

Hot, humid air increases cellular influx during the late-phase response to nasal challenge with antigen

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Summary

Background Inhalation of hot, humid air (HHA: 37 °C, > 95% relative humidity (RH)) partially inhibits the early response to nasal challenge with antigen.

Objective To investigate whether HHA inhibited the late-phase response to nasal challenge with antigen and increased hyper-responsiveness of the nasal mucosa to histamine.

Methods Twenty subjects with seasonal allergic rhinitis, outside of their allergy season, participated in a randomized, 2-way cross-over study. The subjects continuously breathed room air (25 °C, 30% RH) or HHA delivered via a face mask during the entire experiment. Subjects were challenged intranasally with antigen 1 h after beginning conditioning. The response was monitored by symptoms and nasal lavage at 2-h intervals after the last antigen challenge. Eight hours after antigen challenge, nasal challenge with histamine was performed.

Results Exposure to HHA significantly increased nasal mucosal temperature from baseline without affecting nasal secretion osmolality. HHA significantly inhibited antigen-induced sneezes, congestion, pruritus, and human serum albumin levels during the early response to antigen challenge. HHA exposure, however, was associated with an 8-fold increase in the eosinophil influx and a 15-fold increase in the levels of eosinophil cationic protein during the late-phase response compared to room air. There were no significant differences in nasal hyper-responsiveness to histamine during either exposure.

Conclusion HHA partially decreases the early response to nasal challenge with antigen, but dramatically increases eosinophil influx. Increasing eosinophil number had no effects on the hyper-responsiveness to histamine. We speculate that the physical conditions of air differentially impact the stages of allergic inflammation.

Keywords: humidity, heat, nasal challenge, late-phase response, hyper-responsiveness, allergic rhinitis

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Introduction

Antigen challenge of the nose provokes a biphasic clinical reaction in some allergic subjects [1,2]. The early-phase response (EPR) follows within minutes of antigen challenge and is characterized by congestion, rhinorrhea and

sneezing [3]. These symptoms resolve spontaneously and may return between 3 and 10 h after the antigen challenge. A recrudescence of rhinitis symptoms, the late-phase response (LPR), is characterized by cellular infiltration into the nasal mucosa and mediator release. The nose also becomes more sensitive to non-specific stimuli and allergen hours after initial antigen exposure [4].

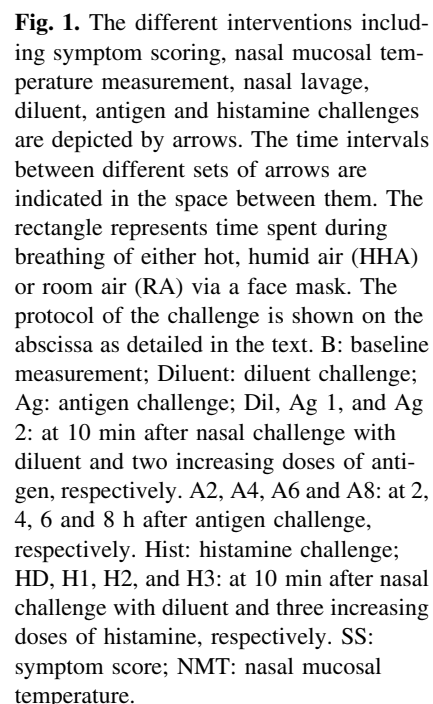
Previous studies have suggested a beneficial effect of supersaturated hot air (42–44 °C, > 95% relative humidity (RH)) on nasal symptomatology in patients with allergic

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All of the above studies investigated the effect of HHA on the EPR to nasal challenge with antigen. In this study, we investigated the effect of HHA on the LPR to antigen challenge and to antigen-induced nasal hyper-responsiveness to histamine. We hypothesized that preconditioning the nasal mucosa of allergic subjects with HHA would inhibit EPR and LPR to antigen challenge and the hyper-responsiveness of the nasal mucosa to histamine.

Subjects

Twenty volunteers with a history of seasonal allergic rhinitis were recruited (15 men and 5 women; ages 19–41 years; mean age, 26 years). Their allergic status was confirmed by a positive skin puncture test to either ragweed or timothy grass, and by a positive nasal challenge with antigen, defined by sneezing two or more times and by a twofold or greater increase in the weight of generated nasal secretions and albumin levels compared to the diluent challenge (0.4% phenol buffered saline). In addition, all subjects had a positive LPR as defined by a twofold or greater increase in the number of eosinophils in nasal secretions 24 h after antigen challenge compared with baseline. Cellular infiltration is the most common characteristic of an LPR. They also reacted to a histamine challenge, which served as a baseline. All subjects were studied out of season and did not take any medications within 2 weeks of the evaluation. Other than allergic rhinitis, the patients had no other



medical problems. The study was approved by the Institutional Review Board of the University of Chicago, and written informed consent was obtained from each subject prior to study entry.

