CC Chemokine Receptor-2 Is Not Essential for the Development of Antigen-Induced Pulmonary Eosinophilia and Airway Hyperresponsiveness

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CC Chemokine Receptor-2 Is Not Essential for the Development of Antigen-Induced Pulmonary Eosinophilia and Airway Hyperresponsiveness

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Monocyte chemoattractant proteins-1 and -5 have been implicated as important mediators of allergic pulmonary inflammation in murine models of asthma. The only identified receptor for these two chemokines to date is the CCR2. To study the role of CCR2 in a murine model of Ag-induced asthma, we compared the pathologic and physiological responses of CCR2−/− mice with those of wild-type (WT) littermates following immunization and challenge with OVA. OVA-immunized/OVA-challenged (OVA/OVA) WT and CCR2−/− mice developed significant increases in total cells recovered by bronchoalveolar lavage (BAL) compared with their respective OVA-immunized/PBS-challenged (OVA/PBS) control groups. There were no significant differences in BAL cell counts and differentials (i.e., macrophages, PMNs, lymphocytes, and eosinophils) between OVA/OVA WT and CCR2−/− mice. Serologic evaluation revealed no significant difference in total IgE and OVA-specific IgE between OVA/OVA WT mice and CCR2−/− mice. Lung mRNA expression and BAL cytokine protein levels of IL-4, IL-5, and IFN-γ were also similar in WT and CCR2−/− mice. Finally, OVA/OVA CCR2−/− mice developed increased airway hyper-responsiveness to a degree similar to that in WT mice. We conclude that following repeated airway challenges with Ag in sensitized mice, the development of Th2 responses (elevated IgE, pulmonary eosinophilia, and lung cytokine levels of IL-4 and IL-5) and the development of airway hyper-responsiveness are not diminished by a deficiency in CCR2. The Journal of Immunology, 2000, 165: 6568–6575.

The recruitment of cells to sites of inflammation is an essential component of the host inflammatory response. Cell recruitment relies on the coordinated action of cell adhesion, cell activation, chemotaxis, and transmigration across the endothelial barrier (1). The chemokines are a superfamily of small, secreted proteins that share the ability to chemoattract leukocytes (2). Chemokines induce cell migration and activation by binding to specific G protein-coupled cell surface receptors on target cells (3). CC chemokine receptor 2 (CCR2) is a member of the CC chemokine receptor family and mediates signal transduction for the monocyte chemoattractant proteins (MCPs)7 MCP-1 (CCL2), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13), and MCP-5 (CCL12) (4–10). CCR2 is the only identified receptor for MCP-1 and MCP-5 and is expressed on many hemopoietic cells, including monocytes, basophils, activated lymphocytes, and dendritic cells (4, 11–13). More recent studies have also demonstrated expression of CCR2 on vascular smooth muscle and endothelial cells (14, 15).

The role of CCR2 in inflammatory and infectious diseases has been investigated using mice with a targeted deletion of this receptor (16–20). CCR2−/− mice developed normally and exhibited no obvious phenotypic abnormalities in the unchallenged state. However, following antigenic challenge and in models of infection, CCR2−/− mice demonstrated significant defects in monocyte recruitment and impaired IFN-γ production, with resultant defects in granuloma formation and host defense against certain intracellular pathogens (e.g., Listeria monocytogenes, Leishmania, and Cryptococcus neoformans) (16–19, 21, 22). Taken together these data support the hypothesis that CCR2 plays an important role in monocyte/macrophage recruitment and in Th1-type inflammatory responses.

The role of CCR2 in models of Th2 inflammation is less well studied. Murine models of asthma have been used to study Th2 inflammatory responses in the lung. These models are characterized by the development of Ag-induced 1) airway hyper-responsiveness (AHR), 2) recruitment of T cells and eosinophils to the airway lumen and lung, 3) elevated levels of Ag-specific IgE in the serum, 4) elevated Th2 cytokines (IL-4, IL-5) in bronchoalveolar lavage (BAL) and lung, and 5) enhanced expression of chemokines with specificities for T lymphocytes and eosinophils in the lung (23–29). We and others have shown that some of the ligands of CCR2 are up-regulated in the murine model of asthma (i.e., MCP-1, MCP-3, and MCP-5) (8, 30–32). Neutralization of MCPs with mAbs has been reported to modulate some aspects of the phenotype induced by allergen exposure. For example, neutralization of either MCP-1 or MCP-5 diminished AHR in Ag-challenged mice.
mice (31). Anti-MCP-1 also diminished leukocyte recruitment to BAL and lung interstitium, while anti-MCP-5 diminished lung interstitial, but not BAL, leukocyte recruitment. To further examine the specific role of CCR2 in an established murine model of asthma, we compared the responses of CCR2<sup>−/−</sup> mice with those of wild-type (WT) littermates following immunization and challenge with OVA.

