Published in final edited form as:

J Allergy Clin Immunol. 2010 April ; 125(4): 889–895.e7. doi:10.1016/j.jaci.2009.09.012.

Effects of Omalizumab on Basophil and Mast Cell Responses using an Intranasal Cat Allergen Challenge

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Abstract

BACKGROUND—Omalizumab treatment suppresses FcεRI expression faster on blood basophils than skin mast cells.

OBJECTIVE—We utilized omalizumab to elucidate the relative contributions of basophil versus mast cell FcεRI activation in a nasal allergen challenge (NAC) model.

METHODS—Eighteen cat-allergic subjects were enrolled in a 3.5-month, double-blind, randomized (3.5:1), placebo-controlled trial of omalizumab using standard dosing. At baseline, subjects underwent NAC with lavage for PGD₂ measurement, skin prick test titration (SPTT), and blood sampling for basophil histamine release (BHR) and basophil IgE/FcεRI measurements. Basophil studies were repeated at day 3 and then weekly until cat allergen-induced BHR was <20% of baseline or until day 45. Baseline visit procedures were repeated after the BHR reduction (mid-study NAC) and at the treatment period's completion (final NAC).

RESULTS—Subjects treated with omalizumab who completed all NACs (n=12) demonstrated significant mean reduction in BHR to an optimal dose of cat allergen by mid-study NAC as compared to baseline (74% decrease, p=0.001). In addition, these subjects demonstrated significant decreases in mean combined nasal symptom scores (50% decrease, p=0.007) and total sneeze counts (59% decrease, p=0.01) by mid-study NAC relative to baseline NAC. In contrast, measures of mast cell response (SPTT and nasal lavage PGD₂) were only significantly reduced by the final NAC. Subjects on placebo (n=4) did not experience a shift in basophil, NAC symptom, or mast cell measures.

CONCLUSIONS—Reduction in nasal symptom scores occurred when the basophil, but not mast cell, response was reduced on omalizumab, implicating a role for basophils in the acute NAC response.

Keywords

IgE; IgE receptors; omalizumab; basophils; mast cells; basophil histamine release; skin prick test titration; nasal allergen challenge; cat allergy

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Capsule summary: A ratio of allergen specific to total IgE of <3% predicts an early onset of basophil hyporesponsiveness to allergen on omalizumab treatment and may also be relevant in predicting the onset of clinical response to omalizumab treatment.

Introduction

At least 20% of individuals in the United States suffer from some form of allergic disease. At the root of these allergic disorders are IgE antibodies that are critical to the induction and perpetuation of allergic responses. IgE arms mast cells and basophils to become capable of activation within seconds of exposure to small quantities of allergens. While the detection of allergen-specific IgE, either by skin testing or *in vitro* assays, is a useful marker of allergic sensitivity, the quantitative relationship between specific IgE levels and allergic responses is far from clear. In addition, the relevance of the percentage of total IgE directed to a particular allergen remains unclear in diagnosing or predicting allergic responses.¹

Omalizumab is a monoclonal antibody directed against IgE and is FDA-approved for use in allergic asthma.² It binds IgE on the same site of the Fc domain as the alpha chain of the high affinity FcεRI, and therefore, blocks the interaction between IgE and mast cells or basophils.^{3, 4} As IgE levels are reduced with omalizumab treatment, FcεRI expression on human basophils is reduced, which leads to eventual reductions in allergen-mediated activation.⁵ This reduction of basophil receptors is pronounced within 7 days of the initial administration⁵ and is reversible once omalizumab is discontinued.⁶ In contrast, omalizumab-induced reductions in skin mast cell FcεRI expression and function is unchanged at day 7 and significantly reduced by day 70.⁷ These actions of omalizumab allow its use as a mechanistic tool in the study of IgE. While these early studies utilized intravenously administered omalizumab (0.015–0.03 mg/kg/IgE (IU/mL)); similar kinetics of reduction of skin test early phase reactions have been seen with subcutaneous dosing (0.016 mg/kg/IgE (IU/mL), where a more careful kinetic revealed significant reductions in skin test responses by day 56.⁸

