Journal of Immunological Methods 384 (2012) 25–32

Contents lists available at SciVerse ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Optimisation of grass pollen nasal allergen challenge for assessment of clinical and immunological outcomes

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ARTICLE INFO

Article history:
Received 30 April 2012
Received in revised form 22 June 2012
Accepted 22 June 2012
Available online 30 June 2012

Keywords:
Nasal allergen provocation
Nasal allergen challenge
Seasonal allergic rhinitis
Grass pollen
Biomarker
Nasal cytokines

ABSTRACT

Nasal allergen challenge can be used to assess the clinical and immunological aspects of rhinitis due to inhalant allergens. We aimed to develop a reproducible technique for grass pollen nasal allergen challenge and to study biomarkers within nasal secretions.

20 Grass pollen allergic individuals underwent nasal challenges with purified Timothy grass allergen. An initial dose-titration challenge was used to determine dose–response characteristics. Subsequently, volunteers underwent 3 further challenges using individualised threshold doses. Symptom scores, visual analogue scores, and peak nasal inspiratory flow (PNIF) were recorded at baseline and up to 6 h after challenge. Nasal secretions were collected at each time point using synthetic filter papers or absorptive polyurethane sponges and analysed for IL-4, -5, -10, -13, IFN-γ, Tryptase and Eosinophil Cationic Protein (ECP).

Challenges gave reproducible symptom scores and decreased PNIF. Tryptase levels in nasal fluid peaked at 5 min after challenge and returned to baseline levels at 1 h. ECP, IL-5, IL-13 and IL-4 levels were increased from 2–3 h and showed progressive increases to 5–6 h. Sponges proved the superior nasal fluid sampling technique.

We have developed a reproducible nasal allergen challenge technique. This may be used as a surrogate clinical endpoint in trials assessing the efficacy of treatments for allergic rhinitis. Tryptase in local nasal secretions is a potential biomarker of the early phase response; ECP and the Th2 cytokines IL-5, -13 and −4 markers of late phase allergic responses. Our model allows correlation between clinical responses and local biomarkers following nasal allergen challenge.

1. Introduction

The prevalence of allergic rhinitis has increased in recent decades (Dahl et al., 2004). It affects over 20% of the population of many European countries (Bauchau and Durham, 2004). Grass pollen allergy is the most common cause of seasonal allergic rhinitis in the UK. Sufferers may experience significant reductions in quality of life, with detrimental impact on work productivity, social activities and school performance (Walker

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doi:10.1016/j.jim.2012.06.013

et al., 2007). Studying both the impact of allergic rhinitis and the efficacy of treatments such as nasal corticosteroids and allergen immunotherapy is complicated by variations in pollen exposure day by day, year by year, and from one individual to another. As a consequence, clinical studies of allergen immunotherapy in particular often require large numbers of participants in order to achieve sufficient power (Bousquet et al., 2011).

Nasal allergen challenges are primarily used as a research tool, both as a surrogate clinical marker of treatment efficacy (Ewbank et al., 2003), and to study the local mechanisms of allergic rhinitis (Naclerio et al., 1985; Durham et al., 1992) and its treatments (Pipkorn et al., 1987; Iliopoulos et al., 1991). They may also be used in clinical practice, including in differentiating between sensitization alone and allergy, identifying suitable patients for allergen immunotherapy, confirming causality in cases of occupational rhinitis, and identifying patients with local IgE in the absence of systemic sensitization (Rondón et al., 2009). Nasal allergen challenge may be undertaken by several methods, each with their own benefits and drawbacks (Melillo et al., 1997). These include nasal sprays, drops, allergen-impregnated filter papers, and even continuous allergen exposure within allergen challenge chambers (Wagenmann et al., 2005; Banfield et al., 2010; Horak et al., 2009). Assessment of clinical responses may be by categorised scoring systems (Bousquet et al., 1987), visual analogue scales (Kim and Jang, 2011), peak nasal inspiratory flow, acoustic rhinometry and rhinomanometry (Holmstrom et al., 1990; Hilberg, 2002). Mechanistic responses may also be measured in several ways. These include collection of nasal fluid using absorptive filter strips (Alam et al., 1992; Nicholson et al., 2011) or lavages (Eckman et al., 2010), and obtaining cells and mRNA by nasal brushings or nasal mucosal biopsy (Jacobson et al., 1999). Again, these methods have relative merits concerning the quality of samples obtained, ease of procedure, reproducibility, cost and patient tolerability.

Given the practical difficulties and low sensitivity of assessing seasonal allergic rhinitis symptoms during natural pollen exposures, we aimed to develop and validate a method of nasal allergen challenge with Timothy grass (*Phleum pratense*) pollen extract in allergic rhinitis sufferers. The technique should be straightforward, acceptable to patients, and reproducible — it could therefore be used as a surrogate clinical outcome for use in trials of interventions for allergic rhinitis. At the same time, we intended to identify a suitable means of capturing nasal secretions and measuring biomarkers within the fluid through a combination of Mesoscale analysis and ImmunoCAP. Several different secretion collection techniques have been used in the past by researchers, without a clear gold-standard method. We chose to compare filter strips made from different synthetic absorptive matrices (SAMs) and open cell polyurethane sponges with the aim of evaluating which was superior for the collection and recovery of protein biomarkers.

