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Understanding the Asthmatic Response to an Experimental Rhinovirus Infection: Exploring the Effects of Blocking IgE

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**Understanding the Asthmatic Response to an Experimental Rhinovirus Infection:
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Conflict of Interest Statement

We are disclosing to the Editor the following relationships which may be considered as potential conflicts of interest and to significant financial contributions to this work. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication with respect to intellectual property. In so doing, we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the

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Abstract: (words = 250)

Background: Rhinovirus (RV) frequently causes asthma exacerbations among children and young adults who are allergic. The interaction between allergen and RV-induced symptoms and inflammation over time is unclear.

Objective: To compare the response to an experimental inoculation with RV-16 in allergic asthmatics and healthy controls, and evaluate the effects of administering omalizumab before and during the infection.

Methods: Two clinical trials were run in parallel. One compared the response to an experimental inoculation with RV-16 among asthmatics with high levels of total IgE versus healthy controls. The other compared the effects of administering omalizumab versus placebo to asthmatics in a randomized, DBPC-investigation. The primary outcome for both trials compared lower respiratory tract symptoms (LRTS) between study groups over the first 4 days of infection.

Results: Frequent comparisons of symptoms, lung function and blood eosinophil counts revealed differences that were more pronounced among allergic asthmatics than controls by days 2 and 3 after RV inoculation. Additionally, an augmentation of upper and LRTS scores occurred among the atopic asthmatics versus controls during the resolution of symptoms ($p < 0.01$ for upper and < 0.001 for LRTS scores). The beneficial effects of administering omalizumab on reducing LRTS and improving lung function were strongest over the first 4 days.

Conclusions: Lower respiratory tract symptoms and blood eosinophil counts were augmented and lung function reduced among allergic asthmatics early after RV inoculation and increased late in the infection during symptom resolution. The effect of administering omalizumab on the response to RV was most pronounced during the early/innate phase of the infection.

Clinical trial numbers: NCT02111772 and NCT02388997

Key messages: The response to an experimental rhinovirus infection was monitored in allergic asthmatics. Frequent assessments for 21 days after virus inoculation revealed:

- that, compared to healthy controls, allergic asthmatics developed augmented lower respiratory tract symptoms, increased blood eosinophil counts and reduced lung function early in the infection (2 to 3 days after virus inoculation).

- that blocking IgE by administering omalizumab decreased these early changes.
- that late in the infection asthmatics with high levels of IgE experienced increased upper and lower respiratory tract symptoms that may warrant treatment.

Capsule summary: Allergic asthmatics experienced an augmented response to an experimental rhinovirus infection very early in the infection and during symptom resolution. The beneficial effect of blocking IgE with omalizumab was most prominent early in the infection.

Key words: asthma, rhinovirus, IgE, allergy, viral infection, anti-IgE, omalizumab.

Abbreviations:

ACT	asthma control test
AUC	area under the curve
DBPC	double blind placebo controlled

173	ER	emergency room
174	FeNO	fractional exhaled nitric oxide
175	IOS	impulse oscillometry
176	LRTS	lower respiratory tract symptoms
177	qPCR	quantitative polymerase chain reaction
178	RV	rhinovirus
179	SAE	serious adverse event
180	SD	standard deviation
181	URTS	upper respiratory tract symptoms
182	UVA	University of Virginia
183	VCU	Virginia Commonwealth University

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189 **Introduction:** Rhinovirus (RV) is the main viral pathogen causing asthma exacerbations
190 among children and young adults¹⁻³. Previous cross-sectional and prospective studies
191 have shown 1) that RV-induced wheezing during infancy is associated with an
192 increased risk for developing asthma⁴⁻⁶, and 2) that RV-induced attacks of wheezing

193 occur predominantly in asthmatics who are allergic even among those who are very
194 young^{2,4,7,8}. These study designs, however, haven't allowed investigators to examine
195 early events that play a central role in the host response to RV before symptoms peak,
196 or late events that influence the resolution or persistence of infection. Currently, the best
197 study design available to explore the kinetics of the host response to RV over time
198 involves the administration of RV experimentally to asthmatic individuals. This study
199 design permits simultaneous sampling of the airway and blood at different time points
200 during the infection to enhance the study of mechanisms and investigate the effects of
201 therapeutic interventions⁹⁻¹².

202 For these reasons, we conducted two complimentary investigations that utilized the
203 same experimental RV-infection study design to evaluate the role of IgE in influencing
204 the asthmatic response to this virus over time during acute symptoms and during
205 symptom resolution. One study focused on comparing the response of highly atopic
206 asthmatic subjects to an experimental inoculation with RV-16 to the response in non-
207 atopic, healthy controls. The other study was a drug intervention study to determine
208 whether the administration of omalizumab (anti-IgE antibody) can diminish the
209 asthmatic response to RV in a randomized, double-blind, placebo-controlled (DBPC)
210 trial. In both investigations, we examined changes in upper and lower respiratory tract
211 symptoms together with frequent assessments of lung function, blood counts and viral
212 load that were monitored following virus inoculation over a period of 21 days.

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227 **Methods:**

228 **Screening for enrollment:** Two screening visits were scheduled to determine eligibility
229 (Figure E1, online). All subjects enrolled were adults (ages 18 to 39). The asthmatics
230 had physician-diagnosed, mild asthma treated with inhaled bronchodilators
231 intermittently. Based on the inclusion/exclusion criteria (Table E1), none were using

anti-inflammatory, controller medications for asthma for at least a month before enrollment. No asthma subject or healthy control experienced a cold or had a positive test for RV by qPCR within 6 weeks prior to RV inoculation.

All asthmatics had a positive methacholine test and an Asthma Control Test (ACT) score > 19 documenting good asthma control during the month before inoculation (Table E1)^{12,13}. Each asthmatic had a total serum IgE $\geq$ 125 IU/mL and a positive prick skin test to a common environmental allergen (Table E1 and E2). All healthy controls had negative prick skin tests, no history of asthma, and a negative methacholine test. All asthma and control subjects had a titer of neutralizing antibody to RV-16 of < 1:4.

Study design: Subjects eligible for participation based on screening were enrolled in one of 2 clinical trials conducted over the same time period. One trial (the **Asthma/Control study**) included 11 asthmatics and 13 non-allergic, non-asthmatic healthy controls; NCT02111772. The other trial (the **Anti-IgE study**) included 20 asthmatics, 10 of whom received omalizumab and the remaining a placebo medication in a randomized, DBPC study conducted over a period of 8 weeks before and for 3 weeks after virus inoculation; NCT02388997. The asthmatics in the Asthma/Control study had total IgE levels that were too high to permit omalizumab dosing and were, therefore, ineligible for the Anti-IgE study. Criteria used to document cold symptoms and to define infection among subjects who participated in these trials are described in Table E2.

The study design for both trials was identical from the time of virus inoculation through 21 days of monitoring (Fig. 1). The main difference between the two investigations was

an 8 week run-in period for the Anti-IgE study to optimize the drug effects of omalizumab before inoculation. The run-in period for the Asthma/Control study was one week. IRB approval and informed consent for screening was obtained at the University of Virginia (UVA) and the Virginia Commonwealth University (VCU), and for the enrollment and evaluation of subjects who participated in these trials at UVA.

Symptom scores: Upper and lower respiratory tract symptom scores (URTS and LRTS scores, respectively) were recorded at home on diary cards twice daily by subjects in both trials. URTS's were evaluated using modified Jackson criteria reported previously⁹. The symptoms evaluated and scale used to judge symptom severity are described on-line (Table E2). LRTS's recorded by participants included cough, wheeze, shortness of breath, chest tightness, and night awakenings using the same severity scale (Table E2).

Procedures and Assessments: Virus inoculation was performed in the evening on day 0, after which each subject was isolated in a hotel room for the first 4 days of the infection. Procedures and assessments (marked by downward arrows in Figure 1) were done at visits scheduled prior to RV inoculation, daily in the morning in the hotel, and during clinic visits scheduled on days 7, 14, and 21. These assessments included a physical examination, measurements of FeNO, complete blood cell counts, lung function (spirometry), and the collection of a nasal wash and blood sample via venipuncture using methods described on-line (Table E2). Impulse oscillometry (IOS), methacholine challenges, and tests for neutralizing antibody to judge sero-conversion to RV-16 were performed as noted in Figure 1 using methods described online (Table E2).

Virus inoculation and determinations of viral load: A pool of RV-16 (a group A serotype) was pre-titered to contain 300 tissue culture infective doses (TCID₅₀) prior to inoculation. This pool was approved for experimental challenges by the FDA (IND# 15162) and for administration together with study drug to asthmatics in the Anti-IgE study (IND# 10510). One-half ml of the pool was administered to each nostril separately on day 0. Viral load assessments in nasal washes were evaluated by qPCR as well as by titers of RV in culture as previously described¹⁵⁻¹⁷.

Administration of study drug: The study drug (omalizumab and placebo) used in the Anti-IgE study was supplied by Novartis Pharmaceuticals, Inc. The dose and frequency of subcutaneous injections of study drug was based on an individual's weight and total IgE level according to the manufacturer's recommendations¹⁸.

Statistical analyses: For both clinical trials, the primary endpoint compared LRTS scores between study groups within each trial during the first 4 days of the infection and over the first 7 days as the secondary endpoint. Sample size estimations for both trials and the randomization plan for the Anti-IgE study are described online (Fig. E1, Fig. E1 legend and Table E2.F). Measurements over time, such as the first 4 days of the infection, were summarized by subtracting off the value taken prior to infection and computing the area under the curve over the time interval. The Wilcoxon signed ranks test was used for within-group comparisons and the Kruskal-Wallis test to evaluate the magnitude of between group differences estimated with distribution-free confidence intervals¹⁹.

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309 **Results:**310 **Patient characteristics**

311 **Asthma/Control study:** The allergic asthmatics and healthy controls were matched for
312 age, sex, and race/ethnicity (Table 1A). The asthmatics had high levels of total serum

IgE and significantly lower ACT scores and FEV₁ (% predicted) values compared to controls at inoculation (day 0). The asthmatics also had significantly higher FeNO levels and blood eosinophil counts than the controls. Control subjects were not atopic.

Anti-IgE study: Subjects in the omalizumab and placebo groups were also matched for age, sex, and race/ethnicity (Table 1B). Their total serum IgE levels were similar and the median levels for both groups were significantly lower than the IgE levels for asthmatics in the Asthma/Control study ($p < 0.001$). At enrollment and at inoculation, the omalizumab treated asthmatics had similar FEV₁ values, but lower FEV₁/FVC ratios than asthmatics in the placebo group (Table 1B). No significant differences between the two groups were noted for ACT scores, methacholine sensitivity, FeNO, or blood eosinophil counts.

Diary cards did not reveal significant changes over time in URTS or LRTS scores between the omalizumab and placebo groups during the 8 weeks of study drug treatment before RV inoculation. Based on Kruskal-Wallis analyses, the mean and standard deviations for URTS scores (AUC results over the entire run-in period) from placebo and omalizumab treated asthmatics were 57.5 (69.2 SD) and 25.3 (13.2 SD), respectively ($p = 0.45$). For LRTS scores the results were 13.1 (16.1 SD) and 16.3 (14.6 SD), respectively ($p = 0.36$). However, as judged by a comparison of ACT scores assessed at 8 weeks and at inoculation, asthma control improved among the asthmatics receiving omalizumab compared to placebo ($p = 0.02$ using Kruskal-Wallis analysis) (Table 1B). FeNO values also declined significantly during the 8 weeks of omalizumab administration compared to placebo treatment ($p = 0.02$), whereas no between group

differences were observed in blood eosinophil counts during the run-in period (Table 1B). Additionally, no significant omalizumab effects on lung function (spirometry values or methacholine reactivity) were observed during the run-in (Table 1B).

Respiratory tract symptoms following RV inoculation:

Changes during the acute infection (days 1-4): URTS scores between study groups in the Asthma/Control study were similar during the first 4 days of infection and the same was true between groups in the Anti-IgE study ($p=0.88$ and 0.17 , respectively) (Figs. 2A and B). LRTS were more prominent and significantly higher by day 2 after inoculation among asthmatics compared to controls in the Asthma/Control study ($p = 0.05$) (Fig. 2C). Area-under-the-curve (AUC) analysis adjusted for baseline, did not reveal significant differences in LRTS scores between groups over the first 4 days of the infection ($p = 0.42$; primary outcome), or over the first 7 days ($p=0.41$; secondary outcome). Notably, the only LRTS recorded by controls during the infection was cough. In the Anti-IgE study, primary and secondary outcome analyses did not reveal significant differences in LRTS scores between the placebo and omalizumab treated asthmatics (Fig 2D).

Development of late symptoms: During the last week of monitoring (days 15 to 21 after inoculation), a significant augmentation of both URTS and LRTS occurred among the asthmatics compared to controls in the Asthma/Control study ($p< 0.01$ and < 0.001 , respectively) (Fig. 2A and C). Additionally, ACT scores declined significantly among the asthmatics (21.8 at inoculation to 19.9 twenty-eight days later; $p=0.02$), whereas controls had ACT scores of 25 at both time points. Among the placebo and omalizumab

treated asthmatics in the Anti-IgE study, URTS and LRTS scores over days 15-21 were not significantly different between the groups, nor did they differ from baseline in either group by day 21 (Fig. 2B and D). ACT scores (23 at day 0 for both groups) did not change significantly from baseline by day 28 (22.5 and 23 for those treated with placebo and omalizumab, respectively).

Changes in lung function and FeNO: Spirometry assessments done in the morning revealed a rapid decline in FEV₁, FEV₁/FVC, and FEF₂₅₋₇₅ on day 1 (12 hours after virus inoculation) in all asthmatic groups participating in both trials, but not among controls in the Asthma/Control study (Fig. 3). Over the first 4 days, spirometry values remained low among asthmatics in the Asthma/Control study and did not return to baseline after 21 days of monitoring. This decline at day 21 was significant for the FEV₁/FVC ratio and FEF₂₅₋₇₅ ($p < 0.05$ and 0.02 , respectively), whereas the decline in spirometry values among controls was not significant (Fig. 3A, C, E and Table E3A). In the Anti-IgE study, the initial decrease in spirometry was transient (one day only) among the asthmatics treated with omalizumab. Their values returned to baseline by day 4. In contrast, spirometry values in the placebo group remained low during the acute infection leading to significant differences in the FEV₁/FVC ratio and FEF₂₅₋₇₅ compared to treatment with omalizumab by day 4 (Fig. 3B, D, and F).

The median provocative dose for methacholine was 0.56 mg/ml (0.07-1.04 intra-quartile range) at inoculation and 0.26 mg/ml (0.07 – 1.41) by day 4 among asthmatics in the Asthma/Control study. This change was not significant ($p = 0.46$). None of the controls developed positive methacholine tests during the infection, and significant changes

were not observed among the asthmatics receiving placebo or omalizumab in the Anti-IgE study (data not shown). Following inoculation, FeNO values trended higher over the first 3 days of the infection and remained significantly higher among asthmatics compared to controls in the Asthma/Control study (Fig. E2). After adjusting for baseline, no significant changes in FeNO were observed during the infection among asthmatic groups in either trial. However, late in the infection a significant increase in this inflammatory marker was observed from day 14 to day 21 among asthmatics in the Asthma/Control study who experienced an augmentation in their URTS and LRTS at the same time (Fig. E2).

Viral load comparisons: After RV inoculation, viral loads were similar between groups in the Asthma/Control study or the Anti-IgE study as judged by qPCR for RV-RNA and by titers of RV in culture (Fig. E3A-D). Seroconversion to RV-16 (i.e., a 4-fold increase in RV-16 specific serum neutralizing antibody) occurred in 91% of the asthmatics and 62% of healthy controls in the Asthma/Control study, and 70% of asthmatics in both treatment groups in the Anti-IgE study 21 days after inoculation ($p > 0.05$ for between group comparisons; data not shown).