Nasal allergen challenge (NAC) has been used to study both the acute and late cellular, biochemical, and physiologic events that are elicited by localized allergen challenge.^{9, 10} These models implicated basophils rather than mast cells in the late phase response based on mediator release signatures, namely a second wave of histamine release (HR) in the absence of PGD₂.¹¹ Using a NAC model, omalizumab significantly suppressed the acute nasal volume reduction induced by NAC after 2 weeks of treatment.¹² In contrast, acute NAC symptoms, such as nasal congestion, are suppressed by 11 weeks¹³ and are more impressive than effects on nasal mediators of the allergic response such as histamine.¹⁴

In this study, we exploited the faster onset of omalizumab's effects on circulating blood basophils' surface IgE and FcεRI relative to tissue mast cells to elucidate the role of the basophil versus the tissue mast cell in a nasal airway allergen. This study is designed to define the kinetics of omalizumab-induced change in basophil allergen-mediated function while monitoring allergen-induced skin and nasal mast cell responses. Given the previous findings, we hypothesized that the acute clinical response to allergen would not be abrogated at the initial time of basophil hyporesponsiveness. We further hypothesized that the ratio of specific-to-total IgE, by controlling the absolute density of antigen-specific IgE on basophils and mast cells, may be a predictor of the acute allergic response, and that this ratio would also predict the ability of omalizumab to blunt allergen challenge responses. Finally, we were interested in the kinetics and magnitude of change in nasal versus skin tissue mast cell allergic responses.

Methods

Study subjects

Adult subjects between 18–50 years of age with a history of cat allergy were recruited by advertising from the greater Baltimore area. Informed consent was obtained via a protocol approved by the Johns Hopkins Hospital Institutional Review Board and the National Institute

of Allergy and Infectious Diseases' Data Safety Monitoring Boards. Subjects met the following inclusion criteria for enrollment: a clinical history of cat-induced allergic rhinitis for at least 2 years with or without mild persistent asthma, serum total IgE 30–700 IU/mL, cat allergen specific IgE > 0.35 IU/mL, basophil histamine release (BHR) to cat allergen $\geq 20\%$ of total leukocyte content (or 10–19% of total leukocyte content AND $> 50\%$ of optimal anti-IgE induced BHR), and a positive NAC to cat allergen as defined by ≥ 5 sneezes. The use of antihistamines, cromolyn, leukotriene modifiers and other non-steroid (azelastine and topical decongestants) nasal medications were allowed, but they were withheld for 5 days prior to each NAC session. For details, see the Methods section in this article's Online Repository at www.jacionline.org.

Study design

The study was a 15-week, double-blinded, placebo-controlled trial of 18 subjects enrolled at the Johns Hopkins Asthma and Allergy Center from July 2007 to September 2008. Eligible subjects were randomly assigned to receive either omalizumab or matched placebo in a 3.5:1 ratio. The dosage of omalizumab was equal to at least 0.016 mg/kg/IgE[IU/mL] per 4 weeks as approved for the treatment of allergic asthma. The overall study design is depicted in Figure 1. The relationship between visit number and study day can be found online (Table E1 and E2 in this article's Online Repository www.jacionline.org). Procedures performed at the baseline visit included: blood sampling for basophil and serum studies, NAC (baseline NAC) with nasal lavage, and skin prick test titration (SPTT) (0.001–10,000 BAU/mL cat allergen, Hollister-Stier lot number K76E7593 containing < 0.03 EU/mL endotoxin units (established by HP Environmental, Herdon, VA using Limulus Lysate test)). Subjects returned for blood sampling at day 3 and then once a week to monitor cat allergen-induced BHR, basophil surface phenotype and serum free IgE levels. When a subject's cat allergen-induced BHR decreased to $< 20\%$ of the baseline value or they reached study day 45; a second NAC, SPTT, and blood sampling were performed (mid-study NAC). At the completion of the treatment period, a final visit (final NAC) repeated all the procedures from the initial visit. Safety labs were also evaluated at screening and again on the day of final NAC.