Experimental protocol

We performed a randomized, 2-way cross-over study comparing the effects of preconditioning the nasal mucosa with HHA (37 °C, > 95% RH) or RA (25 °C, 30% RH) delivered via a face mask on the EPR and LPR to nasal challenge with antigen and antigen-induced hyper-responsiveness to histamine. On the day of challenge, the subjects came to the laboratory and were allowed to rest for 15 min so that equilibration of the nasal mucosa with the environmental conditions of the laboratory was achieved (25 °C, 30% RH). They were randomly assigned to breathe either HHA or RA for 1 h prior to antigen challenge, during antigen challenge, and for 8 h after, including during histamine challenge. At least 2 weeks separated the challenges so that an effect of one challenge on the other was avoided. Analysis of the data to be presented showed no evidence of carryover or period effects. The hypothesis of the study was not presented to the volunteers.

The protocol began with a first series of baseline measurements (Baseline 1: B1) (Fig. 1). Symptoms of nasal congestion, itching and rhinorrhea were recorded by the subjects. The number of sneezes was counted and recorded by the investigator. The nasal mucosal surface temperature was obtained (see below). After that, baseline nasal lavage was performed. Four nasal lavages were then performed to bring mediator levels to a stable baseline. Subjects were then instructed to breathe either HHA or RA for a 1-h conditioning period by inhaling and exhaling through the nose. At the end of the conditioning phase, a second series of baseline measurements, which included symptom evaluation, nasal mucosal temperature measurement and nasal lavage, was made (Baseline 2: B2). After subjects blew their noses to clear any accumulated secretions, antigen challenge was performed followed by a histamine challenge 8 h later.

To investigate whether HHA affected the number of cells in nasal lavage fluid without nasal challenge with antigen, we performed another randomized, 2-way cross-over study in 3 asymptomatic subjects with seasonal allergic rhinitis, all of whom were volunteers who had participated in the present study, and were randomly selected. The numbers of total cells after antigen challenge in these 3 subjects were higher than the median values of the whole group. They continuously breathed HHA or RA in the same way as the present study, except that there were no antigen or histamine challenges during the experiment. Nasal mucosal temperature measurement and nasal lavage were performed

before and 1 h after beginning conditioning and at 2-h intervals after that for a total of 8 h.

Antigen challenge

Antigen challenge was begun with the spraying of 2 puffs (0.15 mL) of 0.05% oxymetazoline hydrochloride (Nostrilla, Ciba Self-Medication, Inc., Woodbridge, NJ, USA) into each nostril. This topical decongestant prevents mucosal congestion that interferes with nasal lavage. Prior experiments have shown that oxymetazoline does not interfere with sneezing or the release of inflammatory mediators during the EPR [3,13]. The effect on the LPR is not fully known. As discussed above, oxymetazoline without antigen does not cause cellular infiltration. Systemic corticosteroids have been shown to reduce eosinophil infiltration in this model [17]. After a 5-minute waiting period, 2 puffs of diluent for the antigen extracts (0.4% phenol buffered saline, Bayer Corporation, Spokane, WA, USA) were sprayed into each nostril to control for non-specific reactivity of the nasal mucosa. After 10 min, symptoms were recorded by the subjects, and the number of sneezes during the past 10 min was recorded by the investigator. A nasal lavage was then performed (Dil). The subjects were then asked to blow their noses to clear any accumulated secretions before starting the next challenge. Two consecutive antigen challenges were performed, 10 min apart, by spraying 2 puffs of either ragweed or timothy grass extracts (Bayer Corporation, Elkhart, IN, USA) at two concentrations (1 : 2000 and 1 : 200 W/V) into each nostril. The same extracts were used throughout the study. Weight per volume (W/V) describes the weight of allergenic source material added to a given volume of extracting fluid. A 1 : 10 extract indicates that the solution contains the extractable material from 1 g of raw material added to each 10 mL glycerol-coca extracting fluid. Symptom score evaluation and nasal lavage were performed 10 min after each challenge (Ag 1 and Ag 2). After that, symptom score evaluation, nasal mucosal temperature measurement and nasal lavage were performed at 2-h intervals for 8 h (A2–A8).

