IL-1β prevents ILC2 expansion, type 2 cytokine secretion, and mucus metaplasia in response to early-life rhinovirus infection in mice

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Abstract

Background: Early-life wheezing-associated respiratory infection with human rhinovirus (RV) is associated with asthma development. RV infection of 6-day-old immature mice causes mucous metaplasia and airway hyperresponsiveness which is associated with the expansion of IL-13-producing type 2 innate lymphoid cells (ILC2s) and dependent on IL-25 and IL-33. We examined regulation of this asthma-like phenotype by IL-1β.

Methods: Six-day-old wild-type or NLRP3−/− mice were inoculated with sham or RV-A1B. Selected mice were treated with IL-1 receptor antagonist (IL-1RA), anti-IL-1β, or recombinant IL-1β.

Results: Rhinovirus infection induced Il25, Il33, Il4, Il5, Il13, muc5ac, and gob5 mRNA expression, ILC2 expansion, mucus metaplasia, and airway hyperresponsiveness. RV also induced lung mRNA and protein expression of pro-IL-1β and NLRP3 as well as cleavage of caspase-1 and pro-IL-1β, indicating inflammasome priming and activation. Lung macrophages were a major source of IL-1β. Inhibition of IL-1β signaling with IL-1RA, anti-IL-1β, or NLRP3 KO increased RV-induced type 2 cytokine immune responses, ILC2 number, and mucous metaplasia, while decreasing IL-17 mRNA expression. Treatment with IL-1β had the opposite effect, decreasing IL-25, IL-33, and mucous metaplasia while increasing IL-17 expression. IL-1β and IL-17 each suppressed Il25, Il33, and muc5ac mRNA expression in cultured airway epithelial cells. Finally, RV-infected 6-day-old mice showed reduced IL-1β mRNA and protein expression compared to mature mice.

Conclusion: Macrophage IL-1β limits type 2 inflammation and mucous metaplasia following RV infection by suppressing epithelial cell innate cytokine expression. Reduced IL-1β production in immature animals provides a mechanism permitting asthma development after early-life viral infection.

Keywords

asthma, IL-1β, IL-25, IL-33, type 2 innate lymphoid cell
INTRODUCTION

Early-life wheezing-associated respiratory infection with human rhinovirus (RV) has been associated with asthma development. We have shown that RV infection of 6-day-old immature mice causes the development of a chronic asthma-like mucous metaplasia phenotype which requires expansion of IL-13-producing ILC2s. IL-1β production, which is deficient in immature mice, attenuates production of IL-25 and IL-33, thereby protecting against rhinovirus-induced asthma development.

1 | MATERIALS AND METHODS

2.1 | RV infection of mice

RV-A1B (ATCC) was partially purified from infected HeLa cell lysates by ultrafiltration using a 100-kD cutoff filter and

GRAPHICAL ABSTRACT

Early-life rhinovirus infection increases epithelial expression of the innate cytokines IL-25 and IL-33, expands (type 2 innate lymphoid cells) ILC2s, and enhances development of an asthma-like phenotype. Rhinovirus causes macrophage (NLR family, pyrin domain containing 3) NLRP3 inflammasome activation and bioactive IL-1β production. IL-1β production, which is deficient in immature mice, attenuates production of IL-25 and IL-33, thereby protecting against rhinovirus-induced asthma development.

Abbreviations: ILC2, type 2 innate lymphoid cell; NLRP3, pyrin domain containing 3.
titered by plaque assay.33 Similarly concentrated and purified HeLa cell lysates were used for sham infection. Six-day-old C57BL/6J mice (Jackson Laboratories), NLRP3−/− and IL-1β−/−mice,34 male or female, were inoculated through intranasal route under Forane anesthesia with RV-A1B (2 × 10⁸ PFU per mouse) or sham HeLa cell lysates. Selected mice were treated with 1 or 10 ng of recombinant mouse IL-1β (R&D Systems) intranasally, or 1 or 2 µg/g body weight of human recombinant IL-1 receptor antagonist (IL-1RA, PeproTech) intraperitoneally 1 hour before RV infection, followed by a half dose of IL-1β or IL-1RA on day 1. IL-1RA is effective against mouse IL-1 receptor. Additional mice were treated with either 1 µg/g body weight of neutralizing antibody to IL-1β, IL-1α (R&D Systems), or isotype control (polyclonal goat IgG, R&D Systems) intraperitoneally 1 hour prior to RV infection. (The same concentration of neutralizing antibody was sufficient to block RV-induced airway inflammation in adult mice.25) Lungs were harvested 1, 7, or 21 days after infection for analysis.