**Materials and Methods**

**Mice**

CCR2<sup>−/−</sup> mice were generated as previously described (16). CCCR2<sup>−/−</sup> and CCCR2<sup>+/+</sup> (WT) littermate controls were generated from matings between heterozygous (CCR2<sup>+/−</sup>) mice of similar genetic background (Sv129×C57BL/6 hybrids). Mice were studied between 6 and 10 wk of age. The mice were bred and housed in a pathogen-free animal facility and were given food and water ad libitum.

**Immunization and challenge protocol**

The sensitization and challenge protocol was previously described (32). Mice were immunized with 10 μg of OVA (Sigma, St. Louis, MO) and 1 mg of aluminum hydroxide i.p. on days 0 and 7. Mice underwent aerosol challenge with either PBS or OVA (5% in PBS) for 20 min/day for 4 days, 7 days after the final immunization. Aerosol challenge was performed by placing mice in a Plexiglas box (dimensions: 22 × 23 × 14 cm) and aerosolizing OVA using a DeVilbiss nebulizer, driven by compressed air. Mice were studied 24 h after the last aerosol challenge.

**Determination of pulmonary resistance and airway responsiveness**

Airway responsiveness was measured as previously described (32, 33). In brief, dose-response curves to methacholine were obtained in anesthetized and ventilated mice 24 h after the last aerosol challenge. Methacholine was administered sequentially in increasing doses (33–1000 μg/kg i.v.). From the relationship between the dose administered and pulmonary resistance (R<sub>p</sub>), the effective dose that resulted in a doubling of R<sub>p</sub> was determined by log-linear interpolation. This dose is referred to as the effective dose required to increase R<sub>p</sub> to 200% of control values (ED<sub>200</sub>R<sub>p</sub>) and was used as a measure of airway responsiveness. Because the doses of agonist are given in geometrically increasing amounts, it is common to log-transform this index.

**Bronchoalveolar lavage**

BAL was performed after the physiological measurements as previously described (32). In brief, the lungs were lavaged with six 0.5-ml aliquots of PBS containing 0.6 mM EDTA. Recovered live cells (trypan blue exclusion) were enumerated in a hemocytometer, and cell differential counts were determined by enumerating macrophages, neutrophils, eosinophils, and lymphocytes on Wright-stained (Leukostat, Fisher Scientific, Pittsburgh, PA) cytocentrifuge preparations. BAL cytokine and eosinophil peroxidase (EPO) were determined in the lavage fluid after recovery from the first 1 ml of instilled PBS/EDTA to avoid overdilution of the BAL fluid components. BAL supernatants were aliquoted and frozen (−80°C) for subsequent cytokine and eosinophil peroxidase (EPO) determinations.

**Histology and immunohistochemistry**

Lungs were harvested after BAL and inflation-fixed to total lung capacity in 10% formalin. Formalin-preserved lung tissue was stained with hematoxylin and eosin using standard protocols. The severity of the inflammatory response was graded semiquantitatively for the following features: overall inflammation, granulomatous inflammation, giant cells, macrophages, lymphocytes, and eosinophils. Each of these features was graded on a scale of 0–3, where 0 represented none, 1 mild, 2 moderate, and 3 severe, based on the severity and extent of the features in the histological sections. The slides were randomized and blinded before grading and were read by one person (F.G.).