Histamine challenge

Histamine challenge was performed 8 h after the last antigen challenge for assessment of nasal hyper-responsiveness. No decongestant was applied because the congestion following a histamine challenge is much less than that following an antigen challenge. After the last nasal lavage (A8), 2 puffs of diluent solution (0.9% normal saline, Baxter Healthcare Corporation, Deerfield, IL, USA) were sprayed into one nostril to control for non-specific hyper-reactivity of the nasal mucosa. Symptom score

evaluation and nasal lavage were performed 10 min later (histamine diluent: HD). Next, three consecutive histamine challenges were performed, 10 min apart, by spraying 2 puffs (0.15 mL) of 0.23 mg/mL, 0.7 mg/mL and 2.2 mg/mL concentrations of histamine diphosphate (Sigma, St Louis, MO, USA) into the same nostril, in which diluent challenge was performed, for delivered quantities of 0.03 mg, 0.1 mg, and 0.3 mg of histamine, respectively. Symptom score evaluation and nasal lavage were performed 10 min after each challenge (H1–H3).

Sneezes and symptom scores

The number of sneezes was counted and recorded by the investigator performing the challenges. Symptoms of runny and stuffy nose for each nostril and a combined sensation of itchy nose and throat were graded on a scale as follows: 0 = no symptoms, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe, and 5 = very severe symptoms. For rhinorrhea and congestion scores, the combined score of the left and right nostril is reported.

Nasal mucosal temperature measurement

A nasal probe was used for measurement of mucosal surface temperature [14] and was calibrated before each use. It was inserted into the anterior nasal cavity using a nasal speculum and a headlight. The sensor was placed in contact with the anterior septal mucosa, posterior to the mucocutaneous junction. The sensor sampled the mucosal temperature for 30 s, and the mean value was used for analysis. Preliminary experiments showed that, after removal of the mask delivering HHA, the nasal mucosal temperature remains unchanged for approximately 3 min (data not shown). The same side of the nasal cavity was used for the temperature measurements throughout the experiment.

Nasal lavage and cell collection

Lavages were performed with 2.5 mL of 37 °C lactated Ringer's solution (Baxter Healthcare Corporation, Deerfield, IL, USA) in each nostril as previously described [3]. The volume of the lavage fluid was recorded, the sample vigorously shaken, and the total number of cells counted with a haemocytometer. The lavage solution was then placed on ice until centrifugation at 400 g for 15 min at 4 °C. The cell pellet was resuspended in enough lactated Ringer's solution to yield a concentration of 100 000 cells per 200 µL. Two hundred microlitres of the suspension were then cytospun onto labelled slides, which were dried, fixed and stained with a Diff-Quick® Stain Set (Dade Behring Inc., Newark, DE, USA). The percentage of

eosinophils, neutrophils and mononuclear cells in 200 cells was counted, and the total number of each cell type was calculated by multiplication of those percentages by the total cell count. If the total number of countable cells was more than 50, but one cell type was not present, the number of that cell type was arbitrarily assigned to be 500. The supernatant was stored at –20 °C until assayed for human serum albumin (HSA) and eosinophil cationic protein (ECP).

Air administration

Air from compressed air tanks (Gas Tech, Inc., Hillside, IL, USA) was delivered through a large tracheal collar placed underneath the chin at a flow rate of 10 L per minute. For HHA, the compressed air was warmed and moisturized by passage through coiled tubing immersed in an Isotemp® water bath (Fisher Scientific, Pittsburgh, PA, USA) and then in a bottle containing distilled water. The temperature of the water bath and the distilled water was adjusted to allow the delivery of air at the mask at 37 °C and more than 95% RH. It was checked by a digital temperature and humidity probe of a digital hygrometer thermometer (Fisher Scientific) placed at the mask. For RA, the compressed air was also modified for the delivery of air at the mask at 25 °C and 30% RH. After preconditioning, the subjects intermittently removed the mask to accommodate the challenge, nasal mucosal temperature measurement and nasal lavage procedure. They then reapplied it and kept breathing the modified air until the end of the experiment.

Nasal secretion osmolality measurement

Osmolality values were measured in the returned lavage fluids (B1, B2, A3–A6) with a Vapro® vapor pressure osmometer (Wescor, Inc., Logan, UT, USA). This instrument provides an accuracy of ± 3 mmol/kg-H₂O. The returned nasal lavage fluid was well homogenized by vigorous shaking. Ten microlitres were then transferred to a filter paper disc. All measurements were performed in triplicate. The average values are reported. This technique has been used previously to demonstrate an increase in osmolality after exposure to cold, dry air [15].

