sensitized mice, 0.5 ml of polyclonal anti-C10 antiserum or normal rabbit serum was delivered into the peritoneal cavity of these mice 2 h prior to challenge. Prior approval for mouse usage in these experiments was obtained from the Animal Welfare Committee of the University of Connecticut Health Center, Storrs, CT.

Anti-C10 Ab generation

Polyclonal anti-C10 antiserum was generated by the multiple site immunization of a New Zealand White rabbit using an Escherichia coli-expressed C10 protein (25, 26). The resulting anti-C10 antiserum was purified over a protein A affinity column, and 10 µl of rabbit anti-C10 antiserum neutralized approximately 20 ng of C10 in vitro (data not shown). This antiserum was titrated by direct ELISA, and no cross-reactivity with the following recombinant murine cytokines and chemokines: IL-1β, TNF-α, IL-4, IFN-γ, IL-10, IL-6, MIP-1α, MCP-1, MIP-1β, RANTES, KC, eotaxin, MIP-2, and MARC (MCP-3). For this and other methods described below, all recombinant murine cytokines and chemokines were obtained from R&D Systems (Minneapolis, MN), Genzyme (Cambridge, MA), or Pepro Tech (Rocky Hill, NJ).

Cell isolation and culture

Alveolar macrophages were isolated from bronchoalveolar lavage (BAL) samples taken from nonsensitized CBA/J mice. BAL samples were obtained using the multiple intratracheal introduction of 1 ml of PBS containing 50 mM EDTA. MC-9 mast cells were grown out from lethally irradiated or whole lung homogenates using a standardized protocol (35). The eosinophils used in this experiment were obtained from the National Institute of Health, Bethesda, MD. Briefly, 1 ml of thioglycolate broth was delivered by i.p. injection into each mouse, and 48 h later the peritoneal cavity of these mice was thoroughly lavaged with normal saline. The collected cells were suspended in RPMI 1640 containing 5% FBS, 2-ME (10 µM), sodium pyruvate (2 mM), 1-glutamine (20 mM), penicillin (100 U), and streptomycin (100 µg/ml). Eosinophils were adhered purified in 175-ml tissue culture flasks for 1 h to yield preparations that contained approximately 85% eosinophilic granulocytes. Contaminating cells were primarily lymphocytes. These cells were suspended at 1 × 10⁶ cells/ml of RPMI-10, and a 100-µl aliquot of this suspension was placed in individual wells of a 24-well microchemotaxis chamber. The upper wells were separated from lower wells containing 10–100 ng/ml of C10 or eotaxin by a 3-µm-pore-size polycarbonate filter (Costar, Pleasanton, CA). Cells were incubated for 4 h at 37°C in a 5% CO₂ incubator, after which the filters were fixed in 4% paraformaldehyde, stained in hematoxylin and eosin, and mounted onto a glass microscope slide for light microscopy visualization. Migrated eosinophils were subsequently quantified in at least twenty 400 fields of view.

Assessment of bronchial hyperresponsiveness

Bronchial hyperresponsiveness was assessed in a Buxco plethysmograph (Buxco, Troy, NY) specifically designed for the low tidal volumes of mice as described previously (36). Sodium pentobarbital (Butler, Columbus, OH; 0.04 mg/g of mouse body weight)-anesthetized mice were intubated and constantly ventilated using a Harvard pump ventilator (Harvard Apparatus, Reno, NV). The following ventilation parameters were employed for each mouse: tidal volume = 0.25 ml, breathing frequency = 120/min, and positive end-expiratory pressure = 5 cm H₂O. Within the sealed plethysmograph chamber that contained transpulmonary pressure (Δ inspire pressure – Δ mouse chamber pressure) and inspiratory volume or flow were monitored online by an adjacent computer. Airway resistance was continuously calculated online by a specialized computer software program (Buxco), and this measurement was the result of the division of the transpulmonary pressure by the change in inspiratory volume. Following a baseline period, mice received 1 µg/mouse of methacholine, and airway responsiveness to this nonelectroselective bronchoconstrictor was again calculated online. Nonsensitized mice exhibited little change in airway resistance following a similar challenge with methacholine. At the conclusion of the assessment of airway responsiveness, a BAL was performed on each mouse. The cell-free supernatant from each BAL sample was frozen at −20°C before chemokine and cytokine ELISA. Whole lungs were then dissected from the thoracic cavity, and snap frozen in liquid N₂ or prepared for histologic analysis.

