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The Role of p38 MAPK in Rhinovirus-Induced Monocyte Chemoattractant Protein-1 Production by Monocytic-Lineage Cells[1]

David J. Hall,[2]* Mary Ellen Bates,† Lasya Guar,† Mark Cronan,* Nichole Korpi,† and Paul J. Bertics†

Viral respiratory infections are a major cause of asthma exacerbations and can contribute to the pathogenesis of asthma. Major group human rhinovirus enters cells by binding to the cell surface molecule ICAM-1 that is present on epithelial and monocytic lineage cells. The focus of the resulting viral infection is in bronchial epithelia. However, previous studies of the cytokine dysregulation that follows rhinovirus infection have implicated monocytic lineage cells in establishing the inflammatory environment even though productive infection is not a result. We have determined that human alveolar macrophages and human peripheral blood monocytes release MCP-1 upon exposure to human rhinovirus 16 (HRV16). Indeed, we have found p38 MAPK activation in human alveolar macrophages within 15 min of exposure to HRV16, and this activation lasts up to 1 h. The targets of p38 MAPK activation include transcriptional activators of the MCP-1 promoter. The transcription factor ATF-2, a p38 MAPK substrate, is phosphorylated 45 min after HRV16 exposure. Furthermore, IκBα, the inhibitor of the transcription factor NF-κB, is degraded. Prevention of HRV16 binding was effective in blocking p38 MAPK activation, ATF-2 phosphorylation, and MCP-1 release. This is the first report of a relationship between HRV16 exposure, MCP-1 release and monocytic-lineage cells suggesting that MCP-1 plays a role in establishing the inflammatory microenvironment initiated in the human airway upon exposure to rhinovirus. ***The Journal of Immunology,*** **2005, 174: 8056–8063.**

Virus infections of the upper respiratory tract are among the most common causes of asthma exacerbations. Therefore, an understanding of the mechanisms underlying virus infection and the associated exacerbations are expected to open up new avenues for therapeutic intervention. There are numerous viruses that can affect the upper respiratory tract including influenza A, coronaviruses, adenoviruses, and parainfluenza viruses (1–5). However, the most common virus detected in the upper airway associated with exacerbations of asthma, is the rhinovirus (6). Human rhinoviruses (HRV)[3] are part of the picornavirus family; small, positive strand RNA viruses whose genetic material is enclosed by an icosahedral protein shell or capsid. The HRV life cycle takes roughly 6 h to complete, with new viruses being detectable after 3 h in a variety of cell types (7, 8). Rhinoviruses can be classified according to the cellular receptor used for attachment and cellular entry. Major group rhinoviruses, including the well studied HRV16, bind to ICAM-1, whereas the minor group viruses, including HRV1A, bind to members of the low density lipoprotein receptor family (9).

Infection of the upper respiratory tract by HRV16 has been demonstrated to occur at localized portions of the epithelia and does not cause widespread lysis of the infected epithelia (1). This observation has suggested that the pathology induced by rhinovirus may be due in part to cytokine dysregulation rather than extensive epithelial necrosis observed in other viral infections. In general, changes in cytokines, chemokines, and other factors following virus infection in asthma subjects are linked to increased bronchial hyperresponsiveness, extensive thickening of the airways, decreased β-adrenergic receptor sensitivity, and dysregulation of the M2 receptor resulting in an increase in cholinergic responsiveness (10). With regard to the cytokine profile of nasal lavage fluid taken during natural and experimental rhinovirus infection, increased levels of multiple cytokines/chemokines was observed including IL-8, RANTES, IFN-γ, IL-1β, and TNF-α (11–14). Another CC chemokine that may be important to the pathophysiology of asthma is MCP-1 (15). MCP-1 is associated with many pathological states, including asthma, atherosclerosis, pulmonary fibrosis, arthritis, sepsis, and chronic bacterial infections (16, 17). In vitro, MCP-1 stimulates recruitment of specific leukocyte subsets, including monocytes, memory T lymphocytes, and NK cells through binding of CCR2 (15). Recent studies have demonstrated that mice lacking CCR2 have a predominant production of Th2 cytokines (18, 19). Targeted deletion of MCP-1 gene in mice has been achieved (20), and it was observed that functional absence of MCP-1 resulted in impaired monocyte recruitment but not polymorphonuclear leukocyte recruitment and rendered the host more susceptible to infection (21, 22).

The major site of HRV infection is the airway epithelium, and hence these cells have been the primary focus of research on virus-induced asthma. However, little work has focused on the participation of other cell types in HRV-induced exacerbations of asthma and how these cells may contribute to the cytokine dysregulation that is observed during virus infection. In fact, monocytic-lineage cells (macrophages, monocytes, and dendritic cells) are the

*Department of Chemistry, Lawrence University, Appleton, WI 54912; and †Department of Biomolecular Chemistry, University of Wisconsin, Madison, WI 53706

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[2] Address correspondence and reprint requests to Dr. David J. Hall, Department of Chemistry, Lawrence University, Appleton, WI 54912-0599. E-mail address: halld@lawrence.edu

[3] Abbreviations used in this paper: HRV, human rhinovirus; BL, bronchial lavage; MOI, multiplicity of infection.