**Flow cytometry**

Flow cytometry was performed as previously described (29). In brief, BAL cells were suspended in staining buffer (PBS with 10% mouse serum) and incubated with the appropriate conjugated Abs at 4°C for 30 min. After washing with PBS, the cells were fixed with 1% paraformaldehyde. Single- and three-color flow cytometries were performed on a FACScan (Becton Dickinson, San Jose, CA) cytometer, and analysis was performed using LYSYS software (Hewlett Packard, Palo Alto, CA).

**Total serum IgE and OVA-specific IgE**

OVA-specific IgE levels were measured by capture ELISA as previously described (32). ELISA microtiter plates were coated with a purified anti-mouse IgE mAb (PharMingen, San Diego, CA) at a concentration of 2 μg/ml and blocked with PBS-10% FCS. Serum samples were diluted in PBS-10% FCS and incubated in the wells for 2 h. After washing with PBS-Tween, biotinylated OVA (10 μg/ml) was added to the wells and incubated for 1 h. The plates were washed with PBS-Tween followed by the addition of avidin alkaline phosphatase (Sigma) for 1 h. The plates were washed with PBS-Tween and distilled water before the addition of the phosphatase substrate. The plates were allowed to develop for 30 min. The plates were read in an ELISA plate reader at OD<sub>405nm</sub>. Total serum IgE was measured by capture ELISA in a manner similar to the detection of OVA-specific IgE. A biotinylated rat anti-mouse IgE (PharMingen) was used to detect captured IgE in place of the biotinylated OVA.

**BAL EPO levels**

BAL EPO levels were measured as previously described (34). One hundred microliters of serum or standard (porcine EPO; ExOxEmis, San Antonio, TX) were plated on 96-well plates and incubated with 100 μl of the assay reaction mixture containing 0.05 M Tris buffer, pH 8.0 (Sigma), 0.0036% H<sub>2</sub>O<sub>2</sub> (Fisher Scientific, Pittsburgh, PA), 0.001% Triton X (Sigma), and 0.18 mg/ml ortho-phenylenediamine (Sigma). The plates were incubated in the dark for 30 min. The reaction was terminated with 50 μl of 4 M H<sub>2</sub>SO<sub>4</sub> per well. The plates were read on an ELISA plate reader at OD<sub>405nm</sub>.

**BAL cytokine ELISAs**

BAL cytokine determinations were performed by capture ELISA according to the protocols provided by the manufacturers (IL-4 and IFN-γ, PharMingen; IL-5, Endogen, Cambridge, MA). The limit of detection of the IL-4 and IFN-γ assays was 10 pg/ml and for the IL-5 assay 5 pg/ml.

**RNA isolation and ribonuclease protection assay (RPA)**

Lung RNA was isolated as previously described (32). In brief, lungs were homogenized in 8 ml of 4 M guanidine thiocyanate, 25 mM sodium acetate (pH 7.0), and 0.1 M 2-ME using a Polytron (Brinkmann Instruments, Westbury, NY). RNA was isolated by gradient density centrifugation over 5.7 M CsCl/25 mM sodium acetate (pH 5.0; 32,000 rpm, 25°C, for 18 h). For the RPA analysis, 5 μg of total RNA was analyzed for cytokine expression using the mCK-1 Th1/Th2 RPA template set (PharMingen). Radiolabeled RNA probes for IL-4, IL-5, IL-10, IL-13, IL-15, IL-9, IL-2, IL-6, IFN-γ, IL-32, and GAPDH were prepared according to the manufacturer’s instructions by in vitro transcription using 100 μCi of 3000 Ci/mmol [α-35S]UTP and T7 RNA polymerase. Following in vitro transcription, the reaction mixture was treated with DNase and the radiolabeled probes were isolated by phenol-chloroform extraction and ethanol precipitation. The radio-labeled probes were dissolved in hybridization buffer and added to each RNA sample at a final concentration of 6 × 104 cpm/μl. Following an overnight incubation at 56°C, single-stranded RNA was digested with RNase and double-stranded protected RNA was purified by phenol-chloroform extraction and ethanol precipitation. Samples were analyzed on a 5% acrylamide/8 M urea gel. After drying, the gel was exposed to film for 18 days.

**Statistical analysis**

Student’s t test (unpaired, two-tailed) was used to calculate significance levels between treatment groups for BAL cell counts and differentials, EPO levels, and IgE levels. A Kruskal-Wallis test was used to compare the methacholine-induced airway responsiveness. Lung histology scores were compared using the Fisher’s exact test. p < 0.05 was considered statistically significant.

