



Effect of exogenous interferons on rhinovirus replication and airway inflammatory responses

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ABSTRACT

Background: Human rhinoviruses (HRVs) are the most common cause of asthma exacerbations. In airway epithelial cells, the primary site of HRV infection, decreased production of interferons (IFNs) may result in greater susceptibility to HRV and worsened symptoms. Thus, exogenous IFN could supplement the innate immune response and provide a treatment for virus-induced asthma exacerbations. Furthermore, the effects of exogenous IFN could be type specific in part because of the cellular distribution of type 1 and type 2 IFN receptors.

Objective: To investigate the effects of exogenous IFNs on HRV replication in bronchial epithelial cells.

Methods: Frozen stocks of primary human bronchial epithelial cells from healthy donors were cultured in monolayers; pretreated (24 hours) with 0.1-ng/mL, 1-ng/mL, or 10-ng/mL doses of IFN- α , - β , - λ 1, or - λ 2; and infected with HRV-1A. Viral replication was quantified using real-time reverse transcription–polymerase chain reaction, and cytokine and chemokine secretion 24 hours after infection was measured by multiplex enzyme-linked immunosorbent assay.

Results: Compared with untreated samples, IFN- α , IFN- β , IFN- λ 1, and IFN- λ 2 (0.1 ng/mL) significantly reduced HRV replication after high- ($P < .02$) and low-dose inoculation ($P < .05$). Similar effects were seen in 1-ng/mL and 10-ng/mL doses of IFN, where HRV replication was significantly decreased in both high- ($P < .001$) and low-dose inoculation ($P < .001$). Treatment with IFNs also enhanced HRV-induced IFN- γ –induced protein 10 secretion ($P < .001$). Finally, treatment with either IFN- λ 1 or IFN- λ 2 significantly increased HRV-induced secretion of RANTES (regulated on activation, normal T-expressed, and presumably secreted) ($P < .05$) but not IL-1 β or vascular endothelial growth factor.

Conclusion: These findings suggest that exogenous IFNs, IFN- λ 1 in particular, warrant further study as a potential therapy for virus-induced asthma exacerbations.

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Introduction

Acute exacerbations are the leading cause of morbidity and mortality associated with asthma, frequently leading to hospitalization and accounting for approximately half of the total health care costs associated with asthma.¹ Asthma exacerbations have been linked to progressive loss of lung function,^{2,3} which may lead to increased asthma severity later in life. Viral upper respiratory tract infections are the most significant risk factor for asthma

exacerbations. In the northern hemisphere, there is a clear correlation between upper respiratory tract infection outbreaks in September and concurrent hospitalizations for asthma exacerbations.⁴ Nearly 85% of acute asthma exacerbations in children and approximately 60% in adults have been linked to the presence of a viral infection through the use of reverse transcription–polymerase chain reaction (RT-PCR),^{5,6} with human rhinoviruses (HRVs) as the most common cause.^{7,8} Although current therapies are effective in controlling day-to-day asthma symptoms, they are less effective at preventing exacerbations,⁹ suggesting that new strategies are needed for exacerbation prevention.

Airway epithelial cells are recognized as the primary site of HRV infection and play an important role in the intrinsic antiviral response.¹⁰ Interferons (IFNs) are critical to host defense against HRV replication. Increased viral replication, decreased epithelial production of IFN- β and IFN- λ , and reduced induction of apoptosis in response to HRV-16 infection have been reported in asthmatic individuals, suggesting a deficient innate immune response in these individuals that may contribute to the risk of viral

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exacerbations.^{11,12} Although not all groups have identified differences in epithelial cell IFN expression between asthmatic patients and healthy controls,^{13,14} deficiencies in peripheral blood IFN- α and - λ responses to viruses have been identified in allergic asthmatic children as well.^{15,16}

Interestingly, treatment with exogenous IFN- β restored induction of apoptosis and inhibited HRV replication in bronchial epithelial cells (BECs) from asthmatic individuals, suggesting potential therapeutic benefit.^{11,17} In an animal model of rotavirus infection, treating suckling mice systemically with IFN- λ 1 led to decreased replication of rotavirus in the gut and less severe viral illness,¹⁸ further supporting the potential utility of exogenous supplementation of IFN as a clinical treatment for viral infections.

