

Interaction between allergy and innate immunity: model for eosinophil regulation of epithelial cell interferon expression

Sameer K. Mathur, MD, PhD^{*,†}; Paul S. Fichtinger, BS^{*}; John T. Kelly, MD^{*}; Wai-Ming Lee, PhD[‡]; James E. Gern, MD[‡]; and Nizar N. Jarjour, MD^{*}

^{*} Division of Allergy, Pulmonary and Critical Care, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

[†] William S. Middleton Veterans Memorial Hospital, Madison, Wisconsin

[‡] Division of Allergy, Immunology, and Rheumatology, Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

ARTICLE INFO

Article history:

Received for publication December 7, 2012.

Received in revised form May 14, 2013.

Accepted for publication May 16, 2013.

ABSTRACT

Background: Eosinophils in asthmatic airways are associated with risk of exacerbations. The most common cause of asthma exacerbations is viral respiratory infections, particularly human rhinovirus (HRV).

Objective: To determine the mechanism by which eosinophils may influence virus-induced responses.

Methods: We used an in vitro coculture model of primary human eosinophils and the BEAS-2B epithelial cell line either stimulated with HRV1A infection or polyinosinic–polycytidylic acid (poly[I:C]). The messenger RNA (mRNA) expression of interferon (IFN) β 1 and IFN- λ 1 was assessed by quantitative reverse-transcriptase polymerase chain reaction and the protein level of IFN- λ 1 by enzyme-linked immunosorbent assay.

Results: Both poly(I:C) and HRV1A infection induced BEAS-2B expression of IFN- β 1 and IFN- λ 1 mRNA. Coculture of eosinophils resulted in suppression of poly(I:C)-stimulated IFN- β 1 and IFN- λ 1 mRNA expression (2.5-fold and 3.6-fold less, respectively). Separation of cells did not block eosinophil regulatory activity. Coculture of eosinophils with HRV1A-infected BEAS-2B cells also suppressed IFN- β 1 and IFN- λ 1 mRNA (5.7-fold and 5.0-fold less, respectively) and reduced IFN- λ 1 protein secretion (1.6-fold decrease). This corresponded to a 34% increase in the quantity of HRV1A virus RNA on coculture with eosinophils. Recombinant transforming growth factor β suppressed IFN- λ 1 from HRV1A-infected BEAS-2B cells. Coculture of eosinophils and BEAS-2B cells induced transforming growth factor β secretion, which may mediate suppression of HRV-induced interferon expression.

Conclusion: Eosinophils suppressed HRV-induced expression of interferons from epithelial cells, resulting in increased quantity of HRV. This represents one mechanism for interaction between allergic inflammation and innate immunity.

© 2013 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

There are numerous studies suggesting that eosinophils are associated with asthma exacerbations.¹ In a study that compared asthma therapeutic adjustments based on the status of airway eosinophilia vs guideline-based management, reduced rates of asthma exacerbations were observed in the patients whose therapy was directed at the reduction of airway eosinophilia.¹ In several clinical trials using anti–interleukin (IL) 5 therapy for the treatment of severe eosinophil-prominent asthma, the reduction of eosinophil levels in the airway and blood was associated with reduced rates of asthma exacerbations.^{2–5} In experimentally induced

human rhinovirus (HRV) infection, baseline sputum eosinophilia was associated with increased severity of respiratory illness.⁶ These findings suggest that eosinophils directly contribute to the development and severity of exacerbations of asthma.

Viral infections, specifically HRV, are the most common cause of asthma exacerbations in both children and adults.^{7,8} A recent study demonstrated that allergic sensitization is a risk factor for HRV-induced wheezing in children, which has been linked to the development of asthma.⁹ Therefore, understanding the mechanism of association between allergic inflammation and innate immunity in the setting of HRV infection may identify targets for the prevention of asthma exacerbations. Type 1 and 3 interferons play a key role in the innate immune response to viral infection. Defects in the expression of these cytokines have been hypothesized to contribute to increased susceptibility of asthma patients to viral infections.^{10,11}

It is now recognized that eosinophils are not simply effector cells that release toxic granule proteins but also regulatory cells capable of influencing and often enhancing local inflammation.¹² In

Reprints: Sameer K. Mathur, MD, PhD, Division of Allergy, Pulmonary and Critical Care, Department of Medicine, University of Wisconsin School of Medicine and Public Health, William S. Middleton Veterans Memorial Hospital, K4/952 CSC, 600 Highland Ave, Madison, WI 53792; E-mail: sm4@medicine.wisc.edu.

Disclosures: Authors have nothing to disclose.

Funding Sources: This research was supported by National Institutes of Health grants HL088594, HL69116, and UL1RR025011.

considering the close clinical relationship between eosinophilia and virus-induced exacerbations, we hypothesized that eosinophils inhibit antiviral responses of airway cells. To test this in an in vitro model, we examined the effects of human eosinophils on interferon expression by BEAS-2B epithelial cells in response to the Toll-like receptor 3 ligand polyinosinic–polycytidylic acid poly(I:C) and in response to HRV infection.

Methods

Cell Purification and Culture

Eosinophils were isolated from peripheral blood obtained from individuals who provided written consent to participate in a University of Wisconsin Institutional Review Board–approved protocol. Individuals with severe or uncontrolled allergic disease or those using systemic steroids were excluded. The peripheral blood was subjected to density centrifugation and immunomagnetic bead–negative selection as previously described, which includes exposure to newborn calf serum.¹³ Eosinophils were more than 98% pure; contaminating cells were typically neutrophils and/or lymphocytes. The BEAS-2B immortalized cell line was cultured in serum-free bronchial epithelial cell growth medium (Lonza, Basel, Switzerland) at 37°C with 5% carbon dioxide and humidified air. The bronchial epithelial cell growth medium was prepared according to manufacturer instructions, which includes hydrocortisone at final concentration of 0.5 µg/mL.