Quantification of leukocytes in BAL

Cells suspended in the BAL were pelleted onto glass slides by cytocentrifugation and subjected to Diff-Quik (Baxter, McGraw Park, IL) staining, and polymorphonuclear and mononuclear cells were then quantified by light microscopy at ×200 magnification.

Chemokine and cytokine ELISA analysis of BAL and whole lung

Murine eotaxin, MCP-1, C10, KC, MIP-2, MARC (mouse MCP-3), MIP-1α, IL-13, and IL-10 were determined in 50-µl supernatant samples from each mouse by using a standardized sandwich ELISA (37). Briefly, Nunc-immuno ELISA plates (MaxiSorp) were coated with the appropriate cytokine capture polyclonal Ab at a dilution of 1–5 µg/ml of coating buffer (0.6 M NaCl; 0.26 M H₃BO₃; 0.08 M NaOH; pH 9.6) for 16 h at 4°C. The rabbit polyclonal Abs directed against murine eotaxin, C10, KC, IL-10, and IL-13 were purchased from R&D Systems. The rabbit polyclonal Abs directed against murine MCP-1, MIP-2, and MARC were purified from the sera of immunized rabbits, as previously described (37). These rabbit polyclonal Abs were used for capture and detection in the ELISA system, and the specificity of each was
confirmed by running a panel of recombinant cytokines and chemokines through each ELISA (see panel listed above). The unbound capture Ab was washed away, and each plate was blocked with 2% BSA-PBS for 90 min at 37°C. Each ELISA plate was then washed with PBS Tween-20 (0.05%; v/v), and 50-μl samples either undiluted or diluted 1/10 were added to duplicate wells and incubated for 1 h at 37°C. Following the incubation period, the ELISA plates were then thoroughly washed and the appropriate biotinylated polyclonal rabbit anti-cytokine Ab (3.5 μg/ml) was added. The polyclonal Abs were biotinylated using an EZ-Link system from Pierce (Rockford, IL). After washing the plates 30 min later, streptavidin-peroxidase (Bio-Rad Laboratories, Richmond, CA) was added to each well for 30 min, and each plate was thoroughly washed again. Chromagen substrate (Bio-Rad Laboratories) was added, and optical readings at 492 nm were obtained using an ELISA plate scanner. Recombinant murine cytokines and chemokines 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.

**Lung histologic analysis**

Whole lungs from *A. fumigatus*-sensitized mice were fully inflated by intratracheal perfusion with 4% paraformaldehyde before dissection from the thoracic cavity and placement in fresh paraformaldehyde for 24 h. Routine histologic techniques were used to paraffin-embed this tissue, and 5-μm sections of whole lung were counterstained with Mayer’s hematoxylin (Mayer & Myles Laboratories, Coopersburg, PA) for the visualization and identification of eosinophils. Inflammatory infiltrates and other histologic changes were monitored around blood vessels and airways using light microscopy at a magnification of ×200.

**Determination of systemic IgE**

Sera from *A. fumigatus*-sensitized mice were analyzed for total IgE before and following intratracheal *A. fumigatus* challenge. Complementary capture and detection Ab pairs for mouse IgE were obtained from Pharmingen (San Diego, CA), and the IgE ELISA was performed according to the enclosed directions. Duplicate sera samples were diluted 1/100, IgE levels in each were calculated from OD readings at 492 nm, and IgE concentrations were calculated from a standard curve generated using rGE (5–2000 pg/ml).

