

Interferon regulatory factor 7 is a major hub connecting interferon-mediated responses in virus-induced asthma exacerbations *in vivo*

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Background: Exacerbations are responsible for a substantial burden of morbidity and health care use in children with asthma. Most asthma exacerbations are triggered by viral infections; however, the underlying mechanisms have not been systematically investigated.

Objective: The objective of this study was to elucidate the molecular networks that underpin virus-induced exacerbations in asthmatic children *in vivo*.

Methods: We followed exacerbation-prone asthmatic children prospectively and profiled global patterns of gene expression in nasal lavage samples obtained during an acute, moderate, picornavirus-induced exacerbation and 7 to 14 days later.

Coexpression network analysis and prior knowledge was used to reconstruct the underlying gene networks.

Results: The data showed that an intricate modular program consisting of more than 1000 genes was upregulated during acute exacerbations in comparison with 7 to 14 days later. The modules were enriched for coherent cellular processes, including interferon-induced antiviral responses, innate pathogen sensing, response to wounding, small nucleolar RNAs, and the ubiquitin-proteasome and lysosome degradation pathways. Reconstruction of the wiring diagram of the modules revealed the presence of hyperconnected hub nodes, most notably interferon regulatory factor 7, which was identified as a major hub linking interferon-mediated antiviral responses.

Conclusions: This study provides an integrated view of the inflammatory networks that are upregulated during virus-induced asthma exacerbations *in vivo*. A series of innate signaling hubs were identified that could be novel therapeutic targets for asthma attacks. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Asthma, exacerbation, picornavirus, rhinovirus, gene expression, gene networks, innate immunity, interferons, systems biology

Asthma exacerbations are acute episodes of wheezing, shortness of breath, cough, and/or chest tightness, and these illnesses are responsible for millions of emergency department visits and thousands of fatalities annually in the United States.¹ Up to 80% of asthma exacerbations are triggered by viral infections, especially with the single-stranded RNA virus rhinovirus (picornavirus family), which causes the common cold.^{2,3} However, the molecular mechanisms that underpin virus-host interactions and provoke asthma attacks are not well understood. Systematic studies are urgently needed to characterize the underlying biology.

Deciphering cellular function and behavior is a challenging task because cellular processes are carried out by networks of interacting genes. These networks are likely to be highly complex in patients with acute asthma because rhinovirus infections and asthma attacks are associated with alterations in the expression of thousands of genes.⁴⁻⁹ A significant advance in the field was the discovery that gene networks are governed by universal organizing principles.¹⁰ Gene network structures are scale free and modular. In scale-free networks the vast majority of genes have few connections, whereas a few genes have many connections, behaving as hubs that hold the network together.¹⁰ The modular property refers to the organization of gene networks into smaller functional modules, which contain sets of genes that work in concert to carry out cellular processes.¹¹⁻¹³ The development of molecular profiling technologies and computational algorithms, which work backward from the observed molecular profiling data to reconstruct the underlying gene networks, now enables the systems-level study of biology.¹⁴ In this study we used coexpression network analysis and bioinformatics to provide an integrated view of the inflammatory modules and hubs that underpin virus-induced exacerbations in asthmatic children *in vivo*.

METHODS

Study population

The study population consisted of 16 children from a larger prospective study of 218 children with mild-to-moderate asthma who were followed for 18 months or until they had an exacerbation. The protocol design and follow-up have been described previously.⁹ Briefly, at enrollment, subjects were assessed by a study physician, and if necessary, adjustments were made to achieve national guideline recommendations for asthma control. When a child experienced symptoms of an exacerbation (cough, dyspnea, chest tightness, and/or wheeze), they were instructed to use albuterol

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Abbreviations used

CHUK:	Conserved helix-loop-helix ubiquitous kinase
FDR:	False discovery rate
IRF:	Interferon regulatory factor
KEGG:	Kyoto Encyclopedia of Genes and Genomes
NF- κ B:	Nuclear factor κ B
NLR:	Nucleotide oligomerization domain–like receptor
STAT:	Signal transducer and activator of transcription
TLR:	Toll-like receptor
WGCNA:	Weighted gene coexpression network analysis algorithm
XBP-1:	X-box binding protein 1

(2 puffs, 90 μ g per puff) administered through a metered-dose inhaler every 20 minutes for up to 1 hour and then every 4 hours, if necessary. A moderate exacerbation was defined as lack of symptom relief after 3 treatments, low peak flow readings (<80% of personal best), or both, and it is noteworthy that these criteria are equivalent to the American Thoracic Society/European Respiratory Society consensus.¹⁵ Participants who met these criteria were scheduled to visit the clinic within 24 hours for collection of nasal lavage samples. This research was approved by the Institutional Review Board of the University of Arizona.

