

Original article

Nasal inflammation and bronchial reactivity to methacholine in atopic children with respiratory symptoms

Background: In atopic subjects, dysfunctions of the upper and lower airways frequently coexist and allergic rhinitis seems to constitute a risk factor for the occurrence of asthma in predisposed individuals.

Aim of the study: To evaluate whether in atopic subjects nasal inflammation could reflect changes in respiratory functions, 11 allergic children, sensitized to house dust mites (HDM), with rhinoconjunctivitis and asthma and 10 nonatopic controls (ctrls) were studied.

Methods: All subjects underwent nasal brushing to detect percentages of nasal eosinophils (Eos %) and intercellular adhesion molecule-1 (ICAM-1) expression by nasal epithelial cells. In the same day pulmonary function tests, i.e. forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), forced expiratory flows at 25–75% of the vital capacity (FEF_{25–75%}) and methacholine (MCh) bronchial inhalation challenge were also evaluated.

Results: Pulmonary function parameters were not significantly different in allergic children and in ctrls ($P > 0.05$), while a significant increase in bronchial reactivity to MCh, expressed as Pd₂₀ MCh, was detected in the former population ($P < 0.05$). As compared with ctrls, allergic children showed elevated Eos % and ICAM-1 expression ($P < 0.05$). When nasal inflammation and pulmonary function parameters were compared, a significant correlation was found between nasal Eos % and bronchial reactivity to MCh ($P = 0.002$).

Conclusions: These data support the concept of significant links between upper and lower respiratory tract involvement in atopic children sensitized to HDM.

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In predisposed individuals, allergic rhinitis may constitute a risk factor for the occurrence of asthma (1, 2) being stronger for patients sensitized to house dust mites (HDM) than for those sensitized to pollens (3, 4).

The concept that, at least in atopy, upper and lower airways should be considered a unique entity influenced by common physiologic processes (1, 4) is supported by recent studies showing tight similarities in tissue inflammatory changes in asthma and rhinitis (4, 5).

However, differences may exist at these two levels (i.e. bronchi and nose) in the extent of the inflammatory changes and in the involvement of the inflammatory cell

products in producing reversible and irreversible damages to the parenchymal structures (5).

In asthma, eosinophilic bronchial inflammation, which may be present also in asymptomatic asthmatic individuals, correlates with baseline lung function and with the increase in airway hyperresponsiveness following allergen challenge (6, 7). Although it is not known whether this 'subclinical' recruitment and activation of leukocytes may lead to irreversible airway remodelling, it has been suggested that evaluation of airway inflammation, together with measurement of pulmonary function, may be useful to identify individuals who may need a closer follow-up and/or a more aggressive treatment (7).

In this context, it could be interesting to know whether the nose could be used as a window on the lower airways, i.e. whether in children with allergic respiratory symptoms, inflammatory changes detected in the nasal mucosa could reflect the degree of asthmatic involvement, i.e. abnormalities in respiratory functions. Nasal brushing was performed in atopic children with rhinoconjunctivitis and intermittent asthmatic symptoms and in control (Ctrl) subjects: the percentages of eosinophils (Eos) and intercellular adhesion molecule-1 (ICAM-1) expression by epithelial

Abbreviations: Ctrs, controls; Eos, eosinophils; FEF_{25–75%}, forced expiratory flows at 25–75% of the vital capacity; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; HDM, house dust mites; ICAM-1, intercellular adhesion molecule-1; IgE, immunoglobulin E; LFA-1, lymphocyte function-associated antigen-1; Mac-1, macrophage antigen-1; MCh, methacholine; mfc, mean fluorescence channel; PD20 methacholine, MCh dose causing a 20% decrease in FEV₁; RAST, radioallergosorbent test; SPT, skin prick test; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

cells were detected and possible correlations with pulmonary function parameters and with response to methacholine (MCh) bronchial inhalation challenge were evaluated.