2.4 | Flow cytometric analysis

Lungs from sham- and RV-treated immature C57BL/6J or IL-1β−/−mice were harvested one or 7 days postinfection, perfused with PBS-containing EDTA, minced, and digested in collagenase IV. Cells were filtered and washed with RBC lysis buffer, and dead cells were stained with PacBlue (Thermo Fisher Scientific). To identify the cellular source of IL-1β, lung cells were harvested 1 day postinfection and stained with fluorescent-tagged anti-CD45, anti-F4/80, and anti-CD11b (all from BioLegend). Cells were subsequently treated with permeabilization buffer (eBioscience) and stained with anti-IL-1β (eBioscience). To identify ILC2s, cells were then stained with fluorescent-tagged antibodies for lineage markers (CD3ε, TCRb, B220/CD45R, Ter-119, Gr-1/Ly-6G/Ly-6C, CD11b, CD11c, F4/80, and FcRα; all from BioLegend), anti-CD25 (BioLegend), and anti-CD127 (eBioscience), as described.3 Cells were fixed, subjected to flow cytometry, and analyzed on an LSR Fortessa (BD Biosciences). Data were collected using FACSDiva software (BD Biosciences) and analyzed using FlowJo software (TreeStar).

2.5 | ILCs culture

Lungs from sham- and RV-treated immature C57BL/6J or IL-1β−/−mice were harvested 7 days postinfection for ILC isolation by flow cytometry (Sony MA900 Cell Sorter). Lung cells were processed as described above and stained with fluorescent-tagged antibodies for lineage markers, CD45 and CD127. Lineage-negative CD45 and CD127 ILCs were plated on round-bottom 96-well plates at 10⁵ cells per well and cultured in RPMI 1640 supplemented with 10% FBS, IL-2, and IL-7 (20 ng/mL each) (R&D Systems). Twenty-four hours later, cells were stimulated with IL-1β (10 ng/mL) + IL-12 (50 ng/mL), IL-25 (50 ng/mL) + IL-33 (50 ng/mL), or IL-1β (10 ng/mL) + IL-23 (50 ng/mL, all from R&D Systems). After 24 hours, cell pellet RNA was extracted for quantitative real-time PCR, as described below.

2.6 | Western blot assay

Lungs were harvested 1 day postinfection, dissolved in lysis buffer, and homogenized for Western blot assay using anti-mouse IL-1β (R&D Systems), anti-mouse caspase-1 (Abcam, Cambridge, MA), anti-mouse NLRP3 (Cell Signaling Technology), and anti-β-actin (Millipore Sigma).
2.7 | Quantitative real-time polymerase chain reaction (qPCR)

After solubilization with Trizol (Invitrogen), RNA was extracted from cells and tissue according to the manufacturer’s recommendations. Purified RNA was processed for first-strand cDNA and qPCR using reverse transcriptase and SYBR green qPCR reagents (ThermoFisher Scientific). For in vivo experiments, mRNA Il1b, Il1b, Il18, Aim2, Nlrp1, Nlrc5, Nlrp3, Nlrp3, Nlrp3b, Nlrc5, Muc5ac, Muc5b, and Gob5 were measured 7 days postinfection; mRNA Il1b, Il1b, Il1b, Il1b, Il1b, Il1b, Il1b, Il1b, Il1b, and Il1b were measured 1 day postinfection; mRNA Il1b, Il1b, Il1b, Il1b, Il1b, Il1b, and Il1b were measured 1 and 7 days postinfection. Expression levels were normalized to GAPDH using the ΔΔCt method. Primers used are described in Table S1. To quantify virus particles, qPCR for positive-strand viral RNA was conducted using RV-specific primers and probes (forward primer: 5′-GTGAAGACCCSCRTGCTGCT-3′; reverse primer: 5′-GCTSCAGGGTTAAGGTTAGCC-3′; probe: 5′-FAM-TGAGTCCCTCGGGCTGAATG-TAMRA-3′).36

2.8 | Measurement of IL-1β, IL-25, and IL-33 protein levels

Lung IL-1β (R&D Systems), IL-25, and IL-33 (Thermo Fisher Scientific) were measured by ELISA. ELISA data were analyzed by BioTek Gen5 software. Total lung protein concentration was measured by BCA protein assay (Thermo Fisher Scientific).

