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Repetitive nasal allergen challenge in allergic rhinitis: priming and Th2-type inflammation but no evidence of remodelling

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Abstract

Repetitive nasal allergen challenge in allergic rhinitis: priming and Th2-type inflammation but no evidence of remodelling

Nara Orban, Mikila R Jacobson, Kayhan T Nouri-Aria, Stephen R Durham, Aarif O Eifan

Background: Local tissue eosinophilia and Th2-cytokines are characteristic features of seasonal allergic rhinitis. Airway-remodelling is a feature of asthma whereas evidence for remodelling in allergic rhinitis (AR) is conflicting.

Objective: By use of a novel human repetitive nasal allergen challenge (RAC) model, we evaluated the relationship between allergic inflammation and features of remodelling in AR.

Methods: Twelve patients with moderate-severe AR underwent 5-alternate day challenges with diluent which after 4-weeks were followed by 5-alternate day challenges with grass pollen extract. Nasal symptoms, Th1/Th2 cytokines in nasal secretion and serum were evaluated. Nasal biopsies were taken 24 hours after the 1st and 5th challenges with diluent and with allergen. Sixteen healthy controls underwent a single challenge with diluent and with allergen. Using immunohistochemistry, epithelial and sub-mucosal inflammatory cells, and remodelling markers were evaluated by computed image analysis.

Results: There was an increase in early and late-phase symptoms after every allergen challenge compared to diluent (both $p < 0.05$) with evidence of both clinical and immunological priming. Nasal tissue eosinophils and IL-5 in nasal secretion increased significantly after RAC compared to corresponding diluent challenges ($p < 0.01$, $p = 0.01$, respectively). There was a correlation between submucosal mast cells and the early-phase clinical response ($r = 0.79$, $p = 0.007$) and an association between epithelial eosinophils and IL-5 concentrations in nasal secretion ($r = 0.69$, $p = 0.06$) in allergic rhinitis. No differences were observed after RAC with regards to epithelial integrity, reticular basement membrane thickness, glandular area, expression of markers of activation of airway-remodelling including α -SMA, HSP-47, extracellular matrix (MMP7, 9 and TIMP-1), angiogenesis and lymphangiogenesis for AR compared to healthy controls.

Conclusion: Novel repetitive nasal allergen challenge in participants with severe persistent seasonal allergic rhinitis resulted in tissue eosinophilia and increases in IL-5 but no structural

changes. Our data support no link between robust Th2-inflammation and development of airway-remodelling in AR.

Key words: Allergic, rhinitis, remodelling, early and late phase response, priming, inflammation, eosinophils, mast cells, lymphangiogenesis, angiogenesis, repetitive allergen challenge

Abstract WORD: 292

Abbreviation

AR: Allergic rhinitis

AUC: Area under the curve

EPR: Early Phase Response

HC: Healthy controls

LPR: Late phase Response

RAC: Repetitive Allergen Challenge

NAC: Nasal allergen challenge

TNSS: Total nasal symptom scores

Introduction

Allergic rhinitis and asthma are widely accepted to be Th2-type airway inflammatory diseases in which production of allergen-specific IgE, accumulation of eosinophils, mast cells and increases in Th2 cytokines are linked to pathophysiology.¹⁻³

In allergic asthma, repeated exposure to airborne allergens is known to result in persistent inflammation, airway epithelial damage and remodelling. In contrast, whether remodelling occurs in allergic rhinitis remains controversial.⁴ A recent statement from the American Thoracic Society reports a lack of consensus regarding importance of multiple features of airway remodelling.⁵ Understanding the mechanisms of airway remodelling and their impact on airway disease, may inform novel therapeutic approaches.

Nasal allergen challenge allows precise study of the time course of clinical, cellular and immune responses following therapeutic interventions with the advantage of small sample sizes.⁶ Repetitive inhaled allergen challenge in asthma has been used to imitate natural allergen exposure whilst being tailored to induce detectable Th2 inflammation and eosinophilia without inducing severe asthma symptoms, hence being used in both translational research and clinical settings.⁷⁻⁹

Nasal allergen provocation in murine models has contributed significantly to our knowledge of pathophysiology and has often been developed with chronic repetitive challenges.¹⁰ It has been questioned whether nasal allergen challenge in a human setting adequately reflects natural environmental exposure to allergens. Hence, longer periods of repetitive allergen challenge and appropriate controls need to be validated in humans.

