Stronger nasal responsiveness to cold air in individuals with rhinitis and asthma, compared with rhinitis alone

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Summary

Background We have previously proposed that, compared with rhinitis alone, the constellation of upper and lower airway allergic disease is a manifestation of a more severe form of a syndrome affecting the entire airway. If this is correct, not only the lower, but also the upper airways of patients with asthma and rhinitis should demonstrate more abnormalities compared with patients with rhinitis alone, including higher sensitivity to irritant factors.

Objective To test the hypothesis that, a previously well-studied natural nasal stimulus, cold, dry air (CDA), produces a stronger response in subjects with allergic rhinitis (AR) and asthma compared with subjects with AR alone.

Methods We performed nasal provocation with CDA on 24 individuals with asthma and rhinitis and 17 with rhinitis alone. Prior to and after the challenge, nasal symptoms were recorded using visual analogue scales and nasal lavages were performed to determine histamine and lysozyme levels.

Results The two groups reacted differently to CDA: after the challenge, patients with rhinitis and asthma reported significantly higher scores for nasal congestion, rhinorrhea and lacrimation. Also in this group, significant increases in histamine and in lysozyme levels in nasal lavage fluids were induced by CDA. In subjects with rhinitis alone, CDA failed to increase histamine or lysozyme levels above baseline. The CDA-induced change from baseline in histamine was significantly higher in the patients with rhinitis and asthma, compared with the rhinitis-only group.

Conclusion Patients with AR and asthma have stronger nasal responsiveness to CDA compared with patients with rhinitis alone. This observation is consistent with the notion that compared with rhinitis alone, the presence of asthma and rhinitis signifies a higher degree of functional abnormality of the entire airway.

Keywords cold air, histamine, lysozyme, nasal responsiveness

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Introduction

The many similarities between the pathologic features of the upper and lower airway mucosa in allergic rhinitis (AR) and asthma suggest that these two illnesses are manifestations of one syndrome in two different parts of the human respiratory tract [1]. It is quite interesting, however, that although the majority of patients with rhinitis do not exhibit clinical evidence of lower airway disease, symptoms of rhinitis are almost invariably present in patients with bronchial asthma [2–6]. In other words, whereas AR may present as an independent entity, asthma rarely does. Based on this observation, we have raised a hypothesis that the presence of lower airway disease is indicative of an overall more severe chronic allergic airway syndrome [1, 7]. If so, functional
abnormalities in both the upper and the lower airways should be more prominent in patients who suffer from rhinitis and asthma, compared with patients with rhinitis alone. Whereas this notion is well-documented when it pertains to the lower airways, little information is available for the nasal passages. Recently, Corren et al. [8] provided evidence supporting our hypothesis by demonstrating that the nasal response to natural cat allergen exposure is significantly greater in individuals with combined rhinitis and asthma compared with those with rhinitis alone.

We opted to address the hypothesis that functional abnormalities in the upper airways are more prominent in individuals with both rhinitis and asthma, compared with those with rhinitis alone, by testing for nasal sensitivity to cold, dry air (CDA), a non-allergic trigger. We chose CDA as the stimulus of interest, because it is the only natural, frequently encountered stimulus among those used in nasal provocation settings [9–11] and because it has been validated as a tool for testing nasal hyper-rersponsiveness [12, 13]. We compared the two groups by performing nasal CDA provocations and obtaining both subjective and objective data to quantify the nasal response.

Materials and methods

Subjects

Two groups, consisting of a total of 41 subjects, 16 females and 25 males (age range: 21–68), were identified among our volunteer database. Twenty-four subjects had rhinitis with asthma, while 17 only reported rhinitis. Clinical nasal sensitivity to CDA was not a selection criterion for any subject. Prior to the subjects’ arrival at the laboratory, they were asked to abstain from certain medications such as bronchodilators, antihistamines and nasal steroids. The washout periods for such medications complied with upheld standards.