2. Materials and methods

2.1. Participants

20 Volunteers were recruited from the allergy clinic and staff at the Royal Brompton Hospital, London. The study was approved by the Cambridgeshire 3 Research Ethics Committee and performed with participants' written informed consent. Inclusion criteria were a history of grass-pollen induced seasonal allergic rhinitis for at least two years and positive skin prick (>3 mm wheal diameter to *P. pratense* extract, ALK-Abello, Denmark) and Timothy grass specific IgE (>0.35 IU/ml). Exclusion criteria were perennial rhinitis, chronic or recurrent sinusitis, current smoking or >5 pack year smoking history, perennial asthma, FEV1 (forced expiratory volume in 1 s) <70% predicted at screening, and previous allergen immunotherapy. The study was conducted outside of the UK grass pollen season. Participants had not used corticosteroids or other anti-allergy medications for at least 2 weeks prior to each study visit.

2.2. Materials used for collection of nasal secretions

Two different synthetic absorptive matrices (SAMs) were used: Accuwik Ultra (fibrous hydroxylated polyester), and 111 (100% cellulose fibres of plant origin) (both from Pall Corporation, USA). These were pre-cut to 35 mm×7 mm with a rounded anterior edge (Parafix Tapes and Conversion Ltd, West Sussex, UK). *Note*: *Accuwik Ultra is no longer manufactured. Leukosorb* (*Pall Corporation*) *is a suitable alternative* (*Hansel T, unpublished data*). A single type of synthetic polyurethane sponge was used (RG 27 grau, Gummi-Welz GmbH & Co., Germany), pre-cut into 20×15×5 mm pieces and sterilized by autoclaving for 20 min at 121 °C prior to use.

2.3. Study design

The study consisted of 4 visits, each separated by at least 3 weeks (Fig. 1). Visit 1 included a screening medical history and examination, plus skin prick tests to Timothy grass (*P. pratense*) and 11 other common aeroallergens, and blood test for Timothy grass-specific IgE and total IgE. Following screening, eligible candidates undertook a graded, up-dosing nasal allergen challenge with purified extract of *P. pratense*. Allergen extract was reconstituted at 100,000 SQ-U/ml (equivalent to 30,000 BU/ml) in albumin-based diluent (ALK-Abello), before dilution in normal saline at the following concentrations: 0, 30, 100, 300, 1000, 3000 and 10,000 BU/ml. Participants received one spray, 100 μl, to each nostril using a Bi-dose nasal applicator device (Aptar Pharma, Germany). Increasing concentrations of allergen were given every 10 min. Immediately before each subsequent dose participants were asked to grade their symptoms according to a verified scoring system: total nasal symptom score (TNSS) (17, 18), a 12 point scale with 4 categories: sneezing, nose running, nose blockage, and itching, each rated from 0 to 3. Additionally, participants graded their overall symptoms on a visual analogue scale (VAS) from 0 to 100 mm, where 0 corresponds to no symptoms and 100 to maximum symptoms. Finally, the best of 3 peak nasal inspiratory flow (PNIF) measures using a Youlten nasal peak flow meter was recorded. Following visit 1, dose–response curves for TNSS, VAS and PNIF were plotted for each participant.

At visit 2 participants underwent a single nasal allergen challenge with the approximate cumulative dose up to and including the first dose provoking a TNSS of ≥5 at visit 1. Prior to the allergen challenge, participants recorded their

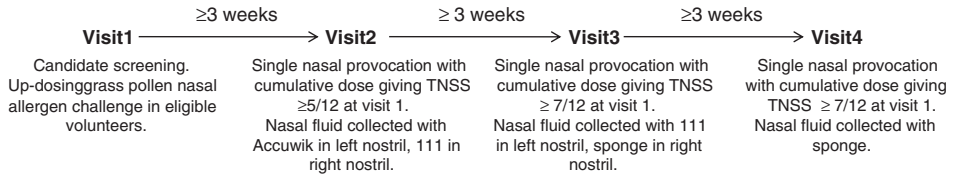

Fig. 1. Flow chart of clinical study. The study was conducted outside of the UK grass pollen season. TNSS, total nasal symptom score; Accuwik, Accuwik Ultra synthetic absorptive matrix; 111, 111 synthetic absorptive matrix; Sponges, polyurethane absorptive sponge material.