The purpose of this study in participants with severe persistent seasonal allergic rhinitis was to investigate the effect of chronic repetitive nasal grass pollen allergen challenge (5 challenges

124 over 2 weeks and controls) on the severity of their allergic rhinitis symptoms, inflammation
125 and priming with a detailed evaluation of evidence for structural remodelling in the nasal
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Methods:

Study Subjects (Please see supplement)

Subjects aged 18-55 years with clinical history of moderate-severe persistent allergic rhinitis according to ARIA guideline¹¹ (please see supplement). The study was approved by the National Ethics committee (ref. 07/Q0404/64) and was performed with the subjects' informed written consent.

Study design

Twelve allergic rhinitis participants and 16 non- allergic healthy controls were studied (Table 1). During the trial period non-allergic healthy participants attended the clinic four times out of season. Each participant underwent a diluent challenge followed 4 weeks later by an allergen challenge. Twenty-four hours after each challenge, participants had a biopsy of their inferior turbinate. During the trial period allergic rhinitis participants attended the clinic 14 times over the course of 8-10 weeks as shown in Fig 1. Allergic rhinitis participants had 7 visits out of pollen season for the diluent phase followed by 7 visits for the allergen challenge phase of the study. For each phase, 5 visits were for the challenge (diluent or allergen) and 2 were for nasal biopsy. Participants completed the symptom score questionnaires at regular intervals for 24 hours prior to and following each challenge. The biopsies took place 24 hours after the first and the last challenge in both phases. Each side of the nose was randomly designated as either side 1 or side 2. This was determined on the first diluent challenge visit by 'flip of a coin' for both allergic and healthy participants. Both sides of the nose were challenged on each challenge visit but only one side of the nose was biopsied. Blood samples and nasal secretions were taken to determine markers of the allergic response immediately prior to the nasal biopsies.

Skin prick test

Skin prick testing was performed as previously described.¹²

Repetitive nasal allergen challenge (RAC), symptoms scores and nasal secretion

RAC and nasal symptoms scores

Nasal provocation was performed using Aquagen-225 diluent for control challenges and Timothy grass pollen freeze-dried extract, *Phleum pratense*, reconstituted in diluent (Aquagen SQ (ALK 225) ALK Abello, Horsholm, Denmark) using a standardised disposable nasal pump spray (Bidose; Valois S.A., Marly le Roi, France), 500 biologic units (BU)/100 µL was administered as a single dose to each nostril.¹³ Symptoms were evaluated on a four-point scale: 0 (no symptoms) to 3 (severe symptoms) for sneezing, nasal discharge, itching and nasal obstruction (Total score range 0-12). Total nasal symptom scores were recorded at screening, before challenge and at 5 minutes, 10 minutes, 30 minutes and 1-24 hours after each RAC (eFig. 1). The effect of RAC was assessed by early phase-TNSS (EPR-TNSS) and late phase reactions-TNSS (LPR-TNSS) area under the curve (AUC).

Nasal secretion procedure, measurements of nasal secretion and serum cytokine levels (please see supplement).

Nasal Biopsies, immunohistochemistry staining and quantification

Nasal biopsies (2.5 mm) were taken from the under surface of the inferior turbinate. Immunohistochemistry was performed using the avidin/biotin complex-alkaline phosphatase (ABC-AP) method as previously described^{12, 14} (Please see supplement).

Statistics

Values are presented as median [IQ 25-75 range] unless otherwise specified. Comparisons for quantitative variables were performed by non-parametrical analysis; Wilcoxon matched-pairs signed-rank test for within group comparisons. Furthermore, remodelling markers were reported as a delta outcome (change from diluent) and comparisons were performed by non-parametrical analysis; Mann-Whitney U test for between groups. The AUC is calculated by taking the per-average of the TNSS AUC measured at 0 to 1 hours (EPR) and the per-hour average of the AUC measured between 1 to 10 hours (LPR). Spearman correlation coefficients were used to assess the relationships between symptoms and biomarkers. Inter-observer reproducibility within 2 standard deviation was measured using Bland-Altman test. All tests were two-tailed. Significance was set at $P < 0.05$. GraphPad Prism 6 Project software was used for analysis.