For coculture experiments, BEAS-2B cells were plated and grown on 6-well tissue culture plates for 48 hours to approximately 50% to 70% confluence. The medium was then replaced, and both 1×10^6 /mL of eosinophils and 25 µg/mL poly(I:C) were added. To separate eosinophils from BEAS-2B cells, Transwell inserts (Corning, Lowell, Massachusetts) were used. For the HRV experiments, HRV1A or HRV16 stocks were prepared by purification of virus grown in HeLa cell suspension cultures with sucrose gradients as previously described.¹⁴ The HRV1A or HRV16 was added at a multiplicity of infection (MOI) of 5 or 20 plaque-forming units (PFU) per cell. For eosinophil cultures with HRV1A alone, 1×10^6 PFU of HRV1A was incubated with 5×10^5 eosinophils with or without 0.1mM *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) (induces degranulation) at room temperature for 3 hours. For the recombinant transforming growth factor (TGF) β1 experiments, 0.1 ng/mL of recombinant TGF-β1 (R&D Systems, Minneapolis, Minnesota) was added. The cultures were incubated for 24 hours at 37°C for poly(I:C) experiments or 34°C for virus experiments. Supernatants were collected and centrifuged to remove debris. BEAS-2B cell lysate was collected for

Table 1

Eosinophil donor characteristics

Characteristic	No. (%) of donors ^a
Total No. of donors	27
Age, mean (range), y	32 (18–49)
Sex	
Male	13 (48)
Female	14 (52)
Allergy diagnoses	
Allergic rhinitis	27 (100)
Asthma	17 (63)
Atopic dermatitis	0
Allergy medications ^b	
Antihistamine	17 (77)
Nasal corticosteroid	2 (9)
Leukotriene modifier	1 (4)
Inhaled corticosteroid	1 (4)
Peripheral eosinophil count, mean (range), cells/µL	203 (53–546)

^aData are presented as number (percentage) of donors unless otherwise indicated.

^bMedication data not available for 5 donors.

RNA purification after rinsing plates with phosphate-buffered saline to remove eosinophils, with greater than approximately 95% removed.

RNA Measurements

Total RNA was isolated according to manufacturer's instructions using an RNeasy mini kit (Qiagen, Valencia, California), including DNase I digestion to remove contaminating DNA. Complementary DNA (cDNA) was synthesized from 0.75 or 1.5 µg of purified RNA according to the manufacturer's instructions using Superscript III (Invitrogen, Carlsbad, California).

Taqman primer and probe sets for target genes (IFN-α1, IFN-β1, and IFN-λ1) and housekeeping/reference genes (β-actin and cyclophilin A) were obtained from a commercial source (Applied Biosystems, Foster City, California). Samples were run in duplicate with each reaction containing Universal Master Mix (Applied Biosystems), the primer and probe set, and cDNA for a total reaction volume of 25 µL. Fluorescence was measured for each cycle using the ABI PRISM 7500 Sequence Detection System or the Step One Plus Real-Time PCR System (both by Applied Biosystems).

Virus Measurements

HRV1A was incubated with eosinophils in RPMI for 3 hours at room temperature. Samples were microfuged and supernatants collected for measurement of infectivity and viral RNA levels. Viral infectivity was measured by a plaque-forming assay as previously

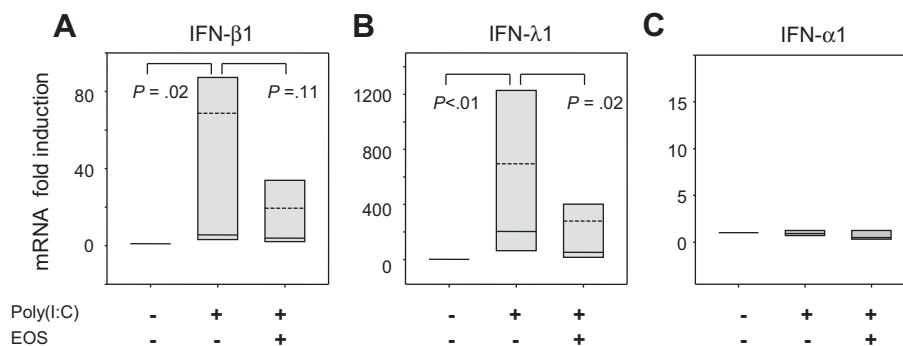


Figure 1. Effect of eosinophil coculture on interferon (IFN) and cytokine expression of polyinosinic–polycytidylic acid (poly(I:C))–stimulated BEAS-2B cells. BEAS-2B cells were cultured for 24 hours with or without poly(I:C), 25 µg/mL, and eosinophils (EOS) as indicated. RNA was extracted from the BEAS-2B cells attached to the culture plate. Quantitative polymerase chain reaction results are presented as fold inductions of unstimulated BEAS-2B cells for the following genes: IFN-β1 (n = 7) (A), IFN-λ1 (n = 8) (B), and IFN-α1 (n = 8) (C). Fold-inductions of messenger RNA (mRNA) are shown with box plots with the median (solid line) and mean (dashed line) values. Error bars represent the 5th to 95th percentiles. P values for statistical comparisons are shown.