Blood eosinophil and neutrophil counts: Blood eosinophil counts increased significantly from baseline by days 2 and 3 following inoculation among the asthmatics compared to controls in the Asthma/Control study ($p = 0.034$ and 0.03 , respectively) (Fig. 4A). Compared to placebo treatment, the increase in blood eosinophil counts was blunted among asthmatics treated with omalizumab, but the between group differences were not significant ($p = 0.79$ by day 2 and by day 3, and $p = 0.08$ by day 4) (Fig. 4B).

Late in the infection (by day 21), median blood eosinophil counts in the Asthma/Control study increased significantly after inoculation among the asthmatics (from 230 to 359 cells/mm³, $p < 0.03$), whereas an increase was not observed among the controls (102 and 99, respectively) (Tables 1A and E3A). By comparison, blood neutrophil counts, which were similar between groups in either trial at inoculation, increased significantly by days 2 and 3 in all study groups ($p < 0.02$ based on fold changes from day 0) (Fig 4C and D). However, no significant differences in neutrophil counts between groups were evident in either clinical trial during the infection. Additionally, no significant differences between groups were detected in either clinical trial in the analyses of other cell types (Table E4A and B).

Post-hoc analysis of LRTS reported during the acute infection (days 1-4): Because not all subjects in this investigation experienced LRTS after inoculation, a post-hoc analysis was conducted to compare the percentage of subjects within each group who developed LRTS during the first 4 days after inoculation. Among the asthmatics, 45% of those in the Asthma/Control study and 40% in the Anti-IgE study experienced LRTS above baseline (Fig. 5 and Fig.E4 to view individual symptom score plots). These asthmatics did not experience severe respiratory symptoms and none required inhaled or oral steroid intervention. By comparison, only one healthy control and one asthmatic treated with omalizumab developed acute LRTS after their inoculation. Altogether there were 9 asthmatics who reported LRTS during the first 4 days who were not treated with omalizumab (Fig. 5). Five were from the Asthma/Control study and 4 from the placebo treated asthmatics in the Anti-IgE study. At inoculation, these asthmatics had

significantly higher blood eosinophil counts than 12 asthmatics from these groups who did not develop LRTS during the first 4 days ($p = 0.04$; Fig. E5A and Table E5A).

After inoculation, the asthmatics who developed LRTS during the acute infection also experienced URTS's that were significantly increased compared to those who did not ($p < 0.02$; Fig E5B and Table E5B). Additionally, IOS assessments on day 4 also revealed a significant increase in peripheral airway resistance (R at 5 and R at 5-20 Hz) and impedance (Z at 5 Hz) among the asthmatics experiencing LRTS compared to those who did not (Fig. E5B and Table E5B; IOS values at enrollment, day 0 and on day 4 are shown in Table E6A and 6B). Higher FeNO values were also observed during the first 4 days among the 9 asthmatics who experienced LRTS (a strong trend; 0.055) (Fig. E5B and Table E5B).

Safety: After enrollment and during the infection, no subject in either trial experienced a SAE, and none required intervention with an inhaled or oral steroid or dropped their FEV_1 by $\geq 20\%$.

Discussion: Following RV inoculation, rapid changes in symptoms, lung function, and blood counts were observed that were more prominent among asthmatics who had high levels of total serum IgE than controls who participated in the Asthma/Control study. Although previous experimental RV challenges have favored sample collection, including bronchoscopic evaluations, 4 days after RV inoculation^{20,21}, the significant

changes reported here were observed 2 to 3 days after inoculation and would be missed in cross-sectional or prospective studies of asthmatics who are evaluated during a natural RV-induced cold. Most striking was that the administration of omalizumab to asthmatics in the Anti-IgE study had its strongest effect in reducing LRTS's and improving lung function during the first 4 days of the infection. Moreover, blocking IgE with omalizumab in the Anti-IgE study resulted in a marked reduction in the percentage of asthmatics who experienced LRTS acutely and the magnitude of differences observed between the omalizumab and placebo treated groups was similar to the differences observed between asthmatics and controls in the Asthma/Control study.

During the 8 weeks of drug administration in the Anti-IgE study, the asthmatics who received omalizumab achieved better asthma control as judged by their ACT scores. They also experienced significant reductions in FeNO during the run-in compared to the placebo group. By the time of RV inoculation, both treatment groups had similar baseline characteristics except for lower lung function (e.g., FEV₁/FVC ratios and FEF₂₅₋₇₅) in the omalizumab group that was also observed at enrollment. After RV inoculation, all asthmatic groups, including those treated with omalizumab, experienced a decline in lung function (FEV₁, FEV₁/FVC, and FEF₂₅₋₇₅) within 12 hours after RV inoculation. This early decline was not observed among healthy controls and was transient for the asthmatics treated with omalizumab whose spirometry values returned to baseline within 4 days. In contrast, spirometry values remained low during the acute infection among those treated with placebo. Blood eosinophil counts were also significantly augmented by days 2 and 3 after inoculation in asthmatics compared to controls in the Asthma/Control study and were blunted by the administration of omalizumab in the Anti-

IgE study. By comparison, blood neutrophil counts increased significantly from baseline in all study groups over the first 2 to 3 days; however, no significant differences in neutrophil counts were observed between groups in either clinical trial during the acute infection.

Currently, information regarding the resolution of symptoms and inflammatory events that occur late in the course of an experimental RV infection is limited. In this investigation, allergic asthmatics in the Asthma/Control study experienced URTS and LRTS that were augmented during the third week of monitoring. Notably, spirometry values in these asthmatics did not return to baseline and their blood eosinophil counts were significantly increased from baseline 21 days after inoculation. These changes have not been reported previously, but are consistent with increased basophil activation that has been described for allergic asthmatics late in the infection²². Taken together, these changes indicate sustained inflammation that in this investigation was not associated with persistent viral replication as judged by qPCR for RV-16 or titers of RV in culture.

Although FeNO levels at inoculation were significantly higher among asthmatics compared to controls in the Asthma/Control study, no significant changes in this biomarker of inflammation were observed after inoculation. The same was true for both asthmatic groups in the Anti-IgE study and for allergic asthmatics challenged with RV as reported previously⁹. Because we have seen FeNO levels that were higher among RV-infected children who were treated for acute asthma than among those testing negative for RV⁷, further studies may be warranted to determine whether RV augments FeNO production during more severe attacks.

Whether allergic asthmatics are more susceptible to frequent infections with RV continues to be of significant interest²³⁻²⁵. Once infected, however, differences in viral load between study groups in this investigation were not evident in either clinical trial in agreement with what has been observed previously^{15,26}. Additionally, the measurements of serum neutralizing antibody to RV-16 in these trials provided evidence that the rates of seroconversion to this virus were not impaired in the atopic asthmatics compared to the healthy controls.

Limitations: For safety reasons, the experimental RV challenges used in these investigations excluded the enrollment of subjects with poorly controlled asthma, or of children who might experience severe RV-induced attacks of asthma. As a result, these trials focused on subjects with mild asthma with outcomes that examined decreased symptom control rather than exacerbations, and the changes in lung function were modest, but informative. It was also difficult to evaluate larger numbers of subjects, because these trials involved repeated assessments over time and screen failures were common. In addition, the use of controller medications by asthmatics was a frequent exclusion factor, and important because these medicines would interfere with the assessment of airway inflammation and confound the evaluation of changes associated with the administration of omalizumab. Subject accrual that led to the results of our clinical trials was higher than anticipated for the Asthma/Control study, but lower than predicted for the Anti-IgE study predominantly because of the high screening to enrollment ratio and an increased drop-out rate during the run-in period than expected (noted in the Fig. E1 legend and described in Table E2.F).

Of note, not all of the asthmatics in the Asthma/Control or Anti-IgE studies developed LRTS after their RV inoculation which has also been reported for asthmatics who experience natural RV infections^{1,27,28}. This confounded the conventional AUC analyses which did not reveal significant differences in LRTS between study groups in either clinical trial over the first 4 days of the infection. By comparison, the post-hoc analysis demonstrated that the percentage of asthmatics who experienced LRTS above their baseline was significantly greater over the first 4 days of the infection among asthmatics compared to controls in the Asthma/Control study. Taken together, the post-hoc analysis reported here may be a better method for distinguishing differences between study groups in the generation of LRTS within 4 days after RV inoculation, including studies that examine therapeutic interventions to modify symptoms.

Strengths: By comparison to other study designs, the experimental RV infection model described here permitted the evaluation of kinetic changes following RV inoculation during the acute infection and symptom resolution. In particular, the rapid changes observed early in the infection before symptoms peaked, highlight the importance of frequent monitoring during the acute infection to provide a better understanding for the events involved in the pathogenesis of RV-induced asthma. Although most subjects who completed the study were Caucasian women, the results from this and our previous experimental challenges have frequently been concordant with results from our cross-sectional studies of children treated in the hospital or ER for acute asthma exacerbations induced by RV^{2,7,29}. Those children were predominantly male and African American. Notably, the results from these children and from our current and previous RV challenges have demonstrated 1) a greater risk for RV-induced symptoms among

asthmatics who have high levels of total serum IgE^{7,9,29}, 2) increased Th2-related airway inflammation observed before an RV infection^{7,9}, and 3) similar viral loads detected in the asthmatic and non-asthmatic airway during an infection¹⁵.

Conclusions: The assessments of symptoms, lung function, and blood counts reported here differentiated the response to RV in allergic asthmatics with high IgE levels compared to healthy controls within 2 to 3 days after RV inoculation. These rapid changes are consistent with previous publications describing gene dysregulation and molecular events that were also augmented early in the infection in the asthmatic host³⁰⁻³². By monitoring the response to RV over 21 days, this investigation also demonstrated augmented URTS and LRTS late in the infection together with reduced lung function and increased blood eosinophil counts among asthmatics in the Asthma/Control study. How long these adverse changes persist following an RV infection and whether they impart an increased risk for subsequent exacerbations and additional deterioration in lung function if left untreated warrants further investigation.

Previous studies have shown that omalizumab has a significant impact on reducing asthma exacerbations, medication requirements and exacerbation risks among children and adults with moderate to severe disease^{28,33-35}. Using the experimental infection model, results from the Anti-IgE trial indicate that the beneficial effects of administering omalizumab are prominent early in the infection (during the first 4 days). Our results further highlight that understanding the atopic status of asthmatic host will be critical in designing studies to decipher the mechanisms of RV-induced attacks of asthma and for evaluating the effects of therapeutic interventions.

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Table I: Patient characteristics

	A. Asthma/Control Study			B. Anti-IgE Study		
	Asthma (n=11)*	Control (n=13)*	p-value	Placebo (n=10)*	Omalizumab (n=10)*	p-value
Mean age (range)	24.5 (19-39)	22.1 (20-27)	0.26	24.1 (18-33)	21.0 (18-28)	0.11
Sex (% female)	73	76	0.81	90	70	0.26
Race/Ethnicity (% Caucasian) [†]	82	100	0.11	60	80	0.33
Total IgE (screening) IU/ml	1045 (726-2128)	19 (8-23)	<0.001	216 (156-248)	240 (156-349)	0.94
# of positive prick skin tests (range)	4-11	0		3-9	1-9	
ACT score enrollment	N/A	N/A		23 (22-24)	22 (21-24)	0.70
ACT score Day 0	21 (21-23)	25 (25-25)	<0.001	23 (22-24)	23 (22-25)	0.59
FEV ₁ (% predicted): Enrollment	92 (82-97)	100 (96-106)	0.01	93 (90-100)	95 (85-105)	0.94
FEV ₁ (% predicted): Day 0	92 (82-96)	102 (98-106)	0.04	94 (89-100)	94 (86-104)	0.94
FEV ₁ /FVC: Enrollment	0.83 (0.77-0.87)	0.86 (0.84-0.89)	0.07	0.86 (0.85-0.88)	0.80 (0.73-0.82)	0.007
FEV ₁ /FVC: Day 0	0.85 (0.77-0.87)	0.85 (0.81-0.89)	0.21	0.87 (0.83-0.89)	0.79 (0.69-0.82)	0.01
FEF ₂₅₋₇₅ (% predicted): Enrollment	90 (65-101)	96 (92-109)	0.11	100 (87-100)	72 (67-98)	0.06
FEF ₂₅₋₇₅ (% predicted): Day 0	82 (60-99)	100 (90-113)	0.03	91 (90-96)	78 (58-92)	0.07
Methacholine PC ₂₀ (mg/ml): Enrollment [#]	0.31 (0.07-0.40)	>16 mg/ml	N/A	0.47 (0.19-0.85)	1.24 (0.57-1.97)	0.13
Methacholine PC ₂₀ (mg/ml): Day 0 [#]	0.56 (0.07-1.04)	>16 mg/ml	N/A	0.88 (0.29-1.55)	1.37 (0.99-3.66)	0.17
FeNO (ppb): Enrollment	48 (28-74)	17 (12-22)	<0.001	25 (18-28)	37 (27-49)	0.09
FeNO (ppb): Day 0	44 (27-80)	15 (10-21)	<0.001	35 (15-69)	22 (9-73)	0.12
Blood eosinophils (cells/mm ³): Enrollment	267 (177-331)	111 (73-168)	0.003	191 (150-238)	140 (119-198)	0.29
Blood eosinophils (cells/mm ³): Day 0	230 (173-270)	102 (61-159)	0.006	210 (168-312)	147 (78-272)	0.29

*Results represent median values with intra-quartile range in parentheses unless otherwise indicated.

[†] Race/Ethnicity of non-Caucasian participants: Two African American asthmatics participated in the Asthma/Control Study. For the Anti-IgE study, 1 African American and 1 Pacific Islander were included in the omalizumab treated group. Two African American subjects, 1 Asian, and 1 Hispanic subject participated in the placebo group.

[#] FEV₁ values did not decline by 20% at methacholine doses > 16 mg/ml for control subjects in the Asthma/Control Study.