Collection of nasal lavage samples

The subjects were instructed to hold their breath and tilt their heads back. Five milliliters of sterile warm 3% saline was instilled into one nostril, and 10 seconds later, the subject tilted the head forward and allowed the saline to drip from the nostril into a sterile cup. The subject was then instructed to blow slightly to maximize saline recovery. The procedure was then repeated in the opposite nostril, and the sample was stored at 4°C. After 30 minutes, the procedure was repeated once more. The samples were processed within 1 hour by using vigorous pipetting to release cells, followed by centrifugation at 800g for 10 minutes. The supernatant was removed, and the cell pellet was immediately stabilized in RNA-Later (Qiagen, Hilden, Germany). Evidence of a picornavirus infection was tested by using RT-PCR (primers: OL27-5'-CGGACACCCAAAGTAG-3'; OL26-5'-GCACTTCTGTTTCCCC-3'), as described previously.⁹

Expression profiling studies

Total RNA was extracted from nasal lavage cells with TRIzol (Invitrogen, Carlsbad, Calif), followed by RNeasy (Qiagen). The RNA samples were labeled and hybridized to Human Gene ST1.0 microarrays (Affymetrix, Santa Clara, Calif) at the Genomics Core facility, University of Arizona. The quality of the microarray data was assessed with the robust multiarray analysis algorithm (positive vs negative area under the curve [mean $\pm$ SD] = 0.74 $\pm$ 0.04; all probe set mean = 6.49 $\pm$ 0.03; all probe set relative log expression mean = 0.33 $\pm$ 0.16). The microarray data were preprocessed in Expression Console software (Affymetrix) with the Probe logarithmic intensity error (PLIER)+16 algorithm (gc background, quantile normalization, iterPLIER).^{9,16} The raw microarray data are available from the Gene Expression Omnibus repository (GSE30326).

Coexpression network analysis

Gene expression levels were compared in paired samples from 16 subjects obtained during an acute virus-induced exacerbation

and 7 to 14 days later to discriminate between relevant signals and noise. Differentially expressed genes were identified by using moderated *t* statistics, and genes that were significantly modulated at a false discovery rate (FDR)–adjusted *P* value of less than .05 were selected for further analysis.¹⁷ A coexpression network was constructed by using the weighted gene coexpression network analysis algorithm (WGCNA).^{14,16} The WGCNA algorithm uses a stepwise analytic process that begins by calculating absolute Pearson correlations for each gene pair across the samples. The correlations were raised to a power (power = 12) to emphasize stronger over weaker correlations. The topological overlap was calculated to quantify the extent at which genes have similar overall correlation patterns with other genes. The topological overlap similarity measure was subtracted from 1 to convert it into a distance measure and analyzed by using hierarchical clustering. Modules were defined from the output of the clustering analysis by using an automated algorithm.¹⁴

Module reconstruction

The list of constituent genes in each module was submitted to the Ingenuity Systems Pathway analysis tool (www.ingenuity.com). The Ingenuity Systems knowledge base is the most comprehensive database available of molecular interactions that have been manually extracted and curated from the literature. The build/connect tool was used to identify all documented molecular relationships between genes (eg, activation, inhibition, expression modulation, protein-DNA interactions, and protein-protein interactions). Genes with no known molecular interactions were removed from the analysis. Hubs were defined as genes that had 10 or more molecular interactions with other genes in the same module.

Bioinformatics

Gene Ontology terms, Swiss-Prot key words, and canonical pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) that were associated with the differentially expressed genes and modules were investigated by using the Database for Annotation Visualization and Integrated Discovery.¹⁸ This database uses a modified Fisher exact test (Expression Analysis Systematic Explorer score) to identify specific biological/functional categories that are overrepresented in the set of genes identified in the microarray study in comparison with a reference set (all genes represented on the Human Gene ST1.0 microarray). As a complementary analysis, canonical pathways from the Ingenuity Systems database were also tested for association, and this analysis was based on the Fisher exact test. The data are presented as unadjusted and Benjamini-Hochberg–corrected *P* values.

