- The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) - Cytokine Analysis of Nasal Fluid Before and After Nasal Allergen Challenges (NAC)

Jenny Thiele (MSc)[1,2], Mena Soliman (MSc, MBChB)[1,2], Lisa Steacy (BSc)[2], Daniel Adams (BSc)[2] and Anne K Ellis (MD, MSc, FRCPC, FAAAAI)[1,2]

Abstract

Rationale: The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) has previously reported biological outcomes following changes to the protocol for nasal allergen challenge (NAC). Using our cumulative allergen concentration (CAC) protocol we investigated whether or not cytokine profiles were reproducible between two separate NAC visits (3 weeks apart).

Methods: 10 ragweed allergic participants were enrolled, all with AR symptoms in ragweed season and a supportive skin test response. During screening, 4-fold incremental concentrations of ragweed allergen were administered until each participant achieved the qualifying symptom score (TNSS ≥ 8/12 and a 50% Peak Nasal Inspiratory Flow (PNIF) reduction). For the subsequent NAC1 visit, (21d later) participants were challenged with one dose of allergen equivalent to the cumulative amount of allergen received during screening. Nasal secretions were collected at baseline, 1hr, 6hr and 24hr post-NAC and cryopreserved. This procedure was repeated at a second NAC visit 21 days later (NAC2). Cytokine levels were determined using the Luminex®x-MAP™Technology.

Results: IL-5 and IL-13 were upregulated at 6hr and 24hr post NAC (Wilcoxon matched-pairs test NAC1: IL-5 p=0.0652 both time points, IL-13 p=0.0273 at 6hr; NAC2: IL-5 p=0.0156 at 24hr, IL-13 p=0.0547 at 6hr). IL-6 was upregulated at 1hr, MCP-1 and MIP-1b were upregulated at 24hr. Other pro-inflammatory cytokines, chemotaxis and growth factors remained mainly unchanged following NAC. In general, cytokine levels appeared slightly lower for NAC2.

Conclusions: Cytokine profiles appear very similar between both NAC visits using a small set of atopic samples. The 24hr time-point was an important addition to the AR-CIC protocol.

Methods

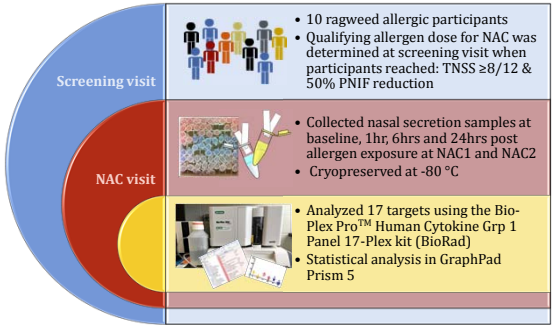

Figure 1. Project workflow

Acknowledgements

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Affiliations

[1] Departments of Medicine and Biomedical & Molecular Science, Queen's University, Kingston, ON, Canada
[2] Allergy Research Unit, Kingston General Hospital, Kingston, ON, Canada

Study Design

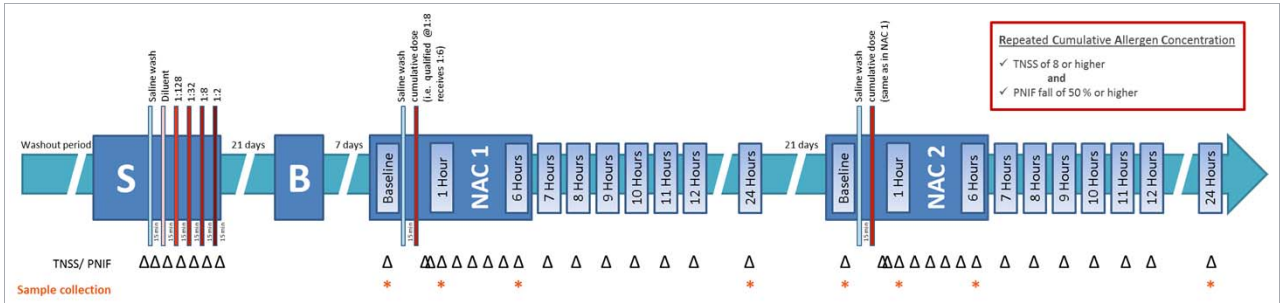

Figure 2. The repeatability of cytokine outcomes in nasal secretions was tested by performing two nasal allergen challenge (NAC) visits 21 days apart from each other. During the screening visit the qualifying allergen dose was determined using incremental concentrations of ragweed allergen until each participant reached their qualifying cut-off (TNSS of ≥8/12 and PNIF fall of ≥50 %). Nasal fluids were obtained during each NAC visit at baseline, 1hr , 6hrs and 24hrs post allergen exposure.

