Rhinovirus Infection Induces Interleukin-13 Production from CD11b-Positive, M2-Polarized Exudative Macrophages

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Abstract

Rhinovirus (RV) causes asthma exacerbations. Previously, we showed that adherent bronchoalveolar cells from allergen-treated mice produce IL-13 when stimulated with RV *ex vivo*, implicating cells of the monocyte/macrophage lineage in viral-induced airway inflammation. In this study, we hypothesized that RV infection of allergen-treated mice results in IL-13 production by CD11b+ exudative macrophages *in vivo*. We sensitized and challenged BALB/c mice with ovalbumin (OVA), after which mice were inoculated with RV or sham HeLa cell lysate. After 1 day, lungs were harvested, and cell suspensions were analyzed by flow cytometry. We repeated this process in IL-13 reporter mice, CD11b-DTR mice in which diphtheria toxin selectively depletes CD11b+ cells, and chemokine receptor 2 (CCR2) null mice. We found that lungs of mice infected with RV alone showed increases in CD45+, CD68+, F4/80+, Ly6C+, and CD11b^{high} cells, indicating an influx of inflammatory monocytes and exudative macrophages. The combination of OVA and RV had synergistic effects on the exudative macrophage number. However, CD11b+ cells from OVA-treated, RV-infected mice showed M2 polarization, including expression of CD206 and CD301 and production of IL-13. Similar results were obtained in IL-13

reporter mice. Diphtheria toxin depleted CD11b+, IL-13-producing cells in OVA-treated, RV-infected, CD11b-DTR mice, decreasing airway inflammation and responsiveness. CD11b+, Ly6C+ cells were reduced in CCR2 knockout mice. We conclude that, in contrast to naive mice, RV infection of mice with allergic airways disease induces an influx of IL-13-producing CD11b+ exudative macrophages bearing M2 macrophage markers. This finding further implicates alternatively activated macrophages in RV-induced asthma exacerbations.

Keywords: alternative activation; asthma; exacerbation; innate immunity

Clinical Relevance

Our studies show that CD11b+, alternatively activated lung macrophages from mice with allergic airways disease produce IL-13 in response to rhinovirus infection and contribute to airways inflammation and hyper-responsiveness. These studies identify the macrophage as a potential therapeutic target for the treatment of viral-induced asthma exacerbations.

Respiratory viral infections are frequent causes of asthma exacerbation. Rhinovirus (RV) is the most common virus isolated. The precise mechanisms by which RV induces asthma exacerbations are not completely known.

Although airway epithelial cells are thought to be the major target of RV infection, cells of the monocyte/macrophage

lineage may also interact with RV. We have noted colocalization of RV with CD68, a sialoadhesin associated with macrophage lineage cells, in the lungs of ovalbumin (OVA)-sensitized and OVA-challenged, RV-infected mice (1) and in human subjects with asthma experimentally infected with RV (2). In OVA-treated mice, depletion of

macrophages using clodronate decreased RV-induced airway inflammation and hyper-responsiveness, suggesting that, in the context of allergic airways disease, macrophages play a direct role in the response to RV (1). In this model, studies of adherent BAL cells using quantitative PCR showed up-regulation of M2 polarization markers and elaboration of

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IL-13 and other type 2 cytokines in response to RV infection *ex vivo* (1).

IL-13, a secreted protein that induces B lymphocyte class switching to IgE and epithelial cell mucus hypersecretion and eotaxin release, is required and sufficient for the asthma phenotype in mice (3, 4). Although IL-13 is produced in large quantities by activated CD4(+) Th2 lymphocytes, other cells may also produce IL-13, including macrophages (1, 5), basophils (6), mast cells (7), dendritic cells (8), type 2 innate lymphoid cells (9, 10), and type 2 myeloid cells (11). In pathologic conditions such as asthma, macrophages and other innate immune cells are ideally situated to respond to viral infections by production of IL-13 and other type 2 cytokines, leading to exacerbation.

The goal of this study is to better define the CD68+, IL-13-producing macrophages observed in our mouse model of RV-induced asthma exacerbation. We hypothesized that OVA sensitization and challenge results in an influx of CD11b+, M2-polarized exudative macrophages into the airway space. We also hypothesized that infection with RV induces release of IL-13 from these cells, increasing airways inflammation and hyper-responsiveness.