RESULTS**Gene expression profiling of virus-induced asthma exacerbations in nasal lavage cells**

To investigate the mechanisms underlying asthma exacerbations, children with mild-to-moderate asthma (*n* = 16) were followed prospectively, and nasal lavage samples were obtained during an acute, picornavirus-induced exacerbation and 7 to 14 days later. The characteristics of the study population are presented in Table I, and it is noteworthy that these were moderate exacerbations defined with criteria equivalent to the American

TABLE I. Characteristics of the study population

Characteristic	Study population
Ethnicity (%)	
Hispanic white	87.5 (14/16)
Non-Hispanic white	12.5 (2/16)
Sex (%)	
Male	62.5 (10/16)
Female	37.5 (6/16)
Age (y), mean $\pm$ SD	9 $\pm$ 3.1
Positive aeroallergen skin test result (%)	68.7 (11/16)
Ever hospitalized for asthma (%)	37.5 (6/16)
FEV ₁ at enrollment (% predicted), mean $\pm$ SD	103.4 $\pm$ 10.9
FEV ₁ at exacerbation (% predicted), mean $\pm$ SD	95.4 $\pm$ 14.2
Time to exacerbation (d), mean $\pm$ SD	151.8 $\pm$ 126.6
Medications at enrollment (%)	
Inhaled corticosteroids	31.25 (5/16)
Combination therapy	25 (4/16)
Leukotriene receptor antagonist	31.25 (5/16)

Thoracic Society/European Respiratory Society consensus.¹⁵ The cellular composition of the acute samples was predominantly macrophages (mean $\pm$ SEM, 83.9% $\pm$ 2.7%), followed by neutrophils (12.3% $\pm$ 2.5%), epithelial cells (2.2% $\pm$ 1.0%), and eosinophils (1.6% $\pm$ 0.5%). The follow-up samples contained a lower proportion of macrophages (72.1% $\pm$ 4.8%, $P = .006$), a higher proportion of epithelial cells (16.6% $\pm$ 4.7%, $P = .005$), and comparable proportions of neutrophils (9.1% $\pm$ 1.2%, $P = .6$) and eosinophils (1.7% $\pm$ 0.8%, $P = .9$).

The samples were labeled and hybridized to microarrays to investigate global changes in the patterns of gene expression. By using Bayesian statistical analyses with FDR adjustment for multiple testing,¹⁷ 1577 probe sets representing 1198 unique annotated genes were differentially expressed during the acute illness in comparison with 7 to 14 days later (FDR < 0.05, see Fig E1 in this article's Online Repository at www.jacionline.org). The vast majority of these genes (1121 genes) were upregulated during the responses, and a smaller subset was downregulated (77 genes). A series of bioinformatics resources were used to interrogate the biological functions associated with the upregulated genes. The functional category "immune response" was the most prominent signature in the data (Gene Ontology and Swiss-Prot databases, see Table E1, A and B, in this article's Online Repository at www.jacionline.org), and this accounted for 10.3% of the gene expression program. Additional signatures that were prominent in the data included genes involved in the regulation of apoptosis (7.26% of genes), defense response (7.44% of genes), response to wounding (6.28% of genes), inflammatory response (4.3% of genes), host-virus interaction (3.77% of genes), proteins found in lysosomes (3.14% of genes), response to virus (2.78% of genes), positive regulation of protein modification (2.6% of genes), innate immune response (2.24% of genes), and chemotaxis (2.15% of genes).

Biological pathways that were upregulated in the responses were investigated by using the KEGG and Ingenuity Systems canonical pathway databases (see Table E1, C and D). Of note, the pathway coverage is different in the KEGG and Ingenuity databases, and thus they provide complementary information about the data. This analysis identified multiple pathways involved in innate pathogen sensing, including the Toll-like receptor (TLR) pathway (eg, myeloid differentiation factor 2 [MD2], TLR2,

