

Autologous serum skin test reactivity in patients with non-allergic asthma

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Summary

Background Inflammatory alterations of respiratory airways have been found in patients with non-allergic asthma, but the triggering event has not been defined. An autoimmune activation of inflammatory cells has been hypothesized.

Objective To evaluate whether histamine-releasing factors are present in sera from non-allergic asthmatics.

Methods Twenty-four patients with non-allergic asthma underwent *in vivo* autologous serum skin test (ASST) and *in vitro* basophil histamine release assay using autologous basophils as well as basophils from normal donors. Twenty-seven subjects with respiratory allergy and three normal subjects were chosen as control.

Results ASST was positive in 14/24 non-allergic asthmatics (58%) whereas it was negative in all 30 control subjects ($P < 0.001$). The serum of only one ASST-positive patient out of 12 (8.4%) induced *in vitro* histamine release from autologous basophils. The serum from another ASST-positive patient induced histamine release from membrane IgE-stripped autologous basophils. Sera from either non-allergic asthmatics or from control subjects did not provoke significant histamine release from basophils from three normal donors.

Conclusion Skin reactivity to autologous serum is common among non-allergic asthmatics, indicating the presence of circulating histamine-releasing factors. However, only in a minority of patients *in vitro* functional evidence of histamine-releasing autoantibodies (anti-FcεRI or anti-IgE) was obtained. The presence of circulating histamine-releasing factors might contribute to initiation/maintenance of inflammation in respiratory airways of non-allergic asthmatics.

Keywords autologous serum skin test, basophils, histamine-releasing factors, mast cells, non-allergic asthma

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Introduction

The term 'intrinsic' asthma was introduced by Rackeman in 1947 to define bronchial asthma which is not triggered by exogenous causes, namely by exposure to aeroallergens [1]. In recent years, the nomenclature has been revised and 'intrinsic' asthma is now known as 'non-allergic' asthma [2]. This definition clearly demonstrates the poor knowledge of the pathomechanism, since the disease is characterized only by a negative attribution (i.e. the lack of allergy). Some clinical features suggest that non-allergic asthma differs from allergic asthma; for example, it has a later onset in life, female preponderance, a more frequent association to nasal polyposis and a more severe clinical course [3]. On the other hand, cellular and molecular studies provide evidence that some common immunopathological mechanisms underlie allergic and non-allergic asthma [4, 5]. Allergic asthma is character-

ized by an inflammatory process of respiratory airways, which is triggered by allergen-induced IgE-mediated mast cell activation [6]. This event is followed by recruitment of inflammatory cells, such as eosinophils and T lymphocytes, and release of pro-inflammatory mediators and cytokines. Analysis of blood and bronchoalveolar lavage cells as well as bronchial biopsies from allergic asthmatics have revealed a predominant activation of T helper type 2 (Th2) lymphocytes with enhanced production of interleukin (IL)-4, IL-5 and IL-13 [7–10]. Non-allergic asthma is also characterized by an inflammatory process of respiratory airways, but a clearcut Th2 activation has not been detected since analysis of cytokine profile has revealed an increased expression of IL-5 and IL-2, but not IL-4 and IL-13 [9, 11].

Some epidemiological and experimental findings suggest that allergic and non-allergic asthma are not clearly distinct immunopathological entities [12]. Burrows et al. showed a strong association between high levels of serum total IgE and asthma either in allergic or non-allergic asthmatics [13]. This observation was confirmed by Beeh et al., who found that elevation of total serum IgE is associated with asthma in non-allergic individuals, supporting the role of IgE as risk factor

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for asthma independent of allergy [14]. Furthermore, a local IgE production has been demonstrated in bronchial biopsies from non-allergic asthmatics [15]. Based on these findings, the possible involvement of IgE in the mechanism of the disease has been postulated in allergic as well as non-allergic asthma. What remains elusive is the event that initiates bronchial inflammation in patients with non-allergic asthma. In recent years, chronic urticaria, formerly considered as 'idiopathic' in the vast majority of patients, was shown to be an autoimmune disorder in a relevant proportion of cases [16]. Intradermal injection of autologous serum was shown to induce a weal-and-flare reaction in 30–60% of chronic urticaria patients [16, 17]. Furthermore, sera from chronic urticaria patients were shown to induce histamine release from basophils *in vitro*. Serum histamine-releasing factor was subsequently identified as IgG autoantibodies specific for the high affinity IgE receptor (FcεRI) present on mast cells and basophils (25% of the patients) or for IgE (5% of the patients) [16]. Thus, some light has been shed on the pathogenesis of a previously 'mysterious' disorder. Based on the observations on chronic urticaria, we hypothesized that a similar mechanism might be operating in non-allergic asthma. Circulating histamine-releasing factors might activate bronchial and pulmonary mast cells, thus initiating or perpetuating bronchial inflammation in non-allergic asthmatics. In this study we sought to investigate the presence of serum histamine-releasing factors in a population of non-allergic asthmatics.

