Eur J Clin Pharmacol (2002) 58: 171–176
DOI 10.1007/s00228-002-0468-z

CLINICAL TRIALS

**Takanobu Shioya · Masahiro Satake · Masaaki Sano
Manabu Kagaya · Akiko Watanabe · Kazuhiro Sato
Takeshi Ito · Nobuaki Ito · Masahiro Sasaki
Mamoru Miura**

Effect of suplatast tosilate, a Th2 cytokine inhibitor, on cough variant asthma

Received: 10 December 2001 / Accepted in revised form: 22 March 2002 / Published online: 18 May 2002

Abstract *Study objective*: Th2 cytokines play an important role in the pathogenesis of asthma. Our study objective was to determine the effect of suplatast tosilate, a Th2 cytokine inhibitor, on patients with cough-variant asthma.
Methods: Twenty patients with cough-variant asthma (CVA) were assigned to a suplatast tosilate (100 mg three times daily) group or a placebo group for 6 weeks in a double-blind randomized study. The cough scores, medication scores, pulmonary function, bronchial hyperresponsiveness to methacholine, cough threshold for capsaicin, percentage of eosinophils and concentrations of eosinophilic cationic protein (ECP) in hypertonic saline-induced (induced) sputum were evaluated. The main outcome measures were capsaicin cough threshold and concentrations of ECP in induced sputum.
Results: In the suplatast group, the cough scores and the medication scores decreased significantly over time. The percentage of eosinophils in induced sputum significantly decreased from $53.5 \pm 5.6\%$ to $13.6 \pm 2.6\%$. The cough threshold for capsaicin improved significantly from 2.72 ± 3.41 μM to 39.7 ± 22.7 μM in the suplatast group. The concentrations of ECP in induced sputum decreased significantly from 435 ± 123 μg/l to 56 ± 34 μg/l in the suplatast group. The bronchial responsiveness to methacholine changed from 8.45 ± 3.43 units to 11.4 ± 3.76 units in the suplatast group.
Conclusions: Suplatast improved the cough scores and the cough threshold for capsaicin in patients with CVA without significant side effects, suggesting the effectiveness of suplatast in the treatment of CVA. Suplatast also decreased the percentage of eosinophils and concentrations of ECP in induced sputum, suggesting improvement in eosinophilic inflammation in patients with CVA. Further pharmacodynamic research is needed to explain the precise mechanism.

Keywords Cough-variant asthma · Cough threshold · Capsaicin

T. Shioya (✉) · M. Satake
Department of Physical Therapy, Akita University College of Allied Medical Science, 1-1-1 Hondo, Akita, 010-8543, Japan
E-mail: shioya@hos.akita-u.ac.jp
Tel.: +81-18-8341111
Fax: +81-18-8846500

M. Sano · M. Kagaya · A. Watanabe · K. Sato · T. Ito
N. Ito · M. Sasaki · M. Miura
The Second Department of Internal Medicine, Akita University School of Medicine, Akita, Japan

Introduction

Cough is one of the main symptoms of respiratory disease. It had been reported that a chronic persistent cough is the only manifested clinical symptom in patients with cough-variant asthma (CVA) [1, 2]. Episodic wheezing is one of the criteria of bronchial asthma, but there is no history of wheezing in CVA patients [1, 2]. Although the precise mechanism responsible for the induction of cough in CVA patients is not clear, bronchodilators such as β2-agonist and theophylline are effective agents in relieving cough [3, 4].

Recent research has clarified that CVA is related to eosinophilic inflammation such as classic asthma [5, 6]. Airway inflammation in asthma has been shown to be associated with persistent infiltration of activated CD4 T lymphocytes with a Th2-like cytokine profile [5]. A possible immunopathological role for Th2 cells in asthma has been postulated based on the part that interleukin (IL)-4, IL-5, and IL-13 play in the stimulation of IgE production, mucosal mastocytosis, and eosinophilia. Evidence of the Th2 cytokines in allergen-induced airway responses has come from manipulation of IL-4 and IL-5 with antibody blockade [6, 7, 8] or gene targeting [8, 9, 10].

