Rhinovirus infection results in stronger and more persistent genomic dysregulation: Evidence for altered innate immune response in asthmatics at baseline, early in infection, and during convalescence

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Abstract

Background

Rhinovirus (HRV) is associated with the large majority of virus-induced asthma exacerbations in children and young adults, but the mechanisms remain poorly defined.

Methods

Asthmatics and non-asthmatic controls were inoculated with HRV-A16, and nasal epithelial samples were obtained 7 days before, 36 hours after, and 7 days after viral inoculation. RNA was extracted and subjected to RNA-seq analysis.

Results

At baseline, 57 genes were differentially expressed between asthmatics and controls, and the asthmatics had decreased expression of viral replication inhibitors and increased expression of genes involved in inflammation. At 36 hours (before the emergence of peak symptoms), 1329 genes were significantly altered from baseline in the asthmatics compared to 62 genes in the controls. At this time point, asthmatics lacked an increase in IL-10 signaling.
observed in the controls. At 7 days following HRV inoculation, 222 genes were significantly dysregulated in the asthmatics, whereas only 4 genes were dysregulated among controls. At this time point, the controls but not asthmatics demonstrated upregulation of SPINK5.

**Conclusions**

As judged by the magnitude and persistence of dysregulated genes, asthmatics have a substantially different host response to HRV-A16 infection compared with non-asthmatic controls. Gene expression differences illuminate biologically plausible mechanisms that contribute to a better understanding of the pathogenesis of HRV-induced asthma exacerbations.

**Introduction**

Human rhinovirus (HRV) infection has been associated with the majority of asthma exacerbations in pediatric patients and with frequent loss of symptom control among asthmatic adults [1–3]. HRV is a positive-sense, single-stranded picornavirus that is subcategorized into A, B, and C strains, with HRV-A and HRV-C genotypes implicated in most exacerbations [4]. The mechanism for the propensity of HRV infection to trigger an asthma exacerbation remains ill-defined.

After 3 years of age, most asthma exacerbations caused by HRV occur in those who are atopic. Moreover, the risk for wheezing with HRV is strongly associated with high levels of total and allergen specific IgE and with the presence of Th2 related airway inflammation prior to an infection [5–7]. Some studies suggest that decreased interferon production in response to HRV infection in the setting of Th2 inflammation may contribute to asthma exacerbation [8]. For example, HRV infection of cultured asthmatic bronchial epithelial cells induced less type I interferon production and resistance to early apoptosis compared to control cells, and this was associated with increased viral replication [9]. Further, decreased production of type I and III interferons in bronchoalveolar lavage cells has been associated with more severe exacerbations in adult asthmatics [10]. Yet, a genome-wide expression analysis of HRV-infected primary bronchial epithelial cells did not reveal any significant differences in interferon expression related to asthma [11].

Following viral exposure, we postulate that gene expression at the epithelial cell level is the earliest response to HRV that, in turn, initiates and influences subsequent events that influence the clinical outcome. Indeed, the presence of Th2 associated inflammation (e.g., increased levels of FeNO and eosinophil cationic protein [ECP]) detected in the asthmatic airway has been proposed to contribute to HRV-induced asthma exacerbation during seasons of increased allergen exposure [7]. Epithelial cells of the asthmatic airway also have an increased number of protease-activating receptors (PAR). The activation of such receptors leads to opening of tight junctions, production of cytokines and chemokines, and degranulation of eosinophils and mast cells [12]. Taken together, we hypothesize that the host response to HRV in the asthmatic airway will be different at the time of initial virus exposure and lead to a unique signature of gene expression that will improve our understanding of asthma attacks caused by HRV.

**Experimental procedures**

**Patient characteristics**

The participants included 5 adults with mild asthma (mean age 25 years; range = 20 to 33 years) and 5 non-atopic adults without asthma (mean age 21.4 years; range = 20 to 23 years).
They were screened and characterized with respect to lung function, atopy, and their asthmatic status prior to enrollment (results shown in Table 1). Inclusion and exclusion criteria were similar to our previous experimental challenges with HRV-A16 [7]. In brief, all asthmatic subjects had physician-diagnosed, mild asthma and used only inhaled bronchodilators for symptom control. Those using inhaled steroids, nasal steroids, cromolyn, nedocromil sodium, ipratropium bromide, or leukotriene modifiers within one month prior to enrollment were excluded, because these medications could alter epithelial cell gene expression and clinical outcome. In keeping with the diagnosis of mild asthma, those who had used oral steroids within 6 weeks prior to enrollment or who were hospitalized or needed treatment in the emergency room for asthma within 3 years of enrollment were excluded. Asthma subjects were also excluded if they had received allergen immunotherapy within the last 3 years or if their ACT score, to judge symptom control during the month before virus inoculation, was less than 19. All asthmatic subjects were atopic as judged by positive skin prick tests (i.e., a response 3 mm greater than a saline control) to common aeroallergens using extracts from Greer Pharmaceuticals (Lenoir, NC). The allergens included dust mite (*D. farinae* and *D. pteronyssinus*), cockroach, cat, dog, *Alternaria*, *Aspergillus*, *Penicillium*, 7 grass mix, Bermuda grass, Eastern tree mix, and ragweed. Each asthmatic subject had a positive methacholine challenge at a concentration ≤ 16mg/mL.

