LPS Modulates Rhinovirus-Induced Chemokine Secretion in Monocytes and Macrophages

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Abstract

Recent studies suggest that both bacteria and rhinoviruses (RVs) contribute to asthma exacerbations. We hypothesized that bacteria might alter antiviral responses early in the course of infection by modifying monocyte-lineage chemokine responses to RV infection. To test this hypothesis, human blood monocytes or bronchoalveolar lavage (BAL) macrophages were treated with RV types A016, B014, A001, and/or A002 in the presence or absence of LPS, and secretion of chemokines (CXCL10, CXCL11, CCL2, and CCL8) and IFN- α was measured by ELISA. Treatment with RV alone induced blood monocytes and BAL macrophages to secrete CXCL10, CXCL11, CCL2, and CCL8. Pretreatment with LPS significantly attenuated RV-induced CXCL10, CXCL11, and CCL8 secretion by 68–99.9% on average ($P < 0.0001$, $P < 0.004$, and $P < 0.002$, respectively), but did not inhibit RV-induced CCL2 from blood monocytes. Similarly, LPS inhibited RV-induced CXCL10 and CXCL11 secretion by over 88% on average from BAL macrophages ($P < 0.002$ and $P < 0.0001$, respectively). Furthermore, LPS inhibited RV-induced signal transducer and activator of transcription 1 phosphorylation ($P < 0.05$), as determined by immunoblotting, yet augmented RV-induced IFN- α secretion ($P < 0.05$), and did not diminish

expression of RV target receptors, as measured by flow cytometry. In summary, major and minor group RVs strongly induce chemokine expression and IFN- α from monocytic cells. The bacterial product, LPS, specifically inhibits monocyte and macrophage secretion of RV-induced CXCL10 and CXCL11, but not other highly induced chemokines or IFN- α . These effects suggest that airway bacteria could modulate the pattern of virus-induced cell recruitment and inflammation in the airways.

Keywords: rhinovirus; LPS; monocytes; macrophages; chemokines

Clinical Relevance

Viral infections and, more recently, bacterial colonization of the airways have been shown to play a role in asthma exacerbations. We show that bacterial product, LPS, alters rhinovirus (RV)-induced secretion of chemokines from monocytic cells. The presence of bacterial products in the airway could therefore alter host immune responses to respiratory viruses, such as RV.

Asthma prevalence has steadily increased globally, making acute asthma exacerbations a major cause of morbidity that entail substantial healthcare costs worldwide (1, 2). Resident airway cells of the lower respiratory tract are consistently in contact

with the external environment, and therefore it is not surprising that the airways are not sterile (3). Interestingly, recent studies have detected asthma-related differences in microbiome composition of the lungs, raising the possibility that they

may alter airway function (4, 5). For example, there is an association between Proteobacteria (Gram-negative bacteria) in the airways and the degree of bronchial hyperresponsiveness in subjects with asthma (4). In addition, a recent study has

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shown that viral and bacterial coinfections resulted in an increased risk of hospital readmission after hospitalization for acute asthma exacerbations (6). In young children, detection of respiratory viruses or common bacterial pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*) are associated with acute wheezing episodes (7–9).

Asthma is often exacerbated by respiratory infections (10, 11). Viral respiratory infections, most commonly caused by rhinoviruses (RVs), contribute to 50–85% of asthma exacerbations (10, 12). RV consists of over 150 different types, classified into three species (A, B, and C) according to their RNA genome sequence (13, 14). RVs from the A and B species can be separated into two different groups (major and minor) based on the cellular host receptor to which they bind. Major group RVs bind intercellular adhesion molecule (ICAM)-1, whereas minor group RVs bind members of the low-density lipoprotein receptor (LDLR) family (13). RV-C types have only recently been discovered, and the receptor target has yet to be identified (15–17).

