
Allergy skin test responses during experimental infection with respiratory syncytial virus

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Background: Allergy skin testing is one of the most frequently performed physician office procedures. Many factors can affect the results of those tests, including the well-defined suppressive effect of systemic antihistamines. False-positive allergen skin test results are known to occur; however, contributing factors are not well understood.

Objective: To determine whether a viral upper respiratory tract infection affects allergy skin test responsiveness.

Methods: We performed skin tests with histamine and a panel of geographically relevant inhalant allergens on 16 adults before and 3, 6, and 21 days after experimental exposure to respiratory syncytial virus (RSV), a virus that causes signs and symptoms of a cold.

Results: The RSV exposure, with and without documented infection, caused increased wheal and flare areas to histamine and allergen and de novo positive allergen test responses in individuals with no measurable responses at baseline. These were noted as late as 21 days after RSV exposure and may be consistent with mediation by up-regulated neurogenic inflammation during RSV infection.

Conclusion: These results may have implications for explaining the cause of such well-known complications of RSV infection as otitis media, bronchiolitis, and asthmatic exacerbation.

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INTRODUCTION

Allergic diseases, including allergic rhinitis and asthma, are increasing in prevalence worldwide.^{1,2} Respiratory viral infections are also common and may contribute to the observed epidemiologic shifts.³ Several studies, including some using a model of experimental viral infections in adults,⁴ highlight the complex interplay between allergens and viruses in the development and expression of diseases characterized by airway inflammation. Allergen sensitization at a young age and specific types of respiratory virus infections in infancy are recognized as risk factors for the later development of asthma.^{5,6} Furthermore, there are differences between atopic and nonatopic individuals in the illness expressions of a viral respiratory tract infection (RTI) and within-subject differences in the responses of the nose and lungs to allergen exposure between periods with and without virus infection.^{7–10} However, to our knowledge, no study has evaluated the possible effects of those infections on allergy skin test responses.

Because of the increasing prevalence and recommendations forthcoming from recently published guidelines,^{1,2} an increasing proportion of the pediatric and adult population is being tested for allergen sensitivities. Although serum testing

for allergen specific IgE is available, skin testing is the preferred method of diagnosis because of its high sensitivity and the immediate availability of diagnostic results. Many factors affect the results of allergen skin testing, including the site of application and the presence or absence of dermatographism or dermatitis.¹¹ Perhaps the best known of these factors is concurrent antihistamine use, which causes false-negative results and necessitates inclusion of a positive histamine control in the test panel. Despite the high prevalence of viral upper RTIs in all the age groups commonly tested for allergies, it is not known whether a concurrent viral upper RTI affects the outcome of allergy skin testing. Either an enhancing or a suppressive effect of concurrent virus infection would suggest added vigilance with respect to each patient's history of viral upper RTI and caution in interpreting the results of allergen skin testing. The purpose of this study was to determine whether a viral upper RTI affects allergy skin test responsiveness. The human experimental infection model was chosen because of the controls it offers,⁴ and respiratory syncytial virus (RSV) was chosen as the infecting agent because it infects almost all children during the first 2 years of life and regularly reinfects and causes common cold symptoms in older children and adults.¹²

MATERIALS AND METHODS

The study protocol was approved by the Human Rights Committee of the Children's Hospital of Pittsburgh. Adults were recruited from the University of Pittsburgh and the surrounding community. Written informed consent was obtained before screening from all the participants who presented for enrollment. Applicants provided a general health

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history, with specific references to allergic diseases, and were given ears, nose, throat, and general physical examinations. Blood specimens were collected for complete blood cell counts and were assayed for a panel of hepatic and renal enzymes, the presence of human immunodeficiency virus antibodies, and preinoculation serum antibody titers to the challenge virus. Urine specimens were collected and submitted for urinalysis. Potential participants were excluded if they had a history of nasal or otologic surgery; a history of cardiovascular or other serious disease; symptomatic upper RTIs in the past 30 days; concurrent nasal or otologic disease; abnormal clinical profiles on urinalysis, complete blood cell counts, or blood enzyme determinations; or seropositivity for human immunodeficiency virus. Potential participants were also excluded if they had a history of asthma, a forced expiratory volume in 1 second less than 80% of predicted, or a 20% decrease in forced expiratory volume in 1 second after provocative inhalation challenge with methacholine at a concentration of less than 25 mg/mL. Women with positive urine pregnancy test results on presentation to the cloister site were also excluded.