Material and methods

Patients

Eleven outpatients, five male and six female [9.1 (5–14) years of age], were enrolled. Inclusion criteria were: (a) rhinoconjunctivitis (8) caused by HDM; (b) intermittent asthmatic symptoms in at least the two previous years; (c) sensitization to *Dermatophagoides pteronyssinus* and/or *D. farinae* assessed by skin prick test (SPT) and/or radioallergosorbent test (RAST). Exclusion criteria were: (a) other sensitizations, including pollens, moulds, pet dander; (b) courses of specific immunotherapy with the same allergens in at least the five previous years; (c) chronic or recurrent inflammation of oral mucosa; (d) low compliance of the patients; (e) systemic immunologic or metabolic disease; (f) malignancies; (g) major anatomic alterations of the upper airways; (h) noncontrolled asthma and severe atopic dermatitis; (i) chronic systemic corticosteroids treatment and (l) forced expiratory volume in 1 s (FEV₁) < 70%. Ten sex- and age-matched healthy subjects, [9.4 (4–17) years], with no history of upper or lower respiratory tract symptoms in at least 4 weeks preceding the evaluation, served as a Ctr group. They had normal pulmonary function parameters, negative results to the standardized SPT and immunoglobulin E (IgE) serum levels < 100 KU/l. The Ethical Committee of Gaslini Institute approved the study and parents/ tutors of children, informed in detail about the experimental procedure and the scope of the study, provided written informed consent.

Diagnosis of allergic sensitization

Aeroallergen sensitization was evaluated by SPT, as previously reported (9). The allergen panels tested included: HDM, *Parietaria officinalis*, mix of Graminae, Compositae, Betulaceae, Oleaceae, mix of *Aspergillus*, *Cladosporium*, *Alternaria tenuis*, cat or dog skin scale allergen extracts (Bayropharm, Milan, Italy). A weal diameter 3 mm larger than the negative Ctr was considered as a positive reaction.

Paper radioimmunosorbent test (PRIST) or Phadebas RAST (Pharmacia, Uppsala, Sweden), respectively, determined serum levels of total IgE and of specific IgE against *D. pteronyssinus* or *D. farinae* (9).

Lung function and methacholine bronchial challenge

All children were able to perform forced expiratory manoeuvres. Forced vital capacity (FVC), FEV₁, forced expiratory flows at 25–75% of the vital capacity (FEF 25–75%) were measured by spirometry (Med Graphics, Pulmonary Function System 1070 series 2; Med Graphics Corporation, St Paul, MN), as previously described (9). A dosimeter device (MEFAR, Brescia, Italy) delivered aerosols of MCh a using the same ampule for each patient, as previously described (9). The MCh dose causing a 20% decrease in FEV₁ (PD₂₀ methacholine) was calculated by interpolation of the dose–response curve (9).

Collection of nasal epithelial and inflammatory cells and evaluation of ICAM-1 expression by nasal epithelial cells

Nasal brushings were performed, as previously described (10). The differential cell count was determined on cytocentrifuge prepara-

tions (Cytospin; Shandon Southern Products Ltd, Runcorn, UK), as previously reported (11). The proportions of inflammatory cells 'contaminating' the epithelial cells were determined by counting 400 cells/sample and were expressed as percentage.

In addition, an aliquot of cell suspensions obtained by nasal brushing were stained with monoclonal antibodies to human ICAM-1 (CD54) (Sera Lab, Crawly Down, UK) fluorescein isothiocyanate-conjugates, as previously reported (11). A monoclonal antibody against human platelets (PTF 19) was used as a negative Ctr preparation. All experiments were performed three times. The intensity of fluorescence was expressed as mean fluorescence channel (mfc) (11).

Statistical analysis

Lung function parameters and ICAM-1 expression are presented as mean $\pm$ SE, whereas percentages are expressed as median values plus lower and upper quartiles. The PD₂₀ MCh values were transformed into logarithms (log PD₂₀ MCh) before statistical analysis. Comparisons are calculated using Student's unpaired *t*-test. Relationships between independent variables were assessed by Pearson correlation test. Statistical significance was considered for *P* < 0.05.

Results

Demographic data and total serum IgE levels of the study population are summarized in Table 1. Pulmonary function parameters were within normal values (95% of predicted values) in all atopic children and similar to Ctrs (*P* > 0.05) (not shown). In contrast, PD₂₀MCh methacholine was significantly lower in atopic patients than in Ctr group (*P* < 0.05) (not shown).

