Epithelial shedding is associated with nasal reactions to cold, dry air

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Background: Cold, dry air (CDA) can cause symptoms of rhinitis and obstructive airway responses. The pathophysiology of these reactions is not understood. One hypothesis is that the respiratory mucosa of individuals with CDA sensitivity cannot compensate for the loss of water that occurs on exposure to the stimulus, leading to epithelial damage.
Objective: To test for an association between nasal reactions to CDA and the number of epithelial cells recovered in nasal fluids.
Methods: Ten CDA-sensitive subjects received nasal provocations with CDA and warm, moist air; 10 CDA-insensitive subjects received CDA; and 10 subjects with allergic rhinitis received allergen and diluent challenges. Nasal lavage cytology was performed at baseline and after the challenge. Symptoms were recorded and histamine, [^{3}H]-N-α-tosyl-L-arginine methyl ester-esterase activity, tryptase, and albumin were assayed in nasal lavages.
Results: A 6-fold increase in nasal lavage epithelial cells was found in the CDA-sensitive group after CDA ($P < .01$), but not after warm, moist air. No changes were observed in the CDA-insensitive group, or after allergen or diluent in allergic rhinitis.
Conclusion: Epithelial cell shedding accompanies clinical responses to CDA in the human nose. This supports the hypothesis that the airway mucosa of CDA-sensitive individuals cannot compensate for the water loss that occurs under extreme conditions leading to epithelial damage.
Clinical implications: A defect in mucosal water homeostasis may need to be considered in individuals who get excessive nasal symptoms when exposed to cold and dry, windy environment. (J Allergy Clin Immunol 2006;117:1351-8.)

Key words: *Hyperosmolarity, hypertonicity, nasal allergen provocation, nasal lavage, nasal challenge*

Nasal sensitivity to cold, dry air (CDA) manifests with symptoms of rhinitis including profuse rhinorrhea and nasal congestion.[1] These symptoms can be reproduced in the laboratory with CDA provocation.[2] Some individuals are exquisitely sensitive to this stimulus and, as a group, patients with nonallergic rhinitis react to CDA more vigorously than healthy controls.[3] CDA sensitivity is also prominent in skiers, probably because, when descending slopes at high speed, they get exposed to a stimulus of high magnitude.[4] Particular interest in the nasal reaction to CDA exists because understanding of its pathophysiology may offer insights into the mechanisms of lower airway reactions to the same stimulus that are quite prominent in asthma.

Early on in the development of the nasal CDA provocation model, it became clear that the best predictor of a nasal reaction to CDA in the laboratory is a clinical history of sensitivity to the natural stimulus. Individuals with severe nasal symptoms in cold, windy weather develop symptoms with experimental provocation and their nasal secretions after exposure to CDA contain increased levels of histamine, sulfidopeptide leukotrienes, and tryptase,[5,6] suggestive of mast cell activation. Also, sensory nerves are involved in the reaction, because provocation through 1 nostril results in bilateral responses.[7] In contrast, individuals who deny nasal symptoms when exposed to cold, windy weather have no clinical response to CDA provocation and show no evidence of mast cell mediator release or neuronal activation.

The mechanistic basis of the difference between CDA-sensitive and insensitive individuals is unknown. We have previously demonstrated that neither the presence of atopy nor nasal responsiveness to histamine predicts CDA responsiveness.[8] In a limited number of experiments, we have found that the osmolarity of the epithelial lining fluid is increased after CDA provocation in the CDA-sensitive

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Abbreviations used
CDA: Cold, dry air
WMA: Warm, moist air

but not in the insensitive group.[9] Also, when both groups undergo nasal challenge with a hyperosmolar solution, CDA-sensitive subjects release significantly more histamine in nasal lavage fluids compared with CDA-insensitive subjects.[8] These observations led us to the general hypothesis that the underlying difference between CDA-sensitive and insensitive individuals relates to the ability of the mucosa to cope with conditions that demand increased water supply to inhaled air or to the epithelial surface, whether inhalation of dry air or application of a hyperosmolar stimulus. If a defect in compensating for water loss exists in the CDA-sensitive individuals, breathing CDA may lead to hypertonicity of the epithelial layer and possibly of the superficial submucosal tissue, resulting in sensory nerve stimulation[10,11] and mast cell activation.[12,13] At the same time, the epithelium can be subject to damage from desiccation and detachment. If, on the other hand, water supply to the epithelial surface under stressful conditions is ample, none of these phenomena should take place, and the subject undergoing CDA provocation should have no reaction to the stimulus.