Total, Free and Cat specific IgE levels

Total and cat allergen specific IgE were measured using the ImmunoCAP-250. Free IgE was measured at the same time as basophil studies using a label-free surface plasmon resonance based technology. For details, see the Methods section in this article's Online Repository at www.jacionline.org.

Basophil Isolation and Flow Cytometry

Venous blood was drawn into syringes containing 10 mM PBS-EDTA and blood basophils were isolated by a single Percoll-based density-gradient centrifugation technique with Accuspin separation tubes (Sigma), as described.¹⁵ For details, see the Methods section in this article's Online Repository at www.jacionline.org.

Basophil Histamine Release

Enriched basophils were enumerated and stimulated for HR using cat allergen 0.1–10 BAU/mL and formyl-met-leu-phe (fMLP, Sigma-Aldrich, 10^{-6} M) in duplicate for 45 minutes at 37 °C using calcium-containing buffers, as described previously.¹⁵ Automated fluorometry was used to measure HR in cell free supernatants.¹⁶ Results for each stimulus are reported as a percentage of the total histamine content found in an aliquot of lysed leukocytes after subtraction of spontaneous HR from cells in buffer alone.

Skin Prick Test Titration

SPTT were performed on the dorsum of the forearms with one-log dilutions of cat allergen (0.001–10,000 BAU/mL cat allergen) using a sterile, metal, bifurcated needle. For details, see the Methods section in this article's Online Repository at www.jacionline.org.

Nasal Allergen Challenge

The cat allergen for each challenge dose was administered as 2 sprays (100 μ L per activation) per nostril. For details, see the Methods section in this article's Online Repository at www.jacionline.org.

Prior to the NAC, baseline measures of nasal symptoms were recorded using a visual analog score (VAS) (1–10) for nasal congestion, rhinorrhea, burning or pain in the nose, and itching in the nose (maximal score 40).¹⁷ A baseline sneeze count over 10 minutes was also recorded. The nasal passages were then washed with five, 5–10 mL lavages of warmed LR followed by administration of oxymetazoline hydrochloride 0.05%, a nasal decongestant. After a diluent nasal challenge, three separate challenges of cat allergen (10, 100, 1000 BAU/mL) were performed at 10-minute intervals. Nasal symptoms and sneezes were recorded every ten minutes after each nasal administration followed by a nasal lavage using 5 mL of LR for mediator measurement.

Nasal lavage PGD₂ measurements

Nasal lavage specimens were shaken to homogenize the constituents. A charcoal-based radioimmunoassay¹⁸ was used to quantify PGD₂ using an ³H-PGD₂ (GE Health Sciences, Piscataway, NJ) and an anti-PGD₂ antibody (Genetex, Irvine, CA). This assay had a sensitivity of approximately 2 pg/mL. For details, see the Methods section in this article's Online Repository at www.jacionline.org.

Statistical design

For continuous variables that are normally distributed we used the student's t test (paired and non-paired) for non-normal distributed data we used two-tailed, Mann-Whitney U test. All statistics mentioned are two-tailed, paired t tests unless otherwise noted, and a $p < 0.05$ was considered statistically significant. All data are expressed as mean $\pm$ SEM unless otherwise stated.

In order to optimize statistical power from the fact that multiple doses of antigen were used for nasal challenge, a single metric of the response, the area-under-the-curve (AUC) was calculated for the dose response curve for PGD₂. To derive statistical significance for the results, a stochastic simulation of the dose response curves was generated using the experimental means and standard deviations as statistical metrics for the simulations. Statistical significance was then calculated from simulated distributions of the AUC metric.

Results

Subject characteristics

Of the eighteen enrolled subjects, 2 of the 14 subjects in the active treatment groups relocated before completion of all NACs. No significant differences were present between baseline characteristics in the 12 active and 4 placebo subjects who completed the study (Table I) or between the initial 14 enrolled subjects in the active group and the placebo group. Data for the 12 subjects on active treatment and the 4 subjects on placebo treatment who completed all 3 study NACs and procedures are reported below with exceptions as noted.

