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Relationship between cholinergic airway tone and serum immunoglobulin E in human subjects

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ABSTRACT: It has recently been shown that immunoglobulin (Ig)E facilitates the cholinergic bronchoconstrictor pathway in human tissue *in vitro*. However, whether this occurs in humans *in vivo* has not been clarified.

In this study, the bronchodilator responses were examined to inhalation of a submaximal dose of the anticholinergic agent oxitropium bromide (600 µg) in normal and allergic subjects with various levels of total serum IgE.

Values of the forced expiratory volume in one second (FEV_1) for all subjects were greater than 80% of predicted, but were negatively correlated with serum IgE levels ($p<0.01$). Oxitropium bromide inhalation induced an increase in FEV_1 that was significantly greater in allergic rhinitis patients with high serum IgE (155±20 mL (mean±SEM), $p<0.05$) than in healthy subjects (64±21 mL) or those with allergic rhinitis but low serum IgE (82±21 mL, $p<0.05$). In contrast, the effects of the inhaled β_2-adrenergic agent orciprenaline sulphate (2.25 mg) were not significantly different among the three groups.

In conclusion, higher serum immunoglobulin E levels were correlated with lower values of the forced expiratory volume in one second, and anticholinergic agents, but not β_2-adrenergic agents, caused more pronounced bronchodilation in subjects with high than in those with low immunoglobulin E levels. These data suggest that serum immunoglobulin E may be one of the factors that determine the airway tone, possibly *via* cholinergic mechanisms.

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Airway calibre is under the control of the autonomic nervous system. Within this system, the parasympathetic (cholinergic) nervous system is the dominant neural bronchoconstrictor mechanism in all animals, including humans, and plays an important role in the regulation of the calibre [1]. An exaggeration of cholinergic systems has been suggested to occur in patients with airway diseases such as asthma because anticholinergic agents can cause a greater bronchodilatory effect in these patients than in healthy subjects [2]. There are several plausible explanations for this phenomenon: increased vagal tone due to reflex mechanisms *via* sensory receptor stimulation by inflammatory mediators, increased acetylcholine release from nerve terminals and an increase in airway smooth muscle responsiveness to acetylcholine [1]. However, the exact mechanism of the cholinergic exaggeration is still unknown.

Because immunological mechanisms are important factors in the pathogenesis of asthma, immunoglobulin (Ig)E may play a role in the airway cholinergic exaggeration. In addition, IgE seems to be one of the determinants of airway calibre in the patients with cough and sputum [3, 4]. Recently, in an *in vitro* study, incubation with IgE was reported to exaggerate the human bronchial contraction elicited by electrical stimulation of vagal nerves without affecting the contractile response to exogenously applied acetylcholine, indicating that IgE can cause vagal cholinergic hyperresponsiveness in human airways *via* facilitation of acetylcholine release at cholinergic efferent pathways *in vivo* [5]. However, it is not clear that this occurs in humans *in vivo*. In this study, the cholinergic airway tone was examined by means of inhalation of submaximal doses of an anticholinergic agent in subjects of various total serum IgE levels. The subjects with a high IgE titre have greater cholinergic airway tone than the subjects with low serum IgE.

Patients and methods

Subjects

Thirty-six patients with allergic rhinitis and 11 healthy subjects took part in the study after giving informed consent. The study was approved by the local ethics committee. Allergic rhinitis was defined as the presence of seasonal or perennial rhinological symptoms and positive

skin test responses or positive allergen-specific IgE to at least one antigen [6]. Healthy subjects were nonasthmatic, nonrhinitic and nonallergic. The clinical characteristics of these subjects are shown in table 1. Allergic rhinitis subjects were divided into two groups, namely low IgE titre (ð250 U·mL^{-1}) and high IgE titre (>250 U·mL^{-1}) groups, because the normal range of IgE in the authors' laboratory is <250 U·mL^{-1}. None of the subjects had a current or past history of bronchial asthma and other chronic respiratory disease [7]. They had no lower respiratory symptoms on the test day or within the previous 3 months. Each subject was measured for forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) using a dry rolling-seal spirometer (OST 80A, Chest Co., Tokyo, Japan) and subjects were excluded from the study if they had obstructive and/or restrictive disorders. Patients with glaucoma or symptoms of bladder-neck obstruction were also excluded.

Protocol

This was a single-blind study, with randomized treatment using two bronchodilators.