2.9 | Human bronchial epithelial cell culture

Airway epithelial cells were isolated from tracheobronchial trappings of unused healthy donor lungs under a protocol approved by the University of Michigan Investigational Review Board (protocol number HUM00000230). Primary airway epithelial cells were cultured in Transwells at air-liquid interface as described previously, with some modifications.37 Briefly, airway epithelial cells were cultured under submerged conditions in complete PneumaCult-Ex Plus medium (Stemcell Technologies) for 1 week. Cells were transferred to Transwells and cultured with complete medium in both basal and apical wells until confluence was reached. Cells were then maintained at air-liquid interface for 3 weeks in PneumaCult-ALI maintenance medium. Cells were infected with sham or RV-A18 at an MOI of 10 for 12 hours. Selected wells were treated with human recombinant IL-1β and IL-17 at concentrations 10 or 30 ng/mL.

2.10 | Quantification and statistical analysis

Data are represented as mean ± standard error. Statistical significance was assessed by unpaired t test, one-way ANOVA, or two-way ANOVA, as appropriate. Group differences were pinpointed by a Tukey multiple comparison test.

3 | RESULTS

3.1 | RV infection activates the inflammasome in vivo in 6-day-old mice

Our recent study showed that RV infection of mature mice induces lung inflammasome priming and activation.20 To examine developmental differences, we collected lungs from RV-infected 6-day-old and 8-week-old mice and measured mRNA and protein expression of IL-1β and IL-1 receptor antagonist (IL-1RA). IL-1β and IL-1RA mRNA and protein expression were increased in RV-infected 6-day-old mice 1 day postinfection (Figure 1A), but expression was significantly lower in immature mice compared to 8-week-old mice. Il1b, Il1b, and Il1b mRNA as well as IL-1β protein peaked at day 1 postinfection in RV-infected 6-day-old mice (Figure 1B). Il25 mRNA was elevated on day 2 after infection and peaked on day 7, consistent with our previous study.10 In addition, mRNA expression of Nlrp1, Nlrc5, and Nlrc5 but not Il1b was increased (Figure 1C). RV increased protein expression of NLRP3 and pro-IL-1β (Figure 1D,E), indicative of the RV-induced priming step. RV also triggered cleavage of pro-IL-1β and caspase-1 and subsequent production of IL-1β and caspase-1 p12 (Figure 1D,E), demonstrating inflammasome activation in the lungs of RV-infected immature mice.
We performed flow cytometry to determine the cellular source of IL-1β. RV-infected 6-day-old mice showed a greater percentage of CD45 + IL-1β + lung cells (Figure 1F), and almost all of them were F4/80 + CD11b + exudative macrophages (Figure 1G). We also examined airway IL-1β and NLRP3 deposition by immunofluorescence. Infection with RV increased airway IL-1β and NLRP3 expression,
with the strongest signal found in F4/80-positive cells, indicative of airway macrophages (Figure 1H). There was less IL-1α and NLRP3 staining in the airway epithelium.

Next, we delivered clodronate- or PBS-containing liposomes to mice intranasally to deplete macrophages as previously described. Twenty-four hours later, mice were inoculated with sham or RV. RV-induced protein expression of NLRP3 and pro-IL-1β as well as production of mature IL-1β and caspase-1 p12 was reduced in clodronate-treated mice (Figure 1I). Clodronate treatment also significantly reduced whole-lung IL-1β mRNA in RV-infected mice (Figure 1J). Together, these data confirm the macrophage to be a major cellular source of inflammasome activation.