Mediator assays

Samples obtained from the same subject on each visit were always measured in the same assay, so that interassay variability was eliminated. HSA was measured by an enzyme-linked immunosorbent assay (ELISA) sensitive to 1 ng/mL of albumin [16]. ECP was assayed by means of a radioimmunoassay technique (Pharmacia ECP RIA,

Pharmacia & Upjohn Diagnostics, Kalamazoo, MI, USA) with a sensitivity of 2 µg/L. Any sample that had a concentration lower than these sensitivities was arbitrarily assigned a value of 0.5 µg/mL for albumin and 1 µg/L for ECP.

Statistical analysis

Because the data were not normally distributed, non-parametric statistics were used for analysis. The effect of different conditions on baseline nasal parameters was examined by the calculation of net changes in nasal mucosal temperature and nasal secretion osmolality, which were calculated by the subtraction of values obtained after 1-h conditioning in the mask from those obtained before. Net changes in other parameters after antigen challenge under the two different conditions (EPR and LPR evaluation) and those after histamine challenge were

calculated by subtracting values obtained after diluent challenge from those after both antigen challenges, or those after three histamine challenges (net change over diluent), and obtaining the sum of these values. The net changes in the influx of neutrophils, eosinophils and mononuclear cells were calculated by subtracting the number of each cell type obtained after 1-h conditioning in the mask from that obtained from 2–8 h after antigen challenge and obtaining the sum of these values. The values of each parameter at all time-points after each exposure were first analysed by Friedman analysis of variance (ANOVA). If a significant difference was found, post-hoc analysis between the two selected values was performed with the Wilcoxon signed rank test. To compare the differences in parameters between both exposures, we compared net changes for each parameter after exposure to each of the two conditions by using the Wilcoxon signed rank test. Correlations were performed by the Spearman Rank method. A *P*-value