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predominant immune cells present in the lumen of the lower airway (7, 23), and the activation of these cells represents one of the first steps in natural immunity toward virus infection. Although monocytic-lineage cells are known to express ICAM-1 and the low density lipoprotein receptor, rhinoviruses are unable to initiate a productive infection. However, it has been observed that rhinovirus exposure elicits a number of responses from peripheral blood monocytes including the release of IL-8 and IL-10 as well as the up-regulation of CD14 on the cell surface (7, 23–26).

In the present study, we determined that when alveolar macrophages are exposed to HRV16, p38 MAPK is activated, and MCP-1 is released. Careful dissection of the p38 MAPK signaling cascade revealed that the transcription factor ATF-2, a known p38 MAPK target, is phosphorylated and that IκBα is degraded after HRV16 treatment. Preventing HRV16 binding to its receptor or introduction of a p38 MAPK inhibitor prevented ATF-2 phosphorylation and MCP-1 release. Furthermore, we demonstrated that both blood monocytes and THP-1 cells can be used as model systems for the difficult-to-obtain alveolar macrophages with respect to this HRV16-induced signaling cascade. These results show, for the first time, that MCP-1 is produced by monocytic lineage cells exposed to HRV16 suggesting that these cells may play an important sentinel role during the initial phase of rhinovirus infection.

Materials and Methods

Reagents

For monocyte preparation, Percoll was purchased from Amersham Biosciences. Sigma-Aldrich was the source for PMA. Protease inhibitor tablets (Complete) were obtained from Boehringer Mannheim, and p38 inhibitor SB203580 was obtained from Calbiochem. Immunoblotting reagents were purchased from a variety of suppliers including Santa Cruz Biotechnology (HRP-conjugated goat anti-rabbit IgG, anti-IkBα Ab), Pierce (Supersignal chemiluminescence substrate reagents), Promega (anti-Active p38 MAPK antisera and anti-ERK antisera), Upstate Biotechnology (anti-Grb2 antisera), and Cell Signaling (anti-phospho-ATF-2 antisera). Anti-CD14 and anti-ICAM-1 was purchased from BD Biosciences.

Cell culture

Alveolar macrophages, human peripheral blood monocytes, and the human promonocytic cell line THP-1 were cultured in RPMI 1640 (Sigma-Aldrich) with 10% FBS (HyClone) and 1% penicillin/streptomycin at 37°C in a humidified incubator with 5% CO_2.

Virus production, purification, inactivation, and tittering

Virus was grown in HeLa cells and subsequently sedimented through a sucrose step gradient to remove contaminants as previously described (27–29). Total virus was determined by infectivity assay (30), and four tubes were inoculated for each 10-fold dilution. Sucrose-purified HRV16 was exposed to irradiation with a 254-nm UV light placed 10 cm above the virus solution. The volume of the virus solution was measured before irradiation, and evaporated volume was replaced with sterile water. No viral infectivity could be detected after irradiation.

Purification of human monocytes

Monocytes were purified from heparinized peripheral blood of volunteer donors as previously described by centrifugation of buffy coat leukocytes through a Percoll density gradient (31). Monocytes were then cultured in 12-well Costar tissue culture plates (1 million cells/well) for 2 h, nonadherent cells were removed by washing the wells with medium, and the remaining adherent cells were cultured for 24 h before treatment with rhinovirus. Purified human blood monocytes were lifted off the plate with Cell Dissolution solution (Sigma-Aldrich), and the cell population was evaluated for CD14-positive cells and viability (annexin V (32)) by flow cytometry. Cell populations were typically 95% viable and 90% CD14 positive. The procedure was approved by the University of Wisconsin-Madison Center for Health Sciences Human Subjects Committee. Informed consent was obtained from each subject before participation.

Isolation of alveolar macrophages

Bronchoscopy and bronchial lavage (BL) were conducted as previously described (33, 34). Briefly, one bronchopulmonary segment was identified, and the fiberoptic bronchoscope was wedged into that segment. The bronchoscope was held in a wedge position, and lavage (60 ml) was performed. This volume was selected to obtain a wash of the airway and to be a likely representative of air space cells and other mediators. The BL fluid return was centrifuged (400*g* for 10 min) to sediment cells. BL cells were washed twice with HBSS containing 2% newborn calf serum. The cell population was evaluated by flow cytometry showing at least 95% viable (annexin V (32)) and 70% CD14 positive. Based on morphological examination (Diff-Quick Scientific Products), the BL fluid cells were typically 87% macrophages with contaminating cells being primarily lymphocytes (7%), neutrophils (1%), and epithelial cells (2%). Macrophages were cultured in 12-well Costar tissue culture plates (1 million cells/well) for 2 h, nonadherent cells were removed by washing the wells with medium, and the remaining adherent cells were cultured for 24 h before treatment with rhinovirus. The procedure was approved by the University of Wisconsin-Madison Center for Health Sciences Human Subjects Committee. Informed consent was obtained from each subject before participation.

ELISA

Sandwich ELISA for MCP-1 was performed with Abs obtained from Endogen as previously described (33, 34). PBS with 0.1% Tween 20 (PBST) was used for all washes. Absorbances were read at 450 nm. MCP-1 concentrations were calculated by interpolation from a standard curve, and all determinations were done in triplicate. Data were summarized as the mean ± SD of triplicate determinations when a representative is shown and mean ± SEM for summaries of experiments on multiple patient samples. Statistical significance of differences between cell treatment conditions was determined by Student's *t* test.