Results

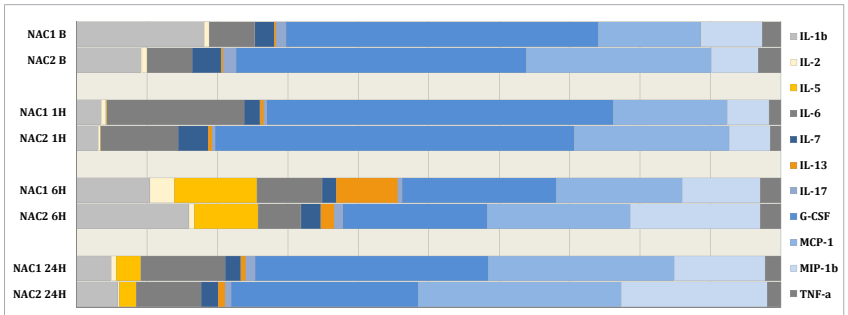

Figure 3. Th2 cytokines IL-5 and IL-13 (shown in orange) were upregulated starting at 6hrs post allergen exposure for both NAC visits. Shown here are the average percentages for all detectable cytokines at NAC1 and NAC2 for 9 participants (one participant withdrew prior to NAC2 and was therefore excluded from the analysis). Overall, the cytokine amounts appeared slightly lower for NAC2, however the relative proportions remained mostly unchanged between NAC1 and NAC2. We detected significantly less IL-1b at baseline; IL-2 at 1hr, 6hr and 24hr; IL-6 at baseline and 1hr; G-CSF at 6hr; MCP-1 at 6hr; and MIP-1b at 1hr in NAC2 samples when compared to NAC1 samples. IL-8 levels (not shown) remained stable between all time points for NAC1 and NAC2.

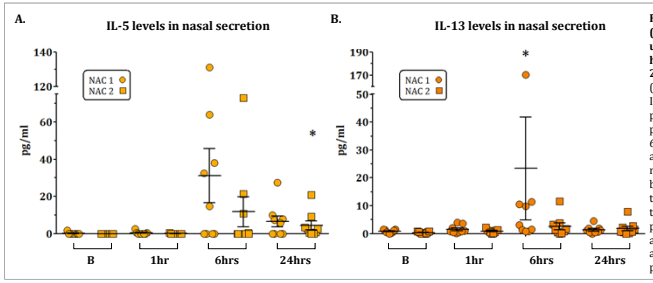

Figure 4. For most participants IL-5 (A.) and IL-13 (B.) levels were undetectable at baseline and 1hr, however increased at 6hrs and 24hrs post allergen exposure (Wilcoxon matched-pairs test NAC1: IL-5 p=0.0652 at 6hr and 24hr, IL-13 p=0.0273 at 6hr; NAC2: IL-5 p=0.0156 at 24hr, IL-13 p=0.0547 at 6hr). Shown are the individual IL-5 and IL-13 levels [pg/ml] with bars representing the group mean (error bars represent the standard error of the mean (SEM) of the group) for a total of 9 participants (one participant withdrew prior to NAC2 and was therefore excluded from the analysis). Statistical analysis was performed using GraphPad Prism 5.

Summary & Discussion

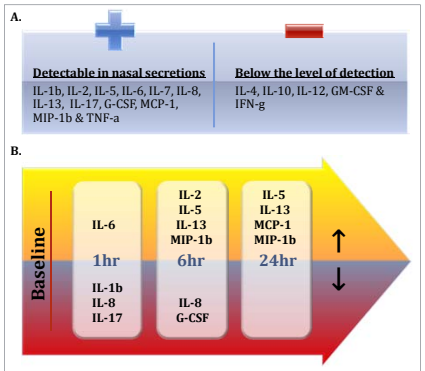

Figure 5. We were able to detect the majority of the 17 cytokine targets in nasal secretions (A.) and their changes after allergen exposure (B.). Only IL-4, IL-10, IL-12, GM-CSF and IFN-g were undetectable in our nasal secretion samples. Besides IL-5 and IL-13 other cytokines such as IL-6 (at 1hr), IL-2 (6hr), MIP-1b (6hr and 24hr) and MCP-1 (24hr) were slightly increased when compared to baseline levels, while IL-1b (1hr), IL-8 (1hr and 6hr), IL-17 (1hr) and G-CSF (6hr) appeared to be slightly downregulated.

The nasal secretion cytokine profiles appear to be very similar in the two NAC visits performed 21 days apart of each other (n=9). This becomes an essential criteria for clinical trials when samples are compared prior to and post treatment. The 24hr time-point was an important addition to the AR-CIC protocol.

The results of this study are in general in agreement with previously reported studies (Scadding *et al.* 2012 & 2015, Nicholson *et al.* 2011). The relatively low levels of cytokines measured however suggest that a different collection material such as a sponge or different filter paper type might yield higher cytokine recovery. The use of protease inhibitors should also be investigated.