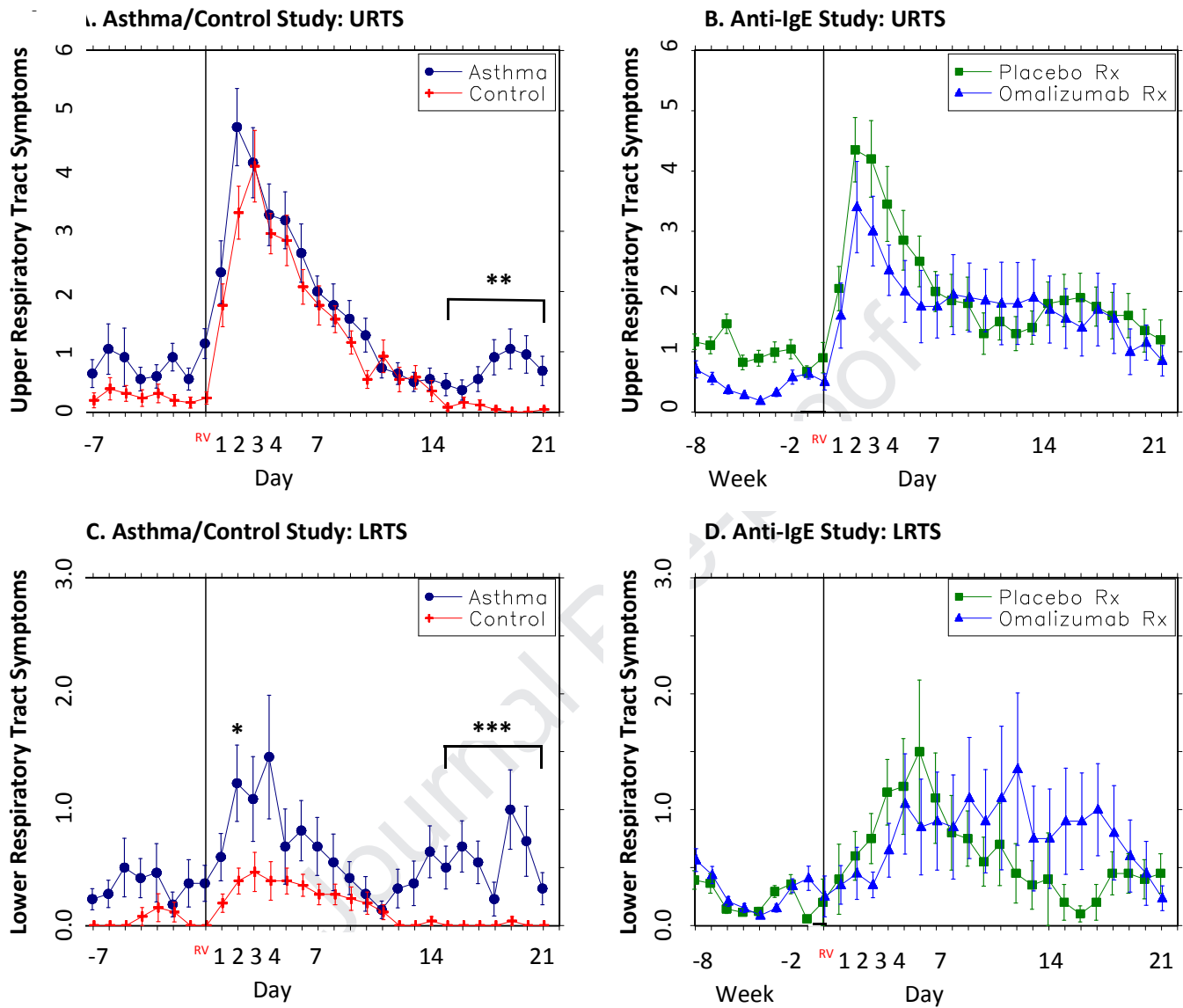
Figure 2. Symptom Scores

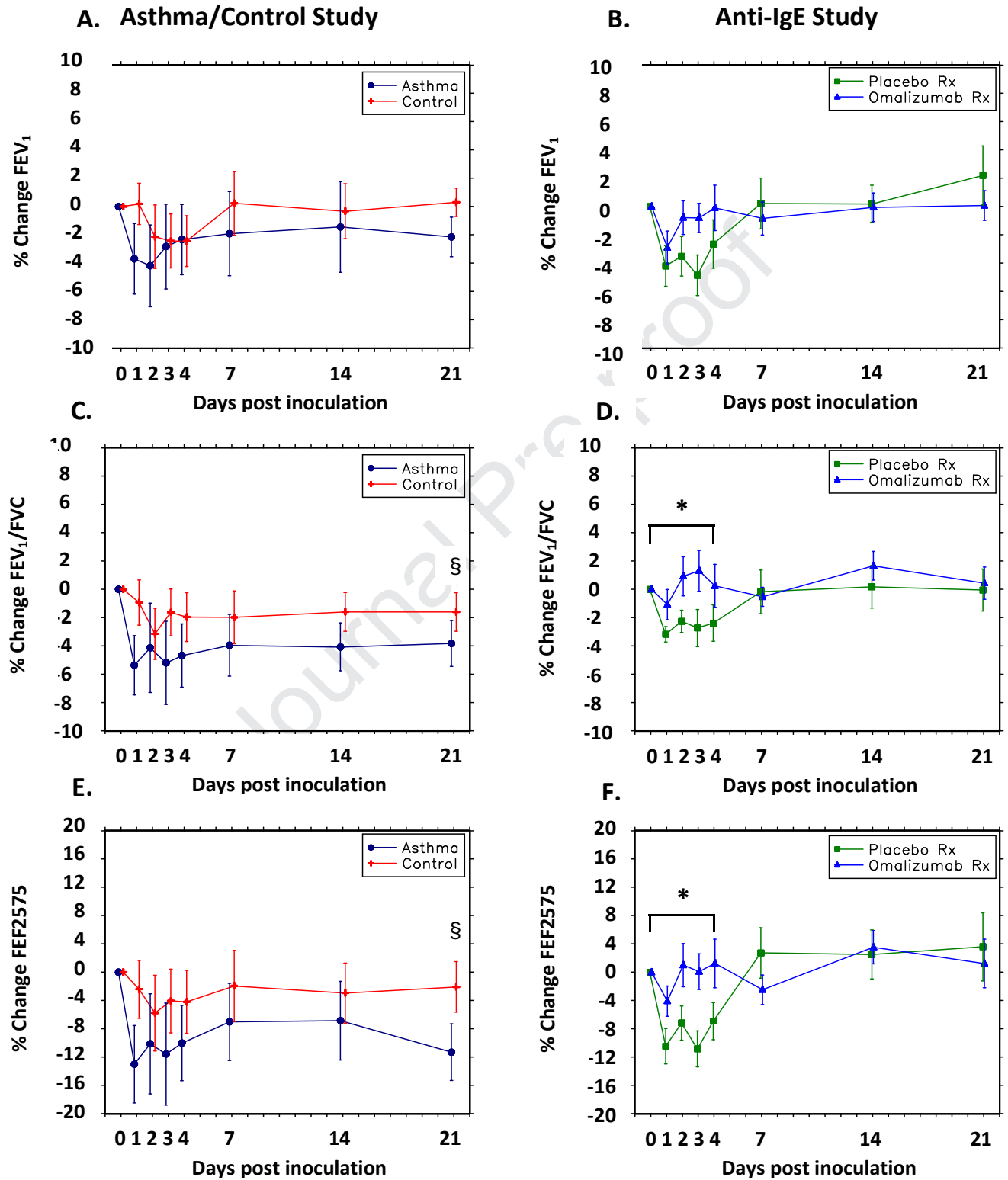
Figure 3. Spirometry

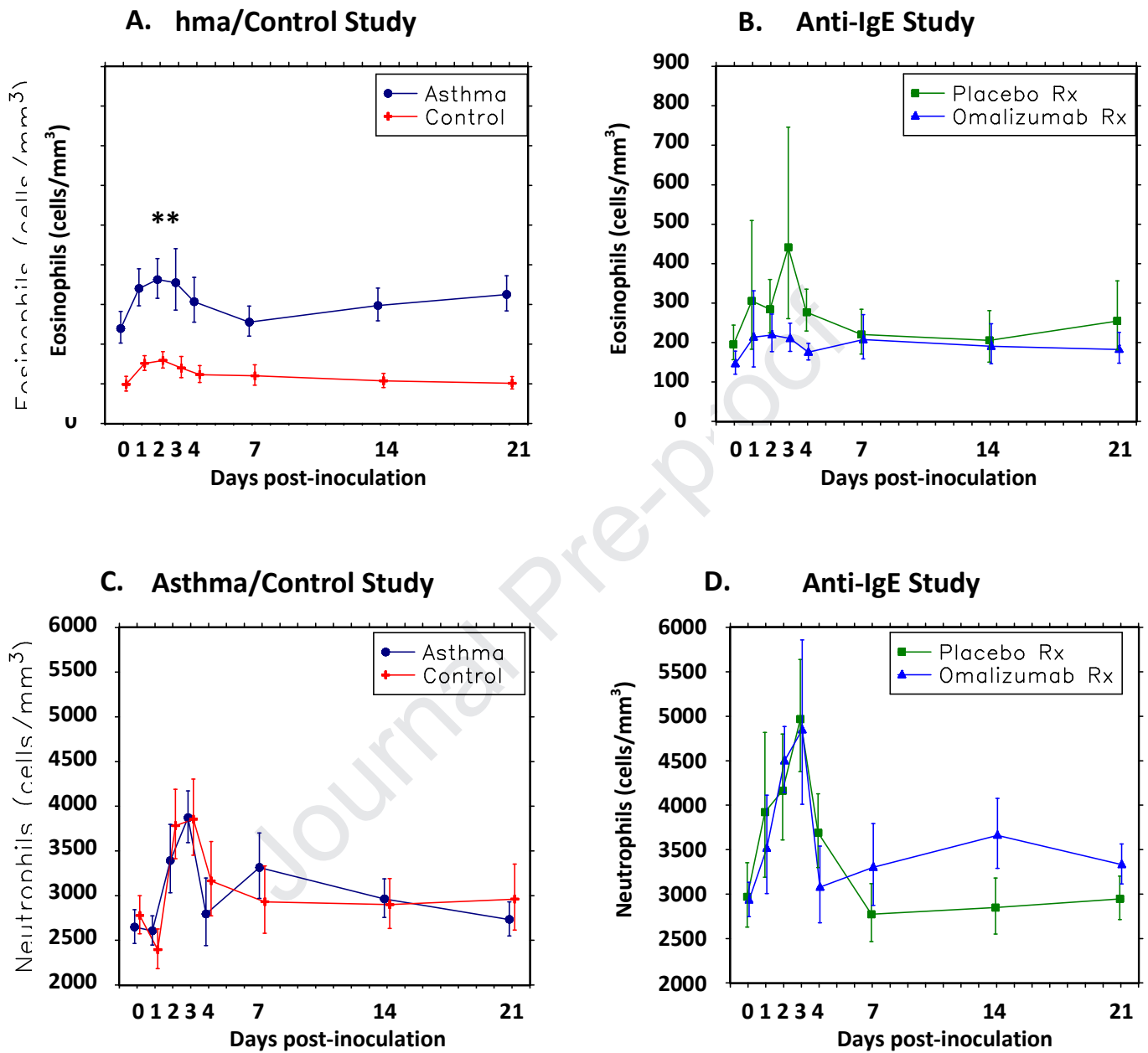
Figure 4. Blood Eosinophil and Neutrophil Counts