**Results**

**Inflammatory cell recruitment in the BAL of WT and CCR2<sup>−/−</sup> mice**

Recovery of cells from the BAL of OVA/PBS WT and CCR2<sup>−/−</sup> control mice revealed a predominance of alveolar macrophages in both groups, without any significant difference between WT and CCR2<sup>−/−</sup> mice (Fig. 1). In OVA/OVA WT and CCR2<sup>−/−</sup> mice, a significant increase in total cells, neutrophils, eosinophils, and lymphocytes was seen compared with their respective OVA/PBS controls (all p < 0.05); alveolar macrophage numbers were not significantly different between OVA/OVA- and OVA/PBS-treated mice (Fig. 1). There was no significant difference in BAL cell
recoveries (total or specific cell types) between OVA/OVA WT and CCR2−/− mice (Fig. 1). These findings indicate that mice deficient in CCR2 had similar numbers of resident BAL cells as WT mice (i.e., OVA/PBS group). In addition, CCR2−/− mice were able to recruit significant numbers of inflammatory cells into the airway lumen following Ag challenge (OVA/OVA) in a manner comparable to WT mice.

Subset analysis of lymphocytes recovered by BAL from OVA/OVA WT and CCR2−/− mice was assessed by flow cytometry to determine whether differences existed in the lymphocyte subpopulations recruited to the airway lumen. There were no differences in the percentages of B220+, CD3+, CD4+, or CD8+ cells in the BALs of OVA/OVA WT and CCR2−/− mice (Fig. 2).

EPO levels in the BAL of WT and CCR2−/− mice

The level of EPO activity in the BAL supernatants of OVA/PBS and OVA/OVA WT and CCR2−/− mice was determined by colorimetric assay (Fig. 3). The levels of EPO activity were significantly higher in OVA/OVA WT and CCR2−/− mice compared with their respective OVA/PBS controls (\( p < 0.01 \) and \( p < 0.003 \), respectively), in agreement with the increased numbers of eosinophils seen in the OVA/OVA groups. There was no difference, however, between the WT and CCR2−/− mice with respect to EPO activity when comparing similarly treated mice.

Lung histology from WT and CCR2−/− mice

Histopathologic examination of lung tissue from OVA/PBS WT and CCR2−/− mice revealed normal lung histology in both groups (Fig. 4, A and B, respectively). Low power views of lung sections from both OVA/OVA WT (Fig. 4C) showed a pleomorphic peribronchial and perivascular inflammation, as has been previously described with this model (32). The lungs from OVA/OVA CCR2−/− (Fig. 4D) mice showed a similar perivascular and peribronchial inflammation. High power views of lungs from OVA/OVA WT (Fig. 4E) and CCR2−/− (Fig. 4F) mice revealed the presence of eosinophils in the inflammatory infiltrates in both groups (arrows). Semiquantitative grading of the slides from the OVA/OVA WT and CCR2−/− mice revealed that the numbers of eosinophils, lymphocytes, macrophages, giant cells, and overall inflammation were similar between the two groups (Table I). In some OVA/OVA WT and CCR2−/− mice, areas of inflammation in the lung periphery consisting primarily of focal collections of macrophages and lymphocytes (granulomatous-like inflammation) were observed (Table I). Granulomatous-like inflammation was absent or mild in 16 of 16 WT mice and 10 of 18 CCR2−/− mice. More severe grades of granulomatous-like inflammation (grades 2 and 3) were seen in 8 of 18 CCR2−/− mice compared with WT mice (Table I; \( p < 0.001 \), by Fisher’s exact test).
Total IgE and OVA-specific IgE in WT and CCR2−/− mice

Immunization and aerosol challenge with OVA was associated with the development of increased titers of both total and OVA-specific IgE in both WT and CCR2−/− mice, compared with their respective OVA/PBS controls (∼ p < 0.05 for both groups; Fig. 5). There was no significant difference in the levels of total and OVA-specific IgE in similarly treated WT and CCR2−/− mice. These data indicate that a deficiency in CCR2 did not affect the ability to induce a Th2-dependent humoral response.