MCP-1 RNA isolation/quantification

After treating monocytes or macrophages with rhinovirus (or LPS), the medium was collected and Trizol reagent (Sigma-Aldrich) was added to the monocytic cells in a 12-well plate. Total RNA isolated as previously described (35) and concentration was determined by absorbance at 260 nm. MCP-1 mRNA was quantified by Invader RNA assay according to the manufacturer's protocol (Third Wave Technologies).

Monocytes stimulation and immunoblotting

Monocytes were treated with various concentrations of HRV16 for times between 5 and 60 min in 12-well tissue culture plates. After treatments, monocytes were suspended in loading buffer and assayed for protein content by BCA assay (Pierce). The cell lysates were diluted with electrophoresis sample buffer, and the proteins were resolved on polyacrylamide slab gels. The lanes were loaded with samples that represented equal amounts of protein. The proteins were transferred to polyvinylidene difluoride membranes and immunoblotted using standard methods (32). The consistency of protein loading in all lanes was confirmed by staining of the polyvinylidene difluoride membrane with amido black following chemiluminography (Supersignal; Pierce) and by subsequent immunoblotting with anti-ERK-1 or Grb-2 antisera. Quantification of immunoblots was done by densitometric analysis of digitized immunoblot images using NIH ImageJ software.

Results

As a first step in this study to confirm previous reports that productive infection does not take place in alveolar macrophages and blood monocytes (7), these two cell types were exposed to sucrose-purified HRV16 at different multiplicities of infection (MOI). The presence of infectious virus was assayed from supernatants at various times postinfection (30). Infectious virus, probably representing unbound inoculum, was detected in virus assays 1 h postinfection. Decreasing amounts of virus were detected at 8 and 12 h postinfection, and no infectious virus was detected in either blood monocytes or alveolar macrophages 24 h postinfection (data not shown). However, HeLa cells infected with HRV16 produced high titers of infectious virus (see *Materials and Methods*). Because no productive infection was detected, we refer to rhinovirus exposure rather than infection in subsequent portions of this study.

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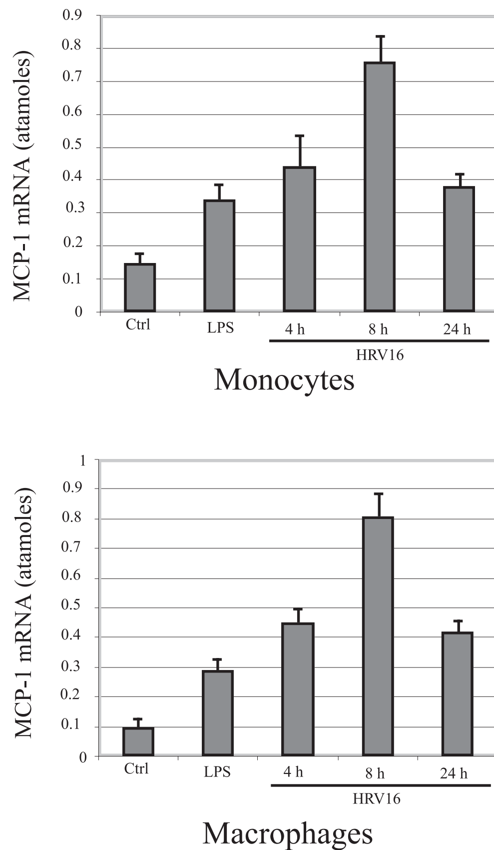

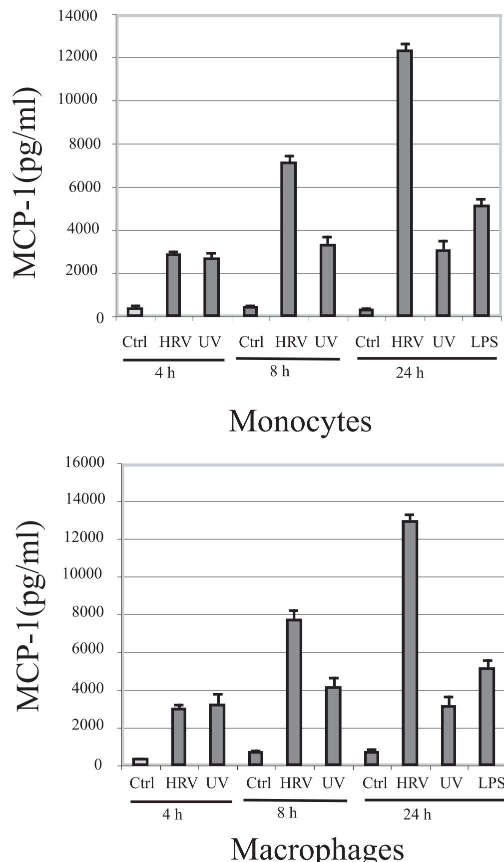

FIGURE 1. MCP-1 mRNA and protein levels in macrophages and monocytes after HRV16 exposure. Alveolar macrophages or monocytes (1×10^6 cells/well) were treated with control vehicle (Ctrl), HRV16 at an MOI of 10, or 100 ng/ml LPS and MCP-1. mRNA or protein was measured. Data are summarized as mean (SD) of triplicate wells. *A*, MCP-1 mRNA concentrations were determined by the INVADER assay and standardized against GAPDH mRNA levels ($n = 3$). *B*, Tissue culture media were recovered after 4, 8, and 24 h of incubation with HRV16 or UV-inactivated HRV16 (UV) and frozen at −20°C. Concentrations of MCP-1 were determined from supernatants by ELISA as described in *Materials and Methods* ($n = 9$).