Non-asthmatic control subjects were individuals with no history of asthma or allergic disease. They were excluded if they had a positive methacholine challenge test or a positive skin prick test to any of the aeroallergens tested. All subjects (asthmatics and controls) were excluded if 1) they had a positive test for serum neutralizing antibody to HRV-A16, 2) they had chronic heart or lung disease (other than asthma), or 3) other chronic illnesses such as primary or secondary immunodeficiency disorders. They were also excluded if they had a 5 pack/year history of smoking or any smoking within the last 6 months, or symptoms of upper or lower respiratory infection during the 6 weeks prior to virus inoculation. The study was approved by the Institutional Review Board at the University of Virginia, and all participants signed informed consent before enrollment.

**Table 1. Patient characteristics.**

| Group   | Age | Sex | FEV1% Predicted | Total IgE | Skin Tests                                      | ACT Score |
|---------|-----|-----|-----------------|-----------|-------------------------------------------------|-----------|
| Asthmatics | 20  | F   | 82              | 726       | Dust mite, cat, dog, mold, tree, grass          | 24        |
|         | 25  | M   | 75              | 623       | Dust mite, cat, dog, mold, tree, grass          | 21        |
|         | 33  | F   | 97              | 1045      | Dust mite, tree, grass, ragweed                | 21        |
|         | 26  | F   | 104             | 1429      | Dust mite, cat, dog, tree, grass               | 22        |
|         | 21  | M   | 94              | 1989      | Dust mite, cat, tree, grass, ragweed           | 23        |
| Controls | 20  | M   | 72              | 18.3      |                                                  | 25        |
|         | 22  | F   | 104             | 5.2       |                                                  | 25        |
|         | 20  | F   | 96              | 34.9      |                                                  | 25        |
|         | 23  | F   | 101             | 23        |                                                  | 25        |
|         | 22  | M   | 104             | 42.5      |                                                  | 25        |

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**Evaluation at enrollment and virus inoculation**

Questionnaires focused on each participant’s past and present medical history were administered at enrollment along with a physical exam, spirometry, methacholine challenge, and a nasal wash to confirm absence of HRV infection through qPCR and culture. A blood sample was obtained to re-evaluate serum neutralizing antibody to HRV-A16 and a total IgE level.
After a 7-day run-in period to monitor baseline values, each participant was inoculated on arrival at a hotel where subjects were isolated in their own room and monitored daily over a 4-day period after inoculation. The inoculation pool of HRV-A16 was prepared according to GMP specifications and approved by the FDA for experimental challenges (IND # 15162). Each participant was inoculated with a total dose of 300 TCID\textsuperscript{50} mL (tissue culture infectious dose 50/mL) after doing a nasal wash at baseline to test for the presence of pre-existing infection with HRV by culture and qPCR.

**Study design**

The study design and timeline is shown in Fig 1. Nasal epithelial samples were obtained after nasal washes (to remove mucous) by gentle scraping of the inferior turbinates within each nostril visualized with a head lamp and a Bionix\textsuperscript{®}, Toledo, OH. disposable nasal speculum. The scrapings were obtained with an ASI RhinoPro\textsuperscript{®}, Arlington Scientific, Inc., Springville, UT. This procedure was done at enrollment (7 days before inoculation (T0)) and at 36 hours after inoculation (T1) in the hotel. A final scraping was obtained 7 days after virus inoculation (T2) during a clinic visit planned at follow-up. RNA extractions from the epithelial cell scrapings were done within 2 hours using the Qiagen AllPrep DNA/RNA Mini Kit\textsuperscript{®}, Hilden, Germany. The nasal washes done prior to the nasal scrapings were used to evaluate viral load and were performed as previously described [5, 13].

Upper airway symptoms were assessed daily on diary cards using the modified Jackson criteria to monitor symptoms of rhinorrhea, sneezing, nasal congestion, sore throat, headache, chills/fevers, and watery/itchy eyes using a scale of 1 through 3, with 1 being mild and clearly present and 3 being severe and interfering with activities [14]. Lower airway symptoms of cough, shortness of breath, chest discomfort, wheezing, and night awakenings were also reported by participants on diary cards daily throughout the study using the same severity scale.
Bioinformatic RNA-seq data analysis