3.2 Inhibition of IL-1β signaling prior to RV infection amplifies ILC2 expansion and development of the asthma-like phenotype in immature mice

To further investigate the role of IL-1β, we employed an antagonist of IL-1 receptor (IL-1RA) and a neutralizing antibody against IL-1β. Again, early-life RV infection induced a mucus metaplasia phenotype, as evidenced by periodic acid-Schiff (PAS) staining and Muc5ac protein deposition in the airway epithelium 21 days after infection (Figure 2A,B). RV infection also expanded the population of lineage-negative CD25+ CD127+ ILC2s 7 days after infection (Figure 2C,D). We have previously shown that ILC2 expansion peaked at 7 days and is maintained 21 days after infection.

In contrast to RV-infected mature mice, IL-1RA treatment augmented RV-induced PAS staining, Muc5ac protein accumulation (Figure 2A,B), and ILC2 expansion (Figure 2C,D). mRNA expression of the ILC2 products IL-5 and IL-13 and the mucus-related genes Muc5ac and Gob5 was also significantly augmented in RV-infected, IL-1RA-treated mice (Figure 2E). mRNA expression was increased in a dose-dependent manner. In addition, IL-25 and IL-33 mRNA and protein expression were induced by RV infection and further increased in the presence of IL-1RA (Figure 2F,G). Levels of IL-25 and IL-33 were measured at days 7 and 1 after infection, respectively, when their production is maximal. These results are consistent with the notion that IL-1β limits development of the mucus metaplasia phenotype via regulation of innate cytokine expression and ILC2 expansion. IL-1RA did not block RV-induced mRNA expression of Tnf, Cxcl1, Cxcl10, or Ifng. However, IL-1RA decreased Il17 mRNA expression (Figure 2H). IL-1RA treatment was associated with a slight increase (0.2 log) in viral copy number (Figure 2I).

Since IL-1RA is a competitive inhibitor of both IL-1α and IL-1β, we employed neutralizing antibodies against IL-1β and IL-1α to specify their individual roles. Consistent with the effects of IL-1RA, anti-IL-1β increased RV-induced mRNA expression of Il5, Il13 and Muc5ac (Figure 2J). Anti-IL-1β had no significant effect on mRNA expression of Tnf, Ifng, or Cxcl10. However, anti-IL-1β decreased Il17 mRNA expression (Figure 2K). Anti-IL-1α also increased mRNA and protein expression of IL-25 and IL-33 (Figure 2L). In contrast, anti-IL-1α had no significant effect on Il25, Il13, Il15, Muc5ac, Gob5, or Il17 mRNA expression (Figure 3I).

3.3 NLRP3 KO increases RV-induced type 2 immune responses in vivo

NLRP3 is required for the RV-induced inflammasome activation but not the priming. We therefore employed NLRP3−/− mice to examine the requirement of NLRP3 for type 2 immune responses and development of mucus metaplasia. Compared to wild-type mice, RV infection of 6-day-old NLRP3−/− mice induced a similar level of pro-IL-1β protein (Figure 3A,B) and Il1b mRNA (Figure 3C), indicating intact inflammasome priming. However, caspase-1 p12 and IL-1β levels were significantly decreased, indicative of impaired IL-1β maturation and secretion (Figure 3A,B). Twenty-one days after RV infection, NLRP3−/− mice showed increased PAS staining and Muc5ac protein accumulation compared to wild-type mice (Figure 3D,E). mRNA expression of Il5 and Il13 as well as the mucus-related genes Muc5ac and Gob5 was significantly increased in NLRP3−/− mice compared to wild-type mice (Figure 3F). IL-25 and IL-33 mRNA and protein expression were also significantly higher (Figure 3G). NLRP3 KO did not block RV-induced mRNA expression of Cxcl1, Cxcl10, or Ifng (Figure 3H). NLRP3 KO mice showed a modest but statistically insignificant increase in viral copy number (Figure 3I). These results further demonstrate that inhibition of IL-1β during RV infection of immature mice increases innate cytokine expression and development of a mucus metaplasia phenotype.
FIGURE 3  NLRP3 KO increases RV-induced innate cytokine expression, type 2 immune responses and mucus metaplasia in 6-d-old wild-type mice. Six-day-old wild-type C57BL/6 and NLRP3−/− mice were inoculated with sham or RV (A and B). One day after infection, whole lungs were homogenized in lysis buffer and subjected to Western blot. Anti-mouse-IL-1β recognizes pro-IL-1β and its bioactive form IL-1β. Anti-mouse-caspase-1 detects both caspase-1 and its cleaved form, caspase-1 p12. Group mean relative expression levels were normalized to β-actin. (N = 3, mean ± SEM, *different from wild-type RV, P < .05, one-way ANOVA.). C. Lung mRNA expression were measured one day after infection. (N = 4, mean ± SEM, *different from sham, †different from WT RV, P < .05, one-way ANOVA). D and E, PAS staining and Muc5ac immunofluorescence were examined 21 d postinfection (bar = 50 µm). Whole-lung mRNA and protein expression were examined 1 or 7 d postinfection. F–H, Il33, Il1b, Nlrp3, Ifng, Tnf, Cxcl1 and Cxcl10 mRNA, and IL-33 protein were examined 1 d postinfection; Il25, Il5, Il13, Muc5ac, and Gob5 mRNA and IL-25 expression were examined 7 d postinfection (N = 4, mean ± SEM, *different from WT sham, †different from WT RV, P < .05, one-way ANOVA). I. RV positive-strand RNA was assessed 24 and 48 h after infection and presented as viral copy number in total lung. (N = 3-4, mean ± SEM, *different from sham, †different from RV, P < .05, one-way ANOVA).
3.4 Effect of IL-1β KO on ILC2 maturation