TLR5, and TLR8), the nucleotide oligomerization domain–like receptor (NLR)/inflammasome pathway (absent in melanoma 2 [AIM2]; caspase 1 [CASP1]; CASP5; heat shock protein 90 [HSP90]; NLR family apoptosis inhibitory protein [NAIP5]; NLR family, CARD domain containing 4 [NLRC4]; and nucleotide-binding oligomerization domain containing 2 [NOD2]), and the cytosolic RNA helicases (melanoma differentiation-associated gene 5 [MDA5], retinoic acid-inducible gene 1 [RIGI], RIG-I–like receptor laboratory of genetics and physiology 2 [LGP2]). Additional immune-related pathways that were identified included the interferon-induced antiviral pathway (eg, interferon regulatory factor 7 [IRF7], IRF9, interferon-stimulated gene 15-kd protein [ISG15], myxovirus resistance 1 [Mx1], 2'-5'-oligoadenylate synthetase 1 [OAS1], protein kinase R [PKR], signal transducer and activator of transcription 1 [STAT1], and STAT2), nuclear factor κ B (NF- κ B) signaling (eg, conserved helix-loop-helix ubiquitous kinase [CHUK], IL-1 receptor–associated kinase 3 [IRAK3], nuclear factor of κ light polypeptide gene enhancer in B cells 1 [NFKB1], NFKB inhibitor β [NFKBIB], v-rel reticuloendotheliosis viral oncogene homolog B [RELB], receptor [TNFRSF]–interacting serine-threonine kinase 1 [RIPK1], TRAF family member–associated NFKB activator [TANK], TANK-binding kinase 1 [TBK1], and TNF receptor–associated factor 3 [TRAF3]), death receptor/apoptosis signaling (eg, BH3 interacting domain death agonist [BID], FAS, granzyme B, perforin-1, TNF-related apoptosis-inducing ligand [TRAIL], and X-linked inhibitor of apoptosis [XIAP]), antigen processing and presentation (cathepsin B [CTSB]; cathepsin L1 [CTSL]; HSP90; legumain [LGMN]; low molecular mass protein 2 [LMP2]; LMP7; LMP10; protein disulfide isomerase family A, member 3 [PDIA3]; transporter-1 [TAP1]; and transporter-2 [TAP2]), and the complement system (complement component 1, q subcomponent, chain C [C1QC]; complement component 1, q subcomponent, chain A [C1QA]; complement component 1, q subcomponent, chain B [C1QB]; complement component 2 [C2]; complement component 3a receptor 1 [C3AR1]; CD59; and serpin peptidase inhibitor, clade G, member 1 [SERPING1]). The primary intracellular protein degradation systems (ubiquitin-proteasome and lysosome pathways) were also strongly overrepresented in the data. The downregulated genes were not significantly enriched for coherent biological functions or pathways; however, multiple olfactory receptors (OR2A1, OR2A4, OR2A7, OR2A9P, OR2M5, and OR7E5P) and speedy homologs (speedy homolog E1 [SPDYE1], SPDYE2, SPDYE5, SDPYE7P, and SPDYE8P) were identified.

Gene coexpression networks underlying virus-induced asthma exacerbations

A coexpression network was constructed by using the WGCNA algorithm, as described in the Methods section, to obtain a holistic view of the exacerbation responses.^{14,16} Briefly, WGCNA uses information derived from the patterns of gene-gene correlations across the samples to reveal the structure of the underlying gene network. As shown in Fig 1, the correlation structure of the coexpression network was characterized by a block-like pattern. This demonstrates that the exacerbation responses had a modular architecture.^{11,12} The WGCNA algorithm identified 8 coexpression modules (modules M1–M8, Fig 1); 7 of these modules were upregulated during exacerbations, and 1 module (M5)