MCP-1 mRNA accumulation and protein secretion in response to HRV16 exposure

The release of IL-8, IL-6, RANTES, and GM-CSF by epithelial cells in response to productive rhinovirus infection was reported previously by several investigators (1, 10, 27, 36). In addition, IL-8 and IL-10 are reported to be released following monocytic lineage cell exposure to rhinovirus (24, 25). We sought to determine whether other cytokines important in immune cell recruitment were elicited by HRV16 exposure. As a method of screening for HRV16-induced alterations in gene expression, human peripheral blood monocytes were treated with HRV16 (MOI of 10) or control buffer. After 4 h, total RNA was isolated, processed, and analyzed by oligonucleotide microarrays (Affymetrix HuGeneFL GeneChip, data not shown). We noted that several transcripts were increased by at least 5-fold: for example, transcripts encoding IFNα isoforms, and several cell surface receptors such as CD44, CD69, and CD54 (preliminary data not shown). MCP-1 was among the most highly induced genes and chose to examine this particular chemokine because of its connections to asthma (12, 37, 38). To confirm the microarray results, total RNA isolated from alveolar macrophages or blood monocytes exposed to HRV16 at an MOI of 10 was examined by INVADER assay. The HRV16 was sucrose purified and tested for the presence of MCP-1 and LPS (data not shown). An increase of MCP-1 mRNA was detected 4, 8, and 24 h postexposure in increasing amounts in both alveolar macrophages and blood monocytes (Fig. 1*A*). Furthermore, ELISA results indicated secretion of MCP-1 into culture supernatants at 4 h postexposure, and peak MCP-1 release was measured 24 h postexposure for both alveolar macrophages and blood monocytes (Fig. 1*B*). After 24 h of incubation, MCP-1 concentrations in tissue culture supernatants of monocytes were (mean ± SEM) 13.6 ± 0.7 ng/ml for HRV16-stimulated cells compared with 0.06 ± 0.06 ng/ml for unstimulated monocytes ($n = 9$, $p < 10^{-10}$). In supernatants from alveolar macrophage after 24 h of stimulation with HRV16 (MOI of 10), MCP-1 concentrations were 16.6 ± 3.1 ng/ml compared with 1.2 ± 0.5 ng/ml without virus addition ($n = 9$, $p < 10^{-6}$).

Although we had previously observed that release of live virus from HRV16-exposed alveolar macrophages and blood monocytes was not detectable, we evaluated whether a virus incapable of replicating (i.e., UV-inactivated virus) would also stimulate MCP-1 release in monocytes and macrophages (Fig. 1*B*). HRV16 was UV-inactivated as described in *Materials and Methods*. Interestingly at early time points, the release of MCP-1 in response to UV-inactivated virus was similar to that of live virus suggesting that replication of the virus genome is not necessary for HRV16 to stimulate MCP-1 production.

Effect of p38 kinase inhibitor on MCP-1 protein production

Previous studies have indicated that the p38 MAPK pathway plays an important role in MCP-1 transcription (39–41). To evaluate the importance of p38 MAPK activity in HRV16-stimulated MCP-1 release from alveolar macrophages, we incubated alveolar macrophages or blood monocytes with HRV16 (MOI of 10) for 24 h in the presence or absence of the p38 MAPK inhibitor SB203580 (10

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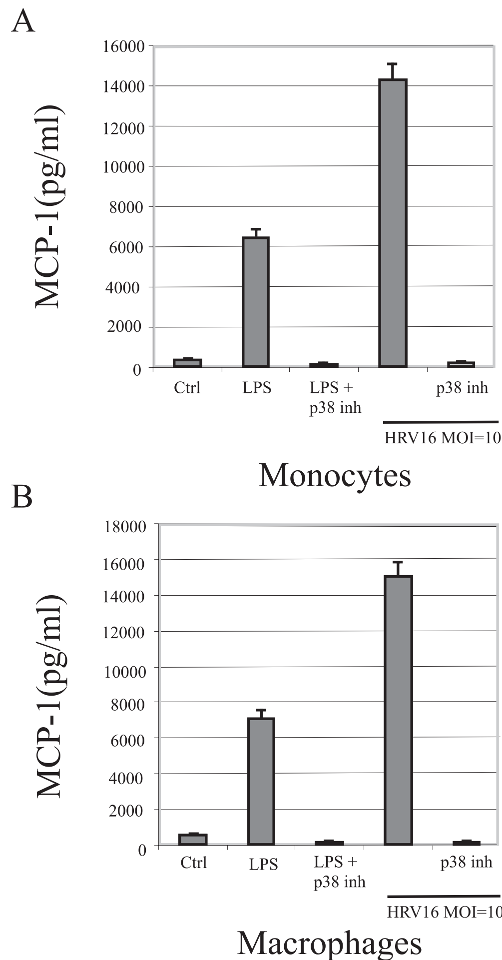

FIGURE 2. Effect of p38 MAPK inhibition on HRV16-stimulated MCP-1 release. Alveolar macrophages or monocytes (1×10^6 cells/well) were treated with HRV16 at an MOI of 10 or 100 ng/ml LPS for 24 h. Cells were pretreated with control buffer or 10 μM SB203580 for 30 min before HRV16 exposure. Concentrations of MCP-1 were determined from supernatants by ELISA. Data are representative (SD of triplicate wells) of three independent experiments.