RNA was isolated from the nasal epithelial samples at the University of Virginia and sent to the University of Cincinnati College of Medicine Genomics, Epigenomics, and Sequencing Core for analysis via RNA-seq. Sequence reads were aligned to the reference human genome (hg19) using the TopHat aligner [15]. Reads aligning to each known transcript were counted and all follow up analyses were performed using Bioconductor packages for next-generation sequencing data analysis [16]. The differential gene expression analysis was performed based on the negative-binomial statistical model of read counts as implemented in the edgeR Bioconductor package [17]. Genes differentially expressed between asthma and control samples before inoculation (time point T0) were identified using the simple generalized linear model with the single factor (asthma = yes or no). Genes differentially expressed after inoculation (time points T1 and T2) in comparison to the baseline (time point T0) were identified by fitting a generalized linear model with both time and subject factors to account for the subject-to-subject variability. These comparisons were made separately for asthma and control samples. P-values were adjusted for multiple testing using the false discovery rates and differential expressions with FDR-adjusted p-value of less than 0.05 were considered statistically significant [18]. To elucidate the most common pathways affected by gene dysregulation, all genes dysregulated at least 1.5 fold were analyzed with QIAGEN’s Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity).

Upper and lower respiratory tract symptom score analyses

Cumulative upper and lower respiratory tract symptom (CURTS and CLRTS, respectively) scores were derived from diary cards. The CURTS and CLRTS scores were computed by numerically adding up the individual upper and lower respiratory tract symptom scores recorded daily by study participants from day 0 (day of HRV-A16 inoculation) to post-inoculation day 7 (T2). The CURTS and CLRTS scores were analyzed by way of negative binomial generalized linear models. Study-group (i.e. the asthmatic or non-asthmatic group) served as the independent variable of each analysis and between-group comparisons were focused on comparing the means of the underlying cumulative symptom score distributions. A two-sided p<0.05 decision rule was used as the between study-group comparison null hypothesis rejection criterion. The software of the PROC GENMOD procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) was used to conduct the cumulative symptom score analysis.

Results
Tests for atopy, Rhinovirus infection, and symptoms

The five asthmatic participants in this study had 4 or more positive tests for allergen specific IgE by prick skin testing and high levels of total IgE (geometric mean [GM] = 1061 IU/ml; range = 623–1989) (Table 1). The 5 controls had negative skin tests and their total IgE levels were low (GM = 20 IU/ml; range = 5–42). Following virus inoculation, HRV titers in cultures from nasal washes were quantified with average viral titers from T0 to T2 ranging from 0.92 to 1.96 titer/mL from the asthmatics. Three of the five controls had HRV isolated in culture, with average viral titers from T0 to T2 ranging from 1.74 to 2.03 titer/mL. The remaining 2 control subjects did not have positive viral cultures, but they had low levels of HRV-A16 detected through qPCR during the study to confirm successful inoculation. The mean CURTS scores over the 7 days of monitoring after RV inoculation were 65 (34–126, 95% confidence limits [CL]) and 32 (16–62, 95% CL) for the asthmatic and non-asthmatic groups, respectively;
The mean CLRTS scores over the same time period were 25 (10–64; 95% CL) and 3 (1.0–9; 95% CL) for the asthmatics and non-asthmatic controls, respectively; \( p = 0.004 \).

**Differences in gene expression between asthmatics and controls evident at baseline**

**Fig. 3** is an overview of the gene dysregulation in asthmatics and controls at baseline and in response to HRV infection. At baseline, 50 genes were differentially expressed at least 1.5 fold.
in asthmatics compared to controls (Table 2). Asthmatics had increased expression of genes involved in inflammation, including interleukin-1 receptor, type 1 (IL1R1), arachidonate 5-lipoxygenase (ALOX5), and tryptase alpha/beta-1 (TPSAB1). Additionally, asthmatics had decreased expression of viral replication inhibitors—interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) and SAM domain and HD domain-containing protein 1 (SAMHD1).

Rhinovirus-16 infection results in increased number of dysregulated genes and more persistent gene dysregulation in asthmatics compared to controls

As evident in Fig 3, there is substantial gene dysregulation in response to HRV infection in both asthmatics and controls, but it is stronger and more persistent in asthmatics. Following inoculation with HRV, gene expression was significantly altered at T1 in asthmatics and controls with changes favoring upregulation in both groups. However, the asthmatic group showed significantly more dysregulated genes compared to controls. From T0 to T1, there

Table 2. Top 30 genes with expression affected at least 3 fold between asthmatics and controls at baseline.