**Data statistical analysis**

All results are expressed as mean ± SEM (SE). All test conditions were completed in duplicate wells of a tissue culture plate. A Student’s t-test was used to determine statistical significance between the control and anti-C10 groups; p < 0.05 was considered statistically significant.

**Results**

Experimental allergic bronchopulmonary aspergillosis is associated with greatly augmented C10 levels in the BAL

Although C-C chemokines have previously been shown to exert significant roles during airway allergic responses to a number of small protein Ags, the expression of chemokines during experimental ABPA was previously uncharacterized. The results from ELISA analysis of BAL cell-free supernatants from our experimental model of ABPA for the presence of C-C chemokines (i.e., MCP-1, eotaxin, MIP-1α, and MARC), CXC chemokines (i.e., MIP-2 and KC), and C10 are shown in Fig. 1. Major elevations above baseline values (measured in BAL from sensitized mice before intratracheal *A. fumigatus* challenge) were observed in BAL levels of C10, MCP-1, and eotaxin, but C10 was increased the greatest to approximately 30 ng/ml by 48 h after intratracheal challenge (Fig. 1). By comparison, MCP-1 and eotaxin levels reached 5 and 1 ng/ml, respectively, over the same period of time (Fig. 1). At 72 h after *A. fumigatus* challenge, BAL levels of all of the chemokines examined had returned to baseline values or were not detected by ELISA. These findings suggested that the allergic airway responses to *A. fumigatus* Ag involved profound changes in C10 generation, particularly in the 48-h period after *A. fumigatus* Ag challenge.

![BAL chemokine levels from A. fumigatus-sensitized mice at various times after intratracheal challenge with A. fumigatus Ag.](Image)

**FIGURE 1.** BAL chemokine levels from *A. fumigatus*-sensitized mice at various times after intratracheal challenge with *A. fumigatus* Ag. Immediately following the determination of the airway hyperresponsiveness to methacholine challenge (see Materials and Methods), mice were intratracheally lavaged with PBS containing 50 mM EDTA. The total volume of the lavage obtained from each mouse was 1 ml. The cells contained in the BAL were pelleted onto microscope slides for quantification, and specific ELISA analyzed the cell-free supernatants. Data are mean ± SE from a minimum of five mice per timepoint.

**Alveolar macrophages, pulmonary fibroblasts, and VSMCs generate C10**

Given the very dramatic elevations in C10 observed in the ABPA mice following *A. fumigatus* challenge, the putative cellular sources of C10 in the lung were next explored. Consistent with previous studies showing that C10 is an IL-4-inducible chemokine in isolated peritoneal macrophages (26), isolated alveolar macrophages from nonsensitized mice were a constitutive source of C10, and IL-4 was the most potent inducer of C10 release by these cells (Table I). Another immune cell, the MC-9 mast cell, was a much poorer constitutive source of C10 (0.38 ± 0.02 ng/ml), and 48/80 activation of these mast cells did not markedly augment their release of C10 (0.44 ± 0.02 ng/ml) above the constitutive level. Our previous studies suggested that structural cells in the lung such as lung fibroblasts and smooth muscle might also generate C10 (38). Thus, the putative contribution of fibroblasts and VSMCs to the overall C10 production within the lung was also examined. As shown in Fig. 2, constitutive and cytokine-inducible C10 production was detected in 24-h cultures of both structural cell types. In VSMCs, only IL-1β, either alone or in combination with IL-4, IFN-γ, or IL-10, enhanced C10 levels approximately 3-fold above levels detected in untreated (i.e., control) cultures (Fig. 2). The presence of either IL-1β, TNF-α, or IL-10 in cultures of pulmonary fibroblasts enhanced C10 production at least 2-fold above