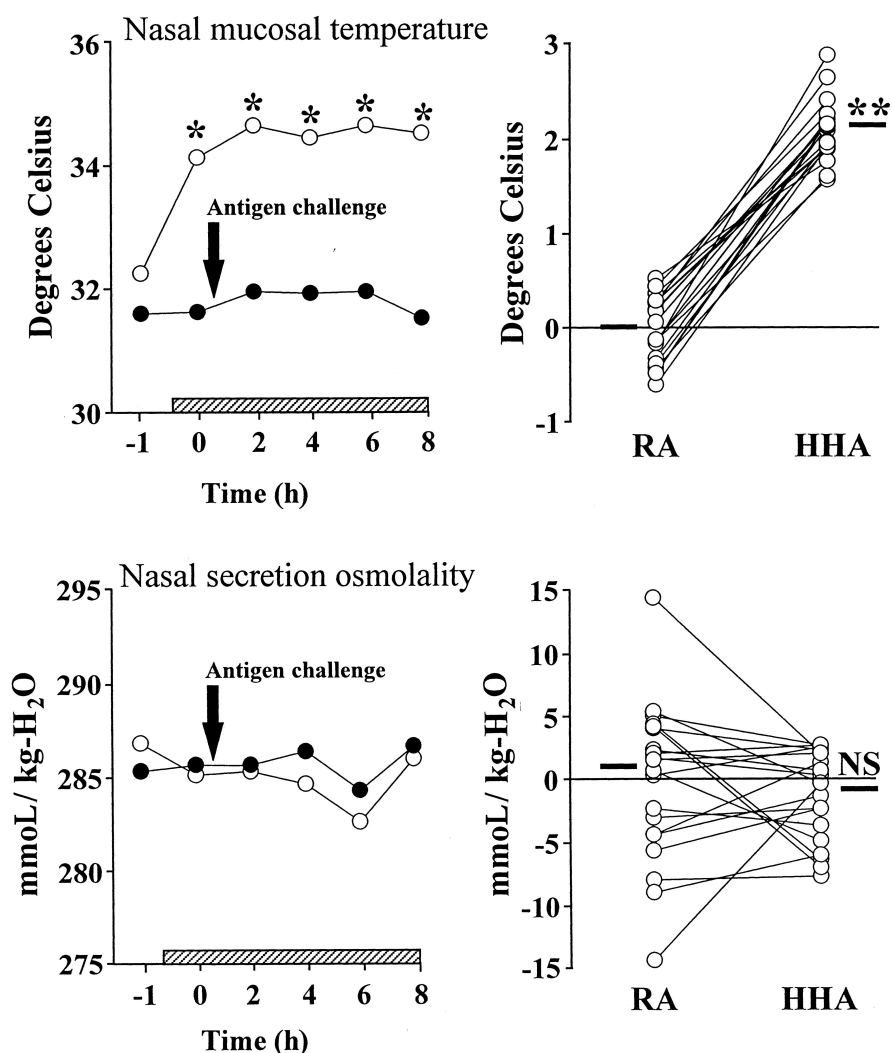


Fig. 2. Nasal mucosal temperature (top) and nasal secretion osmolality (bottom). Left: Values at each of the individual time-points after exposure to hot, humid air (open circles) or room air (filled circles). The time during the experiment is shown on the abscissa. Minus 1 represents the first baseline measurement; zero represents the second baseline measurement obtained after 1 h of inhalation of either type of air followed by measurements at 2-h intervals after the last antigen challenge for a total of 8 h. Data are median for 20 subjects. The shading above the abscissa represents the timing of air administration. Right: Individual data for the net change from baseline after exposure to two different types of air specified on the abscissa. The solid bars represent median values. HHA: hot, humid air, RA: room air. **P* < 0.001; ***P* < 0.0001 compared with RA, NS: not significant.

(two-tailed) < 0.05 was considered to indicate significance. The data are presented as medians, with the 25th–75th percentiles in parentheses.

Results

Inhalation of HHA led to a significant increase in nasal mucosal temperature over baseline without a significant change in the osmolality of the recovered nasal lavage fluid before and 1 h after conditioning, and these persisted throughout the experiment (Fig. 2).

We compared the effect of HHA and RA on the early- and late-phase response to antigen challenge, and on the response to histamine challenge. All of these parameters at baseline were unaffected by the inhalation of either type of air, except for the nasal mucosal temperature. Likewise, there were no significant changes from baseline after diluent challenge. During the EPR, nasal challenges with increasing doses of antigen were accompanied by significant increases in all parameters compared with diluent challenge during both exposures, except for the nasal mucosal temperature and nasal secretion osmolality. HHA

had no effect on allergen-induced rhinorrhea ($P = \text{NS}$), but led to significant reductions in allergen-induced sneezing ($P = 0.02$), pruritus ($P = 0.05$), congestion ($P = 0.01$), and HSA levels ($P = 0.04$) compared to RA (Table 1).

During the LPR, antigen challenge led to significant increases in the number of eosinophils ($P < 0.01$ all vs. B2) (Fig. 3), neutrophils ($P < 0.001$ all vs. B2), mononuclear cells ($P < 0.01$ all vs. B2), congestion score ($P < 0.05$ all vs. Dil), and HSA levels ($P < 0.05$ all vs. Dil) from 2–8 h after antigen challenge (A2–A8) compared to baseline during both exposures. Also, there were significant increases in ECP levels in nasal lavage fluid from 4–8 h after antigen challenge (A4–A8) during HHA exposure and in nasal lavage fluid from 6–8 h after antigen challenge (A6 and A8) during RA exposure compared to baseline (B2). HHA had no effect on allergen-induced rhinorrhea ($P = \text{NS}$), sneezing ($P = \text{NS}$), pruritus ($P = \text{NS}$), congestion ($P = \text{NS}$), or HSA levels ($P = \text{NS}$) (Table 1), but led to significant increases in the number of eosinophils [HHA: 10.8×10^4 (3×10^4 – 26×10^4) vs. RA: 1.3×10^4 (0.3×10^4 – 20×10^4); $P = 0.01$] (Fig. 3), ECP levels