Sixteen patients were enrolled and cloistered in individual rooms at a local hotel for 9 days (study days 0–8).¹³ Approximately 8 hours after admission to the cloister site, the patients were intranasally inoculated with 10⁶ plaque-forming units of a safety-tested clinical isolate of RSV type B (supplied by Brian Murphy, MD, of the National Institute of Allergy and Infectious Disease under a material transfer agreement). Daily during the period of cloister, patients were followed by self-reported symptom scores; ears, nose, throat, and general physical examinations, including vital sign and temperature recording; and evaluation of nasal secretion production. Nasal lavages were performed, and recovered fluids were immediately aliquoted, placed into viral transport medium, and stored at –70°C. Blood samples were obtained on study days 0, 2, 4, 6, 8, and 10 and were assayed for total serum IgE antibodies using the fluorescent alloabsorbent test (FAST; BioWhittaker Inc, Walkersville, MD). Immediate-hypersensitivity skin responses were tested on days 0, 3, and 6 by the modified prick method using standard solutions of relevant inhalant allergens, including ragweed mix, grass mix, English plantain, tree mix, cocklebur, lamb's quarters, pigweed, *Alternaria*, aspergillus mix, *Helminthosporium*, *Hormodendrum*, *Penicillium*, *Epicoccum*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat, and dog (Hollister-Stier Laboratories, Spokane, WA). Positive (histamine) and negative (isotonic sodium chloride) control solutions (Hollister-Stier Laboratories) were also used. During the study, participants refrained from taking any over-the-counter or prescription medications except birth control pills. On study day 21, patients returned to the Children's Hospital of Pittsburgh, and blood samples were obtained for the determination of convalescent serum antibody titers and serum total IgE levels. Allergy skin tests were repeated. One aliquot of lavage fluid from each study day was thawed and assayed for RSV antigen using a commercially available rapid en-

zyme immunoassay (BD Biosciences, Le Pont de Claix, France).¹⁴ The sensitivity and specificity of this assay are 94% and 95%, respectively. A second aliquot was shipped overnight on dry ice to the University of Rochester, thawed, and then inoculated onto HEP-2 cells to detect the presence of RSV using standard techniques.¹⁵ Paired serum samples (baseline and convalescent) were tested for RSV specific IgG levels using an end titration enzyme-linked immunosorbent assay.¹⁵ A patient was considered to be infected if the challenge virus was detected by enzyme immunoassay or culture on at least 1 study day after inoculation or if a 4-fold or greater increase in specific serum antibody titer to the challenge virus between the prechallenge and convalescent samples was documented.

For allergen skin testing, the same operator placed each test at least 2 cm from a neighboring test on the arm using a bifurcated needle to perform the modified prick technique¹¹ and imaged and recorded the results at 20 minutes using ink and transparent tape. The areas of the wheal-and-flare responses were calculated using computerized digitization. A response was considered positive if the wheal area was at least 10 mm² larger than that obtained using the negative control. Repeated tests were applied to alternating forearms, and the exact site of application on the forearm was varied. All skin testing was performed by a single clinical technician (B.A.) who had several years of experience in performing the procedure.

For immediate-hypersensitivity skin testing, the data available for analysis consisted of the measurements of histamine- and allergen-induced wheal and flare areas for all patients recorded before RSV exposure and on days 3 and 6 and for 16 patients on day 21 after RSV exposure. Both measured variables, such as wheal and flare areas for histamine and allergen skin tests, and constructed variables, such as the number of positive responses to the allergen panel and the number of patients with a positive response to each allergen, were compared between the pre-RSV exposure time and each of the 3 postexposure times. All continuous variables were first evaluated for effects of allergy and infection status using a repeated-measures analysis of variance, with variance partitioned for effects of study day, infectious status, and baseline atopic status. For each variable, comparisons of paired time data were made using a 2-tailed Wilcoxon signed rank test for differences in medians, with significance evaluated at $\alpha = .05$. Data are given as mean $\pm$ SD. All statistical procedures were run using a statistical software package (NCSS, Kaysville, UT).

