Gene Expression Profiles during *In Vivo* Human Rhinovirus Infection

Insights into the Host Response

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***Rationale*: Human rhinovirus infections cause colds and trigger exacerbations of lower airway diseases.**

***Objectives*: To define changes in gene expression profiles during *in vivo* rhinovirus infections.**

***Methods*: Nasal epithelial scrapings were obtained before and during experimental rhinovirus infection, and gene expression was evaluated by microarray. Naturally acquired rhinovirus infections, cultured human epithelial cells, and short interfering RNA knockdown were used to further evaluate the role of viperin in rhinovirus infections.**

***Measurements and Main Results*: Symptom scores and viral titers were measured in subjects inoculated with rhinovirus or sham control, and changes in gene expression were assessed 8 and 48 hours after inoculation. Real-time reverse transcription-polymerase chain reaction for viperin and rhinoviruses was used in naturally acquired infections, and viperin mRNA levels and viral titers were measured in cultured cells. Rhinovirus-induced changes in gene expression were not observed 8 hours after viral infection, but 11,887 gene transcripts were significantly altered in scrapings obtained 2 days postinoculation. Major groups of up-regulated genes included chemokines, signaling molecules, interferon-responsive genes, and antivirals. Viperin expression was further examined and also was increased in naturally acquired rhinovirus infections, as well as in cultured human epithelial cells infected with intact, but not replication-deficient, rhinovirus. Knockdown of viperin with short interfering RNA increased rhinovirus replication in infected epithelial cells.**

***Conclusions*: Rhinovirus infection significantly alters the expression of many genes associated with the immune response, including chemokines and antivirals. The data obtained provide insights into the host response to rhinovirus infection and identify potential novel targets for further evaluation.**

Keywords: epithelial cells; antiviral; chemokines; intracellular signaling proteins

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Understanding of the pathogenesis of rhinovirus infections, and of rhinovirus-induced exacerbations of asthma and chronic obstructive pulmonary disease is incomplete.

What This Study Adds to the Field

Rhinovirus infection significantly alters the expression of many genes associated with the immune response, including chemokines and antivirals. The data obtained provide insights into the host response to rhinovirus infection and identify potential novel targets for further evaluation.

Human rhinovirus (HRV) infections are the predominant cause of the common cold, and can trigger the development of otitis media and sinusitis (1–4). HRV infections are also a major risk factor for triggering exacerbations of lower airway diseases, such as chronic obstructive pulmonary disease and asthma (5–7). Although the mechanisms by which HRV infections induce or exacerbate symptoms are not fully understood, the airway epithelial cell is the principal site of HRV infection (8, 9). In contrast to other viral types, such as influenza, HRV infections do not result in any overt epithelial cytotoxicity (10–12). This had led to the hypothesis that viral alterations of epithelial cell biology induce increased airway inflammation, which, in turn, triggers or exacerbates symptoms. Support for this hypothesis has come from several studies demonstrating that epithelial cells produce a number of proinflammatory cytokines and chemokines on HRV infection (12–15), many of which are also detected in airway secretions during *in vivo* infections (13, 15–17). Interestingly, epithelial infection with HRV, both *in vitro* and *in vivo*, also induces the production of molecules such as human β-defensin 2 and inducible nitric oxide synthase, which likely contribute to host defense and antiviral responses (18, 19). Although various individual proteins have been implicated in the pathogenesis of HRV infections on the basis of such studies, there have, thus far, been few attempts to comprehensively assess the host response to HRV infection.