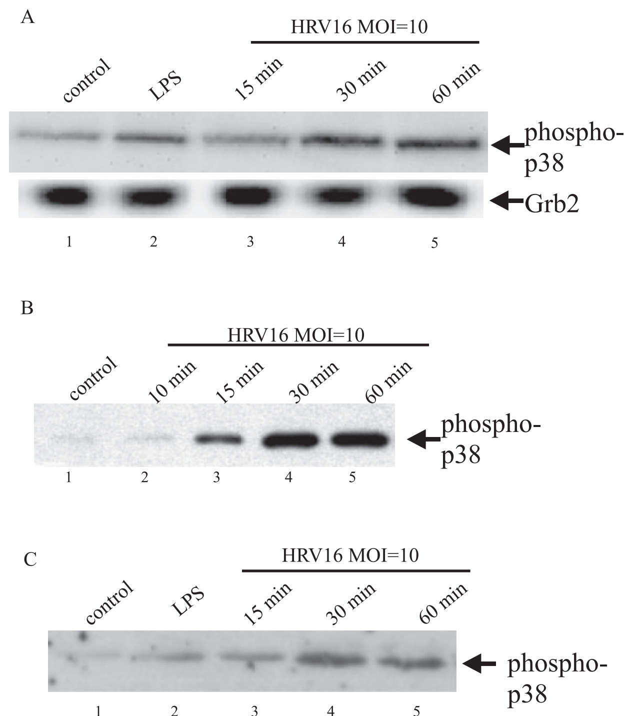

FIGURE 3. Phosphorylation of p38 MAPK in response to HRV16 exposure. Alveolar macrophages, blood monocytes, or THP-1 cells (1×10^6 cells/well) were treated with control vehicle (Ctrl), HRV16, or LPS (100 ng/ml) for the indicated time periods. Cell lysates were generated and protein concentrations were quantified by BCA protein assay. Equal amounts of protein were loaded into each lane and confirmed by immunoblotting for Grb2. Activation of p38 MAPK was visualized by immunoblotting with antiserum raised against the dually phosphorylated activation motif of p38 MAPK. *A*, Lysates of alveolar macrophages, stimulated for various times with HRV16 (MOI = 10), were immunoblotted with anti-active p38 MAPK anti-serum. The observed activation pattern was confirmed in cell lysates from 15 subjects. *B*, Blood monocyte cell lysates stimulated for various times with HRV16 (MOI = 10) were immunoblotted with anti-active p38 MAPK anti-serum. The observed activation pattern was confirmed in 10 subject samples. *C*, THP-1 cells were stimulated for various times with HRV16 (MOI = 10), and the resulting lysates were immunoblotted with anti-active p38 MAPK anti-serum. The observed activation pattern was confirmed in four separate experiments.

μM). The p38 MAPK inhibitor effectively blocked MCP-1 production, induced by virus or LPS (Fig. 2). This decrease in chemokine release was not due to a cytotoxic effect of the inhibitor as assayed by MTT assay or annexin V staining (>98% viable) (42, 43).

p38 MAPK phosphorylation after monocytic-lineage cell exposure to HRV16

Several papers have reported that p38 MAPK is phosphorylated in response to rhinovirus infection of epithelial cells (13, 36). Furthermore, p38 MAPK activation is a necessary event in multiple cell types for subsequent secretion of several cytokines including MCP-1, IL-8, GM-CSF, and IL-6 (13, 36, 44, 45). We hypothesized that HRV16, despite not being able to productively infect alveolar macrophages, activates p38 MAPK in a manner similar to a ligand rather than a virus. To test this hypothesis, human alveolar macrophages, blood monocytes, or THP-1 cells were exposed to HRV16 at an MOI of 10 for up to 60 min. Indeed, HRV16-induced p38 MAPK activation, as determined by immunoblotting with phosphospecific Abs, with an average of 5-fold p38 MAPK activation at 30 min postexposure in each of the three cell types based on densitometry using ImageJ software (Fig. 3). These results confirm that blood monocytes (Fig. 2*B*) and THP-1 (Fig. 3*C*) cells respond to HRV16 treatment in a manner similar to alveolar macrophages (Fig. 3, *lane 4*), suggesting both cell types are adequate model systems for alveolar macrophages when studying the HRV16-induced p38 MAPK signaling cascade. Furthermore, p38 MAPK activation is stimulated in blood monocytes and THP-1 cells in a dose-dependent manner by HRV16 further supporting the hypothesis that HRV functions as a ligand during initial exposure (Fig. 4, *A*, *lanes 2* and *3*, and *B*, *lanes 2–7*).

To evaluate the possibility that LPS contamination of the virus stocks caused p38 MAPK activation, we exploited the fact that HRV16 is incapable of binding to murine ICAM-1 (46). Murine RAW 264.7 macrophages exposed to HRV16 failed to activate ERK1 and ERK2 whereas LPS-stimulated ERK activation within 15 min thereby suggesting that LPS, dsRNA, and other standard contaminants were not responsible for the p38 MAPK activation (data not shown).