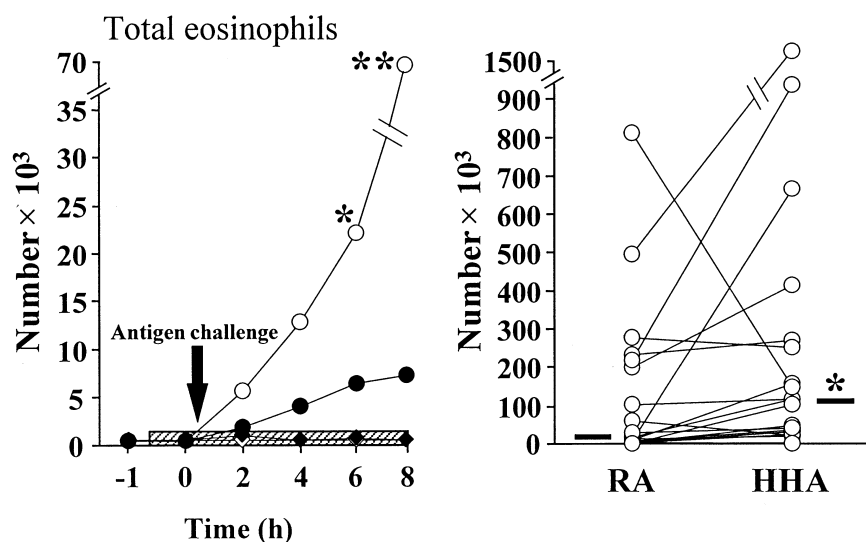


Fig. 3. Eosinophils. Left: Number of eosinophils at each of the individual time-points after exposure to hot, humid air (open circles) or room air (filled circles) in the presence of antigen challenge and after exposure to hot, humid air (open diamonds) or room air (filled diamonds) in the absence of antigen challenge. The time during the experiment is shown on the abscissa. Minus 1 represents the first baseline measurement; zero represents the second baseline measurement obtained after 1 h of inhalation of either type of air followed by measurements at 2-h intervals after the last antigen challenge for a total of 8 h. Data are median for 20 subjects in the presence of antigen challenge (circles) and median for three subjects in the absence of antigen challenge (diamonds). The shading above the abscissa represents the timing of air administration. Right: Individual data for net change in eosinophil influx (the sum of the eosinophils at hours 2, 4, 6 and 8: 4 times the number at hour 0) after exposure to two different types of air specified on the abscissa in the presence of antigen challenge. The solid bars represent median values. HHA: hot, humid air, RA: room air. * $P < 0.05$, ** $P < 0.01$ compared with RA.

Table 1. Symptom and albumin results

Parameter	Early-phase		Late-phase		Hyper-responsiveness	
	RA	HHA	RA	HHA	RA	HHA
Rhinorrhea score	7 (2.5–9.5)	7 (4.5–12)	0 (0–5)	2 (0–3.5)	3.5 (0–7.5)	5 (0–10)
Number of sneezes	17 (10.5–28)	10 (6.5–19)*	0.5 (0–2.5)	0 (0–0.5)	6.5 (5–10)	7.5 (5.5–12)
Pruritic score	4 (1–5.5)	2 (0.5–4.5)*	1 (0–2)	0 (0–2.5)	1.5 (0–2)	0.5 (0–2.5)
Albumin ($\mu\text{g/mL}$)	201 (76.2–427)	70.6 (11–284)*	44.5 (4.6–202)	116 (17.2–196)	210 (128–344)	205 (48.9–366)
Congestion score	6 (4–10.5)	3 (1.5–5.5)*	5 (0–10)	2 (0–11)	2 (0–4.5)	4 (1–6)

Numbers represent the total change from diluent challenge.

Data are reported as medians with 25th–75th percentiles in parentheses.

* $P \leq 0.05$ vs. respective RA.

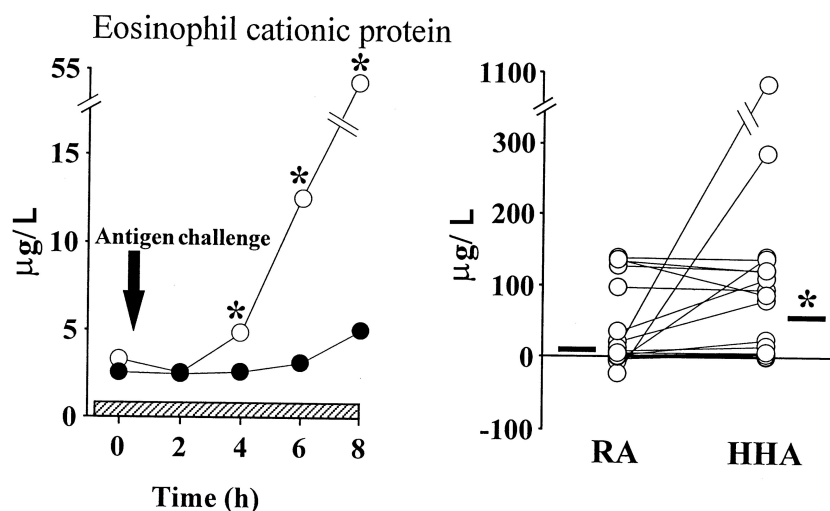
[HHA: $51.5 (5.3–120.1)$ vs. RA: $3.3 (-0.1–50.3)$; $P < 0.05$] (Fig. 4), neutrophils [HHA: $13 \times 10^5 (5 \times 10^5–18 \times 10^5)$ vs. RA: $4 \times 10^5 (2 \times 10^5–16 \times 10^5)$; $P < 0.01$], and mononuclear cells [HHA: $8.3 \times 10^4 (1.9 \times 10^4–23 \times 10^4)$ vs. RA: $1.4 \times 10^4 (0.5 \times 10^4–9 \times 10^4)$; $P < 0.001$] in nasal lavage fluid compared to RA. Significant correlations were found between ECP and the number of eosinophils in both groups (HHA: $r = 0.71$, $P < 0.0001$ vs. RA: $r = 0.71$, $P < 0.0001$).