Table 1. Demographic and clinical children data

No.	Patients	Age (years)	Gender	PRIST (total IgE KU/l)
Atopics				
1	ME	7	M	195
2	BM	11	M	762
3	CM	12	M	356
4	MA	11	F	1443
5	BA	9	F	159
6	AM	7	F	873
7	CE	7	F	341
8	FE	5	F	243
9	PA	8	M	1217
10	MA	9	F	5665
11	TM	14	M	301
Normal healthy controls				
1	SO	11	M	<100
2	RE	14	F	<100
3	CM	8	F	<100
4	SP	5	M	<100
5	SG	8	F	<100
6	AL	9	F	<100
7	PS	17	M	<100
8	BM	7	F	<100
9	RM	4	F	<100
10	EV	11	M	<100

IgE, immunoglobulin E; PRIST, paper radioimmunosorbent test.

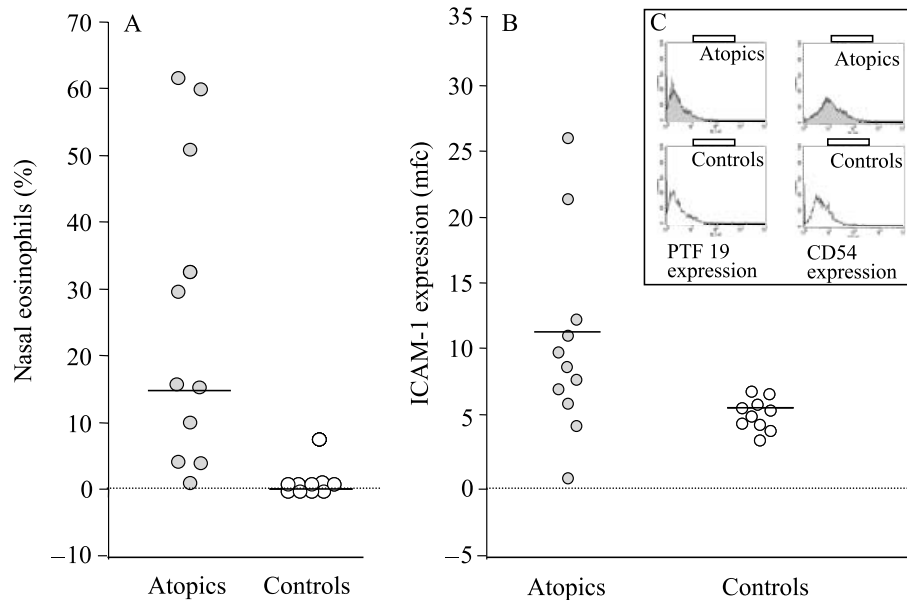


Figure 1. Eosinophil percentage in nasal brushings and constitutive expression of intercellular adhesion molecule-1 (ICAM-1) by nasal epithelial cells in atopic children (●) and in normal healthy controls (○). The two study populations are shown on the abscissa on panel A and on panel B, whereas the eosinophil proportion, expressed as percentage of the total nasal brushing leukocytes, and ICAM-1 expression, expressed as mean fluorescence channel (mfc), are shown on the ordinate on panel A and on panel B respectively. Horizontal bars indicate the median values or the mean values on panel A and on panel B respectively. Panel C reports flow cytometric histograms of PTF19 (anti platelet mAb, as negative control) (upper and lower left panels) and ICAM-1 expression (upper and lower right panels) on nasal epithelial cells, representative of one atopic individual and one normal healthy control.

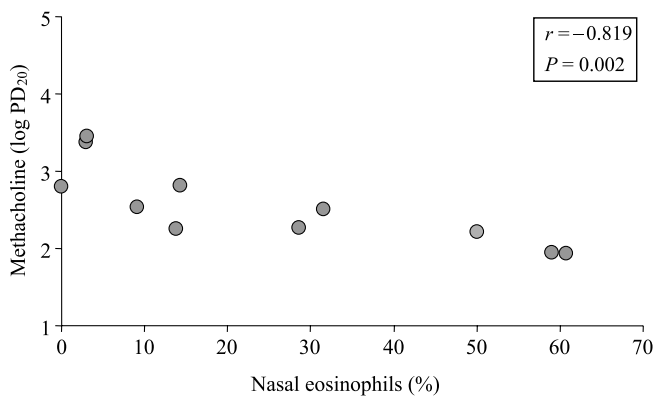


Figure 2. Correlation between nasal brushing eosinophil (Eos) proportion and bronchial reactivity to methacholine (MCh) in atopic children. The Eos proportion, expressed as percentage of the total nasal brushing leukocytes, is shown on the abscissa whereas bronchial reactivity to MCh, expressed as log PD₂₀MCh is shown on the ordinate.