To further investigate the role of IL-1β in ILC2 expansion and development of mucous metaplasia and airway hyperresponsiveness in RV-infected immature mice, we first infected 6-day-old wild-type and IL-1β−/− mice with RV-A1B. In contrast to our results with IL-1RA and anti-IL-1β, IL-1β deficiency blocked RV-induced mRNA expression of Il5, Il13, Muc5ac, and Gob5 (Figure 4A), PAS staining, and Muc5ac protein expression.
Ifng and Il17r1 in wild-type mice but not in IL-1β−/−. In contrast, there was no defect in the Ifn mRNA response to the ILC1 stimuli IL-1β and IL-12. There was no induction of Il17 mRNA. Taken together, these results demonstrate that the absence of IL-1β during development leads to a defect in ILC2 maturation which makes the cells unresponsive to IL-25 and IL-33. This physiologic state contrasts to the absence of IL-1β signaling after RV infection, which promotes ILC2 responses.

3.5 IL-1β protects against RV-induced type 2 immune responses in vivo

We next examined the effects of exogenous IL-1β on RV-induced type 2 immune responses. Two doses of recombinant IL-1β were given intranasally to RV-infected 6-day-old mice; the first dose was given 1 hour prior to infection and the second dose was given 24 hours after infection. One group of mice received 1 ng per dose and a second group