Although IFN- α and - β (type 1) and IFN- λ (type 3) activate similar intracellular signaling pathways and biological activities, type 1 and type 3 IFNs use distinct receptor complexes on the cell surface. Unlike the type 1 high-affinity receptor component, which is broadly expressed, the type 3 high-affinity receptor component is restricted to dendritic cells and cells of epithelial origin.¹⁹ Collectively, these findings suggest that the effect on HRV replication and epithelial antiviral and inflammatory responses may be IFN type dependent.

Taken together, these data suggest that IFNs may be useful in the treatment of respiratory viral infections and associated asthma exacerbations. In this study, we aimed to determine whether exogenous interferons, at physiologic concentrations, inhibit HRV replication and alter inflammatory responses in primary BECs. Further, we sought to determine whether there were differences in the effects of IFN- α , - β , - λ 1, and - λ 2. Some of the findings in this study were previously reported in an abstract.²⁰

Methods

BEC Culture

After the protocol was approved by the University of Wisconsin–Madison Health Sciences institutional review board, human tracheal explants of nonidentifiable lung transplant donors were collected using the methods previously described.²¹ Frozen stocks at passage 0 from 6 donors were cultured at 37°C in monolayers in 75-cm² CellBind flasks (Corning Inc, Corning, New York) in 1 mL of bronchial epithelial growth medium (Lonza, Walkersville, Maryland) to 80% to 90% confluence. BECs were then passaged into 12-well CellBind plates, grown to 65% to 75% confluence, and pretreated for 24 hours with 1 mL of 0.1-ng/mL, 1-ng/mL or 10-ng/mL doses of IFN- α , IFN- β (Sigma-Aldrich Co LLC, St Louis, Missouri), IFN- λ 1, or IFN- λ 2 (PeproTech, Rocky Hill, New Jersey).

Generation of HRV-1A

Because minor receptor group viruses using low-density lipoprotein receptor for cell entry more efficiently infect cultured epithelial cells compared with major group viruses binding to intercellular adhesion molecule 1,²¹ minor group strain HRV-1A was chosen for this study. Purified and concentrated HRV-1A stocks were generated from infected cultures of HeLa cells (Ohio cells) as previously described.^{22,23} To minimize any suppressive effects on gene expression, virus was diluted in bronchial epithelial growth medium with a reduced concentration of hydrocortisone (10^{-8} M) immediately before infection.

BEC Infection With HRV-1A

Twenty-four hours after IFN pretreatment, BECs were infected with either a high (5×10^6 PFU/mL, multiplicity of infection of 10) or low (5×10^5 PFU/mL, multiplicity of infection of 1) inoculum of HRV-1A. After a 2-hour attachment period, the monolayers were washed 3 times with phosphate-buffered saline to remove

unbound virus and incubated in the presence of the appropriate dose and type of IFN. After 24-hour incubation, supernatants were collected and monolayers were lysed using RLT buffer (Qiagen, Hilden, Germany) and stored at -80°C .

Viral Quantification

Total RNA was isolated from cell lysates and purified using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. This was followed by RT (TaqMan; Applied Biosystems, Foster City, California) and quantification of viral replication by real-time PCR in duplicate wells using ABI Prism 7000 sequence detection system (Applied Biosystems). The standard curve was constructed using 10-fold serial dilutions of 10^7 PFU of HRV-1A complementary DNA. The HRV primers and probe were described previously.²⁴

Cytokine and Chemokine Analysis

Interleukin-1 β (IL-1 β), interferon-inducible protein 10 (IP-10 or CXCL-10), RANTES (regulated on activation, normal T-expressed, and presumably secreted) (CCL-5), and vascular endothelial growth factor (VEGF) secretion in the cell supernatant were measured using multiplex cytokine analysis kits (Millipore, Billerica, Massachusetts). Plates were run on the Luminex 100 instrument (Luminex Corporation, Austin, Texas).

