Nasal Allergen Challenge (NAC) Induced Eosinophilia – The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC)

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Abstract

Rationale: The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) is a Canadian initiative with the goal of performing standardized nasal allergen challenge (NAC) to study the effects of therapeutic agents for allergic rhinitis (AR), while allowing the identification of mechanisms and biomarkers of AR. Various NAC protocols have been described previously. The multiple cumulative allergen concentration (MCAC) NAC protocol was shown to produce more robust symptom scores than a cumulative allergen concentration (CAC) protocol. Here we examined NAC-induced eosinophilia for these two protocols.

Methods: 17 atopic and 12 non-atopic participants were enrolled for this study. Atopic individuals presented with AR symptoms in ragweed season and a supportive skin test response. During screening incremental concentrations of ragweed allergen were administered until each participant reached the cumulative symptom cut-off. For the subsequent NAC one week later, ten allergens were challenged with one dose of allergen equivalent to the cumulative amount of allergen each received during screening (CAC). Seven atopic participants reached the cumulative of all preceding allergen doses to the qualifying concentration (QC), followed by the QC 15 minutes later (MCAC). Non-atopics were challenged with a 1:2 ragweed concentration. Nasal lavage samples were collected at baseline, 1 hour (H1) and 6 hours (H6) post NAC to determine differential counts.

Results: The eosinophil fraction was significantly increased in atopics following NAC when compared to non-atopics at both H1 and H6 for the CAC protocol and at H6 for the MCAC protocol. The total cell numbers were observed for MCAC participants at baseline and 1 hour (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.05). Atopy was associated with significantly increased eosinophil fractions (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.05 and P ≤ 0.01) and at 6 hours for the MCAC-protocol (One-way ANOVA, Dunn’s multiple comparison test). Significantly higher eosinophil fractions were noted for MCAC participants at baseline compared to non-atopic participants (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.05 and P ≤ 0.01 respectively) in comparison to non-atopic participants. Shown are average total cell numbers, error bars representing the SD.

Conclusions: Even though the MCAC protocol establishes more robust symptom scores the CAC protocol appears to produce more pronounced eosinophilia.

Study Design & Methods

Figure 1. Both nasal allergy challenge (NAC) designs were comprised of a screening followed by a NAC visit. During the screening visit the qualifying allergen dose was determined individually using incremental concentrations of ragweed allergen until each participant reached their qualifying cut-off (TNSS of ≥7/12 and PFM full of ≥5/12). Nasal lavage samples, to determine total and differential cell counts, were obtained during the NAC visit at baseline, 1 hour and 6 hours post allergen challenge. Total cell counts using trypan-blue were described previously. For the preparation of cytospin slides for each lavage sample which were subsequently fixed and stained using Diff-Quik to obtain differential cell counts. Statistical analysis was performed utilizing the GraphPad Prism Software.

Figure 2. At screening, the majority of atopic participants reached the study inclusion criteria with an allergen dosage of ≤1.2 (CAC 50 % of participants, MCAC 71 % of participants). All non-atopic participants were challenged with a 1:2 dose of ragweed to ensure the absence of symptoms prior to the highest dose.

Figure 3. The total cell number as well as the eosinophil fraction appeared to be increased in atopics following NAC when compared to non-atopics for both CAC and MCAC. Shown are average total cell populations for nasal atopic (top panel) and non-atopic (bottom panel) participant at baseline, 1 hour and 6 hours post allergen challenge (Dunn’s multiple comparison test). The total cell populations contained consisted mostly of neutrophils (N), eosinophils (E), monocytes (M) and a variety of epithelia (Epi) cells.

Figure 4. The eosinophil fraction (%) was significantly increased in atopics following NAC when compared to non-atopics at both 1 hour and 6 hours for the CAC-protocol (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.01) and at 6 hours for the MCAC-protocol (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.05). Moreover, total cell counts increased significantly from baseline to 1 hour for atopic participants of the CAC study (T-test, Wilcoxon matched pairs P = 0.0273) but were not significantly increased when compared to non-atopic participants (One-way ANOVA, Dunn’s multiple comparison test). Significantly higher eosinophil fractions were noted for MCAC participants at baseline compared to non-atopic participants (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.05 and P ≤ 0.01 respectively) in comparison to non-atopic participants. Shown are average total cell numbers, error bars representing the SD.

Summary & Discussion

A variety of NAC protocols have been described previously1,2,3. The multiple cumulative allergen concentration (MCAC) NAC protocol was shown to produce more robust symptom scores than a cumulative allergen concentration (CAC) protocol3. Here we examined NAC-induced eosinophilia for these two protocols in 17 atopic and 12 non-atopic participants. During screening, the majority of atopic participants reached the study inclusion criteria with an allergen dosage of ≤1.2 (CAC 50 % of participants, MCAC 71 % of participants), while all non-atopic participants were challenged with a 1:2 dose of ragweed. At the subsequent NAC one week later, it was noted that the eosinophil fraction was significantly increased in atopics following NAC when compared to non-atopics at both 1 hour and 6 hours for the CAC-protocol (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.01) and at 6 hours for the MCAC-protocol (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.05). Furthermore, total cell counts increased significantly from baseline to 1 hour for atopic participants of the CAC study (T-test, Wilcoxon matched pairs P = 0.0273) but were not significantly increased when compared to non-atopic participants (One-way ANOVA, Dunn’s multiple comparison test). Significantly higher total cell numbers were observed for MCAC participants at baseline and 1 hour (One-way ANOVA, Dunn’s multiple comparison test P ≤ 0.05 and P ≤ 0.01 respectively) in comparison to non-atopic participants. The ragweed NAC doses were optimized for each participant and were independent from the eosinophils observed in atopic individuals. Even though the MCAC protocol establishes more robust symptom scores, the CAC protocol appears to produce more pronounced eosinophilia.

Results

Figure 5. The dosages of ragweed allergen administered during the NAC were catered towards each participant individually and are independent from the eosinophils observed in atopic individuals. All atopic individuals were challenged with a 1:2 dose. Shown are the eosinophil fractions (% of white blood cells) for each participant and time point, color-coded based on their qualifying allergen dose. Even after the MCAC protocol, total cell counts remained high.

Reference


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 J.T. Beckill Foundation and the Department of Medicine at Queen's University.

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