Although there are many immune cells in the lower airway, 80–90% are resident alveolar macrophages (18). Monocytic cells play an important role in immunological surveillance, as they express many cytoplasmic and cell surface innate immune sensors for viral and bacterial pathogens (19). These findings suggest that monocytes and macrophages are likely to play an important role in initiating the immune response to RV infection during the early stages of infection. Although little to no RV replication has been detected in monocytic cells, these cells can respond to RV stimulation through the secretion of a variety of chemokines (10, 20). During RV infections, chemokine responses, such as secretion of CCL5, CXCL8, and CXCL10, correlate with severity of respiratory symptoms (21), and CXCL10 is increased during virus-induced asthma exacerbation (22, 23). In addition, two closely related chemokines, CCL2 and CCL8, have been suggested to contribute to the airway hypersensitivity in subjects with asthma (21, 24, 25), and, although both have been shown to be induced in response to RV in epithelial cells, only CCL2 has previously been shown to be induced in monocytic cells (10, 26).

Despite the growing appreciation for the role of monocytic cells during RV infections and asthma, there are many aspects yet to be elucidated.

When considered together, these findings raise the possibility that airway bacteria might alter the monocytic antiviral response to RV infections. Therefore, we conducted a series of experiments to analyze the chemokine response to RV, and then tested the hypothesis that bacterial products, specifically LPS, modulate monocytic cell chemokine responses to RV infections. A portion of these results have been presented in abstract form (27).

Materials and Methods

Cell Sources

Human bronchoalveolar lavage (BAL) macrophages and peripheral blood were obtained from men and women with atopy (ages 18–50 yr) with or without mild asthma, under informed consent. The study protocol was approved by the University of Wisconsin–Madison, Human Subjects Committee. Exclusion criteria included corticosteroid use within 1 month of screening, smoking, pregnant/lactating women, unstable asthma, and other major health problems.

Purification and Isolation of Cells

Human peripheral blood monocytes and BAL macrophages were purified as previously described (28). Briefly, peripheral blood mononuclear cells, enriched from heparinized whole blood via centrifugation with a Percoll monolayer (1.090 g/ml Percoll; GE Healthcare, Piscataway, NJ), underwent washes with 2% heat-inactivated calf serum/Hanks' balanced salt solution (HBSS; Mediatech, Herndon, VA) to remove platelets. Monocytes were enriched by negative antibody selection (RosetteSep; Stemcell Technologies, Vancouver, BC, Canada) and centrifugation over lymphocyte separation media (Mediatech). The cells were resuspended in monocyte complete medium (MCM): RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin/streptomycin, 2 mM sodium pyruvate, 2 mM L-glutamine. Monocytes, 85–90% pure as determined by flow cytometry (29), were plated and allowed to adhere (2 h, 37°C), then washed twice with HBSS to enhance purity.

BAL macrophages were recovered from BAL fluid after washing with 2% newborn calf serum/HBSS. Average BAL populations contained 94% macrophages based on morphological examination. Cells were resuspended and plated in MCM supplemented with 0.25 µg/ml amphotericin B (Mediatech), incubated (2 h, 37°C), and washed twice with HBSS to enhance purity.

RV Production and Purification

RV A016, B014, A001, and A002 were grown in HeLa cells and purified as previously described (30, 31). The infectivity of the virions in HeLa cell monolayers was determined in tissue culture (plaque forming units).

Treatment Conditions for Chemokine Detection

Blood monocytes were plated (5×10^5 cells/well) in 24-well CoStar plates (Corning Inc., Tewksbury, MA). Monocytes were either (1) cotreated with LPS (*Escherichia coli*, serotype 0111:B4, 0.01–100 ng/ml; Sigma, St. Louis, MO) or vehicle (40 nM Hepes/H₂O; Sigma) and either mock infected (0.00025% HSA/HBSS; Irvine Scientific, Santa Ana, CA) or RV treated (multiplicity of infection = 10) for 8–48 hours (34.5°C); or (2) pretreated (16 h at 37°C) with or without LPS (100 ng/ml), followed by 24 hours (34.5°C) with or without RV. All treatments were diluted in MCM. BAL macrophages were plated and treated similarly to the monocytes, but in 12-well plates.