Statistical Analyses

Repeated-measures analysis of variance models were used to assess the effect of exogenous IFN- α , - β , - λ 1, and - λ 2 on HRV replication and HRV-induced cytokine secretion from airway epithelial cells compared with controls. Logarithmic transformations of the responses were used to meet the assumptions of analysis of variance. If the *P* value of global null hypothesis was significant, then pairwise comparisons were examined (Fisher protected least significantly different test). All *P* values reported are 2-sided. *P* < .05 was the criterion for statistical significance. Analyses were performed in SAS statistical software, version 9.3 (SAS Institute Inc, Cary, North Carolina).

Results

Effect of IFNs on HRV Replication

We first investigated the effects of exogenous IFNs (- α 2, - β , - λ 1, and - λ 2) at 0.1 ng/mL, 1 ng/mL, or 10 ng/mL on HRV-1A replication in BECs infected with either a low- (5×10^5 PFU/mL) or a high- (5×10^6 PFU/mL) dose inoculum of HRV-1A. In the samples infected with the low-dose inoculum, all IFNs significantly reduced HRV replication in a dose-dependent fashion (Fig 1; 0.1 ng/mL [*n* = 5, *P* < .001], 1 ng/mL [*n* = 6, *P* < .001], and 10 ng/mL [*n* = 6, *P* < .001]). In addition, IFN- α 2, - β , and - λ 1 pretreatment led to greater reductions in HRV replication than IFN- λ 2 (*P* < .05) at IFN doses of 0.1 ng/mL and 1 ng/mL. However, at the 10-ng/mL dose of IFN, only IFN- β pretreatment led to greater reductions in HRV replication than IFN- λ 2 (*P* < .05) (Fig 1).

Similarly, all IFNs led to a dose-dependent reduction in HRV replication in samples infected with the high-dose HRV inoculum (Fig 2; 0.1 ng/mL [*n* = 5, *P* < .02], 1 ng/mL [*n* = 6, *P* < .001], and 10 ng/mL [*n* = 6, *P* < .001]). Furthermore, pretreatment with 1 ng/mL of IFN- β and - λ 1 led to greater inhibition of HRV replication than IFN- λ 2 (*P* < .05) (Fig 2).

Effect of IFNs on Inflammatory Responses

Because virus-induced cytokines, chemokines, and growth factors orchestrate airway inflammation and may contribute to clinical symptoms, we also investigated the effects of 1 ng/mL of exogenous IFN- α 2, - β , - λ 1, or - λ 2 on HRV-induced IP-10, RANTES, IL-1 β , and VEGF protein secretion in BECs from healthy donors after 24

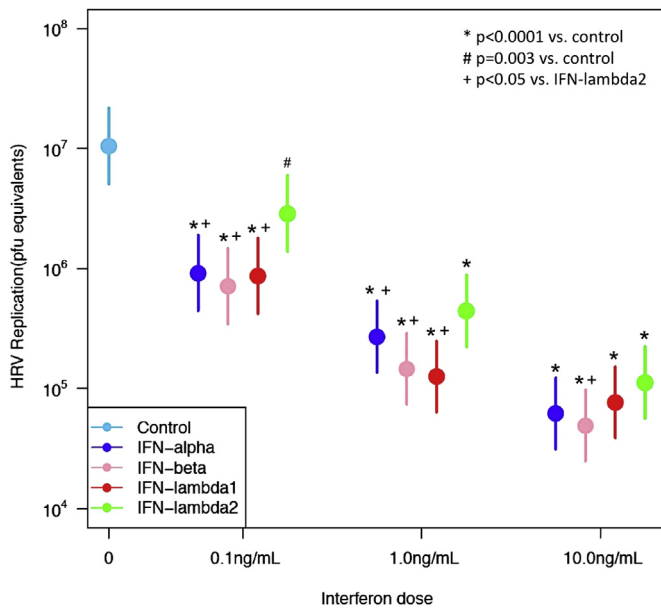


Figure 1. Effect of interferons on human rhinovirus (HRV) replication in bronchial epithelial cells (BECs) infected with low-dose HRV. Dose-response comparison of HRV replication in BECs infected with a low dose (5×10^5 PFU/mL) of HRV-1A. Samples were treated with 0 (infected control), 0.1 (n = 5), 1 (n = 6), or 10 (n = 6) ng/mL of exogenous interferon (IFN) α 2, IFN- β , IFN- λ 1, or IFN- λ 2 before and during infection. * $P < .001$; # $P = .003$; + $P < .05$.

