Macrophage/epithelial cell CCL2 contributes to rhinovirus-induced hyperresponsiveness and inflammation in a mouse model of allergic airways disease

Dina Schneider,1 Jun Young Hong,2 Emily R. Bowman,1 Yutein Chung,1 Deepi R. Nagarkar,2 Christina L. McHenry,1 Adam M. Goldsmith,1 J. Kelley Bentley,1 Toby C. Lewis,1 and Marc B. Hershenson1,2

1Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, Michigan; and 2Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, Michigan

Submitted 8 June 2012; accepted in final form 27 November 2012


Respiratory viral infections are a common cause of asthma exacerbations. A large fraction of viral exacerbations are associated with human rhinovirus (HRV) infection. HRV is a member of the Picornaviridae family. HRV serotypes are classified on the basis of their cellular receptors. Major group viruses attach to intercellular adhesion molecule (ICAM)-1, whereas minor group viruses bind to proteins of the low-density lipoprotein receptor (LDL-R) family. The structural similarity of mouse and human LDL-R proteins allows for experimental infection of mice with minor group HRV and in recent years has led to the establishment of mouse models of HRV infection (1, 23).

Following experimental infection, HRV is detectable in the lower airways of subjects with asthma (9, 20, 28). Although individuals with asthma are not at greater risk of HRV infection than healthy individuals, the frequency, severity, and duration of lower respiratory tract symptoms in asthmatic patients is increased compared with nonasthmatic subjects (6). According to the current paradigm of asthma exacerbation, viral infection of airway epithelial cells induces production of chemokines, thereby recruiting inflammatory cells to the airways. Inflammatory cells, in turn, elaborate cytokines and mediators capable of increasing airways responsiveness. However, this paradigm fails to explain why asthmatic subjects suffer manifestations of lower airways disease after colds while normal subjects do not. We recently observed that allergen sensitization and challenge alters the polarization state of airway macrophages, resulting in an exaggerated chemokine response to HRV infection (21)

The CC chemokines are a class of small (8–10 kDa) chemotactic ligands with two adjacent cysteines near their amino terminus. Recent studies suggest that CC chemokine ligand (CCL)-2/monocyte chemotactic protein (MCP)-1 and its receptor CC chemokine receptor (CCR)-2 play important roles in the pathogenesis of asthma. Studies employing antibody depletion of CCL2 and CCR2-deficient mice demonstrate the requirement of this pathway for allergen-induced airway inflammation and hyperresponsiveness (3, 17). CCR2 blockade prevents Ascaris-induced asthma in monkeys (19). Polymorphisms in the CCL2 gene are associated with asthma in children (33) and adults (12). During asthma exacerbations, peripheral blood monocyte/dendritic cell subpopulations express high levels of CCR2 (32). Finally, we have shown that, in children with asthma, nasal aspirate CCL2 levels increase with natural colds and correlate with respiratory symptoms (15).

In the present study, we show that HRV infection strongly increases CCL2 expression in ovalbumin (OVA)-sensitized and -challenged mice and that upregulation of CCL2 in mice is required for HRV-induced airways inflammation and hyperresponsiveness. These findings demonstrate that HRV-infected macrophages produce CCL2 and suggest a role for CCL2 in HRV-induced exacerbations of allergic airways disease.
Materials and Methods

Generation of HRV. HRV1B and HRV39 (ATCC, Manassas, VA) were grown in HeLa cells, concentrated, partially purified, and titered as described previously (22). Similarly concentrated and purified HeLa cell lysates were used for sham infection. Fifty percent tissue culture infectivity doses (TCID₅₀) were determined by the Spearman-Karber method.

Mice, HRV exposure, and OVA sensitization and challenge. These experiments were approved by the Institutional Animal Care and Use Committee. Animal usage followed guidelines set forth in the “Principles of Laboratory Animal Care” (National Society for Medical Research). Female 8-wk-old BALB/c mice (Jackson Laboratories, Bar Harbor, MA) were inoculated intranasally with 45 pfu of 1 × 10⁶ TCID₅₀/ml HRV1B or an equal volume of sham control. Mice were sensitized intraperitoneally with 200 μl of a 0.5 mg/ml solution of alum and endotoxin-free OVA or PBS (Sigma-Aldrich, St. Louis, MO) on days 1 and 7 and challenged intranasally with 50 μl of a 2 mg/ml solution of OVA or PBS on days 14, 15, and 16. Selected mice were inoculated with HRV1B or sham immediately following the last OVA or PBS treatment. For MCP-1 neutralization, 100 μg of anti-MCP-1 antibody or isotype control hamster IgG (BD Biosciences, San Jose, CA) was administered intraperitoneally on the day of inoculation, and mice were killed the next day. Finally, for selected experiments, C129X1-Il4radm1Tch/j interleukin (IL)-4 receptor knockout mice (Jackson Laboratories) were studied.