Other Immunologic Outcomes

After incubation of blood monocytes with RV, highly expressed mRNA transcripts were identified by microarray. Chemokine and IFN-α secretion were measured by ELISAs. Cell surface expression of ICAM-1 and LDLR were assessed by flow cytometry, and signal transducer and activator of transcription (STAT) 1 expression was determined by immunoblot. Cell viability was tested by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Details of these procedures are provided in the online supplement.

Statistical Analysis

Measurements of chemokine and IFN-α secretion were log transformed to approximate normal distribution. Student's two-tailed paired *t* tests were used to

calculate statistical differences between treatment effects. One-way repeated measures ANOVA (Friedman test) was used to evaluate the effects of different LPS concentrations. Analysis was performed with Prism 5 version 5.01 (GraphPad Software, Inc., La Holla, CA), and a two-sided P value of 0.05 was considered statistically significant.

Results

Monocytes Secrete CXCL and CCL Chemokines in Response to RV Exposure

To identify chemokines highly induced by RV, blood monocytes were incubated with RV A016 (multiplicity of infection = 10), and mRNA expression was determined by a microarray. Among the most highly induced mRNA were multiple chemokines (see Table E1 in the online supplement), and we selected the four most highly expressed chemokines (CXCL10, CXCL11, CCL2, and CCL8; fold increase of 116, 49, 34, and 26, respectively) for further study.

To determine effects of RV-induced chemokine secretion, monocytes purified from human peripheral blood were treated with RV types representing both the major groups (i.e., A016, B014) and minor groups (i.e., A001, A002), as well as representing the A and B species. All four RV types tested caused a significant increase in chemokine (CXCL10, CXCL11, CCL2, and CCL8) secretion after 24-hour treatment (Figure 1). The up-regulation of CXCL10 secretion was the highest (A016-induced GM = 74,880 pg/ml), followed by CCL2 (A016-induced GM = 6,651 pg/ml), CCL8 (A016-induced GM = 6,155 pg/ml), and CXCL11 (A016-induced GM = 677 pg/ml), respectively. These data suggest that major and minor group RVs, which use different receptors to infect cells, induce qualitatively similar monocyte chemokine responses.

LPS Effects on RV-Induced Chemokine Secretion in Monocytic Cells

Peripheral blood monocytes were inoculated with major and minor group RVs for 24

hours in the presence or absence of LPS and the secretion of CXCL10, CXCL11, CCL2, and CCL8 was assessed (Figure 2). LPS cotreatment alone induced secretion of CCL2 and CCL8, and LPS pretreatment alone induced secretion of CCL2, but not the other chemokines. When added as costimulus with A016, B014, or A001, LPS significantly attenuated RV-induced secretion of CXCL10 (Figure 2A, $P \leq 0.0001$) and CXCL11 (Figure 2B, $P < 0.008$). This effect was specific to CXCL10 and CXCL11, as LPS cotreatment significantly ($P < 0.05$) increased A016- and B014-induced CCL2 secretion, and did not attenuate virus-induced secretion of CCL8 (Figures 2C and 2D). Similarly, pretreatment with LPS 16 hours before RV exposure also significantly inhibited RV-induced CXCL10 (Figure 2E, $P < 0.0001$) and CXCL11 (Figure 2F, $P < 0.004$) secretion by a larger magnitude compared with LPS cotreatment. Although LPS pretreatment alone did significantly increase CCL2 secretion, it did not affect RV-induced CCL2 secretion (Figure 2G). In addition, LPS pretreatment significantly

Figure 1. Multiple rhinovirus (RV) types significantly induce chemokine ([A] CXCL10; [B] CXCL11; [C] CCL2; and [D] CCL8) secretion from primary human monocytes. Data are log transformed for normalcy and depicted as individual points representing three different donors; the geometric mean (GM) is represented as a line. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with mock-infected cells, by Student's paired t test.