<table>
<thead>
<tr>
<th>Cytokine Treatment (all cytokines at 10 ng/ml)</th>
<th>C10 Levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11 ± 0.1</td>
</tr>
<tr>
<td>IL-1β</td>
<td>19 ± 3.0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>IL-4</td>
<td>40 ± 0.4*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>15 ± 1.1</td>
</tr>
<tr>
<td>IL-10</td>
<td>16 ± 2.0</td>
</tr>
</tbody>
</table>

* Alveolar macrophages were purified from BAL samples of nonsensitized CBA/J mice. A total of 1.0 × 10⁶ cells were exposed to cytokines at 10 ng/ml for 24 h prior to the removal of cell-free supernatants for ELISA analysis for C10.

* p ≤ 0.05 compared with control.
control levels (Fig. 2). C10 levels were also enhanced between 3- and 5-fold (above control level) in cultures of pulmonary fibroblasts that were incubated with similar combinations of cytokines for 24 h. Taken together, these data indicated that alveolar macrophages and pulmonary structural cells generated C10 in response to inflammatory and immune cytokines.

Airway hyperresponsiveness during experimental aspergillosis is modulated by C10
To evaluate the significance of changes in C10 generation within the allergic airways, C10 was immunoneutralized in *A. fumigatus*-sensitized mice 2 h before intratracheal challenge with *A. fumigatus* Ag. C10 levels in BAL samples from both treatment groups were markedly greater than levels measured in the whole homogenates, consistent with the observation noted above that alveolar macrophages were a major source of C10 particularly following exposure to IL-4 (see Table I). The successful immunoneutralization of C10 using an i.p. injection of anti-C10 antiserum was confirmed in BAL (Fig. 3) and in whole lung homogenates (Fig. 3) removed before and 24 and 48 h after intratracheal *A. fumigatus* Ag challenge. However, in the lung homogenates at 48 h after *A. fumigatus* challenge, C10 levels were not different between the two treatment groups.

Airway responses to methacholine in *A. fumigatus*-sensitized mice immediately before (i.e., at *t* = 0) intratracheal challenge with *A. fumigatus* Ag were not different from responses measured in nonsensitized mice (data not shown). However, at 24 and 48 h after the intratracheal administration of *A. fumigatus* Ag in sensitized mice, airway hyperresponsiveness to methacholine provocation was significantly elevated approximately 3-fold above airway responses measured prior to *t* = 0 (Fig. 4). Immunoneutralization of C10 in *A. fumigatus*-sensitized mice reversed the increase in airway hyperresponsiveness at 24 and 48 h after intratracheal challenge. In addition, airway hyperresponsiveness to methacholine at 48 h post-*A. fumigatus* challenge was significantly lower than responses measured in allergic mice that received normal rabbit serum (Fig. 4).

C10 is an eosinophil chemoattractant
The directed migration or chemotaxis of leukocytes down a chemokine concentration gradient is a classical hallmark of chemokine activation (39). The chemotaxis of eosinophils following chemokine activation is limited to a select group of C-C chemokines that until now did not include C10. However, the present experiments showed that eosinophils from the peritoneal cavity of mice chronically infected with *S. mansoni* exhibited marked chemotactic responses to 100 ng/ml of C10 (Table II). Nevertheless, C10 was not as potent as similar concentrations of eotaxin in the chemotaxis of eosinophils (Table II).

Immunoneutralization of C10 markedly diminishes airway eosinophilia during experimental ABPA
As depicted in Fig. 5, the presence of eosinophils in the BAL of allergic mice that received anti-C10 antiserum was significantly reduced at 24 and 48 h after *A. fumigatus* challenge compared with allergic mice that were pretreated with normal serum. Also at the
24-h timepoint after *A. fumigatus* challenge, significantly fewer lymphocytes and macrophages were present in cytospins of BAL (Fig. 5). However, macrophage counts in the BAL of anti-C10 antiserum-pretreated mice were significantly elevated at 48 h after Ag challenge compared with BAL counts from mice that were pretreated with normal serum. Neutrophil counts were not different between the two treatment groups.