Although microarray analysis has been used to assess gene expression in cultured immortalized human epithelial cell lines exposed to crude rhinovirus preparations (20, 21), it is unclear whether such cells accurately reflect *in vivo* epithelium. A single study has examined responses to HRV infection of primary cultures of human bronchial epithelial cells derived from two donors, using gene chips containing approximately 22,000 probe sets (22). To our knowledge, however, there has been no comprehensive assessment of alterations of gene expression during *in vivo* HRV infections. To gain increased understanding of the response to HRV infection, therefore, we performed a study in which gene expression was assessed by microarray in

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nasal scrapings obtained from subjects before and during experimental infections with HRV-16, as well as from sham-inoculated subjects. Our data provide the first comprehensive picture of the host response to HRV-16 infection *in vivo* and identify several groups of genes that are likely to contribute to the proinflammatory and antiviral responses. To further validate and extend these observations, the gene encoding viperin, which was up-regulated the most among potential antivirals, was selected for further evaluation. The up-regulation of this gene was confirmed by real-time reverse transcription–polymerase chain reaction (RT-PCR) both from nasal scrapings obtained during naturally acquired HRV infections, and in primary cultures of human airway epithelial cells infected with HRV-16. The potential antirhinovirus activity of viperin also was assessed.

METHODS

See the online supplement for additional detail regarding all methods.

Experimental Rhinovirus Infection

A randomized, parallel group study was conducted. One group was inoculated with HRV-16, whereas control subjects were sham inoculated. Nasal epithelial scrapings were collected from alternating nostrils on Day −14 (before inoculation), and 8 hours and 2 days after inoculation, as described (19). From Day 0 to Day 5, subjects underwent daily nasal lavage, and recorded symptom scores as described (16, 23). Viral titers were assessed in nasal secretions, and serum neutralizing antibodies to HRV-16 were assayed before, and 3–4 weeks after, inoculation. The protocol was approved by the Human Investigations Committee of the University of Virginia (Charlottesville, VA) and all volunteers gave written, informed consent.

Microarray Analysis

Total RNA was extracted from nasal scrapings with TRIzol reagent (Invitrogen, Carlsbad, CA) and purified further by passage through RNeasy columns (Qiagen,Valencia, CA). Microarray chips (Affymetrix, Santa Clara, CA) were used to analyze changes in the expression of more than 47,000 transcripts.

Natural Rhinovirus Colds Study

We performed a prospective, cross-sectional study in which subjects reported to the clinic within 36 hours of the onset of cold symptoms. Nasal lavage was performed and a nasal scraping was collected from one nostril. Subjects returned for a retrospective baseline visit 4 weeks later, when lavage was repeated and a nasal scraping was taken from the other nostril. Rhinovirus infections were established by real-time RT-PCR and total RNA was extracted from epithelial scrapings. All subjects gave informed consent and the protocol was approved by the Conjoint Health Ethics Board of the Faculties of Medicine, Nursing and Kinesiology, University of Calgary (Calgary, AB, Canada).

In Vitro Rhinovirus Studies

HRV-16 and WI-38 cells were from the American Type Culture Collection (Manassas, VA). Viral stocks of HRV-16 were generated by passage in WI-38 cells and were purified as described (24). Viral titers were determined with WI-38 cells (25).

Epithelial Cell Cultures

Primary human airway epithelial cells were obtained as described (26). Cells were grown in serum-free growth medium (BEGM; Lonza, Allendale, NJ). Twenty-four hours before stimulation, hydrocortisone was withdrawn from the medium. This hydrocortisone-free medium was used for all experiments.

Viral Infection of Epithelial Cells

Cells were infected with HRV-16 and incubated at 34°C for appropriate times. Total RNA was extracted with TRIzol. Viperin mRNA was quantified by real-time RT-PCR. Specific Stealth siRNA (small interfering RNA), and a matched scrambled control, for viperin were from Invitrogen (Burlington, ON, Canada). To assess the effects of viperin knockdown on viral titers, cells were transfected with specific or control siRNA for 24 hours. After a 24-hour recovery, cells were exposed to HRV-16 for 2 hours, washed, and incubated for 24 hours at 34°C. Viral titers were assessed in recovered supernatants.