Suplatast tosilate (suplatast) ((±)-[2-[4-(3-ethoxy-2-hydroxy-propoxy) phenylcarbamoyl] ethyl] dimethylsulfonium p-toluenesulfonate) is a selective Th2 cytokine inhibitor [11] that suppresses the synthesis of IL-4 and IL-5 in vitro [12, 13] and allergen-induced increases in peritoneal eosinophils in mice [13]. Recently, suplatast has been reported to be effective in the treatment of mild

asthma [14] and in allowing for a dose reduction of inhaled corticosteroids [15], but the effectiveness of suplatast in the treatment of CVA has not been clearly demonstrated. Here we report that suplatast improved the cough scores and cough threshold for capsaicin and induced a reduction in the percentage of eosinophils and eosinophilic cationic protein (ECP) concentrations in hypertonic saline-induced (induced) sputum, suggesting improvement in eosinophilic inflammation in cases of CVA.

Patients and methods

Patients

We studied 20 patients with CVA. The patients were referred to our clinic for chronic cough persisting for longer than 8 weeks but without wheezing or dyspnea. They had no past history of asthma or other respiratory disease. Wheeze or rhonchi were not audible on chest auscultation even at forced expiration. The subjects all had bronchial hyperresponsiveness to inhaled methacholine. Bronchodilators (inhaled β2-agonists and/or oral sustained-release theophyllines) were effective against these patients' coughs. No other apparent causes of cough were present [3, 4]; these patients did not have any signs or symptoms of postnasal drip or gastroesophageal reflux, had not been taking angiotensin-converting enzyme inhibitors, and had normal chest radiograms. Each patient with CVA had been treated with inhaled β2-agonists (used as needed, and this was calculated as the medication points). Treatment with β2-agonists was withheld for 8 h prior to the tests of bronchial responsiveness or pulmonary function. None of the studied subjects had ever taken systemic corticosteroids, cromoglycate, or other anti-allergic agents, and none had smoked within the previous 8 weeks. The study was approved by the ethics committee of our institute, and written informed consent was obtained from all participating subjects.

Measurement of bronchial hyperresponsiveness and cough hyperresponsiveness

Bronchial response to inhaled methacholine (MCh) was assessed with an Astograph (TCK-6100H, Chest, Tokyo, Japan) for measurement of bronchial hyperresponsiveness (BHR). This device uses the forced-oscillation method to measure respiratory resistance and its reciprocal conductance during tidal breathing [16]. Bronchial sensitivity to MCh was expressed as the geometric mean of the lowest concentration of MCh associated with the start of a consistent decrease in conductance (Dmin, in mg/ml (units) of MCh inhalation). Bronchial responsiveness to MCh was expressed as the decrease rate of respiratory conductance (in l/s/cmH2O/min). Cough hyperresponsiveness (CHR) was evaluated using the cough threshold for capsaicin according to the method of Midgren et al. [17, 18]. Briefly, capsaicin (Sigma-Aldrich, St. Louis, Mo., USA) was dissolved in ethanol and diluted with 0.9% NaCl to 0.016, 0.08, 0.4, 2, 10, 50, and 250 μM. Capsaicin was inhaled during tidal breathing from a nebulizer (Nissho, Tokyo, Japan, output 0.5 ml/min, mean mass diameter 5 μm). Concentrations of capsaicin were increased until the patient coughed more than five times. The final concentration was taken as the cough threshold for capsaicin (Ccap, μM) [17, 18].

Measurement of cough scores and medication scores

The patients evaluated their coughing four times a day, every 6 h, and recorded the cough points in a diary. The evaluations were "quite often" (21 times or more) – four points, "often" (11–20 times) – three points, "relatively often" – (six–ten times) – two points, "not often" (five times or fewer) – one point, and "none" – zero points. The total cough points in a day became the cough score.

Inhalation of the β2-agonist (procaterol hydrochloride, 10 μg per puff) was also evaluated according to the standard set by the Japanese Society of Allergology, by counting each inhalation as 0.5 point [18, 19]. Total inhalation points in a day were evaluated as the medication score.

Measurement of ECP

Hypertonic saline-induced (induced) sputum was collected in a silicone-containing tube (SST tube, Becton Dickinson, Mountain View, Calif., USA) and stored at –20°C until measurement. One milliliter of sputum was homogenized and centrifuged at 1300 *g* at 4°C for 10 min, and then the supernatants were analyzed for ECP using a radioimmunoassay kit (Pharmacia Diagnostics, Uppsala, Sweden). The detection limit of this kit is 2.0 μg/l [20].