TNSS, VAS and PNIF before and 30 min after a nasal lavage (SinusRinse, Neilmed, USA). TNSS and PNIF were then repeated at 5, 15 and 30 min after the challenge, then hourly to 6 h post-challenge; VAS was recorded at the same time points, with the exception of 5 min post challenge. At each time point, after the above recordings, 2 Accuwik SAMs were placed back to back and inserted into the left nostril under direct vision using croc forceps and a Thuddicum's nasal speculum. Two 111 SAMs of the same dimensions were placed into the right nostril. SAMs were placed onto the nasal mucosa, beyond the nasal vestibule, and alongside the inferior turbinate. SAMs were left in place for 2 min before removal, and then added to 2 ml centrifuge tubes with indwelling 0.22 μm cellulose acetate filters (Costar Spin-X, Corning, NY, USA). Tubes were kept briefly on ice before being centrifuged at 4500 rcf at 4 °C for 10 min. The isolated fluid was pipetted into eppendorf tubes and stored at −80 °C.

At visit 3 procedures were repeated as for visit 2, except the nasal challenge was given with the approximate cumulative allergen dose provoking a TNSS of ≥7 at visit 1. (If a score of 7 had not been reached during the titration challenge the participant received a single challenge with the approximate total cumulative dose given at visit 1, 100 μl of 15,000 BU/ml allergen extract to each nostril). On this occasion one sponge piece was inserted under direct vision into the right nostril, and two 111 SAMs inserted into the left nostril. For fluid isolation the sponges and 111 SAMs were then centrifuged at visit 2. At visit 4 the same procedures were undertaken at visit 3, except that only nasal sponges were used to collect nasal fluid.

2.4. Analysis of nasal fluid

Measurements of IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12p70, and IL-13 were performed using MSD Human TH1/TH2 7-Plex, Ultra-Sensitive Kit according to the manufacturer's instructions (MS6000 7 spot, Meso Scale Discovery, Maryland, USA). Plates were read using an MSD SECTOR® 6000 instrument. Validation procedures demonstrated that the lower quantification limit for all cytokines was approximately 5 pg/ml, with an assay range of 5–5000 pg/ml in nasal secretions. All measurements were performed on undiluted nasal fluid samples in duplicate and reported as mean values.

Eosinophil cationic protein (ECP) and tryptase measurements were performed on a Phadia/Thermo Fisher Scientific ImmunoCAP instrument according to the supplier's protocol for analysis of these mediators in mucosal secretions (Phadia AB, Uppsala, Sweden). Pilot studies demonstrated that the mediators were detectable in nasal secretion samples diluted at 1:10. All subsequent samples were analysed and diluted at 1:10, after thawing, in assay diluent.

2.5. In vitro spiking experiments

To investigate the recovery of cytokines after absorption to the different synthetic absorptive matrices and sponges, recombinant proteins for each of the 7 human cytokines listed above were diluted in assay diluent or in pooled pre-challenge nasal secretions and applied to the different materials. Fluid was recovered by centrifugation as described above and analysed in the 7-plex MSD cytokine assay. Cytokine concentrations of 0, 55, 167, and 500 μg/ml were investigated and samples were analysed in duplicate.

2.6. Statistical analysis

A commercial software package (Graphpad Prism Version 5.04) was used to perform statistical analyses. Values are presented as observed mean +/− standard error. For clinical outcomes (TNSS, VAS, PNIF) within visit comparisons between baseline and subsequent time points were made by repeated measures one-way ANOVA with Bonferroni correction for multiple comparisons. Between-visit comparisons were made by paired *t*-tests at individual matched time points and by area under the curve (AUC) analyses across the whole time period from challenge (0 h) to 6 h. AUC comparisons were performed as follows: individual patient AUC values were calculated by the software package (Graphpad Prism Version 5.04). Mean and standard error were then calculated for all patients at each visit. Between-visit comparisons were then made by paired *t*-tests. Similar AUC calculations were used to compare cytokine levels recorded with each of the different synthetic absorptive matrices/sponges. Correlations were made by Pearson's correlation coefficient. This was a pilot study and no formal power calculations were made.

3. Results

3.1. Participant demographics

Table 1 summarizes the demographic characteristics of the participants. 20 Volunteers were recruited into the study. 18 Completed all 4 visits. One patient completed only visit 1, then

Table 1
Summary of characteristics of participants recruited into the study.

	Mean	Range
Age	29.85	21–48
Male:female	10:10	
Total IgE (IU/ml)	272.55	3–1214
Timothy grass IgE (IU/ml)	28.26	0.65–>100
Timothy grass skin prick mean diameter (mm)	9.1	5–15

was withdrawn due to pregnancy; 1 patient completed visits 1 and 2, but withdrew from the study due to moving abroad.