| geneid | symbol | name                                         | padj  | fold change |
|--------|--------|----------------------------------------------|-------|-------------|
| 2624   | GATA2  | GATA binding protein 2                       | 0.0212| 66.3627     |
| 1359   | CPA3   | carboxypeptidase A3 (mast cell)              | 0.0400| 64.8943     |
| 64499  | TPSB2  | tryptase beta 2 (gene/pseudogene)            | 0.0003| 52.4161     |
| 7177   | TPSAB1 | tryptase alpha/beta 1                        | 0.0003| 51.6452     |
| 6422   | SFRP1  | secreted frizzled-related protein 1          | 0.0363| 25.5794     |
| 10631  | POSTN  | peristin, osteoblast specific factor         | 0.0472| 21.2008     |
| 2354   | FOSB   | FBJ murine osteosarcoma viral oncogene homolog B | 0.0006| 17.3689     |
| 1958   | EGR1   | early growth response 1                      | 0.0212| 10.2780     |
| 2353   | FOS    | FBJ murine osteosarcoma viral oncogene homolog | 0.0212| 10.1764     |
| 4915   | NTRK2  | neurotrophic tyrosine kinase, receptor, type 2 | 0.0145| 7.3831      |
| 3164   | NR4A1  | nuclear receptor subfamily 4, group A, member 1 | 0.0396| 6.3766      |
| 240    | ALOX5  | arachidonate 5-lipoxygenase                  | 0.0212| 6.3736      |
| 9021   | SOCS3  | suppressor of cytokine signaling 3           | 0.0386| 6.0078      |
| 6550   | SLC9A3 | solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3 | 0.0158| 5.9875      |
| 7832   | BTG2   | BTG family, member 2                         | 0.0039| 5.4052      |
| 55107  | ANO1   | anoctamin 1, calcium activated chloride channel | 0.0000| 5.1866      |
| 857    | CAV1   | caveolin 1, caveola protein, 22kDa           | 0.0212| 4.5104      |
| 7538   | ZFP36  | ZFP36 ring finger protein                    | 0.0181| 4.0869      |
| 9245   | GCNT3  | glucosaminyl (N-acetyl) transferase 3, mucin type | 0.0000| 4.0763      |
| 1549   | CYP2A7 | cytochrome P450, family 2, subfamily A, polypeptide 7 | 0.0341| 4.0437     |
| 56165  | TDRD1  | tudor domain containing 1                    | 0.0080| 3.3343      |
| 1.01E+08 | LOC100996579 | uncharacterized LOC100996579 | 0.0341| -5.6716 |
| 8647   | ABCB11 | ATP-binding cassette, sub-family B (MDR/TAP), member 11 | 0.0039| -5.0905 |
| 285313 | IGSF10 | immunoglobulin superfamily, member 10        | 0.0212| -4.4396 |
| 347    | APOD   | apolipoprotein D                            | 0.0474| -4.2888 |
| 345930 | ECT2L  | epithelial cell transforming 2 like          | 0.0212| -4.1664 |
| 2628   | GATM   | glycine amidotransferase (L-arginine:glycine amidotransferase) | 0.0158| -4.0163 |
| 23007  | PLCH1  | phospholipase C, eta 1                       | 0.0133| -3.8777 |
| 3434   | IFIT1  | interferon-induced protein with tetratricopeptide repeats 1 | 0.0084| -3.8357 |
| 730101 | LOC730101 | uncharacterized LOC730101 | 0.0212| -3.7865 |

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were 1253 genes dysregulated at least 1.5 fold in asthma participants compared to 59 genes in controls (Fig 4A). By T2, gene expression in controls had essentially returned to baseline levels with only 4 genes dysregulated at least 1.5 fold compared to baseline expression (Fig 4B). In contrast, asthmatics continued to have significant gene dysregulation with 178 genes upregulated or downregulated at least 1.5 fold from baseline even 7 days later (Fig 4B). The gene expression from T0 through T2 in asthmatics and controls were categorized into 9 different clusters (Fig 5). A total of 1573 genes were found to have at least 1.5 fold change in expression in all participants of the study—asthmatics or controls—and to be significantly dysregulated (p adj < 0.05). Of this total, 1350 genes were unchanged in controls. Controls had 133 genes that were unchanged from T0 to T1 that then were downregulated from T1 to T2; 31 genes were upregulated from T0 to T1 and then were unchanged from T1 to T2. Asthmatics, on the other hand, had only 295 genes that were unchanged from T0 through T2. 670 genes were upregulated from T0 to T1 and were unchanged from T1 to T2 in asthmatics; 412 genes were upregulated from T0 to T1 and then downregulated from T1 to T2. This cluster analysis further illustrates the increased magnitude and persistence of gene dysregulation in asthmatics compared to controls in response to HRV infection.

The biology of the response to Rhinovirus-16 infection is different in asthmatics compared to controls