Further confirmation of the role of C10 in the tissue eosinophilia associated with experimental ABPA was confirmed from histologic samples derived from mice before *A. fumigatus* Ag challenge (Fig. 6, A and B), at 24 h (Fig. 6, C and D), and at 48 h (Fig. 6, E and F) after this challenge. Little inflammation was present in either the normal serum (Fig. 6A) or anti-C10 antiserum (Fig. 6B) treatment groups before the intratracheal challenge of these Aspergillus-sensitized mice with *A. fumigatus* Ag. After *A. fumigatus* challenge, dramatic histologic changes in the lungs of ABPA mice included major inflammatory infiltrates surrounding various sized airways and blood vessels at 24 h (Fig. 6C) and at 48 h (Fig. 6E) after *A. fumigatus* Ag challenge. The inflammatory infiltrate around the airways of these mice that received normal serum was composed of abundant eosinophils, accompanied by smaller quantities of lymphocytes, macrophages, and neutrophils. Conversely, a

### Table II. Eosinophil chemotactic responses to C10 and eotaxin

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>C10 (ng/ml)</th>
<th>Eotaxin (ng/ml)</th>
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<tr>
<td>1</td>
<td>57 ± 8</td>
<td>131 ± 9</td>
<td>149 ± 4</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>149 ± 4</td>
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<tr>
<td>100</td>
<td></td>
<td>191 ± 15</td>
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<tr>
<td>100</td>
<td></td>
<td>145 ± 15</td>
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</tr>
<tr>
<td>100</td>
<td></td>
<td>288 ± 6</td>
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* Migrated eosinophils were counted on fixed and stained polycarbonate membranes by light microscopy at ×400. Data are the mean ± SE of a minimum of 20 fields of view. Similar findings were obtained in an additional experiment.

FIGURE 4. Effect of anti-C10 antiserum treatment on bronchial hyperresponsiveness in *A. fumigatus*-sensitized mice before and at 24 and 48 h after intratracheal *A. fumigatus* Ag challenge. Sensitized mice received 0.5 ml of anti-C10 antiserum or normal rabbit serum (i.e., control) via an i.p. injection 2 h before the Ag challenge. Bronchial hyperresponsiveness to i.v. methacholine provocation was conducted as described in Materials and Methods. Data are mean ± SE from a minimum of five mice per timepoint in both treatment groups. *, $p \leq 0.05$ compared with mice before *A. fumigatus* Ag challenge (i.e., $t = 0$). †, $p = 0.034$ compared with the corresponding control mice that received normal rabbit serum before *A. fumigatus* Ag challenge.

FIGURE 5. The presence of eosinophils, lymphocytes, neutrophils, and macrophages in BAL from *A. fumigatus*-sensitized mice at 24 and 48 h after intratracheal *A. fumigatus* Ag challenge. Sensitized mice received 0.5 ml of anti-C10 antiserum or normal rabbit serum (i.e., control) via an i.p. injection 2 h before the Ag challenge. BAL counts were made from pelleted whole cell preparations from each mouse immediately after i.v. methacholine provocation (see Materials and Methods). Only macrophages were present in BAL samples from both groups immediately before the intratracheal *A. fumigatus* challenge (data not shown). ‡, $p \leq 0.001$; *, $p \leq 0.05$ compared with the corresponding control mice that received normal rabbit serum before *A. fumigatus* Ag challenge.
clear paucity of inflammatory cells surrounding large airways was observed when ABPA mice received anti-C10 antiserum, most notably at the 24-h (Fig. 6D) and 48-h (Fig. 6F) timepoints after *A. fumigatus* challenge. Thus, these data suggested that C10 has a major effect on the recruitment or movement of eosinophils and other leukocytes into the airways during experimental ABPA.