Entry criteria for rhinitis applied to both groups included: (a) positive skin prick test results to at least one allergen from a panel of 10 allergens (Dermatophagoides, fariac, D. pteronyssinus, ragweed, grasses, trees, alternaria, cladosporium, hormodendrum, cat, dog and cockroach), (b) a subjective rhinitis severity score of at least 1 (on a scale of 0–3) during the month of the study (a score for every month is routinely obtained from all our volunteers upon their first evaluation for participation in laboratory studies). The subjects with asthma and rhinitis were also required to have a diagnosis of asthma and to report chest symptoms (using a scale similar to the above mentioned for rhinitis) during the period of testing. All subjects in the rhinitis alone group had previously undergone a bronchial methacholine provocation and had responded with < 20% reduction in forced expiratory volume in 1 s (FEV1) even after the highest dose (75 mg/mL). In addition, all subjects in this group denied any lower respiratory symptoms other than cough or sputum production. Asthmatics were required to have a methacholine PC20 less than 8 mg/mL.

The study was approved by the Johns Hopkins Bayview Medical Center Institutional Review Board and all subjects offered signed informed consent prior to participation.

Study design

All subjects visited the laboratory on one occasion. Baseline spirometric outcomes were first obtained. Next, seven preliminary nasal lavages using 0.9% saline solution warmed to 37 °C were consecutively performed in accordance to our previously described method [9]. The volume of the first and seventh lavages was 2.5 mL/nostril. The collected fluids from these two washes, labelled Pre 1 and Pre 7, respectively, were saved for analysis. The second through sixth lavages were performed with 5 mL of saline per nostril and were discarded. These preliminary nasal lavages were implemented to reduce pre-existing levels of histamine in nasal secretions to a stable baseline. Next, subjects were asked to wait for a period of 1 h in the laboratory. This period allows the hydration status of the nasal mucosa to return to baseline. We have previously demonstrated that mediators or biochemical markers in nasal secretions do not spontaneously return to pre-lavage levels within this hour [14]. Afterwards, a nasal CDA challenge was performed. This was followed by a nasal lavage of 2.5 mL of saline per nostril at 5 and 15 min after the challenge, labelled CDA 1 and CDA 2, respectively. Nasal symptom scores were obtained from each subject on four occasions, immediately prior to the Pre 1, Pre 7, CDA 1 and CDA 2 nasal lavages.

Nasal cold, dry air provocation

The CDA challenge of 15 min duration was conducted with the use of a facial CPAP mask snugly covering only the nose of the subject, leaving the mouth exposed [9]. This mask was attached to an air tank that delivered 26 L/min of air at 20 psi. The air was cooled to a temperature of –10 to 0 °C after passing through a tube that was immersed in a container filled with 95% ETOH and dry ice. Inspiration was performed nasally, through the mask, while expiration occurred through the mouth. Subjects were advised to breathe at a normal rate and manner and were instructed not to take any deep breaths or talk during the CDA provocation.

Analysis of returned lavage fluids

The returned lavage fluids were collected into polypropylene centrifuge tubes and were vigorously shaken to dissolve the gel phase of nasal secretions. They were then stored on ice, centrifuged for 10 min at 2500 g and 4 °C,
aliquoted and stored at $-20^\circ\text{C}$. One aliquot was used for the measurement of histamine by a previously described spectrofluorometric technique, which has a sensitivity of 1 ng/mL [15]. Measurements below 1 ng/mL were arbitrarily assigned the value of 0.5 ng/mL. The level of lysozyme, which is considered a marker of serous cell glandular activation [16], was determined in a second aliquot of the returned nasal fluids. An ELISA method with a sensitivity of 1 ng/mL was employed [17].

**Symptom evaluation**

Symptom scores, prior to and after the nasal CDA challenge, were obtained using 10 cm long visual analogue scales. The low end of the scale was marked with 'none' and the high end with 'unbearable'. Four symptoms were evaluated separately: (1) runny nose, (2) nasal congestion, (3) watery eyes and (4) nasal burning. At the time-points specified by the protocol, each subject drew a vertical line over the visual analogue scale for each symptom. The distance between the beginning of the scale and the marked line was recorded as the score in cm.

**Data analysis**

In all statistical analyses, two-tailed $P \leq 0.05$ was considered significant. Evaluation of the frequency distribution of the different outcome measures enabled us to employ parametric statistics for symptom scores. However, histamine and lysozyme levels in nasal fluids were not normally distributed and we used non-parametric methodology. We used parametric statistics for demographic comparisons between the two groups.