Cytokine determinations in BAL fluid in WT and CCR2−/− mice

Levels of the Th2 cytokines IL-4 and IL-5 were assessed in BAL fluid samples of OVA/PBS and OVA/OVA WT and CCR2−/− mice (Table II). Levels of IL-4 and IL-5 were below the limit of detection in the OVA/PBS-treated mice of either genotype. Both WT and CCR2−/− OVA/OVA-treated mice showed elevated IL-4 and IL-5 levels in the BAL fluid compared with their respective
There was no difference in BAL IL-4 levels between OVA/OVA WT and CCR2<sup>−/−</sup> mice (p = 0.85). There was a trend toward greater levels of IL-5 in the BAL of CCR2<sup>−/−</sup> mice compared with WT, but this difference did not reach statistical significance (p = 0.09). IFN-γ levels were assessed in the BAL fluid of OVA/OVA WT and CCR2<sup>−/−</sup> mice. Four of seven BAL samples from WT OVA/OVA mice and three of seven samples from CCR2<sup>−/−</sup> OVA/OVA mice had undetectable levels of IFN-γ. The remaining WT samples had IFN-γ levels that ranged from 16–51 pg/ml, while the remaining CCR2<sup>−/−</sup> samples had a range of 13–41 pg/ml. There was no difference in the mean BAL IFN-γ levels between WT and CCR2<sup>−/−</sup> mice.

**RPA analysis of lung mRNA**

To assess the levels of Th1 and Th2 cytokines in the lung tissue, RPA analysis was performed on whole lung RNA isolated from OVA/PBS and OVA/OVA, WT and CCR2<sup>−/−</sup> mice (Fig. 6). Constitutive expression of IL-15 mRNA was seen in all mice. There was increased expression of IL-5, IL-13, and IFN-γ in the OVA/OVA WT and CCR2<sup>−/−</sup> mice compared with their respective OVA/PBS controls. However, no differences were seen between the OVA/OVA WT and CCR2<sup>−/−</sup> mice with respect to the induction of any of the Th1 or Th2 cytokines. There was no induction of IL-2, IL-4, IL-6, IL-9, or IL-10 in any group.

**Airway responsiveness in WT and CCR2<sup>−/−</sup> mice**

OVA/OVA WT mice had significantly increased methacholine responsiveness (as reflected by a lower log ED<sub>200</sub>) compared with OVA/PBS WT controls (p < 0.020; Fig. 7). Similarly, OVA/OVA CCR2<sup>−/−</sup> mice developed significantly enhanced methacholine responsiveness compared with their OVA/PBS controls (p < 0.0006; Fig. 7). There was no significant difference in airway responsiveness between similarly treated WT and CCR2<sup>−/−</sup> mice. These findings indicate that Ag-induced AHR develops in CCR2 cell-deficient mice and that the increased responsiveness was not significantly different from that of similarly treated WT animals.

**Discussion**

A murine model of allergic pulmonary inflammation was used to examine the role of CCR2 in the development of Th2 inflammation and AHR. We found that following systemic immunization and

<table>
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<th>Parameter</th>
<th>None (n)</th>
<th>Mild (n)</th>
<th>Moderate (n)</th>
<th>Severe (n)</th>
<th>p Value&lt;sup&gt;a&lt;/sup&gt; (WT vs CCR2&lt;sup&gt;−/−&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>7</td>
<td>8</td>
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<td>9</td>
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<tr>
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<sup>a</sup> Fisher’s exact test.

**Table II. BAL cytokine levels**

<table>
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<tr>
<th>Group</th>
<th>IL-4 (pg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IL-5 (pg/ml)&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>WT OVA/OVA</td>
<td>30.4 (±7.1)</td>
<td>80.9 (±24.3)</td>
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<tr>
<td>CCR2&lt;sup&gt;−/−&lt;/sup&gt; OVA/OVA</td>
<td>28.5 (±7.2)</td>
<td>175.0 (±65.9)</td>
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</table>

<sup>b</sup> Results are pooled data from two experiments, n = 14 in each group.

<sup>c</sup> p = 0.85, comparing WT to CCR2<sup>−/−</sup>.