We conducted this study to test part of this hypothesis, whether epithelial shedding takes place after CDA nasal challenge and whether this is more prominent in the CDA-sensitive group. Because of the possibility that epithelial shedding may not necessarily be the result of desiccation but of an acute toxic effect of inflammatory mediators on epithelial cells, we added a control group in this study, a group of individuals with allergic rhinitis who received nasal challenges with allergen or its vehicle. Our rationale for this control was that, because the pattern of inflammatory mediator release that is observed in nasal lavage fluids after allergen and CDA challenges is similar,[2,6,14] if these products were the cause of epithelial shedding, epithelial cells in nasal lavage fluids would be found in similar numbers after CDA and allergen challenges.

METHODS

Subjects

We studied 3 groups, 10 volunteers each, ages 20 to 46 years. CDA-sensitive subjects reported rhinorrhea with cold and windy weather and had a positive reaction to a previous CDA challenge.[2] Six of them also had allergic rhinitis. CDA-insensitive subjects reported no symptoms in cold weather and had a previous negative CDA provocation. Four had allergic rhinitis. Volunteers with allergic rhinitis had positive skin tests to grass or ragweed and were tested when asymptomatic, outside pollen seasons. Two of the subjects in the CDA-sensitive group and 3 in the CDA-insensitive group also participated in the allergic rhinitis group. All subjects gave informed consent, and the study was approved by the Johns Hopkins Joint Committee on Clinical Investigation.

Nasal challenges

Fig 1 describes the various provocation protocols in which each group of study subjects participated. CDA-sensitive and CDA-insensitive subjects received a challenge with CDA. The CDA-sensitive group was also challenged with warm, moist air (WMA) to control for the mechanical effects of breathing air through the nose. The allergic rhinitis group received 1 provocation with allergen and 1 with diluent. CDA and WMA provocations were performed as previously described.[2,5,9] Allergen challenges were performed with short ragweed or a mixture of grasses, also with a previously described methodology.[15] Fifty protein nitrogen units of the respective allergen extract were sprayed into each nostril. Within each group, challenges were performed in random order. The minimum period between challenges after WMA or diluent was 48 hours, and after CDA or allergen, 5 days.

Nasal lavages and lavage outcomes evaluations

Nasal lavages were also performed as previously described.[2,14] In each protocol, evaluations of the returned lavage fluids were performed on 4 occasions, PRE A, PRE B, BASELINE, and POST challenge lavages (Fig 1). A set of preparatory (PRE) lavages was first performed to clear pre-existing cells and mediators. Baseline lavages were performed 1 hour before nasal challenge, so that ample time was given for the hydration state of the mucosa to return to its prelavage condition. The time interval between baseline and postchallenge lavages was equal to the interval between the preparatory and baseline lavages (Fig 1). This was meant to control for outcome changes after the respective provocation, reflecting a spontaneous process. On each lavage occasion, we used a pair of lavages, the first with 5 and the second with 10 mL lactated Ringer's warmed to body temperature and divided into the 2 nostrils. Fluids from the 5-mL lavages were assayed for mediators and biologic markers. Measurements of histamine,[16] [^{3}H]-N-α-tosyl-L-arginine methyl ester (TAME)-esterase activity,[17,18] tryptase,[6] and albumin[19] were made by using established assays. The cell pellets from each lavage pair were combined, total cell numbers were obtained with the use of a hemocytometer, and cytospin slides (Shandon, Sewickley, Pa) for differentials were generated. Differential counts were performed after staining with Diff-Quick (American Scientific, McGaw Park, Ill).[20,21] Squamous, basal, and columnar epithelial cells were counted on the stained slides as a single cellular category. Cell differentials were conducted on coded slides.

Symptom scores

Rhinorrhea and nasal obstruction were self-evaluated on 10-cm-long visual analogue scales marked at each end with "no symptoms" and "the worst it has ever been."[22] Evaluations took place at the beginning of each protocol (PRE A) and at the time points when the PRE B, BASELINE, and POST lavage sessions were performed (Fig 1).

Data analysis

The primary outcome of this study was the absolute number of epithelial cells in nasal lavage fluids. However, because the volume of returned lavage fluids in the CDA-sensitive subjects was higher after the CDA challenge compared with the other time points, we decided also to examine the number of epithelial cells per returned lavage volume unit (mL). Because the data distribution was not normal, nonparametric statistics were used, and the results are presented as median values with interquartile ranges. Friedman ANOVA was conducted within each protocol to examine the effect of CDA or allergen in comparison with prechallenge values or to values obtained after the respective negative controls. Post hoc analysis