3.2. Visit 1, up-dosing titration nasal allergen challenge

One participant was able to tolerate a maximum dose of only 1000 BU/ml before stopping due to troublesome symptoms. 4 Patients further tolerated a maximum dose of 3000 BU/ml. All other participants were challenged up to 10,000 BU/ml. Four patients tolerated a maximum dose of 3000 BU/ml.. The modal dose for a TNSS ≥ 7 was 3000 BU/ml, although 3 volunteers failed to reach 7 even at 10,000 BU/ml. Overall, 100 BU/ml was sufficient to cause a significant increase from baseline (Fig. 2A).

A dose of 1000 BU/ml was required to produce a significant fall in PNIF from baseline (Fig. 2B). Further falls were seen at higher allergen doses. A dose of 300 BU/ml was sufficient to produce a significant increase in VAS from baseline (Fig. 2C). There was a close correlation between TNSS and VAS, $r = 0.87$, $p < 0.0001$, and an inverse correlation between TNSS and PNIF, $r = -0.62$, $p < 0.0001$.

3.3. Visits 2–4, clinical data

TNSS peaked at 5 min post-challenge at each visit (Fig. 2D), whereas PNIF reached a nadir at 15–30 min after challenge (Fig. 2E). Peak (5 min) TNSS was greater at visit 3 than visit 2, $p = 0.002$, and at visit 4 versus visit 2 ($p = 0.048$) (paired *t*-test). Area under the curve analyses from baseline to 6 h showed a significant difference between visits 3 and 2, $p = 0.017$. Analysis of VAS showed significantly higher peak scores (15 min) at visit 3 versus visit 2, $p = 0.045$ (paired *t*-test). There were no differences in TNSS, VAS or PNIF between visits 3 and 4.

3.4. Biomarker data

In vitro spiking experiments showed sponges and 111 SAMs to give significantly greater cytokine recovery yield at all concentrations tested for IL-4 and IFN-γ (Supplementary Fig. 1). Results were similar across each of the absorptive materials for IL-10, IL-13, IL-5, IL-2 and IL-12p70. Head to head

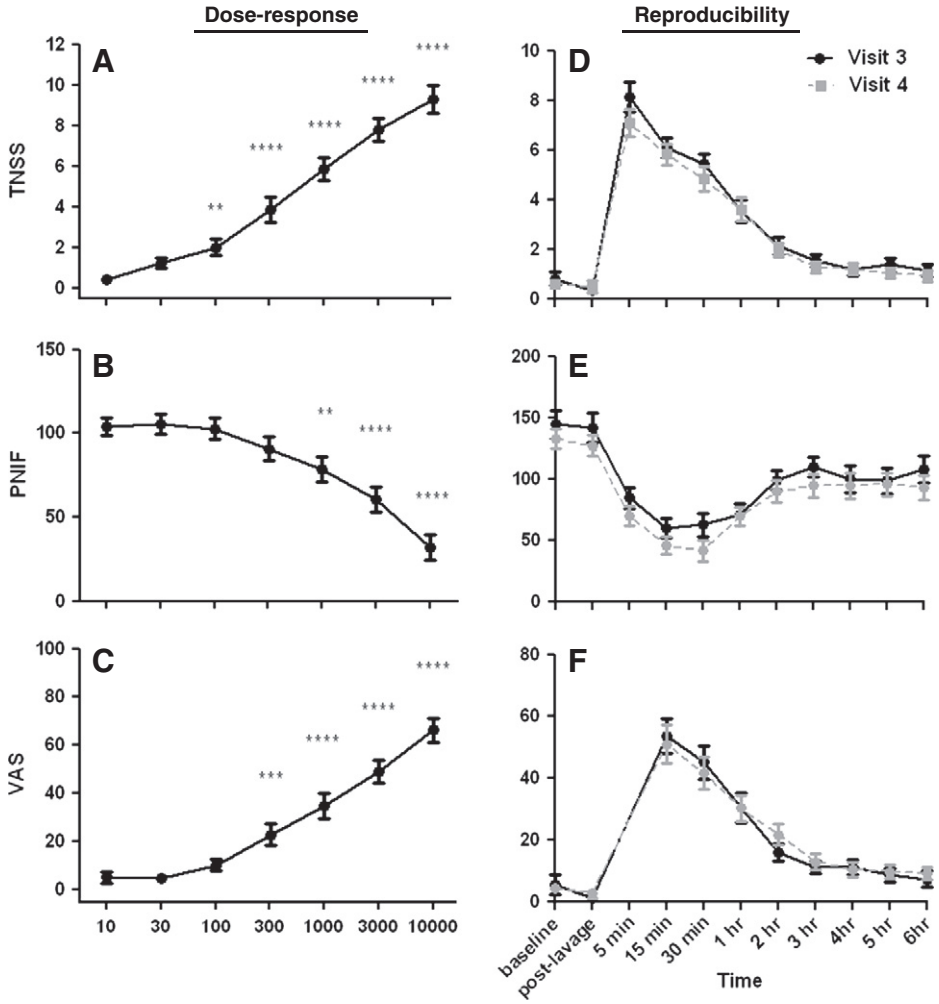

Fig. 2. A–C, dose–response of clinical outcomes at visit 1 during the titration grass pollen nasal allergen challenge. A, total nasal symptom score (TNSS); B, peak nasal inspiratory flow (PNIF); C, visual analogue score (VAS). Recordings were made at 10 min after the preceding challenge dose. Values plotted as mean ± SEM; comparisons versus baseline by repeated measures one-way anova with bonferroni correction; **p<0.01, ***p<0.001, ****p<0.0001. D–F, reproducibility of clinical data at consecutive visits with the same single allergen challenge dose; mean ± SEM at each time point for TNSS, PNIF and VAS at visits 3 and 4.