The primary analysis of the nasal outcome variables was based on the comparison of the two subject groups with respect to the CDA-induced absolute changes from baseline. For this purpose, in accordance to all our previous work with this model, the symptom score data obtained prior to the Pre 7 lavage as well this lavage fluid’s histamine and lysozyme data were considered baseline. Pre 1 lavage data were only used to evaluate whether any major differences between the two groups exist prior to the initiation of the experimental protocols, but were not used in the evaluation of the effect of a nasal challenge. The ‘change from baseline’ approach was appropriate given that we found no baseline differences in nasal symptom scores or in histamine or lysozyme levels in nasal lavage fluids, between the two groups. For ‘between-groups’ analyses, the tests we employed were either the unpaired $t$-test or the Mann–Whitney $U$-test.

Secondary within-group analysis was also performed to assess the effect of nasal CDA provocation. First, we used repeated measures ANOVA for the parametrically evaluated variables and Friedman ANOVA for the non-parametric ones. When ANOVA yielded a statistically significant result, the respective post hoc analysis was performed.

**Results**

Table 1 presents the entry characteristics and demographics of our subject populations. As expected, in

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Table 1. Demographics and entry characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Rhinitis and asthma</th>
<th>Rhinitis without asthma</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female : male)</td>
<td>12 : 12</td>
<td>4 : 13</td>
<td></td>
</tr>
<tr>
<td>Race (African American : Caucasian)</td>
<td>6 : 17*</td>
<td>3 : 12*</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>33.33 ± 2.13</td>
<td>39.29 ± 2.56</td>
<td>0.08</td>
</tr>
<tr>
<td>Baseline FEV$_1$ (% predicted)</td>
<td>82.28 ± 2.95</td>
<td>98.51 ± 2.81</td>
<td>0.0004</td>
</tr>
<tr>
<td>Baseline FVC (% predicted)</td>
<td>92.36 ± 2.81</td>
<td>99.12 ± 3.16</td>
<td>0.12</td>
</tr>
<tr>
<td>FEV$_1$/FVC × 100</td>
<td>75.85 ± 1.66</td>
<td>83.39 ± 1.60</td>
<td>0.003</td>
</tr>
<tr>
<td>Number of positive skin tests (max: 10)</td>
<td>4.92 ± 0.53</td>
<td>4.50 ± 0.68</td>
<td>0.63</td>
</tr>
<tr>
<td>Positive skin tests to dust mites (max: 2)</td>
<td>1.04 ± 0.19</td>
<td>0.69 ± 0.22</td>
<td>0.23</td>
</tr>
<tr>
<td>Positive skin tests to pets (max: 2)</td>
<td>1.08 ± 0.18</td>
<td>0.63 ± 0.22</td>
<td>0.12</td>
</tr>
<tr>
<td>Positive skin tests to pollens (max: 3)</td>
<td>1.92 ± 0.23</td>
<td>2.31 ± 0.22</td>
<td>0.24</td>
</tr>
<tr>
<td>Positive skin tests to cockroach (max: 1)</td>
<td>0.18 ± 0.8</td>
<td>0.20 ± 0.11</td>
<td>0.89</td>
</tr>
<tr>
<td>Rhinitis severity score (for the month of the study)</td>
<td>2.04 ± 0.17</td>
<td>1.70 ± 0.26</td>
<td>0.27</td>
</tr>
<tr>
<td>Pre 1 congestion (max: 10)</td>
<td>1.13 ± 0.26</td>
<td>1.57 ± 0.38</td>
<td>0.33</td>
</tr>
<tr>
<td>Pre 1 rhinorrhea (max: 10)</td>
<td>0.46 ± 0.14</td>
<td>1.28 ± 0.51</td>
<td>0.08</td>
</tr>
<tr>
<td>Pre 1 burning (max: 10)</td>
<td>0.15 ± 0.06</td>
<td>0.30 ± 0.15</td>
<td>0.31</td>
</tr>
<tr>
<td>Pre 1 lacrimation (max: 10)</td>
<td>0.21 ± 0.10</td>
<td>0.96 ± 0.41</td>
<td>0.05</td>
</tr>
<tr>
<td>Pre 1 histamine (ng/mL)</td>
<td>7.86 ± 2.19</td>
<td>6.22 ± 2.99</td>
<td>0.77</td>
</tr>
<tr>
<td>Pre 1 lysozyme (ng/mL)</td>
<td>23.4 ± 6.47</td>
<td>28.26 ± 7.22</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*In addition, the study had two Asian and one Native American subjects.