Figure 5. Subjects who developed LRTS during the first 4 days of the infection

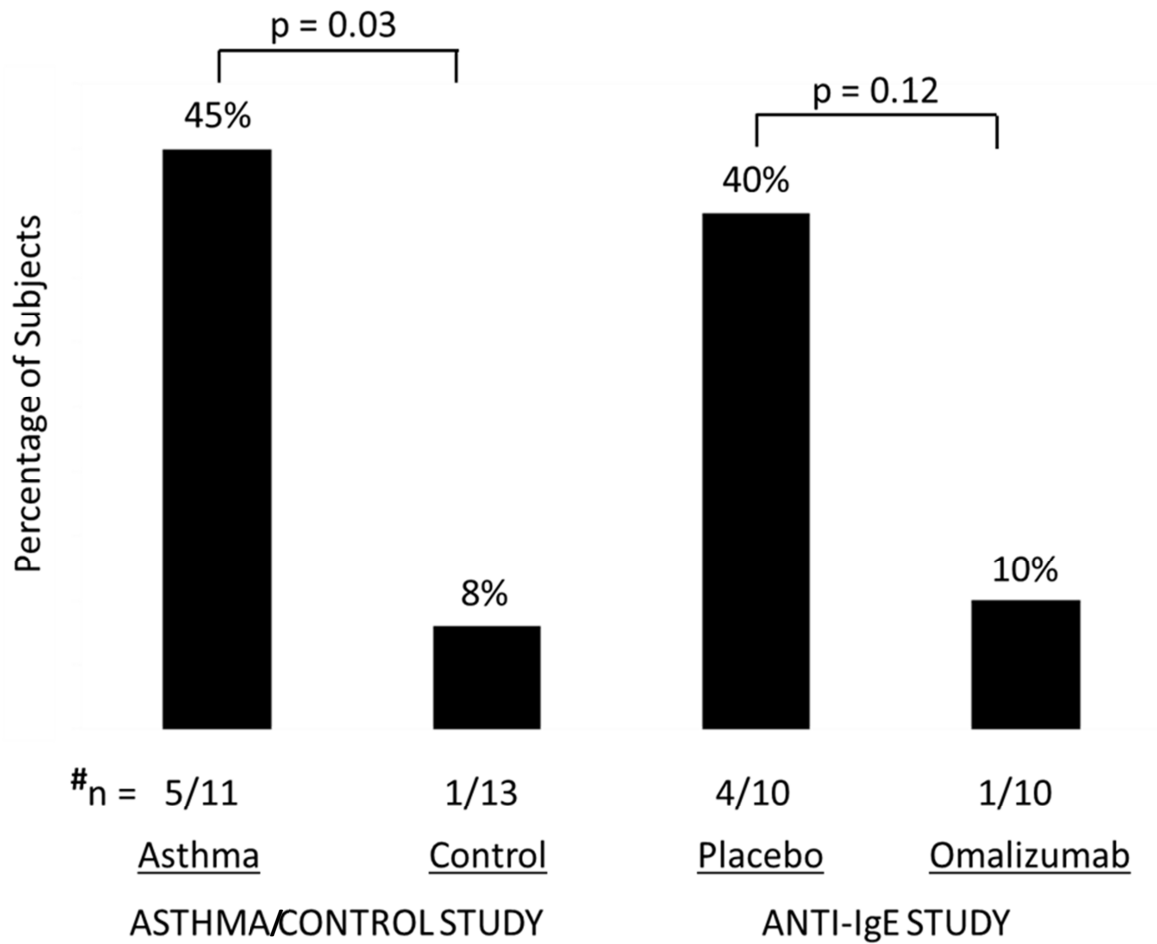


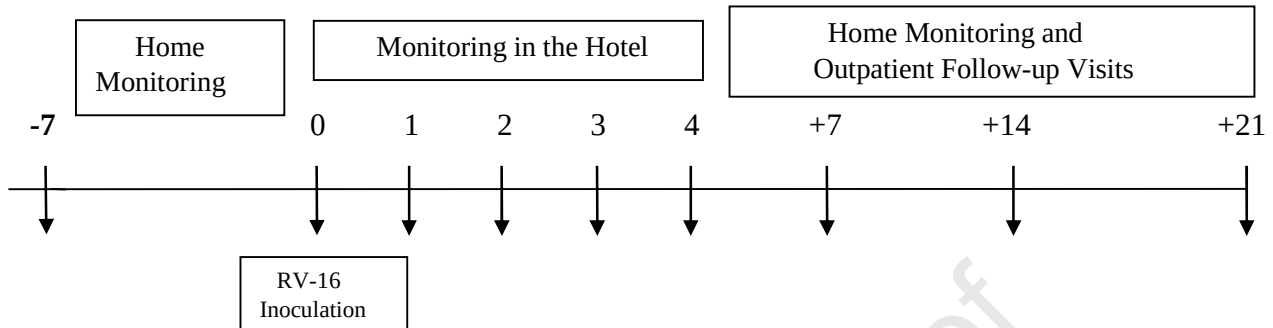
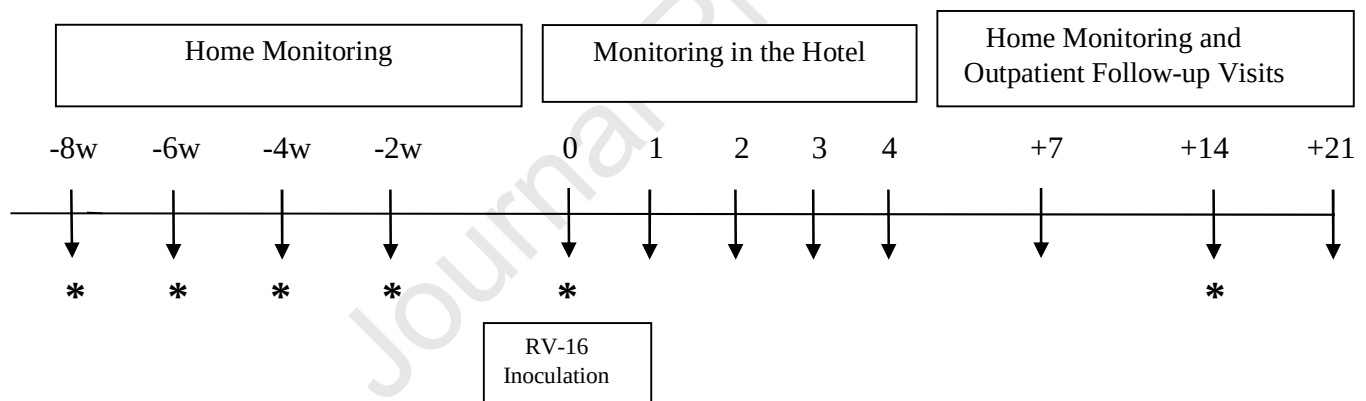
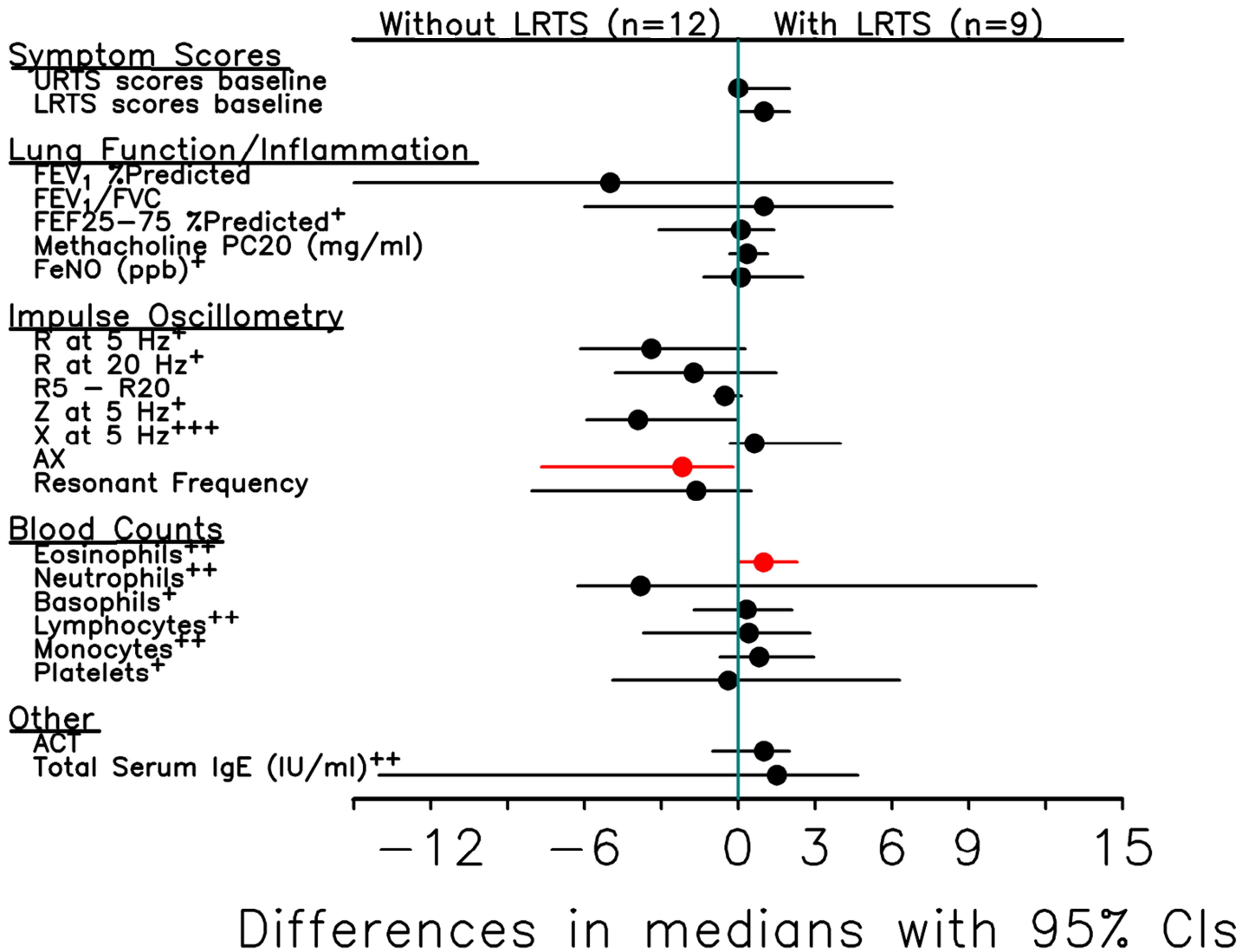
Figure 1 Study diagrams:**A. Asthma/Control Study:****B. Anti-IgE Study (Asthmatics Treated with Omalizumab or Placebo):**

Figure E5. Summary Plots: Comparing the assessments for asthmatics who experienced LRTS (days 0 – 4) in response to RV compared to asthmatics who did not.

A. Comparisons at baseline (day 0) before RV inoculation



B. Comparisons during the acute infection (days 0 – 4 after inoculation)

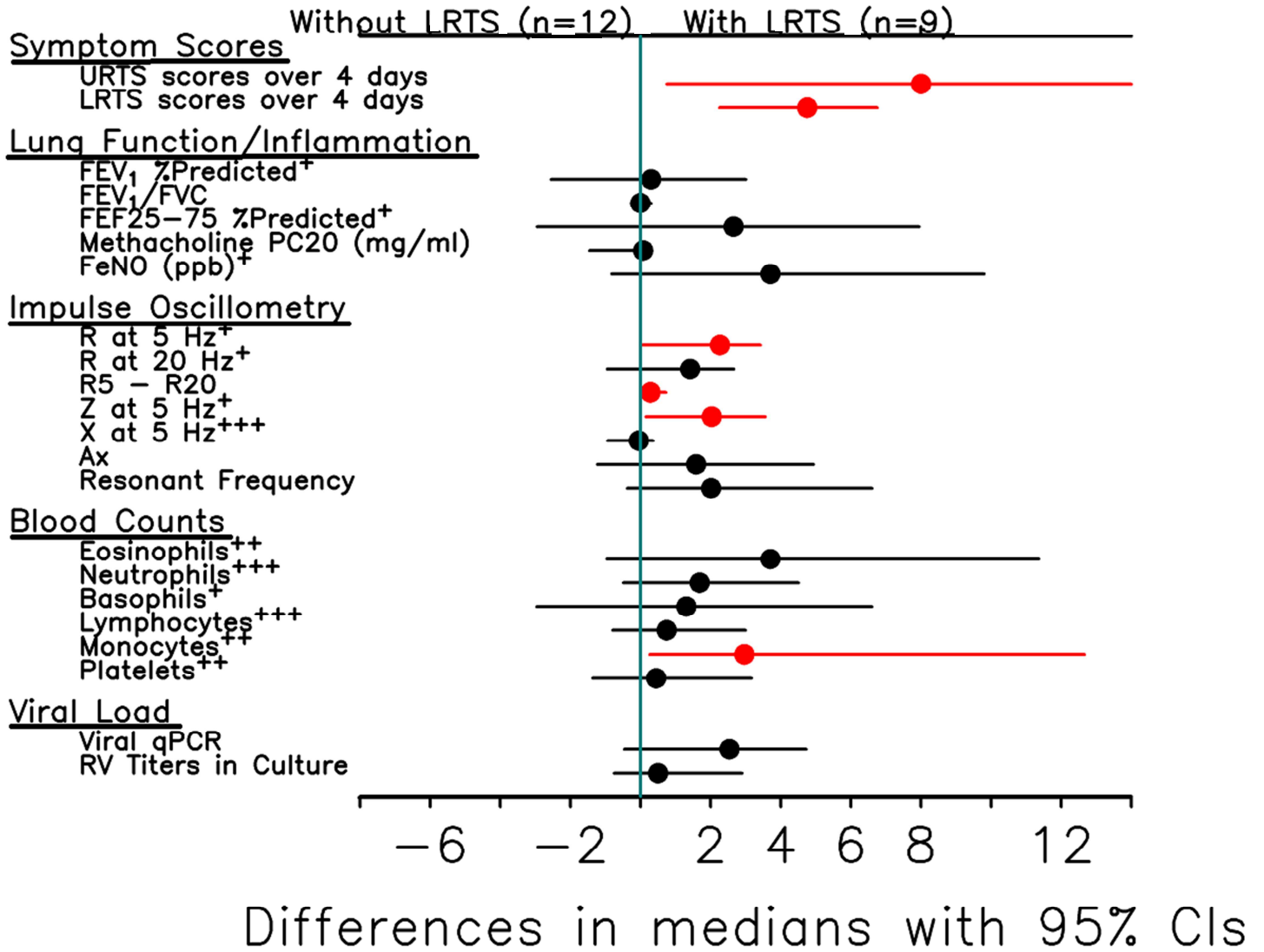


Table E1: Inclusion and Exclusion Criteria:

A. Asthma/Control StudyInclusion Criteria:

- All subjects had physician-diagnosed asthma
- Age 18 to ≤ 40 years of age, any gender, any racial/ethnic origin
- ACT score > 19
- FEV₁ $> 70\%$, or FEV₁/FVC ratio $> 75\%$ for subjects whose FVC values fell between 80 and 87% predicted and whose FEV₁ values were below 70%.
- Positive Methacholine challenge test (i.e. $\geq 20\%$ fall in FEV₁ at a methacholine concentration of ≤ 16 mg/ml) at screening, enrollment, and before RV inoculation (day 0).
- Total serum IgE level measured at screening that was too elevated to meet the recommendations for treatment with Omalizumab.
- Positive test for allergen specific IgE antibody by prick skin testing to allergens associated with current allergen exposure at the time of the RV challenge: e.g., dust mite, *Alternaria*, and/or ragweed for subjects challenged with RV in the fall, or positive tests to tree and/or grass allergens for those challenged in the spring. Sensitization to allergens (e.g., cat or dog) also qualified for enrollment if subjects were currently exposed to these allergens at home.

Exclusion Criteria:

- Subjects with positive test for serum neutralizing antibody to RV-16 at screening (i.e., those with a neutralizing antibody titer $\geq 1:4$) within 6 weeks of RV inoculation were excluded.
- To avoid RV-16 inoculations in subjects with restrictive lung volumes, subjects whose FVC is $< 80\%$ predicted were excluded.
- Chronic heart disease, lung diseases other than asthma, or other chronic illnesses, including primary and/or secondary immunodeficiency.
- Hospitalization or treatment in the ER for asthma during the 3 years before enrollment (unless the treatment only involved the use of a bronchodilator).
- Subjects who had one or more night time awakenings caused by asthma symptoms and/or who used their SABA (albuterol) inhaler for asthma symptoms ≥ 4 days during the week before enrollment, or before RV inoculation.
- Intubation or management in the intensive care unit for an asthma exacerbation
- An upper or lower respiratory tract infection within 6 weeks prior to enrollment
- Previous nasal or sinus surgery within the last 12 months.
- Subjects who have a 5 pack/year history of smoking, or any smoking within 6 months before enrollment.
- Female subjects who are, or who plan to become, pregnant during the study, or who are nursing a baby. To be included in this study, a woman of child-bearing potential must have a negative urine pregnancy test at screening, during the run-in-period, and prior to viral inoculation and agree to use an effective method of

birth control such as, but not limited to, birth control pills, contraceptive foam, diaphragm, IUD, abstinence, or condoms.

- Subjects who used omalizumab within 6 months prior to enrollment, or inhaled corticosteroids, inhaled ipratropium bromide, an inhaled long acting beta agonist, inhaled cromolyn or nedocromil or systemic leukotriene modifiers for their asthma on a daily basis within 4 weeks prior to enrollment, or subjects using nasal corticosteroids on a daily basis within 4 weeks prior to enrollment. Subjects currently receiving beta-adrenergic blocking agents.
- Subjects currently receiving allergen immunotherapy (IT), or who received allergen IT within the last 3 years.
- Hemoglobin <11.5 g/dL for non-African American subjects or hemoglobin < 11.0 g/dL for African American subjects detected during screening or within 6 weeks of enrollment.
- Absolute neutrophil count (ANC) < 1500 cells/mm³ (or 1.5 K/uL) detected during screening, or within 6 weeks of enrollment.

B. Anti-IgE Study:

Inclusion Criteria: Same as for the Asthma/Control study.

Exclusion criteria: Same as for the Asthma/Control study, except for the total serum IgE criteria established to meet the recommendations proposed by Novartis for the administration of omalizumab^{E1}.

Table E2: Procedures and Assessments:**A. Screening Procedures:**First visit:

- A questionnaire, focused on the inclusion and exclusion criteria (listed in Table E1), was administered to collect demographic information and to evaluate subject characteristics used to support the diagnosis of mild asthma.
- Blood (7 ml) was obtained by venipuncture. Serum was analyzed for total IgE using the Phadia ImmunoCap assay (Uppsala, Sweden) and for neutralizing antibody to RV-16 as determined by standard microtiter assays^{E2}.

Second visit:

- Prick skin tests were performed using extracts of aeroallergens from Greer Pharmaceuticals (Lenoir, NC). Allergen extracts used for testing included dust mite (*D. farinae* and *D. pteronyssinus*), cockroach, cat, dog, *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Helminthosporium*, Eastern tree mix, oak, 7-grass mix, Bermuda grass, and short ragweed allergens. Skin test responses measuring 3 mm wheal $\geq$ a saline control were read as positive.
- Bronchial reactivity to methacholine consistent with the diagnosis of asthma was determined by calculating the provocative concentration causing a 20% fall in FEV1 (PC₂₀) at a methacholine concentration of < 16 mg/ml^{E3}.

B. Home monitoring:

- Diary cards were used by subjects to record their upper and lower respiratory tract symptom scores twice daily before breakfast and bedtime beginning the day of enrollment until the day of RV inoculation and over the 21 days of monitoring during the infection. The upper respiratory tract symptoms (URTS) recorded included sneezing, nasal discharge, nasal obstruction, sore throat, headache, malaise, or chills, using a modification of the Jackson criteria as previously described^{E4}. Each symptom was scored on a severity scale of 0 to 3 (i.e. 0 = symptoms not present; 1 = mild, but clearly present; 2 = moderate and uncomfortable; and 3 = severe, interfering with sleep or activity). Lower respiratory symptoms (LRTS) monitored included cough, chest tightness, wheeze, and shortness of breath and night awakenings due to respiratory tract symptoms. Each LRTS symptom was recorded using the same severity scale utilized to evaluate URTS.

C. Administration of study drug (omalizumab and placebo):

- Injections of either omalizumab (150 to 300 mg) or placebo were given monthly, or bi-monthly, depending on the subject's weight and total IgE level using the

prescribing guidelines provided by the manufacturer^{E1}. The dose (mg) and dosing frequency was determined by total serum IgE level (IU/mL) measured before the start of treatment and body weight (kg).

- The placebo used was provided by Novartis Pharmaceuticals (Cambridge, MA) and contained the same mixture of inactive excipients, in quality and quantity, as used for the drug product.

D. Inoculation with RV-16:

A nasal wash was performed before inoculation for assessments of RV by qPCR and titers of virus in culture. Each subject was inoculated with 0.5 ml of RV type-16 in each nostril (divided into 2 instillations of 0.25 ml per naris) after obtaining the pre-inoculation nasal wash. The RV-16 virus pool used for inoculation was diluted in HANK's balanced salt solution and pre-titrated to contain 300-500 TCID₅₀/ml (tissue culture infectious dose 50/ml).