Figure 2. LPS modifies RV-induced chemokine secretion in primary human blood monocytes. Peripheral blood monocytes were cotreated with or without LPS (100 ng/ml) and with or without RV (multiplicity of infection [MOI] = 10) for 24 hours at 34.5°C. Supernatants were analyzed for the presence of (A) CXCL10, (B) CXCL11, (C) CCL2, and (D) CCL8 by ELISA. Monocytes were pretreated with or without LPS (100 ng/ml, 16 h at 37°C), followed by mock infection or RV (MOI = 10, 24 h at 34.5°C). Supernatants were analyzed for the presence of (E) CXCL10, (F) CXCL11, (G) CCL2, and (H) CCL8 by ELISA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Student's paired t test. The data are log transformed for normalcy and are summarized as boxplots (whiskers: 10th–90th percentile), each representing cells from 10 different donors.

decreased RV-induced CCL8 secretion from blood monocytes (Figure 2H, $P < 0.002$). Overall, these findings indicate that the effects of LPS are similar whether the exposure is simultaneous with, or prior to, RV stimulation on RV-induced CXCL10, CXCL11, and CCL2 secretion. No significant differences in monocyte metabolic activity were observed between any of the treatment conditions used, demonstrating that cell viability is unaffected by LPS and RV cotreatment (Figure E1).

To test these relationships in airway cells, human BAL macrophages were either cotreated with LPS and RV or pretreated with LPS for 16 hours and then incubated for 24 additional hours with RV (Figure 3). LPS cotreatment alone significantly increased CXCL10 and CXCL11 secretion from BAL macrophages in the absence

of RV. Although LPS cotreatment of mock-infected cells induced CXCL10 and CXCL11 secretion ($P = 0.0002$), LPS cotreatment significantly attenuated RV B014- and A002-induced CXCL10 (Figure 3A, $P < 0.03$) and CXCL11 (Figure 3B, $P < 0.03$). In addition, LPS cotreatment decreased A016-induced CXCL10 secretion (from a GM of 140,494 pg/ml to a GM of 44,848 pg/ml; $P = 0.07$). Interestingly, LPS pretreatment appeared to cause a more robust effect on RV-induced CXCL10 and CXCL11 secretion than LPS cotreatment (Figures 3E and 3F). LPS pretreatment significantly attenuated RV A016-, B014-, and A002-induced CXCL10 ($P < 0.002$) and CXCL11 ($P < 0.0001$) secretion in BAL macrophages, which is similar to peripheral blood monocytes. In the absence of RV, BAL macrophages alone secreted large amounts of CCL2

(GM = 4,200 pg/ml) and minor amounts of CCL8 (GM = 10 pg/ml), and LPS cotreatment and LPS pretreatment alone enhanced both CCL2 and CCL8 secretion (Figures 3C, 3D, 3G and 3H, $P < 0.04$). Although LPS cotreatment slightly reduced A002-induced CCL2 secretion, both LPS cotreatment and pretreatment generally maintained RV-induced CCL2 or CCL8 secretion from BAL macrophages (Figures 3C, 3D, 3G, and 3H).

Effects of Treatment Time and LPS Dose on RV-Induced Chemokine Secretion

We next evaluated effects of timing and dose on interactions between simultaneous LPS and RV infection with respect to chemokine secretion. A016 treatment for 8–48 hours stimulated CXCL10 secretion (Figure 4A, $P < 0.01$). LPS cotreatment significantly