Wherever applicable, data are presented with mean ± SEM. The last six parameters (Pre 1) represent visual analog scale-based symptom scores recorded prior to the first nasal lavage, as well as mediator/marker levels in the first nasal lavage fluids on the study day.

FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; max., maximum.
asthmatics, FEV₁ and forced vital capacity (FVC), as well as their ratio, were lower than in the group with rhinitis alone (significance was reached for FEV₁ and FEV₁/FVC). Skin test results showed no significant differences between the two groups. Also, the two groups were not significantly different with respect to the rhinitis severity score for the month in which the study was conducted or with respect to the visual analogue scale-based individual symptom scores prior to the initiation of the study protocol (Pre 1). The only exception to this was the symptom score for eye tearing which was significantly higher in the subjects with rhinitis alone. This score being less than 1 for both groups (on a 0–10 scale), dampens any concern regarding this difference. Also, the levels of histamine and lysozyme in the Pre 1 lavage fluids were not different between the two groups. Of note, there exists a difference in gender numbers between the two groups where the rhinitis and asthma group comprised of 12 females and 12 males while the rhinitis group had 4 females and 13 males. As there is no report of a gender difference with regards to nasal CDA sensitivity, there is no reason to suspect that the gender imbalance may have influenced our results.

Figure 1 shows the changes in the average symptom scores from Pre 7 lavage baseline to the 5 min post CDA time-point (CDA 1) for nasal congestion, rhinorrhea, burning, and lacrimation. Baseline values did not differ between the two groups (for nasal congestion: \( P = 0.62 \), rhinorrhea: \( P = 0.44 \), burning: \( P = 0.87 \), and lacrimation: \( P = 0.06 \)). Subjects with rhinitis and asthma had consistently higher symptomatic response to CDA as compared with subjects with rhinitis alone. The difference between the two groups was statistically significant for nasal congestion, rhinorrhea and lacrimation and showed a trend for burning (\( P = 0.09 \)). When the changes from baseline at the 15 post-CDA time-point (CDA 2) were considered, the asthmatic subjects still demonstrated a stronger response but the differences between the two groups were only significant for rhinorrhea (\( P = 0.0008 \), data not shown). Within-group data analysis (secondary analysis) showed significant increases from baseline at the CDA 1 time-point in all symptoms and in both groups. At the CDA 2 time-point, however, whereas the increase from baseline was still statistically significant with regards to congestion and rhinorrhea in the rhinitis and asthma group, no symptoms were different from baseline in the rhinitis only group indicating a shorter duration of the nasal response to CDA (data not shown).

Figure 2 shows the average changes in nasal lavage histamine and lysozyme levels from baseline to CDA 1. Baseline (Pre 7) values did not differ between the two groups (\( P > 0.24 \) for both markers). The CDA-induced change in histamine levels was significantly higher in the group of patients with asthma compared with the group with rhinitis alone (\( P = 0.02 \)). The difference between the groups was not statistically significant for the CDA effect on lysozyme (\( P = 0.61 \)). Within-group analysis of the...
biologic markers showed that, in the group of subjects with rhinitis and asthma, a statistically significant CDA-induced increase from baseline was present at the 5 min time-point (CDA 1) for both histamine ($P = 0.01$) and lysozyme ($P = 0.01$); for the latter marker, this increase was still significant at the 15 min time-point (CDA 2). In contrast, subjects with rhinitis alone showed no significant increase in histamine (ANOVA, $P = 0.80$) or lysozyme ($P = 0.07$) over baseline in their nasal lavage fluids after the CDA provocation.

**Discussion**

In this study, subjects with rhinitis and asthma, compared with non-asthmatics, exhibited more severe nasal symptoms and had higher increments in the levels of biochemical markers in returned lavage fluids following nasal CDA provocation. This indicates that, in individuals with asthma, not only lower but also upper airway responsiveness is stronger, compared with non-asthmatics with rhinitis alone. This study is in agreement with the recent report by Corren et al. [8], who also found the nose of asthmatics to be more reactive than that of individuals with rhinitis alone, but with allergen as the nasal stimulus.