We next more carefully examined the differences in the biologic response to HRV-A16 in asthmatics versus controls. Tables 3–6 list the 30 most dysregulated genes in asthmatics and controls at T1 versus T0 and T2 versus T0 (complete listing of dysregulated genes is detailed in S1 File). Pathway analyses revealed that most of the dysregulation in both asthmatics and controls involves the innate and early adaptive immune response to HRV infection including IL-6 signaling [FOS, IL18RAP, IL1B, and JUN], dendritic cell maturation [including ICAM1, IL1RN, JAK2, STAT1,STAT4, TNF, TLR2, TLR4, NFKB2, and PIK3R5], granulocyte adhesion/diapedesis [including ICAM1, ITGB2, CCL3L1, CCL3L3, CXCL1, MMP2, MMP13, C5AR1, CLDN10, and CXCR4], interferon signaling [STAT1, JAK2, IFIT1, IFIT3, IFITM1, IFITM2, IFITM3, OAS1, and TAP1], and B-cell development [including CD19, IL7R, CD86, PTPRC, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DQA, HLA-DRB1]. There were several notable differences between asthmatics and controls. The regulatory IL-10 pathway was upregulated in controls at T1, with significant increase in expression of SOCS3, a suppressor of cytokine signaling. This increase in IL-10 signaling was not evident in asthmatics at T1 or T2. Also, in controls, SPINK5, which has been implicated in epithelial maintenance and repair, was induced 5.3 fold at T2 compared to baseline expression. This increase in SPINK5 was not evident in asthmatics.

Discussion

Our data demonstrate that the biology of the host response to HRV is fundamentally different in asthmatics compared to controls with striking differences in epithelial cell gene expression during the early, innate phase of the infection before symptoms peak. Asthmatics demonstrated increased magnitude and persistence of gene dysregulation, as well as distinct differences in the quality of the response following infection with HRV-A16 (Fig 6).

Consistent with reports that increased levels of inflammatory biomarkers are detected in the upper and lower airway of asthmatics before an experimental challenge with HRV [7], baseline differences in gene expression associated with inflammatory pathways were also significantly increased among the asthmatics compared to the controls. These baseline differences may contribute to an increased susceptibility to viral infection in keeping with the observation
HRV infection induces stronger gene dysregulation in asthmatics.

Fig 4. A. Heat map illustrating gene dysregulation in controls (left) and asthmatics (right) at T1 versus T0. The baseline samples of one asthmatic and one control patient were not of sufficient quality and were not included in analysis. The sample from T1 of one asthmatic was not of sufficient quality and was also excluded. B. Heat maps illustrating gene dysregulation in controls (left) and asthmatics (right) at T2 versus T0. The normalized RNA-seq raw counts for SPINK5 in controls at baseline and at T2 ranged from 2.6 to 35.3 and are reflected in the heat map with green representing lower values and red representing higher values.

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that asthmatics had decreased expression of viral replication inhibitors compared to controls. We observed in asthmatics increased expression of genes involved in inflammation—interleukin-1 receptor, type 1 (IL1R1), arachidonate 5-lipoxygenase (ALOX5), and tryptase alpha/beta-1 (TPSAB1). Tryptase is an activator of PAR-2, which in turn causes weakening of tight junctions within the epithelial layer, allowing for easier access to receptors used by HRV to infect epithelial cells and contributing to the severity and persistence of symptoms [12, 19].

As with prior studies, gene expression in both asthmatics and controls skewed towards activation of the innate and early adaptive immune response, including interferon signaling and granulocyte adhesion/diapedesis, after HRV inoculation [20]. Additionally, pathway analysis showed an early emergence of IL-10 signaling pathway in the control group from T0 to T1, which was absent in the asthmatic group from T0 to T1 and even from T0 to T2. While nasal mucosal samples do not exclude infiltrating cells such as monocytes, a major producer of IL-10, Guajardo et al has previously shown that such samples are composed mainly (> 92%) of respiratory epithelial cells [21]. IL-10 is a regulatory cytokine with dominant immunosuppressive
effects resulting in inhibition of IL-12 production, reduced expression of co-stimulatory molecules and Class I MHC, suppression of production of pro-inflammatory cytokines including IL-2, TNF-alpha, and IL-5, and increased production of anti-inflammatory cytokines including IL-1Ralpha and VEGF [22]. Indeed, impaired IL-10 responses have been associated with HRV-induced asthma exacerbations [23]. Further, several studies have shown that polymorphisms in the IL-10 promoter are associated with asthma, possibly due to decreased IL-10 production [24–28].

Although the asthmatics had markedly persistent gene dysregulation following HRV infection, there were a few genes that were notably induced during the convalescent recovery phase (T2) in control subjects. One of 4 genes that was induced in controls at T2 compared to baseline was SPINK5, which has been intensely studied in asthma due to its location within chromosome 5q31-33, a region shown in genome-wide linkage scans to be linked to asthma and atopy [29]. SPINK5 encodes an inhibitor of multiple serine proteases, including plasmin, neutrophil elastase, and trypsin, and mutations in this gene have been associated with Netherton syndrome [29]. SPINK5 polymorphisms/haplotypes have also been associated with asthma susceptibility in numerous populations [30–32]. The upregulation of SPINK5 in controls at T2 suggests it may be important in epithelial repair. While a recent study involving the transfection of A549 cells (adenocarcinomic human alveolar basal epithelial cells) with SPINK5