in vivo comparison of Accuwik Ultra and 111 SAMs at visit 2 suggested 111 to be superior for all mediators (Supplementary Figs. 2 and 3).

The pattern of mediator secretion was similar at visits 2–4. Tryptase levels peaked at 5 min, corresponding with the peak in early phase symptom scores (Fig. 3A). This was followed by a fall back to baseline values by 2 h. Conversely, ECP levels peaked at 5 h post allergen challenge (Fig. 3B). Sponges proved a better absorptive medium than 111 SAMs, both in terms of recovered concentration of tryptase and ECP (area under the curve, $p<0.05$), and regarding the volumes of neat fluid recovered (area under the curve, $p<0.01$) (Fig. 3C).

Following nasal allergen challenge, there were significant increases in IL-13 and IL-5, and a trend toward an increase in IL-4 ($p<0.013$, 0.024, and 0.052, respectively, 5 h versus baseline, unpaired *t*-test, Fig. 4A–C). IFN-γ levels were highly variable without an obvious pattern (Fig. 4D). Mean IL-10 values peaked at approximately 110 pg/ml at 5–6 h post challenge using sponges; conversely there was no clear pattern for secretions collected using 111 SAMs (data not shown). Overall, sponges appeared to be the preferable absorptive medium for nasal fluid

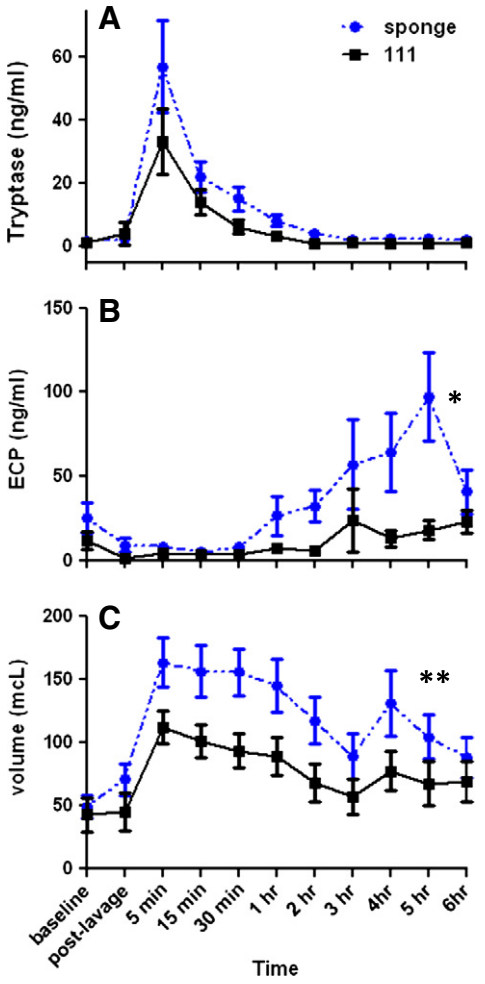

Fig. 3. Observed mean ± SEM at each time point for Tryptase in ng/ml (A), ECP in ng/ml (B), and total recovered nasal fluid volume in μl (C) at visit 3 for sponges and 111 synthetic absorptive matrices. *$p<0.05$, **$p<0.01$ for area under curve analyses, sponge versus 111.

cytokines with AUC being significantly greater than 111 SAMs for IL-5 ($p<0.001$) and IL-13 ($p<0.01$).

Cytokine levels recorded with nasal sponges at visit 4 followed a similar pattern to visit 3 with increasing levels of IL-4, IL-5 and IL-13 from 2 h after challenge onwards (Supplementary Fig. 4). Mean values for IL-5 and IL-13 from 4 h onwards were greater at visit 4 then visit 3, although area under the curve analyses did not reveal a significant difference between the two visits in any of the cytokines measured.

IL-5 levels at 5 h significantly correlated with symptoms measured by VAS at the same time point ($r=0.676$, $p=0.011$; Fig. 5A). There was an extremely close correlation between IL-5 and IL-13 levels in nasal fluid across all time points ($r=0.94$, $p<0.0001$; Fig. 5B), as well as between IL-5 and ECP ($r=0.72$, $p<0.0001$) and IL-5 and IL-4 ($r=0.79$, $p<0.0001$).