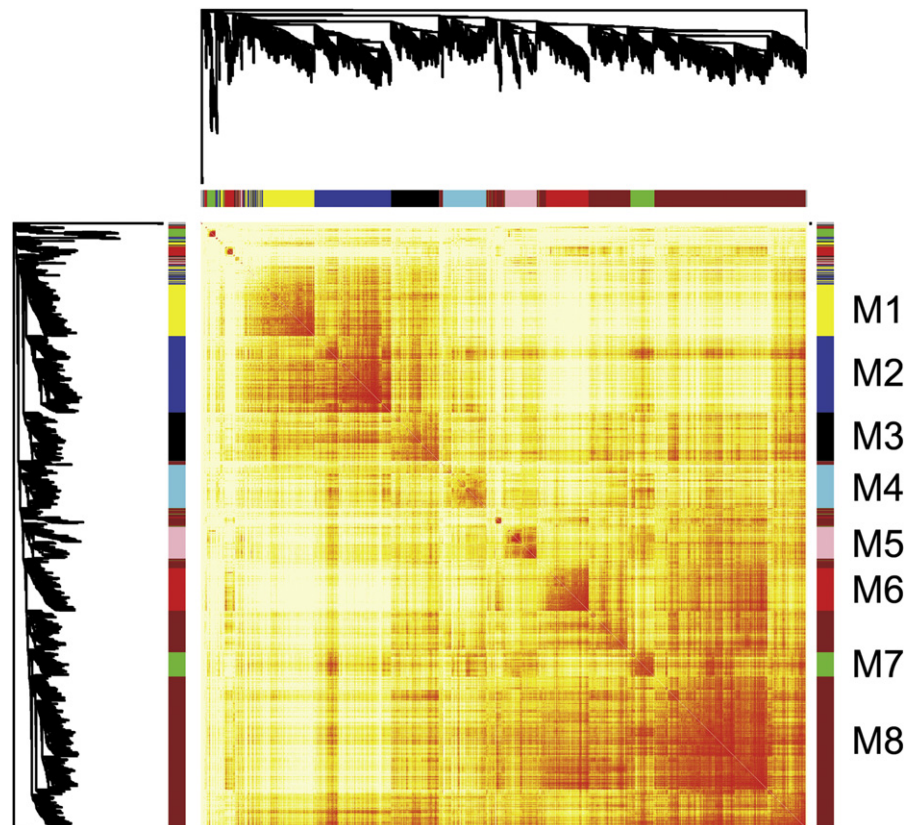


FIG 1. Coexpression network underlying virus-induced asthma exacerbations *in vivo*. Nasal lavage samples were obtained from asthmatic children ($n = 16$) during an acute virus-induced exacerbation and 7 to 14 days later. Gene expression was profiled on microarrays, and a coexpression network was constructed by using the WGCNA algorithm. The figure shows the strength of connections between genes (derived from pairwise gene-gene correlations across the samples), and stronger correlations are indicated by the increasing red intensity. The genes were arranged by means of hierarchical clustering, and the red block-like structures represent clusters/modules of highly coexpressed genes; 8 modules were identified (M1-M8).

was downregulated (see Fig E2 in this article's Online Repository at www.jacionline.org).

The first module contained 158 genes, and this module was significantly enriched for genes involved in the response to wounding, the immune response, endocytosis, chemotaxis, cell adhesion, lysosome proteins, and the complement system (see Table E2, A, in this article's Online Repository at www.jacionline.org). The second module contained 197 genes, and dominant biological signatures in this module included metallothioneins, interferon signaling, antiviral defense, antigen processing and presentation, and the protein ubiquitination pathway (see Table E2, B). The third module consisted of 120 genes, including hydrolases, proteasome subunits, and genes involved in virus-host interactions (see Table E2, C). The fourth module contained 98 genes, which were mainly involved in lysosome degradation pathways and lipid metabolism (see Table E2, D). Modules M5 and M6 contained 46 and 29 genes, respectively, and although these modules were not significantly enriched for known biological processes (data not shown), module M5 contained the olfactory receptors and speedy homolog receptors mentioned above, and module M6 contained a series of small nucleolar RNAs (small nucleolar RNA A20 [*SNORA20*], *SNORA22*, *SNORA23*, *SNORA28*, *SNORA36A*, *SNORA40*, *SNORA49*, *SNORD32A*, *SNORD32B*, *SNORD35A*, *SNORD45A*, *SNORD57*, *SNORD80*, and *SNORD82*, $P = 2.4 \times 10^{-6}$). Module M7

contained 76 genes, and this module was enriched with genes involved in immune responses (see Table E2, E). Module M8 contained 475 genes, and this module was enriched for genes involved in innate pathogen sensing (TLR pathway, NLR pathway, RNA helicases, and inflammasomes), NF- κ B signaling, and cell death (see Table E2, F).

Reconstruction of the network modules

The above analyses suggested that exacerbation responses are modular and additionally that coherent immunologic processes are enriched to a certain extent within specific modules. However, the mechanistic insight obtained above is limited because coexpression networks are based on correlations and not functional data. To obtain more detailed information in this regard, we used molecular interaction data from the Ingenuity Systems Knowledge Base to reconstruct the "wiring diagram" of the modules. The result for module M2, which contained the interferon-induced antiviral genes and is illustrated in Fig 2 (see Fig E3 and Table E3 in this article's Online Repository at www.jacionline.org for the annotated gene list), revealed that interferon regulatory factor 7 (*IRF7*) was the most highly interactive gene, harboring 45 functional interactions with other genes. *STAT1* was also a major hub within this module, harboring 27 functional interactions. Additional hubs in this module included *STAT2*,

