

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

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Abstract

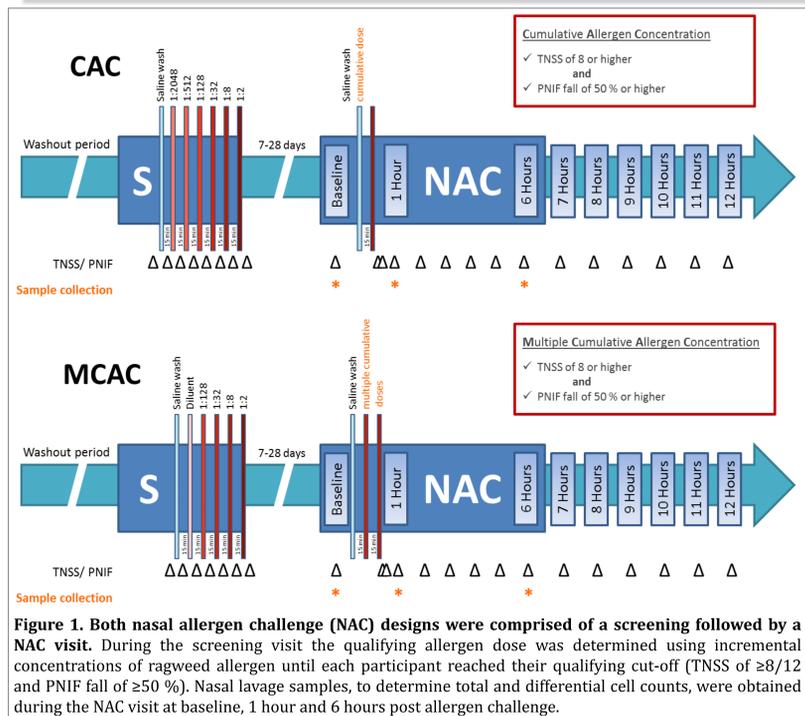
Rationale: The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) is a Canadian initiative with the goal of performing standardized nasal allergen challenge (NAC) to study the effects of therapeutic agents for allergic rhinitis (AR), while allowing the identification of mechanisms and biomarkers 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 qualifying symptom cut-off. For the subsequent NAC one week later, ten atopics were challenged with one dose of allergen equivalent to the cumulative amount of allergen each received during screening (CAC). Seven atopics received 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 (1H) and 6 hours (6H) post NAC to determine differential cell counts.

Results: The eosinophil fraction was significantly increased in atopics following NAC when compared to non-allergics at both 1H and 6H for the CAC-protocol and at 6H for the MCAC-protocol.

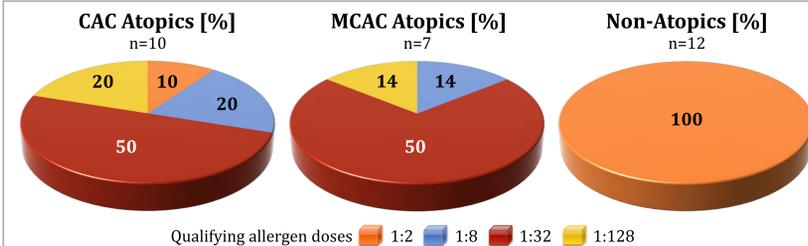
Conclusions: Even though the MCAC protocol establishes more robust symptom scores, the CAC protocol appears to produce more pronounced eosinophilia.

Study Design & Methods



17 atopic (10 CAC; 7 MCAC) and 12 non-atopic participants were enrolled for this study. Allergen dosages were determined individually during the screening visit as described above. Nasal lavage samples were collected during the NAC visit at baseline, 1 hour and 6 hours post allergen challenge. Total cell counts using trypan-blue were determined prior to 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.

Results



Qualifying allergen doses: 1:2 (orange), 1:8 (blue), 1:32 (red), 1:128 (yellow)

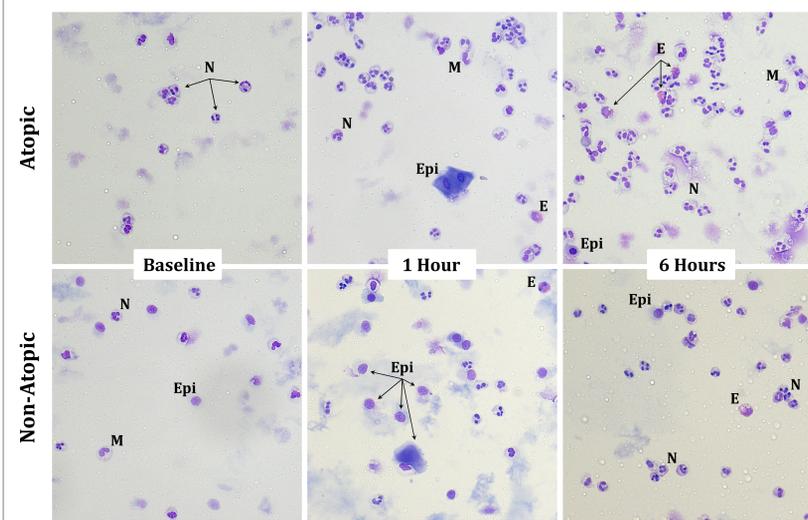


Figure 3. The total cell number as well as the eosinophil fraction appeared to be increased in atopics following NAC when compared to non-atopics for both, CAC and MCAC. Shown are differentially stained nasal cell populations for one atopic (top panel) and one non-atopic (bottom panel) participant at baseline, 1 hour and 6 hours post allergen challenge (200X magnification). The nasal cell populations examined consisted mostly of neutrophils (N), eosinophils (E), monocytes (M) and a variety of epithelia (Epi) cells.