Materials and Methods

Animals

BALB/c mice, C57BL/6 mice, C.129S4(B6)-*Il13^{tm1(YFP/cre)Lky}/J* YetCre-13 IL-13 reporter mice (12), B6.FVB-Tg(ITGAM-DTR/EGFP)34Lan/J CD11b diphtheria toxin receptor (DTR) transgenic mice (13), and B6.129S4-*Ccr2^{tm1Ifc}/J* chemokine receptor 2 (CCR2) null mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed in the pathogen-free facility within the animal care unit at University of Michigan Unit of Laboratory Medicine.

Antigen Sensitization and Challenge, RV Infection

Female mice (6–8 wk old) were sensitized intraperitoneally with 50 μ l of 2 mg/ml endotoxin-free OVA in PBS mixed with alum or PBS control on Days 0 and 7. Mice were also sensitized intranasally with 50 μ l of 2 mg/ml OVA on Days 0 and 7. Mice were challenged with OVA or PBS intranasally on Days 12 and 13. Mice were

inoculated with 50 μ l 1×10^8 PFU/ml RV1B or an equal volume of HeLa cell extract (sham control) on Day 14. Finally, selected mice were sensitized and challenged with 100 μ g of *Dermatophagoides pteronyssinus* (house dust mite [HDM]) extract in 50 μ l PBS (Greer Labs, Lenoir, NC) by intranasal installation on Days 0, 7, 14, 15, and 16. Mice were infected with RV1B on Day 17 and studied 1 day after infection.

Depletion of CD11b+ Cells

Twenty-four hours after the last OVA challenge, CD11b-DTR mice were treated with 50 ng diphtheria toxin (DTX) intraperitoneally immediately before RV infection.

Flow Cytometry

One day after inoculation with RV or sham, lungs were perfused with 5 mM EDTA in PBS and minced. Minces were digested with 10 mg/ml collagenase and 100 μ g/ml DNase cocktail in Dulbecco's Minimal Essential Medium (Life Technologies, Grand Island, NY) for 1 hour at room temperature. Samples were washed with red blood cell lysis buffer and kept on ice in media containing 10% serum. Cell suspensions were stained with fluorescent-labeled antibodies against various leukocyte surface markers (CD45, CD68, F4/80, CD11b, CD11c, Ly6C, CD206, and CD301) or yellow fluorescent protein (YFP). Antibodies were purchased from EBiosciences (San Diego, CA) or Biolegend (San Diego, CA). Antibody-stained cells were washed, fixed in 4% paraformaldehyde, and analyzed on a flow cytometer (Canto2; Becton-Dickinson, San Jose, CA). Results were analyzed using FlowJo software (TreeStar, Ashland, OR).

Detection of Intracellular Cytokines

Digested lung cells were stimulated with Cell-Stimulation Cocktail Buffer for 3 hours at 37°C, washed, fixed, and permeabilized with Cell Permeabilization Buffer (both from EBiosciences). Cells were then incubated with phycoerythrin-conjugated anti-IL-13, washed, fixed, and analyzed by flow cytometry.

Histology and Immunohistochemistry

Lungs were fixed with 4% formaldehyde overnight. Paraffin sections (5 μ m thick) were processed for fluorescence microscopy as described (1). Hematoxylin and eosin

staining was performed on adjacent sections.

Analysis of Airway Resistance

Airway resistance was measured as described (14). Mice were anesthetized, intubated, and ventilated with a Buxco FinePointe operating system (Wilmington, NC). To assess airways responsiveness, mice were administered increasing doses of nebulized methacholine.

Statistical Analysis

Data were represented as mean $\pm$ SEM. Statistical significance was assessed by unpaired *t* test or by one-way or two-way ANOVA as appropriate. Differences were pinpointed by the Newman-Keuls multiple comparisons test.