RESULTS

Sixteen healthy adults (9 men and 7 women; 13 whites and 3 nonwhites; age, 32.4 ± 7.1 ; age range, 23–45 years) were enrolled after providing written informed consent. Based on baseline allergy skin testing (≥ 1 positive skin test responses to inhalant allergens), 8 (50%) of the patients were classified as atopic and 8 as nonatopic. Eleven patients (5 atopic and 6 nonatopic) had evidence of RSV infection (viral detection on

at least 1 day after exposure or a 4-fold increase in specific serum antibodies). Results for the primary variables related to infection, signs, symptoms, and pathophysiologic features of experimental RSV type B infection were previously described,¹⁰ and the present study is limited to the effects of RSV on the measures of allergy.

Total serum IgE measurements were significantly different between atopic (123 ± 80 IU/mL) and nonatopic (45 ± 36 IU/mL) patients at baseline ($P < .01$). No patterned change in these levels was observed for nonatopic patients, but a non-significant increase was noted for atopic patients on days 8 and 10 only (peak, 146 ± 112 IU/mL).

At no time did any individual have a wheal or flare reaction to the immediate skin test with diluent, but all showed marked responses to histamine. Baseline wheal and flare areas measured 14.7 ± 11.1 and 398.6 ± 159.5 mm² in allergic patients and 17.7 ± 8.3 and 269.0 ± 261.4 mm² in nonallergic patients, respectively. Between-group differences were not statistically significant. Analysis of variance identified a significant effect of study day ($P < .03$) but not of allergic or infection status on the wheal and flare responses to histamine (Figure 1). Compared with baseline, there was a significant increase in the mean wheal response to histamine on days 3, 6, and 21 and in the flare response on days 6 and 21 ($P < .03$).

Figure 2 shows the cumulative distribution of patients as a function of the number of antigens provoking a positive wheal response. Of the 8 nonatopic patients, only 3 had negative wheal reactions to all antigens in the panel on all 4 test days. The numbers of positive wheal responses for the patients were 1.7 ± 2.3 , 4.4 ± 3.8 , 3.6 ± 3.5 , and 3.9 ± 3.6 at baseline and on days 3, 6, and 21, respectively. All pairwise comparisons with baseline were significant ($P < .01$). This pattern of increased responses was not different between groups with and without RSV recovery from the nasal lavage.

Figure 3 shows the percentage of patients ($n = 16$) who had a positive wheal response to each antigen on each of the

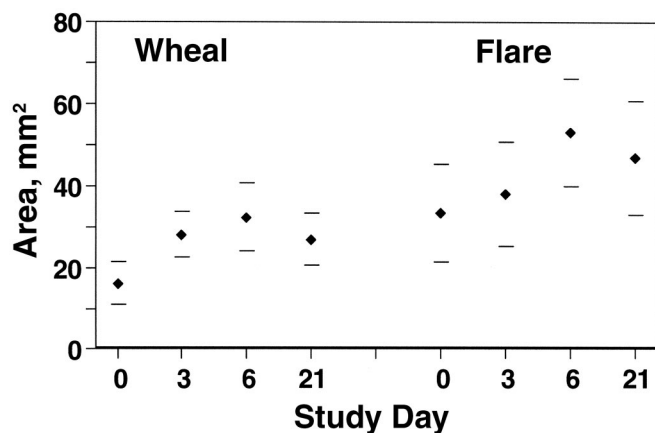


Figure 1. Average histamine wheal and flare (divided by 10) areas for tests performed at baseline and on days 3, 6, and 21 after respiratory syncytial virus exposure. Error bars represent 95% confidence intervals.

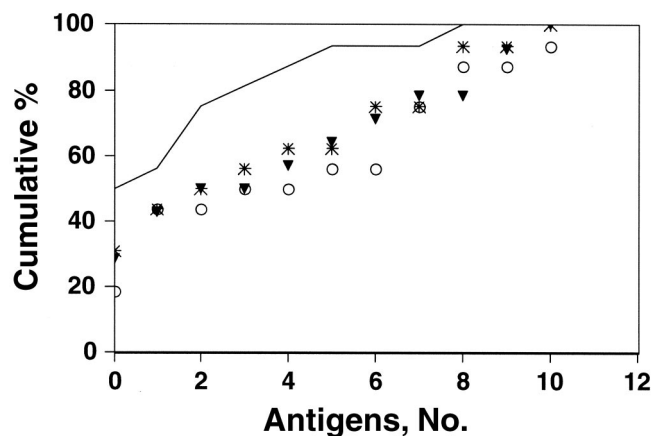


Figure 2. Cumulative distribution of patients for each test day as a function of the number of antigens provoking a positive skin response. The line indicates baseline; circles, day 3; asterisks, day 6; and triangles, day 21.