Figure 3. LPS modifies RV-induced chemokine secretion in primary human bronchoalveolar lavage (BAL) macrophages. BAL macrophages were cotreated with or without LPS (100 ng/ml) and with or without RV (MOI = 10) for 24 hours at 34.5°C. Supernatants were analyzed for the presence of (A) CXCL10, (B) CXCL11, (C) CCL2, and (D) CCL8 by ELISA ($n = 9$). BAL macrophages were pretreated with or without LPS (100 ng/ml, 16 h at 37°C), followed by mock infection or RV treatment (MOI = 10, 24 h at 34.5°C). Supernatants were analyzed for the presence of (E) CXCL10, (F) CXCL11, (G) CCL2, and (H) CCL8 by ELISA ($n = 8$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Student's paired t test. The data are log transformed for normality and are summarized as boxplots (whiskers: 10th–90th percentile).

attenuated A016-induced CXCL10 at both 24 and 48 hours. A different pattern was observed for CCL2. Either LPS or A016 alone induced increasing amounts of CCL2 over time, whereas LPS and A016 cotreatment induced a significantly ($P < 0.05$) more robust secretion of CCL2 compared with A016 alone at both 24 and 48 hours (Figure 4B).

To determine if the effect of LPS on RV-induced chemokine secretion was dose dependent, CXCL10 and CCL2 secretion from monocytes was measured in the presence or absence of A016 and increasing doses of LPS (0–100 ng/ml). A016-induced CXCL10 secretion (Figure 4C) was inversely proportional to concentrations of LPS ($P = 0.007$), whereas LPS alone increased modest amounts of CXCL10 secretion ($P = 0.02$). On the other hand, LPS and A016 had additive effects on CCL2 secretion (Figure 4D, $P = 0.01$).

Major and Minor Group RV Receptor Expression in Response to Stimuli in Monocytes

Cell surface receptors for minor and major group RVs are LDLR and ICAM-1, respectively (13). To test whether LPS effects on CXCL10 and CXCL11 were due to down-regulation of target receptors for RV, we cultured primary human monocytes with LPS, A016, and/or A001 for 24 hours and analyzed ICAM-1 and LDLR expression. After gating for live, CD14⁺ cells, GM fluorescence intensity was determined for each receptor. Rather than diminishing expression, LPS stimulation enhanced the expression of ICAM-1, irrespective of RV cotreatment (Figure 5A). Treatment with A001 alone also enhanced the expression of ICAM-1, whereas A016 alone did not have a significant effect. Similarly, treatment with RV and/or LPS enhanced LDLR expression (Figure 5B). These data indicate that

treatment of monocytes with major and minor group RVs or LPS does not diminish the expression of RV target receptors, ICAM-1 and LDLR.

Phosphorylation of STAT1 in Monocytes in Response to LPS and RV A016

One of the pathways involved in RV A016-induced CXCL10 secretion is the Janus kinase (JAK)/STAT pathway (28). To determine whether LPS inhibition of RV-induced CXCL secretion was mediated through the JAK/STAT pathway, monocytes were cotreated with RV with or without LPS, and RV-induced phosphorylated STAT1 (pSTAT1) was determined 2 or 4 hours later (Figure 6A). At 2 hours (Figure 6B), LPS significantly induced pSTAT1, whether alone or in combination with A016, whereas RV A016 treatment alone did not significantly induce

Figure 4. LPS modification of RV-induced chemokine release from primary human monocytes is dose dependent. Open circles, mock infected; open triangles, LPS treated; solid circles, A016 treated; solid triangles, LPS and A016 cotreated. Supernatants were analyzed for the presence of chemokine secretion by ELISA. All values are means $\pm$ SEM using cells from six different donors. (A) CXCL10 and (B) CCL2 time-dependent release, * P < 0.05, ** P < 0.01 A016 compared with LPS and A016 by Student's paired t test. LPS concentrations were significantly related to (C) CXCL10 and (D) CCL2 secretion (P values by one-way repeated measures ANOVA [Friedman test]).

detectable phosphorylation of STAT1 above mock-infected cells. At 4 hours (Figure 6C), RV A016 induced more robust pSTAT1 compared with LPS alone. Interestingly, cotreatment of monocytes with LPS and A016 at 4 hours significantly diminished RV-induced pSTAT1. These

data indicate that pSTAT1 induction occurs more rapidly with LPS treatment versus RV treatment. In addition, LPS cotreatment with A016 at 4 hours significantly attenuates RV-induced pSTAT1 signaling in monocytes, without altering the overall STAT1 available in the cells.

