Hiroshima J. Med. Sci.
Vol. 48, No. 4, 105~110, December, 1999
HIJM 48-18

Pranlukast, a Cysteinyl Leukotriene Antagonist, Reduces Serum Eosinophil Cationic Protein Levels in Patients with Asthma

Shinichi ISHIOKA, Soichiro HOZAWA, Yoshinori HARUTA, Keiko HIYAMA, Akihiro MAEDA and Michio YAMAKIDO

Second Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan.

ABSTRACT

Cysteinyl leukotrienes (cysLTs) are considered to be important mediators involved in bronchial asthma and the ensuing eosinophilic inflammation. We evaluated the effects of pranlukast, a potent and selective cysLT receptor antagonist, on the clinical course and serum eosinophil cationic protein (ECP) levels of 10 asthmatic patients. A four-week administration of pranlukast increased the morning peak expiratory flow (PEF) ($p=0.007$) and decreased as-needed β_2-agonist use ($p=0.021$). Changes in the morning PEF inversely correlated with those in the serum ECP levels ($r=-0.80$, $p=0.0057$). These results suggest that cysLTs are important mediators involved in eosinophilic inflammation, a major pathophysiologic feature of bronchial asthma in humans.

Key words: *Bronchial asthma, Cysteinyl leukotriene receptor antagonist, Pranlukast, Serum eosinophil cationic protein*

Regardless of the type or severity, the underlying pathologic basis of bronchial asthma has been most recently characterized by chronic airway inflammation[20,34]. Airway inflammation arises from many factors, including chemical mediators generated by resident airway cells and recruited leukocytes. In particular, cysteinyl leukotrienes (cysLTs) including LTC4, LTD4, and LTE4, metabolites of arachidonic acid via the 5-lipoxygenase pathway, make up the material known as slow-reactive substance of anaphylaxis (SRS-A). This was previously considered to play an important role in the pathophysiology of bronchial asthma[6,27]. Moreover, cysLTs are potent mediators that induce bronchoconstriction[1,4,8], airway microvascular leakage, edema[39], mucus secretion[19] and eosinophil recruitment to the airway[9,16]. Since pathophysiologic features of bronchial asthma can be explained by the action of cysLTs, cysLTs are considered to be the most important mediators involved in bronchial asthma. Therefore, the anti-asthmatic drugs that alter the action or production of cysLTs are expected to show therapeutic potential.

Pranlukast is a novel, potent and selective cysLT receptor antagonist which is orally active[24]. It has been shown to inhibit LTC4-and LTD4-induced bronchial contractions in guinea pigs[24] and humans[23,40], and also inhibit antigen-induced bronchoconstriction in guinea pigs sensitized with ovalbumin[24], and to attenuate allergen-induced early and late phase bronchoconstrictions in asthmatic patients[14,31]. Furthermore, pranlukast has been shown to antagonize LTD4-induced microvascular leakage and eosinophilic influx into the airways in guinea pigs[35]. Moreover, the anti-asthmatic effects of pranlukast have been confirmed clinically[5,13,30].

Eosinophilic infiltration of the asthmatic airway is the most characteristic finding of bronchial asthma that differentiates asthma from other inflammatory airway diseases. Eosinophils are considered, therefore, to be the primary effector cells causing airway inflammation in bronchial asthma[7,10]. Activated eosinophils are the major cells that produces cysLTs[17,29] and which release granule proteins such as major basic protein (MBP) and eosinophil cationic protein (ECP), thereby damaging the airway epithelium[12,29]. Eosinophilic inflammation is considered to be a major component of airway inflammation in bronchial asthma. In recent years, serum ECP levels have been reported to reflect the presence of primed eosinophils and to serve as a clinical parameter for predicting the degree of eosinophil activation[36]. They are reported also to be a good marker for evaluating eosinophilic inflammation in bronchial asthma[3,21]. To date, few reports

Address correspondence to: Shinichi Ishioka, MD, Second Department of Internal Medicine, Hiroshima University School of Medicine, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan.
Tel: (81) 82–257–5196, FAX: (81) 82–255–7360