4. Discussion

This study demonstrates a dose-responsive, reproducible method of grass pollen nasal allergen challenge. Clinical outcomes are recorded using two symptom scoring methods as well as an objective measure of nasal patency, PNIF. The technique allows a detailed time course analysis of inflammatory mediators within nasal fluid. Nasal tryptase is a suitable biomarker of the early allergic response, whilst IL-5, IL-13 and ECP reflect Th2-cell and eosinophil activation during late phase allergic inflammation.

We have used a well-validated symptom score (Kim and Jang, 2011), and a visual analogue score (Holmstrom et al., 1990). Results between the two correlated closely. Moreover, there was a strong inverse correlation between symptoms and PNIF. The model proved dose-responsive (during the titration challenge, and from visit 2 to visit 3) and reproducible (between visits 3 and 4). This technique therefore has the potential to be used as a primary clinical outcome for studies of seasonal allergy. It should provide greater power, thereby reducing patient numbers, compared to trials using in-season symptom diaries, and allow studies to be conducted both in and out of season. In addition, direct comparisons between clinical data and immunological readouts are possible, giving the potential to identify clinically meaningful biomarkers.

With regards to techniques used to collect and study mediators in nasal fluid, key considerations include acceptability to patients, collection of adequate volumes and adequate concentration to allow the planned immunoassays. Generally, techniques involving direct absorbance from the mucosa provide optimum results (Klimek and Rasp, 1999; Lü and Esch, 2010; Riechelmann et al., 2003). Accordingly, we planned to compare two such methods: use of synthetic filter strips (SAMs) and polyurethane sponges.

Following allergen challenge, we found an early (5 min) rise in tryptase and gradual increases in ECP and Th2 cytokines from 2 to 3 h onwards. These results are, qualitatively, in agreement with those of several other researchers. Tryptase levels have been found to increase within 10–15 min of allergen challenge (Rondón et al., 2009; Jacobi et al., 1998). Conversely, ECP levels increase more gradually, being elevated at 6 and 24 h (Rondón et al., 2009; Miadonna et al., 1999). This correlates with elevated eosinophil numbers seen in the nasal mucosa from 6 to 8 h after allergen exposure (Banfield et al., 2010; Nouri-Aria et al.,

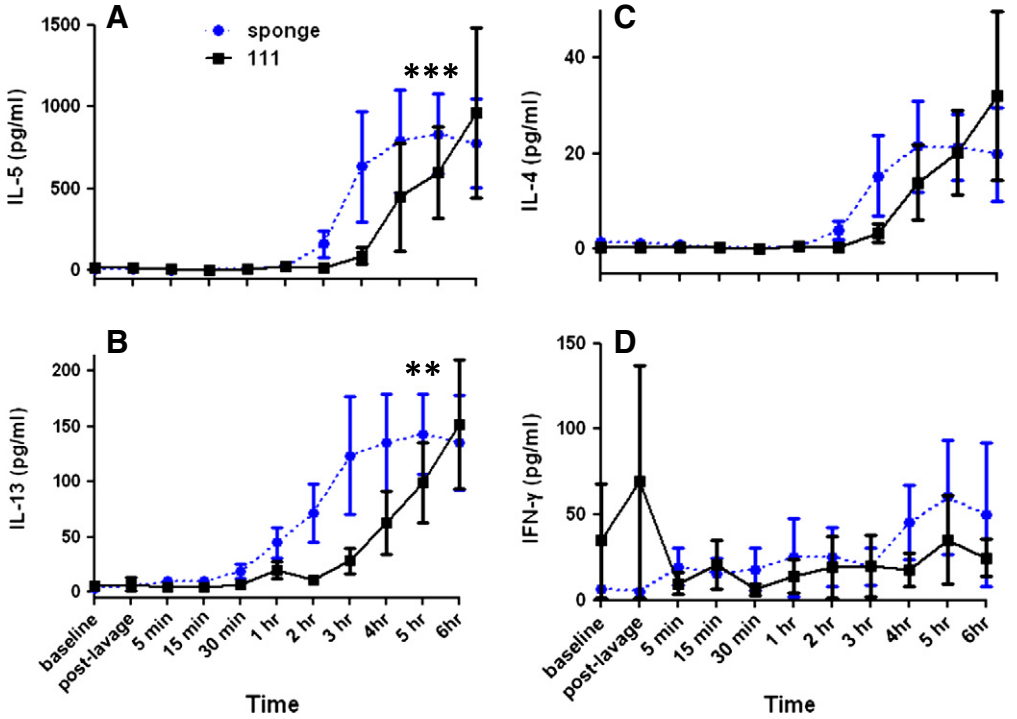

Fig. 4. A–D, observed mean ± SEM of IL-5, IL-13, IL-4, IFN-γ, and IL-10 (all pg/ml), at each time point at visit 3, as recorded with sponges and 111 synthetic absorptive matrices. **p<0.01, ***p<0.001 for area under curve analyses, sponge versus 111.