Study design

During a 2-week run-in period (baseline), patients were only allowed to take inhaled β2-agonist to treat their coughing. In the 6-week double-blind treatment period, we randomly assigned patients to commercially available suplatast tosilate (IPD, CAS 94055–76–2; Taiho Pharmaceutical Co. Ltd., Japan; 100 mg per capsule three capsules daily) group or a placebo (identical in taste and appearance to suplatast tosilate) group. Randomization was performed by one author (T.S.) who also held the subjects to s predetermined schedule and who was not involved directly in the care of any patient. Drugs were given to the patients by pharmacy personnel of the hospitals who were unaware of the study and hence did not know who was receiving what drug. The code was not broken during the study.

Patients were allowed to take procaterol from a metered-dose inhaler (10 μg per dose). All patients visited an outpatient clinic every 2 weeks throughout the study. On the first visit, we recorded demographic details. Each patient recorded in a booklet all medications taken throughout the study, as well as symptoms (cough) of asthma, morning and evening peak expiratory flow rate (PEFR, best of three attempts before taking medication), and the use of supplemental β2-agonist inhalation. At each visit, physicians recorded changes in medication, other illness, and asthma exacerbations, and they calculated the diurnal variation in peak flow (highest evening value minus lowest morning value as a percentage of the highest), which reflects bronchial reactivity.

At the end of the baseline period, and at weeks 2, 4, and 6 of treatment, forced vital capacity (FVC) and forced expiratory volume in 1s (FEV1) were recorded using a Chestac-55V (Chest, Tokyo, Japan). The percentage of eosinophils and ECP concentrations in induced sputum were measured at the baseline period and week 6.

To assess safety, we did hematological, biochemical, and urine analyses, and a 12-lead resting electrocardiogram (ECG) every 2 weeks during the baseline and treatment periods. Adverse events were recorded daily and were reviewed by the physician at every study-related visit to our institution.

Statistical analysis

All variables were analyzed for change from the reference value. We used Student's *t*-test to analyze within and between groups, and P less than 0.05 was taken as statistically significant.

Results

Patients' baseline characteristics are given in Table 1. Distribution of sex, age, FVC, FEV1, blood eosinophils,

Table 1. Patient characteristics. *FVC* forced vital capacity, *FEV1* forced expiratory volume in 1 s, *FEV1%* FEV1 as a percent of FVC (FEV1/FVC), *Ccap* capsaicin cough threshold, *Dmin* geometric mean of the lowest concentration of methacholine associated with the start of a consistent decrease in conductance [in mg/ml (units) of inhalation], *WBC* white blood cells

	Placebo ($n=10$)	Suplatast ($n=10$)
Sex (male/female)	3/7	3/7
Mean age (years)	45.2 ± 5.9 (22–69)	44.1 ± 5.2 (24–65)
FVC (l)	3.24 ± 0.85	3.35 ± 0,93
FEV1 (l)	2.74 ± 0.68	2.83 ± 0.76
FEV1/FVC (%)	82.5 ± 2.31	84.1 ± 2.55
WBC (/μl)	6891 ± 421	7162 ± 510
Blood eosinophils (%)	8.9 ± 2.8	8.1 ± 3.5
Serum IgE (IU/ml)	479 ± 323	458 ± 362
Dmin (units)	8.45 ± 3.43	9.18 ± 3.52
Ccap (μM)	2.74 ± 3.41	2.52 ± 3.53

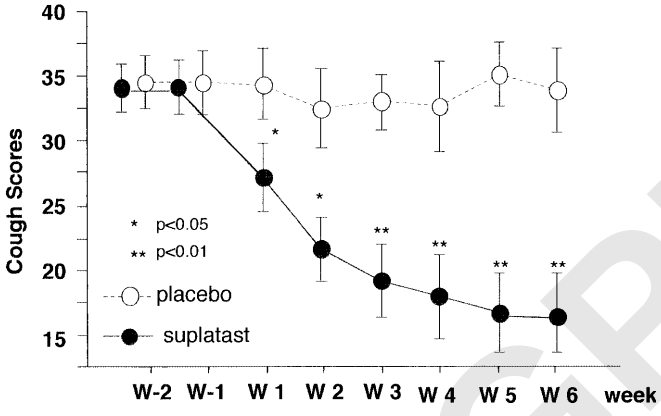

Fig. 1. Changes in cough scores. The cough scores in the suplatast group were significantly decreased at week 1, week 2, week 3, week 4, week 5, and week 6 in comparison with week –2 (*$P<0.05$, **$P<0.01$)

serum IgE, BHR (Dmin), and cough hyperresponsiveness (Ccap) did not differ significantly between the two groups.