Statistical Analysis

Levels of gene transcript expression at baseline were compared between groups by analysis of variance (ANOVA). Analysis of covariance, with main effects for sex and group, was used to compare data for each postinoculation visit. For *in vitro* studies, data were analyzed by ANOVA with appropriate post hoc analysis. The Wilcoxon matched-pairs signed-ranks test was used for nonparametric data.

RESULTS

Experimental *in Vivo* Rhinovirus Infection Study

Forty-two subjects were initially randomized to the inoculation groups. Seven of these subjects were excluded from final gene expression data and statistical analysis. Of these seven, four subjects were excluded because, although they had negative titers of neutralizing antibodies to HRV-16 at Day −14, they had titers greater than 2 in blood samples drawn on Day 0 immediately before inoculation. One subject in the HRV-16–inoculated group was removed from the analysis because the subject did not become infected, as judged both by no viral shedding in nasal secretions and a failure to produce serum neutralizing antibodies 20–30 days postinoculation. Two subjects in the control group were also deleted from analysis because they developed natural rhinovirus infections (not HRV-16). Thus, data from 35 subjects, 17 in the infected group and 18 control subjects, were used for data analyses. The subjects in each group were demographically well balanced, as shown in Table 1.

Subjects infected with HRV-16 developed symptomatic colds. As shown in Figure 1, mean total symptom scores were first increased on Day 1 after infection, and peaked on Day 3. As expected, variations in responses occurred among subjects, with peak symptoms for individuals ranging from Day 2 to Day 4. The sham-inoculated group did not show significant increases in symptom scores.

Gene Expression Analysis

Changes in gene expression in epithelial scrapings obtained 8 hours after either sham or HRV-16 inoculation, compared with baseline, showed essentially no differences between groups other than those expected by chance. By contrast, on analysis of samples obtained 2 days after inoculation, 11,887 gene transcripts representing 6,530 discrete genes were found to be significantly different between the HRV-16–inoculated and sham-inoculated groups. The complete data set is publicly

TABLE 1. PATIENT DEMOGRAPHICS

	HRV-16 Group	Control Group
Subjects, n	17	18
Age, yr: mean (±SD)	20.2 (1.5)	21.1 (1.8)
Sex		
Females, n	11	12
Race, no.		
Whites	13	15
Asians	1	2
Blacks	1	0
Hispanics	1	0
Multiracial	1	1

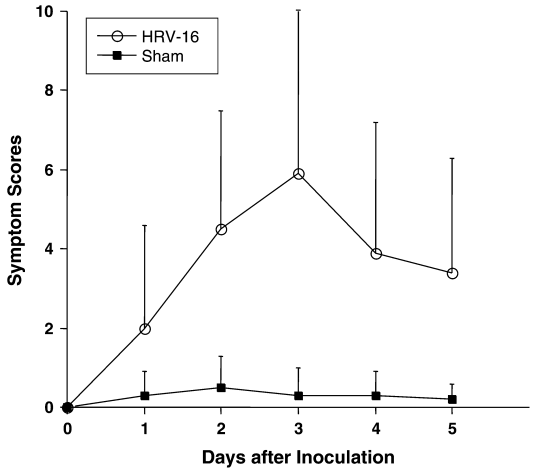

Figure 1. Total daily symptom scores (means ± SD) of subjects infected with human rhinovirus (HRV)-16 and sham-infected control subjects.