**Immunoneutralization of C10 attenuates eotaxin and MCP-1 levels in BAL and whole lung**

While the previous data suggested that C10 directly affected the recruitment of leukocytes to the allergic airway, we next examined the possibility that changes in recruitment were partly the consequence of C10 modulating the generation of other chemotactic cytokines within the lung. To test this postulate, specific ELISA examined MCP-1 and eotaxin levels in BAL and whole lung homogenates. These C-C chemokines were also elevated with C10 in the BAL from *A. fumigatus*-challenged mice (see Fig. 1), and both have been shown to be major participants in other eosinophil-mediated airway allergic models (36, 40). Immunoneutralization of C10 in ABPA mice significantly inhibited the levels of eotaxin and MCP-1 present in BAL and whole lung homogenates before and at 24 h after intratracheal *A. fumigatus* challenge (Fig. 7).

**C10 does not affect systemic IgE levels**

A hallmark of clinical ABPA is increased systemic IgE levels (43). Similar to the clinical condition, the murine model of ABPA used in the present study showed similar dramatic changes in serum levels of IgE (Fig. 9). Although lower levels of IgE were observed before and at 48 h after *Aspergillus* intratracheal challenge, these changes did not reach statistical significance. These findings are interesting when considering previous studies showing that allergic responses to *A. fumigatus* proceed even in the absence of IgE (12).

**Discussion**

ABPA complicates asthma (4), and results in lung destruction due to a complex interaction between pulmonary resident cells such as epithelium, alveolar macrophages, mast cells, fibroblasts, and smooth muscle, and infiltrating leukocytes such as eosinophils and T cells (19). The evolution of this cellular milieu within the allergic lung is dependent upon the expression of adhesion molecules and numerous soluble mediators, including the recently described chemotactic cytokines or chemokines (18, 40). The present
The study demonstrates that the endogenously generated C10 chemokine modulates many of the pulmonary features of experimental ABPA. Among the chemokines analyzed in BAL and whole lung homogenates, C10 was present at the highest levels during the inflammatory response to an intratracheal *A. fumigatus* challenge. Based on the present in vitro observations, this increase may have been the consequence of C10 production by alveolar macrophages, vascular smooth muscle, and fibroblasts within the inflamed lung. Compared with *A. fumigatus*-sensitized and challenged mice that received normal rabbit serum, the neutralization of C10 using an anti-C10 antiserum abrogated the bronchial hyperresponsiveness and eosinophilia that followed intratracheal *A. fumigatus* delivery in sensitized mice. Diminished C10 levels also significantly attenuated the presence of lymphocytes in the BAL, and the levels of MCP-1, eotaxin in BAL, and whole lung homogenates. Decreased C10 was also associated with significantly lower IL-13 in whole lung homogenates, but lung levels of IL-10 and serum IgE were not changed. Thus, C10 exerts a prominent role during the allergic airway response to *A. fumigatus* challenge.

Sources of C10 include a diverse array of cells from macrophage (26) to trigeminal ganglia (44). Based on our previous studies of a Th2-type pulmonary granulomatous response in the lung...
response to also explain, in part, the decreased C10 levels measured in these allergen-challenged lungs. In addition, the absence of recruited leukocytes in the lungs, pulmonary resident cells are also excellent sources of both chemokines (32, 50). Furthermore, the lack of endogenous C10 may have directly affected the ability of these cells to generate MCP-1 and eotaxin. IL-13 is a product of T cells and alveolar macrophages (51) that induces Ig production, B cell proliferation, and monocye differentiation. In addition, IL-13 and IL-4 share the same cell activation pathway (52), which may explain why allergic responses to A. fumigatus develop normally in mice genetically deficient in IL-4 (8). Consistent with the decrease in lymphocyte recruitment in anti-C10 antiserum-treated mice, less IL-13 was also detected in whole lung homogenates from these mice. IL-10 appears to exert a prominent role during pulmonary responses to A. fumigatus that relates to the ability of this cytokine to balance the Th1 and Th2 cytokine responses in this model (13). The presence of IL-10 also suppresses the oxidative burst response, but enhances the phagocytotic activity of mononuclear cells exposed to Aspergillus (53). In the present study, IL-10 levels in whole lung homogenates were significantly attenuated as the allergic response progressed, but additional changes in IL-10 were not observed in the ABPA model when C10 was inhibited. The relative impact of reduced pulmonary IL-10 during the progression of the allergic response to A. fumigatus is not presently apparent, and warrants further study. In addition, the mechanism through which C10 modulates the synthesis of MCP-1, eotaxin, and IL-13 within the allergic lungs deserves further attention.