Our conclusions regarding the difference in nasal CDA responsiveness between the two groups are mostly based on subjective symptomatology. However, the use of visual analogue scales renders these measurements quite reliable [18]; also, the differences we observed were quite striking in that the response to CDA in the group of subjects with rhinitis and asthma was consistently around twice that of the subjects with rhinitis alone (Fig. 1). Given that baseline symptomatology, as well as the overall severity of rhinitis for the month the study was performed, did not differ between the two groups (Table 1), their difference in the response to CDA is an even more robust observation.

Moreover, the differences in subjective symptomatology are accompanied by a significant difference in histamine release in nasal lavage fluids (Fig. 2), a marker that we have found to be indicative of the strongest responses to nasal CDA challenge [9]. The lack of a significant difference between the two groups in the CDA-induced effect on the concentration of lysozyme in nasal secretions could be interpreted as indicative of no difference in serous gland responsiveness. On the other hand, only in the group of subjects with asthma were the post-CDA lysozyme levels in nasal lavages significantly elevated, compared with baseline. This raises the possibility that a difference between the two groups does, indeed, exist and that our inability to detect it may be a matter of inadequate statistical power.

It is important to discuss potential mechanisms behind the difference in the severity of the nasal response to CDA between individuals with rhinitis and asthma and individuals with rhinitis alone. It is likely that the nasal reaction to CDA is a manifestation of nasal hyper-responsiveness of non-specific nature [19], merely a reflection of the same phenomenon that is well recognized in the lower airways. In previous work, only presented in abstract form, we have shown that, compared with healthy controls, subjects with perennial AR experience stronger reactions to nasal CDA provocation [12]. Also, we have reported that 24 h after an allergen provocation, the reactivity of the nasal mucosa to CDA is increased [20]. Braat et al. [13] have found CDA to be a better tool than histamine in differentiating subjects with non-AR from controls. These findings indicate that nasal CDA challenge could be considered a tool to measure nasal hyper-responsiveness. However, nasal provocations with other agents such as methacholine, histamine, or bradykinin also need to be performed under the same experimental settings to test whether our observation reflects a global hyper-responsiveness state of the nasal mucosa in individuals with asthma, compared with those with rhinitis alone. This is because, unlike other stimuli, the effect of CDA in causing release of histamine, and in other studies, tryptase [21] may reflect a more specific mast cell response caused by (a) increased mast cell releasability, (b) increased mast cell numbers in the nasal mucosa of patients with rhinitis and asthma or (c) decreased homeostatic ability of the mucosa which allows CDA to induce a state of hyper-osmolarity leading to mast cell degranulation [11]. In the context of the latter possibility, work by Assanassen et al. [22] has demonstrated that the nasal mucosa of asthmatics has decreased ability to humidify inhaled air, compared with that of subjects with perennial rhinitis alone. Given that CDA is also known to cause lower airway reactions with high specificity for asthma [23], it is possible that the upper and lower airways of asthmatics have reduced ability to condition inhaled air leading to increased susceptibility to CDA. We have previously found that the osmolarity of nasal secretions increases from baseline during nasal breathing of CDA only in subjects who develop nasal symptomatology to this stimulus [11].

Our findings have both pathophysiologic and clinical significance. The data subscribe to the notion that has gained support among investigators in the field, that asthma represents a ‘total airway disease’ [24–26]. They are also supportive of the more general model that we have put forward [1] according to which, within the context of a chronic inflammatory airway syndrome, the presence of rhinitis and asthma indicates the upper end of the syndrome’s severity spectrum, whereas the presence of rhinitis alone indicates the lower end. As, according to the model, the syndrome affects the entire airway mucosa, it is projected that, in parallel with the lower airways, the nose of asthmatics will have more functional abnormalities compared with the nose of individuals who only have rhinitis. The clinical significance of our findings is that the rhinitis of patients with asthma is an important entity that requires the attention of clinicians as it may be, in general, of worse nature than that of patients who do not have asthma.
References

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