available in the GEO (Gene Expression Omnibus) public repository (http://www.ncbi.nlm.nih.gov/geo/) (accession no. GSE11348) in a format that complies with the Minimal Information About a Microarray Experiment (MIAME) guidelines. Using an arbitrary threshold of a twofold change, 471 transcripts showed at least a 2-fold increase in expression in response to HRV infection, whereas 201 transcripts were decreased to an expression level of 0.5-fold or less relative to control. As shown in Table 2, a substantial number of highly induced genes fell into discrete functional categories. Several of the most highly induced genes were chemokines of both the C-C and C-X-C families that could regulate inflammatory cell recruitment. A substantial number of interferon-inducible genes were also up-regulated. Overlapping with this category were a variety of gene products with known antirhinoviral functions, such as members of the 2′,5′-oligoadenylate synthase family, as well as potential novel antiviral molecules, such as viperin. Other groups of induced genes were those associated with the regulation of cytokine and viral signaling, and genes associated with adhesion processes and structural remodeling. Interestingly, a smaller group of genes, not shown in Table 2, and which are associated with apoptotic functions, were induced. These included programmed cell death 1 ligand 1 (induced 7.67-fold), apoptosis regulator BCL-G (induced 2.57-fold), and caspases 1 and 4 (both induced 2.06-fold). By contrast to the induced genes, there were less clear patterns in the genes that were down-regulated in subjects with HRV-16 infections. Examination of the 20 genes down-regulated to the greatest extent (Table 3), for example, revealed no obvious functional groupings, other than the three structurally unrelated receptors (prostaglandin F receptor, IL-5 receptor, and scavenger receptor class A, member 3).

Further Evaluation of Viperin Induction *In Vivo* and *In Vitro*

Viperin has been reported to have antiviral actions against several viral types (27–29), but its effects on rhinovirus replication have not yet been defined. Given that this was the gene that was most induced in the class of potential antivirals, we chose to evaluate further the role of this gene in HRV infections. Initially we examined correlations between viperin gene expression and symptom scores in the subjects with experimental HRV-16 infections. Viperin expression was significantly correlated with rhinorrhea ($r = 0.801$, $P = 0.001$), sneezing ($r = 0.589$, $P = 0.034$), and chilliness ($r = 0.580$, $P = 0.038$). Correlations with nasal obstruction ($r = 0.510$), malaise ($r = 0.270$), headache ($r = 0.235$), and cough ($r = -0.298$) were not statistically significant. To validate and extend the microarray data, we also conducted a study examining viperin expression by real-time RT-PCR in nasal epithelial scrapings obtained from subjects with naturally occurring HRV infections. Samples were obtained from nine otherwise healthy, nonallergic subjects (mean age, 44 yr; age range, 21–59 yr; four female) reporting to the clinic with symptomatic HRV infections, as confirmed by real-time RT-PCR detection of viral RNA. Samples were also obtained from the same subjects 4 weeks later, when they had been symptom free for more than 2 weeks and nasal lavages were negative for HRV by real-time RT-PCR. As shown in Figure 2, levels of viperin mRNA were higher in all subjects during HRV infection. Median levels of viperin mRNA were 208 ag (attograms) from epithelial scrapings obtained from subjects when symptomatic, compared with 17 ag at baseline.

We have previously reported that more than 95% of cells obtained in nasal scrapings are epithelial cells (30). Nonetheless, it was possible that viperin derived from contaminating cells. To establish whether HRV infection directly induces epithelial expression of viperin, we infected primary cultures of human

