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Experimental common cold increases mucosal output of eotaxin in atopic individuals

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Key words: airway; allergy; eotaxin; infection; inflammation.

Background: In view of recent observations demonstrating that rhinovirus infections are associated with increased local activity of eosinophils, we hypothesized that eotaxin, a selective eosinophil chemoattractant, may be involved in eosinophil recruitment/activation in common cold infections.

Methods: Twenty-three patients with seasonal allergic rhinitis were inoculated with human rhinovirus 16 (HRV 16) outside the pollen season. Sequential diluent and allergen (10^3 SQ-U) challenges, each followed by nasal lavage, were carried out about 3 months before and 4 days after virus inoculation.

Results: Seventeen patients developed common cold and were further analyzed. Lavage fluid levels of eotaxin were increased at the height of active common cold (day 4 after inoculation) both at baseline (after diluent challenge) ($P < 0.01$) and acutely after allergen challenge ($P < 0.05$).

Conclusions: Common cold symptoms after nasal HRV 16 inoculation are associated with increased nasal mucosal output of eotaxin, suggesting that this chemokine is partly responsible for recruitment/activation of airway mucosal eosinophils in common cold infections.

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We recently observed that experimental common cold conditions were associated with much increased mucosal output of eosinophil cationic protein (ECP). This sign of viral inoculation-induced nasal eosinophil activity was evident in both allergic (1) and nonallergic (2) individuals. Many mechanisms may be involved in the dynamic interplay between virally evoked airway inflammation and inducement and/or aggravation of eosinophilic conditions. Pluripotent cytokines, such as interleukin-8 (IL-8) and interferon- γ (IFN- γ), and plasma-derived proteins with

potential eosinophil attraction and activation effects (3–5), are increased in the common cold (1, 6, 7). Moreover, at the height of human rhinovirus 16 (HRV 16) inoculation-evoked common cold symptoms, a significant correlation between nasal levels of IL-8 and ECP has been recorded (1, 2). Although IL-8 may contribute to the nasal eosinophilia that occurs in common colds, the possible involvement of other more potent and specific eosinophil regulator mechanisms should be explored.

The possibility that eosinophilia is caused by cytokine-mediated inhibition of eosinophil apoptosis is of interest (8). However, the cytokines involved, e.g., granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-5 (IL-5), are induced in allergy rather than in viral infection (6, 9). Furthermore, since our interest is focused on *in vivo* events, it must also be noted that apoptotic eosinophils have not been clearly detected in diseased airway tissues *in vivo* (10).

Eotaxin is a recently discovered chemokine with potent eosinophil attraction and activation properties (11–15). Differing from other chemokines, including IL-8, eotaxin selectively increased chemotaxis *in vitro* of eosinophils, but not of other leukocytes (13). Eotaxin may regulate physiological homing of eosinophils to the airway mucosa (16), and it may be involved in the pathogenesis of rhinitis and asthma. In allergic airway disease conditions, local eotaxin levels are elevated (17–19), and airway eotaxin levels may be reduced by topical steroid treatment (19). We hypothesized that rhinovirus inoculation-induced experimental disease of the human upper respiratory tract might affect the local eotaxin levels. As an extension of a previous report (1) involving subjects with seasonal allergic rhinitis, the present study demonstrates increased nasal lavage fluid levels of eotaxin in subjects developing symptomatic common cold after inoculation with HRV 16. Additionally, the present study explores the relationship between nasal lavage fluid levels of eotaxin and ECP.

Material and methods

Study design and patients

Nasal inoculation with HRV 16 was performed out of season in 23 patients with seasonal allergic rhinitis. Inclusion criteria were a history of seasonal allergic rhinitis (verified by skin prick test to birch and/or timothy pollen allergen), no history of chronic airway disease, no infectious nasal disease for the 2 months preceding each allergen challenge

series, a negative skin prick test to perennial allergen, and no history of smoking. Exclusion criteria were nasal polyps, septal deviation of clinical significance, known impairment of liver and kidney function, pregnancy or nursing, drug treatment, and current desensitization therapy. Sequential nasal diluent and allergen challenges were carried out before and on day 4 after inoculation, and were followed by nasal lavages. Nasal lavage fluid levels of eotaxin were measured. (Clinical and pathophysiological observations from the present study have been reported elsewhere [1].)