E. Procedures and assessments performed during each study visit and daily in the hotel:

- Spirometry: Spirometry was done in the morning using a Koko Spirometer (Inspire Health Care, Louisville, CO) approved and standardized by the American Thoracic Society (ATS).
- Impulse Oscillometry (IOS): IOS was performed on a Jaeger IOS Analyzer; Viasys Healthcare, Hoechst, Germany using methods previously described^{E5}.
- FeNO was done by having subjects exhale for 12 seconds into a portable hand held FeNO monitor NioxMino® Nitric Oxide analyzer (Aerocrine AB, Sweden).
- Nasal Wash Procedure: As previously described (but with nasal wash solution volumes adjusted for adults)^{E6}, a small amount (2 ml) of phosphate buffered saline (PBS), pH 7.4 was instilled into each nostril using a Mucosal Atomization Device (Wolfe Tory Medical, Inc; www.wolfetory.com). Each nostril was treated separately with the subject sitting upright and holding their breath. Wash fluid and secretions were aspirated with gentle suction through a BBG Nasal Aspirator (Codan, US Corp., Santa Ana, CA) inserted into the anterior nares. Two ml of PBS containing 0.25% gelatin for stabilization of protein in the wash fluid was aspirated through the suction device to rinse any residual secretions in the trap. The combined washes from both nares resulted in volumes of 4 to 5 ml of fluid and secretions per nasal wash. After the wash; a plastic transfer pipette was used to vigorously mix secretions with wash fluid. One ml of this mixture was transferred to a vial containing veal infusion broth (Bartels Viral Transport Medium, Trinity Biotech, USA) which was used to measure titers of RV in culture. The remaining wash solution and

secretions were frozen and stored at -80°C for qPCR-based viral analyses and for immunoassays.

- **CBC and differential cell count:** Differential cell counts were done on a SYSMEX XN counter (Sysmex America, Inc. in Lincolnshire, IL).
- **Asthma Control Questionnaire (ACT):** The validated ACT questionnaire was administered to all subjects in the Asthma/Control study and the Anti-IgE study as part of the assessment of LRTS's before and after the infection^{E7}. The ACT questionnaire was completed a) when subjects in the Asthma/Control study were admitted to the hotel on the day of RV inoculation and b) when subjects in the Anti-IgE study were enrolled 8 weeks before the RV challenge, at the clinic visit 4 week clinic visit before the RV challenge, and on the day of RV inoculation in the hotel. One week after subjects in either study completed the 21 days of monitoring (i.e. 28 days after RV inoculation), subjects came to clinic to fill out the ACT questionnaire and to receive their payment for participation.

F. Sample size estimates:

- **Asthma/Control Study:** Sample size estimate was based on the results of our previous RV challenge study which indicated that if 7 participants completed the trial in each of the study groups (asthma and controls), there would be 80% power to detect a significant difference, with a 2-sided significance level of 5%, when the mean difference in cumulative lower respiratory tract symptom (LRTS) score over the first 4 days after RV infection was equal to 3.8. The power was computed based on simulation, using the permutation distribution of the two-sample Wilcoxon test. Even though the sample size indicated 7 participants per group, the approved IRB protocol allowed for an enrollment of up to 14 participants per study group.
- **Anti-IgE Study:** Sample size estimations indicated that if 17 participants completed the study in each study group the results would yield 80% power, with a 2-sided significance level of 5%, to achieve the primary outcome if the mean difference in cumulative LRTS scores over the first 4 days was equal to 2.4. A 20% dropout rate was anticipated. The power was based on the Wald test in a generalized linear model for a negative binomial outcome, using the LRTS score on the day prior to inoculation as a covariate. As noted in the disposition of subjects screened (Fig. E1), the dropout rate for the Anti-IgE study was higher than 20% (i.e., 35%). There was also a high screening to enrollment ratio of 15:1 to identify subjects eligible for participation in the Anti-IgE study. In part this high ratio resulted from the limitations of total serum IgE levels needed for participation in order for subjects to receive the study drug (omalizumab). Thus, 20 subjects completed the study and were included in the data analysis.

G. Criteria used to define infection and for including subjects in the data analysis for the Asthma/Control and Anti-IgE studies:

- The criteria to define infection included a positive test for RV-16 by qPCR or titers in culture during the first 4 days of the infection after virus inoculation. All subjects in both clinical trials met these criteria, and,
- All subjects in both clinical trials had cumulative URTS scores during the first 4 days of the infection that were greater than their cumulative scores during the 4 days prior to RV inoculation.

Table E3A. Differences late in the infection (Day 21) among asthma and control subjects: Asthma/Control Study.

	Asthma		Control		Asthma – Control	
	n	Median (Q1, Q3)	n	Median (Q1, Q3)	*Difference (95% CI)	P value
Late in the Infection (Day 21)						
FEV1 (% predicted)	11	85.0 (80.0, 98.0)	13	100 (96.0, 104)	-15.0 (-22.0, -1.0)	0.026
FEV1/FVC ratio	11	0.8 (0.8, 0.8)	13	0.8 (0.8, 0.9)	0.0 (-0.1, 0.0)	0.037
FEF25-75 (% predicted)	11	68 (58, 94)	13	96.0 (85.0, 109)	-28.0 (-43.0, -8.0)	0.013
R at 5 Hz (% predicted)	11	136.8 (109, 154)	13	108 (93.6, 124)	28.8 (-0.2, 48.7)	0.046
R at 20 Hz (% predicted)	11	124.4 (118, 156)	13	117.6 (104, 132)	6.8 (-4.6, 32.4)	0.125
R at 5 Hz - R at 20 Hz	11	0.6 (0.4, 1.2)	13	0.5 (0.1, 0.6)	0.1 (0.0, 0.8)	0.068
Z at 5 Hz (% predicted)	11	148.6 (113, 157)	13	112.9 (96.2, 130)	35.7 (-0.2, 55.1)	0.046
A _x	11	5.3 (2.9, 10.3)	13	2.3 (1.8, 3.1)	3.0 (0.0, 6.2)	0.046
X at 5 Hz (% predicted)	11	-425 (-1057, 619)	13	-927 (-1489, -288)	502 (-160, 1458)	0.111
Resonant Frequency	11	13.0 (12.1, 19.5)	13	10.0 (9.1, 10.7)	3.0 (-0.2, 5.8)	0.052
FeNO (ppb)	11	51.0 (35.5, 104)	13	17.0 (13, 20)	34.0 (16.5, 87.5)	<0.001
Eosinophils	11	359 (231, 435)	13	99 (80.0, 150)	260 (131, 324)	<0.001
Neutrophils	11	2678 (2171, 3560)	13	2926 (2176, 4108)	-248 (-1430, 590)	.339
Basophils	11	40.3 (19.2, 50.9)	13	39.5 (19.3, 51.3)	0.8 (-12.5, 22.8)	0.750
Lymphocytes	11	1690 (1432, 2210)	13	1452 (1309, 1758)	238 (-68, 648)	0.111
Monocytes	11	451 (349, 551)	13	412 (321, 547)	39 (-142, 167)	0.750
qPCR (log copies)	11	3.1 (2.7, 3.3)	13	3.3 (3.2, 3.4)	-0.1 (-0.4, 0.1)	.339
RV titers	11	-0.5 (-0.5, -0.5)	13	-0.5 (-0.5, -0.5)	0.0 (0.0, 0.0)	.358

Table E3B. Differences late in the infection (Day 21) among placebo and omalizumab treated subjects: Anti-IgE Study.

	Placebo		Omalizumab		Placebo – Omalizumab	
	n	Median (Q1, Q3)	n	Median (Q1, Q3)	*Difference (95% CI)	P value
Late in the Infection (Day 21)						
FEV1 (% predicted)	10	97.0 (92.0, 107)	10	90.0 (88.0, 104)	7.0 (-7, 15)	0.427
FEV1/FVC ratio	10	0.9 (0.8, 0.9)	10	0.8 (0.7, 0.8)	0.1 (0.0, 0.2)	0.006
FEF25-75 (% predicted)	10	92.0 (86.0, 123)	10	80.0 (58.0, 89.0)	12.0 (0.0, 43)	0.041
Methacholine PC ₂₀ (mg/ml)	10	1.0 (0.7, 2.0)	9	2.1 (0.4, 5.1)	-1.0 (-4.4, 0.7)	0.514
R at 5 Hz (% predicted)	9	106.7 (89.3, 120)	10	113.5 (105, 159)	-6.8 (-65.3, 13.0)	0.288
R at 20 Hz (% predicted)	9	111.5 (105, 121)	10	126.3 (122, 174)	-14.8 (-68.6, -2.8)	0.027
R at 5 Hz - R at 20 Hz	9	0.6 (0.1, 0.7)	10	0.2 (-0.1, 0.5)	0.4 (-0.2, 0.8)	0.220
Z at 5 Hz (% predicted)	9	109.9 (96.4, 131)	10	116.4 (106, 164)	-6.5 (-66.9, 17.6)	0.228
A _x	9	3.3 (2.4, 5.4)	10	2.4 (1.6, 4.0)	0.8 (-0.7, 3.8)	0.153
X at 5 Hz (% predicted)	9	-663 (-953, 1188)	10	-679 (-1000, -144)	15.4 (-2195, 3846)	0.683
Resonant Frequency	9	12.3 (9.9, 14.0)	10	10.0 (8.8, 12.3)	2.3 (-2.0, 4.0)	0.236
FeNO (ppb)	10	31.0 (16.5, 80.0)	10	22.0 (16.5, 51.0)	9.0 (-13.5, 41.0)	0.384
Eosinophils	9	160 (102, 511)	10	129 (110, 271)	31.0 (-111, 401)	0.624
Neutrophils	9	2698 (2645, 2938)	10	3533 (3060, 3669)	-835 (-1148, 358)	0.221
Basophils	9	42.0 (32.0, 52.0)	10	41.0 (33.0, 73.0)	1.0 (-31.0, 12.0)	0.653
Lymphocytes	9	1859 (1622, 2022)	10	1830 (1548, 2188)	29 (-482, 362)	0.806
Monocytes	9	429 (390, 571)	10	480 (400, 690)	-51.0 (-268, 171)	0.683
qPCR (log copies)	10	2.6 (2.4, 2.7)	10	2.8 (2.2, 3.4)	-0.2 (-0.8, 0.4)	0.406
RV titers	10	-0.5 (-0.5, -0.5)	10	-0.2 (-0.5, -0.5)	0.0 (0.0, 0.0)	0.317

Results show medians and interquartile ranges for each group.

* denotes the difference in median values between groups.

p values are based on between group differences; significant p values ($p \leq 0.05$) are noted in bold.

Table E4A. Blood cell counts at inoculation (day 0) and during the acute infection (days 1-4) among asthma and control subjects: Asthma/Control Study.

	n	Asthma Median (Q1, Q3)	n	Control Median (Q1, Q3)	Asthma – Control *Difference (95% CI) P value
Inoculation (Day 0)					
WBC	11	5380 (4420, 5670)	13	4800 (4710, 5330)	580 (-610, 870) 0.706
Eosinophils	11	230 (173, 270)	13	102 (61, 159)	128 (41, 191) 0.006
Neutrophils	11	2697 (2209, 3190)	13	2581 (2436, 3306)	116 (-672, 524) 0.543
Lymphocytes	11	1712 (1461, 2147)	13	1529 (1439, 1860)	183 (-150, 476) 0.296
Basophils	10	32 (22, 51)	12	32 (20, 48)	0 (-17, 19) 0.792
Monocytes	11	411 (352, 568)	13	399 (319, 549)	12 (-107, 142) 0.643
Platelets	11	253 (216, 285)	13	251 (232, 290)	2 (-43, 41) 0.931
Acute infection (Days 1–4)					
WBC	11	4890 (1860, 8120)	13	3520 (2450, 4020)	1370 (-1470, 4600) 0.339
Eosinophils	11	306 (172, 561)	13	201 (113, 264)	105 (-22, 445) 0.099
Days 1–2	11	129 (103, 230)	13	85 (52, 127)	44 (1, 145) 0.034
Days 1–3	11	259 (165, 486)	13	155 (115, 191)	104 (5, 324) 0.030
Neutrophils	11	1791 (1001, 3582)	13	2099 (745, 3040)	-308 (-1876, 1689) 0.977
Days 1–2	11	334 (-136, 495)	13	372 (6, 657)	-38 (-666, 538) 0.839
Days 1–3	11	1093 (477, 1577)	13	1223 (446, 1737)	-130 (-1143, 825) 0.716
Lymphocytes	11	1200 (184, 3877)	13	498 (-39, 1540)	702 (-424, 2356) 0.173
Days 1–2	11	827 (347, 1822)	13	341 (178, 896)	486 (-102, 1118) 0.077
Days 1–3	11	1247 (347, 3344)	13	227 (132, 1361)	1020 (-264, 1833) 0.068
Basophils	10	21 (-19, 69)	12	32 (-10, 45)	-12 (-43, 52) 0.843
Monocytes	11	640 (186, 1152)	13	395 (114, 724)	245 (-356, 595) 0.582
Platelets	11	32 (-44, 108)	13	-4 (-13, 14)	36 (-39, 110) 0.354

Table E4B. Blood cell counts at inoculation (day 0) and during the acute infection (days 1-4) among placebo and omalizumab treated asthmatics: Anti-IgE Study.

	n	Placebo Median (Q1, Q3)	n	Omalizumab Median (Q1, Q3)	Placebo – Omalizumab *Difference (95% CI) P value
Inoculation (Day 0)					
WBC	10	5150 (4940, 5810)	10	5510 (4500, 6390)	-360 (-1250, 8300) 0.734
Eosinophils	10	171 (168, 312)	10	142 (78, 272)	29 (-51, 176) 0.290
Neutrophils	10	2781 (2333, 3469)	10	2760 (2622, 3245)	21 (-778, 681) 0.880
Lymphocytes	10	1671 (1592, 1962)	10	1940 (1440, 2410)	-269 (-667, 300) 0.880
Basophils	10	31 (28, 42)	10	31 (30, 58)	0 (-19, 11) 0.880
Monocytes	10	371 (341, 628)	10	471 (419, 540)	-100 (-152, 192) 0.450
Platelets	10	257 (204, 298)	10	262 (206, 310)	-5 (-55, 84) 0.910
Acute infection (Days 1–4)					
WBC	10	2530 (1490, 8360)	10	3310 (-560, 5830)	-780 (-4180, 5360) 0.597
Eosinophils	10	185 (122, 447)	10	90 (84, 142)	95 (-46, 363) 0.082
Days 1–2	9	72 (28, 171)	7	60 (32, 119)	12.0 (-153, 139) 0.791
Days 1–3	9	98 (28, 171)	7	44.5 (40, 119)	53.5 (-189, 278) 0.791
Neutrophils	10	1836 (769, 7960)	10	1676 (631, 6558)	160 (-4722, 5300) 0.705
Days 1–2	9	829 (298, 2042)	7	1185 (1152, 2011)	-356 (-2082, 1583) 0.560
Days 1–3	9	829 (551, 2932)	7	1205 (1168, 3653)	-376 (-3355, 1747) 0.368
Lymphocytes	10	-260 (-1001, 1153)	10	-600 (-961, 816)	340 (-773, 1432) 0.705
Days 1–2	9	346 (-228, 651)	7	440 (-301, 632)	-94 (-766, 936) 0.958
Days 1–3	9	346 (-228, 829)	7	298 (-509, 632)	48.0 (-668, 1026) 0.491
Basophils	10	6.5 (-8, 23)	10	2.0 (-4, 29)	4.5 (-36, 23) 0.880
Monocytes	10	427 (192, 1001)	10	562 (25, 73)	-135 (-382, 444) 0.940
Platelets	10	-36.0 (-58, -10)	10	-24.5 (-33, -8.0)	-11.5 (-52, 21) 0.384

Cell counts are reported as cells/mm³. Results show median and intra-quartile ranges for each study group.

* denotes the difference in median values between groups.

p values are based on between group differences; significant p values ($p \leq 0.05$) are noted in bold.

During the acute infection (days 1-4) the values represent AUC analysis of medians adjusted for day 0 and the same for days 1-2 and 1-3 analyses for eosinophils, neutrophils, and lymphocytes.