Focused gene arrays of mouse lung lysates. Lung RNA from mice of OVA/sham and OVA/HRV groups was subjected to a targeted PCR array examining mouse inflammatory cytokines (SA Biosciences, Frederick, MD).

Quantitative real-time PCR. Whole lung, macrophage, and epithelial cell RNA was extracted with an RNeasy kit (Qiagen, Valencia, CA), and mRNA was analyzed for chemokine gene expression by quantitative real-time PCR. Signals were normalized to GAPDH and expressed as fold increase.

Measurement of CCL2 protein. CCL2 protein from lung lysates and bronchoalveolar lavage (BAL) macrophage supernatants was measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Lung inflammation. To quantify inflammatory cells, lung digests were obtained by mincing the tissue, proteolysis in collagenase type IV (Gibco Invitrogen, Carlsbad, CA), and straining through a 70-μm nylon mesh (BD Falcon, San Jose, CA), as described (24). The resulting pellet was treated with red blood cell lysis buffer (BD Pharmingen, San Diego, CA), and leukocytes were enriched by spinning the cells through 20% Percoll (Sigma-Aldrich). Lung pellet cytospins were stained with Diff-Quick (Dade Behring, Newark, DE), and counts were determined from 200 cells.

Immunohistochemistry and confocal fluorescence microscopy. Lungs were fixed with 10% formaldehyde overnight and paraffin embedded. Blocks were sectioned at a thickness of 5 μm, and each section was deparaffinized, hydrated, and stained. Sections were incubated with Alexa Fluor (AF)-555-conjugated goat anti-mouse JE/MCP-1 (R&D), AF633- rat anti-mouse CD68 (AbD Serotec, Raleigh, NC), guinea pig antiserum against HRV1B (ATCC), or AF-conjugated isotype control IgGs. Antiserum was partially purified by incubation with nitrocellulose-bound HeLa cell proteins and passing through an affinity resin containing non-denatured mouse lung protein. Repurified antibody was directly conjugated to AF488. The control used was AF488-conjugated guinea pig antiserum. Nuclei were stained with 4',6-diamidino-2-phenylindole. Images were visualized using a Zeiss Axioplan microscope equipped with and digital AxiosCamMR charge-coupled device camera.

Flow cytometry. Freshly isolated aliquots of lung mince were stimulated for 5 h with phorbol 12-myristate,13-acetate and ionomycin in the presence of brefeldin A, washed, fixed, permeabilized, and incubated with anti-mouse CCL2 and anti-mouse CD11b, a surface protein marker on macrophages.

Table 1. Description of human subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Indication for Bronchoscopy</th>
<th>Postoperative Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>Chest pain</td>
<td>Bronchomalacia, gastroesophageal reflux</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Chronic cough</td>
<td>Gastroesophageal reflux</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>Chronic cough</td>
<td>Tracheomalacia, swallowing dysfunction</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>Cough, wheeze</td>
<td>Tracheobronchomalacia, bronchitis (Streplococcus pneumoniae, Moraxella catarrhalis)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Persistent pulmonary infiltrate</td>
<td>Abnormal airway anatomy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2/MCP-1</td>
<td>4.1015</td>
<td>(1.35, 6.85)</td>
<td>0.01</td>
</tr>
<tr>
<td>CCL7/MCP-3</td>
<td>5.0258</td>
<td>(1.52, 8.53)</td>
<td>0.01</td>
</tr>
<tr>
<td>CCL4/MIP-1B</td>
<td>4.0677</td>
<td>(0.56, 7.58)</td>
<td>0.03</td>
</tr>
<tr>
<td>CCL19/MIP38</td>
<td>2.691</td>
<td>(1.35, 4.03)</td>
<td>0.02</td>
</tr>
<tr>
<td>CCL20/MIP3α</td>
<td>5.8024</td>
<td>(0.00001, 11.63)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Note:** Additional tables and figures may be present in the full version of the document, which is not included here.
marker for exudative macrophages (16). Filtered and washed cells were stained and subjected to flow cytometry, as described (2, 26, 30). Cells were analyzed on an LSR II flow cytometer (BD Biosciences). Data were collected using FACSDiva software (BD Biosciences) with automatic compensation and analyzed using FlowJo software (Tree Star, Ashland, OR). Isotype-matched irrelevant control monoclonal antibodies were tested simultaneously in all experiments.