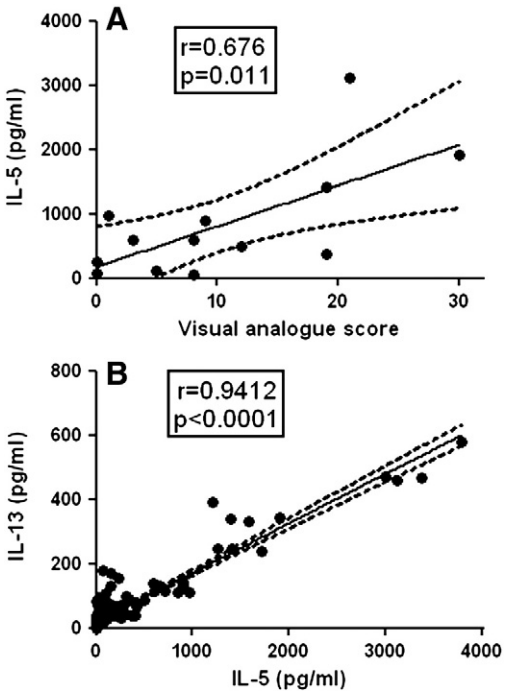

Fig. 5. A, IL-5 (pg/ml) at 5 h recorded using sponges correlates with visual analogue score at the same time point at visit 3. B, correlation between IL-5 and IL-13 levels recovered from sponges during visit 3 across all time points. Correlations by Pearson's correlation coefficient.

2000). Nasal fluid Th2 cytokine levels also increase some hours after challenge: IL-5 at 6–9 h (Nicholson et al., 2011; Linden et al., 2000); IL-13 at 6–8 h (Erin et al., 2005a; Nicholson et al., 2011); and IL-4 at 5–6 h (Wagenmann et al., 2005). Again, these findings correlate with histological mRNA results (Nouri-Aria et al., 2000). IFN-γ and IL-10 have received less attention, but Bensch et al. (2002) found an increase in nasal fluid IL-10 at 6 h post allergen challenge.

Conversely, there is considerable discrepancy within the literature concerning quantitative results for nasal biomarkers. Such variability is likely to be largely accounted for by different nasal fluid collection techniques (particularly whether or not these involve dilution of nasal fluid) and the different immunoassays used. Regarding tryptase levels, two allergen challenge studies found levels to peak at less than 2 ng/ml (Rondón et al., 2009; Allocco et al., 2002); whereas another study found levels of 6 μg/ml (6000 ng/ml) in the absence of challenge (Lü and Esch, 2010). Our mean level, at 5 min post allergen, was 43 ng/ml. Klimek and Rasp (1999) reported an approximate normal range for ECP in non-allergic individuals as 4–51 ng/ml; yet an allergen challenge study in atopics found mean levels of 20 ng/ml and 40 ng/ml at 6 and 24 h post-challenge respectively (Miadonna et al., 1999). Finally, much higher levels have been reported in nasal polyp patients, with mean levels of approximately 300 μg/l (300 ng/ml) (Gevaert et al., 2006). In comparison, our mean level for ECP at 5 h post challenge was 97 ng/ml.

Similar variability is found for cytokine levels. Peak post challenge IL-5 levels of approximately 10 pg/ml (Erin et al., 2005a) and 900 pg/ml (Linden et al., 2000) have been reported. An unchallenged, in-season level of 80 pg/ml was found in birch pollen allergic patients (Klimek et al., 1999); unchallenged levels

of 30–50 pg/ml were seen in nasal polyp patients (Gevaert et al., 2006). Our mean IL-5 level of 833 pg/ml at 5 h post-challenge is most comparable to that of Linden et al. (2000). They also used an allergen challenge and absorptive filter papers. IL-13 levels peaked at just 1 pg/ml post-challenge in a study employing nasal lavage (Erin et al., 2005a); but individual patient peak values of approximately 500 pg/ml have been recorded using Accuwik Ultra filter strips (Nicholson et al., 2011). We saw a mean level of 143 pg/ml for IL-13 at 5 h.

IL-4 levels have been typically difficult to measure, with samples frequently below the minimum level of detection. Wagenmann et al. (2005) did report a significant increase in IL-4, but with a peak median level below 0.5 pg, quoted as a total amount accounting for dilution of samples in elution fluid. We found a mean of 21 pg/ml at 5 h, whilst mean baseline level was less than 2 pg/ml. However, due to high variability, including several apparent non-secretors, this difference narrowly failed to reach statistical significance. Concerning IFN-γ, Klimek et al. (1999) found levels of 7 pg/ml in unchallenged allergics in season, increasing to 17 pg/ml following birch pollen immunotherapy. Our mean level was 60 pg/ml at 5 h post challenge. Finally, Bensch et al. (2002) recorded peak IL-10 level of approximately 5 ng/ml (5000 pg/ml); our mean level at 5 h was 112 pg/ml.