### Table 3. Genes with expression affected at least 3 fold in control group from T0 to T1.

| geneid  | symbol | name                                               | padj       | fold change |
|--------|--------|---------------------------------------------------|------------|-------------|
| 4060   | LUM    | lumican                                           | 0.020943   | 72.8022     |
| 342510 | CD300E | CD300e molecule                                   | 0.008487   | 38.3880     |
| 3553   | IL1B   | interleukin 1, beta                               | 0.003612   | 21.7846     |
| 1278   | COL1A2 | collagen, type I, alpha 2                         | 0.000348   | 21.7134     |
| 414062 | CCL3L3 | chemokine (C-C motif) ligand 3-like 3              | 0.026042   | 17.5723     |
| 6349   | CCL3L1 | chemokine (C-C motif) ligand 3-like 1              | 0.026042   | 17.5723     |
| 1960   | EGR3   | early growth response 3                           | 0.0124     | 12.4203     |
| 5329   | PLAUR  | plasminogen activator, urokinase receptor          | 0.033343   | 9.6363      |
| 1959   | EGR2   | early growth response 2                           | 0.048586   | 9.2946      |
| 4313   | MMP2   | matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) | 0.00553    | 7.4013      |
| 1289   | COL5A1 | collagen, type V, alpha 1                         | 0.043756   | 7.2466      |
| 9021   | SOCS3  | suppressor of cytokine signaling 3                | 1.27E-06   | 7.1697      |
| 64092  | SAMS1  | SAM domain, SH3 domain and nuclear localization signals 1 | 0.026322   | 7.1446      |
| 2353   | FOS    | FBJ murine osteosarcoma viral oncogene homolog    | 0.022499   | 6.7704      |
| 6503   | SLA    | Src-like-adaptor                                   | 0.017101   | 6.2597      |
| 5999   | RGS4   | regulator of G-protein signaling 4                | 0.012113   | 5.8468      |
| 5788   | PTPRC  | protein tyrosine phosphatase, receptor type, C     | 0.030499   | 5.2359      |
| 84913  | ATOH8  | atonal homolog 8 (Drosophila)                     | 0.007565   | 4.2610      |
| 8807   | IL1RAP | interleukin 18 receptor accessory protein          | 0.012113   | 4.2399      |
| 133    | ADM    | adrenomedullin                                     | 0.009749   | 3.8967      |
| 51297  | BPIFA1 | BPI fold containing family A, member 1             | 0.005879   | 3.8852      |
| 11151  | CORO1A | coronin, actin binding protein, 1A                 | 0.012414   | 3.8204      |
| 1907   | EDN2   | endothelin 2                                       | 0.015707   | 3.8022      |
| 92304  | SCGB3A1| secretoglobin, family 3A, member 1                 | 2.08E-08   | 3.6013      |
| 7538   | ZFP36  | ZFP36 ring finger protein                          | 0.012292   | 3.3842      |
| 4322   | MMP13  | matrix metalloproteinase 13 (collagenase 3)        | 0.12414    | 3.2228      |
| 7031   | TFF1   | trefoil factor 1                                   | 0.001698   | 3.0703      |

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expression vectors resulted in increased production of IL-6, IL-8, and RANTES, transfection also resulted in increased susceptibility to cell death [33]. As destruction of infected cells is key for antigen presentation in the immune response to active viral infections, increased SPINK5 expression may be a protective measure. Additionally, studies measuring SPINK5 in nasal epithelial tissue revealed that low SPINK5 expression was associated with chronic rhinosinusitis with and without nasal polyps [34] and among those with chronic rhinosinusitis and aspirin intolerance [35]. The association between atopic diseases and polymorphisms or decreased expression of SPINK5 suggests a significant role for this protease inhibitor in epithelial cell maintenance and repair.