LPS Increases A016-Induced IFN- α Secretion from Monocytes

RV induces IFN- α secretion, and autocrine binding of IFN- α to the type I IFN receptor leads to activation of the JAK/STAT pathway (10, 32). In addition, treatment of monocytes with IFN- α

Figure 5. Effects of LPS and RV cotreatment on major and minor group RV receptor expression on monocytes. CD14⁺ monocyte expression of (A) intercellular adhesion molecule (ICAM)-1 and (B) low-density lipoprotein receptor (LDLR) is reported as GM fluorescence intensity (gMFI). * P < 0.05, ** P < 0.01, *** P < 0.001 compared with mock-infected cells by Student's paired t test. The data are summarized as boxplots (whiskers: 10th–90th percentile) using cells from five to six different donors.

Figure 6. LPS reduces RV-induced phosphorylated signal transducer and activator of transcription 1 (pSTAT1) in primary human peripheral blood monocytes. (A) Representative immunoblot images for pSTAT1, total STAT1, and actin. (B) Summary of 2-hour cotreatment ($n = 10$). (C) Summary of 4-hour cotreatment ($n = 8$). Data were normalized to total actin levels, and fold changes in pSTAT1 were in respect to mock-infected cells. All values are means $\pm$ SEM using cells from different donors. * $P < 0.05$, *** $P < 0.001$ compared with A016; ## $P < 0.01$, ### $P < 0.001$ compared with mock-infected cells by Student's paired t test.

induces CXCL10 secretion (28). We therefore tested for effects of LPS on RV-induced IFN- α secretion. Monocytes that were treated with LPS alone showed little to no secretion of IFN- α at 8 and 24 hours (Figure 7). A016 alone induced IFN- α secretion after 24 hours ($P = 0.02$). In contrast, LPS and A016 cotreatment induced significant IFN- α secretion earlier (by 8 h), and LPS significantly augmented A016-induced IFN- α secretion at both time

points (Figures 7A and 7B, $P = 0.01$ and 0.03). These findings suggest that, whereas LPS diminishes RV-induced pSTAT1 at 4 hours, this effect is unlikely due to a reduction in RV-induced IFN- α secretion.

Discussion

Our results demonstrate that primary blood monocytes and BAL macrophages secrete

CXCL10, CXCL11, CCL2, and CCL8 in response to all the RV types tested. Furthermore, LPS modifies monocytic cell response to RV exposure, in a chemokine-specific manner. LPS cotreatment inhibits RV-mediated CXCL10 and CXCL11, but either has no effect or else potentiates RV-mediated CCL2 and CCL8. These findings were demonstrated in human peripheral blood monocytes as well as in human BAL macrophages. Interestingly, LPS augments RV-induced IFN- α secretion, yet diminishes RV-induced pSTAT1 in the blood monocytes. These findings suggest that the quantity of Gram-negative bacteria in the airway could influence the immunological response to RV infections. Because asthma is associated with increased bacterial colonization (4), our findings suggest that monocytic chemokine responses to RV could be distinct.

Recent findings have demonstrated that, after *in vivo* RV infections in humans, RV colocalizes with macrophages in the tissues, suggesting a direct role for macrophages in antiviral responses (33). In addition, Rajan and colleagues (34) have shown that the source of monocytic cells influences RV-induced cytokine and chemokine secretions from epithelial cells. This is in agreement with results of our previous study demonstrating that monocytic cells can regulate epithelial cell responses to RV infections (29). We have demonstrated that monocyte-epithelial cell cocultures have more robust RV-induced CXCL10 secretion compared with the monocultures. Interestingly, conditioned medium from RV-treated monocytic cultures enhanced epithelial cell CXCL10 responses to RV (29). When considered