Subjects and methods

Twenty-four patients with non-allergic asthma (12 men and 12 women, mean age $\pm$ SEM 47.2 ± 2.6 years), were selected for the study at the Respiratory Disease outpatient clinic of the Fatebenefratelli Hospital of Milan. Non-allergic asthma was diagnosed on the basis of clinical story, respiratory function tests and negativity of skin prick tests and/or specific IgE determinations (UniCAP RAST, Pharmacia, Uppsala, Sweden). Serum total IgE concentration was measured in all patients by UniCAP system (Pharmacia). All asthmatic subjects had a 20% decrease of forced expiratory volume in 1 s following inhalation of <8 mmol methacholine or bronchial obstruction at basal spirometry with reversibility after inhalation of 200 µg albuterol. A detailed family and personal history was collected for each patient. Asthma was classified as intermittent, mild persistent, moderate persistent or severe persistent according to Global Initiative for Asthma (GINA) workshop report [18]. Coexistence of non-allergic rhinitis was diagnosed when non-allergic asthmatics complained of symptoms such as nasal congestion, rhinorrhoea, itching/sneezing and impairment of smell, and two or more of these symptoms were present for more than 1 h on most days [19]. One week before the study systemic therapies, including anti-leukotrienes and corticosteroids, were suspended. Patients with respiratory infections at the time of the investigation were not recruited. None of the patients was taking antihistamines at the time of the investigation.

The study protocol included two visits for each patient. On the first occasion clinical story was collected and physical examination, spirometry, methacholine provocation test and

skin prick or RAST tests were performed. If the patients were considered eligible for the study, they underwent a second visit during which venous blood was drawn and autologous serum skin test (ASST) was performed. Furthermore, aliquots of blood were used for histamine release assay from autologous and/or homologous basophils.

Twenty-seven subjects with respiratory allergy (either rhinitis or asthma or both) and three non-allergic non-asthmatic subjects were chosen as control.

The hospital ethical committee approved the study protocol. Patients and controls were informed about the aims of the study and gave their informed consent.

Skin tests

Both patients and controls underwent intradermal test with 0.05 mL of sterile, fresh autologous serum and with saline (0.9% w/v NaCl) as negative control. Readings were taken at 20 min. Only an unequivocal weal formation in response to serum injection was considered as a positive test. Skin tests were considered positive when the weal diameter was at least 3 mm and were graded according to the following scale: 3–4 mm+, 4–5 mm++, >5 mm+++.

Basophil histamine release assay

In vitro serum histamine-releasing activity was assessed in 24 patients with non-allergic asthma and 7 control subjects using autologous basophils as well as basophils from normal donors, as previously described [17]. Sera were tested with autologous leucocytes and with leucocyte suspensions from three normal donors whose basophils were previously shown to release 30% of total histamine content on challenge with an optimal dose of goat polyclonal anti-human IgE (Sigma Chemicals, St Louis, MO, USA; 10 µg/mL). In addition, in order to detect the presence of functionally active anti-FcεRI autoantibodies, basophil histamine release was evaluated after stripping of membrane-bound IgE with 10 mmol/L lactic acid (pH 3.9, incubation for 3.5 min at room temperature), a procedure that allows dissociation of FcεRI-bound IgE without damaging basophil functional activity [20]. Histamine concentration in the cell supernatant was measured by an automated fluorometric method [21]. Results were expressed as net percent histamine release over total histamine content. A 5% release cut-off value was used.

Statistical analysis

Fisher's exact test was used in order to compare occurrence of ASST positivity among non-allergic asthmatics and control subjects. A *P*-value lower than 0.05 was considered significant.

Results

Patients

The characteristics of the patients are shown in Table 1. Six subjects out of 24 (25%) had a positive family history of asthma, two subjects (8%) had a positive family history of allergy, and 18 subjects (75%) had coexisting non-allergic rhinitis. Mean serum total IgE concentration was $135.93 \pm$

Table 1. Characteristics of the non-allergic asthmatic patients included in the study