Nasal rhinovirus inoculation

HRV 16 (10^5 – 10^6 TCID₅₀ per ml) was diluted 1:20 in phosphate-buffered saline at 4°C to a concentration of 5×10^3 – 5×10^4 TCID₅₀ per ml. With the subjects in a supine and extended neck position, 0.25 ml of this solution was administered to each nasal cavity (study day 0). A further 100 µl of the solution was administered to each nasal cavity with a spray device.

Determination of infection

In previous virus inoculation studies (7, 20), we have noticed that seropositive, infected subjects may be without symptoms. The present study focused on common cold with clear symptoms. Hence, subjects with and without common cold were distinguished exclusively by symptoms, which were scored and entered into diaries by the subjects before and once daily for 5 days after inoculation. Nasal blockage, sneezes, rhinorrhea, muscle ache, fever, headache, and sore throat were each scored on a four-point scale, i.e., 0 for no symptoms, 1 for mild symptoms, 2 for moderate symptoms, and 3 for severe symptoms. The criteria for the development of common cold was a total diary score of more than 12 points and a convincing progress of the diary score from days 2 to 5 after inoculation; i.e., a 4-point increase for at least 2 consecutive days. The score is a modification of that suggested by Beare & Reed (21). Furthermore, as an adjunct, the subjects were asked about their own opinion of whether they had developed a cold or not. The classification of the subjects, in terms of development of common cold, was carried out after the clinical part of the study was finalized, but before the nasal lavage fluid levels of eotaxin were analyzed.

Nasal allergen challenges and lavages

Approximately 3 months before and at day 4 after inoculation, nasal challenges with diluent and allergen (10^3 SQ-U [standard quantity units]) (Aquagen[®], ALK, Denmark) were carried out. Two 30-s isotonic saline lavages were carried out before the actual challenge and lavage series in order to create a consistent baseline (removing gross material that may have accumulated on the nasal mucosa for an unknown period of time). Nasal lavages were carried out after each diluent and allergen challenge with a nasal pool-device containing 15 ml isotonic saline (22). The lavage fluids were kept in the nasal cavity for 10 min. The recovered lavage fluid was centrifuged (105 g, 10 min, 4°C), and aliquots were prepared from the supernatants and frozen (-30°C) for later analysis.

Analysis

The nasal lavage fluid levels of eotaxin were measured with a commercially available ELISA (R&D Systems, UK). The detection level of the assay is <5 pg/ml.

Statistics

Differences in nasal lavage fluid levels of eotaxin between observations before and after nasal virus inoculation were examined with the Wilcoxon signed rank test. $P < 0.05$ was considered significant. Data are presented as means $\pm$ SEM.

Results

Seventeen patients developed significant common cold symptoms. The individual nasal symptoms and the grouping of the present subjects have been published previously (1).

The levels of eotaxin in nasal lavage fluids obtained after diluent challenge in these infected patients suffering from clinical common colds were increased ($P < 0.01$, cf before inoculation) (Fig. 1). The allergen challenge was also associated with greater lavage fluid levels of eotaxin after inoculation ($P < 0.05$, cf before inoculation) (Fig. 1). However, the magnitude of the eotaxin increase after allergen challenge was not significantly different from that seen after diluent challenge (Fig. 1). No statistically significant correlations were observed between cumulative nasal symptoms (1) and levels of eotaxin in nasal lavages

obtained after diluent ($r_s = 0.31$) and allergen ($r_s = 0.31$) challenge after virus inoculation.

The nasal lavage fluid levels of ECP have been published previously (1). A weak but statistically significant correlation was observed between nasal lavage fluid levels of ECP and the present eotaxin levels after allergen challenge before virus inoculation ($r_s = 0.62$, $P < 0.05$). No statistically significant correlations were observed after diluent challenge before inoculation ($r_s = 0.07$), after diluent challenge after inoculation ($r_s = 0.26$), or after allergen challenge after inoculation ($r_s = 0.32$).