Figure 7. LPS augments RV-induced IFN- α secretion in primary human monocytes. Peripheral blood monocytes were cotreated with LPS (100 ng/ml) and A016 (MOI = 10) or the respective vehicle controls for either (A) 8 hours or (B) 24 hours at 34.5°C. Supernatants were analyzed for the presence of IFN- α by ELISA. * $P < 0.05$, ** $P < 0.01$ by Student's paired t test. The data are log transformed for normalcy and are summarized as boxplots (whiskers: 10th–90th percentile) using cells from six different donors.

together, these findings provide evidence that RV interacts with monocytic cells *in vivo*, which, in turn, secrete chemokines and modify epithelial cell responses to RV exposure.

If these effects also occur *in vivo*, how would LPS affect the course of an RV infection? CXCL10 and CXCL11 are ligands for the receptor, CXCR3, which is commonly found on activated T cells and natural killer T cells. Thus, these chemokines are thought to promote viral clearance (21, 23). CCL2 primarily binds CCR2, found on the surface of a variety of myeloid cells, including monocytes, macrophages, dendritic cells, eosinophils, and neutrophils (24, 25, 35). CCL8 can bind to several receptors: CCR-1, -2, -3, -5 (21, 24, 25). CCR3 is highly expressed on eosinophils, and its expression in the airways is increased in asthma (21). CCL2 and CCL8 levels are elevated in asthmatic airways, and likely contribute to inflammatory responses that promote exacerbations of asthma (21, 26, 36–39). These findings suggest that LPS attenuation of RV-mediated CXCL10 and CXCL11 secretion could impair viral clearance (35, 40). In contrast, LPS does not inhibit RV-induced CCL2 and CCL8 secretion, and these two chemokines have been linked to airway obstruction and exacerbations of asthma. Because LPS did not diminish RV-induced IFN- α secretion, these findings imply that LPS could impair RV-induced antiviral responses downstream of IFN- α secretion, while maintaining or augmenting proinflammatory effects in the airway.

A study by Oliver and colleagues (41) demonstrated that infectious RV reduces BAL macrophage responsiveness to LPS. Specifically, they found that RV pretreatment impaired LPS-induced secretion of TNF- α and IL-8, and impaired the ability of macrophages to phagocytose *E. coli* particles. Our findings, when considered together with those of the Oliver study, suggest that the presence of RV and bacterial products in the airway leads to altered BAL macrophage responsiveness to either stimuli.

The effect of LPS on RV-induced CXCL10, CXCL11, and CCL2 secretion was consistent regardless of whether LPS exposure was before RV stimulation or whether LPS was costimulated with RV in both the monocytes and the BAL macrophages. Whereas this was also true for RV-induced CCL8 secretion from BAL

macrophages, LPS timing-dependent differences were observed in blood monocytes. RV-induced CCL8 secretion was unaffected by LPS cotreatment, but was inhibited by LPS pretreatment in blood monocytes (Figures 2D and 2H). Interestingly, prior exposure of the monocytic cells to LPS appeared to exaggerate LPS-induced attenuation of RV-induced CXCL10 and CXCL11 secretion compared with LPS and RV cotreatment. Whereas BAL macrophages are differentiated cells, blood monocytes can differentiate depending on their environment. LPS is commonly used *in vitro* to differentiate blood monocytes into various types of macrophages. Therefore, it is reasonable that the differences observed between LPS pretreatment and cotreatment are due to LPS-induced low levels of monocyte differentiation.

The similar induction of chemokine secretion by all RV types tested (major and minor), suggests that the overall pattern of chemokine responses is independent of RV type and RV receptor use. Furthermore, RV and/or LPS treatment increased the expression of RV target receptors, ICAM-1 and LDLR. Together, these data suggest that attenuation of RV-induced CXCL10 and CXCL11 secretion is not explained by LPS effects on cell surface receptors, and implies that LPS instead modifies RV-induced intracellular signaling.