Table 1. Clinical characteristics of subjects

No.	Age (yr)	Sex	Type[a]	Severity	Eos[b] (%)	IgE[c] RIST (IU/ml)	IgE[c] RAST	Complication[d]	Treatment[e] BDP (μg/d)	Treatment[e] Theo (mg/d)	Treatment[e] PSL (mg/d)
1	59	F	Ext	Moderate	5.0	645.8	None	AR	300	800	–
2	74	M	Ext	Moderate	11.2	621.7	JC	AR	300	–	–
3	48	F	Ext	Moderate	4.7	150.0	JC	AR	200	200	2.5
4	27	M	Ext	Mild	4.8	100.2	JC	AR	–	400	–
5	21	M	Ext	Mild	13.3	941.9	HD, mite, JC	AR	–	–	–
6	16	F	Ext	Mild	4.5	110.7	HD, mite, JC	AR	–	400	–
7	65	F	Ext	Moderate	12.0	11.2	HD, mite	AR	400	400	–
8	54	F	Ext	Mild	4.0	213.0	JC	None	–	400	–
9	53	F	Ext	Moderate	1.9	83.0	HD, mite	None	400	400	–
10	54	F	Int	Moderate	1.1	30.4	None	None	400	200	–

[a]Ext=extrinsic, Int=intrinsic, [b]Eos=peripheral eosinophil, [c]RIST=radioimmunosorbent test, RAST=radioallergosorbent test, JC=Japanese ceder, HD=house dust, [d]AR=allergic rhinitis, [e]BDP=beclomethasone dipropionate, Theo=theophilline, PSL=prednisolone. In addition, all patients were treated with on-demand β_2-agonist inhalation.

describing the involvement of cysLTs in eosinophilic inflammation associated with bronchial asthma in humans have been published. Therefore, in order to assess the clinical contribution of cysLTs in eosinophil activation and ensuing eosinophilic inflammation in bronchial asthma in humans, we administered pranlukast, a potent and selective orally active cysLT receptor antagonist, to patients with asthma. We evaluated the relationship between the clinical efficacy of pranlukast treatment and serum ECP levels.

MATERIALS AND METHODS

Study Population

The subjects comprised 10 asthmatic patients (4 with mild and 6 with moderate asthma) aged 16 to 74 years (average 47.1 years). Three men and 6 women had extrinsic asthma and one woman had intrinsic asthma (Table 1). The forced expiratory volume in one second ($FEV_{1.0}$) of each patient improved by more than 15% after inhalation of 300 μg salbutamol sulfate. All the patients were non-smokers who developed mild wheezing and chest tightness rather than asthma attacks. These patients had been treated with as-needed β_2-agonist inhalation in addition to the following agents, without changes, for 6 months before starting this study: beclomethasone dipropionate (BDP, 200–800 μg/day) by inhalation in 6 patients, orally administered slow-release theophylline (200–800 mg/day) in 8, and orally administered prednisolone (2.5 mg/day) in one. Except in one case, corticosteroids had not been administered systemically to these patients for at least 6 months before starting the study. No patient suffered a viral infection during the 2 months prior to the start of the study. Concomitant medication was continued without modification throughout. The purpose of this study was explained carefully, after which informed consent was obtained from each patient. Furthermore, the study was approved by the ethics committee of our hospital.

Study Design

The total duration of this study was 6 weeks. Patients took 225 mg of pranlukast orally twice daily for 4 weeks after a 2-week trial period. All patients visited the outpatient clinic every two weeks and kept a booklet in which all medication was recorded daily during the course of the study. Morning and evening peak expiratory flow (PEF) values (the best of 3 attempts before taking medication) and the amount of as-needed inhaled β_2-agonist were recorded daily. The ASSESS™ peak flow meter (Health Scan Products Inc., Cedar Grove, NJ, USA) was used to measure PEF.