Results

Influx of CD11b+ Macrophages into the Lungs of Mice Undergoing OVA Sensitization/Challenge and RV Infection

Whole lung cells were harvested from wild-type BALB/c mice treated with PBS or OVA for 13 days and then infected overnight with either sham or RV. Lung cells were stained with antibodies against leukocyte surface markers and analyzed using flow cytometry. Cells were gated according to size, complexity, and CD45 for leukocytes and then F4/80 for macrophages (Figure 1A). CD45+, F4/80^{high} macrophages were then analyzed for CD11b and CD11c. PBS-treated, sham-treated mice showed mostly CD11c+, CD11b– cells, typical of residential macrophages (15). Cells from RV-treated mice showed a substantial influx of CD11c+, CD11b^{high} cells, consistent with exudative macrophages (16–18). Similar results were found in OVA-treated mice and in OVA-treated, RV-infected mice (Figures 1A and 1B). When total cells were calculated, there was a synergistic increase in CD11b^{high} cells in OVA-treated, RV-infected mice (Figure 1C). When we examined the Ly6C expression of CD11b^{high} cells, RV-infected animals showed an increase in Ly6C+ inflammatory monocytes (Figure 1B). Together, these data demonstrate that RV infection causes an influx of inflammatory monocyte and exudative macrophages into the lung, which is increased in mice with preexisting allergic airways disease.

Figure 1. Rhinovirus (RV) infection increases the influx of macrophages into the lungs. Female BALB/c mice were sensitized with ovalbumin (OVA) or PBS control for 13 days before infection with RV or sham control. Twenty-four hours later, the mice were killed, and the lungs were digested and analyzed on flow cytometry. (A) Analysis of lung cells for size, complexity (side scatter), CD45, and F4/80. (B) Cell populations from each treatment group (PBS sham, PBS RV, OVA sham, and OVA RV) were examined for expression of CD11c and CD11b to identify monocyte lineage cells that were either resident macrophages (high CD11c, low CD11b) or exudative macrophages (high CD11c, high CD11b). High CD11b cells from RV-infected mice were also high for Ly6C. The result of incubation with isotype control antibodies is also shown. (C). Group mean data for percent and total CD45⁺, F4/80⁺, CD11b⁺ cells ($n = 3$ mice per group). *Different from PBS sham ($P < 0.05$, one-way ANOVA). †Different from OVA sham ($P < 0.05$, one-way ANOVA). FSC-A, forward scatter area; FSC-W, forward scatter width; SSC, side scatter.

OVA-Sensitization and Challenge Induces M2 Polarization of CD11b+ Cells in the Lungs

M2 macrophages play a role in asthma (19–21). We also demonstrated that OVA sensitization and challenge increases alternative activation of BAL macrophages (1). We next tested whether CD11b^{high} cells bear characteristics of M2-polarized macrophages. CD11b+ cells were examined for coexpression of CD206 and CD301, both of which are associated with M2 macrophages (22). Lungs from RV-infected mice showed a relatively small number of CD206– and CD301+ cells within the CD11b^{high} population, with the majority of CD11b+ cells being CD206 and CD301 negative (Figures 2A and 2B). In contrast, lungs from OVA-exposed mice showed a substantial increase in CD206+ and CD301+ cells within the CD11b^{high} population, and the number of M2 macrophages was further increased in the OVA-treated, RV-infected group (Figures 2A and 2B). These findings indicate that RV infection of naive mice induces infiltration of conventional macrophages into the lung, whereas RV infection of allergen-sensitized and allergen-challenged mice increases the number of M2 polarized lung macrophages.

To confirm that the effect of RV was due to intact virus rather than proteins from infected HeLa cells, we spun lysates from infected HeLa cells through a 100-kD cutoff membrane to exclude viral particles. (This is different from sham infection, which contains proteins from uninfected HeLa cells.) In contrast to RV, this filtrate had no effect on lung infiltration by CD11b+ cells or lung inflammation (Figure 2C).

CD11b+ Cells from OVA-Treated Mice Produce IL-13

Lung cells were stained with antibodies against leukocyte surface markers, permeabilized, and stained with antibody to detect intracellular IL-13. Lungs of mice treated with RV alone showed a minimal increase in CD11b+, IL-13+ cells compared with control mice (Figures 3A and 3B). In contrast, mice treated with OVA showed a significant increase in IL-13-producing macrophages. Finally, there was a synergistic increase in IL-13-positive cells in lungs from OVA-treated and RV-infected lungs. We examined the

expression of macrophage markers on IL-13-producing CD11b+ cells (Figure 3C). CD11b+, IL-13+ cells were high in CD68, F4/80, CD206, and CD301 staining, indicating that M2-polarized, CD11b+ exudative macrophages contribute to IL-13 production after allergic sensitization and viral infection.