Table E5A. Comparison of assessments at inoculation (day 0) between untreated asthmatics who experienced LRTS over days 0-4 versus those who did not.

	Asthmatics with LRTS		Asthmatics without LRTS		With LRTS - Without	
	n	Median (Q1, Q3)	n	Median (Q1, Q3)	*Difference (95% CI)	P value
URS total	9	0.0 (0.0, 2.0)	12	0.0 (0.0, 0.0)	0.0 (0.0, 2.0)	0.294
LRS total	9	1.0 (0.0, 2.0)	12	0.0 (0.0, 1.0)	1.0 (0.0, 2.0)	0.162
FEV ₁ (% predicted)	9	89.0 (84.0, 99.0)	11	94.0 (87.0, 100)	-5.0 (-15, 6.0)	0.403
FEV ₁ /FVC ratio	9	86.0 (82.0, 87.0)	11	85.0 (80.0, 88.0)	1.0 (-6.0, 6.0)	0.879
FEF ₂₅₋₇₅ (% predicted)	9	90.0 (75.0, 96.0)	11	89.0 (78.0, 101)	1.0 (-31.0, 14.0)	0.879
Methacholine PC ₂₀ (mg/ml)	8	0.7 (0.1, 1.2)	12	0.4 (0.1, 0.5)	0.3 (-0.3, 1.1)	0.316
FeNO	9	2.8 (2.5, 5.1)	12	2.7 (1.8, 4.8)	0.1 (-1.3, 2.5)	0.499
R at 5 Hz (% predicted)	8	99.1 (94.3, 121)	12	133.0 (103, 163)	-33.9 (-61.6, 2.7)	0.076
R at 20 Hz (% predicted)	8	121.2 (114, 131)	12	138.7 (115, 144)	-17.4 (-48.1, 14.9)	0.165
R at 5 Hz - R at 20 Hz	8	0.1 (0.0, 0.3)	12	0.6 (0.1, 0.9)	-0.5 (-0.9, 0.1)	0.090
Z at 5 Hz (% predicted)	8	103.1 (95.5, 128)	12	142.2 (109, 174)	-39.1 (-59.2, 0.0)	0.054
X at 5 Hz (% predicted)	8	14.3 (-363, 1111)	12	-610.5 (1729, 332)	624.8 (-318, 3993)	0.076
Ax	8	2.0 (0.7, 2.7)	11	4.2 (3.0, 9.7)	-2.2 (-7.7, -0.2)	0.048
Resonant Frequency	8	10.1 (7.9, 11.6)	12	11.8 (10.2, 16.3)	-1.6 (-8.1, 0.5)	0.064
Eosinophils	9	270 (230, 436)	12	171 (160, 240)	99.0 (0.0, 230)	0.043
Neutrophils	9	2399 (2281, 4081)	12	2781 (2037, 2960)	-382 (-627, 1162)	0.776
Basophils	9	32.3 (30.9, 43.1)	12	29.0 (20.0, 41.0)	3.3 (-17.2, 21.0)	0.455
Lymphocytes	9	1712 (1568, 1910)	12	1671 (1533, 1962)	41.0 (-370, 280)	0.887
Monocytes	9	452 (371, 639)	12	371 (341, 442)	81.0 (-71.0, 295)	0.213
Platelets	9	253 (216, 292)	12	257 (194, 275)	-4.0 (-49.0, 63.0)	0.696
ACT	9	23.0 (21.0, 23.0)	12	22.0 (21.0, 23.0)	1.0 (-1.0, 2.0)	0.884
Total IgE (IU/ml)	9	623, (233,749)	12	473 (156, 1989)	150 (-1402, 467)	0.887

Results show medians and interquartile ranges for each study group.

* denotes the difference in median values between groups.

p values are based on between group differences; significant p values ($p \leq 0.05$) are noted in bold.

There were 9 Asthmatics (not treated with Omalizumab) who developed LRTS above baseline (5 from the Asthma/Control Study and 4 from the Placebo group of the Anti-IgE Study). The 12 Asthmatics who did not experience symptoms included 6 each from the Asthma/Control Study and Anti-IgE studies.

The results showing differences in medians in this table were used to generate the summary plot shown in Fig. 5A.

Table E5B. Comparison of assessments over days 0-4 between untreated asthmatics who experienced LRTS versus those who did not.

	Asthmatics with LRTS		Asthmatics without LRTS		With LRTS - Without	
	n	Median (Q1, Q3)	n	Median (Q1, Q3)	*Difference (95% CI)	P value
URS total	9	13.3 (9.5, 19.8)	12	5.3 (4.0, 9.5)	8.0 (0.8, 14.5)	0.017
LRS total	9	4.8 (3.5, 6.3)	12	0.0 (0.0, 0.3)	4.8 (2.3, 6.8)	<0.001
FEV ₁ (% predicted)	8	-4.0 (-13.5, -1.0)	10	-18 (-26, 1.5)	14.0 (-8.5, 28.5)	0.183
FEV ₁ /FVC ratio	8	-0.1 (-0.2, 0.0)	10	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.3)	0.722
FEF ₂₅₋₇₅ (% predicted)	8	-26.5 (-36.0, -6.5)	10	-30.5 (-56.0, -8.5)	4.0 (-17.0, 72.5)	0.307
Methacholine PC ₂₀ (mg/ml)	9	0.0 (-0.3, 0.0)	10	-0.1 (-0.2, 1.2)	0.1 (-1.5, 0.2)	0.288
FeNO	9	102.5 (96.5, 173)	12	65.5 (43.4, 90.3)	37.0 (-8.3, 98.0)	0.055
R at 5 Hz (% predicted)	8	11.8 (6.5, 21.9)	12	-10.8 (-19.9, 9.3)	22.6 (0.5, 34.2)	0.037
R at 20 Hz (% predicted)	8	9.2 (-6.4, 20.1)	12	-5.0 (-25.2, 4.0)	14.1 (-9.5, 26.6)	0.190
R at 5 Hz - R at 20 Hz	8	0.2 (0.1, 0.3)	12	0.0 (-0.5, 0.2)	0.3 (0.0, 0.7)	0.045
Z at 5 Hz (% predicted)	8	11.2 (4.9, 20.6)	12	-9.0 (-19.0, 9.0)	20.2 (1.5, 35.6)	0.031
X at 5 Hz (% predicted)	8	-136.4 (-1171, -50.0)	12	-78.9 (-503, 33.3)	-57.4 (-939, 359)	0.396
Ax	8	1.8 (-0.3, 3.0)	11	0.2 (-2.4, 1.5)	1.6 (-1.2, 4.9)	0.248
Resonant Frequency	8	1.8 (0.1, 4.7)	12	-0.2 (-1.9, 1.6)	2.0 (-0.4, 6.6)	0.064
Eosinophils	9	887 (512, 1525)	11	517 (331, 566)	370 (-95.5, 1136)	0.138
Neutrophils	9	7361 (6305, 9347)	11	5674 (3952, 8327)	1687 (-488, 4504)	0.119
Basophils	9	84.0 (49.2, 107)	10	71.0 (39.0, 83.2)	13.0 (-29.6, 66.0)	0.288
Lymphocytes	9	4558 (3575, 5717)	11	3814 (1476, 4369)	744 (-794, 2993)	0.425
Monocytes	9	1371 (1232, 2134)	11	1075 (748, 1208)	296 (26.5, 1266)	0.030
Platelets	9	488 (246, 655)	11	444 (217, 542)	44.0 (-136, 317)	0.342
Viral PCR	9	4.7 (2.8, 6.9)	12	2.2 (1.7, 3.8)	2.5 (-0.5, 4.7)	0.155
Viral Titer	9	5.3 (4.4, 7.3)	12	4.8 (4.3, 5.1)	0.5 (-0.8, 2.9)	0.594

Results show medians and interquartile ranges for each study group.

* denotes the difference in median values between groups.

p values are based on between group differences; significant p values ($p \leq 0.05$) are noted in bold.

There were 9 Asthmatics (not treated with Omalizumab) who developed LRTS above baseline (5 from the Asthma/Control Study and 4 from the Placebo group of the Anti-IgE Study). The 12 Asthmatics who did not experience symptoms included 6 each from the Asthma/Control Study and Anti-IgE studies.

The results showing differences in medians in this table were used to generate the summary plot shown in Fig. 5B.

Table E6A. IOS Assessments at enrollment, day 0 (at inoculation), and on day 4 (adjusted for day 0 values): Asthma/Control Study.

	Asthma		Control		Asthma – Control	
	n	Median (Q1, Q3)	n	Median (Q1, Q3)	*Difference (95% CI)	P value
Enrollment (Day -7)						
R at 5 Hz (% predicted)	11	143 (112, 118)	13	108 (105, 134)	35 (1.5, 71)	0.026
R at 20 Hz (% predicted)	11	132 (108, 187)	13	127 (118, 146)	5 (-14, 47)	0.339
R at 5 Hz – R at 20 Hz	11	0.75 (0.42, 1.46)	13	0.41 (0.29, 0.49)	0.34 (0.10, 1.05)	0.011
A _x	11	4.92 (2.68, 10.68)	13	2.6 (1.46, 3.65)	2.32 (0.58, 6.26)	0.014
X at 5 Hz (% predicted)	11	-691 (-957, 433)	13	-817 (-1489, -322)	126 (-432, 1142)	0.369
Z at 5 Hz (% predicted)	11	145 (118, 193)	13	112 (108, 140)	33 (3.5, 77)	0.022
Resonant Frequency	11	13.0 (11.7, 16.3)	13	10 (9, 11.1)	3 (1.2, 5.9)	0.006
Inoculation (Day 0)						
R at 5 Hz (% predicted)	10	124 (114, 171)	13	106 (97, 128)	18 (-7, 57)	0.137
R at 20 Hz (% predicted)	10	135 (115, 166)	13	129 (109, 134)	7 (-14, 45)	0.321
R at 5 Hz – R at 20 Hz	10	0.32 (0.08, 1.25)	13	0.16 (-0.04, 0.66)	0.16 (-0.42, 0.97)	0.292
A _x	9	4.03 (1.30, 9.73)	13	2.56 (1.42, 3.69)	1.47 (-0.92, 7.68)	0.271
X at 5 Hz (% predicted)	10	-388 (-573, 616)	13	-991 (-1617, -325)	603 (-78, 1912)	0.072
Z at 5 Hz (% predicted)	10	136 (122, 178)	13	110 (102, 136)	26 (-5, 57)	0.154
Resonant Frequency	10	11.6 (9.7, 16.3)	13	9.9 (8.7, 11.9)	1.7 (-1.5, 6.3)	0.172
Acute infection (Day 4)						
R at 5 Hz (% predicted)	10	7.27 (-1.12, 22)	13	-7.28 (-16, 4)	14.6 (-2.62, 35)	0.063
R at 20 Hz (% predicted)	10	4.03 (-5.75, 22)	13	-8.06 (-12, 8)	12 (-1.92, 32)	0.107
R at 5 Hz – R at 20 Hz	10	0.13 (-0.51, 0.62)	13	0.02 (-0.11, 0.08)	0.11 (-0.47, 0.60)	0.292
A _x	9	-0.27 (-2.35, 3.56)	13	-0.19 (-0.66, 0.23)	-0.08 (-2.58, 4.03)	0.920
X at 5 Hz (% predicted)	10	-232 (-619, -37.5)	13	11 (-233, 207)	-243 (-710, 1.3)	0.055
Z at 5 Hz (% predicted)	10	7.14 (4.65, 19.33)	13	-9.89 (-23, 5.0)	17 (-0.36, 40)	0.072
Resonant Frequency	10	-0.39 (-1.05, 6.85)	13	-0.21 (-0.9, 0.38)	-0.18 (-1.4, 6.6)	0.620

Results show median and intra-quartile ranges for each group. R at 5, R at 20, X at 5, and Z at 5 Hz were measured in cm H₂O/L/s and are shown as % predicted values. Ax and Resonant Frequency assessments were measured in cm H₂O/L and 1/s, respectively.

*denotes the difference in median values. p values are based on between-group differences; significant p values (p ≤ 0.05) are noted in bold.

Table E6B. IOS assessments at enrollment, day 0 (at inoculation), and on day 4 (adjusted for day 0 values): Anti-IgE Study.

	Placebo		Omalizumab		Placebo – Omalizumab	
	n	Median (Q1, Q3)	n	Median (Q1, Q3)	*Difference (95% CI)	P value
Enrollment (Day -56)						
R at 5 Hz (% predicted)	9	103 (94, 119)	10	120 (117, 129)	-17 (-33, 3.0)	0.086
R at 20 Hz (% predicted)	9	109 (89, 128)	10	141 (136, 146)	-31 (-57, -8.0)	0.034
R at 5 Hz – R at 20 Hz	9	0.46 (0.17, 1.01)	10	0.21 (0.03, 0.48)	0.25 (-0.19, 0.95)	0.221
A _x	9	3.04 (2.40, 6.37)	10	2.29 (1.95, 3.31)	0.75 (-0.43, 5.13)	0.165
X at 5 Hz (% predicted)	9	-563 (-874, 811)	10	-750 (-957, -284)	187 (-793, 2146)	0.683
Z at 5 Hz (% predicted)	9	110 (100, 123)	10	126 (122, 132)	-16 (-32, 6.0)	0.086
Resonant Frequency	9	10.4 (9.4, 17.2)	10	9.5 (9.2, 11.1)	0.9 (-1.0, 6.8)	0.288
Inoculation (Day 0)						
R at 5 Hz (% predicted)	10	103 (94,133)	10	122 (118, 153)	-19 (-41, 5.0)	0.199
R at 20 Hz (% predicted)	10	122 (118, 139)	10	133 (126, 162)	-11, (-43, 9.5)	0.174
R at 5 Hz – R at 20 Hz	10	0.26 (0.13, 0.63)	10	0.35 (0.33, 0.54)	-0.09 (-0.39, 0.16)	0.273
A _x	10	2.74 (2.05, 4.23)	10	3.26 (1.79, 4.08)	-0.52 (-1.97, 1.35)	0.821
X at 5 Hz (% predicted)	10	-363 (-768, 1225)	10	-493 (-1018, -277)	130 (-600, 3177)	0.450
Z at 5 Hz (% predicted)	10	109 (96, 142)	10	130 (121, 153)	-21, (-44, 5.4)	0.151
Resonant Frequency	10	10.2 (10.1,11.8)	10	10.8 (10.3, 12.7)	-0.54 (-2.9, 1.4)	0.406
Acute infection (Day 4)						
R at 5 Hz (% predicted)	10	0.87 (-11, 12)	9	4.08 (-23, 8.0)	-3.21 (-17, 35)	0.514
R at 20 Hz (% predicted)	10	-4.98 (-12, 9.1)	9	-6.41 (-26, 8.0)	1.42 (-26, 34)	0.683
R at 5 Hz – R at 20 Hz	10	0.15 (-0.04, 0.23)	9	0.03 (-0.40, 0.15)	0.12 (-0.10, 0.64)	0.191
A _x	10	0.46 (0.20, 1.78)	9	0.11 (-0.25, 0.15)	0.35 (-0.09, 3.23)	0.086
X at 5 Hz (% predicted)	10	-56 (-250, 33)	9	-29 (-97, 151)	-27 (-717, 217)	0.624
Z at 5 Hz (% predicted)	10	1.46 (-9.0, 11)	9	0.58 (-21, 14)	0.87 (-13, 33)	0.327
Resonant Frequency	10	0.53 (0.04, 3.8)	9	-0.46 (-1.5, 1.2)	0.99 (-0.7, 4.6)	0.086