The progressive improvement of cough scores was significant in the suplatast group (Fig. 1). In the suplatast group, the cough scores were decreased significantly (24.5 ± 6.1 at week 1, 19.8 ± 5.5 at week 2, 17.1 ± 6.2 at week 3, 16.3 ± 5.8 at week 4, 15.6 ± 6.5 at week 5, 14.4 ± 5.5 at week 6) in comparison with the value of 33.4 ± 4.8 at week –2, whereas in the placebo group there was no significant difference in change from the baseline. In the suplatast group, the medication scores decreased significantly (7.5 ± 0.4 at week 2, 6.9 ± 1.1 at week 3, 6.2 ± 0.6 at week 4, 6.0 ± 0.5 at week 5, 5.8 ± 0.4 at week 6) in comparison with the values of 8.6 ± 0.7 at week –2, whereas in the placebo group there was no significant difference in change from the baseline (Fig. 2).

The percentage of sputum eosinophils significantly decreased from 53.5 ± 5.6% to 13.6 ± 2.6% ($P<0.01$, baseline vs week 6, data are not shown). The concentrations of ECP changed significantly from 435 ± 123 μg/l to 55 ± 34 μg/l in the suplatast group ($P<0.05$, baseline vs week 6) and from 758 ± 78 μg/l to 1250 ± 480 μg/l in the placebo group ($P>0.10$, baseline vs week 6, Fig. 3).

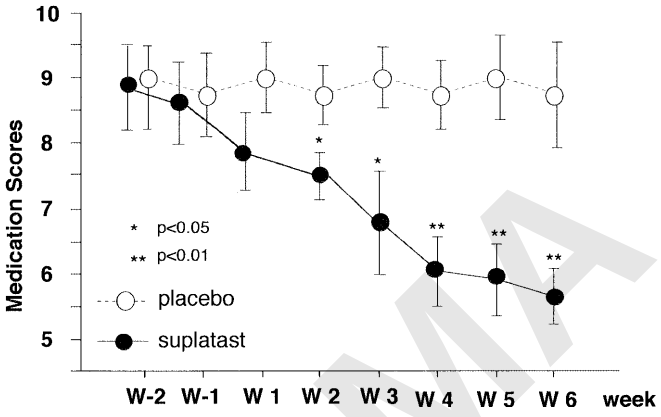

Fig. 2. Changes in medication scores. The cough scores in the suplatast group were significantly decreased at week 2, week 3, week 4, week 5, and week 6 in comparison with week –2 (*$P<0.05$, **$P<0.01$)

The cough threshold for capsaicin changed from 2.72 ± 3.41 μM to 39.7 ± 22.7 μM in the suplatast group ($P<0.05$, baseline vs week 6) and from 2.52 ± 3.53 μM to 2.48 ± 4.65 μM in the placebo group ($P>0.10$, baseline vs week 6, Fig. 4). The airway responsiveness to MCh (Dmin) changed from 8.45 ± 3.43 units to 11.4 ± 3.76 units in the suplatast group ($P>0.10$, baseline vs week 6) and from 9.18 ± 3.52 units to 8.96 ± 4.67 units in the placebo group ($P>0.10$, baseline vs week 6, Fig. 5).

The morning and evening PEFR (l/min) did not change from the baseline period in either the suplatast group or the placebo group. The same was true for the FVC and FEV1 values.

No adverse events were recorded in the study subjects' diaries. No changes from baseline values were noted with respect to the physical examination results for blood pressure, pulse rate, ECG, or the hematological, biochemical, and urinary analysis data.