Given the variability in levels of both early and late phase mediators reported within the literature it is not possible to establish normal ranges at present. Whilst the immunoassays used have doubtlessly become more sensitive in recent years, the differing fluid collection and protein recovery techniques are perhaps most responsible for these discrepancies. For example, Erin et al. collected nasal fluid by both lavage and filter strips in the same individuals, and analysed samples using identical multiplex assays (Erin et al., 2005b). IL-5 and IL-13 levels were between 5 and 10 times greater using the filter technique; IL-4 levels were below the limit of detection using lavages but showed a clear late phase increase with filters. Other researchers have reported that sponges provide levels of mediators generally 10× higher than nasal lavage (Riechelmann et al., 2003). Establishing an optimum and consistent approach between researchers in this regard is therefore crucial. This will greatly assist the development of local biomarker assays of inflammation and treatment efficacy.

In interpreting our results, we should consider the possibility that frequent insertion of materials into the nose may have a mechanical/irritative effect, and that this may, in part, be responsible for clinical and laboratory results. Regarding the former, symptom scores were recorded prior to insertion of SAMs/sponges at each time point, meaning immediate physical effects did not impact on patients' scores. Moreover, symptoms peaked clearly in relation to allergen exposure, 5 min after challenge, and had returned to near normal levels by 6 h, despite repeated interventions by this stage. With regard to effects on biomarker levels, the early rise in tryptase at 5 min, followed by a fall to baseline levels by 2 h, suggests an allergic rather than a physical/mechanical effect. Riechelmann et al. (2003) provide further evidence: they used alpha-2-macroglobulin and lactate dehydrogenase as markers of plasma exudation and tissue injury, and found no differences following use of filter strips or polyurethane sponges when compared to nasal lavage or blowing.

There remain some limitations to our model. First, we recorded a number of dry samples, particularly at pre-challenge time points. However, the available measurements indicate that mediator levels (out of season) prior to allergen challenge are very low. Thus, baseline data might be replaced by a fixed value. Unfortunately we lost statistical power due to these non-results. Secondly, we chose to insert SAMs/sponges for just 2 min, whereas others have used 10 min (Riechelmann et al., 2003; Klimek et al., 1999). In preliminary studies we found little benefit of increasing our sampling time. Additionally, a short insertion time allows frequent sampling, particularly in the early phase. Third, we sampled nostrils individually (in order to compare different absorptive media) rather than pooling results from both sides, therefore not taking into account the natural nasal cycle as well as reducing the total volume of fluid collected. For future studies, we intend to use sponges bilaterally and pool secretions. Fourth, recordings beyond 6 h post allergen challenge may have given additional clinical and immunological data on the late phase reaction (Rondón et al., 2009; Miadonna et al., 1999). In part, the 6 h time period was enforced due to practical considerations, but we intend to measure up to 10 h post challenge in another cohort of patients. Finally, with regards to use of this technique in interventional studies, a good correlation between nasal challenge symptom scores and seasonal symptom severity cannot be assumed. In consequence, efficacy of a treatment demonstrated by diminution of nasal challenge response may not necessarily equate to an improvement in seasonal symptoms. We plan to address these issues in future studies.

The advantage of nasal fluid analysis in the study of allergic rhinitis is that it is a non-invasive procedure that allows evaluation of responses at the relevant site. Nasal biopsy allows assessment across the full depth of the mucosa and maintains tissue architecture, allowing the cellular source of mediators to be examined (Durham et al., 1992; Banfield et al., 2010; Nouri-Aria et al., 2000). However, this is a moderately invasive procedure, time consuming and not amenable to frequent repetition to give a time course analysis. It may also discourage potential volunteers from participating in studies. Alternatives include the use of nasal scrapings (Meltzer et al., 1994) or brushings (Lopez-Guisa et al., 2012) to provide cytological, rather than histological, data and/or mRNA for analysis by PCR. Whilst these also may provide excellent data, it is our experience that these procedures are less tolerable to patients than simple fluid absorption — of particular importance if repeat sampling to provide a time-course analysis is planned.

Given that our technique is simple to perform, non-invasive, and provides undiluted samples for biomarker analysis, we believe that it is the most useful single approach in studies of nasal allergen provocation. The reproducibility of clinical scores elicited by our nasal provocations makes this a potential means of assessing responses to treatments for seasonal allergic rhinitis without dependence on the pollen season. We plan to use this model to further assess the mechanisms of allergic inflammation in the nasal mucosa, to investigate the local mechanisms of allergen immunotherapy and allergen tolerance, and to identify local biomarkers which may be used both to predict and monitor responses to immunotherapy.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jim.2012.06.013.

Acknowledgements

The authors are grateful to Dr Pablo Rodríguez del Río for his helpful advice given during the planning of this study, and to Dr Melina Makatsori for practical assistance during the study.

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