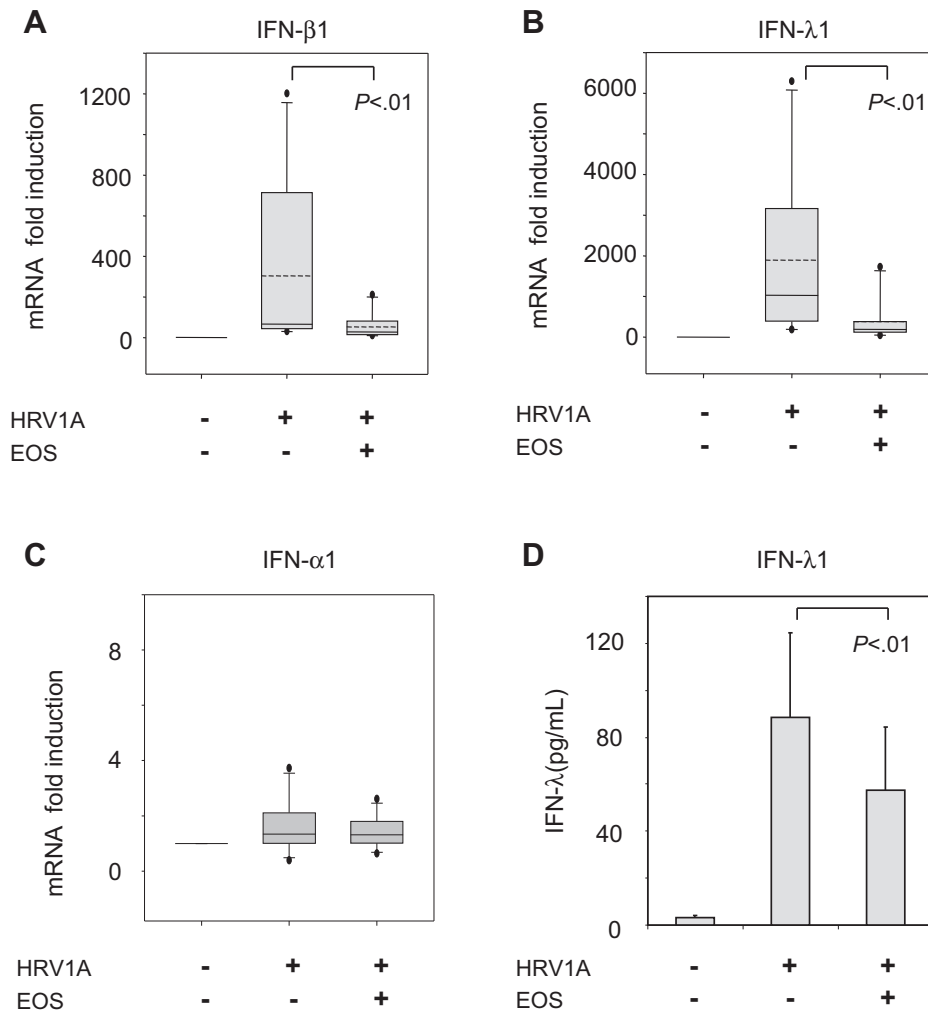


Figure 2. Effect of eosinophil coculture on interferon (IFN) expression of human rhinovirus 1A (HRV1A)-infected BEAS-2B cells. BEAS-2B cells were infected with HRV1A at 20 plaque-forming units (PFU) per cell for 24 hours with or without eosinophils (EOS) as indicated. RNA was extracted from the BEAS-2B cells attached to the culture plate. Quantitative polymerase chain reaction results are presented for IFN- β 1 (A), IFN- λ 1 (B), and IFN- α 1 (C) as fold inductions of unstimulated BEAS-2B cells ($n = 11$). Fold-inductions of messenger RNA (mRNA) are shown with box plots with the median (solid line) and mean (dashed line) values. Error bars represent the 5th to 95th percentiles. Percentage expression is shown as the means with error bars representing the SEM. D, BEAS-2B cells were infected with HRV1A at 20 PFU/cell for 24 hours with or without EOS as indicated. Supernatants were collected and enzyme-linked immunosorbent assay results for IFN- λ protein are shown as the means, with error bars representing the SEM. P values for statistical comparisons are shown.

described.¹⁵ Briefly, HeLa cell monolayers in 60-mm plates were infected with 10-fold dilutions of culture supernatants at room temperature for 1 hour, agar and nutrient overlays were added, and then the samples incubated at 35°C for 3 days for plaque development. Cells were fixed and stained to quantify plaques. Viral RNA levels were measured with quantitative polymerase chain reaction with a pan-HRV primer set using an ABI Prism 7500 (Applied Biosystems) as previously described.^{16,17} The HRV1A virus quantification was based on a standard curve of cDNA corresponding to known amount of HRV1A virions.

Protein Measurements

IFN- λ 1 (IL-29) and TGF- β 1 protein in culture supernatants was measured according to the manufacturer's instructions using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems). Notably, activation of latent TGF- β 1 in supernatants was performed using 1N hydrochloride followed by neutralization with 1.2 N sodium hydroxide and 0.5M HEPES before assay performance. Samples were run in duplicate, and the sensitivity ranged from 1.7 to 15.4 pg/mL.

Statistical Analysis

Statistical analyses were performed using SigmaPlot software (Systat Software, Chicago, Illinois). RNA data were analyzed after log transformation. Comparisons were made using a paired t test or Wilcoxon sign ranked test for parametric and nonparametric data, respectively. Statistical significance was defined as $P < .05$.

Results

Eosinophil Donor Characteristics

As indicated in Table 1, the eosinophils used for all the experiments were purified from 27 donors 18 to 49 years old (mean age, 32 year; 14 female). All had allergic rhinitis, 17 had a diagnosis of asthma, and none had atopic dermatitis. The donors generally had mild disease, mostly managed with antihistamines. Only one donor was using an inhaled steroid controller medication. The mean eosinophil count was 203 cells/ μ L. Of note, different subsets of these donors were used for each experiment, and demographics of the respective subsets were similar.