Patient no.	Initials	Age	Sex	Family history	Rhinitis	Total IgE (kU/L)
1	C. A.	66	F	Asthma	Yes	912
2	C. F.	35	F	Asthma and allergy	Yes	29.1
3	L. P.	41	F	Allergy	No	14.9
4	T. M.	43	F	None	Yes	13.1
5	M. L.	40	F	None	Yes	101
6	T. P. T.	52	F	Asthma	Yes	15.4
7	B. A.	28	M	None	Yes	19.1
8	G. G.	58	M	Asthma	No	5.39
9	D. V.	45	M	None	No	272
10	V. G.	62	M	None	Yes	50.5
11	C. S.	38	F	None	Yes	162
12	S. V.	35	M	None	Yes	5.6
13	P. A.	35	M	None	Yes	49.5
14	K. E.	48	F	None	Yes	134
15	P. S.	63	F	None	Yes	28.4
16	B. E.	68	M	None	Yes	54.8
17	O. M.	67	F	Asthma	Yes	40.2
18	C. An.	68	F	None	No	8.45
19	D. I.	30	F	Asthma	Yes	55.6
20	C. Ab.	36	M	None	Yes	87.7
21	O. A.	46	M	Asthma	No	479
22	F. G.	45	M	None	Yes	638
23	C. Ang.	38	M	None	No	39.7
24	G. M.	46	M	None	Yes	46.9

46.24 kU/L (mean $\pm$ SEM), with levels above the normal range (>100 kU/L) in seven patients.

Skin test and basophil histamine release assay

The results of *in vivo* and *in vitro* assays for serum histamine-releasing activity are reported in Table 2. ASST was positive in 14 asthmatics out of 24 (58%) whereas it was negative in all 30 controls ($P < 0.001$). Intradermal injection of saline did not induce the formation of a weal with a diameter above 3 mm neither in patients nor in controls. Among the subjects with positive reaction to ASST, there were eight women (57%). The diameter of the weal reaction was ≥ 5 mm in 10 patients and 4 mm in four patients. ASST positivity was not associated with severity of asthma. As shown in Table 2, among patients with severe persistent asthma, three were ASST-positive and four ASST-negative; furthermore, among patients with moderate persistent asthma, two were ASST-positive and three were ASST-negative.

The serum of only one out of 12 ASST-positive patients (8.4%) induced significant histamine release (22.6% of total histamine content) from autologous basophils. The serum from another ASST-positive patient out of four tested induced significant histamine release (36.9%) from autologous basophils treated with lactic acid (Table 3). No significant histamine release was observed when basophils from three normal donors were stimulated with sera from non-allergic asthmatics. Furthermore, no functional evidence for the presence of anti-Fc ϵ RI autoantibodies was obtained when lactic acid-treated basophils from two normal donors were stimulated with sera from 18 non-allergic asthmatics. Control

Table 2. Severity of asthma and results of *in vivo* and *in vitro* assays of serum histamine-releasing factors

Patient no.	Severity of asthma	ASST	% Histamine release	
			Autologous basophils	Homologous basophils
1	Severe persistent	3+	0	3
2	Mild persistent	Neg.	0	3.5
3	Severe persistent	Neg.	0	2.4
4	Mild persistent	1+	0	1.8
5	Moderate persistent	Neg.	0	2.3
6	Moderate persistent	2+	0	2.2
7	Intermittent	1+	0	2
8	Mild persistent	1+	0	2.4
9	Mild persistent	1+	0	1.4
10	Severe persistent	2+	0	1.8
11	Mild persistent	2+	0	1.7
12	Mild persistent	Neg.	0	2.1
13	Moderate persistent	Neg.	2.5	1.8
14	Mild persistent	3+	2.7	1.7
15	Mild persistent	2+	22.6	2.1
16	Severe persistent	Neg.	0	3.2
17	Moderate persistent	Neg.	4.6	3.7
18	Severe persistent	2+	0	2.8
19	Moderate persistent	2+	2.8	2.1
20	Mild persistent	2+	4.9	1.6
21	Severe persistent	Neg.	1.2	2.1
22	Severe persistent	Neg.	2.2	0
23	Mild persistent	Neg.	3.3	2.1
24	Mild persistent	2+	1.8	1.6

ASST, autologous serum skin test; Neg., negative.

Results of histamine release from homologous basophils represent the mean values of three different experiments performed with basophils of normal donors. Histamine release was considered positive when above 5%.

Table 3. Results of *in vivo* and *in vitro* histamine release assays performed in four non-allergic asthmatic patients

Patient no.	ASST	% Histamine release from autologous basophils			
		Untreated		Lactic acid treated	
		Serum	Anti-IgE	Serum	Anti-IgE
18	2+	0	17.1	36.9	9.5
19	2+	2.8	18.1	1.3	3.3
20	2+	4.9	7.6	2	1
21	Neg.	1.2	13.3	4.3	6.3

In vitro histamine release from autologous basophils was evaluated either before or after lactic acid-stripping of membrane IgE. Treatment with lactic acid lowered basophil response to anti-IgE, but enabled serum from patient 18 to trigger histamine release.
Neg., negative

sera from normal and allergic subjects did not provoke any significant histamine release from homologous basophils.