TABLE 2. SELECTED GENES MARKEDLY INDUCED BY HRV-16 INFECTION

Gene	Description	Fold Induction
Chemokines		
CCL2	Chemokine (C-C motif) ligand 2	55.00
CCL8	Chemokine (C-C motif) ligand 8	43.80
CXCL11	Chemokine (C-X-C motif) ligand 11	37.40
CXCL10	Chemokine (C-X-C motif) ligand 10	25.50
CXCL13	Chemokine (C-X-C motif) ligand 13	14.80
CXCL9	Chemokine (C-X-C motif) ligand 9	12.90
CCL20	Chemokine (C-C motif) ligand 20	12.00
Interferon pathways		
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	11.40
GBP1	Guanylate-binding protein 1 (interferon-inducible)	11.10
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1	8.82
G1P2	Interferon, α-inducible protein (clone 1F1-15k)	7.61
IFIT4	Interferon-induced protein with tetratricopeptide repeats 4	7.56
IL28B	Interleukin 28B (interferon, lambda 3)	5.56
IRF7	Interferon regulatory factor 7	4.16
Potential antiviral		
CIG5	Viperin	6.72
NOS2A	Nitric oxide synthase 2A (inducible)	6.07
OAS3	2′,5′-Oligoadenylate synthase 3	4.72
OASL	oligoadenylate synthase like	4.57
OAS2	2′,5′-Oligoadenylate synthase 2	4.35
MX2	Myxovirus (influenza virus) resistance 2	4.26
OAS1	2′,5′-Oligoadenylate synthase 1	3.78
MX1	Myxovirus (influenza virus) resistance 1	3.29
PLSCR1	Phospholipid scramblase 1	2.53
Signaling		
SOCS1	Suppressor of cytokine signaling 1	31.70
SOCS2	Suppressor of cytokine signaling 2	6.93
MDA5	Melanoma differentiation antigen 5	3.69
RIGI	Retinoic acid–inducible protein I	3.45
SOCS3	Suppressor of cytokine signaling 3	2.91
Adhesion/remodeling		
ICAM-1	Intercellular adhesion molecule 1 (CD54)	8.48
HAPLN3	Hyaluronan and proteoglycan link protein 3	6.43
MMP12	Matrix metalloproteinase 12	4.70
EPSTI1	Epithelial stromal interaction 1	4.45
TNC	Tenascin C	2.80

TABLE 3. THE 20 GENES MOST DOWN-REGULATED BY HRV-16 INFECTION

Gene	Description	Fold Control
PTGFR	Prostaglandin F receptor (FP)	0.15
ADH6	Alcohol dehydrogenase 6 (class V)	0.16
SEC14L3	SEC14-like (*S. cerevisiae*)	0.20
C20orf85	Chromosome 20 open reading frame 85	0.22
IL5RA	Interleukin 5 receptor, α	0.23
WDR17	WD repeat domain 17	0.24
FLJ21687	PDZ domain containing, X chromosome	0.25
ELF5	E74-like factor 5 (ets domain transcription factor)	0.26
HMGCS2	3-Hydroxy-3-methyglutaryl-coenzyme A synthase 2 (mitochondrial)	0.27
CHAD	Chondroadherin	0.27
TRIM3	Tripartite motif-containing 3	0.29
OMG	Oligodendrocyte myelin glycoprotein	0.29
PCDH19	Protocadherin 19	0.30
USP2	Ubiquitin-specific protease 2	0.30
BRG4	B-cell translocation gene 4	0.30
SATB2	SATB family member 2	0.31
SCAR3	Scavenger receptor class A, member 3	0.31
C6orf162	Chromosome 6 open reading frame 162	0.32
RTDR1	Rhabdoid tumor deletion region gene 1	0.32
STK33	Serine/threonine kinase 33	0.33

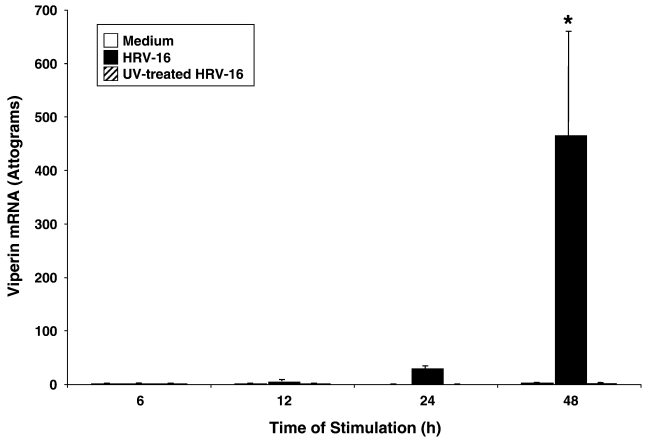

Figure 3. Infection of primary cultures of human bronchial epithelial cells with intact, but not ultraviolet-inactivated, human rhinovirus (HRV)-16 induces time-dependent expression of viperin mRNA ($P <$ 0. 05 by analysis of variance [ANOVA]). Cells were derived from three different tissue donors. The *asterisk* indicates a significant increase ($P <$ 0.02 in each case) compared with all other time points.