Results

Subjects

The demographic characteristics are summarised in Table 1. Twenty-eight subjects (12 AR [F:M; 4:8, median age 27] and 16 normal controls [F:M; 10:6, median age 29]) were included for repetitive grass pollen allergen challenge study. Two (16%) AR subjects were sensitized to perennial allergens without symptoms. Eight percent of AR patients also had previous history of eczema and 16 % had mild seasonal asthma (Table 1).

Symptoms after repetitive nasal allergen challenge

Participants with seasonal allergic rhinitis had bothersome seasonal total symptoms scores compared to healthy control (Median [IQ]; 7 [4-9] vs 0, $p<0.0001$, Table 1). Response to repetitive allergen challenge was assessed by monitoring the early phase-TNSS (EPR-TNSS, 0-1 hour) and late phase reactions-TNSS (LPR-TNSS, 1-24 hours).

Early phase symptoms (TNSS 0-1hr):

The early phase TNSS increased significantly in repetitive allergen challenge group after each challenge when compared to the corresponding diluent challenge in the same participants for each of the 5 paired repetitive challenges ($p<0.05$, respectively) throughout 11 days. The early phase TNSS increased gradually from the initial allergen challenge reaching a peak after the third challenge ($p=0.01$, median [IQ]; 4.7 [3-6] vs 3.5 [2-5]), and then remained consistently high for the final 2 provocation tests compared to control challenges (Fig. 2A). Control participants showed minimal increases in their early phase TNSS after the initial control challenge but no change after allergen challenge ($p=0.03$, median [IQ]; 0.7 [0.5-0.8] vs 0 [0-0.2]) (Fig. 2 A), likely related to a minor irritant effect of the procedure with adaptation on re-challenge.

Late phase symptoms (TNSS 1-24hr): A significant increase in late-phase TNSS was observed after all 5 repeated allergen doses when compared to corresponding diluent challenges at each time point. The magnitude of LPR-TNSS also increased gradually from the initial allergen challenge progressively over the 5 challenges and was significantly higher after the 5th allergen challenge compared to the first and third challenges ($p=0.01$ and $p=0.02$, respectively). Normal control participants demonstrated no changes in LPR-TNSS after allergen when compared to diluent (Fig. 2 B, eFig 1 A-B).

Eosinophils: Total nasal tissue eosinophils increased after both the first and fifth allergen challenge compared to corresponding diluent challenges ($p=0.01$ and $p<0.01$, respectively) (Fig 3A, Table 2). This increase was mainly due to increases in submucosal tissue eosinophils ($p<0.01$ and $p=0.03$, respectively) (Table 2), with a trend for an increase in epithelial eosinophil counts ($p=0.06$) after the fifth allergen challenge but not for the first challenge when compared to corresponding diluent. No changes in nasal eosinophils were observed in healthy controls (Table 2).

Mast cells and basophils: In participants with allergic rhinitis there were no changes in the numbers of mast cells or basophils after either the 1st or 5th nasal allergen challenge when compared to the corresponding diluent challenge or with responses in normal control participants (Table 2). However, a highly significant correlation was observed between the early-phase (0-60 min) TNSS after the first allergen challenge and the number of nasal submucosal mast cells ($r=0.79$, $p=0.007$) (Fig 2C).

Cytokines in nasal secretion and serum cytokines

Nasal cytokines: In allergic participants, IL-5 concentrations were markedly and consistently increased after the 5th repeated allergen challenge (day 11) compared to diluent challenge ($p=0.01$, Fig 3B). There was a trend for the increased nasal levels of IL-5 after the 5th challenge

to correlate with the corresponding nasal epithelial eosinophil counts ($r=0.62$, $p=0.06$; Fig 3C). In contrast, nasal IL-4 concentrations were low, at or below the limit of detection for the assay and no significant alterations were observed after all 5 allergen challenges for the allergic groups. There were no significant changes in concentration of IL-10, IL-13 or IFN- γ after the last (5th) dose of repetitive allergen challenge compared to its diluent. After the initial dose of allergen challenge in allergic participants there were no significant changes observed in all nasal cytokines when compared to their diluent. Likewise, no changes in levels of nasal cytokines were detected in healthy controls after allergen challenge compared to diluent (Table 2).

Serum cytokines: No changes in serum concentrations of IL-4, IL-5, IL-10 nor IFN- γ were observed after allergen challenges within allergic participants when compared to diluent challenges (Table 2). There were no changes in concentration of serum IL-13 after the last (5th) dose of repetitive allergen challenge compared to its diluent. In normal control subjects there was a small reduction in serum IL-4 concentration ($p=0.04$) after allergen compared with diluent, otherwise no changes in levels of serum IL-5, IL-10 nor IFN- γ cytokines were detected in healthy controls after allergen challenge compared to diluent (Table 2).