Discussion

The present study, involving atopic subjects without active allergic disease (seasonal allergic rhinitis out of season), has demonstrated that common cold symptoms produced by experimental HRV 16 inoculation are associated with increased mucosal output of eotaxin. The present observation suggests a mechanism by which rhinovirus infections may provoke eosinophilic airway inflammation.

The nasal pool-technique employed in this study effectively but atraumatically lavages almost the entire nasal mucosal surface area (22). Without evoking acute luminal entry of molecules, as occurs during plasma exudation responses, the present diluent challenge experiments demonstrated that viral infection was associated with a more than twofold increase in mucosal output of eotaxin, compared to the corresponding preinfection baseline data. The achieved levels of eotaxin in common cold, of the order of 20 pg/ml, may be compared to those observed in

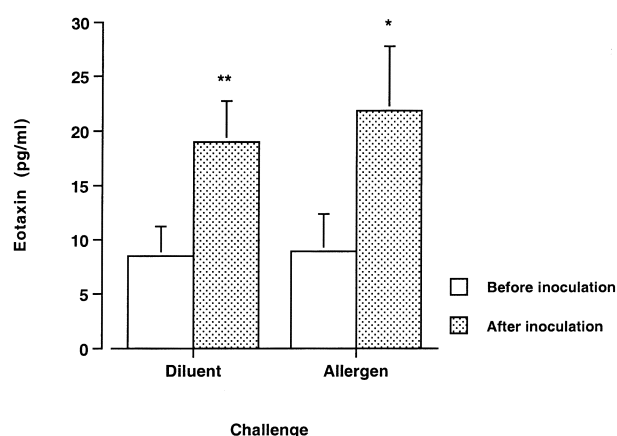


Figure 1. Levels of eotaxin in nasal lavages obtained after diluent (baseline) and allergen challenge before and 4 days after nasal HRV 16 inoculation in subjects that developed common cold symptoms.

* $P < 0.05$; ** $P < 0.01$.

bronchoalveolar lavage fluids obtained in a preliminary study involving seven asthmatic individuals (18). The volume that was retrieved after administration of 50 ml fluid into the right middle lobe was concentrated 10 times; with a sandwich ELISA method of assay, eotaxin levels of 400–800 pg/ml were then determined in asthma (18). Whether common cold infections evoke increased eotaxin levels also in the lower airways is a matter for future studies. This possibility warrants investigation since HRV 16-induced infections commonly provoke episodes of asthma (23), and since it may be hypothesized that eotaxin's ability to recruit and activate eosinophils (11–15) may be involved in the eosinophil activation that is known to occur in HRV 16-inoculation-evoked common cold (1, 2). This is also of interest because eotaxin may selectively recruit Th₂ cells, a possible contributory mechanism in inducing the wheezy state (24).

Repeated allergen exposures may increase mucosal output of eotaxin (19). Eotaxin levels did not differ between diluent and allergen challenges, suggesting that, in this acute challenge study, it was only the common cold infection that induced the increased eotaxin levels. It is possible, however, that an acute plasma exudation response, evoked within minutes by the present mode of allergen challenge in

patients with seasonal allergic rhinitis (1), could have brought eotaxin molecules from mucosal interstitial spaces to the mucosal surface. Indeed, acute plasma exudation responses have previously been observed to bring mucosal cytokines and ECP into the airway lumen (1). The poor effect of allergen challenge on eotaxin levels in this study may thus suggest that freely movable eotaxin molecules may not abound in the airway mucosal tissue in the present patients, irrespective of the common cold status.

Rhinovirus infections are a major health problem as well as a major risk factor for exacerbation of serious and common eosinophil airway disease conditions. The present observation of increased eotaxin levels identifies a potentially important link between common cold and eosinophilic inflammation and may further support the notion that eotaxin generation and actions should be considered potential targets for therapeutic interventions.

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