In patients with clinical ABPA, elevations of IgE correlate both with allergic inflammation of the airways and with bronchial hyperresponsiveness (2). Whereas a central role for IgE in the pathogenesis of the eosinophilic inflammation as well as in the obstructive airway physiology associated with ABPA has been suggested, experimental ABPA proceeds normally in IgE-deficient mice (12). The present data demonstrate that although C10 effectively abolishes many of the features of experimental ABPA, this chemokine does not appear to affect serum levels of IgE. Therefore, these findings suggest that C10 may not participate in the sensitization process to A. fumigatus Ags.

Chemoskines have been shown to exert defined effects on leukocyte recruitment to the allergic airways and the development of airway hyperresponsiveness in experimental models (36, 40, 54). The role of chemokines in the pathogenesis of ABPA is less well defined, but the results from the present study show that the C10 chemokine exerts a prominent role in the development of lung inflammation and bronchial hyperresponsiveness. Further examination of the role of these types of chemokines in clinical and experimental ABPA may be warranted.

Other observations in the ABPA model during the suppression of endogenous C10 activity indicated that C10 possibly modulates the levels of other mediators previously shown to be present during or to contribute to the development of allergic responses. MCP-1 and eotaxin are two examples of a proallergic mediator (36, 40, 49). Although the diminution of MCP-1 and eotaxin levels observed in the present study may reflect the reduction in recruited leukocytes in the lungs, pulmonary resident cells are also excellent sources of both chemokines (32, 50). Furthermore, the lack of endogenous C10 may have directly affected the ability of these cells to generate MCP-1 and eotaxin. IL-13 is a product of T cells and alveolar macrophages (51) that induces Ig production, B cell proliferation, and monocye differentiation. In addition, IL-13 and IL-4 share the same cell activation pathway (52), which may explain why allergic responses to A. fumigatus develop normally in mice genetically deficient in IL-4 (8). Consistent with the decrease in lymphocyte recruitment in anti-C10 antiserum-treated mice, less IL-13 was also detected in whole lung homogenates from these mice. IL-10 appears to exert a prominent role during pulmonary responses to A. fumigatus that relates to the ability of this cytokine to balance the Th1 and Th2 cytokine responses in this model (13). The presence of IL-10 also suppresses the oxidative burst response, but enhances the phagocytotic activity of mononuclear cells exposed to aspergillus (53). In the present study, IL-10 levels in whole lung homogenates were significantly attenuated as the allergic response progressed, but additional changes in IL-10 were not observed in the ABPA model when C10 was inhibited. The relative impact of reduced pulmonary IL-10 during the progression of the allergic response to A. fumigatus is not presently apparent, and warrants further study. In addition, the mechanism through which C10 modulates the synthesis of MCP-1, eotaxin, and IL-13 within the allergic lungs deserves further attention.

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References


FIGURE 9. Serum IgE levels in A. fumigatus-sensitized mice before and at 24 and 48 h after intratracheal A. fumigatus Ag challenge. Sensitized mice received 0.5 ml of anti-C10 antiserum or normal rabbit serum (i.e., control) via an i.p. injection 2 h before the Ag challenge. Sera from both treatment groups were analyzed by specific ELISA for total IgE. Data are mean ± SE from a minimum of five mice per timepoint in both treatment groups.