Measurement of Serum ECP Levels

Serum ECP levels were measured at the end of the trial and treatment periods, that is, before and after pranlukast administration. Blood samples were collected by venepuncture using an SST tube (Becton Dickinson, Tokyo, Japan), allowed to clot at room temperature for 60 min, centrifuged at 1,350 × g for 10 min; serum was collected and stored at –20°C. The ECP levels of these serum samples were measured collectively using a radioimmunoassay (RIA) kit (Pharmacia Upjohn, Tokyo, Japan).

Statistical Methods

The mean morning and evening PEF values and number of puffs of as-needed β_2-agonist were calculated every 2 weeks. The mean values of these parameters during the trial period were used as reference values. These variables, during trial and treatment periods were compared using the Wilcoxon signed rank test. The serum ECP level obtained at the end of the trial period, before starting treatment, was used as the reference value and compared with the value after treatment using the Wilcoxon signed rank test. All the

values are expressed as means ± standard errors of the mean (SEM). Differences at p values of less than 0.05 were considered to be statistically significant. In this study, treatment with pranlukast was considered to be clinically effective when the morning PEF values increased by more than 5% and the amount of β_2-agonist inhaled decreased during the 3rd and 4th weeks of the treatment period compared with the values of the parameters obtained during the trial period.

RESULTS

The Effects of Pranlukast on the PEF and As-needed Inhaled β_2-Agonist

The mean morning PEF was 356.9 ± 31.9 liters/min during the 2-week trial period, increasing significantly to 383.8 ± 28.6 liters/min during the first 2 weeks (weeks 1–2, p=0.015) and 385.6 ± 26.8 liters/min during the next 2 weeks (weeks 3–4, p=0.007) of pranlukast treatment (Fig. 1). The mean evening PEF was 400.0 ± 34.8 liters/min during the 2-week trial period and did not change significantly after pranlukast administration (415.4 ± 35.9 liters/min during weeks 1–2 and 417.7 ± 37.3 liters/min during weeks 3–4 of pranlukast treatment). As-needed use of inhaled β_2-agonist during the 2-week trial period was 15.7 ± 6.3 puffs/week and it decreased significantly to 9.1 ± 3.7 puffs/week during weeks 1–2 (p=0.028) and 9.6 ± 4.5 puffs/week (p=0.021) during weeks 3–4 of pranlukast treatment (Fig. 2).

Based on the above changes in the variables, increase of the morning PEF by more than 5% and decrease of as-needed use of inhaled β_2-agonist during weeks 3–4 of pranlukast treatment were observed in 7 of the 10 subjects. This suggested the clinical effectiveness of pranlukast.

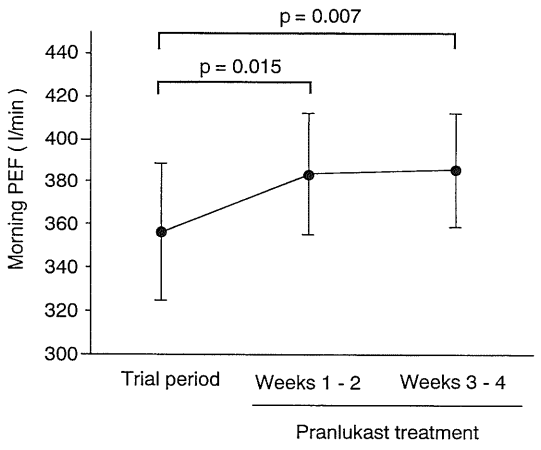

Fig. 1. Morning peak expiratory flow (PEF) before (Trial period) and after pranlukast treatment (Weeks 1–2 and Weeks 3–4). The mean morning PEF value during each 2-week period was calculated for 10 asthmatics and expressed as means ± SEM.