Subjects attended the clinical laboratory on two occasions at least 7 days apart to eliminate any possibility of carry-over effects. On each day, FVC and FEV_1 were measured and then the subjects inhaled the anticholinergic agent oxitropium bromide (600 μg; Japan Boehringer Ingelheim, Kobe, Japan) or the β_2-adrenergic agent orciprenaline sulphate (2.25 mg; Japan Boehringer Ingelheim) in random order by metered-dose inhaler. Thirty minutes after each inhalation, FVC and FEV_1 were again measured. The doses of oxitropium bromide [8] and orciprenaline sulphate [9] and lung function measuring time were chosen to cause almost maximal bronchodilation by each drug according to the previous papers and this effect was confirmed in a preliminary study.

Total serum IgE was analysed using the fluoroenzymatic immunoassay (FEIA) technique (Pharmacia Diagnostics, Sweden). The sensitivity of the IgE level was 2–5000 U·mL^{-1} and 250 IU·mL^{-1} was defined as the cut-off for elevated IgE.

Nasal symptoms were assessed as follows: rhinorrhoea, nasal blockage, sneezing, nasal itching and loss of sense of smell. The methods of scoring each symptom were as follows. Rhinorrhoea was scored as: without rhinorrhoea (score of 0), number of times nose is blown was <5 time·day^{-1} (1), 5–10 times·day^{-1} (2), or >10 times·day^{-1} (3). Nasal blockage was scored: without nasal blockage (0), with nasal blockage but without nasal breathing disturbance (1), nasal breathing disturbance by nasal blockage (2), or nasal breathing impossible because of nasal blockage (3). Sneezing was scored as: without sneezing attack (0), number of sneezing attacks was <5 times·day^{-1} (1), 5–10 times·day^{-1} (2), or >10 times·day^{-1} (3). Nasal itching was scored as: without nasal itching (0), or with nasal itching (1). Loss of sense of smell was scored as: normal sense of smell (0), weak sense of smell (1), intermediate smell between 1 and 3 (2), or no sense of smell (3). The nasal symptom scores of two allergic rhinitis groups are shown in table 2.

Table 1. – Characteristics of study subjects

	Healthy subjects	Allergic rhinitis IgE ð250	Allergic rhinitis IgE >250
Age yrs	31.5±2.2	31.2±3.5	26.6±2.7*
Sex M/F	6:5	11:6	10:9
Total serum IgE IU·mL^{-1}	37.3±11.5	124±15.6	531±43.9
FVC % pred	122±2.7	115±3.0	106±2.3*†
FEV_1 % pred	114±3.0	105±2.9*	98.4±2.7*
FEV_1/FVC	89.5±1.3	86.8±1.0	88.3±1.7

Values are means±SEM. M: male; F: female; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second. *: $p<0.05$ compared with the values of the healthy subjects; †: $p<0.05$ compared with the values of the allergic rhinitis patients with a low immunoglobulin (Ig)E level. Differences between means were analysed by one-way analysis of variance (ANOVA) followed by Scheffé's test and the Student's t-test.

Table 2. – Nasal symptom score

	Low IgE group	High IgE group
Rhinorrhoea	2.0±0.3	1.5±0.3
Nasal blockage	1.4±0.2	1.7±0.2
Sneezing	1.1±0.3	1.4±0.3
Nasal itching	0.3±0.2	0.3±0.1
Loss of sense of smell	0.8±0.3	0.5±0.2
Total	5.5±0.8	5.4±0.9

Values are means±SEM. IgE: immunoglobulin E. Total shows the sum of all scores of nasal symptoms.

Statistical analysis

Data are expressed as means±SEM. Comparisons of mean data among groups were performed by one-way analysis of variance (ANOVA) followed by Scheffé's test and Student's t-test for unpaired data as *post hoc* tests. A linear regression analysis was performed using the method of least squares. A p-value <0.05 was considered significant.

Results

Baseline FEV_1 values (% pred) of healthy and allergic rhinitis subjects are shown in figure 1a. All values were within normal limits but the values were significantly decreased according to the increase in serum IgE levels ($p<0.01$). Age and sex did not have a significant relationship with the %FEV_1 values (data not shown). Figure 1b shows the %FEV_1 values after oxitropium bromide inhalation. These values also significantly decreased as serum IgE levels increased ($p<0.05$), but the slope of the regression line became less steep after oxitropium bromide inhalation compared with that before oxitropium bromide inhalation; the slope of the regression line for FEV_1 before and after oxitropium bromide was -0.021±0.007 and -0.017±0.007, respectively.