<sup>d</sup> p = 0.09, comparing WT to CCR2<sup>−/−</sup>.
repeated airway challenges, the percentages and numbers of recovered leukocytes from BAL were not different between WT and CCR2−/− mice. Following challenge with PBS, the BAL of CCR2−/− mice was composed primarily of alveolar macrophages (>98%), as was seen in WT mice, and suggested that CCR2−/− mice have normal numbers of resident macrophages in the airway lumen. These data are consistent with the findings regarding peritoneal macrophages in CCR2−/− mice, where normal numbers of resident macrophages were demonstrated at baseline (16–18). Following Ag sensitization and challenge, CCR2−/− mice developed significant increases in total BAL cells compared with mice challenged with PBS alone. The composition of the inflammatory cell infiltrate (i.e., quantity of macrophages, PMNs, lymphocytes, and eosinophils) in CCR2−/− mice was not significantly different from that in similarly treated WT mice, suggesting that the lack of CCR2 does not alter the recruitment of any of the cell populations in the BAL in this Th2-type inflammatory model.

Previous studies in CCR2−/− mice have demonstrated diminished macrophage/monocyte recruitment in murine models of Th1-type granulomatous inflammation (16–18). The lack of any differential recruitment of macrophages in CCR2−/− mice in our model is probably due to the nature of the inflammatory response in this model (i.e., Th2-type inflammation), which may be less dependent on recruited monocytes than a Th1 response. CCR2 is expressed on activated T lymphocytes, and many of the ligands for CCR2 (e.g., MCP-1, -2, and -3) are chemotactic for these cells (35). As such, one might have expected a differential recruitment of lymphocytes to the BAL in CCR2−/− mice. However, the BAL differentials and the flow cytometric analysis of BAL lymphocytes demonstrated no difference in the recruitment of T or B lymphocytes in Ag-challenged CCR2−/− mice compared with WT mice. These data suggest that alternate chemoattractants are recruiting lymphocytes to the lung in this model. Likewise, eosinophil recruitment to the airways was not affected by a deficiency in the CCR2 receptor. These data demonstrate that the recruitment of eosinophils to the airways is not dependent on the presence of CCR2 in this model and suggest that the signals required to recruit eosinophils to the lung and airway of these mice are intact.

AHR was assessed in CCR2−/− and WT mice to determine the role of CCR2 in regulating this physiological response in the murine model. We found that CCR2−/− mice had baseline airway responsiveness (i.e., OVA/PBS group) similar to WT mice. Following Ag challenge, CCR2−/− mice developed increased airway responsiveness compared with their own OVA/PBS controls; however, their responsiveness was not significantly different from that in OVA/OVA WT mice. These data demonstrate that CCR2 is not critical for the development of increased airway responsiveness following repeated aerosol Ag challenge in sensitized mice.

BAL cytokine levels and lung cytokine mRNA expression were analyzed to assess whether differences existed between WT and CCR2−/− mice in an allergic model of inflammation. Cytokine responses have been reported to be altered in CCR2−/− mice in models of Th1-type inflammation and infection (16–19, 21, 22). Diminished production of IFN-γ has been reported and has been associated with defects in delayed-type hypersensitivity responses, granuloma formation, and defective host immune response to intracellular pathogens. Further studies have suggested that the expression of CCR2 plays an important role in determining the polarization of T cell responses to intracellular pathogens, favoring a Th2 response when CCR2 was lacking (19, 22). If the predominant cytokine defect in CCR2−/− mice is a diminished production of IFN-γ, we might have expected to see a Th2-permissive effect in our model. We noted a trend toward higher levels of IL-5, but not IL-4, cytokines in the BAL fluid; however, this did not reach statistical significance, and there was no difference in the pulmonary eosinophilia, total and OVA-specific IgE production, lung cytokine mRNA expression, or AHR.

In a model of Th2-granulomatous inflammation (schistosomal Ag-elicited), CCR2−/− mice displayed diminished expression of some Th1 (IFN-γ) and Th2 (IL-4) cytokines in the lungs and lymphoid tissue, but granuloma formation was not inhibited (36). In our studies both OVA/OVA WT and CCR2−/− mice developed the characteristic peribronchial and perivascular inflammation with lymphocytes and eosinophils that characterizes this model. The protocol used in our studies also led to the development of small areas of granulomatous-like inflammation in the lungs of both OVA/OVA WT and CCR2−/− mice. These areas were characterized by focal collections of lymphocytes and macrophages. Interestingly, OVA/OVA CCR2−/− mice developed a greater degree of granulomatous-like inflammation in the lung compared with WT mice following OVA inhalation. The difference in inflammatory response in the lungs of CCR2−/− mice warrants further investigation.