Effects of Pranlukast on Serum ECP Levels

The mean serum ECP level before pranlukast treatment of all 10 subjects was 14.0 ± 2.3 μg/liter and did not change significantly after treatment (11.2 ± 3.0 μg/liter). However, the mean serum ECP level of the 7 patients in whom pranlukast administration was clinically effective decreased significantly from 15.5 ± 2.8 to 8.3 ± 1.8 μg/liter after pranlukast treatment (p=0.018), while that of the 3 patients in whom pranlukast administration was not clinically effective did not change significantly after pranlukast treatment (Fig. 3).

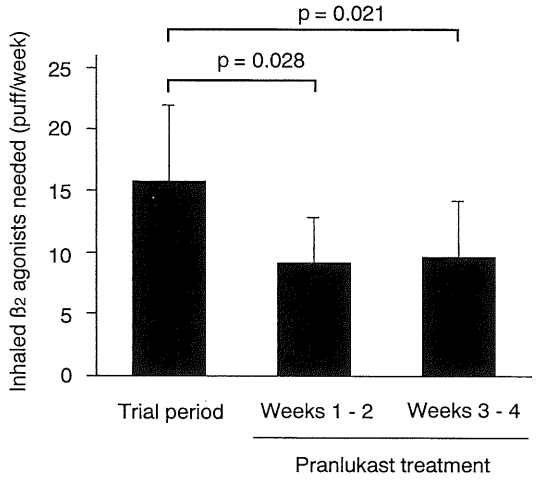

Fig. 2. The amount of inhaled β_2-agonist needed before (Trial period) and after pranlukast treatment (Weeks 1–2 and Weeks 3–4). The number of puffs of β_2-agonist needed during each 2-week period was calculated for 10 asthmatics and expressed as means ± SEM.

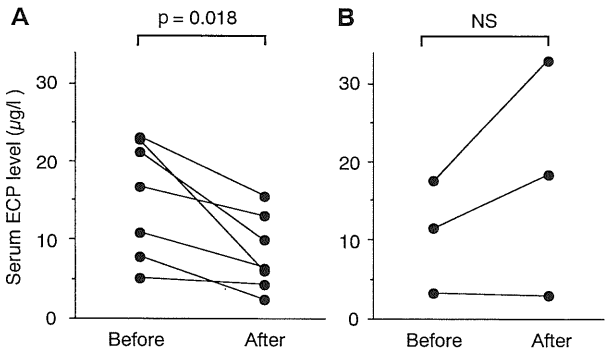

Fig. 3. Serum eosinophil cationic protein (ECP) levels before and after pranlukast treatment for 4 weeks in clinically effective patients (A, n=7) and ineffective patients (B, n=3). Pranlukast treatment was considered to be clinically effective when the morning peak expiratory flow (PEF) increased by more than 5% and the amount of inhaled β_2-agonist decreased during the 3rd and 4th weeks of the treatment period compared with the corresponding values during the trial period.

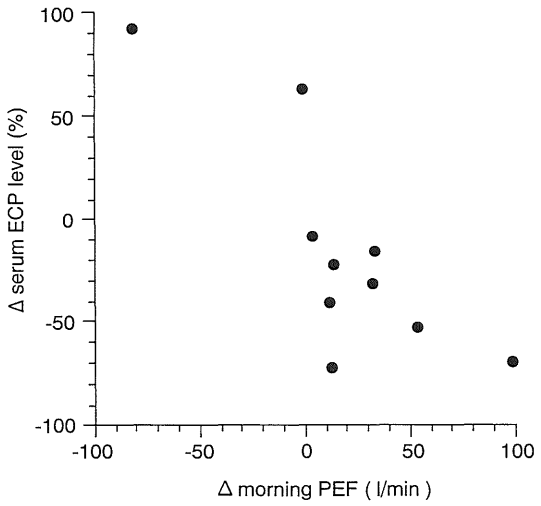

Fig. 4. Relationship between the changes in the morning peak expiratory flow (ΔPEF) and serum eosinophil cationic protein level (ΔECP) following pranlukast treatment (n=10). An inverse correlation was observed (r=–0.80, p=0.0057). ΔPEF=morning PEF after treatment-morning PEF before treatment. ΔECP=(serum ECP level after treatment-serum ECP level before treatment)/serum ECP level before treatment × 100.