Effect of ICAM-1 Ab, pH, and WIN compound on HRV-stimulated p38 MAPK activation in monocytes

To determine whether p38 MAPK phosphorylation was mediated by HRV16 interaction with the HRV major group receptor,

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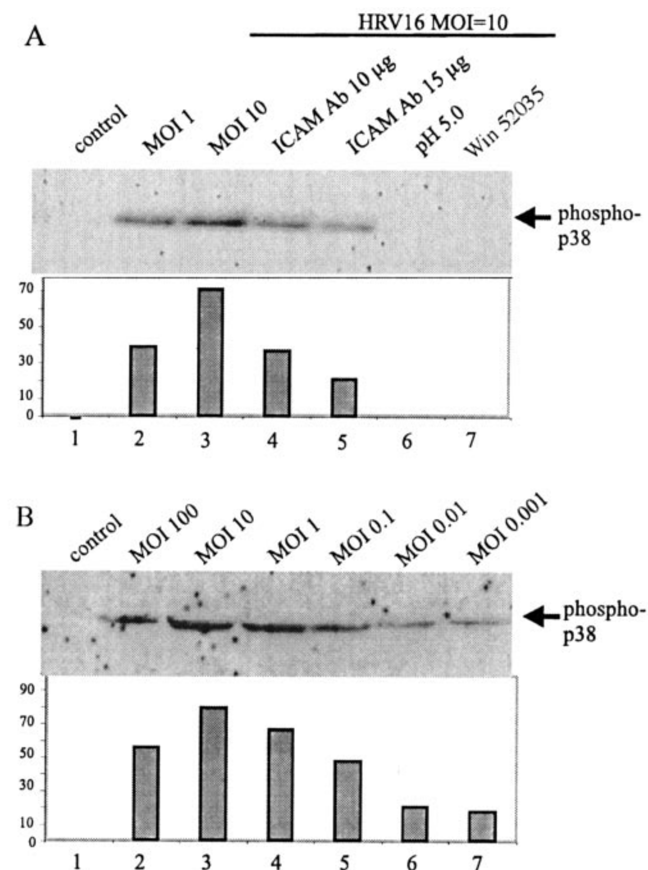

FIGURE 4. Effects of disruption of HRV16 interaction with ICAM-1 by prior treatment of cells with anti-ICAM Ab, or pretreatment of the HRV16 at pH 5.0 or with WIN-52035. *A*, Monocytes (1×10^6 cells/well) were treated with control buffer (*lane 1*) or HRV16 at an MOI of 1 or 10 (*lane 2* or *3*) for 30 min. To reduce the interaction between HRV16 and its putative receptor, the monocytes were pretreated with 10 or 15 μg of anti-ICAM-1 Ab (*lanes 4* and *5*) before HRV16 exposure. In addition, monocytes were stimulated with HRV16 that had been pretreated with pH 5.0 buffer to disrupt viral capsid structure or WIN-52035 (20 μg/ml) (*lanes 6* and *7*). Cell lysates were generated, and protein assays performed. Equal protein was loaded and immunoblotted. Anti-phospho-p38 Ab detected dually phosphorylated, active p38 MAPK ($n = 5$). Densitometry (graphed in arbitrary units) was performed using ImageJ software. The control density was subtracted from each sample lane measured. *B*, THP-1 cells (1×10^6/well) were treated with HRV16 at different MOIs for 30 min. Cell lysates were generated and immunoblotted. Anti-phospho-p38 Ab detected dually phosphorylated, active p38 MAPK ($n = 3$). Densitometry (graphed in arbitrary units) was performed using ImageJ software. The control density was subtracted from each sample lane measured.

ICAM-1, several approaches were used. Previous studies have shown that anti-ICAM-1 Ab can effectively block HRV16 from binding to the ICAM-1 receptor (47, 48). Monocytes were pretreated with two different concentrations of anti-ICAM-1 Ab for 30 min (10 and 15 μg/ml) followed by 30 min of exposure to HRV16 at an MOI of 10, (Fig. 4*A*, *lanes 4* and *5*). The anti-ICAM-1 Ab partially (~50% by densitometry using ImageJ software) attenuated the virus-induced p38 MAPK phosphorylation (Fig. 4*A*, *lane 3*). Because receptors are often activated by Ab binding (49, 50), we sought other means to disrupt interaction between HRV16 and ICAM-1. These techniques included prevention of the HRV16-ICAM-1 interaction by degrading the virus capsid at low pH (51, 52) or inhibiting virus binding with the pharmacologic agent WIN52035 (29). As shown in Fig. 4*A*, HRV16 that was inactivated by low pH or WIN52035 was unable to activate p38 MAPK in monocytes. These studies provide evidence that p38 MAPK activation by HRV16 is mediated by interaction with ICAM-1.