RV1B Infection of HDM-Exposed Mice Induces IL-13 Production from CD11b+ M2-Polarized Macrophages

We performed similar experiments in mice sensitized and challenged through the airways with HDM, a more physiologic allergen. HDM was administered on Days 0, 7, 14, 15, and 16, and mice were infected with RV1B on Day 17. HDM-treated, RV-infected mice showed a synergistic increase in CD11b^{high} cells (Figure 4A). After HDM treatment, CD11b+ cells showed increased expression of the M2 markers CD206 and CD301 (Figure 4B) and produced IL-13 (Figures 4C and 4D).

YetCre-13 Transgenic Mice Show Activation of the IL-13 Promoter in CD11b+ Exudative Macrophages

YetCre-13 mice express a YFP-Cre fusion protein in IL-13-expressing cells. We exposed these mice to the same OVA sensitization/challenge and RV infection protocol as described for wild-type mice. Intracellular IL-13 production was assessed using an anti-YFP antibody (Figure 5A). YFP levels were the highest in CD11b+ cells from OVA-treated, RV-infected lungs. In addition, using a conjugated anti-IL-13, we found that the high YFP population in our flow analysis corresponded to the IL-13-producing cells. The one exception was the OVA-treated, RV-infected mice, which showed some autofluorescent YFP+, IL-13-negative cells.

We also examined activation of the IL-13 promoter by immunofluorescence microscopy, using antibodies against YFP and CD11b (Figure 5B). Lungs from PBS, sham-treated mice showed no staining. Lungs from RV-infected and from OVA-treated mice showed rare double-positive cells. Lungs from OVA-treated, RV-infected mice showed abundant colocalization of YFP and CD11b. Together, these findings are consistent with the notion that exudative macrophages are a major source of IL-13.

Depletion of CD11b+ Cells Decreases RV-Induced Airway Inflammation and Hyper-Responsiveness

We have shown that exudative macrophages high in CD11b, CD68, F4/80, CD206, and CD301 are increased in the lungs of OVA RV mice and a source of IL-13. We next investigated whether CD11b+ cells are required for RV-induced airway inflammation and hyper-responsiveness. We used mice containing a transgene encoding the simian DTR and green fluorescent protein under the control of the human CD11b promoter. CD11b-DTR mice were sensitized and challenged with OVA as described above and treated with DTX or PBS before RV infection. Lungs from OVA-treated, RV-infected mice given DTX before RV infection showed substantially fewer green fluorescent protein-expressing cells compared with those that did not receive DTX treatment (Figure 6A), indicating the loss of CD11b cells upon DTX treatment. DTX treatment also induced a loss of IL-13-positive cells, and hematoxylin and eosin staining showed reduced airway inflammation in the lungs of DTX-treated mice (Figure 6B). DTX treatment significantly reduced bronchoalveolar neutrophils, eosinophils, macrophages, and lymphocytes (Figure 6C). Finally, DTX-treated, OVA-exposed, RV-infected mice showed less airways responsiveness than PBS-treated, OVA-exposed, RV-infected mice at the 10 and 20 mg/ml methacholine doses (Figure 6D). Together, these results show that CD11b cells are required for RV-induced exacerbation of allergic airways disease.

CCR2 Is Required for Migration of Inflammatory Monocytes to the Lung

Exudative macrophages are derived from inflammatory monocytes recruited from the circulation in a CCR2-dependent manner (16, 17). We therefore examined the effects of CCR2 knockout on RV-induced lung inflammation. Compared with C57BL/6 mice, the number of F4/80+ CD11b+ Ly6C+ cells were reduced in RV-infected and in OVA-treated, RV-infected mice (Figure 7A). OVA-treated, RV-infected mice also showed reduced histologic evidence of lung inflammation (Figure 7B). CCR2–/– mice also showed reduced airways responsiveness (Figure 7C). Together, these results are consistent with the notion that exudative macrophages