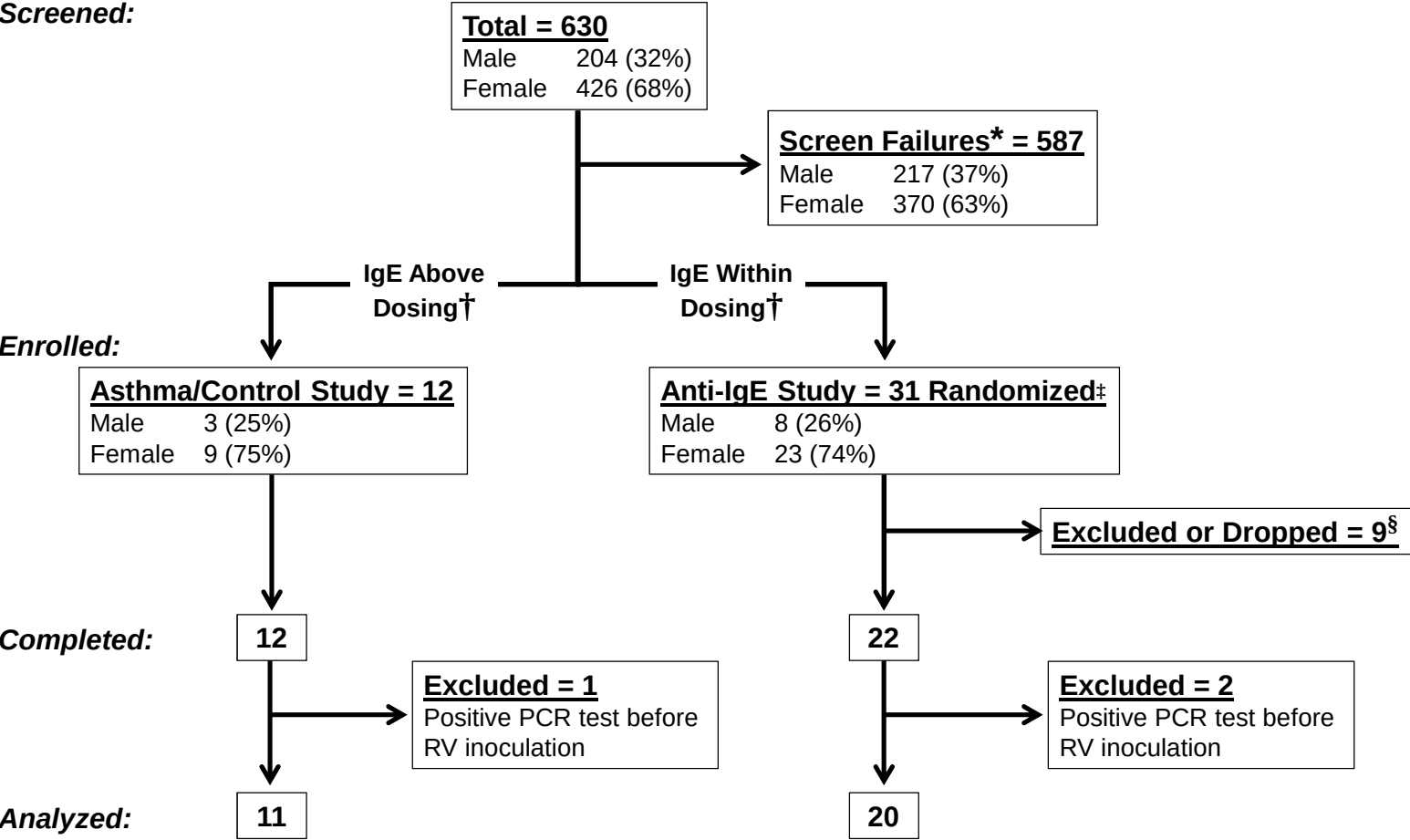
Results show median and intra-quartile ranges for each group. R at 5, R at 20, X at 5, and Z at 5 Hz were measured in cm H₂O/L/s and are shown as % predicted values. Ax and Resonant Frequency assessments were measured in cm H₂O/L and 1/s, respectively.

* denotes the difference in median values. p values are based on between-group differences; significant p value (p ≤ 0.05) are noted in bold.

Figure E1. Disposition of Subjects Screened for the Asthma/Control and Anti-IgE Trials (Flow Diagrams)

A. Asthmatics Participating in the Asthma/Control and Anti-IgE Studies:

Screened:



*Screen Failures Based on Exclusion Criteria (Table E1)	
Total serum IgE < 125 IU/mL	224
Using controller medications	209
Neutralizing antibody to RV-16 ≥ 1:4	112
Chronic medical condition or lost to follow up	31
Negative methacholine test	10
Negative skin tests	1

B. Non-Asthmatic Control Subjects Participating in the Asthma/Control Study

Screened:

Total = 147	
Male	58 (39%)
Female	89 (61%)

Screen Failures* =	
133	
Male	54 (41%)
Female	79 (59%)

Enrolled:

Asthma/Control Study = 14	
Male	4 (29%)
Female	10 (71%)

Completed:

14

Excluded = 1	
Uninfected ¶	

Analyzed:

13

***Screen Failures Based on Exclusion Criteria (Table E1)**

Total serum IgE > 50 IU/mL	58
Neutralizing antibody to RV-16 ≥ 1:4	52
Chronic medical condition or lost to follow up	16
Positive skin tests	7

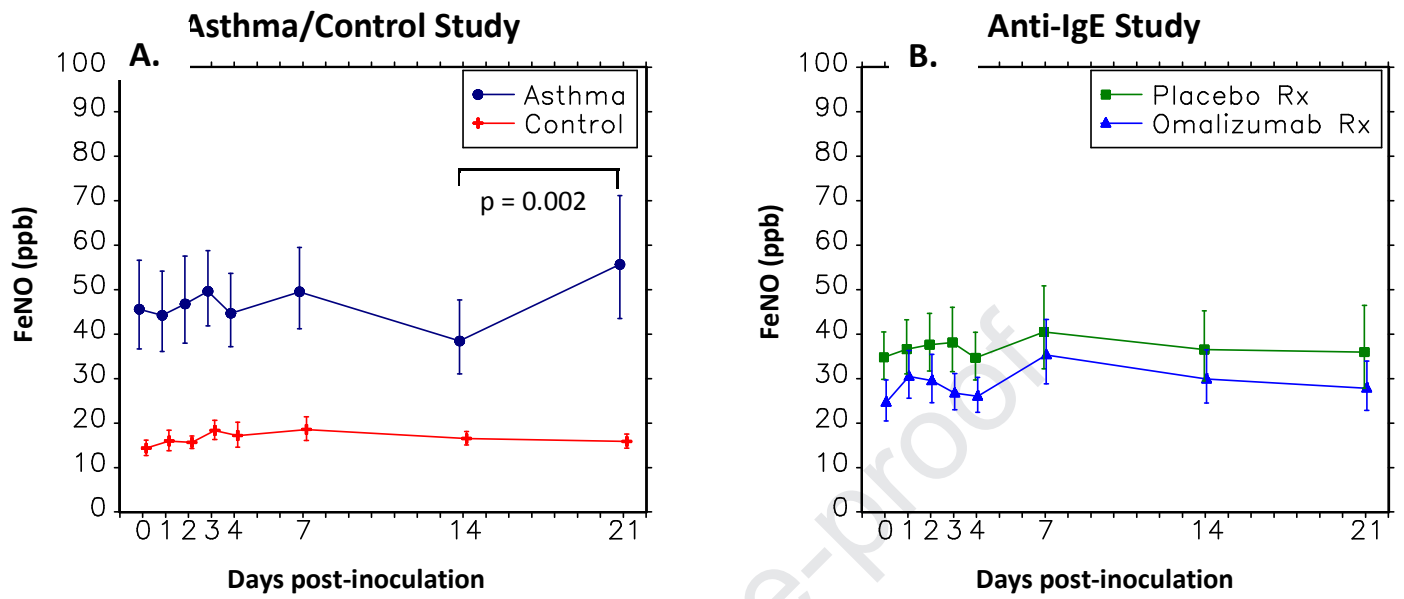
Figure E2. FeNO Measurements

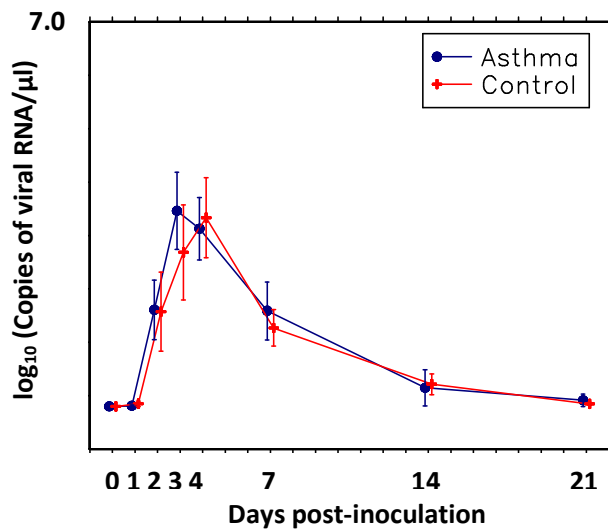
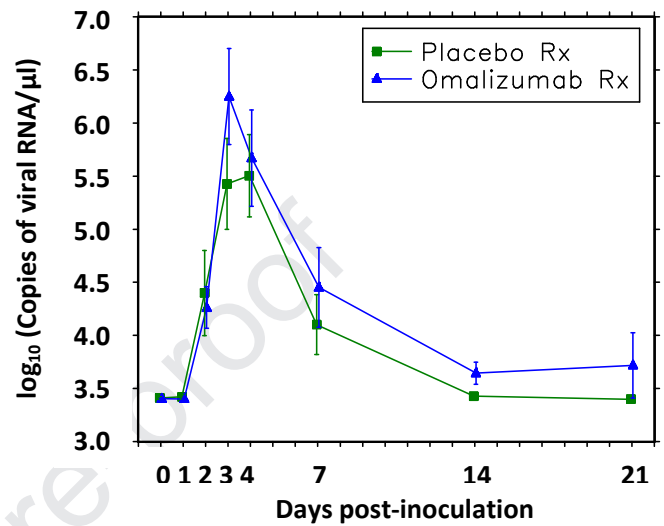
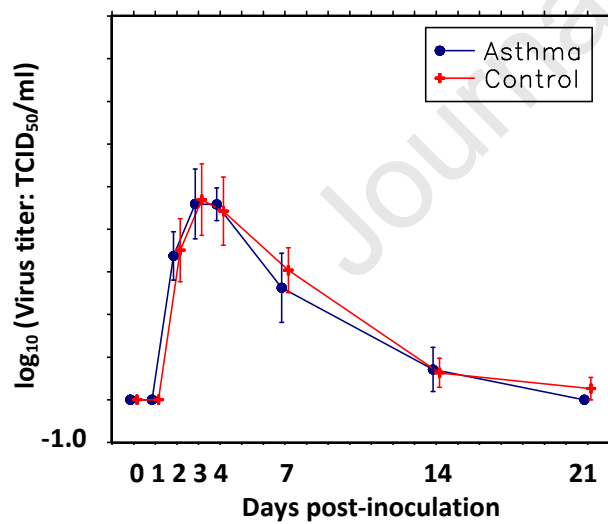
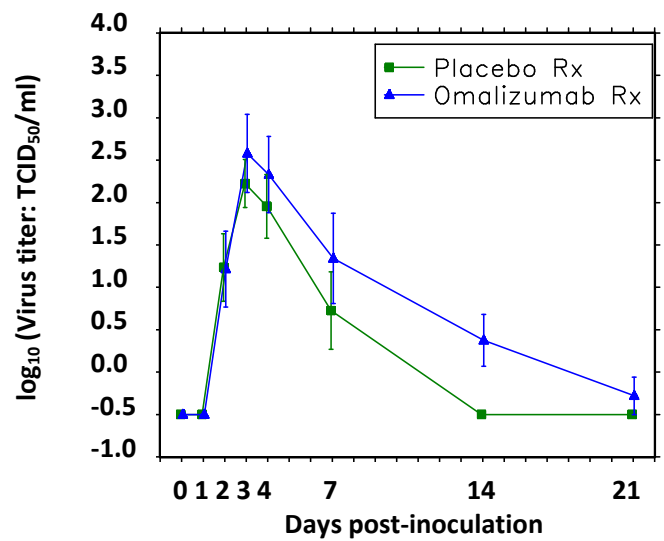
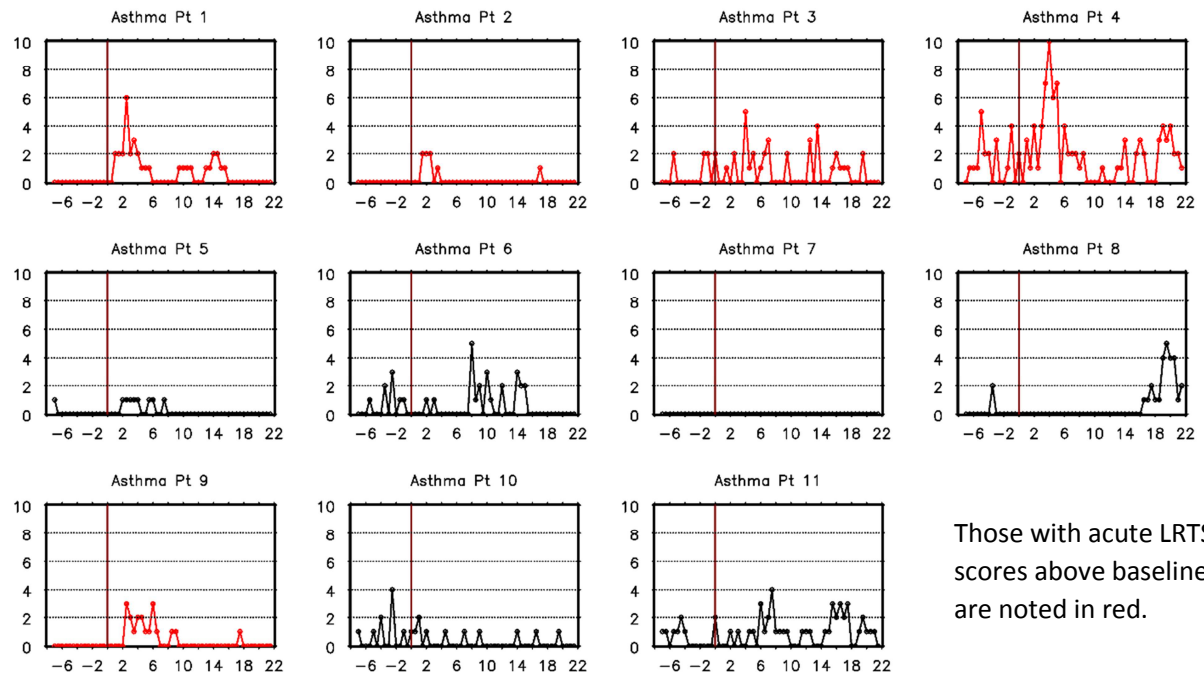
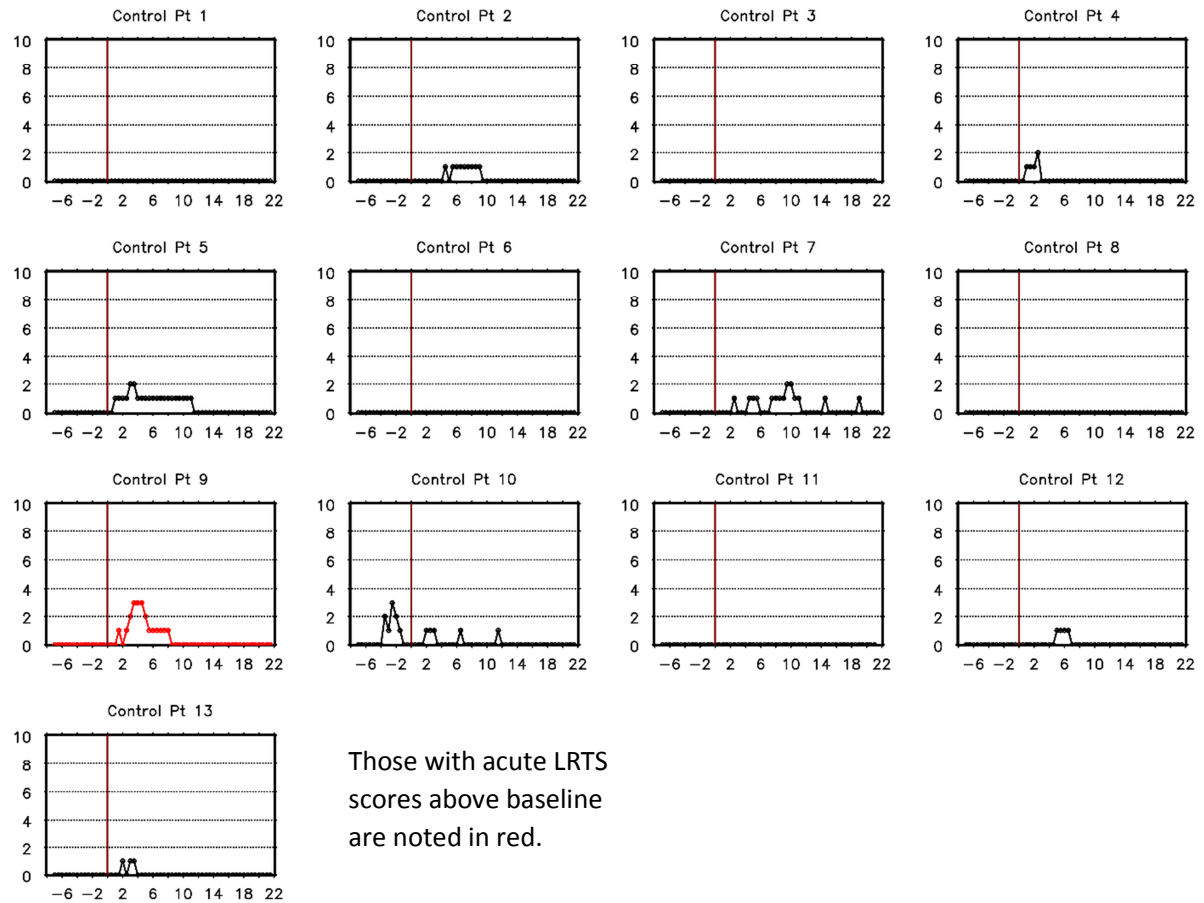
Figure E3. Viral Load**A. Asthma/Control Study****B. Anti-IgE Study****C. Asthma/Control Study****D. Anti-IgE Study**