hours (n = 6). The addition of exogenous IFNs resulted in significant upregulation of HRV-induced IP-10 secretion compared with infected control ($P < .001$) (Fig 3A). The IFN- λ 1-treated samples had the highest levels of HRV-induced IP-10, although this difference was only statistically significant compared with IFN- α 2 ($P = .04$).

IFN- λ 1 and - λ 2 also enhanced HRV-induced RANTES secretion, and a similar trend was noted to treatment with IFN- β (Fig 3B).

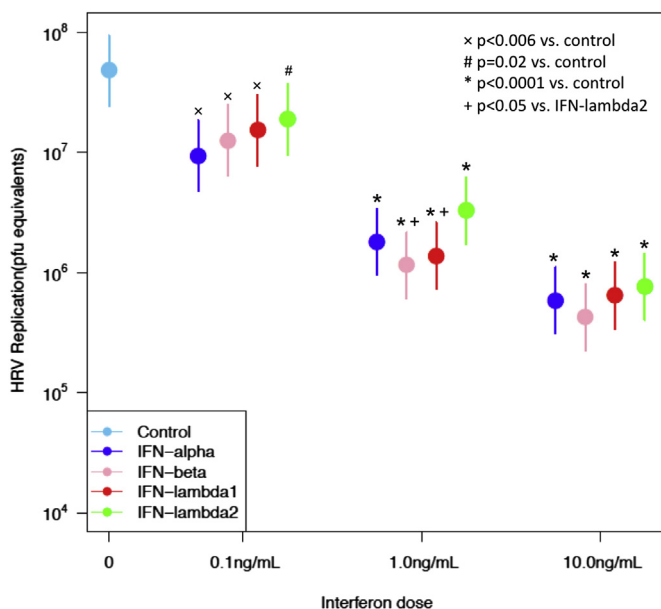


Figure 2. Effect of interferons on human rhinovirus (HRV) replication in bronchial epithelial cells (BECs) infected with high-dose HRV. Dose-response comparison of HRV replication in BECs infected with a high dose (5×10^6 PFU/mL) of HRV-1A. Samples were treated with 0 (infected control), 0.1 (n = 5), 1 (n = 6), or 10 (n = 6) ng/mL of exogenous interferon (IFN) α 2, IFN- β , IFN- λ 1, or IFN- λ 2 before and during infection. * $P < .001$; # $P = .02$; + $P < .05$; $\times P < .006$.

Treatment with interferons did not significantly alter HRV-induced IL-1 β or VEGF secretion (data not shown).

Discussion

Through the use of in vitro culture of primary BECs from healthy individuals, we found that exogenous interferons effectively inhibit HRV replication in a dose-dependent manner, while also altering the inflammatory response to the virus. Samples treated with 0.1-ng/mL doses of IFN had a 0.5-log reduction in HRV replication compared with control. Similar results were seen at the 1- and 10-ng/mL doses of IFN, where HRV replication was reduced by more than 1-log compared with control. Further, both IP-10 and RANTES production were upregulated in response to HRV infection in the presence of IFN.

It has been reported that exogenous IFN- β inhibits HRV replication in cultured BECs.^{11,17} However, the effects of exogenous IFN- λ 1 and IFN- λ 2 on HRV replication have not been previously reported and are of interest in light of the deficient HRV-induced IFN- λ production that has been described in allergic asthmatic individuals.^{12,15} In this study, we demonstrated that IFN- λ 1 was as effective as IFN- β in inhibiting HRV replication. This is intriguing based on the restriction of type 3 interferon receptors to epithelial and dendritic cells,¹⁹ suggesting that exogenous IFN- λ 1 could have fewer systemic adverse effects than treatment with type 1 interferons. Indeed, early studies of IFN- λ 1 in hepatitis C infection suggest similar efficacy to other interferons with fewer adverse effects.²⁵