Time-course of IκBα degradation after exposure of alveolar macrophages and blood monocytes to HRV16

IκBα is the protein responsible for the retention of the transcription factor NF-κB in the cytoplasm. Following stimulus-dependent degradation of IκBα, NF-κB translocates into the nucleus and promotes the transcription of numerous genes including MCP-1 (53, 54). To determine whether IκBα is degraded following HRV16 exposure, alveolar macrophages and monocytes respectively were incubated with HRV16 at an MOI of 10 for times ranging from 10 to 60 min and then the mass of IκBα in the cell lysates was evaluated by immunoblotting (Fig. 5, *A*, *lanes 2–4*, and *B*, *lanes 4–6*). Basal levels of IκBα were visible in lysates from unstimulated cells (Fig. 5, *A* and *B*, *lane 1*) and a time-dependent degradation was observed beginning 15 min after HRV16 exposure with maximal degradation (decrease of 90%, $n = 5$) after 30 to 45 min (Fig. 5, *A*, *lane 3*, and *B*, *lane 5*). The amount of IκBα degradation is comparable to that seen following 15 min of stimulation with LPS (100 ng/ml), a potent activator of NF-κB (Fig. 5*A*, *lane 2*).

Exposure of alveolar macrophages, blood monocytes, and THP-1 cells to HRV16 affects ATF-2 phosphorylation

The family of stress-activated kinases including p38 MAPK and JNK are serine/threonine kinases known to phosphorylate a multitude of transcription factors including CREB, c-Jun, and ATF-2 (40, 41, 55). Because, ATF-2 phosphorylation was implicated in MCP-1 transcription in other cell systems (40, 41), the following experiments were conducted to determine whether ATF-2 is phosphorylated in human alveolar macrophages, blood monocytes, or THP-1 cells following HRV16 stimulation. Each cell type was

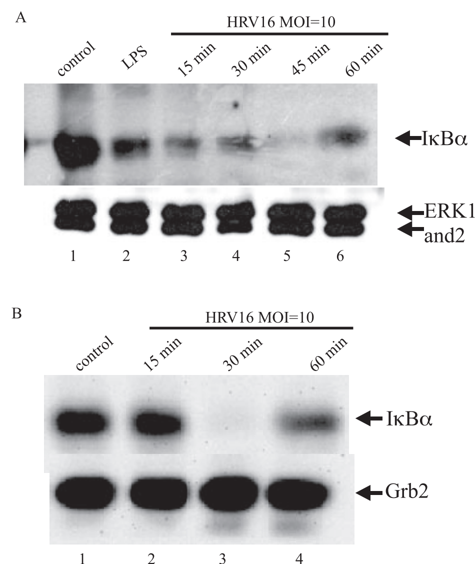

FIGURE 5. Effect of HRV16 exposure on the degradation of IκBα. *A*, Alveolar macrophages (1×10^6 cells/well) were treated with HRV16 at an MOI of 10 or 100 ng/ml LPS for 15–60 min. *B*, Monocytes (1×10^6 cells/well) were treated with HRV16 at an MOI of 10 or 100 ng/ml LPS for 5–60 min. Cell lysates were generated, and protein assays performed. Immunoblotting detected total IκBα. Cell lysates were generated, and protein assays performed. Equal protein was loaded and immunoblotted. Equal protein loading was assured by immunoblotting the same sample with anti-Grb2 or anti-ERK1 and ERK2 Ab. Data are representative of five independent experiments.

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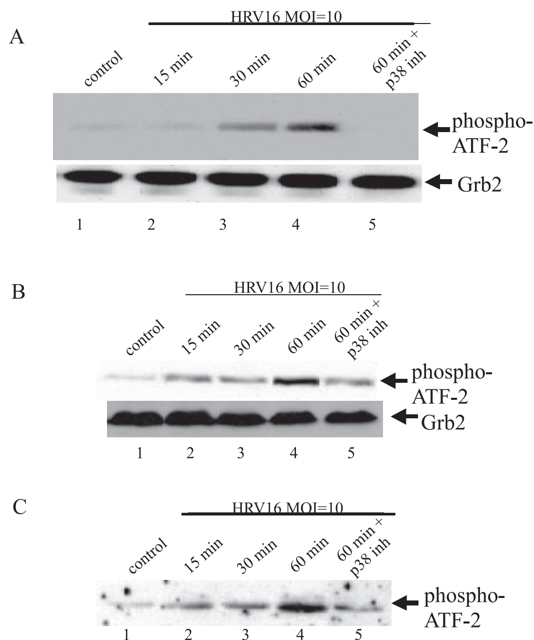

FIGURE 6. Effect of HRV16 exposure on ATF-2 phosphorylation. *A*, Alveolar macrophages (1×10^6 cells/well) were treated with HRV16 at an MOI of 10. Cells were pretreated with control buffer or 10 μM SB203580 (p38 inhibitor) for 30 min before HRV16 exposure. *B*, Monocytes (1×10^6 cells/well) were treated with HRV16 at an MOI of 10. Cells were pretreated with control buffer or 10 μM SB203580 for 30 min before HRV16 exposure. *C*, THP-1 cells (1×10^6 cells/well) were treated with HRV16 at an MOI of 10. Cells were pretreated with control buffer or 10 μM SB203580 for 30 min before HRV16 exposure. Cell lysates were generated, and protein assays performed. Equal protein was loaded and immunoblotted with anti-phospho-ATF-2 Ab. Equal protein loading was assured by immunoblotting the same membrane with anti-Grb2 Ab. Experiments with each cell type were performed five times with similar results.

incubated (15–60 min) with HRV16 (MOI of 10). For each cell type, ATF-2 phosphorylation was observed beginning 30 min after HRV16 exposure with maximal phosphorylation after 60 min (Fig. 6, *lanes 3* and *4*). In contrast, untreated cells and cells pretreated for 60 min with the p38 MAPK inhibitor SB203580 (10 μM) (Fig. 6, *lane 5*) showed little ATF-2 phosphorylation demonstrating the efficacy of the inhibitor. These data suggest that ATF-2 is a downstream substrate of the p38 MAPK pathway following the HRV16 exposure of alveolar macrophages, blood monocytes, or THP-1 cell line.