Effect of repetitive allergen challenge on upper airway remodelling

No significant changes in epithelial integrity, reticular basement membrane thickness nor glandular area (epithelial mucus content measured by epithelial area occupied by goblet cells (%), and mucus (%) staining area in submucosa) were detected in allergic participants after either the 1st or 5th allergen challenges compared to healthy controls (eTable 1). Similarly, no differences in expression of markers of activation of airway-remodelling including α -SMA, HSP-47 and extracellular matrix (MMP7, 9 and TIMP-1) nor angiogenesis or

lymphangiogenesis were observed for either AR or healthy controls after allergen challenge compared to corresponding diluent, with no differences between groups (eTable 1).

Discussion

Repetitive grass pollen allergen challenge performed out of season in participants with moderate-severe allergic rhinitis resulted in typical immediate and late-phase responses. Clear evidence of priming was observed with alternate-day allergen challenges resulting in increases in the magnitude of the immediate (0-60 min) nasal response that peaked at the 3rd challenge performed after 5 days compared to the first challenge and in the magnitude of the late-phase response (1-24 hours) that peaked at the 5th challenge after 11 days. The number of mucosal mast cells correlated with the magnitude of the IgE-dependent early-phase nasal symptom response. Allergen-induced late-phase nasal symptoms were accompanied by increases in the Th2 cytokine IL-5 in nasal secretion that correlated with parallel increases in the number of nasal mucosal eosinophils on day 11. Despite these clear symptomatic responses with nasal priming and Type 2 inflammation, no significant alterations in markers of remodelling, including epithelial integrity, mucus glands, blood vessel and lymphatic vascular changes nor evidence of fibroblast deposition were observed. This contrasts with typical remodelling that occurs after even a single allergen inhalation challenge in asthma. and suggests that in contrast to anti-IgE or targeting Th2 cytokines, therapeutic strategies against remodelling in severe allergic rhinitis are unlikely to be successful.

In order to eliminate confounding factors such as use of anti-inflammatory therapies and variability of allergen concentration during natural exposure, allergen challenges were performed outside the pollen season when patients were asymptomatic and off all anti-allergic medication. The repetitive allergen challenge procedure and associated repetitive sampling of nasal fluid and repeat nasal biopsies in the same subjects was safe and well-tolerated. A further strength of the study was the inclusion of well-selected severe allergic rhinitis patients, longitudinal comparisons, the control of each of 5 repetitive challenges with 5 temporally

identical control diluent challenges in the same individuals, the inclusion of sampling of the mucosa after a first single allergen as well as repetitive 5 alternate day challenges in the same individuals and the inclusion of a washout period of at least 4 weeks between the sequences of diluent and allergen challenges in order to avoid carry-over effects. Finally, healthy subjects were also used as negative control participants in order to control for the effects of allergen in the absence of atopic status and to control for effects of the clinical procedures and/or any temporal changes independent of the clinical protocol.

Previously, several studies have been conducted on the effect of repeated nasal allergen challenge and its relation with early and late allergic responses, dynamics of inflammatory cells and treatment effects.¹⁵⁻¹⁸ Studies in rhinitis have mainly detected the EPR after allergen challenge with only few reports of the LPR.¹⁹⁻²² The nasal LPR is less distinct post-nasal challenge than the late phase bronchial response in asthmatics after inhaled allergen challenge.²³ The dominant nasal late nasal symptom that persists is nasal blockage and to a lesser extent rhinorrhoea. This variability of detecting clear-cut late nasal responses in studies involving either single or repetitive allergen challenge may be attributable to factors such as the lack of dynamic change in the nose, the duration of repetitive allergen challenge and the duration of symptom assessment after allergen challenge that varies from a few hours up to 24 or 48 hours.⁶ In this study in which nasal symptom scores were collected for 24 hours, more than 50% of participants developed a late-phase nasal response. There was no accumulation of local nasal basophils but a strong correlation between numbers of mast cells and early phase response was observed indicating the importance of mast cell activity during the early phase reaction and corroborating previous findings. Despite this correlation there was no significant difference observed when mast cell numbers were compared following allergen and diluent challenge. There was a significant increase in magnitude of LPR after the third dose of repeated allergen challenge (6 days after an initial challenge) with an increase in local late phase reaction

inflammatory responses eosinophil and IL-5 expression which is in line with previous findings.²⁴