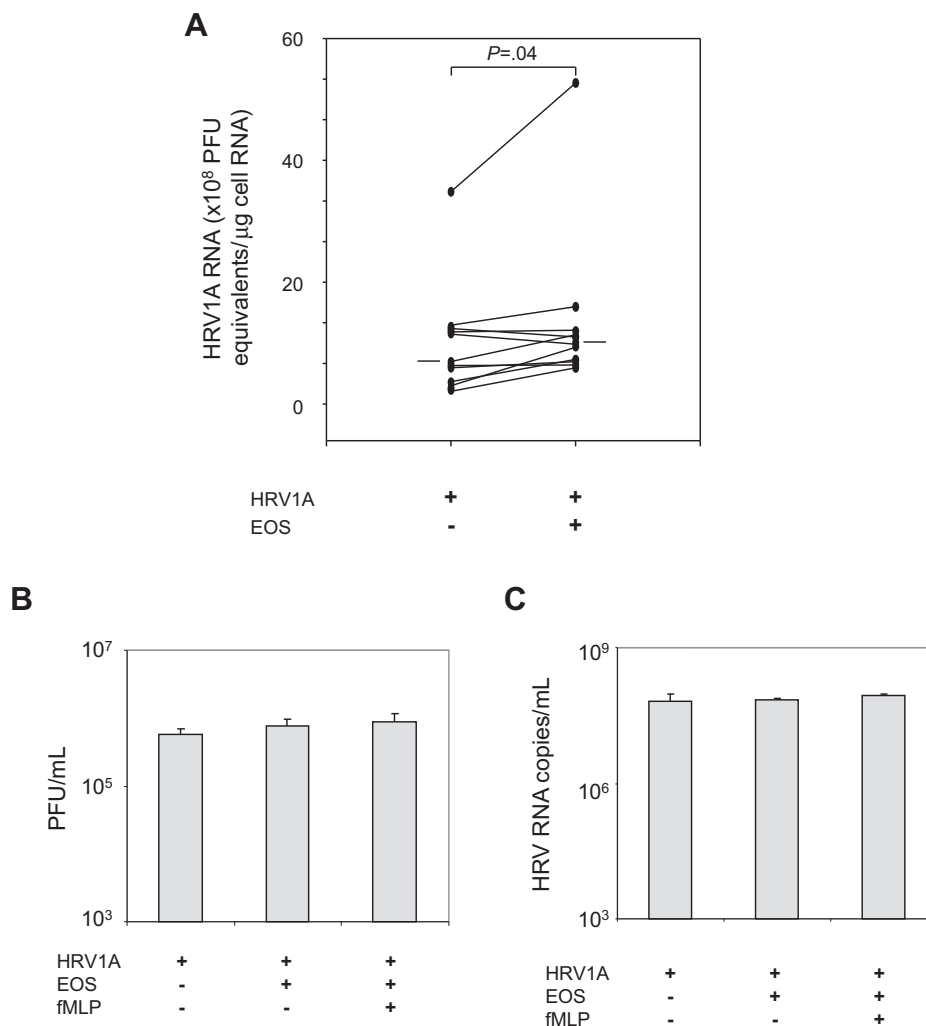


Figure 3. Effect of eosinophil (EOS) coculture on human rhinovirus 1A (HRV1A). A, BEAS-2B cells were infected with HRV1A at 20 plaque-forming units (PFU) per cell for 24 hours with or without EOS as indicated. RNA was extracted from the BEAS-2B cells and reverse-transcriptase polymerase chain reaction performed for viral RNA levels with a standard curve to determine HRV1A RNA levels (PFU equivalents) per microgram of cellular RNA. Median HRV1A RNA levels are shown as horizontal bars. P value for statistical comparison is shown. Supernatants were collected for analyses of PFU (B) and copies of HRV RNA (C) from cultures of HRV1A (multiplicity of infection 1), EOS (1×10^6 cells/mL), and *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) (0.1 mM) incubated for 3 hours.

Effect of Eosinophils on Poly(I:C) Stimulation of BEAS-2B Cells

BEAS-2B cells were stimulated with poly(I:C), and mRNA expression was induced for IFN- β 1 (mean [SD], 69-fold [44-fold]; $P = .02$) and IFN- λ 1 (mean [SD], 690-fold [370-fold]; $P < .01$) (Fig 1A and 1B, respectively). However, IFN- α 1 expression was essentially unchanged (1.5-fold) with poly(I:C) stimulation (Fig 1C). Coculture with eosinophils, in the absence of Transwells with potential for cell-cell contact, suppressed poly(I:C)-stimulated mRNA expression of IFN- λ 1 (2.5-fold decrease, $P = .02$), and a similar trend was observed for IFN- β 1 (3.6-fold decrease, $P = .11$). Coculture of eosinophils did not affect the poly(I:C)-induced mRNA expression of IFN- α 1.

To determine whether the eosinophil-mediated suppression of poly(I:C)-stimulated gene expression from the BEAS-2B cells was due to soluble mediators or dependent on cell contact, the cocultures were performed in the presence of a semipermeable membrane (Transwell) to physically separate the cell populations. Despite the lack of cell-to-cell contact, eosinophil-mediated suppression of IFN- β 1 and IFN- λ 1 was evident (eFig 1). This finding suggests that eosinophils suppress BEAS-2B interferon expression via a soluble mediator.

Effect of Eosinophils on HRV Infection of BEAS-2B Cells

Next, we tested for effects of eosinophils on HRV-induced interferon expression using a minor receptor (low-density lipoprotein receptor) group (HRV1A) and a major receptor (intercellular adhesion molecule 1) group (HRV16) virus. There was greater induction of IFN- β 1 (338-fold vs 247-fold) and IFN- λ 1 (2,010-fold vs 890-fold) with the use of HRV1A compared with HRV16 (eFig 2). Furthermore, the eosinophil-mediated suppression of their expression was observed with 2 different concentrations of virus (MOIs = 5 and 20 PFU/cell); however, it was more pronounced at the higher concentration. Therefore, we chose to perform additional experiments with HRV1A at an MOI of 20 PFU/cell, which is consistent with our previous studies.^{18,19}

Similarly, as shown in Figure 2, A–C, HRV1A infection of BEAS-2B cells also induced both IFN- β 1 (mean [SD], 304-fold [133-fold]) and IFN- λ 1 (mean [SD], 1,890-fold [630-fold]). Coculture with eosinophils suppressed HRV1A-induced IFN- β 1 mRNA (mean [SD], 53-fold [20-fold]; $P < .01$) and IFN- λ 1 mRNA (mean [SD], 380-fold [160-fold]; $P < .01$) compared with responses of epithelial cell monocultures. IFN- α 1 was also examined; however, its expression was not greatly induced (3-fold), and there was no significant effect

on coculture with eosinophils. To determine whether the changes in gene expression corresponded to changes in protein production, IFN- λ 1 and IFN- β secretion was examined. Infection of BEAS-2B with HRV1A induced IFN- λ 1 protein secretion (mean [SD], 89 [36] pg/mL) that was suppressed by eosinophil coculture (1.6-fold decrease, $P < .01$, Fig 2D). IFN- β protein levels were below the limits of detection by ELISA.

Because one role of interferons is to block virus replication, we examined whether the coculture of eosinophils and corresponding suppression of IFN- β 1 and IFN- λ 1 affected HRV1A virus levels in infected BEAS-2B cells. There was a significant 34% increase in the quantity of HRV1A virus RNA in infected BEAS-2B cells on coculture with eosinophils ($P = .04$, Fig 3A).