Mouse bronchoalveolar inflammatory cells and macrophage culture. BAL was performed using 1 ml PBS. BAL fluid from PBS- and OVA-treated mice was seeded in 12-well plates. Macrophages were purified by plastic adherence (7, 14). Cytospins were stained and counted as described above. Diff-Quick staining showed adherent cells to consist of >90% macrophages, with the rest of the cells being neutrophils. Remaining cells were resuspended in RPMI (Invitrogen), stimulated for 2 h with sham or HRV1B (multiplicity of infection, 5.0), and RNA was harvested 24 h after infection. Selected cultures were pretreated with 30 ng/ml IL-4 and IL-13 (Peprotech, Rocky Hill, NJ).

Respiratory system resistance. Airway responsiveness was assessed by measuring changes in respiratory system resistance with nebulized methacholine. Resistance was measured using intubated plethysmography (Buxco, Wilmington, NC).

Airway epithelial cell culture. BEAS-2B human bronchial epithelial cells, a SV-40-transformed airway bronchial epithelial cell line, were purchased from ATCC. Cells were grown on collagen-coated (5 µg/cm²) plates in bronchial epithelial growth medium (Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum, L-glutamine, and penicillin/streptomycin (Gibco). Cells were purified by plastic adherence (7, 14), infected with HRV39 (multiplicity of infection, 5.0), and cultured for 8 h. CCL2 mRNA was quantified by qPCR.

Analysis of BAL macrophages from children with asthma. BAL macrophages were obtained from children undergoing flexible bronchoscopy at the University of Michigan C.S. Mott Children’s Hospital (Table 1). All procedures were done for clinical indications. Collection of extra BAL fluid was approved by the University of Michigan Institutional Review Board. Ten patients were studied, five with asthma and five with other conditions. BAL cells were seeded in 96-well plates at 1 × 10⁵ cells/well in 100 µl RPMI medium supplemented with 10% fetal bovine serum, l-glutamine, and penicillin/streptomycin (Gibco). Cells were purified by plastic adherence (7, 14), infected with HRV39 (multiplicity of infection, 5.0), and cultured for 8 h. CCL2 mRNA was quantified by qPCR.

Data analysis. Data are represented as means ± SE. For analysis of human BAL cell responses to RV infection, CCL2 mRNA values were normalized for GAPDH and log transformed. Statistical significance was assessed by unpaired t-test, one-way ANOVA, or two-way ANOVA, as appropriate. Group differences were pinpointed by Bonferroni post hoc test.

RESULTS

Focused gene array in OVA-sensitized and -challenged mice. To determine the proinflammatory cytokines involved in HRV-induced airway inflammation in OVA-sensitized and -challenged mice, we performed a focused gene array on the lung tissues of OVA/sham and OVA/HRV-treated mice. Mice were infected with HRV1B on the day of the last OVA or PBS challenge, and lung tissue was harvested 1 day later. Compared with lungs from OVA/sham mice, lungs from OVA/RV mice showed significant increases in the mRNA expression of MCP-1/CCL2, MCP-3/CCL7, macrophage inflammatory protein (MIP)-1α/CCL4, MIP-
3β/CCL19, and MIP-3α/CCL20 (Table 2). CCL2 mRNA and protein expression was increased in the lungs and BAL fluid, respectively, of OVA/RV mice (Fig. 1).