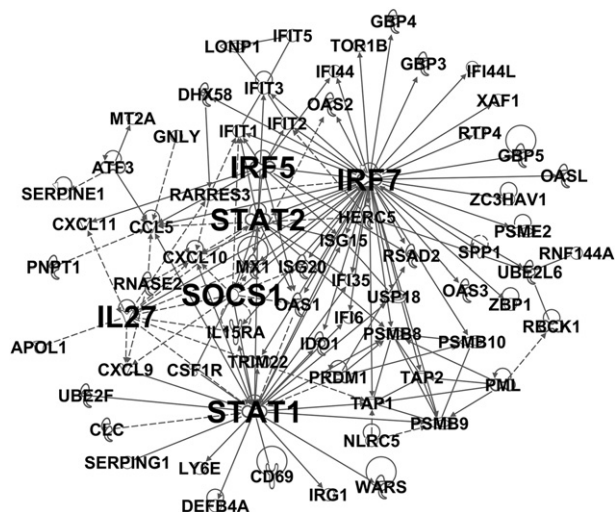


FIG 2. Reconstruction of module M2 identifies *IRF7* as a major regulator of interferon-mediated antiviral responses. The wiring diagram of module M2 from Fig 1 was reconstructed by using molecular interaction data from prior studies. See Fig E3 (in this article's Online Repository at www.jacionline.org) for the definition of the of the line types connecting the genes and Table E3 for the list of component genes in the module.

suppressor of cytokine signaling 1 (*SOCS1*), IL-27 (*IL27*), and *IRF5* (13, 12, 12 and 10 interactions, respectively). Of note, these hubs are all known regulators of interferon-induced antiviral responses (Table II).^{19,32}

A reconstruction of module M8 is shown in Fig 3 (see Fig E3 and Table E4 in this article's Online Repository at www.jacionline.org for the annotated gene list). This module contained genes involved in innate pathogen sensing, NF- κ B signaling, and cell death. *NFKB1* and *STAT3* were the dominant hubs in this module, with 20 functional interactions each. Additional hubs in this module included X-box binding protein 1 (*XBPI*), I κ B kinase- α (*CHUK*), *CASP1*, Retinoblastoma-1 (*RBI*), TANK-binding kinase 1 (*TBK1*), and *FAS* (15, 13, 12, 12, 11, 10 interactions, respectively). These hubs were mainly involved in pathogen recognition receptor-mediated signaling pathways, regulation of apoptosis, or both (Table II).

DISCUSSION

Acute exacerbations have a substantial effect on health care use and treatment costs for children with asthma. Although inhaled corticosteroids can reduce the frequency of exacerbations, they cannot entirely prevent them, and they are not effective at controlling neutrophilic inflammation, which is increasingly recognized as playing a major role in pathogenesis.³³ New drugs are urgently needed. However, selection of drug targets based on oversimplified, gene/factor-centric paradigms of inflammatory mechanisms, which focus on the role of individual effector molecules without taking into account the broader molecular context (ie, local network topology and interaction partners), is likely to be suboptimal.³⁴⁻³⁶ In contrast to previous studies in this area that have identified differentially expressed genes, pathways, or both,⁴⁻⁸ this is the first study in which the gene networks associated with picornavirus-induced asthma exacerbations have been identified. Our findings illustrate several important principles, which might stimulate further research in this area. First, we demonstrated that an intricate, modular^{9,16,37-39} inflammatory

program consisting of more than 1000 genes was upregulated during asthma exacerbations in comparison with 7 to 14 days later. Second, we showed that the modules were enriched with coherent cellular and immunologic processes. Finally, reconstruction of the modules with molecular interaction data from prior studies,⁴⁰ revealed the presence of hyperconnected hub nodes. This suggests a network structure that is tolerant to random perturbations from variations in genes and the environment but vulnerable to the targeted removal of hubs.^{16,41} These hubs therefore represent treatment targets for asthma attacks.