Discussion

Recent research indicates that the pathophysiology of CVA is eosinophilic inflammation of the airway. Anatomic and physiologic research has shown that the cough receptors that mark the starting point of the cough reflex pathway comprise rapidly adapting stretch receptors (RASR) located mainly in the central airways, and bronchial or pulmonary c-fibers found in the peripheral airways [21]. Recently, suplatast has been shown to have immunoregulatory effects [11, 12, 13]. Suplatast inhibits IgE production and degranulation of basophils or mast cells induced by IgE, and it suppresses production of IL-4 and -5 by Th2 cells [11, 12]. Suplatast specifically inhibits the production of IgE antibodies,

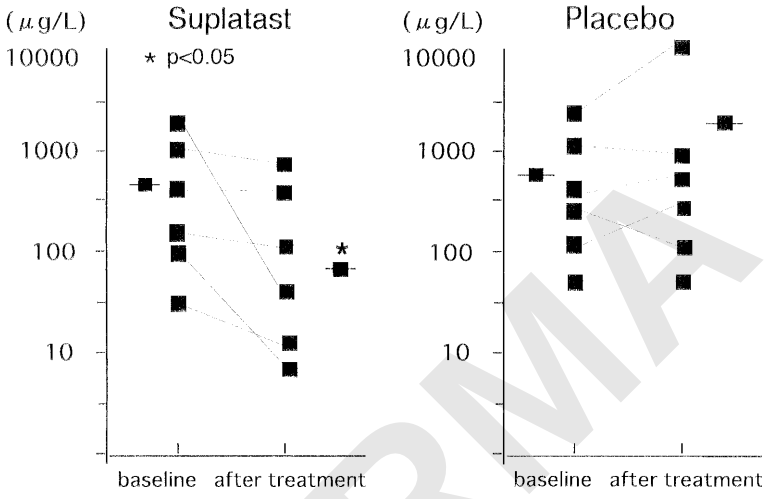

Fig. 3. Changes of eosinophilic cationic protein (ECP) in hypertonic saline-induced sputum. Concentrations of ECP in hypertonic saline-induced sputum were significantly deceased after treatment with suplatast ($P < 0.05$). The number of patients was six for each group

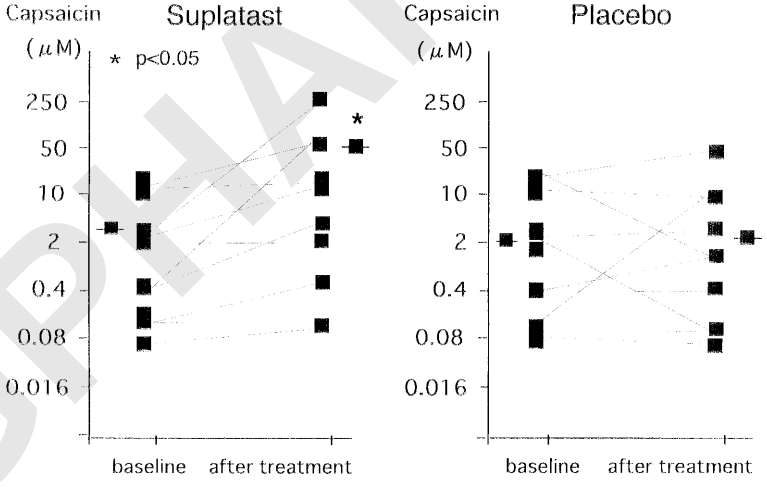

Fig. 4. Changes of cough threshold for capsaicin (Ccap). Cough threshold for capsaicin in the suplatast group was significantly decreased after treatment with suplatast ($P < 0.05$)