As with previous reports that repetitive allergen challenge induces priming,²⁵⁻²⁷ our model produced significant increases in the magnitude of nasal symptoms after the third dose of repeated allergen challenge, six days after an initial challenge. Priming phenomenon was evidenced by an increase in tissue eosinophils and IL5 cytokines in nasal secretion after the fifth repetitive allergen challenge. Interestingly, there was a correlation between eosinophils migrating into the epithelium and IL5 in nasal secretions after persistent exposure allergen challenge. The current study provides insight into the priming phenomenon confirmed by clinical symptoms coinciding with physiological/immunological changes at the tissue level. Other studies have shown that the nasal priming phenomenon appears to be sensitive to topical corticosteroids that likely act by suppressing local Th2 cytokines release and associated tissue eosinophilia.^{28, 29}

In contrast to allergic rhinitis, single allergen inhalation challenge in asthma has been shown to increase lower airway remodelling.^{30, 31} In one study, methacholine inhalation challenge induced late features of remodelling, suggesting that mechanical factors accompanying bronchoconstriction *per se* and independent of allergen may provoke remodelling of the lower airways in asthma. As there is a large variability of individual response to allergen during natural allergen exposure, it might be difficult to assess the effect of inflammation on the local target organ. We used our novel human-nasal repetitive allergen challenge model out of natural pollen season to evaluate the relationship between chronic local Th2 inflammation and tissue remodelling.

Several repetitive chronic challenge models of asthma have been shown to induce local IL5 and eosinophilia in both mice and humans with both being associated with remodelling³²⁻³⁴ in

some, but not all studies,³⁵⁻³⁷ and recent murine studies using eosinophil deficient mice and a transgenic mouse model of chronic Th2 driven inflammation with overexpression of IL-5 and eotaxin have shown that eosinophil degranulation and IL-5 may not be a driving source of pulmonary damage and/or remodelling.^{38, 39} Furthermore, trials using anti-IgE, anti-IL5 and anti-CCR3 to target eosinophils and Th2 inflammation in animal models were promising, but clinical trials have only demonstrated efficacy on asthma symptoms in subsets of asthmatics but not remodelling..⁴⁰⁻⁴²

In the present study, the close correlation between mast cell numbers and the magnitude of the early response is consistent with the known IgE-mast cell associated dependency of the immediate allergic response. The increases in local IL-5 after the fifth nasal allergen challenge that paralleled nasal mucosal eosinophilia (but no increase in basophils) highlights the role of Th2-cytokines and eosinophils in the genesis of late-phase responses. However, in contrast to the lower airways, even repeated nasal challenge with repeated late responses and persistent chronic type 2 inflammation was insufficient to provoke features of upper airway remodelling in allergic rhinitis as observed in asthma. These findings are consistent with those of a recent study of the upper airway performed during natural allergen exposure in patients with moderate-severe persistent allergic rhinitis¹². It was shown that despite the presence of severe disease and persistent upper airway allergic inflammation there was no evidence of nasal structural remodelling. These results might support the notion that either the upper airways respond differently to allergic inflammation than the lower airways or there are factors other than eosinophils or Th2 cytokines that contribute to tissue remodelling in lower airways.

In conclusion, the present study demonstrated that repetitive allergen challenge in humans induces early and late phase allergic response and priming. Moreover, a marked Th-2 mediated inflammation involving Th2 cytokine IL-5 and eosinophils was demonstrated. This study

391 confirms that there is a strong association between Th2 inflammation and persistent allergic
392 responsiveness but not structural nasal airway remodelling.

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Legends:

Figure 1: Flow chart of study design. Definition of abbreviations: TNSS= Total nasal symptom score. *Dil 1-5*: Diluent challenges-dose 1 to 5. *Grass 1-5*: Timothy Grass allergen challenges-Dose 1 to 5; *AR*: Seasonal Allergic Rhinitis; *HC*: Healthy controls; *V1*-Recruitment visit, *V2*-Repetitive diluent challenge visit; *V3*- Repetitive allergen challenge.

