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

Cory M. Hogaboam,2* Chad S. Gallinat,† Dennis D. Taub,‡ Robert M. Strieter,‡ Steven L. Kunkel,* and Nicholas W. Lukac*†

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 minor due in large 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, making it unique from other chemokines (24), and this additional exon is necessary for a significant portion of the biologic activity of this molecule (25). C10 is chemotactic for B cells and CD4+ T cells (26), and is highly homologous to human chemokines such as MIP-16 (27), CC18 (28), HCC-1, and HCC-2 (29). These human chemokines all possess similar affinity for CC chemokine receptor 1 and promote T cell and monocyte chemotaxis. Previous studies indicated that unlike numerous other chemokines, C10 is IL-4– but not LPS-inducible in macrophages and requires de novo protein synthesis 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.
Materials and Methods

Murine 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 (Gree Laboratories, Lenoir, NC) dissolved in 0.2 ml of IFNa (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 Ag dissolved in normal saline via the intranasal route. Four days after treatment, lungs were harvested and minced, and the resulting suspension was used for the intranasal challenge. Prior approval for mouse usage in these studies was obtained from University Laboratory Animal Medicine (ULAM) facility at the University of Michigan Medical School (Ann Arbor, MI).

Anti-C10 Ab generation

Polyclonal anti-C10 antisera 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 antisera was purified over a protein A affinity column, and 10 ng of rabbit anti-C10 antisera neutralized approximately 20 ng of C10 in vitro (data not shown). This antisera 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 through the multiple intratracheal introduction of 1 ml of PBS containing 50 mM EDTA. MC-9 mast cells (ATCC CRL 8306) represent a mature, nontransformed mast cell line, and these cells were maintained in culture with IL-3 supplementation and DMEM growth medium containing 1% (v/v) antibiotic-antimycotic and 15% (v/v) FBS (DMEM-15). Fibroblasts and vascular smooth muscle cells (VSMCs) from nonsensitized CBA/J mice were grown out from lung using techniques previously described in detail elsewhere (32, 33). Briefly, pulmonary fibroblasts were grown out from mechanically dispersed whole lungs in 175-mm tissue culture flasks containing DMEM-15. VSMCs were grown from explants of large pulmonary vessels in 60-mm tissue culture dishes in the presence of 2D collagen. Confluent cultures of these cells exhibit a “hill and valley” appearance, and confluent cultures of these cells exhibited a myofibroblast-type phenotype (34). Each well in a six-well tissue culture plate was seeded with approximately 1×10⁵ fibroblasts or VSMCs. Twenty-four hours later, the DMEM growth medium was replaced with RPMI containing 10% FBS (RPMI-10) containing IL-1β, TNF-α, IL-4, IFN-γ, or IL-10 at 10 ng/ml. Cytokine combinations of IL-1β + IL-4, IL-1β + IFN-γ, IL-1β + IL-10, TNF-α + IL-4, TNF-α + IFN-γ, or TNF-α + IL-10 (10 ng/ml each) were also added to other wells. Twenty-four hours after the addition of cytokines to cultured fibroblasts and VSMCs, cell-free supernatants were removed for the measurement of C10 levels by ELISA.

Eosinophil chemotaxis

A modified Boyden chamber technique was used to quantify eosinophil chemotactic responses to C10 and eotaxin, a potent and selective eosinophil chemotactic (35). The eosinophils used in this experiment were isolated from mice chronically infected with Schistosoma mansoni (these mice were obtained from the National Institute of Health, Bethesda, MD). Briefly, 1 ml of thioglycollate 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 adherence purified in 175-mm tissue culture flasks for 1 h to yield preparations that contained approximately 85% eosinophil granulocytes. Contaminating cells were primarily monocytes. These cells were stained weakly for actin, suggesting a myofibroblast-type phenotype. Eosinophils were adhesion purified in 175-mm tissue culture flasks for 1 h to yield preparations that contained approximately 85% eosinophil granulocytes. Contaminating cells were primarily monocytes. These cells were stained weakly for actin, suggesting a myofibroblast-type phenotype. Eosinophils were adhesion purified in 175-mm tissue culture flasks for 1 h to yield preparations that contained approximately 85% eosinophil granulocytes. Contaminating cells were primarily monocytes. These cells were stained weakly for actin, suggesting a myofibroblast-type phenotype.
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-emboss 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 rIgE (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 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.

![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.](http://www.jimmunol.org/)

**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>
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* Alveolar macrophages were purified from BAL samples of nonsensitized CBA/J mice. A total of 1.0 × 10^6 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 fibro-
blasts that were incubated with similar combinations of cytokines 
for 24 h. Taken together, these data indicated that alveolar mac-
rophages 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. fumiga-
tus Ag. C10 levels in BAL samples from both treatment groups 
were markedly greater than levels measured in the whole homog-
enates, 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 immunoneutraliza-
tion of C10 using an i.p. injection of anti-C10 antiserum was con-
firmed 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 sensi-
tized mice, airway hyperresponsiveness to methacholine provoca-
tion was significantly elevated approximately 3-fold above airway 
responses measured prior at $t = 0$ (Fig. 4). Immunoneutralization 
of C10 in A. fumigatus-sensitized mice reversed the increase in 
airway hyperresponsiveness at 24 and 48 h after intratracheal chal-
lenge. 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 se-
rum (Fig. 4).

C10 is an eosinophil chemoattractant

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

FIGURE 3. Effects of anti-C10 antiserum treatment on pulmonary C10 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. C10 was measured in cell-free BAL samples and in whole lung homogenates taken from allergic mice immediately following the determi-
nation of bronchial hyperresponsiveness (see 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 the corresponding control mice that received normal rabbit serum before A. fumigatus Ag challenge.
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
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 \textit{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 \textit{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 \textit{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 \textit{Aspergillus} intratracheal challenge, these changes did not reach statistical significance. These findings are interesting when considering previous studies showing that allergic responses to \textit{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
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...
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 monocyte 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 antisera-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 phagocytic 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.

Chemokines 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.

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