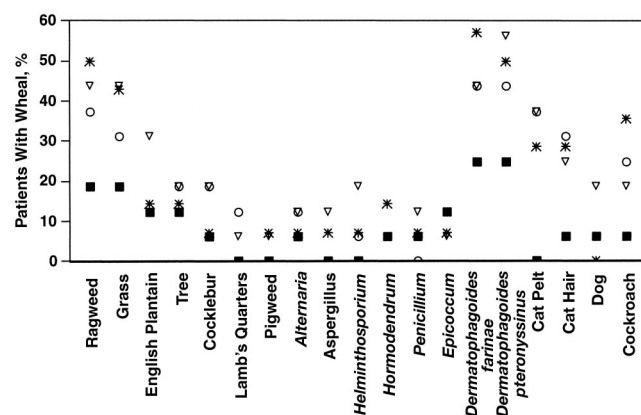


Figure 3. Patients ($n = 16$) with positive skin test responses to each allergen for tests conducted before and on days 3, 6, and 21 after respiratory syncytial virus exposure. Squares indicate baseline; triangles, day 3; circles, day 6; and asterisks, day 21.

4 test days. These results show that the increased number of responses after RSV exposure was an across-the-board effect and was not limited to individual allergens or classes of allergens (eg, seasonal). In general, for individuals with positive responses to specific allergens at baseline, the mean wheal and flare areas provoked by those allergens were increased after RSV exposure (data not shown). As with the histamine responses, this increased responsiveness was not affected by postexposure recovery of RSV from the nasal lavage.

DISCUSSION

The results of this study show that RSV exposure can enhance the wheal and flare responses to type I allergic hypersensitivity skin tests in atopic individuals. Also, individuals with no responses to those tests before RSV exposure can develop measurable responses after exposure. Indeed, of pa-

tients manifesting negative responses at baseline, approximately 60% developed positive responses using the same criteria at some time during the acute and convalescent period after RSV exposure. We are unaware of previous studies describing this unexpected phenomenon, an omission most likely related to the inherent difficulties in conducting clinical studies of naturally acquired human viral infections.⁴ These difficulties include (1) identification of patients after illness presentation, which precludes knowledge of preinfection status; (2) the asymptomatic and unsuspected nature of many upper respiratory tract virus infections, which confounds accurate assignment of patients into comparison groups; (3) similarities in illness presentation for many upper respiratory tract viruses and insensitive clinical tests, which prevent definitive identification of the etiologic agent; and (4) variability in the "incubation" period between infection and illness, which makes inoculation onset indeterminable. The significance of these limitations is demonstrated in the present study, where neither overt symptoms nor signs of illness or recovery of RSV from nasal washings was prerequisite for the development of augmented allergy skin test responses. This emphasizes the importance of highly controlled clinical studies that use experimental virus exposure and close follow-up of patients with respect to infection, illness expression, and complication for elucidating the less salient effects of upper respiratory tract virus infections.

Allergy skin testing is one of the most frequently performed physician office procedures, and it is being used with increasing frequency to differentiate between allergic and nonallergic triggers for the development of rhinitis and asthma.^{1,2,16} Many factors can affect the results of these tests, as illustrated by the dampening effect of systemic antihistamine therapy.¹¹ Although most described factors cause false-negative results, irrelevant or false-positive allergen skin test results may occasionally occur.^{11,17} One study reported that clinical sensitivity, defined as a positive response to nasal challenge and natural pollen exposure, was observed in only 46% of individuals with a positive skin prick test response to timothy grass.¹⁷ The etiology of these responses heretofore lacked satisfactory explanation, and it has been attributed to irritant reactions or impurities, contaminants, or nonspecific mast cell secretagogues in the extracts.¹¹ The results of the present study suggest that RSV exposure is one possible cause of these false-positive test results. Other possible explanations, including the presence of dermatographism and the enhancement of a reaction by a neighboring skin test, are unlikely due to control for these factors in the present study.