Effect of Eosinophils on HRV Infectivity

To determine whether eosinophils have anti-HRV activity that could diminish interferon responses, we cultured HRV1A with resting eosinophils or eosinophils that were treated with fMLP to induce degranulation. The virus suspension was then retested for HRV1A infectivity (Fig 3B) and HRV1A RNA levels (Fig 3C). Eosinophils had no significant effects on HRV infectivity. Furthermore, we confirmed that eosinophil-derived neurotoxin (EDN) was released and there was increased RNase activity in the culture supernatants with eosinophils (eFig 3).

Role of TGF- β 1 in Eosinophil-Mediated Suppression of Interferon Expression

To determine whether TGF- β could suppress IFN- β 1 and IFN- λ 1 mRNA expression in our system, recombinant TGF- β 1 was added to BEAS-2B cells infected with HRV1A for 24 hours (Fig 4A). TGF- β 1 had no effect on IFN- β 1 mRNA but significantly suppressed IFN- λ 1 mRNA (65% decrease).

We next tested whether eosinophils induced BEAS-2B cells to produce TGF- β and whether this induction was enhanced with HRV infection. As shown in Figure 4B, BEAS-2B cells secreted similar amounts of TGF- β with or without HRV infection (mean [SD], 67 [8] pg/mL vs 60 [9] pg/mL). Coculture with eosinophils increased TGF- β 1 secretion either with HRV1A infection (mean [SD], 60 [9] pg/mL to 110 [17] pg/mL; $P < .01$) or without HRV1A infection (mean [SD], 67 [8] pg/mL to 113 [18] pg/mL; $P = .01$).

Discussion

Our study examined the effects of eosinophils on the expression of interferons from BEAS-2B epithelial cells. We demonstrated that eosinophils can suppress poly(I:C) and HRV-stimulated expression of IFN- β 1 and IFN- λ 1 from BEAS-2B cells, without requirement for cell-cell contact. Consequently, eosinophil coculture increased HRV replication in BEAS-2B cells. The diminished expression of IFN- β 1 and IFN- λ 1 is therefore not attributable to eosinophils directly inhibiting HRV infectivity. Rather, our studies suggest that eosinophil secretion of TGF- β 1, and perhaps other eosinophil-derived mediators, can suppress virus-induced interferon responses in epithelial cells.

It has been proposed that reduced interferon responses may contribute to virus-induced exacerbations of asthma²⁰ and that asthma may be associated with a deficiency in type 1 and type 3 interferons.^{10,11} Other studies, however, have not found asthma-related differences in interferons.¹⁸ TGF- β can inhibit the expression of IFN- α and IFN- β from HRV-infected fibroblasts.²¹ A recent study also demonstrated TGF- β inhibition of IFN- β and IFN- λ .²² Our data suggest that TGF- β 1 production by airway eosinophils in asthma could provide another mechanism for in vivo deficiency in type 1 and 3 interferons. Reduced interferon responses could in turn lead to increased viral replication, more extensive lower airway virus infection and inflammation, and increased likelihood

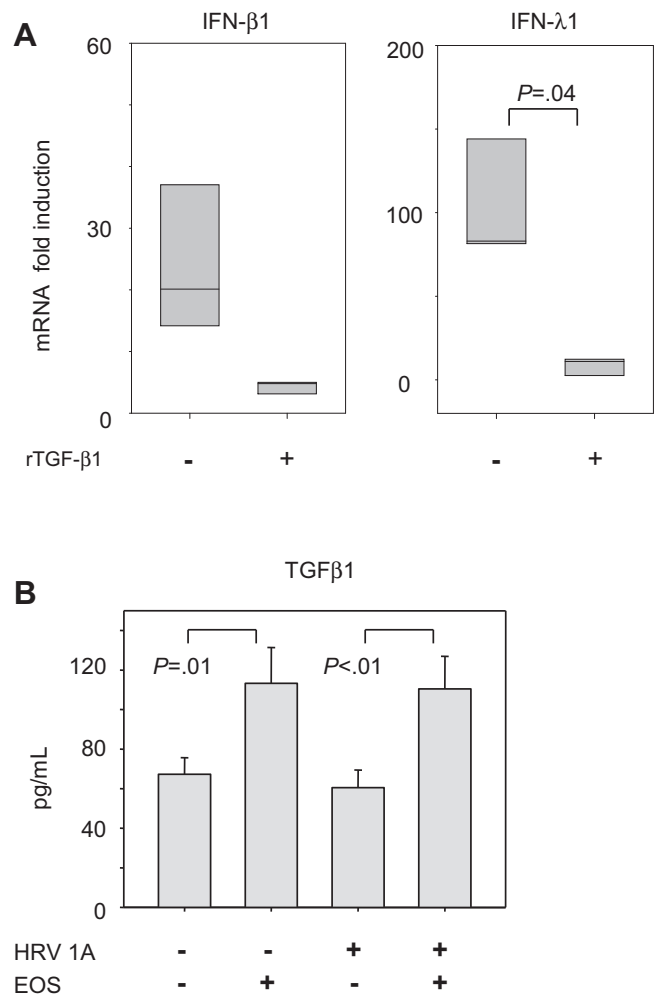


Figure 4. Effect of recombinant transforming growth factor β 1 (rTGF- β 1) and levels of TGF- β 1 on coculture with eosinophils (EOS). A, BEAS-2B cells were infected with human rhinovirus 1A (HRV1A) at 20 plaque-forming units (PFU) per cell for 24 hours with or without 0.1 ng/mL of rTGF- β 1. Quantitative polymerase chain reaction results for IFN- β 1 and IFN- λ 1 ($n = 3$) are shown as fold inductions of unstimulated BEAS-2B cells presented as box plots, with the solid line as the median and the dashed line as the mean. P values for statistical comparisons are shown. B, BEAS-2B cells were untreated or infected with HRV1A at a multiplicity of infection of 20 PFU/cell for 24 hours with or without EOS as indicated. Enzyme-linked immunosorbent assay results for the culture supernatants are shown as the mean of TGF- β 1 ($n = 9$), with error bars representing the SEM. P values for statistical comparisons are shown.

of airway obstruction and an exacerbation of symptoms in patients with asthma. Although we were only able to detect the decrease in IFN- λ 1 protein, it is possible a similar reduction is occurring with IFN- β , which is typically expressed at lower levels than IFN- λ .²²

Eosinophils have antiviral effects under some circumstances.^{23–26} For example, the eosinophil granule proteins EDN and eosinophil cationic protein (ECP) exhibit ribonuclease activity, which can diminish the infectivity of respiratory syncytial virus.²⁶ It has been reported that activated eosinophils express intercellular adhesion molecule 1, which can bind HRV16 and subsequently present HRV antigens to T cells.²⁷ Thus, eosinophils could simply sequester HRV and prevent their interaction with the cocultured BEAS-2B cells. In addition, eosinophil granule protein ribonuclease activity caused decreased infectivity of respiratory syncytial virus.²⁶ However, whether EDN and ECP ribonuclease activities can diminish infectivity of HRV had not previously been examined. We were not able to detect any antiviral effects in vitro in either activated or resting eosinophils.