bronchial epithelial (HBE) cells *in vitro*. HRV-16 infection induced a significant ($P < 0.05$ by ANOVA) time-dependent induction of viperin mRNA in HBE cells (Figure 3). There was little or no expression of viperin under basal culture conditions. Induction of viperin mRNA was first observed by 12 hours postinfection with HRV-16 and increased until 48 hours after infection. Induction at 48 hours was significantly increased ($P <$ 0.02) compared with all other time points. Induction of viperin is dependent on HRV replication, as HRV-16 rendered replication deficient by treatment with ultraviolet light as described (15) did not induce significant expression of viperin at any time point studied.

To determine whether viperin plays a role in host defense against HRV infection, we used siRNA knockdown. We first compared the effects of transfection of sequence-specific siRNA, with transfection of scrambled control siRNA and exposure to transfection reagent alone, on HRV-induced epithelial expression of viperin. Because the absolute magnitude of viperin mRNA induction by HRV-16 varied markedly among HBE cells derived from different donors, data were normalized with the induction levels seen in HRV-16–infected cells exposed to transfection reagent alone, expressed as 100% for each experiment. As shown in Figure 4A, scrambled control siRNA did not affect HRV-16–induced viperin expression, whereas viperin-specific siRNA reduced HRV-16–induced viperin gene expression by a mean of 90%. In individual experiments, knockdown ranged between 84 and 95%. A comparison of the effects of each type of treatment on HRV-16 replication showed that, whereas scrambled siRNA had no significant effect on viral titers, knockdown of viperin led to a significant increase ($P < 0.02$ in each case) in HRV replication compared with both transfection reagent alone and scrambled siRNA (Figure 4B). In each of four experiments, viral titer more than doubled in the setting of viperin knockdown. Despite this effect on viral titers in cell supernatants, knockdown of viperin did not significantly affect levels of the chemokine IP-10 (IFN-γ–inducible protein-10), in these same samples. Levels of IP-10 in supernatants from cells exposed to transfection reagent alone were 1,070 ± 678 pg/ml (mean ± SD, n = 4) compared with 861 ± 473 pg/ml in the setting of viperin knockdown.

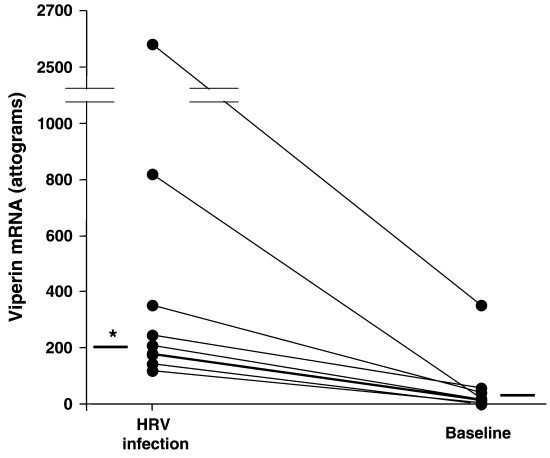

Figure 2. Viperin mRNA expression levels are significantly increased in nasal epithelial scrapings of nine subjects with naturally occurring symptomatic human rhinovirus (HRV) infections compared with subsequent symptom-free baseline. *Asterisk* indicates a significant increase ($P < 0.01$) compared with baseline by Wilcoxon matched-pairs signed-rank test.