The anticholinergic agent oxitropium bromide increased the FEV_1 in all subjects. The degree of increase in FEV_1 was greater in allergic rhinitis subjects with a high IgE titre (155±20 mL) than in healthy subjects (64±21 mL) and allergic rhinitis subjects with a low IgE titre (82±21 mL) (fig. 2a). In contrast, the effect of the β_2-adrenergic agent orciprenaline sulphate on FEV_1 was the

same in all groups (fig. 2b). The increases in FEV1 values after inhalation of the β_2-adrenergic agent were 66±37 mL (in healthy subjects), 74±47 mL (in low IgE titre allergic rhinitis) and 91±32 mL (in high IgE titre allergic rhinitis).

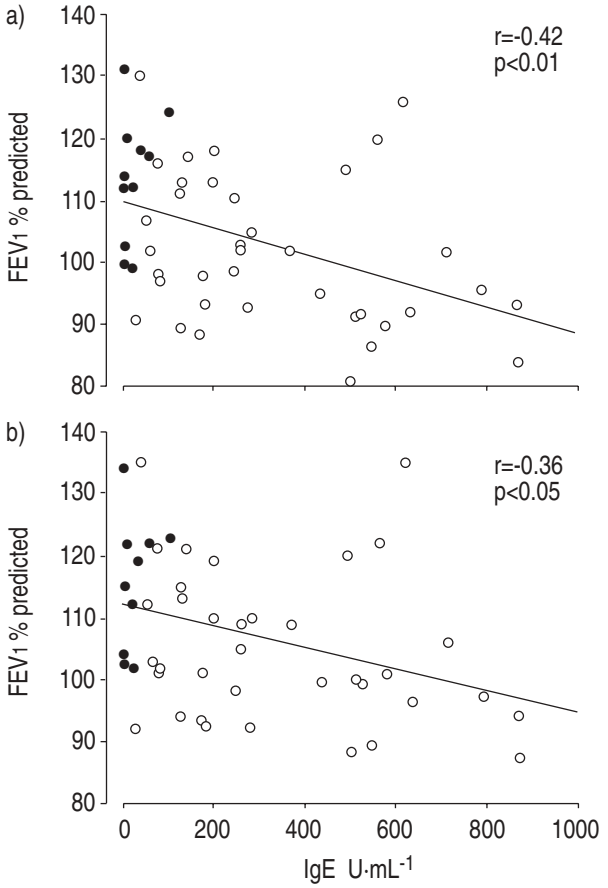

Fig. 1. – Relationship between total serum immunoglobulin (Ig)E and forced expiratory volume in one second (FEV1 in % predicted) for a) before and b) after anticholinergic agent inhalation. ●: healthy subjects; ○: patients with allergic rhinitis. r: correlation coefficient; the line and p-value correspond to the fitted regression equation for all subjects.

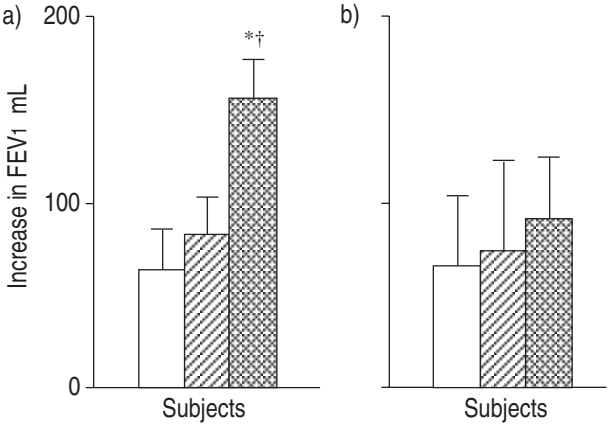

Fig. 2. – Changes in forced expiratory volume in one second (FEV1) by a) anticholinergic and b) β_2-adrenergic agent. All values are means± SEM. ☐: healthy subjects; ▨: allergic rhinitis with low immunoglobulin (Ig)E; ▩ : allergic rhinitis with high IgE. *: $p<0.05$ compared with the values of healthy subjects; †: $p<0.05$ compared with the values of the allergic rhinitis patients with normal IgE level. Differences between means were analysed by one-way analysis of variance (ANOVA) followed by Scheffé's test and Student's t-test.

Discussion

The results demonstrate that the serum IgE levels were negatively correlated with the %FEV1 values in healthy and allergic rhinitis patients without lower airways diseases. In addition, the anticholinergic agent-induced bronchodilator effect was greater in subjects with high serum IgE levels than in those with low IgE levels, suggesting that serum IgE may affect the airway calibre *via* cholinergic mechanisms in humans *in vivo*.