Analysis of the proportions of inflammatory cells demonstrated similar percentages of lymphocytes, monocytes and neutrophils ($P > 0.05$) (not shown), but increased Eos proportions in atopic children [14.29 (6.12–40.79)], as compared with Ctrs [0 (0–5.85)],

($P = 0.008$) (Fig. 1A). Similarly, adhesion molecule evaluation on nasal epithelial cells demonstrated a significant upregulation of ICAM-1 expression in atopic children (10.45 ± 2.77) as compared with Ctrs (5.21 ± 0.31), ($P = 0.048$) (Fig. 1B and C).

In atopic children, a significant correlation was demonstrated between nasal Eos proportions and log PD₂₀ MCh ($P = 0.002$), (Fig. 2). In contrast, both in atopic children and in Ctrs, no correlations were found between Eos proportions and pulmonary function parameters or between ICAM-1 expression and pulmonary function parameters or log PD₂₀ MCh ($P > 0.05$) (not shown).

Discussion

Evaluating atopic children sensitized to HDM and with respiratory symptoms, we have shown increased ICAM-1 expression by nasal epithelial cells and elevated nasal Eos percentages. Nasal eosinophilia correlated which bronchial hyperresponsiveness to MCh.

Although a variety of leukocytes are increased in number and are activated in allergic rhinitis, the inflammatory cells representing the hallmark of this condition are metachromatic cells and Eos (3, 8). Indeed, the presence of elevated nasal Eos number here reported is in agreement with the observation that these cells and their

mediators are found in nasal secretions of patients allergic to HDM, even in symptom-free periods (3, 12).

Eos can be recruited and activated at sites of disease activity (11) through a complex mechanism including not only the response to chemotactic signals but also the interaction between surface adhesion molecules, expressed by circulating cells, and counter-receptors, expressed by vascular endothelial cells (13). Eos express a variety of adhesion molecules including the β_2 -integrin lymphocyte function-associated antigen-1 (LFA-1) and macrophage antigen-1 (Mac-1), and the α_4 -integrin very late antigen-4 (VLA-4) (13). The VLA-4 and its endothelial cell counter-receptor, vascular cell adhesion molecule-1 (VCAM-1) appear to play an essential role in the initial adherence of activated Eos, while LFA-1, Mac-1 and their counter-receptor, ICAM-1, are important for Eos extravasation (13).

In this context, an increased expression of ICAM-1 in response to cytokines may represent a mechanism by which airway epithelial cells adhere to and interact with inflammatory and immuno-effector cells in the respiratory mucosa of atopic patients (12, 13). Consistent with previous reports (14), we found an increased expression of ICAM-1 on nasal epithelial cells.

We also found a statistically significant correlation between nasal Eos percentages and bronchial responsiveness to MCh. In asthma, the relationship between

eosinophilic inflammation and airway hyperresponsiveness or airflow limitation is still unclear (6). However, in allergic individuals, significant correlations have been demonstrated between total serum IgE or blood Eos and bronchial responsiveness to MCh (14) and between the decrease in circulating Eos following allergen inhalation challenge, and the degree of late asthmatic response and changes in bronchial responsiveness to histamine (15). The observations here reported, that are in agreement with those observed in experimental animals (16), further support the hypothesis of a link between a 'more systemic' allergic reaction and enhanced bronchial responsiveness in allergic individuals with respiratory symptoms.

Although we did not explore the mechanisms underlying the systemic link between the inflammatory processes occurring in the nose and in the bronchi in atopic children, the results here reported support the concept that the evaluation of upper airway inflammation may reflect and give additional insight on the lower airway involvement.

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