Each histamine challenge performed 8 h after antigen challenge was accompanied by significant increases in the number of sneezes ($P < 0.001$), rhinorrhea score

($P < 0.01$), congestion score ($P < 0.01$), pruritic score ($P < 0.05$), and HSA levels in nasal lavage fluid ($P < 0.01$) compared with diluent challenge during both exposures. After antigen challenge, these responses to histamine significantly increased compared to those in baseline histamine screening ($P < 0.05$ for both HHA and RA) as indicated by a higher number of sneezes and higher levels of albumin in nasal lavage samples. HHA had no effect on the histamine response after antigen challenge as indicated by non-significant differences in rhinorrhea ($P = \text{NS}$), sneezing ($P = \text{NS}$), pruritus ($P = \text{NS}$), congestion ($P = \text{NS}$), and albumin levels ($P = \text{NS}$) compared to RA (Table 1).

Fig. 4. Eosinophil cationic protein (ECP).

Left: Levels of ECP at each of the individual time-points after exposure to hot, humid air (open circles) or room air (filled circles). The time during the experiment is shown on the abscissa. Zero represents the second baseline measurement obtained after 1 h of inhalation of either type of air followed by measurements at 2-h intervals after the last antigen challenge for a total of 8 h. Data are median for 20 subjects. The shading above the abscissa represents the timing of air administration. Right: Individual data for net change in ECP (the sum of the eosinophils at hours 2, 4, 6 and 8: 4 times the number at hour 0) after exposure to two different types of air specified on the abscissa. The solid bars represent median values. HHA: hot, humid air, RA: room air. * $P < 0.05$ compared with RA.



In the control experiment, in which subjects ($n = 3$) were exposed to the same condition except no antigen challenge was performed, HHA increased nasal surface temperature [HHA: $1.9 (1.8-2.1)$ vs. RA: $-0.1 (-0.4-0.3)$] for 8 h without affecting the number of either type of cells in nasal lavage fluid, compared to RA [Eosinophils [HHA: $0 (0-6.1 \times 10^3)$ vs. RA: $0 (-0.3 \times 10^3-5.8 \times 10^3)$], neutrophils [HHA: $-2.2 \times 10^4 (-19 \times 10^4-5.7 \times 10^4)$ vs. RA: $0 (-22 \times 10^4-1.9 \times 10^4)$], mononuclear cells [HHA: $2.5 \times 10^3 (-6.5 \times 10^3-7.7 \times 10^3)$ vs. RA: $-0.5 \times 10^3 (-11 \times 10^3-0)$]]. However, the number of total cells and the number of each cell type in the control experiment (without antigen challenge) were statistically less than those after antigen challenge during both exposures ($P < 0.01$ for all).

Discussion

Since the EPR to antigen is only one component of the allergic response and does not always predict the clinical allergic response, we investigated whether HHA had a favourable effect on the LPR and hyper-responsiveness to histamine. To our surprise, a reduction in the EPR was not accompanied by a reduction in the LPR. In contrast, the number of cells increased during the LPR. Furthermore, the increase in cells was not associated with a further increase in the response to histamine.

Regardless of the conditions of inhaled air, nasal challenge with antigen led to EPR and LPR as well as an increased response to histamine, consistent with previous studies [1-3,16-24]. Our data demonstrated that HHA delivered by mask significantly inhibited allergen-induced sneezes, congestion and HSA levels during the EPR, consistent with previous studies [9,11,12]. The most likely mechanism underlying the beneficial effects of HHA in the reduction of early allergic response is the modification of nasal mucosal temperature since our results showed that the inhalation of HHA led to a significant increase in nasal mucosal temperature without a significant change in nasal secretion osmolality. In support of this concept, an increase in nasal surface temperature without a change in nasal secretion osmolality was induced by the warming of feet and has been shown to reduce the early allergic response to nasal challenge with antigen [14]. 37°C and more than 95% RH air would be expected to prevent evaporation and deposit water on the nasal mucosa [25,26] because the surface temperature is normally 32°C [27]. The condensation of warm water would be expected to raise the surface temperature [28], which we demonstrated.