Basophil surface IgE and FcεRI expression

Complete data for all twelve active recipients are available through day 21, as four subjects qualified for mid-study NAC prior to day 45 (see below for details of days to mid-study NAC). For placebo recipients, complete data was collected at all visits and all subjects underwent NAC at ~day 45.

As compared to baseline, blood basophils of subjects receiving omalizumab had an average 30% decline in surface IgE presence after 3 days of treatment ($p=0.001$) and 87% decline by 10 days of treatment ($p<0.0001$). An 11% reduction in basophil surface FcεRI receptors was noted after 3 days of omalizumab ($p=0.02$) and 78% reduction after 10 days ($p<0.0001$). By the conclusion of the treatment period (day 105), surface IgE and surface FcεRI were reduced 95% and 84%, respectively ($p<0.0001$) (data not shown). Placebo recipients showed minimal fluctuation in these surface measures during the study. In comparison to basophil surface IgE levels in subjects treated with omalizumab, serum free IgE levels as a percentage of total IgE measured at the same visit were 9.72% ($\pm 2.76\%$ SEM, $n=12$) by day 3 and 9.15% ($\pm 3.56\%$ SEM, $n=12$) by day 10 (data not shown).

Cat allergen induced BHR

The average cat-allergen induced BHR was similar between the active treatment ($35.3\pm 3.7\%$) and placebo groups ($36.4\pm 15.1\%$) at baseline for the 1 BAU/mL dose ($p=0.71$, two-tailed Mann-Whitney test). The concentration of cat allergen leading to optimal BHR at baseline was 0.1 BAU/mL for 8 subjects (7 active and 1 placebo subjects) while and 1 BAU/ml for the other 8 subjects (5 active and 3 placebo). Among omalizumab recipients, the average percent reduction of cat allergen-induced BHR at the *optimal* dose was 74% ($p=0.001$, $n=12$) by mid-study NAC and 84% ($p<0.0001$) by the final NAC compared to the baseline NAC. The group mean cat-allergen induced BHR response was first noted to be significantly decreased at the 0.1 BAU/mL dose after 3 days of omalizumab treatment ($p=0.03$, $n=10$) and at the 1 BAU/mL dose by day 18 ($p=0.013$, $n=11$) (Figure 2, **Panel A**). By the end of the study, the overall group mean reduction in cat allergen-induced BHR compared to baseline was 89% ($n=10$) at 0.1 AU/ml, 61% at 1 AU/mL ($n=12$), and 6% at 10 AU/ml ($n=12$).

No significant changes in cat allergen (Figure 2, **Panel B**) occurred in the placebo treated group, and no significant changes occurred in HR to fMLP for either the active treatment or placebo groups (data not shown).

Relationship of specific-to-total IgE ratios to basophil response

Given that a reduction in cat allergen BHR of $>80\%$ from baseline was required before the mid-study NAC, we also examined the relationship between the percentage of cat specific IgE to total serum IgE at baseline and the time to presentation for the mid-study NAC among omalizumab treated recipients. The average day for presentation to the mid-study NAC was 37 ± 3 days in subjects on omalizumab. A positive correlation was noted between the percentage of cat specific to total IgE and the time to mid-study NAC in days ($R^2=0.57$, $p=0.0046$, $n=12$, two-tailed, Spearman correlation) (Figure 3). All subjects with a cat specific to total IgE ratio of $<3\%$ ($n=4$) achieved a $>80\%$ decrease in BHR to all doses of cat allergen by day 30. In contrast, subjects with a cat specific to total IgE ratio $>3\%$ ($n=8$) did not achieve the required $>80\%$ fall to all doses of cat allergen the week prior to the mid-study NAC, and by the end of the study the number of these subjects with a $>80\%$ decrease in cat allergen induced BHR was 7 of 8 for the 0.1 BAU/mL dose, 3 of 8 for the 1 BAU/mL dose, and 1 of 8 for the 10 BAU/mL dose.