Enhanced allergen- and nonallergen-provoked upper and lower airway responses during and after virus infection were previously documented.¹⁸ For example, infection of allergic patients with rhinovirus 16 caused increased lower airway hyperreactivity and increased the frequency of late-phase allergic asthmatic responses,⁷ effects possibly attributable to up-regulation of mediator release¹⁹ or tissue eosinophil infiltration.²⁰ Also, lower airway hyperresponsiveness and enhanced airway sensitization to allergen were demonstrated in

a murine model of RSV infection,²¹ and bronchial hyperactivity after early childhood bronchiolitis, mainly due to RSV infection, has been well documented.²² Studies^{8,9} in our laboratories documented increased upper airway responses to histamine and to cold air exposures during respiratory virus infection. The results of the present study extend these observations to include RSV as the viral stimulus and the skin as the hyperresponsive target organ.

One shortcoming of the present study is the lack of a control group that did not get inoculated with RSV. Inclusion of such a group would have allowed us to explore the possibility that serial skin testing alone accounted for some of the increased sensitivity. Other shortcomings relate to failure to correlate enhanced skin test results with each patient's history of rhinitis or other manifestations of allergy and failure to measure RSV specific IgE antibodies or to perform skin testing with RSV antigen.

The mechanism(s) underlying RSV-induced augmentation of allergen skin test responses is not known. It is possible that increased production of specific IgE antibody or increased histamine releasability, both of which were observed for in vivo or in vitro virus infections, could play a role.^{19,23} The de novo development of positive allergen skin test responses supports RSV stimulation of allergen specific IgE antibody production, and earlier studies reported increases in RSV specific IgE antibodies in infants with RSV bronchiolitis.^{24,25} Interestingly, RSV bronchiolitis during the first year of life was an important risk factor for the development of sensitization to common allergens during the subsequent 2 years.²⁶ Other studies showed that RSV infection provokes pro-IgE T_H2 cytokine production,²⁷⁻³⁰ and studies in mice^{31,32} and in human T-cell lines³³ showed that RSV protein G alone induced these T_H2-like responses. However, our observation that the histamine skin response was also increased and our failure to detect significant increases in total serum IgE antibodies during RSV infection argue against an exclusive role for augmented IgE antibody production as driving the observed effect. Measurement of specific serum IgE levels in the present trial would have helped clarify this issue; however, this was not performed owing to financial constraints. Alternatively, it is conceivable that RSV infection could have augmented mast cell releasability; however, this was also not performed owing to financial constraints.

Histamine- and allergen-induced wheals are largely due to local histamine-mediated effects on vascular permeability,³⁴ whereas flare responses were shown to be mediated by neurogenic inflammatory pathways that involve axonal release of substance P.^{35,36} Substance P has marked effects on vascular permeability and endothelium-dependent vasodilation via its high affinity for the neurokinin 1 receptor subtype,³⁷ and skin reactivity to substance P is greater in allergic asthmatic patients compared with controls.³⁸ The development of neurogenic inflammation during an RSV infection is well described for the rat.^{39,40} There, up-regulation of neurokinin 1 receptors was demonstrated in the lower airways of infected animals after endotracheal inoculation and was observed despite the

absence of recoverable virus or viral antigens in the proximal airways.⁴¹ A specific role for RSV protein G or SH in increasing pulmonary substance P and neurogenic inflammation was also suggested.⁴² The observation of effects at sites distal to the area of infection suggests mediation by circulating cytokines, such as granulocyte-macrophage colony-stimulating factor, interleukin 6,⁴³ RANTES,⁴⁴ or interleukin 11.⁴⁵ Indeed, elevated local nasal and systemic levels of a variety of cytokines and proinflammatory mediators were reported for upper RTI with rhinovirus, influenza virus, and RSV.^{46–51} For these reasons, we hypothesize that the effect of RSV exposure on the skin test responses is mediated by up-regulated neurogenic inflammation, and this is a focus of the present study.

In summary, the present study shows that experimental RSV infection in a few adults caused de novo and enhanced skin responses to histamine and allergens. This phenomena may be consistent with RSV up-regulation of pathways related to neurogenic inflammation, but this mechanism remains speculative and needs to be evaluated in future studies. Nonetheless, it is interesting to speculate that the nonlocal effects of neurogenic inflammation could extend to other regions, such as the lungs and middle ears, and thus explain the cause of such well-known complications of RSV infection as otitis media, bronchiolitis, and asthmatic exacerbation.

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