Relationship between Morning PEF and Serum ECP Levels

The changes in the morning PEF (ΔPEF=morning PEF after treatment-morning PEF before treatment) and the changes in the serum ECP levels {ΔECP=(serum ECP level after treatment-serum ECP level before treatment) / serum ECP level before treatment × 100} of all 10 subjects showed a significant inverse correlation (r=–0.80, p=0.0057, Fig. 4).

DISCUSSION

In this study, pranlukast, a potent and selective cysLT receptor antagonist, exerted clinical effects in patients with asthma: pranlukast increased morning PEF and reduced the as-needed use of inhaled β_2-agonist. These clinical effects of pranlukast were related to reduced serum ECP levels. After pranlukast treatment, changes in the morning PEF inversely correlated with changes in the serum ECP levels.

Based on the results of previous animal and *in vitro* experiments, it is suggested that cysLTs are important mediators involved in bronchial asthma[8,11,17,27]. Clinically, cysLTs have been detected in plasma[25], urine[18,32], and bronchoalveolar lavage fluid (BALF)[37,38] obtained from patients with asthma attack and after antigen challenge. Moreover, inhalation of cysLTs evoked bronchoconstriction in both normal and asthmatic subjects[1,4] and increased bronchial hyperresponsiveness[15,22]. In recent years, based on the theory that cysLTs are important mediators involved in bronchial asthma, selective cysLT receptor antagonists such as pranlukast, zafirlukast, and montelukast, have been developed to control the action of endogenous cysLTs. These selective cysLT receptor antagonists inhibited allergen-induced early and late asthmatic responses (EARs and LARs)[14,33] and showed antiasthmatic effects in clinical trials[5,13,26,28,30].

Chronic airway inflammation in bronchial asthma is mainly due to eosinophilic inflammation[7,10]. It occurs even in patients with stable asthma[10], and activated eosinophils are the major cells that produce cysLTs[17,29]. In view of the importance of cysLTs in bronchial asthma, attention has been focused on the relation between cysLTs and eosinophilic inflammation. Pranlukast attenuated LTD4-induced eosinophil influx into the airways of guinea pigs[35] and biopsy analysis showed that inhalation of LTE4 by asthmatic subjects resulted in marked eosinophilic infiltration of the airways[16]. Therefore, cysLTs are considered to induce eosinophil recruitment into the airway.

Serum ECP was reported to be composed mainly of ECP released by eosinophils during the clotting process after blood collection and it reflects the releasability of peripheral primed eosinophils[36]. In asthmatic patients, it was reported that serum ECP level correlates with the ECP concentration in BALF[2]. Clinically, the serum ECP level is considered to be a good marker for evaluating eosinophil activation and ensuing eosinophilic inflammation in bronchial asthma[3,21]. Tamaoki et al.[30] reported that adding pranlukast prevented asthma deterioration and a rise in the serum ECP level provoked, in well-controlled asthmatic patients receiving high-dose inhaled corticosteroid, by a 6-week reduction of the inhaled dose by half. This was a finding compatible with our results.

In this study, we enrolled asthmatic patients with relatively mild symptoms such as mild wheezing and chest tightness, and who did not suffer asthmatic attacks. We did not include asthmatic patients with moderate to severe symptoms because the action of various chemical mediators would probably overlap in such patients. The presence of chronic airway inflammation has been observed even in patients with stable asthma[10] and various chemical mediators may be released continuously by the asthmatic airway. Some of these mediators may be involved in the development of eosinophilic inflammation resulting from activation of eosinophils. Although patients with bronchial asthma show clinically indistinguishable symptoms, patients are considered to have biochemically heterogenous diseases in which different mediators are pathophysiologically important. In this study, serum ECP levels of all 10 subjects did not decrease significantly after pranlukast administration. However, the serum ECP levels of