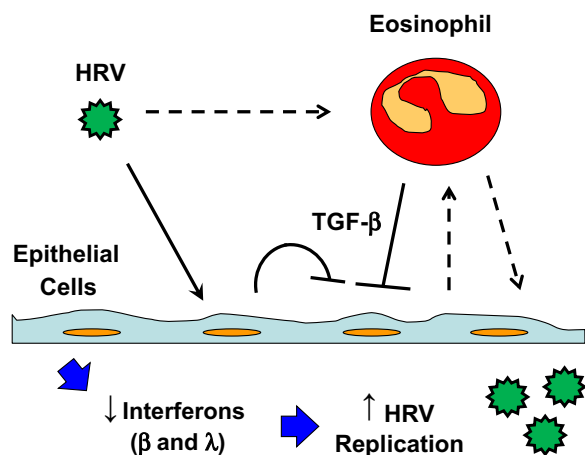


Figure 5. Schematic of proposed eosinophil regulatory activity. Epithelial cells are shown being infected by human rhinovirus (HRV) in the presence of eosinophils. The eosinophil release of transforming growth factor (TGF) β along with epithelial cell endogenous TGF- β is shown to suppress the epithelial expression of IFN- β and IFN- λ , which enables increased virus replication that could lead to more severe inflammation and asthma symptoms. The dashed line between HRV and eosinophils represents a possible activation of eosinophils directly by HRV. The dashed lines between the eosinophil and epithelial cells represent possible release of unidentified mediators from epithelial cells to regulate eosinophil function and release of unidentified mediators from eosinophils to further regulate epithelial cell activity.

Eosinophils secreted TGF- β 1 and/or stimulated epithelial secretion of additional TGF- β 1 at about the same level regardless of the presence of HRV. In our experiments, we did not prime or preactivate the eosinophils. In an allergic airway, the airway eosinophils are likely primed or activated, resulting in an increased capacity to release TGF- β . Because the stimulation of eosinophils by cytokines, including IL-3, IL-4, and IL-5, can increase TGF- β release,²⁸ we speculate greater suppression of IFN- λ 1 may occur in vivo.

Most studies of virus-induced inflammatory responses use monocultures of epithelial cells, mononuclear cells, or granulocytes, which do not reflect cell-cell interactions that occur in vivo. We describe an in vitro coculture system that can be used as a model to examine cell-cell interactions that occur in patients with allergic asthma during HRV infections. A limitation of this study was the use of the BEAS-2B cell line instead of primary epithelial cells. However, BEAS-2B cells have been useful as a model system, particularly in the study of HRV-induced signaling.^{29–31} In fact, our findings are in general agreement with a recent study reporting that TGF- β -mediated suppression of both IFN- λ and IFN- β in primary bronchial epithelial cells.²² Another limitation of in vitro coculture studies is reliance on reagents that may be important for one cell but have consequences for the other cell type. For example, serum-free media was used for cocultures, which is important to maintain the epithelial phenotype and prevent transition to fibroblasts; however, serum is also typically important for long-term survival and function of eosinophils. Similarly, hydrocortisone was included in the coculture media for the epithelial cells but can also affect eosinophil survival and function. We confirmed that the lack of serum and inclusion of hydrocortisone did not affect eosinophil survival; however, it remains possible that there are effects on eosinophil function.

The eosinophil has been postulated to be a regulator of local immunity and/or remodeling or repair, also referred to as the LIAR hypothesis.¹² With respect to this hypothesis, we and others have previously found that human eosinophils are capable of enhancing cytokine secretion from activated T cells.^{27,32–34} Our results suggest that eosinophil secretion of cytokines, such as TGF- β , and other eosinophil-dependent mechanisms regulate virus-induced

interferon responses and thereby affect virus replication and clearance of virus. As shown in Figure 5, eosinophils may release TGF- β due to HRV stimulation or signaling from epithelial cells. TGF- β is also released from epithelial cells, and this may be further increased by signaling from eosinophils. The net increase in TGF- β and potentially other mediators from eosinophils suppress the expression of IFN- β and IFN- λ . The decreased levels of interferons enable greater virus replication, which in turn can stimulate increased inflammatory responses, leading to more severe HRV illnesses in patients with airway eosinophilia. This may, in part, explain the clinical observations of lower asthma exacerbation risk on reduction of airway eosinophils by inhaled corticosteroids or mepolizumab.

Acknowledgments

We gratefully acknowledge Beth Schwantes, Michelle Wolff, and Evelyn Falibene for their support in the purification of eosinophils.

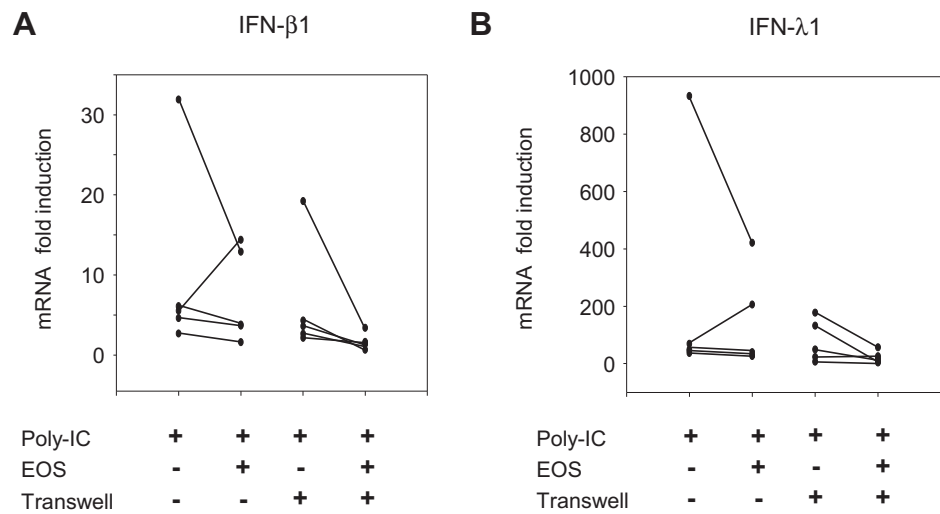
Supplementary Data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.anai.2013.05.010>.