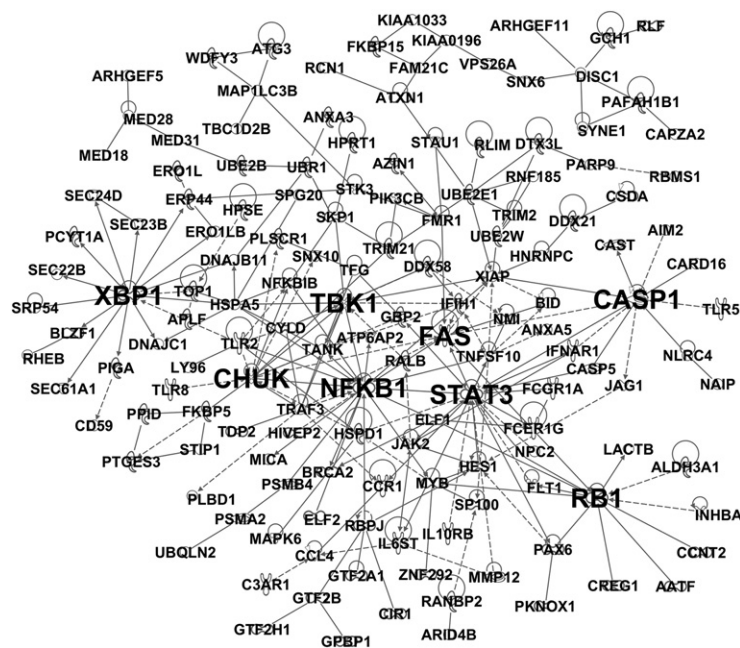
Innate immune responses to viruses are initiated when viral proteins and nucleic acids are detected by pattern recognition receptors. This activates intracellular signaling cascades that converge on the NF- κ B and IRF families of transcription factors, which translocate to the nucleus and switch on inflammatory and antiviral programs.^{19,42} Our findings provide a modular view of this paradigm, and in particular show that modules involved in "innate pathogen sensing" (module M8, Fig 3) and "interferon-induced antiviral responses" (module M2, Fig 2) are upregulated during virus-induced asthma exacerbations. The innate pathogen-sensing module contained a diverse set of pathogen recognition receptors (TLRs, nod-like receptors/inflammasomes, RIG-I-like receptors, and the IFI200 family) arranged around a series of innate signaling hubs. Previous studies have shown that the dominant hubs in this module (*NFKB1* and *STAT3*) are essential for mounting effective innate immune responses while limiting collateral damage.²⁰⁻²³ It is also noteworthy that the other hubs in this module have established roles in innate immunity (Table II).²⁴⁻²⁷

The interferon-induced antiviral module contained multiple genes downstream of interferon signaling, including archetypal antiviral effectors (*ISG15*, *OAS1-OAS3*, *Mx1*, and promyelocytic leukemia [*PML*]), chemokines (chemokine [C-X-C motif] ligand 9 [*CXCL9*], *CXCL10*, *CXCL11*, and *RANTES*), genes involved in antigen processing and presentation (*TAP1*, *TAP2*, *LMP2*, *LMP7*, and *LMP10*), and an extended cohort of genes (DEAD [Asp-Glu-Ala-Asp] box polypeptide 60 [*DDX60*]; interferon-induced protein 44-like [*IFI44L*]; interferon, α -inducible protein 6 [*IFI6*]; 2'-5'-oligoadenylate synthetase-like [*OASL*]; receptor transporter protein 4 [*RTP4*]; Mab-21 domain containing 1 [*MB21D1*]; Moloney leukemia virus 10 homolog [*MOV10*]; *RTP4*; solute carrier family 25, member 28 [*SLC25A28*]; tripartite motif containing 14 [*TRIM14*]; and unc-93 homolog B [*UNC93B1*]) that inhibit viral replication in high throughput assays.⁴³ The hubs that were identified in this module are all known regulators of interferon-induced antiviral responses (Table II).²⁸⁻³² *IRF7* was by far the most dominant hub identified in this module and in the entire analysis. *IRF7* is potentially induced by rhinovirus infections and is a master regulator of the antiviral response.^{32,44,45} *IRF7* normally resides in the cytoplasm at low levels in an inactive form, but during viral infections, signaling through pattern recognition receptors trigger phosphorylation and translocation of *IRF7* to the nucleus, where it activates expression of type I interferons. Type I interferon signaling through *STAT1* and *STAT2* in turn activates *IRF7* transcription, which further amplifies interferon expression, and thus *IRF7* mediates positive-feedback amplification of antiviral responses.⁴⁶

This study has limitations that should be acknowledged. The study population consisted of asthmatic children experiencing symptoms of a moderate virus-induced exacerbation but did not include a control group of healthy children experiencing