FIGURE 5 IL-1β−/− mice demonstrate deficient ILC2 maturation. Six-day-old wild-type C57BL/6 and IL-1β−/− mice were inoculated with sham or RV. Lungs were collected from sham or RV-infected wild-type or immature mice, and cell suspensions were sorted for Lin-CD45 + CD127+ ILCs. Sorted ILCs were stimulated with combinations of type 1 (IL-1β + IL-12), type 2 (IL-25 + IL-33), and type 3 (IL-1β + IL-23) stimuli. The cell pellet was collected for mRNA expression by quantitative PCR (N = 3/group). (*different from wild-type mock, †different from stimulated wild type, P < .05 one-way ANOVA)
FIGURE 6  IL-1β treatment is protective against RV-induced type 2 inflammation. Six-day-old wild-type C57BL/6 mice were inoculated with sham or RV in combination with recombinant mouse IL-1β. A-C, Whole-lung mRNA and protein were assessed 1 d (Cxcl1, Cxcl2, Tnfα, and Il13) or 7 d (Il5, Il13, Il17, Il25, IFng, Muc5ac, and Gob5) postinfection. D, RV positive-strand RNA was assessed 24 h after infection and presented as viral copy number in total lung. (N = 3-4, mean ± SEM, *different from sham, P < .05; † different from RV, P < .05, one-way ANOVA). E, Two days postinfection, lungs were stained for IL-33 (red), IL-25 (green), RV VP3 protein (red), and nuclei (DAPI, black). Scale bar, 50 μm. IL-25 and IL-33 were quantified as the fraction of epithelium that was positively stained, measured by NIH ImageJ software (N = 4, mean ± SEM, *different from sham, P < .05; † different from RV, P < .05, one-way ANOVA). F, PAS staining and Muc5ac immunofluorescence were examined 21 d postinfection (bar = 50 μm). PAS and Muc5ac were quantified as the fraction of epithelium that was positively stained, measured by NIH ImageJ software (N = 4, mean ± SEM, *different from sham, P < .05; † different from RV, P < .05, one-way ANOVA).
of mice received 10 ng per dose. Seven days after infection, mice
treated with exogenous IL-1β showed decreased RV-induced mRNA
eexpression of Ifi25 and Il13 as well as mucus-related genes Muc5ac and
Gob5 (Figure 6A). On the other hand, exogenous IL-1β increased RV-
induced Il18 and Il17 mRNA, and there was no effect of IL-1β on Tnfa,
Cxc11, or Cxcl2 (Figure 6B). IL-1β treatment had no significant effect on
viral copy number (Figure 6C). In addition, IL-1β inhibited lung Il25
and Il33 mRNA and protein expression (Figure 6D). IL-1β also attenu-
ated IL-25 and IL-33 deposition but not RV immunoreactivity in the
airway epithelium (Figure 6E). Twenty-one days after infection, IL-1β-
treated, RV-infected mice showed significantly reduced PAS staining
and Muc5ac expression in the airways (Figure 6F).

3.6 | IL-1β inhibits RV-induced human epithelial-
derived innate cytokine expression

Innate cytokines are produced by the airway epithelium in response to
allergens, pathogens, pollutants, and toxic compounds. To determine the
effects of IL-1β on the epithelial Il25 and Il33 expression, we infected
human bronchial epithelial cells with RV in combination with human re-
combinant IL-1β or IL-17. RV infection increased mRNA expression of
Il25, Il33, and Muc5ac but not Cxc11, Cxcl8, or Cxcl10 (Figure 7). Both
IL-1β and IL-17 suppressed RV-induced mRNA expression of Il25, Il33,
and Muc5ac. IL-1β and IL-17 had no effect in sham-infected cells (data
not shown). IL-1β and IL-17 treatment significantly decreased viral copy
number (Figure 7). Together, these data suggest that IL-1β prevents type
2 inflammation and asthma development following early-life viral infec-
tion by suppressing epithelial cell innate cytokine expression.

4 | DISCUSSION

Early-life respiratory viral infection has been associated with asthma
development. In a prospective study of Finnish infants hospitalized
for wheezing, RV was the most common virus isolated and was
significantly associated with a diagnosis of asthma 6 years after hospi-
talization.39 In the University of Wisconsin Childhood Origins of
Asthma Study, infants with a family history of allergy and wheezing-
associated illnesses with RV were more likely to develop asthma
than infants with allergen sensitization or infection with respiratory
syncytial virus.40,41 The association between asthma and wheezing
illnesses with RV was still present at age 13 years.1 Similarly, infants
in the Netherlands Generation R study with bronchitis, bronchioli-
tis, and pneumonia before 3 years of age were more likely to have
lower lung function and asthma at 10 years of age.2 In the latter
study, allergic sensitization did not factor into the associations seen.
These data are consistent with the notion that early-life RV infec-
tions induce a nonallergic asthma phenotype.3,42 It has also been
suggested that early-life RV infections could drive the development
of atopic sensitization and subsequent allergic airways disease.4

Rhinovirus infection of 6-day-old immature mice causes the de-
velopment of a chronic asthma-like mucous metaplasia phenotype
which is associated with expansion of IL-13-producing ILC2s and de-
pendent on Il25 and Il33.8-10,36 We recently found that RV-induced
inflammation and asthma development following early-life viral infec-
tion by suppressing epithelial cell innate cytokine expression.
innate cytokine expression. These results are consistent with a previous study showing that Heligmosomoides polygyrus bakeri-induced IL-1β expression suppresses intestinal epithelial cell IL-25 and IL-33 production. In the latter case, suppression of epithelial IL-25 and IL-33 production attenuates parasite expulsion, allowing pathogen chronicity. In the case of early-life viral infection, IL-1β appears to play a protective role, limiting expression of innate cytokines, type 2 cytokins and mucus-related genes and blocking the development of mucous metaplasia. Finally, we found that, compared to mature mice, immature mice show reduced IL-1β production in response to RV infection, consistent with the notion that a limited IL-1β response permits development of the mucous metaplasia phenotype.