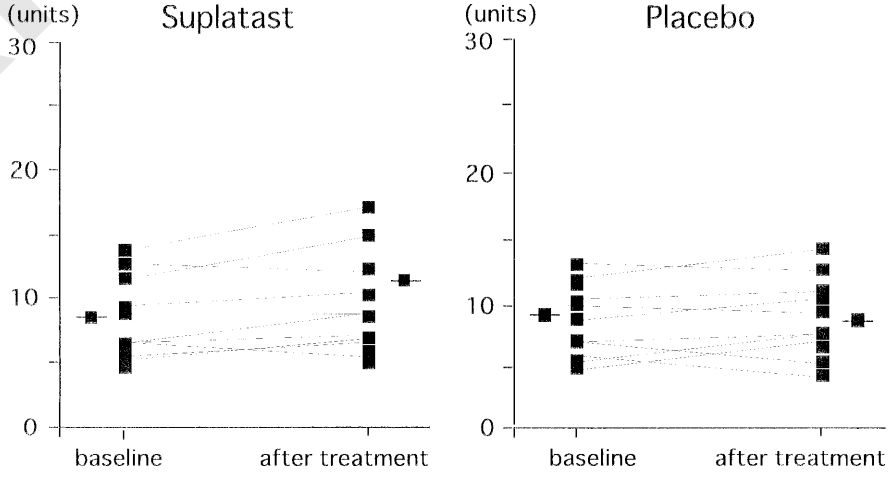

Fig. 5. Changes of bronchial responsiveness to methacholine (Dmin). Bronchial responsiveness to methacholine in the suplatast group was decreased, but these changes were not statistically significant

but not IgG and IgM, in mice, and it also inhibits eosinophil infiltration in an in vivo mouse model by suppressing Th2 cells [13]. Treatment of patients suffering from mild asthma with suplatast has confirmed that suplatast significantly reduces eosinophil infiltration in the sputum and bronchial epithelium [14]. Also, suplatast has been shown to allow a reduction in dose of inhaled corticosteroid in cases of steroid-dependent asthma [15]. Tsubura et al. [22] reported moderate improvement in 52.9% of adult bronchial asthma patients 5–6 weeks after treatment with suplatast at a dose of 300 mg/day.

In this study, treatment with suplatast, a selective Th2 cell cytokine inhibitor, markedly reduced patients' persistent cough and also resulted in significant improvement in cough and medication scores. There also was a marked reduction in the number of eosinophils and ECP concentrations in induced sputum, suggesting an improvement in eosinophilic inflammation in the patients with CVA.

Because pulmonary function is not usually reduced in patients with CVA [2, 3, 4], it is very difficult to evaluate the effect of the drug by using the usual pulmonary function data from patients with CVA [3, 4]. We used cough scores, the medication scores from patient diaries, and the capsaicin cough threshold to evaluate CHR in patients with CVA. We also used the percentage of eosinophils and the concentrations of ECP in induced sputum for the evaluation. Administration of suplatast improved significantly the cough scores and the medication scores 1 week after treatment with suplatast, and the cough threshold for capsaicin improved significantly 6 weeks after treatment. These data suggest that the evaluation of cough scores and medication scores as well as the cough threshold for capsaicin are good indicators for evaluation of the severity CVA.

Ccap, an objective indicator of CHR, improved significantly from 2.72 μM to 39.7 μM after treatment with suplatast. Dmin, an objective indicator of BHR, changed from 8.45 units before treatment to 11.4 units after treatment with suplatast, but these changes were not statistically significant, suggesting that suplatast may have improved CHR and but not BHR in patients with CVA. Recently, researchers suggested that cough responsiveness and bronchial responsiveness are independent factors from each other [23, 24]. Our results supported this hypothesis and suggest that suplatast may improve CHR more than BHR by inhibiting eosinophilic inflammation of the cough RASR itself. However, further basic research is also needed to explain the precise mechanism by which suplatast inhibits cough in patients with CVA.

Suplatast appeared to reduce eosinophilic airway inflammation by suppressing the production of Th2 cell-derived inflammatory cytokines such as IL-4 and -5, as suggested by the marked reduction in the percentage of eosinophils and ECP concentrations in induced sputum observed in our patients. However, inflammatory cytokines such as IL-4 and -5 in the sputum need to be evaluated to verify this hypothesis. The usefulness and mechanism of action of suplatast in relation to cough in CVA should be explored in more detail by conducting a large-scale, case-controlled study of the drug at multiple institutions to investigate CHR and BHR and inflammatory cytokines in patients with CVA.

Acknowledgements This work was supported in part by a Grant-in-Aid for Scientific Research C (12670546) from the Ministry of Education, Science and Culture, Japan. The authors thank Prof. T. Suzuki and Dr. E. Sato, Department of Pharmacy, Akita University Hospital, for preparing capsaicin for this study.