the 7 patients in whom pranlukast administration was clinically effective did decrease significantly. Furthermore, analysis of the whole group demonstrated a significant inverse correlation between the changes in the morning PEF and those in the serum ECP levels after pranlukast treatment. Therefore, in asthmatic patients in whom pranlukast administration is effective for controlling the action of endogenous cysLTs, cysLTs are considered to play an important role in the major pathophysiologic features of bronchial asthma, such as eosinophil activation and the ensuing eosinophilic inflammation. By contrast, in asthmatic patients in whom pranlukast administration is ineffective for controlling the action of endogenous cysLTs, mediators other than cysLTs may make a greater contribution to the pathophysiology of bronchial asthma. In future, the cloning of cysLT receptors may elucidate the detailed mechanisms responsible for the action of cysLTs on eosinophils.

CONCLUSIONS

Pranlukast, a potent and selective cysLT receptor antagonist, was administered to patients with bronchial asthma. The clinical effects were related to serum ECP level reduction, a clinical parameter for evaluating eosinophilic inflammation. The results of our study, together with previous findings, suggest that cysLTs, which exert proinflammatory effects in humans, are important mediators contributing to the main pathophysiologic features of bronchial asthma, such as eosinophil activation and the ensuing eosinophilic inflammation.

ACKNOWLEDGEMENTS

Part of this study was supported by a research grant from the Japanese Ministry of Health and Welfare.

(Received August 11, 1999)
(Accepted November 1, 1999)

REFERENCES

1. **Adelroth, E., Morris, M.M., Hargreave, F.E. and O'Byrne, P.M.** 1986. Airway responsiveness to leukotrienes C4 and D4 and to methacholine in patients with asthma and normal controls. N. Engl. J. Med. **315:** 480–484.
2. **Adelroth, E., Rosenhall, L., Johansson, S.A., Linden, M. and Venge, P.** 1990. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. The effects of antiasthmatic treatment with budesonide or terbutaline. Am. Rev. Respir. Dis. **142:** 91–99.
3. **Ahlstedt, S.** 1995. Clinical application of eosinophilic cationic protein in asthma. Allergy Proc. **16:** 59–62.
4. **Barnes, N.C., Piper, P.J. and Costello, J.F.** 1984. Comparative effects of inhaled leukotriene C4, leukotriene D4, and histamine in normal human subjects. Thorax **39:** 500–504.
5. **Barnes, N.C. and Pujet, J.C.** 1997. Pranlukast, a novel leukotriene receptor antagonist: results of the first European, placebo controlled, multicentre clinical study in asthma. Thorax **52:** 523–527.
6. **Borgeat, P. and Samuelsson, B.** 1979. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxyeicosatetraenoic acid. J. Biol. Chem. **254:** 2643–2646.
7. **Bousquet, J., Chanez, P., Lacoste, J.Y., Barneon, G., Ghavanian, N., Enander, I., Venge, P., Ahlstedt, S., Simony Lafontaine, J., Godard, P. and Michel, F.B.** 1990. Eosinophilic inflammation in asthma. N. Engl. J. Med. **323:** 1033–1039.
8. **Dahlen, S.E., Hedqvist, P., Hammarstrom, S. and Samuelsson, B.** 1980. Leukotrienes are potent constrictors of human bronchi. Nature **288:** 484–486.
9. **Diamant, Z., Hiltermann, J.T., van, R.E., Callenbach, P.M., Veselic, C.M., van, d. Veen. H., Sont, J.K. and Sterk, P.J.** 1997. The effect of inhaled leukotriene D4 and methacholine on sputum cell differentials in asthma. Am. J. Respir. Crit. Care Med. **155:** 1247–1253.
10. **Djukanovic, R., Roche, W.R., Wilson, J.W., Beasley, C.R., Twentyman, O.P., Howarth, R.H. and Holgate, S.T.** 1990. Mucosal inflammation in asthma. Am. Rev. Respir. Dis. **142:** 434–457.
11. **Drazen, J.M. and Austen, K.F.** 1987. Leukotrienes and airway responses. Am. Rev. Respir. Dis. **136:** 985–998.
12. **Frigas, E., Motojima, S. and Gleich, G.J.** 1991. The eosinophilic injury to the mucosa of the airways in the pathogenesis of bronchial asthma. Eur. Respir. J. Suppl. **13:** 123s–135s.
13. **Grossman, J., Faiferman, I., Dubb, J.W., Tompson, D.J., Busse, W., Bronsky, E., Montanaro, A., Southern, L. and Tinkelman, D.** 1997. Results of the first U.S. double-blind, placebo-controlled, multicenter clinical study in asthma with pranlukast, a novel leukotriene receptor antagonist. J. Asthma **34:** 321–328.
14. **Hamilton, A., Faiferman, I., Stober, P., Watson, R.M. and O'Byrne, P.M.** 1998. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early-and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. J. Allergy Clin. Immunol. **102:** 177–183.
15. **Kaye, M.G. and Smith, L.J.** 1990. Effects of inhaled leukotriene D4 and platelet-activating factor on airway reactivity in normal subjects. Am. Rev. Respir. Dis. **141:** 993–997.
16. **Laitinen, L.A., Laitinen, A., Haahtela, T., Vilkka, V., Spur, B.W. and Lee, T.H.** 1993. Leukotriene E4 and granulocytic infiltration into asthmatic airways. Lancet **341:** 989–990.
17. **Lewis, R.A., Austen, K.F. and Soberman, R.J.** 1990. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. N. Engl. J. Med. **323:** 645–655.