Figure 2. OVA treatment and RV infection induce M2 polarization of lung macrophages. (A) CD45+ cell populations from whole lung digests that were high in CD11b and forward scatter (size) were analyzed for expression of M2 markers CD206 and CD301. The result of incubation with isotype control antibodies is also shown. (B) Group mean data for CD206+, CD301+ and CD206-, CD301- cells among the treatment groups ($n = 3$ mice per group). *Different from PBS sham ($P < 0.05$, one-way ANOVA). †Different from OVA sham ($P < 0.05$, one-way ANOVA). (C) Lysates from infected HeLa cells were spun through a 100-kD cutoff membrane to exclude viral particles and administered intranasally to BALB/c mice. In contrast to RV, this filtrate had no effect on lung infiltration by CD11b+ cells or lung inflammation.

require CCR2 for recruitment to the lung and that exudative macrophages contribute to RV-induced airways inflammation.

Discussion

The mechanisms by which RV and other viral infections cause asthma exacerbation

are not completely known. In this report, we characterized the role of the macrophage in mice sensitized and challenged with OVA and then infected with RV. We found that RV infection of naive mice, or of mice with allergic airways disease, increased the influx of CD45+, CD68^{high}, Ly6C+, CD11b^{high}, and CD11c^{high} cells

into the lungs. In the context of OVA sensitization and challenge, these cells carried the M2 polarization markers CD206 and CD301 and produced IL-13. Depletion of CD11b+ cells reduced RV-induced airway inflammation and responsiveness. Finally, knockout of CCR2 blocked the recruitment of CD11b+ cells.

Figure 3. OVA treatment and RV infection increase the number of CD11b⁺, IL-13-producing exudative macrophages in the lung. Lung cells from each treatment group were subjected to intracellular staining of IL-13. (A) Analysis of CD11b surface expression and IL-13 production by flow cytometry. The result of incubation with isotype control antibodies is also shown. (B) Group mean data ($n = 3$ mice per group). *Different from PBS sham ($P < 0.05$, one-way ANOVA). †Different from OVA sham ($P < 0.05$, one-way ANOVA). (C) In-depth analysis of CD11b⁺, IL-13⁺ cell populations for F4/80, CD206, CD68, and CD301. PE, phycoerythrin.

These findings suggest that myeloid leukocytes play a major role in the virus-induced exacerbation of allergic airways disease in mice.

CD11c⁺ and CD11b[−] resident alveolar macrophages are readily apparent in PBS-treated, sham-infected mice. However, after RV infection, a new population of CD11c[−], CD11b^{+/+} cells is recruited to the lung. The number of these cells is further increased when RV infection is preceded by OVA sensitization and challenge. These cells carry the surface markers of exudative macrophages (16–18). Previous studies have demonstrated influx of these cells into the lungs after influenza infection (16) and bleomycin administration

(17). More recently, CD11c^{high}, CD11b⁺ cells were shown to be recruited to the lung after LPS administration (18). Finally, Ly-6C⁺ monocytes and exudative macrophages have been implicated in CCR2-dependent lung fibrosis after type II cell alveolar injury (23). Whereas resolution of acute LPS-induced inflammation was characterized by restoration to a homogenous population of CD11c^{high}, CD11b[−] cells, Src homology 2 domain-containing inositol-5-phosphatase (SHIP)-1 null mice developed spontaneous chronic pulmonary inflammation characterized by CD11c^{high}, CD11b⁺ alveolar macrophages and surrounding collagen deposition and alveolar wall

thickening. These data suggest the possibility that recurrent viral infection could lead to the development of chronic airways disease and/or remodeling. We did not examine the clearance of CD11c^{high} CD11b⁺ exudative macrophages from the lung after RV infection.

A shift of classically activated M1 macrophages to an M2 alternative activation phenotype is associated with an altered pattern of phagocytic receptors and secretory repertoire (reviewed in Reference 24). We found that CD11c^{high}, CD11b⁺ exudative macrophages expressed the M2 alternative activation markers CD206 (mannose receptor) and CD301 (galactose-type C-type lectin) and

Figure 4. RV1B infection of house dust mite (HDM)-exposed mice induces IL-13 production from CD11b⁺ M2-polarized macrophages. HDM was administered on Days 0, 7, 14, 15 and 16, and mice were infected with RV1B on Day 17. (A) Flow cytometric analysis of CD11b^{high} cells. (B) CD11b⁺ cells were analyzed for the M2 surface markers CD206 and CD301. (C) IL-13 intracellular staining of CD11b⁺ cells. The results of incubation with isotype control antibodies are also shown (A–C). (D) Group mean data ($n = 3$ mice per group). *Different from PBS sham ($P < 0.05$, one-way ANOVA). †Different from OVA sham ($P < 0.05$, one-way ANOVA).