In this study, in addition to their antiviral effects, we found significant effects of the interferons on HRV-induced proinflammatory IP-10 and RANTES. RANTES is a known chemoattractant and activator for eosinophils, serving as an inflammatory mediator. IP-10, a chemoattractant for macrophages, dendritic cells, and T lymphocytes, promotes adhesion of T lymphocytes to epithelial cells and has gained increasing interest because of its association with HRV infections and asthma exacerbations.^{26–28} Wark and colleagues²⁸ reported that serum IP-10 levels were elevated in asthmatic patients with HRV-induced asthma exacerbations, with higher levels associated with more severe bronchial obstruction. Interestingly, in our study, although all interferons enhanced IP-10 production, IFN- λ 1 had the most prominent effects on both IP-10 and RANTES secretion after HRV infection of BECs.

The role of type 1 IFNs in the antiviral community was discovered in 1957, but it was not until 2003 that type 3 IFNs were discovered.²⁹ As a relatively newly identified antiviral cytokine, much remains to be learned about the role of IFN- λ 1 in allergic disease and asthma. A recent clinical study by Miller and colleagues³⁰ found greater levels of IFN- λ 1 in nasal secretions of wheezing children with more severe HRV-induced exacerbations, despite replicating prior findings of deficient IFN- λ 1 levels at baseline in vivo and in vitro after HRV infection of nasal epithelial cells of wheezing children. These findings, taken together with the effects of IFN- λ 1 on HRV replication and inflammatory responses in our study, support further study of this antiviral cytokine in asthma.

The enhanced production of HRV-induced IP-10 and RANTES seen with exogenous interferons in our study was not found in a recent study of effects of IFN- β on HRV replication and inflammatory responses in bronchial epithelial cells.¹⁷ Cakebread and colleagues¹⁷ reported that IFN- β treatment diminished HRV-induced IP-10 and RANTES production in BECs from asthmatic individuals. Interestingly, IFN- β also caused a significant increase in IP-10 protein expression above baseline values in the absence of HRV infection. Methodologic differences between the 2 studies include strain of HRV (HRV-1A vs HRV-1B), time point of measurement (24 vs 72 hours), phenotype (asthma vs no asthma),