References

1. Glauser FL (1972) Variant asthma. Ann Allergy 30:457–459
2. Corrao WM, Braman SS, Irwin RS (1979) Chronic cough as the sole presenting manifestation of bronchial asthma. N Engl J Med 300:633–637
3. Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F (1998) Eosinophilic inflammation in cough variant asthma. Eur Respir J 11:1064–1069
4. Irwin RS, Boulet L-P, Cloutier MM et al (1998) Managing cough as a defense mechanism and as a symptom. A consensus panel report of the American College of Chest Physicians. Chest 114:133S–181S
5. Kon OM, Kay AB (1999) T cell and chronic asthma. Int Arch Allerg Immunol 118:133–135
6. Kung TT, Stelts DM, Zurcer JA (1995) Involvement of IL-5 in a murine model of allergic pulmonary infiltration: prophylactic and therapeutic effect of anti-IL-5 antibody. Am J Respir Cell Mol Biol 13:360–365
7. Coyle AJ, Legros G, Berant C (1995) Interleukin-4 is required for the induction of Th2 mucosal immunity. Am J Respir Cell Mol Biol 13:54–59
8. Kips JC, Tournoy KG, Pauwels RA (2001) New anti-asthma therapies: suppression of the effect of interleukin (IL)-4 and IL-5. Eur Respir J 17:499–506
9. Brusselle G, Kips J, Joos G, Bluethmann H, Pauwels R (1995) Allergen-induced airway inflammation and bronchial responsiveness in wild-type and interleukin-4 deficient mice. Am J Respir Cell Mol Biol 12:254-259
10. Foster PS, Hogan SP, Ramsay AJ, Martthaei KI, Young IG (1996) Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity and lung damage in a mouse asthma model. J Exp Med 183:195–210
11. Koda A, Yanagihara Y, Matsuura N (1991) IPD = 1151T: a prototype drug of IgE antibody synthesis modulation. Agents Actions Suppl 34:369–378
12. Yamaya H, Basaki Y, Togawa M, Kojima M, Kiniwa M, Matuura N (1995) Down-regulation of Th2 cell mediated murine peritoneal eosinophilia by antiallergic agents. Life Sci 19:1647–1654
13. Yanagihara Y, Kiniwa M, Ikizawa K, Shida T, Matsuura N, Koda A (1993) Suppression of IgE production by IPD-1151T (Suplatast Tosilate),a new dimethylsulfonium agent: regulation of human IgE response. Jpn J Pharmacol 61:31–39
14. Sano Y, Miyamoto T, Makino S (1997) Anti-inflammatory effect of suplatast tosilate on mild asthma. Chest 12:862–863
15. Tamaoki J, Kondo M, Sakai N, Aoshiba K, Tagaya E, Nakata J, Isono K, Nagai A (2000) Effect of suplatast tosilate, a Th2 cytokine inhibitor, on steroid-dependent asthma: a double blind randomized study. Lancet 356:273–278
16. Takishima T, Hida W, Sasaki H, Suzuki S, Sasaki T (1981) Direct-writing recorder of the dose–response curves of the airway to methacholine, clinical application. Chest 80:600–606
17. Midgren B, Hansson L, Karlsson J-A, Simonsson BG, Persson CGA (1992) Capsaicin-induced cough in humans. Am Rev Respir Dis 146:347–351
18. Shioya T, Ito N, Sasaki M, Kagaya M, Sano T, Shindo T, Kashima M, Miura M (1996) Cough threshold capsaicin increases by azelastine in patients with cough variant asthma. Pulm Pharmacol 9:59–62
19. Japanese Society of Allergology (1995) Committee on the definition, treatment and management of bronchial asthma. Guidelines for the diagnosis and management of bronchial asthma. Allergology 50[Suppl]:1–42
20. Venge P (1993) Serum measurement of eosinophilic cationic protein (ECP) in bronchial asthma. Clin Exp Allergy 23[Suppl 2]:3–7
21. Widdicombe JG (1995) Neurophysiology of the cough reflex. Eur Respir J 8:1193–1202

22. Tsubura E, Kobayashi S, Makino S (1992) Clinical evaluation of IPD-1151T on adult patients with bronchial asthma (in Japanese). Pharmacol Ther 20:3221–3242
23. Fujimura M, Sakamoto S, Kamio Y, Matsuda T (1992) Cough receptor sensitivity and bronchial responsiveness in normal and asthmatic subjects. Eur Respir J 5:291–295
24. Fujimura M, Kamio Y, Hashimoto T, Matsuda T (1994) Cough receptor sensitivity and bronchial responsiveness in patients with only chronic non-productive cough in view of effect of bronchodilator therapy. J Asthma 31:463–472