All subjects who displayed an optimal dose of cat allergen induced HR at 0.1 BAU/mL had a cat specific to total IgE ratio $>3.5\%$ (group mean $9.8\pm 2.1\%$, $n=8$), whereas 2 of 8 subjects with

optimal dose of cat allergen at 1 BAU/mL had a cat specific IgE >3.5% (4.1% and 4.2%) (group mean $2.0 \pm 0.5\%$; compared to 0.1 BAU/mL group, $p=0.0006$, Mann Whitney test). The specific-to-total IgE ratio had predictive value with respect to the position of the optimum; with a sensitivity of 88% and a specificity of 88%, a cat specific-to-total IgE ratio of >4.3% could predict an optimum of 0.1 BAU/ml.

Nasal Allergen Challenge

There were also no significant differences in baseline NAC total symptoms and sneezes between omalizumab and placebo groups.

Total group average VAS nasal symptoms scores were significantly decreased in omalizumab treated subjects by mid-study NAC (13.1 ± 3.1) relative to baseline NAC (26.0 ± 4.4) ($p=0.007$), which represented a 50% group mean reduction. A further significant decrease in group average total VAS occurred at the final NAC (mean 4.7 ± 1.3) relative to mid-study ($p=0.002$), which represented an 82% group mean reduction from baseline (Figure 4. **Panel A**). There were no significant changes in average total VAS in the placebo treated subjects at either the mid-study or final NAC Figure E1 in this article's Online Repository www.jacionline.org). An allergen dose-response relationship to symptom scores could be observed for both active and placebo treated subjects.

In the omalizumab recipients, the total number of sneezes counted after all three cat allergen doses at the baseline NAC (19.2 ± 3.5) were significantly decreased at the time the mid-study NAC (7.8 ± 1.9) ($p=0.01$, $n=12$) and represents a 59% group mean reduction. A further significant decrease in total number of sneezes occurred at the final NAC (2.9 ± 0.8) relative to mid-study NAC ($p=0.006$, $n=12$) and represents an 85% group mean reduction from the baseline NAC (Figure 4. **Panel B**). In contrast, there were no significant changes in average total sneezes in the placebo treated subjects from baseline NAC to mid-study or final NAC. (Figure 4. **Panel C**).

Among omalizumab treated subjects, there was no significant correlation between the percent of cat specific to total IgE and the reduction in total VAS or sneezes from baseline to either the mid-study or final NAC (data not shown).

Nasal lavage PGD₂ levels

Two subjects (one omalizumab treated and one placebo) did not have PGD₂ measured due to technical problems. Nasal lavage PGD₂ was not significantly different from baseline at the time of the mid-study NAC in the actively treated subjects. However, there was a significant decrease in PGD₂ measurement by the time of the final NAC for the cumulative nasal lavages measured ($p<0.001$) (Figure 4, **Panel D**). There were no significant changes in nasal lavage PGD₂ in the placebo treated subjects ($n=3$; Figure E2 in this article's Online Repository www.jacionline.org). No significant trends were seen in nasal lavage histamine measurements relative to the dose of cat allergen administered or the time on active treatment with omalizumab.

Cat Allergen SPTT

There were no significant reductions in cat allergen SPTT by the mid-study NAC in the actively treated subjects. The average SPT wheal and flare diameter was significantly ($p<0.05$) reduced at the final NAC compared to baseline in omalizumab treated subjects ($n=12$) at the 10, 100, 1000, and 10,000 BAU concentrations (Figure 5 **Panels A** and Figure E3 in this article's Online Repository www.jacionline.org). The group average wheal diameter for the highest concentration of tested cat allergen (10,000 BAU) at baseline was 4.8 ± 0.5 mm compared to the final NAC wheal diameter of 3.3 ± 0.5 mm and represents a 43% group mean reduction

($p < 0.05$). The average flare diameter for the 10,000 BAU concentration of cat allergen at baseline was 21.3 ± 2.5 mm compared to the final NAC flare diameter of 13.4 ± 2.3 mm and represents a 37% group mean reduction ($p < 0.05$). A one to two log shift in sensitivity between baseline and final measures of wheal and flare diameters could be observed for cat allergen SPTT. No significant changes were observed for placebo treated subjects at any time point relative to baseline (Figure 5 **Panels B** and Figure E4 in this article's Online Repository www.jacionline.org).

