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Rhinovirus Specific IgE Can Be Detected in Human Sera

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Capsule summary

Rhinovirus specific IgE is specifically detected in the sera of exposed humans, and may provide insight into the mechanisms through which viral infections may drive development and exacerbate of atopic diseases.

Keywords

Rhinovirus; IgE; Asthma; Atopy; viral immune response

To the Editor

Respiratory viral infections, such as those from respiratory syncytial virus (RSV) or human rhinovirus (HRV), have been implicated in the development of asthma and atopic disease, as well as in causing asthma exacerbations.¹ However, the mechanisms through which viruses lead to atopic disease and asthma exacerbations remain elusive.

Using a mouse model and the rodent parainfluenza virus type 1 (Sendai virus, SeV), we have shown that development of post-viral atopic disease requires crosslinking of FcεRI on lung dendritic cells by antiviral (i.e., anti-SeV) IgE.² Humans studies have documented that anti-RSV IgE is made as part of the antiviral response to RSV infection, and that the level of IgE correlated with the subsequent risk for wheeze.³ Other viral infections appear to also drive antiviral IgE production, such as anti-H1N1 IgE, which was shown to persist even after clearance of the initial viral infection.⁴

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Human rhinovirus is a nonenveloped, single stranded RNA virus belonging to the family *Picornaviridae* and is one of the most prevalent respiratory viral pathogens associated with development of asthma. In fact, in at least one study, HRV infection conferred a greater risk of asthma than did RSV.⁵ However, it is not known if humans make antiviral IgE against HRV.

Indirect evidence that anti-HRV IgE may play a role in asthma exacerbations comes from the recent Inner-City Anti-IgE Therapy for Asthma (ICATA) study, where, removal of IgE by treatment with omalizumab markedly and significantly abrogated exacerbations of asthma.⁶ Therefore, we hypothesized that that part of the immune response to HRV would include the development of antiviral anti-HRV IgE.

While HRV infection is ubiquitous, there are over 150 HRV serotypes with many more distinct HRV strains circulating in the human population. These HRV strains belong to three genetic clades (A, B, and C). Rhinoviruses elicit serotype-specific antibody responses, and individuals are typically infected repeatedly with different strains.⁷ In order to test our hypothesis and to avoid issues involving differences in serotypes, we utilized a lab strain of HRV (HRV-39, ATCC VR-340) that is not found naturally circulating, and developed an enzyme-linked immunosorbent assay (ELISA) to detect anti-HRV39 specific IgE in the sera from subjects that had or did not have a history of being exposed to HRV39.

The study protocol was approved by the institutional review board (IRB) at the participating institutions, and peripheral blood was obtained following informed consent. HRV39 was propagated in HeLa cells to a concentration of 2×10^7 PFU/mL similar to what we have done with other *Picornoviruses*.⁸ UV-inactivated virus at a 1:10 dilution in phosphate buffered saline (PBS), or control (PBS alone), was fixed to the bottom of wells of 96-well EIA/RIA plates (Costar 9018) overnight at 4°C. After overnight incubation the plates were washed with PBS/0.05% Tween 20, blocked with PBS and 10% fetal calf serum (FCS), and then incubated for 2 hours at room temperature with 0.1 mL subject serum samples. Adult subjects with (n=8) or without (n=2) neutralizing antibodies to HRV39 were compared to cord blood serum (n=4) from deidentified donors as a negative control.⁹ Five subjects had an atopic history (2 with allergic rhinitis, 1 with mild asthma, 1 with atopic dermatitis and allergic rhinitis, and 1 asymptomatic with a history of positive skin testing), and 3 of these had neutralizing antibodies to HRV39 (the 3 subjects with a history of allergic rhinitis). The plates were then washed and incubated for an additional hour at room temperature with 0.1 ml of biotin-labeled goat anti-human IgE (Kirkegaard and Perry 16-10-04) diluted 1:500 in PBS/10%FCS. Following an additional washing, 0.1 ml of ExtraAvidin-Peroxidase (Sigma, E2886) diluted 1:500 in PBS/10%FCS was added and incubated for 30 min. Tetramethylbenzidine was then used to develop the staining, and the resulting optical density (OD) at 450nm was read on a plate reader (Bio-Tek Instruments ELx808 Absorbance Microplate Reader). Total serum IgE level in the subject's serum was also determined using a commercially available ELISA kit (eBioscience 88-50610-22) according to the manufacturer's instructions.