Summary & Discussion

A variety of NAC protocols have been described previously^{1,2,3,4}. The multiple cumulative allergen concentration (MCAC) NAC protocol was shown to produce more robust symptom scores than a cumulative allergen concentration (CAC) protocol⁵. Here we examined NAC-induced eosinophilia for these two protocols on 17 atopic and 12 non-atopic participants. During screening, the majority of atopic participants reached the study inclusion criteria with an allergen dosage of 1:32 (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 $P \leq 0.01$) and at 6 hours for the MCAC-protocol (One-way ANOVA, Dunn's multiple comparison test $P \leq 0.05$). 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 $P \leq 0.05$ and $P \leq 0.01$ respectively) in comparison to non-atopic participants. The ragweed NAC doses were optimized for each participant and were independent from the eosinophilia observed in atopic individuals. Even though the MCAC protocol establishes more robust symptom scores, the CAC protocol appears to produce more pronounced eosinophilia.

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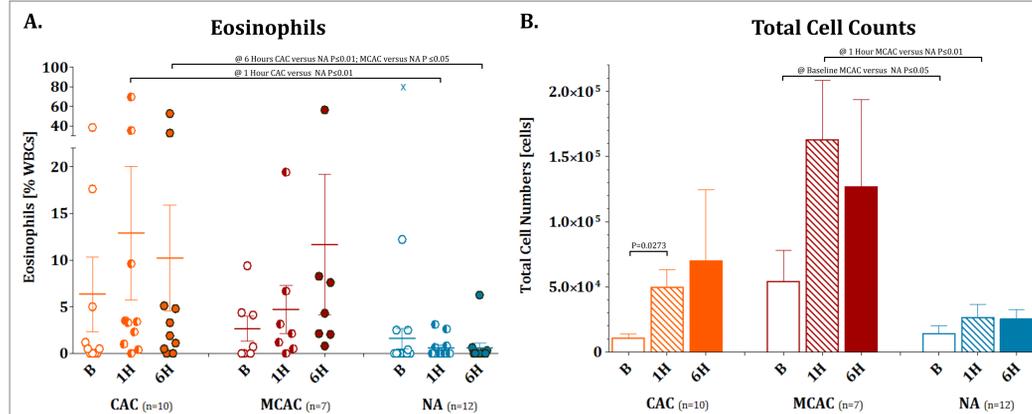


Figure 4. The eosinophil fraction (A) was significantly increased in atopics following NAC when compared to non-atopics at both 1 hour and 6 hours for the CAC-protocol (One-way ANOVA, Dunn's multiple comparison test $P \leq 0.01$) and at 6 hours for the MCAC-protocol (One-way ANOVA, Dunn's multiple comparison test $P \leq 0.05$). Shown are individual eosinophil percentages [% of white blood cells] with bars representing the group mean (error bars represent the standard error of the mean (SEM) of the group). Total cell counts (B.) 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 $P \leq 0.05$ and $P \leq 0.01$ respectively) in comparison to non-atopic participants. Shown are average total cell numbers, error bars representing the SEM.

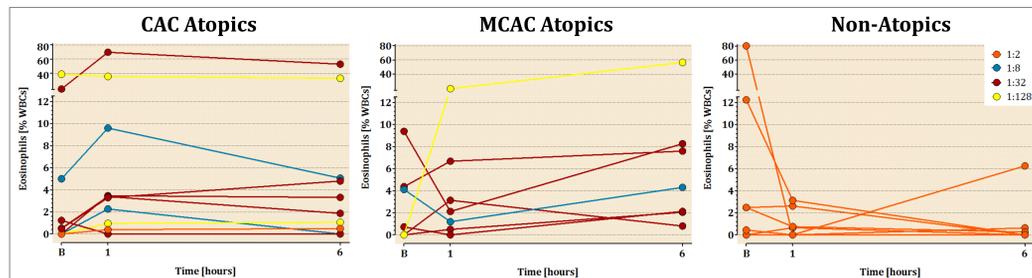


Figure 5. The dosages of ragweed allergen administered during the NAC were catered towards each participant individually and are independent from the eosinophilia observed in atopic individuals. All non-atopic individuals were challenged with a 1:2 dose. Shown are the eosinophil fractions [% of white blood cells] for each participant and time point, color-coded based on their qualifying allergen dose. Even though the MCAC establishes a more robust symptom score, the CAC appears to produce more pronounced eosinophilia.

References

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