TABLE II. Biological functions of the hubs identified in modules M2 and M8

Symbol	Function/pathway
Module M2	
<i>IRF7</i>	<i>IRF7</i> is a master regulator and amplifier of type I interferon-induced antiviral responses. ³²
<i>STAT1</i>	<i>STAT1</i> mediates signaling by type I, type II, and type III interferons and induces an antiviral gene expression program. ^{19,28}
<i>STAT2</i>	<i>STAT2</i> mediates signaling by type I, type II, and type III interferons and induces an antiviral gene expression program. ^{19,28}
<i>SOCS1</i>	<i>SOCS1</i> is a negative regulator of IFN- α/β receptor 1 (IFNAR1)/STAT1 signaling and limits the duration of interferon-induced antiviral responses. ²⁹
<i>IL27</i>	<i>IL27</i> promotes T _H 1 differentiation and type I interferon-induced antiviral responses. ³⁰
<i>IRF5</i>	<i>IRF5</i> knockout mice have deficient type I interferon responses and are susceptible to infections with DNA and RNA viruses. ³¹
Module M8	
<i>NFKB1</i>	<i>NFKB1</i> encodes the NF- κ B p50 subunit. Knockdown of <i>NFKB1</i> attenuates TLR-induced inflammatory responses. ¹⁹ <i>NFKB1</i> has both proinflammatory and anti-inflammatory functions ^{22,23} and is involved in regulation of apoptosis.
<i>STAT3</i>	Mutations in <i>STAT3</i> underlie hyper-IgE syndrome, which is associated with recurrent pulmonary infections. ²⁰ <i>STAT3</i> protects epithelial cells from apoptosis during pulmonary viral infections. ²¹
<i>XPB1</i>	<i>XPB1</i> is a transcription factor that is activated by the endoplasmic reticulum stress sensing kinase endoplasmic reticulum-to-nucleus signaling 1 (<i>IRE1</i>). Knockdown of <i>XPB1</i> attenuates TLR2/TLR4-induced (PAM ₃ CSK ₄ /LPS) inflammatory responses ²⁴ and poly(I:C)-induced antiviral responses. ²⁵
<i>CHUK</i>	<i>CHUK</i> is a component of the I κ B kinase complex, which activates NF- κ B signaling by modulating inhibitory proteins (I κ B) that sequester NF- κ B in the cytoplasm. ²⁶
<i>CASP1</i>	Caspase-1 activation is triggered by inflammasomes and is essential for processing pro-IL-1 β and pro-IL-18 into bioactive forms. ²⁷ <i>CASP1</i> is involved in regulation of apoptosis.
<i>RB1</i>	RB1 is a tumor suppressor protein involved in cell-cycle checkpoint/arrest and regulation of apoptosis.
<i>TBK1</i>	<i>TBK1</i> is an I κ B kinase-related kinase that couples viral detection through pathogen recognition receptors to activation of <i>IRF3</i> and <i>IRF7</i> . ²⁶
<i>FAS</i>	<i>FAS</i> has a central role in regulation of programmed cell death.

**FIG 3.** Reconstruction of the innate pathogen-sensing module M8. The wiring diagram of module M8 from Fig 1 was reconstructed by using molecular interaction data from prior studies. See Fig E3 for the definition of the line types connecting the genes and Table E4 for the list of component genes in the module.

symptoms of a cold. Therefore the analyses cannot differentiate between variations in gene network patterns that are associated with viral infection as opposed to those that are specific to exacerbations. Follow-up studies in a much larger sample will be required to determine how variations in gene network patterns

underpin phenotypic patterns and to examine how disease cofactors, such as atopy, modify exacerbation responses and disease severity. The expression profiling studies were based on cells obtained from nasal lavage fluid and not the airways, and distinct cellular and molecular mechanisms might be operating in these

compartments. The nasal lavage samples comprised a mixed cell population, variations in which might potentially confound the analyses. The modules were reconstructed by using molecular interaction data from prior studies, which could have limited relevance to the current study. Therefore detailed mechanistic studies will be required to define the precise function of the hubs in the direct content of virus-induced exacerbations. Finally, because the analytic strategy incorporated prior knowledge, genes for which there are no functional interaction data available cannot be interpreted. Notwithstanding these limitations, this study provides a modular view of the inflammatory networks that are upregulated during virus-induced exacerbations in asthmatic children *in vivo*. Moreover, a cohort of innate signaling hubs exemplified by *IRF7* were identified, which are logical therapeutic targets for asthma exacerbations.

Clinical implications: This study provides a modular view of the inflammatory networks that are upregulated during virus-induced asthma exacerbations *in vivo*. *IRF7* was identified as a major hub connecting interferon-mediated antiviral responses.

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