A mechanistic role for type III interferon-λ_1 in asthma exacerbations mediated by human rhinoviruses

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ONLINE DATA SUPPLEMENT

Supplemental Methods.

In vitro infection of primary nasal epithelial cells. Primary nasal epithelial cells were collected midturbinate using flocked swabs (Copan). Cells were eluted in 4ml of small airways epithelial cell growth media (SAGM) with supplements of 2ml of bovine pituitary extract, 0.5ml of insulin, 0.5ml of hydrocortisone, 0.5ml of gentamicin, 0.5ml of retinoic acid, 5ml of bovine serum albumin fatty acid-free, 0.5ml of transferrin, 0.5ml of triiodothyronine, 0.5ml of human epidermal growth factor and 0.5ml of epinephrine (Lonza). The media was supplemented with 1% amphotericin–B (Sigma) and 2% heat inactivated fetal bovine serum (FBS, Atlanta Biological Inc). Cells were cultured in T25 tissue culture flasks with 2% SAGM media until 80-90% confluent, and then split into 24 well plates. Cells were incubated at 37°C and media changed every two days until cells reached 80-90% confluence. Virus was grown in MRC-5 cells, harvested, and frozen in aliquots. MRC-5 cells were grown and prepared identically to virus production for use as control. Four hours prior to infection, supernatant was poured off and fresh media (2% SAGM) was added. Twelve wells were infected with 100µl/well of HRV16A at 2.1 x 10^4 pfu/ml and 12 wells with inoculated with MRC-5 supernatant as control. Plates were rocked at room temperature for 1 hour, 1ml/well of fresh media (2%SAGM) was added, supernatant was collected, and cells were placed in lysis buffer (Roche). This was designated time point 0. The remaining wells were then

incubated at 37°C and supernatants and cell fractions collected at 1.5, 24 and 48 hours. 24-well plates of A549 cells were also grown in parallel. Twelve wells were infected with HRV16A strain and 12 with MRC-5 supernatant as above, and supernatant and cells were collected at time points 0, 1.5, 24 and 48 hours. Cells were placed in lysis buffer and RNA was extracted using MagnaPure (Roche). Real-time RT-PCR for HRV was performed as previously described. Cytokine supernatant proteins were analyzed by ELISA (R&D Systems DY1598B) and mRNA was measured using real-time RT-PCR (Applied Biosystems 438739M).

Supplementary Table 1. Socioeconomic status in study participants.

Population under the poverty line * (%)	71
Household overcrowding ** (%)	59
Public water system (%)	79
Sewer drain in house (%)	42
Literacy in parents (%)	
-Complete elementary school	63
-Complete high school	23

* income less than US$ 161/month
** more than three people per room

References:

1. National Institute of Statistics and Census, Ministry of Economy, Argentina. Censo nacional de población, hogares y viviendas. 2001.
2. Latinoamerican Foundation of Economic Research. Valorización de la canasta básica alimentaria y canasta básica total. 2010.

Supplementary Table 2. Baseline asthma status.*

	Days with Symptoms	Nights with Symptoms	FEV1
Severe Persistent	Continual	Frequent	≤60%
Moderate Persistent	Daily	≥5/month	60-80%
Mild Persistent	3-6/week	3-4/month	≥80%
Mild Intermittent	≤2/week	≤2/month	≥80%

*From the National Asthma Education and Prevention Program of the National Heart, Lung, and Blood Institute, and the National Center for Environmental Health of the Centers for Disease Control and Prevention MMWR: Recommendations and Reports, Vol. 52/No. RR-6, March 28, 2003.

Supplementary Table 3. Modified functionalized Global Initiative for Asthma (GINA) asthma exacerbation severity scheme.

	Mild	Moderate	Severe	Respiratory Arrest Imminent
Breathlessness	When walking Can lie down Talks in sentences	When talking Prefers sitting Talks in phrases	At rest Sits hunched forward Talks in words	At rest Difficult to speak
Respiratory Rate Normal: **1-5 years <40/min** **>6 years <30/min**	Increased	Increased	Increased	Decreased
Alertness		Agitated	Agitated	Drowsy, unresponsive, somnolent
Accessory Muscle Use	No	Yes	Yes	
Wheeze	Expiratory Only	Inspiratory and expiratory	Inspiratory and expiratory	None
Pulse/min	<100	100-120	>120	Bradycardia
SaO2 on Room Air*	>95%	91-95%	<90%	

***PaO2/PaCO2 and post-treatment FEV1 were not assessed in all patients and thus were not include in modified score.**

Based on the 2006 GINA report: www.ginasthma.org.

Supplementary Table 4. Allergy Specific IgE Test System (MyAllergyTest® System) for measurement of Serum-specific IgE antibodies to 10 common allergens.

Aeroallergens Tested
Timothy Grass
Bermuda Grass
Mountain Cedar
Short Ragweed
Alternaria (mold)
Cat Hair
Mite (pteronyssinus)
Food Allergens Tested
Milk
Egg White
Wheat

Supplementary Table 5. Comparison of epidemiologic and clinical characteristics between patients analyzed for IFN-λ1 or not.

	All Children	***All Children***		
	(n=409)	Children not analyzed for IFN-λ_1 (n=212)	Children analyzed for IFN-λ_1 (n=197)	p-value
Age	8.77	8.46 (8.07, 8.84)	9.11 (8.71, 9.51)	0.020
Female	174 (42.54%)	83 (39.15%)	91 (46.19%)	0.150
Baseline Severity Mild Intermittent Mild Persistent Moderate Severe	 99 (24.21%) 132 (32.27%) 167 (40.80%) 11 (2.61%)	 42 (19.81%) 66 (31.33%) 99 (46.70%) 5 (2.36%)	 57 (28.93%) 66 (33.50%) 68 (34.52%) 6 (3.05%)	0.056
Prior Asthma Hospitalizations 0 1 $\geq$ 2	 156 (38.24%) 53 (12.99%) 199 (48.77%)	 87 (41.04%) 27 (12.74%) 98 (46.23%)	 69 (35.03%) 26 (13.20%) 102 (51.78%	0.443
Wheezing Yes No	 200 (48.90%) 209 (51.10%)	 101 (47.64%) 111 (52.36%)	99 (50.25%) 98 (49.75%)	0.597
Mother with asthma	69 (16.87%)	32 (15.09%)	37 (18.78%)	0.320
Mother with allergies	55 (13.45%)	30 (14.15%)	25 (12.69%)	0.665
Human Rhinovirus positive	180 (46.04%)	90 (46.39%)	90 (45.69%)	0.889