In our study, IL-1RA reduced, and IL-1β enhanced, IL17 transcription. IL-17 also attenuated epithelial cell innate cytokine expression. It is therefore possible that the effect of IL-1β was at least partially mediated by IL-17. IL-17 treatment attenuates ovalbumin-induced Th2-mediated allergic airways disease. Among the innate immune cells, γδ T cells and type 3 innate lymphoid cells produce IL-17 in response to IL-1β. We have found that, in adult mice, RV infection expands these cell populations, though to a lesser extent than enterovirus-D68 infection. A subpopulation of ILC2s that can convert into IL-17-producing NKp44-ILC3-like cells has recently been identified. However, in our study lung ILCs did not produce IL-17 in response to IL-1β and IL-23 stimulation. IL-1β also increased expression of the canonical type 1 cytokine IFN-γ, which we have shown to directly suppress ILC2 function. In contrast to IL-1β, IFN-γ had no effect on lung IL-25 or IL-33 production.

While in vitro studies of RV-induced inflammasome activation have focused on airway epithelial cells, we found that CD11b+ F4/80+ macrophages in the airway lumen and subepithelium produce IL-1β in response to RV infection in vivo. CD11b+ exudative macrophages are recruited to the lung following RV infection, influenza infection, and LPS administration. These data are consistent with previous work showing that caspase-1 inflammasome activation in the hematopoietic, but not stromal, compartment was required to induce protective antiviral immunity in influenza-infected mice. These data provide support to the concept that airway macrophages, ILC2s, and other innate immune cells are an important source of pro-inflammatory cytokines following RV infection, interacting with airway epithelial cells to determine the final response to RV infection.

We found that IL-1β tended to reduce viral copy number whereas IL-1β blockade tended to increase vRNA. It is therefore conceivable that IL-1β inhibits the RV-induced mucous metaplasia phenotype by decreasing viral load. However, IL-1β administration increased IL-17 mRNA and IFN-γ expression, demonstrating that the inhibitory effect of IL-1β on type 2 gene expression and mucous metaplasia was not due to a general suppression of viral-induced responses. In addition, changes in vRNA were small, rarely reaching statistical significance and reaching at most 0.3 log in IL-1β KO mice). Finally, IL-1β did not appear to reduce RV immunoreactivity in the airway epithelium (Figure 6E).

One unexpected aspect of our study is the contradictory effect of IL-1β KO mice compared to inhibition or activation of IL-1β signaling prior to RV infection. Studies of cultured ILCs from immature mouse lungs showed that IL-1β KO block ILC2 maturation, as evidenced by reduced mRNA expression of Il17b, Il1r1, and Gata3 and insensitivity to IL-25 and IL-33 stimulation. Recent studies have shown that ILC2s cultured in the presence of IL-1β show increased IL-5 and IL-13 production as well as increased mRNA expression of Il17b and Il1r1, which encode unique subunits of the IL-25 and IL-33 receptors, respectively.

The immature immune system is qualitatively different from that of adult, refractory to type 1, and permissive to type 2 responses. Infection of mice with RV induces an age-dependent immune response in the airways. Early-life RV infection, but not adult infection, increases expression of IL-4, IL-5, IL-13, IL-25, and IL-33. In contrast, induction of the type 1 cytokines IFN-γ, IL-12 p40, and TNF-α is diminished in neonates compared to adults. In this context, upregulation of the macrophage IL-1β response pulls the immune response toward a mature antiviral response and away from a pro-asthmatic phenotype. Further insight into this pathway may lead to therapeutic interventions against asthma development.

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CONFICT OF INTEREST
The authors declare no competing interests.

ETHICS STATEMENT
Mouse work was approved by the University of Michigan Animal Care and Use Committee, protocol #PRO00006118, and performed according to the 2011 Guide for the Care and Use of Laboratory Animals.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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