Discussion

These findings show that skin reactivity to injection of autologous serum is common among patients with non-

allergic asthma: 14 patients out of 24 showed an unequivocal weal and flare reaction upon injection of autologous serum. A skin reactivity to autologous serum has been described first in chronic urticaria patients [22] and subsequently in patients with multiple drug allergy syndrome [23] and/or non-steroidal antiinflammatory drugs hypersensitivity [24]. ASST positivity has been ascribed to the presence of anti-FcεRI or anti-IgE autoantibodies or to other undefined histamine-releasing factors. According to Sabroe et al. [25], ASST is a screening test for anti-FcεRI or anti-IgE autoantibodies, although there is a proportion of chronic urticaria patients with positive ASST and negative *in vitro* assays for histamine-releasing autoantibodies. The detection of these histamine-releasing autoantibodies is hampered by the lack of standardized immunoassays; therefore a functional assay using peripheral blood basophils is usually employed to determine their presence. However, the results of ASST and *in vitro* functional assays with basophils do not overlap. In our population of non-allergic asthmatics we found a high proportion of patients with positive ASST, but a low proportion of patients with positive *in vitro* assays. Serum from one ASST-positive patient induced histamine release from untreated autologous basophils, suggesting the presence of anti-IgE-autoantibodies. Serum from another ASST-positive asthmatic patient induced histamine release from membrane IgE-stripped basophils, indicating the presence of anti-FcεRI autoantibodies.

ASST positivity in asthmatic patients does not seem to be a non-specific phenomenon because no reactivity to intradermal injection of autologous serum was found in healthy or allergic control subjects. Non-specific formation of vasoactive mediators (for example bradykinin) during clot formation is in fact unlikely since ASST was negative in all control subjects. In agreement with our observations, Grattan et al. [22] found ASST positivity in 7 out of 12 chronic urticaria patients and in none of 19 healthy controls; Sabroe et al. [25] also found that ASST was positive in only one out of 40 healthy subjects. In contrast to these findings, Mari [26] observed that ASST was positive not only in 47% of the adults and 84% of the children with allergy-like respiratory symptoms, but also in 40% of adult patients with respiratory allergy and in 45% of healthy adult subjects. This striking difference might be because of differences in ASST interpretation and serum samples preparation.

As far as the discrepancy between *in vivo* and *in vitro* tests is concerned, we suggest that it could be because of the presence of serum histamine-releasing factors which are active only on skin mast cells but not on blood basophils. A hitherto undefined mast-cell-specific histamine-releasing factor has been detected in chronic urticaria patients and might be present in asthmatic patients as well [27]. A heterogeneity of skin mast cells and blood basophils regarding sensitivity to activating stimuli has been described. For instance, substance P, platelet-activating factor, opioids, compound 48/80, complement anaphylatoxins, free immunoglobulin light chains and stem cell factor can induce directly histamine release from skin mast cells, but not from blood basophils [28–30]. The low percentage of positive basophil histamine release assay could be also because of the difficulty to create optimal *in vitro* conditions for the detection of histamine-releasing factors. For example, intrinsic basophil releasability

and saturation of membrane IgE receptors represent two factors conditioning the capacity of the assay to detect the presence of histamine-releasing autoantibodies or other histamine-releasing factors. Membrane IgE stripping by lactic acid can increase the sensitivity of the test, allowing the detection of anti FcεRI antibodies-containing sera. However, evidence for anti-FcεRI autoantibodies was found only in one patient out of four tested using autologous basophils and in none of the 18 patients whose sera were tested on IgE-stripped basophils from two normal donors. Interestingly, the presence of circulating anti-FcεRI antibodies in asthmatic subjects has been reported by Kinet [31], who found that sera from four out of 10 asthmatic subjects contained anti-FcεRI antibodies, and two of them were functionally active on basophils.

A clinical finding emerging from our study is that most patients with non-allergic asthma also complained of rhinitic symptoms, mainly nasal obstruction and rhinorrhoea. This indicates that rhinitis and asthma symptoms often coexist in patients with non-allergic asthma, suggesting that non-allergic asthma is a systemic disorder affecting upper and lower airways. The concept of one airway disease, which has been proposed for allergic asthma, might indeed be valid for non-allergic asthma as well [32]. It is puzzling however, that non-allergic asthmatics usually do not experience urticaria; probably, in these patients only respiratory airways are the target of the inflammatory process. On the other hand, chronic urticaria patients with positive ASST and circulating anti-FcεRI or anti-IgE autoantibodies usually do not suffer from chronic asthma.

In summary, the results of our study demonstrated that skin reactivity to intradermal injection of autologous serum is common in non-allergic asthmatics. In a small proportion of patients functional evidence of serum histamine-releasing autoantibodies was found. The role of these autoantibodies and/or other histamine-releasing factors remains to be defined, but they have the potential to trigger and maintain inflammatory alterations in the airways.

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