DISCUSSION

The current study represents the first comprehensive assessment of alterations of gene expression during experimental *in vivo* HRV infections. Given that the epithelium is the primary site of HRV infection, we elected to use nasal epithelial scrapings as a relatively noninvasive procedure to obtain RNA that is predominantly of epithelial origin. Data from animal models suggest that, after epithelial denudation, a fully intact, redifferentiated epithelium is observed within 5 days (31). We, therefore, planned the protocol such that baseline samples were taken 14 days before inoculation, to ensure that the epithelium would have 16 days to return to normal before sampling the same nostril a second time. The sham-inoculated group also served as a control for changes due solely to repeated scrapings. The time required for a complete replication cycle of human rhinovirus to occur is 5 to 10 hours (32). We chose, therefore, to perform nasal epithelial scrapings 8 hours after inoculation, in

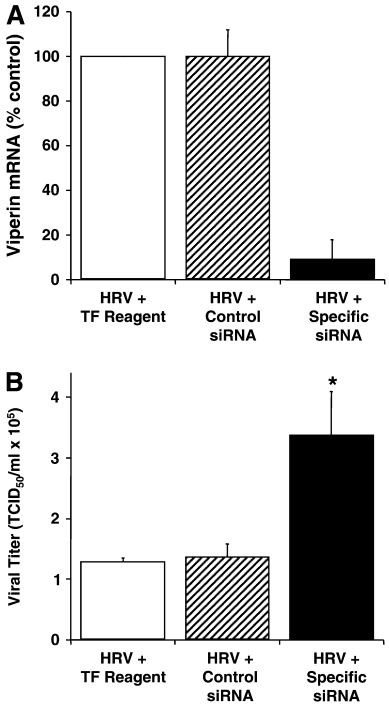

Figure 4. Effects of small interfering RNA (siRNA) knockdown in primary epithelial cells derived from four different tissue donors. (*A*) Sequence-specific siRNA, but not a scrambled version of the same sequence, inhibits viral induction of viperin mRNA expression. (*B*) Sequence-specific siRNA, but not a scrambled version of the same sequence, leads to significantly increased human rhinovirus (HRV) replication as assessed by viral titer assay ($P < 0.05$ by ANOVA). The *asterisk* indicates a significant increase ($P < 0.02$ in each case) compared with both of the other treatment groups.

an attempt to identify genes induced early in the infectious process that may play a key role in disease pathogenesis. Unfortunately, there were no significant differences in gene expression profiles between the two groups at this early time point. Potential explanations for this relate mainly to differences between *in vitro* and *in vivo* infections. In cell culture, cells tend to be in the same growth phase and are exposed to doses of virus that simultaneously infect all cells with appropriate HRV receptors. Examination of *in vivo* rhinovirus infections by either *in situ* hybridization or immunohistochemistry, however, demonstrates that the pattern of epithelial infection is patchy (9, 33), and may be initiated at different point sites in the airway. Indeed, sampling of multiple sites in the nasal cavity after experimental infections found that virus was detectable most often in the nasopharynx and progressed anteriorly (34). Given this distribution, and the patchiness of infection, it is likely, therefore, that few infected cells were sampled from the inferior turbinate at this early time point.

In samples obtained 2 days after HRV infection, however, expression levels of more than 11,000 transcripts, representing 6,530 discrete genes, were altered compared with sham inoculation. At this time point after infection, it obviously becomes difficult to ascribe changes that are directly induced by viral signaling, as compared with those induced via feedback actions of products (e.g., cytokines) induced by viral actions. Nonetheless, the fact that differences were observed between infected and sham-inoculated control subjects implies that changes observed in gene expression relate directly to viral pathogenesis.

As is apparent from Table 3, there were few discrete functional groupings that could be discriminated among those genes that were down-regulated. On the other hand, several major categories of genes were up-regulated by HRV infections. The induction of numerous chemokines was expected on the basis of prior literature. Epithelial cells are known to be a major source of many of the chemokines listed in Table 2, as well as others reported in the complete database. Some, such as CXCL10, previously have been shown to be induced on HRV infection both *in vitro* and *in vivo* (15), whereas others, such as CCL2 and CXCL11, are known to be induced by other viral types (35). By contrast, the marked induction of some chemokines, such as CXCL13, the unique ligand for the CXCR5 receptor, was quite surprising. This ligand, for example, is normally considered to be restricted to lymphoid organs, where it regulates dendritic cell and lymphocyte homing (36). Detection in epithelial scrapings obtained from the turbinate implies a broader distribution of this chemokine, and further studies are needed to define the role of CXCL13 in the pathogenesis of HRV infections.