In the present study, the degree of β_2-adrenergic agent-mediated bronchodilation was not significantly different among the three groups (healthy subjects and allergic rhinitis patients with low and high serum IgE), while the responses to the anticholinergic agent differed according to the serum IgE titres. The bronchodilatory effect of anticholinergic agents is specific to constrictor responses involving cholinergic pathways [2, 10]: these agents are not particularly effective against an allergen challenge, do not block the late response [11] and do not inhibit the release of mediators from mast cells [12]. In contrast, β_2-adrenergic agents inhibit bronchoconstriction irrespective of the spasmogen involved [10]. Therefore, it seems likely that the cholinergic pathway itself is affected by serum IgE titre.

There are several possible explanations for the hyperreactivity of the airway cholinergic mechanisms observed in the present study. Recently, it has been reported that incubation with IgE can enhance the electrically stimulated vagal nerve-mediated contractile response without affecting the responses to exogenously applied acetylcholine in human airways *in vitro* [5]. In the tissues incubated with IgE, acetylcholine release from cholinergic nerves after electrical stimulation also showed a greater increase, and muscarinic M_2-receptor-mediated inhibition of acetylcholine release from vagal nerves was diminished, indicating that IgE can cause cholinergic hyperresponsiveness in human airways by facilitating neurotransmission through the cholinergic efferent nervous pathway, presumably *via* autoreceptor M_2 dysfunction. This mechanism, which was observed in the *in vitro* study, may also work in the present *in vivo* study.

Another explanation for the cholinergic hyperresponsiveness is the facilitation of the cholinergic hyperresponsiveness reflex, possibly due to nasal sensory nerve activation [1]. Because the patients with allergic rhinitis participating in the present study had nasal symptoms in all cases, an increase in nasal afferent receptor discharge by the inflammatory mediators may be involved in the mechanisms. However, in patients with allergic rhinitis, the nasal symptoms were not significantly different between the groups with low and high IgE (table 2). It has been reported that nasal sensory nerve activation does not influence respiratory tract calibre [13, 14]. Therefore, it is unlikely that this mechanism is involved in the cholinergic hyperresponsiveness observed in patients with high IgE levels.

An alternative explanation for the cholinergic hyperresponsiveness is an increase in airway responsiveness to acetylcholine. A clinical report suggested that, even without asthma, subjects with allergic rhinitis show bronchial hypersensitivity to cholinergic agents compared with healthy subjects [15]. A basic report has also shown that IgE

enhances airway smooth muscle contractility to spasmogen in guinea-pigs *via* direct interaction between the IgE and airway smooth muscle cell membrane potential [16]. More directly, it has been reported that the airway responsiveness to the cholinergic agent methacholine is very strongly linked to the serum IgE levels in subjects who have no atopic history [17]. This evidence suggests that IgE-mediated cholinergic hyperresponsiveness may be due to the increase in airway smooth muscle responsiveness to acetylcholine. Further study is needed to clarify this issue.

It has been reported that the serum IgE level is negatively correlated with airway calibre in patients with lower airway diseases [3, 4]. In the present study, it was shown that this is also the case in the subjects without lower respiratory tract diseases. As mentioned above, the cholinergic component seems to be involved in this phenomenon. However, even after pretreatment with the anticholinergic agent, %FEV1 values were still negatively correlated with the serum IgE levels, although the correlation between the %FEV1 and the IgE values was weaker than that of the values without anticholinergic agent administration. Taken together, serum IgE may enhance airway smooth muscle tone not only *via* cholinergic mechanisms but also through other mechanisms.

In conclusion, this study has demonstrated that higher serum immunoglobulin levels correlate with lower values of the forced expiratory volume in one second in healthy and allergic rhinitis patients, and that an anticholinergic agent but not a β_2-adrenergic agent caused a more pronounced bronchodilatory effect in subjects with high levels than in those with low levels of immunoglobulin E. These data indicate that immunoglobulin E itself may affect the airway tone *via* cholinergic hyperresponsiveness. This *in vivo* study and the previous reports suggest that the facilitation of acetylcholine release from the vagal nerve efferent pathway [5] and/or the increased responsiveness to acetylcholine at the airway smooth muscle [15–17] are the likely causes. The precise mechanisms, whether the effect of immunoglobulin E on the hyperresponsiveness is receptor-operated or not, and what kind of immunoglobulin E fragment, such as Fc or Fab, is involved in the phenomenon, remain to be elucidated.

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