**FIGURE 6.** Th1 and Th2 cytokine mRNA expression in WT and CCR2−/− mice. RPA was performed on whole lung RNA isolated from OVA/PBS and OVA/OVA, WT and CCR2−/− mice. Th1 and Th2 cytokine mRNA expression was determined using the mCK-1 RPA template (PharMingen) as described in Materials and Methods. Each lane represents the RNA from a single mouse lung from a representative experiment.
and p, required to increase lung resistance (R_L) to 200% of the control value/CCR2 (reported in a separate model of allergic pulmonary inflammation). Levels of infection with intracellular parasites (19, 22). Recent observation that MCP-1-deficient mice respond differently to immunologic challenges compared with CCR2 receptors (elevated IgE, pulmonary eosinophilia, and cytokine levels of IL-4 and IL-5) and the development of Th2 responses (31). Neutralization of MCP-5 by mAb diminished the recruitment of all leukocyte subsets in the lung interstitium and BAL in sensitized mice following repeated airway challenges in sensitized mice, and was also associated with a decrease in AHR. Our experiments suggest that in the absence of the receptor for MCP-1 and MCP-5 (i.e., CCR2), airway inflammation and AHR are not diminished. The apparent differences between our findings and those of Gonzalo may be explained by the fact that neutralization of the ligand produces effects different from those resulting from a life-long absence of the receptor. Alternatively, ligand neutralization may simply result in a phenotype distinct from that occurring from deletion of the receptor. This is supported by the recent observation that MCP-1-deficient mice respond differently to immunologic challenges compared with CCR2 mice. MCP-1-deficient mice have been reported to have a defect in the development of Th2 responses (37). This is in contrast to CCR2 mice, which favor a polarization toward a Th2 phenotype in models of infection with intracellular parasites (19, 22).

The role of CCR2 in the development of AHR has been reported in a separate model of allergic pulmonary inflammation (38). Campbell et al. found partially diminished airway responsiveness in CCR2 mice following sensitization and challenge with cockroach allergen. The diminished airway resistance in CCR2 mice was accompanied by diminished BAL histamine levels, suggesting a role for pulmonary mast cells in this process. Interestingly, as in our studies, pulmonary eosinophilia did not differ between WT and CCR2 mice. The authors concluded that the diminished AHR was not dependent on pulmonary eosinophilia, but, rather, on a mast cell-dependent pathway (38). The differences in AHR in CCR2 mice seen in our experiments compared with those reported by Campbell et al. may be a consequence of methodologic differences in the models. In murine models of asthma the end points of AHR and pulmonary eosinophilia can develop by at least two distinct pathways: 1) a mast cell-dependent pathway and 2) a CD4+ T cell-dependent pathway (39, 40). The protocol used for sensitization and challenge may determine the relative importance of mast cell- vs T cell-mediated processes in regulating tissue eosinophilia and AHR. Mast cells and IgE are believed to play an important role in models where sensitization and challenge are relatively attenuated (40–42). In contrast, in protocols that use repeated airway challenges in sensitized mice, AHR and pulmonary eosinophilia develop independently of IgE, B cells, and mast cells, suggesting a primary role for T cells in these models (32, 42–45). This may explain the differences seen between our studies (multiple challenge model) and those of Campbell et al. (single-challenge model) (38).

In summary, previous studies have shown that CCR2 plays an important role in Th1-type inflammatory responses and may dictate the polarization of T cell responses toward a Th2 phenotype during infection with intracellular parasites. In a murine model of allergic pulmonary inflammation, the development of Th2 responses (elevated IgE, pulmonary eosinophilia, and cytokine levels of IL-4 and IL-5) and the development of AHR were not significantly affected by the absence of CCR2, suggesting that this receptor is not essential for the development of these responses.

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**References**