produced IL-13, a type 2 cytokine. These findings indicate that exudative macrophages are alternatively activated, perhaps under the influence of IL-4. IL-4 has been shown to induce alternative macrophage activation *in vitro* (25). SHIP-1 null mice show M2 skewing of alveolar macrophages and lung pathology

reminiscent of human Th2-skewed asthmatic lungs (18, 26). IL-13 is required and sufficient for the asthma phenotype in mice (3, 4). We therefore hypothesized that CD11c^{high}, CD11b⁺ exudative macrophages are required for RV-induced airway inflammation and hyper-responsiveness.

To test the requirement of CD11b⁺ cells for RV-induced airway inflammation and responsiveness, we used CD11b-DTR mice. These transgenic mice have a DTX-inducible system that transiently depletes macrophages in various tissues. CD11b-DTR mice were sensitized and challenged with OVA treated with DTX

Figure 5. Demonstration of IL-13 production by CD11b⁺ cells using yellow fluorescent protein (YFP) IL-13 transgenic mice. Mice that are transgenic for YFP protein on IL-13 promoter were sensitized and challenged with OVA and infected with RV as performed previously. (A) Whole lung cells were first analyzed for CD11b and YFP and subsequently examined for IL-13 production. Group mean data for CD11b⁺, YFP⁺, and IL-13⁺ cells ($n = 3$ mice per group). *Different from PBS sham ($P < 0.05$, one-way ANOVA). †Different from OVA sham ($P < 0.05$, one-way ANOVA). (B) Immunofluorescence microscopy images of lung sections from the YFP IL-13 mice from each treatment condition (original magnification: $\times 200$). Green: YFP protein; red: CD11b; black, nuclei. Inset, original magnification: $\times 630$.

Figure 6. CD11b⁺ exudative macrophages from OVA-treated, RV-infected mice contribute significantly to airway inflammation. Mice heterozygous for diphtheria toxin receptor (DTR) fused with green fluorescent protein (GFP) were exposed to OVA and RV in the presence or absence of diphtheria toxin (DTX). (A) Flow analysis to detect the presence of GFP⁺, CD11b⁺, and DTR⁺ cells from various treatment groups in combination with the detection of intracellular IL-13. Group mean data are also shown ($n = 3$ mice per group). *Different from PBS sham ($P < 0.05$, one-way ANOVA). †Different from OVA RV ($P < 0.05$, one-way ANOVA). (B) Tissue sections from each group were stained with hematoxylin and eosin (original magnification: $\times 100$). (C) Group mean data of BAL cells from mice treated with various combinations of PBS, OVA, sham, RV, and DTX ($n = 3$ mice per group). *Different from PBS sham ($P < 0.05$, one-way ANOVA). †Different from comparable condition without DTX ($P < 0.05$, one-way ANOVA). (D) Airway resistance for each group was measured after treatment with 0, 5, 10, and 20 mg/ml methacholine ($n = 3$ –4 mice per group). *Different from all other groups ($P < 0.05$, two-way ANOVA).

Figure 7. CCR2 is required for migration of inflammatory monocytes to the lung. (A) Whole lung cells were first analyzed for CD45 and F4/80 by flow cytometry, and positive cells were subsequently examined for CD11b and Ly6C surface expression. (B) Compared with C57BL/6 mice, OVA-treated, RV-infected CCR2 knockout (KO) mice showed reduced histologic evidence of lung inflammation. Data are representative of three individual experiments. (C) Airway resistance for each group was measured after treatment with 0, 5, 10, and 20 mg/ml methacholine ($n = 3-4$ mice per group). *Different from all other groups ($P < 0.05$, two-way ANOVA).

before RV infection. Depletion of CD11b cells attenuated airway inflammation and responsiveness in OVA-treated, RV-infected mice, demonstrating that CD11b⁺ cells are required for

RV-induced airway responses. Finally, CCR2 KO mice, which fail to recruit inflammatory monocytes and exudative macrophages into the lung (16, 17), also showed reduced RV-induced

inflammation. Because CD11b-DTR and CCR2^{-/-} mice were bred on a C57BL/6 background, we used C57BL/6 mice as controls for these experiments. Compared with BALB/c mice, we found similar levels

of airway inflammation induced by OVA and RV, consistent with previous data showing that C57BL/6 mice are capable of showing exacerbations of allergen-induced airway inflammation after RV infection (27).