Our data also demonstrated induction of a number of genes generally referred to as interferon-responsive genes, several of which also were previously observed to be up-regulated on HRV infection of cultured epithelial cells (22). Induction of these genes is a common response pattern to many viral infections, although it must be noted that several viruses can induce such genes in an interferon-independent manner by direct viral activation of steps in the interferon signal transduction pathway (37–39). The detection of a limited number of genes associated with apoptotic pathways was of interest because, although some studies suggest that infection of cell lines with high doses of some rhinovirus serotypes can lead to apoptosis (40), the role of these processes in the pathogenesis of HRV infections *in vivo* remains unclear. Induction of genes associated with cell signaling, or adhesion and remodeling, was also observed. As was the case with chemokine gene induction, in each of these categories, genes induced included a mix of the expected and surprising. Indeed, novel genes in a variety of categories will require further evaluation to assess their role in disease pathogenesis.

As a first step in this regard, we chose to focus further on the group of genes associated with antiviral responses, because supplementation of endogenous antiviral responses may hold therapeutic potential. Once again, genes such as inducible nitric oxide synthase have been implicated in the antiviral response to HRV infection (19, 30, 41), while members of the 2′,5′-oligoadenylate synthase family activate the RNase L pathway, which is known to be important in defense against picornaviruses (42). HRV infection also induced molecules whose roles in defense against HRV infections are unknown. These include phospholipid scramblase 1, which has no known direct antiviral activity but has been reported to potentiate the antiviral activity of interferons (43). Of particular interest, however, was that viperin, a protein with known antiviral effects against several viruses (27–29), was the most highly induced gene in this category. We confirmed by real-time RT-PCR that this gene was induced *in vivo* in subjects with naturally acquired rhinovirus infections. We did not identify the individual viral serotypes responsible for each infection, but it is reasonable to assume that serotypes besides HRV-16 were causative agents, implying a general, rather than serotype-specific, induction of viperin by rhinoviruses. We also used real-time RT-PCR to confirm that epithelial cells were at least partially responsible for levels of viperin in nasal scrapings, because HRV-16 infection of primary cultures of human epithelial cells induced viperin expression in a replication-dependent manner. Finally, we determined that knockdown of HRV-induced viperin expression enhanced HRV-16 titers released from infected epithelial cells. These data clearly show that viperin has an inhibitory role in HRV replication. It must be noted, however, that substantial viral titers were still produced 24 hours after infection in cells with a normal viperin response, and knockdown of viperin did not significantly affect production of the chemokine IP-10. This suggests that viperin is only one component of the antiviral response, or that it plays a more important role later after infection, because induction of viperin in epithelial cultures was still increasing up to 48 hours after infection, whereas titers were measured 24 hours after infection. The lack of effect on IP-10 production may suggest that levels of

intracellular viral replication intermediates, such as double-stranded RNA, are not altered, despite the reduced release of virions into the cell supernatants. Consistent with this, one study has shown that viperin inhibits influenza virus replication by blocking viral release at the level of the plasma membrane by perturbing lipid rafts (28).

In summary, these studies represent the first comprehensive evaluation of changes in gene expression during experimental HRV infection *in vivo*. The data identify several groups of genes that are likely to contribute to disease pathogenesis, as well as to host defense and antiviral responses. We also demonstrated that viperin is induced in epithelial cells infected with HRV both *in vivo* and *in vitro*, and that this induction occurs in a manner that is dependent on viral replication. Finally, we showed that viperin is a component of the host antiviral response to HRV infection. Overall, these data provide novel insights into the host response to HRV infection and identify several novel candidate genes that require further study to clarify their role in disease pathogenesis.

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