We anticipated that a reduction in the EPR would be accompanied by a decrease in the LPR. Surprisingly, HHA significantly increased inflammatory cells (eosinophils, neutrophils and mononuclear cells) and levels of ECP during LPR compared with RA exposure. To our

knowledge, this is the first study to show that decreasing the early response does not decrease the late reaction.

There were no significant differences in the percentages of each cell type between HHA and RA exposures, suggesting that HHA increased cellular influx without selective recruitment of either type of cell. After HHA exposure, eosinophil influx and ECP levels during the LPR were 8-fold and 15-fold greater than those after RA exposure, respectively. The increase in eosinophils, as well as in their secretory product, ECP, suggests that HHA influenced not only the trafficking of eosinophils within the nasal mucosa but also their state of activation.

In our study, there were significant increases in congestion score and albumin levels during LPR and in antigen-induced hyper-responsiveness to histamine 8 h after challenge. However, there were no significant differences in congestion score, albumin levels, and nasal hyper-responsiveness between either exposure, raising the question about the role of increased eosinophils in LPR and nasal hyper-responsiveness. Although eosinophils can contribute mediators to the inflammatory milieu of the LPR, their precise role at the inflammatory site is still unknown. Some studies showed no correlation between the increase in nasal reactivity and the number of eosinophils or the ECP levels [29,30]. Furthermore, some antihistamines were able to inhibit the increased nasal reactivity to methacholine observed 24 h after allergen challenge without affecting an increase in total eosinophils [31,32]. In addition, a murine model for antigen-induced bronchial hyper-reactivity and airway eosinophilia demonstrated no role of eosinophils in the development of airway hyper-reactivity [33,34]. These data suggest that late-phase symptoms, eosinophil influx, ECP levels and hyper-responsiveness after antigen challenge are not necessarily integrally linked in a causal relationship.

Our data do not explain the mechanisms by which HHA increased leucocyte recruitment after antigen challenge. Mast cells have been shown to contribute to eosinophil recruitment by producing interleukin (IL)-5 and tumour necrosis factor (TNF)- α , which have actions relevant to eosinophil activation and recruitment [35,36]. If HHA has an inhibitory effect on other cytokines as it has on histamine release during EPR [11,12], this end-point theoretically should lead to a decreased eosinophil influx into the nasal mucosa. Since HHA increased eosinophil influx during LPR, this explanation seems unlikely.

Other possible mechanisms for the increased leucocyte recruitment after HHA exposure are the effect of increased temperature on the rate of leucocyte locomotion, leucocyte chemotactic factors and the formation of heat shock proteins (HSPs). Human neutrophils [37-39] and lymphoblasts [40] have been shown to move significantly faster with increasing temperatures.

Some studies have shown that the generation and release of eosinophil chemotactic factor, an uncharacterized substance with eosinophil chemotactic activity generated by neutrophils, is temperature dependent with its activity being greater at higher temperatures [41,42].

HSPs formation may amplify allergic inflammation by increasing the processing and presentation of antigens or by HSPs serving themselves as antigens [43]. Exposure to elevated temperature has also been shown to induce the expression of HSPs in human endothelial cells [44]. Up-regulation of HSPs on vascular endothelium induced by increased nasal surface temperature may activate T cells [45] and lead to increased cellular influx into the nasal mucosa. Moreover, enhanced temperature stimulated endothelial expression, not only of HSPs, but, concomitantly, of ICAM-1 [46]. Possibly, coexpression of HSPs and increased ICAM-1 after inhaling of HHA synergistically increase leucocyte influx into the nasal mucosa.

In summary, we have shown that HHA partially inhibited the EPR to antigen challenge. In contrast, HHA increased the LPR to antigen challenge by increasing cellular influx without affecting the nasal hyper-responsiveness to histamine. The mechanisms by which HHA increases cellular influx are unknown. Importantly, we have shown that the nasal mucosal surface temperature in response to environmental air conditions influences the allergic inflammatory reaction and so further demonstrated the complexity of allergic inflammation.

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