**CCL2 neutralization in vivo.** Based on studies implicating CCL2 in human asthma as well as asthma exacerbations (12, 15, 32, 33), we sought to examine the contribution of CCL2 to HRV-induced airway responsiveness by administering neutralizing antibody to mouse CCL2. OVA-treated mice were given one systemic injection of anti-CCL2 on the day of HRV inoculation. Control mice were treated with the isotype control. Mice were harvested for analysis on day 1 after HRV1B infection. As previously shown for BAL cells (21), HRV-infected OVA-sensitized and -challenged mice showed increased lung macrophages and eosinophils compared with sham-infected OVA-treated mice (Fig. 2A). Anti-CCL2 treatment significantly reduced lung macrophages and eosinophils in OVA-treated, HRV-infected mice. No reduction in neutrophils or lymphocytes was observed (data not shown). Finally, compared with IgG, anti-CCL2 significantly reduced the airways responsiveness of HRV-infected OVA mice (Fig. 2B).

![Fig. 3. CCL2 colocalizes with CD68/macrophages and HRV1B in OVA-sensitized and -challenged mice.](image-url)

**Fig. 3.** CCL2 colocalizes with CD68+ macrophages and HRV1B in OVA-sensitized and -challenged mice. A: PBS or OVA-treated mice were inoculated with sham or HRV, and sections were stained for HRV1B (green), CD68 (blue), and CCL2 (red) or isotype control IgGs. Merged images are shown for PBS/sham (A), PBS/HRV (B), OVA/sham (C), and OVA/RV (D). Colocalization is white (arrowheads). For OVA/RV lungs, individual stains for CCL2 (E), HRV1B (F), and CD68 (G) are shown. H: flow cytometric analysis of CCL2+, CD11b+ lung cells. I: group mean data for flow cytometric analysis. *Different from OVA/sham, P < 0.05, ANOVA.
Administration of anti-CCL2 did not affect the airways responsiveness of OVA/sham mice. Together, these data suggest that, following HRV1B infection of OVA-sensitized and -challenged mice, CCL2 is required for maximal macrophage and eosinophilic chemotaxis to the airways, as well as airways cholinergic responsiveness.

Production of CCL2 by CD68-positive macrophages in OVA/HRV-treated lungs. To investigate the cellular source of CCL2 production in vivo, we examined the lungs of PBS/sham-, PBS/HRV-, OVA/sham-, and OVA/HRV-treated mice by fluorescence microscopy. Antibodies to CCL2, HRV1B, and the macrophage cell surface marker CD68 were used for colocalizations (Fig. 3, A–G). Lungs from PBS/HRV-infected mice showed HRV1B-positive epithelial cells and macrophages, and modest CCL2 staining, indicating that HRV infection alone does not induce a robust CCL2 response. Sections from OVA/sham lungs showed moderate CCL2 staining in the airway epithelial cell layer and lamina propria and rare CD68-positive macrophages. In contrast, sections from the HRV-infected OVA-treated mice showed colocalization of CCL2 and HRV1B in CD68-positive cells, consistent with the notion that lung macrophages express CCL2 in response to HRV1B infection.

We examined the CCL2 production of CD11b+ lung macrophages in vivo by flow cytometry. CD11b is a surface marker for exudative macrophages (16). Compared with PBS-treated, sham-inoculated mice, only HRV-infected OVA-treated mice showed a significant increase in CCL2+ cells (Fig. 3, H and I).

Macrophage HRV1B infection in vitro. To further investigate the production of CCL2 in HRV-infected macrophages, plastic-adherent BAL cells from PBS- and OVA-treated mice were seeded in culture and infected with sham or HRV1B for 24 h. Although macrophages from both PBS- and OVA-treated mice showed a significant increase in CCL2 mRNA expression following HRV1B infection, macrophages from allergen-sensitized and -challenged mice showed a significantly higher response (Fig. 4A). Macrophages from allergen-treated mice showed a significantly higher CCL2 protein response to HRV infection (Fig. 4B).