18. **Manning, P.J., Rokach, J., Malo, J.L., Ethier, D., Cartier, A., Girard, Y., Charleson, S. and O'Byrne, P.M.** 1990. Urinary leukotriene E4 levels during early and late asthmatic responses. J. Allergy Clin. Immunol. **86:** 211–220.
19. **Marom, Z., Shelhamer, J.H., Bach, M.K., Morton, D.R. and Kaliner, M.** 1982. Slow-reacting substances, leukotrienes C4 and D4, increase the release of mucus from human airways in vitro. Am. Rev. Respir. Dis. **126:** 449–451.
20. **National Institutes of Health, National Heart, Lung and Blood Institute.** 1995. Global Initiative for Asthma (Global strategy for asthma management and prevention. NHLBI/WHO workshop report). **95:** 3659.
21. **Niimi, A., Amitani, R., Suzuki, K., Tanaka, E., Murayama, T. and Kuze, F.** 1998. Serum eosinophil cationic protein as a marker of eosinophilic inflammation in asthma. Clin. Exp. Allergy **28:** 233–240.
22. **O'Hickey, S.P., Hawksworth, R.J., Fong, C.Y., Arm, J.P., Spur, B.W. and Lee, T.H.** 1991. Leukotrienes C4, D4, and E4 enhance histamine responsiveness in asthmatic airways. Am. Rev. Respir. Dis. **144:** 1053–1057.
23. **O'Shaughnessy, T.C., Georgiou, P., Howland, K., Dennis, M., Compton, C.H. and Barnes, N.C.** 1997. Effect of pranlukast, an oral leukotriene receptor antagonist, on leukotriene D4 (LTD4) challenge in normal volunteers. Thorax **52:** 519–522.
24. **Obata, T., Katsube, N., Miyamoto, T., Toda, M., Okegawa, T., Nakai, H., Kosuge, S., Konno, M., Arai, Y. and Kawasaki, A.** 1985. New antagonists of leukotrienes: ONO-RS-411 and ONO-RS-347. Adv. Prostaglandin Thromboxane Leukot. Res. **15:** 229–231.
25. **Okubo, T., Takahashi, H., Sumitomo, M., Shindoh, K. and Suzuki, S.** 1987. Plasma levels of leukotrienes C4 and D4 during wheezing attack in asthmatic patients. Int. Arch. Allergy Appl. Immunol. **84:** 149–155.
26. **Reiss, T.F., Altman, L.C., Chervinsky, P., Bewtra, A., Stricker, W.E., Noonan, G.P., Kundu, S. and Zhang, J.** 1996. Effects of montelukast (MK-0476), a new potent cysteinyl leukotriene (LTD4) receptor antagonist, in patients with chronic asthma. J. Allergy Clin. Immunol. **98:** 528–534.
27. **Samuelsson, B., Dahlen, S.E., Lindgren, J.A., Rouzer, C.A. and Serhan, C.N.** 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science **237:** 1171–1176.
28. **Spector, S.L., Smith, L.J. and Glass, M.** 1994. Effects of 6 weeks of therapy with oral doses of ICI 204,219, a leukotriene D4 receptor antagonist, in subjects with bronchial asthma. ACCOLATE Asthma Trialists Group. Am. J. Respir. Crit. Care Med. **150:** 618–623.