LPS-mediated attenuation of RV-induced CXCL10 and CXCL11 occurs within hours, and is maintained, suggesting that LPS alters one or more of the early RV-induced signaling pathways. RV induces the secretion of IFN- α , which acts in an autocrine manner through the type I IFN receptor to activate the JAK/STAT1 pathway, leading to the secretion of CXCL10 (28). Here, we demonstrate that, although LPS diminishes RV-induced pSTAT1, it increases RV-induced IFN- α . Therefore, the mechanism by which LPS reduces RV-induced pSTAT1 and CXCL10/CXCL11 is likely to be downstream of IFN- α secretion. One possible mechanism by which LPS could reduce the phosphorylation of STAT1 may relate to the ability of LPS to rapidly phosphorylate STAT1. LPS stimulation of macrophages can induce the secretion of IFN- β , which activates the type I IFN receptor and promotes phosphorylation of STAT1 (42, 43). By inducing pSTAT1, LPS costimulation with RV could either divert

activated STAT1, or lead to its down-regulation, and thereby diminish the pool of activated STAT1 available for RV-induced signaling. This mechanism could therefore contribute to LPS attenuation of RV-induced genes that are STAT1 dependent, such as CXCL10 and CXCL11. Furthermore, LPS can activate multiple mitogen-activated protein kinase signaling pathways in human monocytes that induce secretion of CCL2 (44, 45). Thus, LPS effects on signaling pathways are likely to direct the selective effects on RV-induced chemokine secretion. Alternatively, suppressor of cytokine signaling (SOCS) family proteins directly inhibit the phosphorylation of STATs by inhibiting JAK catalytic activity and by marking it for proteasomal degradation, and both LPS and IFN can induce SOCS-1 and -3 under some experimental conditions (46). It is possible that the combination of LPS and RV increases SOCS levels, which would diminish STAT1 phosphorylation.

In this study, we used multiple purified RV types, representing both the major and minor groups as well as RV A and B species, allowing for characterization of RV-induced signaling in a general context. Recent studies have shown differences in virulence and illness severity across RV species (14, 47). Thus, it is advantageous to use representative RV types in experimental models. In addition, we used human monocytes and BAL macrophages, which demonstrate that LPS has similar immunomodulating effects on blood and airway cells. One limitation of this study is the use of monocytic cells from donors with atopy or atopic asthma. We selected these donors because of our interest in mechanisms of RV-induced inflammation; however, it is possible that effects of LPS on virus-induced chemokine secretion in individuals without atopy could have distinct features. Another limitation to consider is that, whereas LPS is a single component, bacteria are complex organisms, and more work is needed to determine the effects of whole bacteria on RV-induced airway inflammation.

Several clinical studies provide evidence that bacteria may contribute to asthma. Martin and colleagues (48) have shown that lower airway colonization or infection by atypical bacteria is more common in adults with asthma compared with healthy control subjects. Furthermore, studies by Hilty and colleagues (8) and

Huang and colleagues (4) have shown that subjects with asthma have characteristic airway microbial flora compared with healthy individuals, and that these differences strongly associate with airway disease. In addition, a recent study by Wark and colleagues (6) demonstrated that bacterial and RV coinfection is an independent predictor of more severe acute asthma exacerbations, and that coinfections increase the likelihood for hospital readmission, thus providing clinical support for the immunomodulating effects of bacteria in RV-induced asthma.

In summary, we demonstrate a mechanism by which endotoxin exposure can alter the mononuclear cell immune response to RV infections. RV infections are a substantial cause of asthma exacerbation, and with increasing appreciation that airway bacteria are also related to asthma, it is critical to understand how microbial products can alter airway immune responses. Additional studies to determine how the effects of LPS on RV-induced signaling *ex vivo* correspond to physiologic responses

in vivo could aid in the development of future therapeutics. ■

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

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