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 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® Bio-Plex ProTM technology.

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® Bio-Plex ProTM 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, chemokinas 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 CIC protocol.

Results

Summary & Discussion

This study was supported by the Allergy, Genes and the Environment Networks of Centres of Excellence, the Division of Allergy & Immunology at Kingston General Hospital, the Spar Endowment Funds and the Department of Medicine at Queen’s University.

Acknowledgements

This study was supported by the Allergy, Genes and the Environment Networks of Centres of Excellence, the Division of Allergy & Immunology at Kingston General Hospital, the Spar Endowment Funds and the Department of Medicine at Queen’s University.

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

Figure 1. Project workflow

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 ≥ 8/12 and PNIF fall ≥ 50%). Nasal fluids were obtained during each NAC visit at baseline, 1hr, 6hrs and 24hrs post-allergen exposure.

Figure 3. 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-5 at baseline, IL-8 at 1hr, 24hr; IL-17 at baseline, 1hr, 6hr and 24hr; G-CSF at all time points for NAC1 and NAC2 samples when compared to NAC1 samples. IL-6 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=0.0723 at 6hr and 24hr; IL-13 p=0.0723 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 (ggplot2) with bars representing the group mean (error bars represent the standard error of the mean). The mean fold change (%) is calculated 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.

Figure 5. We were able to detect the majority of the 17 cytokine targets in nasal secretions (%). The lower the changes after allergen exposure (%). Only IL-4, IL-10, IL-12, GM-CSF and IFN-γ were undetectable in our nasal secretion samples. Besides IL-5 and IL-13 other cytokines such as IL-1β, IL-2, IL-6, IL-12, GM-CSF and IFN-γ were undetectable in our nasal secretion samples. Besides IL-5 and IL-13 other cytokines such as IL-1β, IL-2, IL-6, IL-12, GM-CSF and IFN-γ were undetectable in our nasal secretion samples. Besides IL-5 and IL-13 other cytokines such as IL-1β, IL-2, IL-6, IL-12, GM-CSF and IFN-γ were undetectable in our nasal secretion samples. Besides IL-5 and IL-13 other cytokines such as IL-1β, IL-2, IL-6, IL-12, GM-CSF and IFN-γ were undetectable in our nasal secretion samples.