Discussion

The most numerous immune cells present in the airway lumen are monocytic-lineage cells (macrophages, monocytes and dendritic cells). The participation of macrophages and monocytes in rhinovirus-induced inflammatory responses is incompletely understood. In this study, we present data demonstrating that MCP-1 mRNA and protein is produced by both alveolar macrophages and blood monocytes in response to HRV16 (Fig. 1). These studies also suggest that the mechanism of MCP-1 mRNA transcription incorporates the activation of p38 MAPK (Fig. 3) and transcription factors ATF-2 (Fig. 6) and NF-κB. Indeed, we demonstrate that a p38 MAPK inhibitor blocked HRV-induced MCP-1 protein production (Fig. 2) and ATF-2 phosphorylation (Fig. 6). Furthermore, intact HRV16 particles capable of binding ICAM-1 contributed to p38 MAPK phosphorylation (Fig. 4*A*) in a dose-dependent manner (Fig. 4*B*). Finally, our findings support the idea that, with respect to selected aspects of the signaling initiated by HRV16, blood monocytes and the cell line THP-1 are reasonable models for alveolar macrophages, which are difficult to obtain and often impure.

Other studies have described IκBα degradation and p38 MAPK activation in response to HRV16 infection of epithelial cells (53, 56, 57). Although, the majority of these former studies focus on a time frame of 2–12 h postexposure, a couple of studies observed p38 MAPK activation in the first hour after exposure (13, 44, 58). In our study, HRV16 activated p38 MAPK within 15 min despite the fact that there was no productive viral infection. This time course suggests that HRV16 acts as a ligand of the ICAM-1 molecule to cause cell activation. We determined that p38 MAPK activation was dependent on intact virus capsid (Fig. 4*A*), affected by the amount of virus used for stimulus (Fig. 4*B*) and triggered by replication-deficient UV-inactivated virus (Fig. 1).

In cell types that do support a productive viral infection (HeLa, A549, BEAS-B2), viral RNA replication is not observed until at least 1.5 h after initial exposure (7, 8, 59) and virus particle release is not observed until 2.5 h postexposure. Therefore protein kinase R, a kinase that is activated by viral dsRNA replication intermediate, is often invoked as the kinase responsible for p38 MAPK phosphorylation (36, 60–63). Because our studies demonstrate HRV16-induced p38 MAPK activation within 60 min of HRV16 exposure, protein kinase R is probably not the kinase responsible for p38 MAPK activation, because the endocytosis and release of HRV16 genomic RNA has been determined to take at least 1 h (46, 52).

Of the multitude of downstream effectors for the p38 MAPK pathway, we chose to examine the transcription factor ATF-2 because it is known to stimulate the transcription of MCP-1 (40, 41). We demonstrated that ATF-2 is phosphorylated after alveolar macrophage, blood monocyte, and THP-1 cell exposure to HRV16 and that the p38 inhibitor SB203580 can suppress this phosphorylation (Fig. 6).

A second important contributor to the transcription of MCP-1 is NF-κB (64, 65). Indeed, NF-κB activation occurs during respiratory syncytial virus exposure through redox-sensitive degradation of IκBα (66). The present study determined that IκBα was degraded within 45 min of alveolar macrophages and blood monocytes exposure to HRV16 (Fig. 5) although it was not a redox-sensitive mechanism (data not shown). This is the earliest documented degradation of IκBα by HRV suggesting that viral replication is not involved and that NF-κB may act as a potential regulator of MCP-1 transcription.

This particular study is unique because it demonstrates, for the first time, that MCP-1 is released from monocyte-lineage cells via rhinoviral-stimulated p38 MAPK activation. Indeed, we demonstrate that blood monocytes, alveolar macrophages, and THP-1 cells respond in a like manner with p38 MAPK and ATF-2 phosphorylation (Figs. 3 and 6). This is significant in understanding HRV-induced inflammation for two reasons. First, MCP-1 appears to shift the immune response toward the Th1 spectrum (21, 22), and second, the primary receptor for MCP-1, CCR2, is expressed on immune cells that do not exhibit a robust chemotactic response to other cytokines and chemokines released in response to HRV infection. Indeed, the primary role of MCP-1 is to attract NK cells, dendritic cells, monocytes, and macrophages. Thus, monocytic-lineage cells attracted to the site of infection are likely stimulated by virus through ligand binding, thereby further promoting and enhancing the inflammatory response. However, future studies are needed to determine whether MCP-1 is necessary for the quick resolution of HRV infection as it is for other pathogens (21, 22). The results present the idea that cell types other than epithelial cells contribute to viral exacerbations of asthma, as well as causing the symptoms of the common cold.

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Disclosures

The authors have no financial conflict of interest.

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