As shown in figure 1A, anti-HRV39 IgE could be detected in all samples to varying degrees. Importantly, this was specific and dose dependent (figure 1B). Significantly higher levels

were found in the serum of subjects with neutralizing antibodies to HRV39 (OD 0.62–2.95) compared to those with no neutralizing antibodies (OD 0.73–0.99, $p=0.026$) or cord blood (OD 0.43–0.62, $p=0.0039$). It is interesting to note that patients with no known exposure to the virus generally had lower OD values than those with reported exposure (note dotted line in Figure 1A). Whether this is due to cross-reactivity between rhinovirus serotypes or distant exposure is unknown. There was no relationship between atopic status and presence of HRV39 specific IgE (1.69 ± 0.29 (mean OD $\pm$ sem) for non-atopic subjects versus 1.41 ± 0.43 for atopic subjects, $p=0.60$; in figure 1 compare triangles (atopic subjects) and circles (non-atopic subjects)). As shown in figure 1B (right panel), the ELISA is specific for HRV39, as addition of HRV39 to subject's serum significantly inhibited binding to the level seen in subjects without an exposure history. We believe OD values above 1.5 represent presence of anti-HRV39 IgE, while those between 1.5 and 1.0 are indeterminate, and below 1.0 indicate no evidence of anti-HRV39 IgE. Importantly, total IgE levels did not correlate with anti-HRV39 specific IgE (figure 1C, Pearson $p>0.05$).

To our knowledge, this study is the first to show that IgE antibodies against rhinovirus (at least a laboratory strain) can be found in the sera of human subjects who have been exposed to that serotype, and adds to a growing body of evidence supporting a role for antiviral IgE as part of the natural antiviral immune response. It is important to stress that these data alone do not support the hypothesis that antiviral IgE is causative in the development of post-viral atopic disease, but do begin to provide potential mechanistic explanations for why omalizumab might reduce asthma exacerbations outside of the pollen season. Further, based on our studies in mice, these data support the model where IgE against a respiratory virus drives the de novo development of post-viral atopic disease (like asthma), and provides some tantalizing evidence that the pathways identified in mice might be operative in humans.

These data provide the initial support for further investigations focusing on pre/post exposure to HRV (and other respiratory viruses) and the role of antiviral IgE in the immune response to respiratory viruses, as well as the development and exacerbation of atopic diseases. We acknowledge the limitation of the small sample size, as well as the issue of potential cross-reactivity of anti-HRV39 IgE with other HRV strains. It is interesting to note that anti-HIV-1 IgE has been shown to inhibit HIV-1 production in infected PBMC cultures.¹⁰ Whether antiviral IgE is a purposeful or maladaptive immune response, it nonetheless appears to contribute to the exacerbation and perhaps development of atopic disease. Taken together, our study helps to shed light on the role viral infections might play in the development and exacerbation of atopic disease, and provides some additional rationale for future studies exploring therapeutic strategies to reduce or prevent the development of atopic disease.

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Abbreviations

RSV Respiratory syncytial virus

HRV	Human rhinovirus
SeV	Sendai virus
ELISA	Enzyme-linked immunosorbent assay
OD	Optical density

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Figure 1. Human Rhinovirus-39 specific IgE can be detected in the sera of individuals with a known exposure to the virus

(A) Optical density (450 nm) of anti-HRV39 IgE from the sera of individuals with neutralizing antibodies to HRV39 (red, n=8), no neutralizing antibodies (blue, n=2), cord blood (green, n=4), or control blanks (black, n=3). Individuals with history of atopic disease are indicated in triangles, while those without are in circles. Significantly more anti-HRV39 IgE was detected in those subjects with known exposure to the virus. Data compared using an unpaired t test with Welch's correction. (B) Left panel is representative dilutions of samples with neutralizing antibodies (red) compared with cord blood (green). Right panel represents neutralization of one sample (mean $\pm$ sem). The white bar is the optical density (OD) from the undiluted sample, while the black bars were diluted 1:2. The addition of HRV39 (+) to the diluted sample significantly reduced the OD. (C) Total serum IgE levels did not correlate with anti-HRV39 specific IgE (colors and symbols as in (A)). Pearson $R^2 = 0.30$; $p > 0.05$.