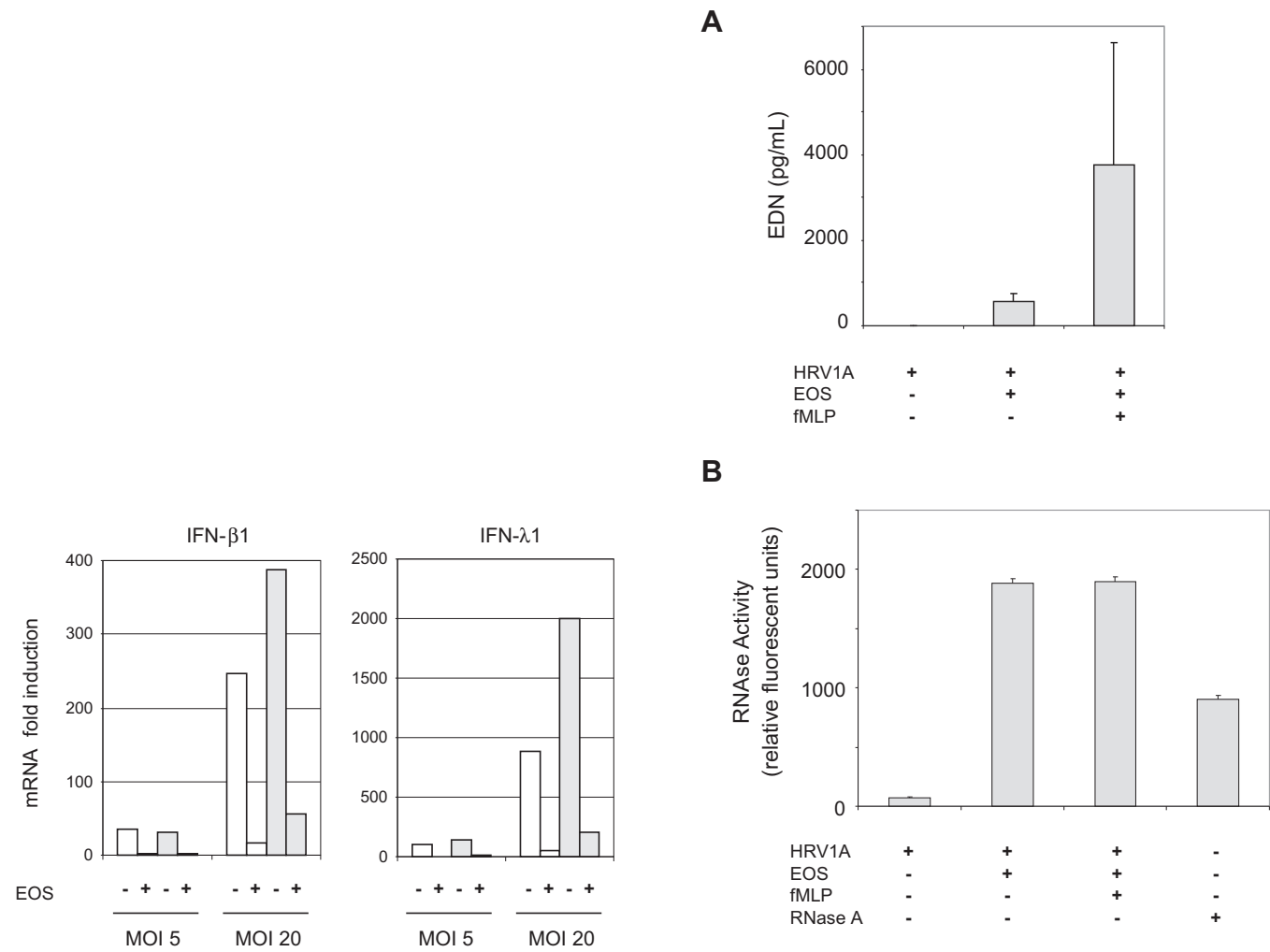
References

- [1] Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet*. 2002;360:1715–1721.
- [2] Flood-Page P, Swenson C, Faierman I, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med*. 2007;176:1062–1071.
- [3] Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med*. 2009;360:973–984.
- [4] Nair P, Pizzichini MMM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med*. 2009;360:985–993.
- [5] Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet*. 2012;380:651–659.
- [6] DeMore JP, Weisshaar EH, Vrtis RF, et al. Similar colds in subjects with allergic asthma and nonatopic subjects after inoculation with rhinovirus-16. *J Allergy Clin Immunol*. 2009;124:245–252.
- [7] Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral-infections in exacerbations of asthma in 9–11 year-old children. *BMJ*. 1995;310:1225–1229.
- [8] Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ*. 1993;307:982–986.
- [9] Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med*. 2008;178:667–672.
- [10] Wark PAB, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med*. 2005;201:937–947.
- [11] Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med*. 2006;12:1023–1026.
- [12] Lee JJ, Jacobsen EA, McGarry MP, Schleimer RP, Lee NA. Eosinophils in health and disease: the LIAR hypothesis. *Clin Exp Allergy*. 2010;40:563–575.
- [13] Yamamoto H, Sedgwick JB, Busse WW. Differential regulation of eosinophil adhesion and transmigration by pulmonary microvascular endothelial cells. *J Immunol*. 1998;161:971–977.
- [14] Lee WM, Wang W. Human rhinovirus type 16: mutant V1210A requires capsid-binding drug for assembly of pentamers to form virions during morphogenesis. *J Virol*. 2003;77:6235–6244.
- [15] Lee WM, Monroe SS, Rueckert RR. Role of maturation cleavage in infectivity of picornaviruses: activation of an infectiousome. *J Virol*. 1993;67:2110–2122.
- [16] Mosser AG, Vrtis R, Burchell L, et al. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *Am J Respir Crit Care Med*. 2005;171:645–651.
- [17] Denlinger LC, Sorkness RL, Lee WM, et al. Lower airway rhinovirus burden and the seasonal risk of asthma exacerbation. *Am J Respir Crit Care Med*. 2011;184:1007–1014.
- [18] Bochkov YA, Hanson KM, Keles S, Brockman-Schneider RA, Jarjour NN, Gern JE. Rhinovirus-induced modulation of gene expression in bronchial epithelial cells from subjects with asthma. *Mucosal Immunol*. 2010;3:69–80.
- [19] Shi L, Manthei DM, Guadarrama AG, Lenertz LY, Denlinger LC. Rhinovirus-induced IL-1 β release from bronchial epithelial cells is independent of functional P2X7. *Am J Respir Cell Mol Biol*. 2012;47:363–371.
- [20] Johnston SL. Innate immunity in the pathogenesis of virus-induced asthma exacerbations. *Proc Am Thorac Soc*. 2007;4:267–270.