Among omalizumab treated subjects, there was no significant correlation between the percent of cat specific to total IgE and the reduction in SPT wheal and flare from baseline to either the mid-study or final NAC (data not shown).

Discussion

In this study, we demonstrate a refined kinetics of serum and cellular changes by omalizumab. The expected ~80% reduction in both basophil surface IgE and FcεRI expression in subjects 10 days after receiving omalizumab followed the expected decline in IgE (> 90% in free IgE by day 3). Of note, significant, but modest reductions in cat allergen induced BHR occurred as early as day 3 at the 0.1 BAU/mL dose (20% decline) and by day 18 at the 1 BAU/mL dose (35% decline) compared to greater reductions in surface bound IgE, which was reduced by 30% and 87% at day 3 and 18, respectively.

We also found that the percentage of cat specific to total IgE <3% was a predictor of a decline in basophil responsiveness to cat allergen by day 30 while patients were receiving omalizumab. In these enrolled subjects with a positive NAC, this percentage <3% was also associated with a less sensitive basophil response to cat allergen *in vivo*. However, the ratio of cat specific to total IgE was not associated with the magnitude of decline in symptoms score or reduction in SPT wheal/flare size during either the mid-study or final NAC in patients receiving omalizumab. This observation may be explained by the longer time to mid-study NAC in subjects with higher percentages of cat specific to total IgE. By the final NAC, subjects had received omalizumab for a sufficient duration to clearly effect both basophil and tissue mast cell responses. Future studies should more thoroughly examine the impact of allergen specific to total IgE as a predictor of the early and ultimate clinical impact of omalizumab on subject's symptoms to a particular allergen.

Acute NAC responses (VAS and sneezes) were suppressed ~50% at the time of basophil hyporesponsiveness although a further suppression to ~80% occurred by the end of the treatment period relative to baseline. Nasal lavage PGD₂ measurements, a measure of mast cell activation, were not suppressed at the time of basophil hyporesponsiveness to cat allergen. Another measure of mast cell function, SPT wheal and flare response, was also not suppressed at the time of basophil hyporesponsiveness. These findings are striking and suggest that the acute allergic response to cat allergen appears at least partly dependent on the reduction of basophil allergen response given the stable measures of mast cell function (PGD₂) at a time of depressed clinical nasal response to allergen. Alternatively, it is possible that basophil hyporesponsiveness may serve as a surrogate for process leading to a change in the end-organ allergic response or a surrogate for changes in another cellular compartment. Although we did not directly assess nasal mast cell numbers or surface FcεRI density, the measures of PGD₂ achieved at mid-study NAC were stable. Shortcomings of this study include limited measures of nasal mast cell biology other than PGD₂ levels. It seems less likely this would support either a heightened per mast cell PGD₂ production in the case of a marked reduction in nasal mast cell population at the mid-study NAC. These findings also suggest that IgE depletion with omalizumab leads to a change in tissue responsiveness to basophil and mast cell mediators independent of, or in addition to, suppressed mediator release, since a decrease occurred in

nasal scores at each dose of nasal allergen challenge. Recently, the critical role of the basophil in both acute and chronic allergic response has been better defined in mouse models.¹⁹ Overall, the basophil has emerged as a initiator of certain allergen-dependent responses in the skin and also a key cell in the production of a Th2 response.

More recently, mechanistic studies using the same omalizumab dosing strategy as our present study have been published. Noga et al. demonstrated a 29–96% reduction in BHR to the optimal dose of allergen (dust mite, cat or dog) after 16 weeks of treatment with omalizumab, but no kinetic to this reduction was studied.²⁰ Lin et al.¹² demonstrated a reduction in serum free IgE by 96% by day three, a sustained 70% reduction in basophil FcεRIα expression by day 14, and a significant reduction (~30%) in the acute response to intranasal ragweed challenge by day 7–14 and a further decrease by day 35–42 (~60%). However, in contrast to our study, no concomitant mast cell studies were present in that particular study. Hanf et al.¹⁴ demonstrated a 90% reduction in nasal symptoms scores to allergen challenge (variety of allergens) after 16 weeks of omalizumab treatment. Similar to Hanf et al., we were not able to measure a consistent rise in nasal lavage histamine or tryptase content (data not shown) following NAC unlike others¹⁰.