Figure E4. Lower Respiratory Tract Symptoms (LRTS) recorded by subjects on their diary cards for:

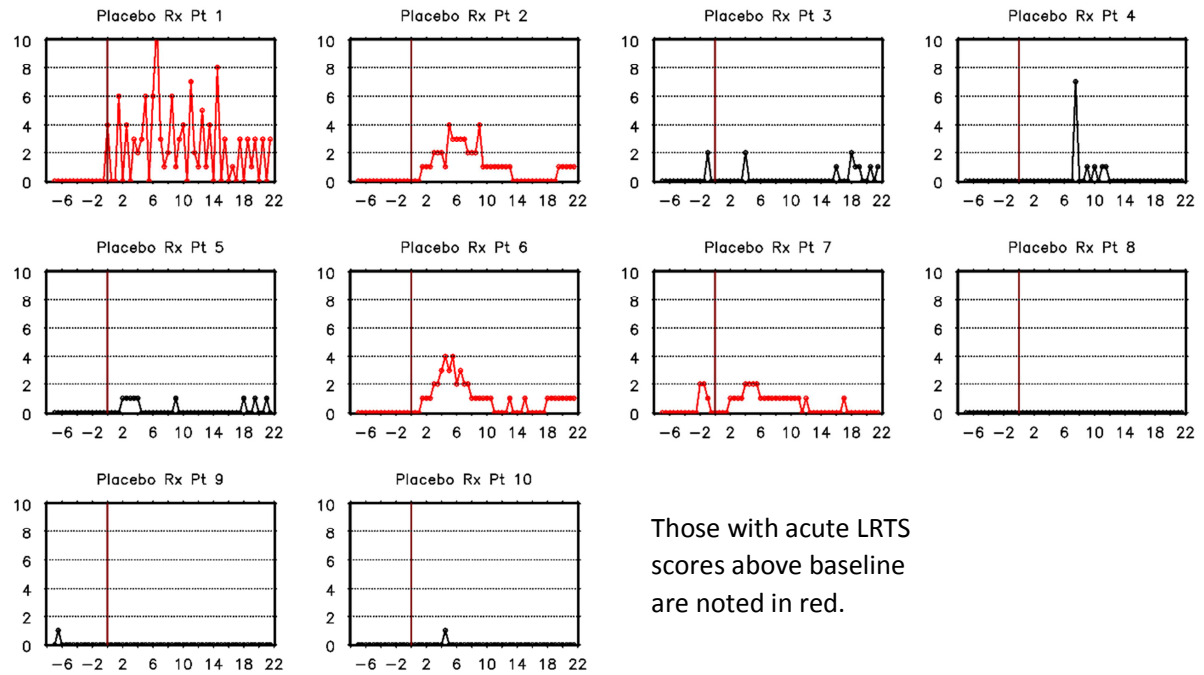
A. Asthma/Control Study: Asthmatics (n=11)



B. Asthma/Control Study: Healthy controls (n=13)



C. Anti-IgE Study: Placebo-treated asthmatics (n=10)



D. Anti-IgE Study: Omalizumab-treated asthmatics (n=10)

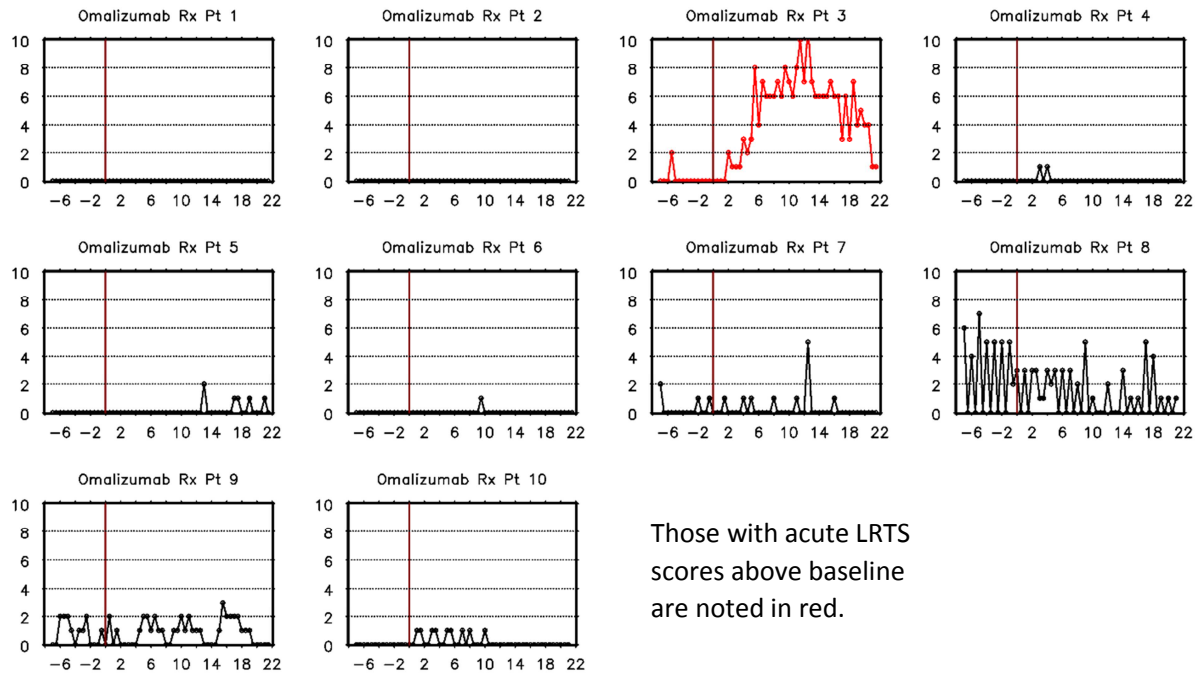


Figure 1. Study Design Diagrams. URTS and LRTS scores were recorded twice daily at home on diary cards by subjects participating in the Asthma/Control and Anti-IgE studies. The study design for both trials were identical following RV inoculation. Physical examination, spirometry, FeNO measurements, and sample collection (nasal washes and blood) were done on study days marked by downward arrows prior to and following RV inoculation. Methacholine challenges were done on days 0, 4, and 21; IOS assessments on days 0, 4, 7, and 21; and tests for neutralizing antibody to RV-16 on days 0 and 21. Stars under the Anti-IgE study diagram indicate days when study drug (placebo or omalizumab) was administered.

Figure 2. Symptom scores. Mean and standard errors are shown for URTS and LRTS scores. After adjusting for baseline symptoms, AUC comparisons for URTS scores between groups over the first 4 days following inoculation were 10.5 (8.3) and 10.4 (7.0) for asthma and control subjects, respectively, ($p=0.88$; **Panel A**); and 10.0 (5.2) and 7.3 (6.6) for scores from the placebo and omalizumab treated asthmatics, respectively, ($p=0.17$; **Panel B**). AUC comparisons for LRTS scores over the first 4 days (primary outcome) were 2.7 (4.3) and 1.0 (2.1) for asthma and control subjects, respectively, ($p=0.42$; **Panel C**), and 2.1 (2.6) and 0.4 (1.8) for the placebo and omalizumab treated asthmatics, respectively, ($p=0.09$; **Panel D**). **Late symptoms:** Significant differences were observed over days 15-21 between groups in the Asthma/Control study for both URTS and LRTS scores. * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$. The differences for URTS and LRTS over days 15-21 between groups in the Anti-IgE study were not significant ($p=0.54$ and 0.91 , respectively). There were also no significant differences between the placebo and omalizumab treated groups in LRTS scores recorded on days 15, 16, and 17 (unadjusted, or adjusted for scores on day 0) ($p > 0.05$ for all comparisons).

Figure 3. Lung function changes (spirometry). By day 1, FEV₁ values declined by 3% and 0% from baseline (day 0) among asthmatics and controls in the Asthma/Control Study, respectively. They decreased by 5% and 3% among asthmatics treated with placebo and omalizumab in the Anti-IgE study ($p < 0.04$ and 0.05 , respectively). Over the first 4 days, percent change differences were significant between placebo and omalizumab groups for the FEV₁/FVC ratio and FEF₂₅₋₇₅ measurements (panels D and F) and also early in the infection between groups over the first 2, 3, and 4 days after inoculation ($p < 0.04$, 0.03 , and 0.02 , respectively). By day 21, significant reductions from baseline (day 0) occurred in FEV₁/FVC and FEF 25-75 among asthmatics in the Asthma/Control study, but not among the controls or the asthmatic study groups in the Anti-IgE study.

Figure 4. Blood Eosinophil and Neutrophil counts. Geometric mean values with standard error bars are shown. * $p < 0.05$.

Figure 5. Percentage of subjects who developed LRTS over the first 4 days of the infection within each study group. # = number of subjects who developed LRTS/total number of subjects within each group. Individual records from diary cards showing which subjects reported LRTS above their baseline within each study group are shown in Fig. E4.

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Figure E1. Disposition of Subjects Screened for the Asthma/Control and Anti-IgE Trials. The ratio of subjects screened to subjects enrolled was 15:1 for asthmatics and 10:1 for healthy controls. Enrollment dates for the Asthma/Control study were 10/30/13 – 4/27/17. Enrollment dates for the Anti-IgE study were 2/14/13 – 5/4/17.

†The dosing range of study drug (omalizumab or placebo) used to determine eligibility for enrollment in the Anti-IgE study was based on each individual's weight and total serum IgE, according to the manufacturer's recommendations (1).

‡ Randomization lists were generated using permuted block randomization with random block sizes of size 2,4,6, or 8. The randomization was stratified by season, with separate randomization lists generated for RV challenges scheduled for the spring and fall.

§ Subjects who were excluded or who dropped out during the 8-week run-in period included those who had: Low Hb, n=3; conflicts with school or work, n=2; developed neutralizing antibody to RV-16 before inoculation, n=1; a negative methacholine test before RV challenge, n=1; a minor concussion unrelated to the study, n=1; or an FEV1 <70% before inoculation, n=1.

¶ Among the non-asthmatic controls, one subject had negative tests for virus (culture and qPCR), did not develop cold symptoms, and did not sero-convert following RV inoculation.

Figure E2: FeNO measurements for asthmatics and controls in the Asthma/Control Study (**Panel A**) and for placebo and omalizumab treated asthmatics in the Anti-IgE study (**Panel B**). On day 0 (the day of RV inoculation), FeNO levels among asthmatics (Asthma/Control study) were significantly higher than levels among controls (values shown in Table 1A in the text). During the infection, however, no significant changes from baseline were observed in FeNO levels among asthmatic groups in either clinical trial. In parallel with their increase in LRTS during the last week of infection, FeNO levels among asthmatics in the Asthma/Control study were lower on day 14, but then increased significantly by day 21 (median values: 11.5 versus 44.5 ppb on days 14 and 21 respectively, $p = 0.002$).

Figure E3. Viral load comparisons in nasal washes: Similar viral loads during the acute infection (days 0-4) were observed in both clinical trials by qPCR tests for RV-16 ($p=0.56$; asthmatics vs controls based on AUC analyses, [panel A], and $p=0.29$; placebo vs omalizumab treatment groups [panel B]). Similarly, no significant differences in viral loads were detected as judged by titers of RV in culture ($p=0.79$; asthmatics vs controls [panel C], and $p=0.45$; placebo vs omalizumab treatment groups [panel D]). Values for both qPCR and viral titer measurements were transformed to logarithmic 10 scales for analysis. Vertical lines at data points represent standard error bars.

Figure E4: Lower Respiratory Tract Symptoms (LRTS) recorded by subjects on their diary cards for: **A.** asthmatics in the Asthma/Control study; **B.** healthy controls in the Asthma/Control study; **C.** placebo-treated asthmatics in the Anti-IgE study; **D.** omalizumab-treated asthmatics in the Anti-IgE study. Symptom scores are noted on the y-axis. Study days prior to and after RV inoculation are noted beneath the x-axis. Vertical lines indicate the day of RV inoculation.

Subjects who developed acute LRTS over the first 4 days above baseline are highlighted in red. Subjects who experienced a LRTS score > 2 for two or more days in succession after inoculation and who had cumulative scores that were greater than their LRTS scores during the 4 days before inoculation were identified as subjects who developed LRTS in response to RV inoculation.

Figure E5: Summary plots for comparisons between untreated asthmatics who developed LRTS over the first 4 days of the infection versus asthmatics who did not. There were 9 asthmatics (not treated with omalizumab) who developed LRTS above baseline (5 from the Asthma/Control Study and 4 from the placebo group in the Anti-IgE Study). The 12 asthmatics who did not experience symptoms included 6 each from the Asthma/Control and Anti-IgE studies.

A. Comparison of baseline characteristics on the day of inoculation. **B.** Comparison of changes over the first 4 days after RV inoculation (AUC analysis adjusted for baseline). None of the asthmatics included in the comparisons received omalizumab. Median AUC values with 95% CI's were used to create these summary plots. Data showing the median values with intra-quartile ranges for asthmatics who did versus those who did not develop LRTS are shown in Tables E5A (baseline comparisons) and B (comparisons during the acute infection). When indicated, units of measurement were divided by 10 (+), 100 (++), or 1000 (+++) in order to permit comparisons of each assessment on summary plots using the same scale.

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