Figure 2: A) Early phase response (EPR) area under the curve (AUC) post-repetitive allergen or diluent challenge in allergic rhinitis. *D1-5*: Diluent doses; *A1-5*: Allergen challenge doses; *ND*: Normal healthy controls single diluent challenge, *NA*: Normal healthy controls single allergen challenge. B) Late phase response (LPR)-AUC post-repetitive allergen or diluent challenge in allergic rhinitis. *D1-5*: Diluent doses; *A1-5*: Allergen challenge doses; *ND*: Normal healthy controls single diluent challenge, *NA*: Normal healthy controls single allergen challenge. C) Significant correlation between mast cells in nasal submucosa and early phase response (total nasal symptoms scores-AUC) after 1st dose of allergen challenge. D) Immunoreactivity and localization of mast cells stained using Tryptase antibody.

Figure 3: A) Total eosinophil counts in nasal tissue post repetitive allergen challenge compared to diluent for both allergic rhinitis and healthy controls. B) Th2 cytokine (IL-5) levels from nasal fluid post repetitive allergen challenge compared to diluent for both allergic rhinitis and healthy controls. C) Correlation between IL-5 levels from nasal fluid and nasal tissue epithelial eosinophils post 5th dose of repetitive allergen challenge. D) Immunoreactivity and localization of eosinophils stained using major basic protein antibody (MBP). E) Panel E show the nasal tissue eosinophils in an allergic rhinitis subject who underwent repetitive diluent and allergen challenges and biopsies taken at day 1 and day 11 for both post diluent and allergen challenges. Biopsy specimens were immunostained with MBP.

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Table 1: Demographics		
	AR (n=12)	Normal controls (n=16)
Age yrs (Median-Range)	27 (23:32)	29 (24:42)
Gender F/M	4:8	10:6
TNSS ****	7 (4-9)	0
TQoL ****	2 (1.2-3)	0
Eczema %	8	0
Asthma %	16	0
Timothy SPT/mm² ****	53 (33-70)	0
Specific IgE grass IU/L ****	17 (7-44)	0
Total IgE IU/L **	78 (34:175)	11 (5.5:51)

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555 *P values: ****: <0.0001, **: <0.01*

556 (median range): 25-75 centiles

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Table 2: Nasal tissue, secretion inflammatory cells and cytokines, and serum cytokines

	Seasonal allergic rhinitis				Normal Healthy control		<i>p values</i>		
	1 st dose Diluent	1 st dose Allergen	5 th dose Diluent	5 th dose Allergen	Diluent Normal	Allergen Normal	1 st Dose Diluent v Allergen	5 th Dose Diluent v Allergen	Healthy controls Diluent v Allergen
	Epithelial Inflammatory cells (medians 25-75 centile)								
Eosinophils	0 (0)	0 (0-16)	0 (0)	10 (0-157)	0 (0)	0 (0)	0.5	0.06	0.5
Mast cells	0(0-3)	0(0-3.8)	0 (0-8.3)	0 (0)	0 (0)	0 (0-9)	0.63	0.5	0.06
	Submucosal Inflammatory cells (medians 25-75 centile)								
Eosinophils	0 (0)	53 (12-150)	0 (0)	20 (0-85)	0 (0-0.6)	0 (0-0.6)	<0.01	0.03	0.81
Mast cells	29 (2-50)	32 (13-38)	12 (4-38)	9 (0-37)	24 (8-45)	23 (14-41)	1.00	0.57	0.61
Basophils	0	0(0-8)	0	0 (0-4)	0	0	0.12	0.12	0.8
	Total epithelial and submucosal Inflammatory cells (medians 25-75 centile)								
Eosinophils	0 (0-9)	53 (12-166)	0 (0-2.8)	32 (5-292)	0 (0-1)	0 (0-0.6)	0.01	<0.01	0.57
Mast cells	28.5 (5-50)	34 (13-49)	12.6 (4-51)	9.5 (0-46)	24 (8.5-45)	35 (23-48)	0.91	0.57	0.20
	Nasal secretion cytokines (medians 25-75 centile)								
IL-4	0.4(0.2-1)	0.5(0.3-1.5)	0.6(0.1-1.1)	0.8(0.3-2)	0.6(0.3-0.8)	0.4(0.3-0.6)	0.9	0.4	0.4
IL-5	4.5(1.5-8)	6(3-16)	6(1.5-8)	9.6(5.7-34)	3.7(2.4-6)	4.3(3-5.2)	0.4	0.01	0.7
IFN-γ	3(1-4)	2(1-5)	3(1-4)	2.5(1-3)	4.6(2.6-7)	4.8(3-6)	0.85	0.38	0.78
IL-10	3.5(1.8-13)	3.7(2.3-12)	6.8(1.8-20)	7.1(1.7-16)	7.8(4.8-12)	11(8-18)	0.49	0.7	0.07
	Serum secretion cytokines (medians 25-75 centile)								
IL-4	12 (1-54)	28 (1-137)	20 (1-106)	26 (2-132)	14 (0-72)	4.5 (1-33)	0.7	0.2	0.04
IL-5	8 (0-81)	12 (0-210)	7 (1-88)	9 (1-183)	5 (0-111)	4 (0-43)	0.4	1.0	0.2
IFN-γ	50 (4-322)	97 (4-921)	61 (2-481)	70 (4-854)	40 (1-449)	22 (0-154)	0.31	0.72	0.76
IL-10	15 (2-113)	21 (2-334)	14 (2-195)	16 (2-255)	14 (3-141)	12 (2-61)	0.67	0.57	0.31