### Table 4. Top 30 genes with expression affected at least 3 fold in asthmatics from T0 to T1.

| geneid | symbol | name                                      | padj      | fold change |
|--------|--------|-------------------------------------------|-----------|-------------|
| 3552   | IL1A   | interleukin 1, alpha                      | 1.71E-05  | 28.92492495 |
| 402665 | IGLON5 | IgLON family member 5                     | 0.002964315 | 26.55078527 |
| 719    | C3AR1  | complement component 3a receptor 1        | 4.17E-05  | 26.09216702 |
| 4973   | OLR1   | oxidized low density lipoprotein (lectin-like) receptor 1 | 0.000737414 | 25.21195402 |
| 6373   | CXCL11 | chemokine (C-X-C motif) ligand 11         | 2.34E-10  | 22.43688977 |
| 4064   | CD180  | CD180 molecule                            | 0.03869994 | 21.74115708 |
| 23601  | CLEC5A | C-type lectin domain family 5, member A    | 0.000460589 | 20.90985398 |
| 6347   | CCL2   | chemokine (C-C motif) ligand 2             | 0.001554308 | 20.54486259 |
| 2865   | FFAR3  | free fatty acid receptor 3                 | 1.65E-05  | 18.54119076 |
| 101928513 | CATIP-AS1 | CATIP antisense RNA 1                   | 0.005560741 | 15.54512675 |
| 5806   | PTX3   | pentraxin 3, long                         | 1.99E-07  | 14.73660109 |
| 6351   | CCL4   | chemokine (C-C motif) ligand 4             | 3.02E-10  | 13.67747639 |
| 160622 | GRASP  | GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein | 0.008466801 | 13.672469691 |
| 9560   | CCL4L2 | chemokine (C-C motif) ligand 4-like 2      | 7.20E-07  | 13.43126991 |
| 388372 | CCL4L1 | chemokine (C-C motif) ligand 4-like 1      | 9.04E-07  | 13.33820322 |
| 5329   | PLAUR  | plasminogen activator, urokinase receptor | 7.87E-08  | 13.28421895 |
| 10578  | GNLY   | granulysin                                | 0.04395143 | 12.75855934 |
| 221692 | PHACTR1| phosphatase and actin regulator 1         | 0.016054301 | 12.71174252 |
| 3576   | CXCL8  | chemokine (C-X-C motif) ligand 8           | 1.35E-09  | 12.70191061 |
| 3575   | IL7R   | interleukin 7 receptor                     | 2.51E-05  | 12.5753077 |
| 10563  | CXCL13 | chemokine (C-X-C motif) ligand 13          | 0.041386656 | 12.1909315 |
| 56165  | TDRD1  | tudor domain containing 1                 | 0.000775926 | 12.09246229 |
| 100128385 | FAM225B | family with sequence similarity 225, member B (non-protein coding) | 0.030859801 | 11.91060151 |
| 8013   | NR4A3  | nuclear receptor subfamily 4, group A, member 3 | 0.001564312 | 11.82961672 |
| 7772   | ZNF229 | zinc finger protein 229                    | 0.046632528 | 11.7866035 |
| 1493   | CTLA4  | cytotoxic T-lymphocyte-associated protein 4 | 0.033675632 | 11.71145859 |
| 400680 | LINC00664 | long intergenic non-protein coding RNA 664 | 2.79E-06  | 11.59893421 |
| 23769  | FLRT1  | fibronectin leucine rich transmembrane protein 1 | 0.003228573 | -23.25413453 |
| 25787  | DGCR9  | DiGeorge syndrome critical region gene 9 (non-protein coding) | 0.020863424 | -18.17084882 |
| 152    | ADRA2C | adrenocorticotropin hormone alpha C         | 0.002794832 | -13.37477796 |

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### Table 5. Genes with expression affected at least 3 fold in control group from T0 to T2.

| geneid | symbol | name                                      | padj      | fold change |
|--------|--------|-------------------------------------------|-----------|-------------|
| 84913  | ATOH8  | atonal homolog 8 (Drosophila)              | 0.034015  | 3.796982    |
| 6035   | RNASE1 | ribonuclease, RNase A family, 1 (pancreatic) | 0.018146  | 5.157555    |
| 11005  | SPINK5 | serine peptidase inhibitor, Kazal type 5   | 1.51E-08  | 5.268909    |

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The RV challenge model has been helpful in other investigations to explore mechanistic questions focused on the asthmatic response to RV [11, 23, 36–38]. To our knowledge, however, this is the first report of changes in gene expression in response to HRV observed well before symptoms peak among asthmatics who, in our study, experienced more significant

The table below lists the top 30 genes with expression affected at least 3 fold in asthmatics from T0 to T2.