CD11b, also known as integrin α_M , compromises part of the heterodimeric integrin $\alpha_M\beta_2$ (macrophage-1 antigen). CD11b is expressed on monocytes, macrophages, dendritic cells, granulocytes, and natural killer cells. We therefore cannot say whether the effects of CD11b depletion were strictly due to a reduction in exudative macrophages. Other CD11b+ cells could play a role in RV-induced airway inflammation, including CD11b+, CD11c+ dendritic cells (28–30). However, these cells do not express CD68 or F4/80, as we observed in our study. To confirm the potential role of IL-13-producing macrophages in our model, perhaps a better approach would be to use macrophage/neutrophil-specific IL-4 receptor α -deficient mice (31).

M2 alternatively activated macrophages have been implicated in the pathogenesis and exacerbation of asthma. Adoptive transfer of bone marrow-derived macrophages was sufficient to enhance Th2-dependent, OVA-induced allergic lung inflammation (20). Infection of mice with Sendai virus was shown to induce production of IL-13 by alveolar and interstitial macrophages (5). Bronchoalveolar lavage samples from patients with asthma show increased numbers of IL-13+ macrophages compared with healthy control subjects (5). Immunohistochemistry of biopsy specimens taken from the lungs of patients with asthma and healthy control

subjects demonstrated an increase in M2 macrophages in the lungs of patients with asthma (21). Regarding viral-induced exacerbation, we have shown that RV-induced exacerbation of allergic airways disease in mice requires the involvement of lung macrophages (1). In addition, studies of adherent BAL cells using quantitative PCR showed up-regulation of M2 polarization markers and elaboration of IL-13 and other type 2 cytokines in response to RV infection *ex vivo*. We now confirm that RV increases the number of CD68+, CD11b+, CD206+, CD301+, and IL-13 cells, producing alternatively activated macrophages in the lungs of allergen-sensitized and -challenged mice.

We did not examine the precise mechanism by which macrophages are alternatively activated in RV-infected mice. Tumor-associated macrophages polarize to M2 at the level of the bone marrow progenitor cell before migration to the lung (32). Consistent with this, peripheral blood monocytes isolated from children undergoing viral-induced asthma exacerbations had elevated expression of alternatively activated macrophage signature genes (33). We have recently shown that, in the context of OVA sensitization and challenge, IL-4 receptor activation is required for M2 polarization of lung macrophages (34). Levels of the IL-4 receptor ligands IL-4 and IL-13 are likely to be enriched in the lungs of OVA-sensitized, RV-infected mice, consistent with intrapulmonary polarization.

Regarding general aspects of our animal model, we recognize that infection of mice with a human virus presents challenges. As with RSV mouse models (35),

species differences partially restrict viral replication, thereby requiring a higher inoculum. However, we have shown viral replication and IFN responses in response to RV infection. Also, we believe the distinct nature of human RV infection, which causes minimal cytotoxicity (36, 37) and infects relatively few cells in the airway compared with other respiratory viruses (38–40), argues in favor of the RV model despite the relatively low levels of replication. Another potential concern relates to its dependence on minor group viruses. However, the effects of RV1B infection on wild-type mice are indistinguishable from those of RV16, a major group virus, on transgenic human ICAM-1 mice (41). Major and minor group viruses induce nearly identical patterns of gene expression in cultured airway epithelial cells (42). Analysis of all RV genomes revealed that HRV1 and 16 are highly homologous and respond similarly to antiviral compounds (43), implying that the distinction between some major and minor group strains may not be clinically relevant.

In summary, our data provide further evidence of the role of alternatively activated macrophages in RV-induced exacerbations of allergic airways disease in mice. We have shown colocalization of RV with CD68, a sialoadhesin associated with macrophage lineage cells, in human subjects with asthma experimentally infected with RV (2). Further studies are needed to determine whether these cells play a role in the exacerbation of human asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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