29. **Sur, S., Adolphson, C.R. and Gleich, G.J.** 1993. Eosinophil, Biochemical and cellular aspects, p.169–200, In Mosby-Year Book, St. Louis.
30. **Tamaoki, J., Kondo, M., Sakai, N., Nakata, J., Takemura, H., Nagai, A., Takizawa, T. and Konno, K.** 1997. Leukotriene antagonist prevents exacerbation of asthma during reduction of high-dose inhaled corticosteroid. The Tokyo Joshi-Idai Asthma Research Group. Am. J. Respir. Crit. Care Med. **155:** 1235–1240.
31. **Taniguchi, Y., Tamura, G., Honma, M., Aizawa, T., Maruyama, N., Shirato, K. and Takishima, T.** 1993. The effect of an oral leukotriene antagonist, ONO-1078, on allergen-induced immediate bronchoconstriction in asthmatic subjects. J. Allergy Clin. Immunol. **92:** 507–512.
32. **Taylor, G.W., Taylor, I., Black, P., Maltby, N.H., Turner, N., Fuller, R.W. and Dollery, C.T.** 1989. Urinary leukotriene E4 after antigen challenge and in acute asthma and allergic rhinitis. Lancet **1:** 584–588.
33. **Taylor, I.K., O'Shaughnessy, K.M., Fuller, R.W. and Dollery, C.T.** 1991. Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. Lancet **337:** 690–694.
34. **U.S. Department of Health and Human Services, Public Health Service, National Institute of Health.** 1992. International consensus report on diagnosis and management of asthma.
35. **Underwood, D.C., Osborn, R.R., Newsholme, S.J., Torphy, T.J. and Hay, D.W.** 1996. Persistent airway eosinophilia after leukotriene (LT) D4 administration in the guinea pig: modulation by the LTD4 receptor antagonist, pranlukast, or an interleukin-5 monoclonal antibody. Am. J. Respir. Crit. Care Med. **154:** 850–857.
36. **Venge, P.** 1993. Serum measurements of eosinophil cationic protein (ECP) in bronchial asthma. Clin. Exp. Allergy **2:** 3–7.
37. **Wardlaw, A.J., Hay, H., Cromwell, O., Collins, J.V. and Kay, A.B.** 1989. Leukotrienes, LTC4 and LTB4, in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. J. Allergy Clin. Immunol. **84:** 19– 26.
38. **Wenzel, S.E., Larsen, G.L., Johnston, K., Voelkel, N.F. and Westcott, J.Y.** 1990. Elevated levels of leukotriene C4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. Am. Rev. Respir. Dis. **142:** 112–119.
39. **Woodward, D.F., Weichman, B.M., Gill, C.A. and Wasserman, M.A.** 1983. The effect of synthetic leukotrienes on tracheal microvascular permeability. Prostaglandins **25:** 131–142.
40. **Yamaguchi, T., Kohrogi, H., Honda, I., Kawano, O., Sugimoto, M., Araki, S. and Ando, M.** 1992. A novel leukotriene antagonist, ONO-1078, inhibits and reverses human bronchial contraction induced by leukotrienes C4 and D4 and antigen in vitro. Am. Rev. Respir. Dis. **146:** 923–929.