| geneid   | symbol | name                                  | padj        | fold change |
|----------|--------|---------------------------------------|-------------|-------------|
| 10578    | GNLY   | granulysin                            | 0.023905745 | 19.41766265 |
| 5079     | PAX5   | paired box 5                          | 0.024519067 | 18.71066511 |
| 2537     | IFI6   | interferon, alpha-inducible protein 6 | 5.10E-06    | 15.51483751 |
| 4283     | CXCL9  | chemokine (C-X-C motif) ligand 9       | 0.043209055 | 8.50763183  |
| 5551     | PRF1   | perforin 1 (pore forming protein)      | 0.024519067 | 7.64010167  |
| 91543    | RSAD2  | radical S-adenosyl methionine domain containing 2 | 9.98E-12 | 7.56697850 |
| 3434     | IFIT1  | interferon-induced protein with tetratrico peptide repeats 1 | 0.024776552 | 7.00849278  |
| 10964    | IFI44L | interferon-induced protein 44-like     | 0.001195079 | 5.75513534  |
| 6362     | CCL18  | chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated) | 0.01629478  | 5.62378525  |
| 9636     | ISG15  | ISG15 ubiquitin-like modifier          | 7.68E-05    | 5.21592391  |
| 5653     | KLK6   | kallikrein-related peptidase 6         | 0.019548496 | 4.86813902  |
| 129607   | CMPK2  | cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial | 0.002773796 | 4.741074774 |
| 3437     | IFIT3  | interferon-induced protein with tetratrico peptide repeats 3 | 0.002006977 | 4.27697455  |
| 710      | SERPING1 | serpin peptidase inhibitor, clade G (C1 inhibitor), member 1 | 0.000537538 | 4.17732423 |
| 79931    | TNIP3  | TNFAIP3 interacting protein 3           | 0.000432613 | 4.10550274  |
| 80320    | SP6    | Sp6 transcription factor               | 0.02127551  | 4.07723526  |
| 27074    | LAMP3  | lysosomal-associated membrane protein 3 | 0.008830902 | 3.86218513  |
| 348      | APOE   | apolipoprotein E                      | 4.41E-06    | 3.84662089  |
| 7453     | WARS   | tryptophanyl-riRNA synthetase          | 0.000534318 | 3.822353718 |
| 100126311| MIR147B| microRNA 147b                         | 4.86E-18    | 3.65218463  |
| 8771     | TNFRSF6B | tumor necrosis factor receptor superfam, member 6b, decoy | 0.000551508 | 3.64304083  |
| 3433     | IFIT2  | interferon-induced protein with tetratrico peptide repeats 2 | 0.003161937 | 3.64082415  |
| 4481     | MSR1   | macrophage scavenger receptor 1        | 0.013181546 | 3.57867619  |
| 641517   | DEFB109P1B | defensin, beta 109, pseudogene 1B      | 0.030905088 | -27.09924  |
| 284654   | RSPO1  | R-spondin 1                           | 0.006456265 | -5.278907  |
| 101927560| LOC101927560 | uncharacterized LOC101927560         | 4.11E-05    | -4.316873  |
| 100616209| MIR4461| microRNA 4461                         | 7.68E-05    | -4.285838  |
| 128102   | HSD3BP4 | hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 4 | 0.002899914 | -4.12954  |
| 1592     | CYP26A1 | cytochrome P450, family 26, subfamily A, polypeptide 1 | 0.000130986 | -3.80011  |
| 102465432| MIR6723 | microRNA 6723                         | 0.027519068 | -3.601905  |

The RV challenge model has been helpful in other investigations to explore mechanistic questions focused on the asthmatic response to RV [11, 23, 36–38]. To our knowledge, however, this is the first report of changes in gene expression in response to HRV observed well before symptoms peak among asthmatics who, in our study, experienced more significant

Fig 6. Summary of gene dysregulation in asthmatics versus controls from T0 to T2 (left) and summary of differences in innate immunity, including the epithelial barrier (right). These differences may contribute to increased magnitude and persistence of gene dysregulation in asthmatics in response to HRV infection.

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lower respiratory tract symptoms and sustained gene expression that was still augmented compared to the non-asthmatics 7 days after virus inoculation. Our study was limited by a small sample size, and the gene expression analysis could not be completed for two participants—one control and one asthmatic—due to samples of insufficient quality. Because of the broad inclusion and exclusion criteria required to find subjects who are eligible for participation, it is difficult to enroll larger numbers of mild asthmatics who are seronegative to the strain of virus used for inoculation and who are not using controller medications in these studies. However, the dysregulation of genes noted at 36 hours, and the persistent changes at 7 days, was striking among the asthmatics and these results are unlikely to be altered by including data from more subjects. For example, epithelial cell gene expression has been examined in response to HRV-A16 in nasal scrapings from healthy, non-asthmatic subjects who tested positive for HRV in cultures of their nasal washes. As compared to the 1263 genes dysregulated at least 1.5 fold among the asthmatics in our study, significantly fewer genes were dysregulated in nasal scrapings at 48 hours in that study (i.e., 471 genes showed a $\geq$ 2-fold increase in gene expression and 201 genes decreased $\geq$ 0.5-fold) [39]

In the future, further studies would be desirable to examine gene expression in epithelial cell samples obtained at earlier time points after HRV inoculation. A longer follow-up period (e.g., 3 to 4 weeks after inoculation) would also be beneficial to determine how long the gene dysregulation in asthmatics persists and to evaluate any differences in relation to the adaptive immune response. Additionally, the enrollment of atopic subjects without asthma would be of interest, along with selected samples collected for gene expression analysis from the lower airway.

In summary, asthmatics have a substantially altered host response to HRV-A16 infection in terms of magnitude, quality, and persistence when compared to non-asthmatic controls. Differences in gene expression were apparent at baseline, during infection, and during the onset of convalescence/repair. At baseline, increased expression of inflammatory genes and decreased expression of viral replication inhibitors was evident in asthmatics, and emergence of IL-10 signaling early in infection was evident only in controls. During convalescence/repair, upregulation of *SPINK5* was seen only in controls underscoring the role of this protease inhibitor in the recovery phase. These key differences provide novel mechanistic insights into how HRV infection may contribute to asthma exacerbation and highlight previously unrecognized pathways that may be important targets for therapeutic intervention.

### Supporting information

**S1 File. Complete list of dysregulated genes.** This excel workbook details the complete list of dysregulated genes in both asthmatics and controls.

(XLSX)

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