In summary, these findings support the importance of the basophil, either directly being involved in the acute response to allergen during NAC or as a biomarker of the end organ nasal clinical allergic responses. The kinetic of basophil loss of responsiveness to allergen in the setting of omalizumab was also elucidated. The ratio of cat allergen specific to total IgE <3%, a predictor of early basophil hyporesponsiveness to allergen, may also be important in predicting the onset of clinical response to omalizumab treatment and is in need of future study.

Key message

Reduction in nasal symptom scores occurred when the basophil, but not mast cell, response was reduced on omalizumab, implicating a role for basophils in the acute NAC response.

Acknowledgments

Grant funding: Drs. Saini and Bochner received support as Cosner Scholars in Translational Research from Johns Hopkins University. This work was supported by the Asthma and Allergic Disease Research Centers grant U19AI070345 from the National Institutes of Health.

Abbreviations used

BHR	Basophil Histamine Release
fMLP	formyl-met-leu-phe
HR	Histamine release
LR	Lactated Ringer's solution
NAC	Nasal allergen challenge
SPT	Skin Prick Test
SPTT	Skin Prick Test Titration
VAS	visual analog scale

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Figure 1.

Overall study design. *Mid-study NAC and SPTT occurred when cat allergen BHR is <20% of baseline or ~day 45.

Panel A

Panel B

Figure 2.

Cat-allergen induced BHR. Cat-allergen induced basophil histamine response in omalizumab (**Panel A**, n=12) and placebo recipients (**Panel B**, n=4) relative to the day of study. All comparisons are within each group relative to the response measured at baseline (day 0) by two-tailed paired t test. Error bars – S.E.M. *p<0.05, **p<0.01, #p<0.001, ‡p<0.0001

Figure 3.
The percent of cat-allergen specific IgE to total IgE is correlated to the timing of the mid-study NAC for subjects receiving omalizumab.

Panel A

Panel B

Panel C

Panel D

Figure 4.

Average VAS score in omalizumab treated (**Panel A**, n=12) subjects grouped according to the dose of allergen and NAC visit. Average sneeze counts in omalizumab treated (**Panel B**, n=12) and placebo treated subjects (**Panel C**, n=4) grouped according to the dose of allergen and NAC visit. PGD₂ levels in nasal lavage for each NAC allergen dose at the three NAC time points in actively treated subjects (**Panel D**, n=11). Baseline NAC – closed circles, Mid-study NAC – closed squares, Final NAC – open circles. .

Error bars – S.E.M. *p<0.05, **p<0.01, #p<0.001, ‡p<0.0001

Panel A

Panel B

Figure 5.

Average SPT flare size in omalizumab (n=12; **Panel A**) and placebo (n=4; **Panel B**) treated subjects at the time of indicated NAC. Error bars – S.E.M. *p<0.05, two-tailed, paired t test compared to baseline SPT value.

Tables I

Baseline characteristics of the study subjects

Characteristic	Active N=12	Placebo N=4
Mean Age (range)	30.6 (24–47)	32.3 (25–49)
Gender (male sex %)	42%	25%
Mean SPT Cat Wheal mean ($\pm$ SEM)	4 ($\pm$ 0.6)	4 ($\pm$ 0.8)
Mean SPT Cat Flare mean ($\pm$ SEM)	20 ($\pm$ 1.4)	23 ($\pm$ 3.1)
Number of subjects with seasonal SPT reactivity	10	3
Mean total IgE mean ($\pm$ SEM)	146.3 ($\pm$ 41.9)	146.8 ($\pm$ 83.3)
Mean cat specific IgE mean ($\pm$ SEM)	6.3 ($\pm$ 1.5)	2.9 ($\pm$ 1.2)
Mean % Cat specific/Total IgE mean ($\pm$ SEM)	10% ($\pm$ 1.8%)	3.9% ($\pm$ 2.1%)

Abbreviations used: SPT – skin prick test, SEM – standard error of the mean