To determine whether the enhanced CCL2 response to HRV1B infection was due in part to Th2 cytokine exposure in vivo, macrophages from PBS-treated mice were infected with HRV1B in the presence of IL-4 and IL-13. IL-4/IL-13 pretreatment significantly increased CCL2 mRNA expression (Fig. 4A), suggesting the sufficiency of Th2 cytokines in this response. Similarly to primary BAL cells, RV1B infection of

![Graphs](http://ajplung.physiology.org/)
the alveolar macrophage cell line MH-S induced CCL2 mRNA expression only when cells were pretreated with IL-4/IL-13 (Fig. 4C). To determine the effects of Th2 cytokine exposure on the CCL2 response of airway epithelial cells, human airway epithelial cell cultures were infected with HRV39 in the absence or presence of IL-4 and IL-13. IL-4/IL-13 treatment significantly increased HRV39-induced CCL2 mRNA expression (Fig. 4D). Finally, to determine the requirement of IL-4 receptor signaling for HRV-induced macrophage CCL2 expression, IL-4 receptor knockout mice were treated with OVA and BAL macrophages infected with HRV ex vivo. Compared with wild-type mice, cells from IL-4 receptor knockout mice showed reduced HRV-induced CCL2 mRNA expression (Fig. 4E). In contrast, expression of tumor necrosis factor (TNF)-α, a type I cytokine, was not reduced (Fig. 4F).

Response of human BAL macrophages to HRV infection. We cultured BAL macrophages from 10 children, 5 with asthma and 5 without. BAL macrophages were purified by adherence and infected with HRV39 in culture for 8 h. RNA was extracted and assayed for CCL2 (Fig. 5). Compared with cells from nonasthmatic patients, HRV39-induced CCL2 mRNA expression was significantly higher in cells from asthmatic patients. Compared with sham-inoculated cells, macrophages from asthmatic subjects showed a significant increase in CCL2 mRNA after inoculated with replication-capable HRV39.

Discussion

CCL2 and its receptor CCR2 play important roles in the pathogenesis of asthma (3, 12, 17, 19, 33). Recent studies also suggest the importance of CCL2 and related chemokines in asthma exacerbations. Plasma CCL2 and CCL13/MCP-4 concentrations are higher in patients with chronic stable asthma than in normal subjects and further increased in individuals with an acute asthma exacerbation (4, 13). Nasal aspirate CCL2 (15), CCL7, and CCL13 (29) levels are increased in children with asthma suffering from respiratory viral infections, and concentrations of CCL7 and CCL13 significantly correlate with the number of recruited nasal macrophages. However, the cellular source of CCL2, the precise role of CCL2 in inflammatory cell recruitment, and the requirement of CCL2 for viral-induced airways hyperresponsiveness, among other questions, have not been examined. In the present study, we show that HRV infection strongly increases CCL2 expression in OVA-sensitized and -challenged mice, leading to additional airways inflammation and responsiveness. Moreover, CCL2 is required for this response, since neutralization of CCL2 decreased airway responses almost to levels produced by OVA sensitization and challenge alone.

Immunohistochemical stains, flow cytometry, and ex vivo studies showed that, in contrast to lung macrophages from naïve mice, macrophages from OVA-exposed mice express CCL2 in response to HRV1B infection. Pretreatment of both mouse lung macrophages and epithelial cells with the type 2 cytokines IL-4 and IL-13 increased HRV-induced MCP-1 expression, suggesting that exposure to these Th2 cytokines plays a role in the altered HRV response. Finally, bronchoalveolar macrophages from wheezing children expressed significant levels of CCL2 upon ex vivo exposure to HRV1B. Therefore CCL2, produced by macrophages and epithelial cells, may play an important role in the pathogenesis of viral-induced asthma exacerbations.

The capacity of airway epithelial cells to produce CCL2 (31), particularly in response to respiratory syncytial virus infection (25), has been previously demonstrated. In the present study, fluorescence microscopy showed CCL2 expression in the airway epithelial cells of OVA-sensitized and -challenged mice. However, CCL2 was also highly expressed in CD68-positive lung macrophages from HRV-infected, OVA-challenged mice. Furthermore, adherent BAL cells from OVA-treated mice and children with asthma, but not nonasthmatic subjects, expressed CCL2 mRNA in response to HRV infection ex vivo. In agreement with these results, it has been previously reported that human alveolar macrophages and peripheral blood monocytes release CCL2 in response to HRV16 (11). However, we show for the first time that CD68+, CD11b+ macrophages produce CCL2 in vivo and that BAL macrophages from allergen-sensitized and -challenged mice respond more vigorously to HRV infection than naïve macrophages. Finally, we found that adherent BAL cells from children with asthma also demonstrate a CCL2 response to HRV infection ex vivo. Together, these data suggest that macrophages may play a unique role in viral exacerbations.