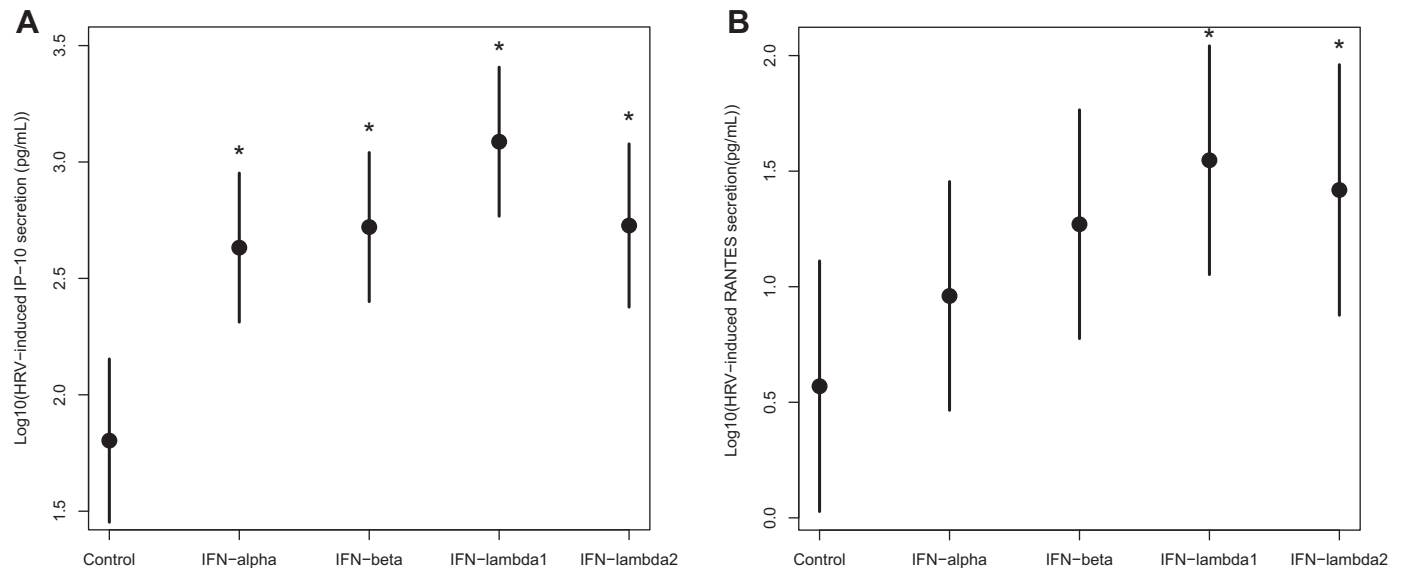


Figure 3. Effect of interferons on cytokine and chemokine secretion in bronchial epithelial cells (BECs) infected with low-dose human rhinovirus (HRV). A, Comparison of interferon-inducible protein 10 (IP-10) secretion (log scale with 95% confidence interval) measured in cell supernatant of BECs from healthy donors ($n = 6$) treated as infected control or with a 1-ng/mL dose of exogenous interferon (IFN) α 2, IFN- β , IFN- λ 1, or IFN- λ 2 before and during low-dose (5×10^5 PFU/mL) infection of HRV-1A. Samples treated with IFN- α 2, IFN- β , IFN- λ 1, or IFN- λ 2 revealed elevation in IP-10 protein secretion compared with infected control. * $P < .005$. B, Comparison of RANTES (regulated on activation, normal T-expressed, and presumably secreted) protein secretion (log scale with 95% confidence interval) measured in cell supernatant of BECs from healthy donors ($n = 6$) treated as infected control or with a 1-ng/mL dose of exogenous IFN- α 2, IFN- β , IFN- λ 1, or IFN- λ 2 before and during low-dose (5×10^5 PFU/mL) infection of HRV-1A. Samples treated with IFN- λ 1 or IFN- λ 2 revealed significant elevation in RANTES protein secretion compared with infected control. * $P < .05$.

and source of the airway epithelial cells (bronchial explants vs brushings). However, we performed a separate experiment and found greater enhancement of IP-10 and RANTES at 72 hours after HRV infection in IFN-treated samples, suggesting that duration of HRV infection does not explain the difference among studies (data not shown). Of note, similar to our findings for IFN- β , Korpi-Steiner and colleagues³¹ previously demonstrated that HRV-induced IP-10 production was upregulated when BECs and monocytes were cocultured and that these effects could be reduced by blocking type 1 IFN receptors, suggesting that IP-10 release is regulated by type 1 IFN signaling.

Strengths of our study include the use of primary BECs, as opposed to cell lines, and the multiple doses of HRV and interferons used for direct comparison of the antiviral and inflammatory effects. In this study we used doses of 0.1, 1.0, and 10 ng/mL of IFN, whereas clinical studies typically administer higher doses intranasally.^{32,33} Therapeutic targeting of the lower airway, rather than intranasal delivery, could be important for limiting adverse effects and controlling asthma exacerbations. In addition, we tested effects of 4 different interferons within the same study. The sample size of these experiments was modest, but the findings were quite reproducible among cell samples from different donors. On the basis of these findings in BECs from healthy donors, further study of the antiviral and inflammatory effects of interferons in cells from asthmatic patients, with a focus on IFN- λ 1 in particular, is warranted.

In conclusion, we have found that exogenous type 1 and 3 interferons significantly inhibit HRV replication in primary BECs in a dose-dependent manner. IFN- λ 1 is of particular interest based on similar efficacy, with the potential advantages of a more targeted response and fewer adverse effects.²⁵ Further studies are warranted to explore the physiologic significance of the enhanced HRV-induced inflammatory responses seen with exogenous IFN- λ 1 in our study. Taken together, exogenous administration of IFNs could represent a viable approach for the prevention of asthma exacerbations.

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