eTable 1: Summary of airway remodelling markers in upper airways between severe persistent allergic rhinitis and healthy controls post repetitive allergen challenge.

Measurement	Allergic rhinitis (n=12) (medians 25-75 centile)		Healthy controls (n=16) (medians 25-75 centile)	p-Values	
	Delta change Day 1 NAC (After 1 st dose of NAC)	Delta Change Day11 NAC (After 5th dose of RAC)	Delta Change Healthy	Day 1 NAC AR vs HC	Day 11 AR vs HC
Epithelial Integrity	-1.0 (-1.87 to 0.5)	0 (-1.0 to 0.37)	0 (-0.5 to 1.0)	0.07	0.35
Total Glandular (Epithelial & Submucosal glands) % Area	-11.1 (-20.4 to 17.8)	13.88 (-32.9 to 23.53)	-3 (-8.5 to 7.4)	0.48	0.45
RBM Thickness, μm	-1.4 (-4.6 to 0.74)	-2.1 (-3.3 to 2.75)	0.4 (-1.58 to 2.29)	0.06	0.26
CD31⁺ Cells/mm²	19.3 (-75.9 to 203.5)	-32.8 (-207.7 to 164.1)	31.78 (-78.8 to 233.9)	1.0	0.37
CD31⁺ Blood vessels/mm²	-4.36 (-31.1 to 23)	0 (-23.1 to 25.4)	2.1 (-10.7 to 25)	0.55	0.64
CD31⁺ Blood vessels % vascular area	0 (-1.5 to 0.8)	-0.8 (-3.7 to 0.3)	-0.8 (-1.7 to 1.4)	0.79	0.41
Mean size blood vessels (μm^2)	-175 (-772 to 249)	-350 (-1685 to 850)	-310 (-695 to 289)	0.81	0.56
D2-40⁺ Cells (mm²)	0 (-8.2 to 6.6)	0 (-1.36 to 36.4)	0 (-2.5 to 27.8)	0.55	0.92
D2-40⁺ Lymphatic vessels (mm²)	0 (0 to 1.5)	0 (-0.96 to 2.43)	0 (-0.8 to 4.7)	0.89	0.68
D2-40⁺ % Lymphatic vascular area	0 (-0.27 to 0.1)	0 (0.14 to 0.18)	0 (-0.01 to 0.1)	0.64	0.92
VEGF-A⁺ vessels (mm²)	0.92 (-1.5 to 14.3)	-0.5 (-8.6 to 11.9)	6 (-4 to 13.6)	0.88	0.5
MMP-7	0 (-15 to 16.6)	0 (-3.6 to 2.1)	0 (-1.2 to 14.1)	0.99	0.58
MMP-9	7.5 (0 to 38.5)	-0.83 (-36.9 to 10.7)	-0.2 (-3.7 to 12.5)	0.20	0.59
TIMP-1	3.7 (0 to 92.5)	0 (-2.0 to 4.4)	2.5 (0 to 15.7)	0.26	0.17

HSP-47+ Fibroblast/mm²	112.9 (8 to 155)	64.2 (-3.0 to 145.6)	16.4 (0.82 to 91.8)	0.07	0.49
Alpha-SMA	33.3 (-140 to 97.5)	-8.3 (-54.4 to 8.3)	10.3 (-278.1 to 107.9)	0.77	0.48