- [21] Thomas BJ, Lindsay M, Dagher H, et al. Transforming growth factor-beta enhances rhinovirus infection by diminishing early innate responses. *Am J Respir Cell Mol Biol*. 2009;41:339–347.
- [22] Bedke N, Sammut D, Green B, et al. Transforming growth factor-Beta promotes rhinovirus replication in bronchial epithelial cells by suppressing the innate immune response. *PLoS One*. 2012;7:e44580.
- [23] Adamko DJ, Yost BL, Gleich GJ, Fryer AD, Jacoby DB. Ovalbumin sensitization changes the inflammatory response to subsequent parainfluenza infection: eosinophils mediate airway hyperresponsiveness, M-2 muscarinic receptor dysfunction, and antiviral effects. *J Exp Med*. 1999;190:1465–1477.
- [24] Rosenberg HF, Domachowske JB. Eosinophils, eosinophil ribonucleases, and their role in host defense against respiratory virus pathogens. *J Leuk Biol*. 2001;70:691–698.
- [25] Phipps S, Lam CE, Mahalingam S, et al. Eosinophils contribute to innate antiviral immunity and promote clearance of respiratory syncytial virus. *Blood*. 2007;110:1578–1586.
- [26] Domachowske JB, Dyer KD, Bonville CA, Rosenberg HF. Recombinant human eosinophil-derived neurotoxin/RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. *J Infect Dis*. 1998;177:1458–1464.
- [27] Handzel ZT, Busse WW, Sedgwick JB, et al. Eosinophils bind rhinovirus and activate virus-specific T cells. *J Immunol*. 1998;160:1279–1284.
- [28] Elovic AE, Ohyama H, Sauty A, et al. IL-4-dependent regulation of TGF- α and TGF- β 1 expression in human eosinophils. *J Immunol*. 1998;160:6121–6127.
- [29] Zaheer RS, Proud D. Human rhinovirus-induced epithelial production of CXCL10 is dependent upon IFN regulatory factor-1. *Am J Respir Cell Mol Biol*. 2010;43:413–421.
- [30] Skevaki CL, Christodoulou I, Spyridaki IS, et al. Budesonide and formoterol inhibit inflammatory mediator production by bronchial epithelial cells infected with rhinovirus. *Clin Exp Allergy*. 2009;39:1700–1710.
- [31] Lau C, Castellanos P, Ranev D, Wang X, Chow CW. HRV signaling in airway epithelial cells is regulated by ITAM-mediated recruitment and activation of Syk. *Protein Pept Lett*. 2011;18:518–529.
- [32] Liu LY, Mathur SK, Sedgwick JB, Jarjour NN, Busse WW, Kelly EAB. Human airway and peripheral blood eosinophils enhance Th1 and Th2 cytokine secretion. *Allergy*. 2006;61:589–597.
- [33] Weller PF, Rand TH, Barrett T, Elovic A, Wong DTW, Finberg RW. Accessory cell-function of human eosinophils: HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1- α expression. *J Immunol*. 1993;150:2554–2562.
- [34] Odemuyiwa SO, Ghahary A, Li Y, et al. Cutting edge: human eosinophils regulate T cell subset selection through indoleamine 2,3-dioxygenase. *J Immunol*. 2004;173:5909–5913.



eFigure 1. Gene expression analysis in Transwell separated cultures. BEAS-2B were cultured at the bottom of a well and stimulated with poly-IC 25 μ g/mL. A Transwell insert was placed above the BEAS-2B cells where indicated, with or without eosinophils (EOS). RNA was purified from the BEAS-2B cells attached to the culture plate. Quantitative PCR results were normalized to unstimulated BEAS-2B cells and shown as scatter plots of paired samples connected with a line for the following genes: (A) IFN- β 1 (n = 5) and (B) IFN- λ 1 (n = 5). Statistical analysis of these data normalized to the poly-IC stimulated epithelial cells demonstrated significant decreases in the expression of IFN- β and IFN- λ with eosinophils separated by transwells ($P < .01$ and $P = .04$, respectively).



eFigure 2. Optimizing HRV strain and multiplicity of infection. Quantification of mRNA fold inductions of IFN- β 1 (left panel) and IFN- λ 1 (right panel) following 24 hour infection of BEAS-2B cells with HRV16 (white bars) or HRV1A (gray bars) at the indicated MOI of 5 or 20. Eosinophils (1×10^6 /mL) were co-cultured as indicated (n = 1).

eFigure 3. Evidence of degranulation and RNase activity upon direct culture of eosinophils with HRV1A. The HRV1A (MOI 1), eosinophils (5×10^5 cells), and fMLP (0.1mM) were cultured together as indicated for 3 hours and supernatants collected (n = 3). (A) EDN protein levels. (B) RNase activity, with an RNase A assay control. The upper limit of activity detection was approximately 2000 RFU; therefore, fMLP treatment may have induced greater levels of activity.