The precise mechanism by which allergen sensitization and challenge enhances the CCL2 response of adherent BAL cells to HRV infection is unclear. To examine the potential role of the type 2-dominant allergic environment, we incubated cells from naïve mice with IL-4 and IL-13. Naïve mouse BAL macrophages infected in the presence of IL-4 and IL-13, two prototypical Th2 cytokines, increased the CCL2 response to HRV compared with medium alone, demonstrating the sufficiency of these cytokines to upregulate macrophage chemokine expression. However, the response was not as robust as that observed in cells isolated from OVA-treated mice. It is conceivable that additional Th2 cytokines are required for the response. Similar results were obtained in a mouse macrophage cell line, consistent with the notion that macrophages indeed
contribute to the observed upregulation of CCL2 responses. Type 2 cytokines increased the RV39 response of cultured human airway epithelial cells, suggesting that, in addition to macrophages, epithelial cells contribute to the heightened CCL2 production in response to HRV infection. Finally, to test the requirement of IL-4 and IL-13 for heightened HRV-induced CCL2 responses in BAL macrophages, we examined the effect of OVA treatment on IL-4 receptor knockout mice. We showed that IL-4 receptor knockout significantly reduces alveolar macrophage production of CCL2 in response to ex vivo HRV infection. In contrast, there was no effect of the knockout on ex vivo production of TNF-α, a type 1 cytokine. Because the entire CCL2 response was not blocked in the knockout cells, we conclude that IL-4/IL-13 signaling is at least partially required for the augmentation of CCL2 responses in OVA-treated mice.

CCL2 was first identified as a monocyte chemoattractant and activating factor (18, 34). However, CCL2 is also a chemoattractant for eosinophils and dendritic cells. Eosinophils express CCR2, and CCL2 induces eosinophil calcium mobilization and chemotaxis (8). Furthermore, in an OVA mouse model of allergic airways disease, CCL2 neutralization reduces eosinophil chemotaxis by 80% (10). In our study, CCL2 neutralization reduced the number of macrophages and eosinophils in the lung tissue of HRV-infected, OVA-treated mice, confirming the role of CCL2 in the chemotaxis of these cells to the airways. Because, as we show here, macrophages themselves produce CCL2, it is plausible to speculate that HRV infection sets up a positive feedback loop for CCL2-mediated inflammatory cell chemotaxis to the lung. In addition to CCL2, macrophages may be producing additional inflammatory mediators that attract eosinophils to the airways. Indeed, we have found that macrophages from HRV-infected, OVA-treated mice also produce eotaxin-1/CCL11 (21).

We would like to add a few comments about our mouse model of HRV infection. We (22) and others (1) have found that a much higher viral titer is required to infect mice compared with humans. This is to be expected, since differences in the homology of viral receptors and intracellular signaling mechanisms are likely to restrict viral infection and replication in mice. Nevertheless, we have clearly shown that human HRV1B replicates in mouse lungs, as evidenced by: 1) the presence of negative-strand viral RNA in the lungs of inoculated mice; 2) transmissibility of RV infection from the lung homogenates of inoculated mice to cultured HeLa cells; and 3) the induction of a robust lung interferon response (22). Replication-deficient ultraviolet-irradiated virus has none of these effects. In addition, because of the dissimilarity between human and mouse ICAM-1, our animal studies were limited to HRV1B, a minor group virus. However, the effects of HRV1B infection on wild-type mice are indistinguishable from those of HRV16, a major group virus, on transgenic human ICAM-1 mice (1). Major and minor group viruses induce nearly identical patterns of gene expression in cultured airway epithelial cells (5). Analysis of all HRV genomes revealed that HRV1 and -16 are highly homologous and respond similarly to antiviral compounds (27), implying that the distinction between some major and minor group strains may not be clinically relevant.

We conclude that, in the context of allergic airways disease, HRV-infected macrophages and epithelial cells elaborate CCL2, which is required for airways hyperresponsiveness. These data suggest a role for CCL2 in HRV-induced exacerbations of asthma.

GRANTS
This work was supported by National Institutes of Health Grants HL-081420 (M. B. Hershenson) and ES-016769 (T. C. Lewis).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES