Effects of KP-496, a Novel Dual Antagonist for Cysteinyl Leukotriene Receptor 1 and Thromboxane A₂ Receptor, on Sephadex-Induced Airway Inflammation in Rats

Masakazu ISHIMURA,^{*,a} Takashi MAEDA,^a Sayuri KATAOKA,^a Masahiro SUDA,^a Shigeo KUROKAWA,^a and Yoshiyuki HIYAMA^b

^aPharmacology Department, Central Research Laboratories, Kaken Pharmaceutical Co., Ltd.; 14 Shinomiya, Minamigawara-cho, Yamashina-ku, Kyoto 607–8042, Japan; and ^bPharmacokinetics and Safety Research Department, Central Research Laboratories, Kaken Pharmaceutical Co., Ltd.; 301 Gensuke, Fujieda, Shizuoka 426–8646, Japan.

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Bronchial asthma is characterized by chronic airway inflammation. Eosinophils are involved in airway inflammation and play crucial roles in asthma. There is accumulating evidence to suggest contributions of cysteinyl leukotrienes (cysLTs) and thromboxane (TX) A₂ to the recruitment of eosinophils into lung in asthmatics. KP-496 is a novel dual antagonist for CysLT receptor type 1 and TXA₂ receptors. The aim of this study was to evaluate the anti-inflammatory effects of KP-496 on Sephadex-induced airway inflammation. Sephadex suspension was intratracheally injected into rats. Amounts of regulated on activation, normal T cell expressed and secreted (RANTES) and eotaxin, and numbers of infiltrating cells in bronchoalveolar lavage fluid were measured 24 and 48 h after Sephadex injection, respectively. KP-496 (30, 100 µg/head) was intratracheally administered to rats 1 h before and 7 h after Sephadex injection. KP-496 and prednisolone (10 mg/kg, *per os*) exhibited significant inhibitory effects on infiltration of total cells and eosinophils into lung. Production of RANTES was significantly inhibited by KP-496 and prednisolone. Production of eotaxin was significantly inhibited by prednisolone. KP-496 also inhibited the production of eotaxin, though this effect was not significant. These results demonstrate that KP-496 exhibited the anti-inflammatory effects by inhibiting infiltration of inflammatory cells and productions of RANTES and eotaxin.

Key words dual antagonist; cysteinyl leukotriene; thromboxane A₂; airway inflammation; eosinophil

Bronchial asthma is characterized by chronic airway inflammation. Eosinophils are involved in airway inflammation and play crucial roles in asthma. Various types of mediators which recruit and activate eosinophils are involved in the development of airway inflammation.

The cysteinyl leukotrienes (cysLTs, including LTC₄, LTD₄, and LTE₄) which are lipid mediators generated from arachidonic acid are potent bronchoconstrictors.¹⁾ CysLT type 1 receptor (CysLT₁) antagonists including montelukast,²⁾ pranlukast,³⁾ and zafirlukast,⁴⁾ exhibit beneficial effects on the clinical symptoms of asthma. In addition to bronchoconstriction, cysLTs are implicated in airway inflammation and induce eosinophil recruitment to the lung. Aerosolized cysLTs elicit recruitment of eosinophils into lung in guinea pigs, which is blocked by CysLT₁ antagonists.^{5,6)} It has also been reported that LTD₄ challenge increased the percentage of eosinophils in sputum from asthmatics.⁷⁾ CysLT₁ antagonist reduced sputum and mucosal eosinophils in asthmatics.^{8,9)}

In addition to CysLT₁ antagonists, the thromboxane (TX) A₂ receptor (TP) antagonist seratrodast¹⁰⁾ is available in Japan. TXA₂ is a lipid mediator generated from arachidonic acid. TXA₂ is thought to be an important mediator in the development of asthma because of its potent bronchoconstrictive activity.¹¹⁾ Moreover, there is accumulating evidence to suggest that TXA₂ is involved in airway inflammation.^{12,13)} In asthmatics, seratrodast significantly decreases the number of submucosal eosinophils and concentration of eosinophil cationic protein (ECP) in sputum.^{14,15)}

Although the mechanism by which cysLTs and TXA₂ induce recruitment of inflammatory cells is not fully clear, these findings suggest contributions of cysLTs and TXA₂ to

the recruitment of eosinophils into lung in asthmatics. KP-496 (2-{N-[4-(4-chlorobenzenesulfonylamino)butyl]-N-{3-[(4-isopropylthiazol-2-yl)methoxy]benzyl}sulfamoyl}benzoic acid) is a novel dual antagonist for CysLT₁ and TP.¹⁶⁾ Inhaled KP-496 exhibited significant inhibitory effects on immediate airway response (IAR), late airway response (LAR), and airway hyperresponsiveness (AHR).¹⁷⁾ However, anti-inflammatory effect of KP-496 is not investigated.

Sephadex-induced airway inflammation is a convenient model for evaluating the effects of potential anti-inflammatory compounds. Instillation of Sephadex induced significant eosinophil recruitment into lung, which was associated with increased expression of chemokines, cytokines, and lipid mediators.^{18–20)} Eosinophils play important roles in airway inflammation, and the C-C chemokines, regulated on activation, normal T cell expressed and secreted (RANTES) and eotaxin, contribute to eosinophil recruitment, survival, and activation.^{21,22)}

In the present study, we evaluated the anti-inflammatory effects of KP-496 on Sephadex-induced airway inflammation in rats by measuring the recruitment of inflammatory cells into lung and the amounts of RANTES and eotaxin in lung. In the previous study, we evaluated the *in vivo* antagonistic activities of inhaled KP-496, suspecting that inhalational administration would maximize the pharmacological properties of KP-496. Thus KP-496 was administered intratracheally in the present study.

MATERIALS AND METHODS

Animals Male Sprague Dawley rats (six-week-old,

* To whom correspondence should be addressed. e-mail: ishimura_masakazu@kaken.co.jp

150–200 g) were purchased from Charles River Japan, Inc. (Yokohama, Japan). The handling and treatment of the animals were in accordance with the guidelines of the Japanese Association for Laboratory Animal Science (1987).

Drugs and Chemicals A dry powder formulation of KP-496 (KP-496DPI) containing 3 and 10% of KP-496 and vehicle for KP-496DPI were prepared by Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan). Prednisolone (50 mg of water-soluble prednisolone) was purchased from Shionogi & Co., Ltd. (Osaka, Japan). Methylcellulose (MC) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Urethane was purchased from Sigma (St. Louis, MO, U.S.A.). Sephadex™ G75S Superfine was purchased from Amersham Biosciences (Uppsala, Sweden).

Drug Administration Vehicle and KP-496DPI were weighed into silicone tubes (1 mg per tube). A compressor (PARI GmbH, Starnberg, Germany) was connected to an air hose and a three-way stopcock. A silicone tube containing the test compound was connected to the other end of the three-way stopcock. A tracheal cannula for intratracheal administration was connected to the silicone tube. Animals were anesthetized with ether and the tracheal cannula was inserted into the trachea from the oral cavity noninvasively. It was confirmed that treatment with ether had no effect on inflammatory cell recruitment and expression of chemokines. The test compound was sprayed by rapidly and fully opening the three-way stopcock connected to the compressor and sending compressed air from the compressor. The amounts of KP-496 administered were 30 µg/head and 100 µg/head, respectively, when 3% and 10% of KP-496DPI were administered. The prednisolone was dissolved with 0.5% MC and administered orally. Vehicle for KP-496 intratracheally (i.t.) and 0.5% MC [*per os* (*p.o.*)] were administered in the sham and control groups. KP-496DPI (3 and 10%, i.t.) and 0.5% MC (*p.o.*) were administered in the KP-496 group (30 µg/head and 100 µg/head group, respectively). Vehicle for KP-496 (i.t.) and prednisolone (10 mg/kg, *p.o.*) were administered in the prednisolone group. The drugs were administered 1 h before and 7 h after intratracheal administration of Sephadex following the procedures shown below.

Sephadex Injection Sephadex was prepared as a suspension (5 mg/ml) by soaking in saline. Animals were anesthetized with ether and a tracheal cannula, the Surfloflash 18G×2 (Terumo Corporation, Tokyo, Japan), was inserted into the trachea from the oral cavity noninvasively and connected to a 1 ml syringe containing Sephadex, and Sephadex was injected (5 mg/kg, i.t.).

Measurement of Cells The animals were anesthetized with urethane [1 g/kg, intraperitoneally (i.p.)] and sacrificed

48 h after Sephadex injection. The trachea was exposed and cannulated. The airway lumen was lavaged by flushing with phosphate buffered saline (PBS) (–) 4 times (4, 5, 6, and 7 ml each), and bronchoalveolar lavage fluid (BALF) was recovered. The BALF was centrifuged (200×*g*) for 10 min at 4 °C. The cell pellet was resuspended in 0.5 ml of PBS (–). Total cell number was counted with a particle counter (CDA-500, Sysmex Corporation, Kobe, Japan). The cytospin preparations were stained with Giemsa, and more than 200 cells were counted to determine the cell differential.

Measurement of RANTES and Eotaxin by Enzyme-Linked Immunosorbent Assay (ELISA) The animals were anesthetized with urethane (1 g/kg, i.p.) and sacrificed 24 h after Sephadex injection. BALF was recovered as described above. Supernatant of BALF was concentrated by ultrafiltration (1500×*g*, 390 min, 4 °C) using the Centriplus YM-3 (Billerica, MA, U.S.A.). Concentrated sample was dried using a centrifugal evaporator (300 min, 25 °C) and dissolved in PBS (–) to prepare 10-fold concentrated BALF. The amounts of RANTES and eotaxin were measured using ELISA Kit (Biosource Co., Ltd., Camarillo, CA, U.S.A. and R & D Systems, Minneapolis, MN, U.S.A., respectively).

Statistical Analysis The same experiment was conducted twice and the reproducibility of the results was confirmed. In this study, the values of 2nd experiment were expressed as the mean±S.D. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple range test and Student's *t*-test, as appropriate. Values at *p*<0.05 were considered significant.

RESULTS

Effects of KP-496 on Inflammatory Cell Recruitment

Numbers of inflammatory cells in BALF were measured 48 h after intratracheal injection of Sephadex. Total cell number and numbers of all types of cells in the control group were significantly increased compared with the sham group (*p*<0.01, Table 1). Eosinophils, monocytes/macrophages, neutrophils, and lymphocytes in the control group were increased respectively 615-fold, 2.3-fold, 20.9-fold, and 9.9-fold compared with the sham group. The KP-496 (100 µg/head) group and prednisolone (10 mg/kg) group exhibited significant inhibition of numbers of infiltrating total cells, eosinophils, monocytes/macrophages, and lymphocytes compared with the control group (Fig. 1, Table 1). Infiltration of all types of cells except neutrophils was decreased in the KP-496 (30 µg/head) group, though not to significant extents.

Effects of KP-496 on Amounts of RANTES and Eotaxin in BALF

The amount of RANTES and eotaxin in

Table 1. Effect of KP-496 on Infiltration of Inflammatory Cells into BALF in Sephadex-Induced Airway Inflammation

Group	Dose	Cell numbers (×10 ⁵ cells in BALF)				
		Total cells	Eosinophils	Monocyte/macrophages	Neutrophils	Lymphocytes
Sham	—	5.34±1.55	0.01±0.01	5.18±1.52	0.09±0.05	0.07±0.04
Control	—	20.48±11.04 ^{##}	6.15±4.60 ^{##}	11.76±6.15 ^{##}	1.88±1.47 ^{##}	0.69±0.59 ^{##}
Prednisolone	10 mg/kg (<i>p.o.</i>)	10.48±2.44 ^{**}	0.60±0.59 ^{**}	7.64±2.30 [*]	2.11±2.00	0.14±0.08 [*]
KP-496	30 µg/head (i.t.)	17.12±4.05	4.29±1.24	9.62±1.94	2.75±2.29	0.46±0.33
	100 µg/head (i.t.)	10.89±2.51 ^{**}	2.34±1.02 ^{**}	7.16±2.32 [*]	1.12±0.77	0.27±0.13 [*]

Results are expressed as cell numbers (×10⁵ cells) in BALF. Each value represents the mean±S.D. of ten animals. ^{##}*p*<0.01 compared with sham (Student's *t*-test), ^{*}*p*<0.05 and ^{**}*p*<0.01 compared with control (Dunnett's multiple range test).

Fig. 1. Effects of KP-496 on the Number of Infiltrating Total Cells (A) and Eosinophils (B) in BALF in Sephadex-Induced Airway Inflammation Model

Drugs were administered to rats 1 h before and 7 h after Sephadex injection. The number of total cells and eosinophils in BALF was measured 48 h after Sephadex injection. Results are expressed as cell numbers ($\times 10^5$ cells) in BALF. Each value represents the mean $\pm$ S.D. of ten animals. ^{##} $p < 0.01$ compared with sham (Student's *t*-test) and ^{**} $p < 0.01$ compared with control (Dunnett's multiple range test).

Fig. 2. Effect of KP-496 on Production of RANTES in Sephadex-Induced Airway Inflammation Model

Drugs were administered to rats 1 h before and 7 h after Sephadex injection. The amount of RANTES in BALF was measured 24 h after Sephadex injection. Results are expressed as RANTES level in BALF. Each value represents the mean $\pm$ S.D. of ten animals. ^{##} $p < 0.01$ compared with sham (Student's *t*-test), ^{*} $p < 0.05$ and ^{**} $p < 0.01$ compared with control (Dunnett's multiple range test).

BALF was measured 24 h after intratracheal injection of Sephadex. The amount of RANTES in the control group increased significantly compared with the sham group ($p < 0.01$, Fig. 2). Production of RANTES was significantly inhibited in the KP-496 (100 μ g/head) group and prednisolone (10 mg/kg) group compared with the control group ($p < 0.05$ in the KP-496 group and $p < 0.01$ in the prednisolone group). The amount of RANTES also decreased in the KP-496 (30 μ g/head) group, though not to a significant extent. The amount of eotaxin in the control group increased significantly compared to the sham group ($p < 0.05$, Fig. 3).

Fig. 3. Effect of KP-496 on Production of Eotaxin in Sephadex-Induced Airway Inflammation Model

Drugs were administered to rats 1 h before and 7 h after Sephadex injection. The amount of eotaxin in BALF was measured 24 h after Sephadex injection. Results are expressed as eotaxin level in BALF. Each value represents the mean $\pm$ S.D. of ten animals. [#] $p < 0.05$ compared with sham (Student's *t*-test) and ^{**} $p < 0.01$ compared with control (Dunnett's multiple range test).

Production of eotaxin was significantly inhibited in the prednisolone group compared to the control group ($p < 0.01$), while the KP-496 (100 μ g/head) group exhibited a tendency toward inhibition of production of eotaxin ($p = 0.080$). The amount of eotaxin also decreased in the KP-496 (30 μ g/head) group, though not to a significant extent.

DISCUSSION

In the present study, we investigated the effects of KP-496 on Sephadex-induced airway inflammation in rats. KP-496 inhibited the recruitment of inflammatory cells, especially eosinophils, into lung. KP-496 also reduced the amount of RANTES and eotaxin in lung.

Sephadex-induced airway inflammation is characterized by increase in number of eosinophils. The exact mechanism of airway inflammation caused by intratracheal instillation of Sephadex is not clear, but macrophage activation through phagocytosis, mast cell degranulation and T cell activation are thought to be involved in eosinophilic inflammation.^{18,23)} The increase in eosinophil numbers is accompanied by increase in amounts of chemokines and Th2 cytokines in lung and AHR.^{24,25)} Airway inflammation including eosinophil recruitment and AHR are characteristic features in asthmatics. Chemokines and Th2 cytokines play important role in the development of airway inflammation. It had been demonstrated that the degree of AHR is related to severity of asthma symptoms.²⁶⁾ Therefore, this model is thought to reflect important aspects of asthma and is useful and convenient for investigation of the anti-inflammatory effects of small compounds. In fact, many groups have investigated the anti-inflammatory effects of small compounds and steroids using this model.^{20,27–29)}

The cysLTs are potent eosinophil chemoattractants and promote eosinophil survival.^{30,31)} CysLTs also induce up-regulation of their own receptor, CysLT₁, and CysLT₁ antagonist inhibited the up-regulation of CysLT₁ induced by cysLTs.^{32,33)} The CysLT₁ antagonistic activity of KP-496 thus contributes to the inhibition of recruitment of eosinophils.

In addition to cysLTs, RANTES and eotaxin are eosinophil activating and chemoattractant chemokines. In fact, numbers of cells expressing eotaxin and RANTES were sig-

nificantly increased, and enhanced expression of C-C chemokine receptor 3 (CCR3) was observed in asthmatics.^{34,35} Furthermore, there is a strong relationship between the expression of eotaxin and severity of asthma.³⁵

It has been reported that cysLTs activate nuclear factor (NF)- κ B and induce RANTES production from isolated lung mononuclear cells, which might in turn cause recruitment of eosinophils into the lung.³⁶ The CysLT₁ antagonists, montelukast and pranlukast, inhibited the activation of NF- κ B.^{36–38} The CysLT₁ antagonistic activity of KP-496 should thus reduce the production of RANTES by inhibition of NF- κ B activation and prevent the recruitment of eosinophils into lung.

Interleukin (IL)-5 also plays crucial role in eosinophil activation and survival. In the present study, we did not investigate the effect of KP-496 on IL-5 production. But, it has been demonstrated that CysLTs induced IL-5 production from inflammatory cells and CysLT antagonists inhibited IL-5 production.^{33,39,40} Additionally, some results suggested that IL-5 and eotaxin might promote the recruitment of eosinophils into lung cooperatively.^{41,42} Thus, it can be expected that CysLT₁ antagonistic activity of KP-496 might contribute to inhibit the recruitment of eosinophils through inhibiting IL-5 production and cooperative action of IL-5 with eotaxin.

It is not clear if TXA₂ has eosinophil chemoattractant activity like cysLTs. However, some groups have reported the involvement of TXA₂ in airway inflammation.^{10,12,13} Hoshino *et al.*¹⁴ reported that administration of seratrodast to asthmatics reduced the number of activated eosinophils in bronchial mucosa and the production of the C-C chemokines RANTES and eotaxin. Although the mechanisms by which TXA₂ induced production of these chemokines are unclear in detail, it has been reported that TXA₂ analogue induced the production of RANTES.⁴³ Thus, C-C chemokines might be produced in part by direct stimulation *via* TP, and antagonism by seratrodast and KP-496 of TP might contribute to the inhibition of their production.

These findings and those of previous reports suggest that KP-496 exhibit the anti-inflammatory effects by inhibiting infiltration of inflammatory cells into lung and productions of RANTES and eotaxin. In addition to these results, KP-496 exhibited anti-inflammatory effects on a guinea pig chronic airway inflammation model (manuscript in preparation).

According to the GINA guideline, inhaled corticosteroids (ICS) are the first choice for control of asthma. However, it is known that ICS do not inhibit the production of cysLTs.^{44,45} It has also been reported that CysLT₁ antagonists and a TP antagonist exhibit additive effects with ICS.⁴⁶ Moreover, there is some evidence that inhibition of multi-mediators, including KP-496, provides more potent effects on airway obstruction than inhibition of single mediators.^{17,47,48} It can thus be expected that KP-496 will provide new therapeutic approach and choice such as combination of ICS and KP-496 to asthmatics.

REFERENCES

- Dahlen S. E., Hedqvist P., Hammarstrom S., Samuelsson B., *Nature* (London), **288**, 484–486 (1980).
- Jones T. R., Labelle M., Belley M., Champion E., Charette L., Evans J., Ford-Hutchinson A. W., Gauthier J. Y., Lord A., Masson P., McAuliffe M., McFarlane C. S., Metter K. M., Pickett C., Piechuta H., Rochette C., Rodger I. W., Sawyer N., Young R. N., Zamboni R., Abraham W. M., *Can. J. Physiol. Pharmacol.*, **73**, 191–201 (1995).
- Obata T., Okada Y., Motoishi M., Nakagawa N., Terawaki T., Aishita H., *Jpn. J. Pharmacol.*, **60**, 227–237 (1992).
- Krell R. D., Aharony D., Buckner C. K., Keith R. A., Kusner E. J., Snyder D. W., Bernstein P. R., Matassa V. G., Yee Y. K., Brown F. J., Hesp B., Giles R. E., *Am. Rev. Respir. Dis.*, **141**, 978–987 (1990).
- Muñoz N. M., Douglas I., Mayer D., Herrreiter A., Zhu X., Leff A. R., *Am. J. Respir. Crit. Care Med.*, **155**, 1398–1403 (1997).
- Underwood D. C., Osborn R. R., Newsholme S. J., Torphy T. J., Hay D. W., *Am. J. Respir. Crit. Care Med.*, **154**, 850–857 (1996).
- Diamant Z., Hiltermann J. T., Van Rensen E. L., Callenbach P. M., Veselic-Charvat M., Van Der Veen H., Sont J. K., Sterk P. J., *Am. J. Respir. Crit. Care Med.*, **155**, 1247–1253 (1997).
- Nakamura Y., Hoshino M., Sim J. J., Ishii K., Hosaka K., Sakamoto T., *Thorax*, **53**, 835–841 (1998).
- Pizzichini E., Leff J. A., Reiss T. F., Hendeles L., Boulet L. P., Wei L. X., Efthimiadis A. E., Zhang J., Hargreave F. E., *Eur. Respir. J.*, **14**, 12–18 (1999).
- Ashida Y., Matsumoto T., Kuriki H., Shiraishi M., Kato K., Terao S., *Prostaglandins*, **38**, 91–112 (1989).
- Svenssen J., Strandberg K., Tuvemo T., Hamberg M., *Prostaglandins*, **14**, 425–436 (1977).
- Itoh K., Mukaiyama O., Satoh Y., Yamaguchi T., Iizuka Y., *Eur. J. Pharmacol.*, **239**, 159–169 (1993).
- Shi H., Yokoyama A., Kohno N., Hirasawa Y., Kondo K., Sakai K., Hiwada K., *Eur. Respir. J.*, **11**, 624–629 (1998).
- Hoshino M., Sim J., Shimizu K., Nakayama H., Koya A., *J. Allergy Clin. Immunol.*, **103**, 1054–1061 (1999).
- Fukuoka T., Miyake S., Umino T., Inase N., Tojo N., Yoshizawa Y., *J. Asthma*, **40**, 257–264 (2003).
- Ishimura M., Kataoka S., Suda M., Maeda T., Hiyama Y., *Allergol. Int.*, **55**, 403–410 (2006).
- Ishimura M., Suda M., Morizumi K., Kataoka S., Maeda T., Kurokawa S., Hiyama Y., *Br. J. Pharmacol.*, **153**, 669–675 (2008).
- Haddad El-B., Underwood S. L., Dabrowski D., Birrell M. A., McCluskie K., Battram C. H., Pecoraro M., Foster M. L., Belvisi M. G., *J. Immunol.*, **168**, 3004–3016 (2002).
- Harrington P. M., Newton D. J., Williams C. M., Hunt J. A., Dearman R. J., Kimber I., Coleman J. W., Flanagan B. F., *Int. J. Exp. Pathol.*, **80**, 177–185 (1999).
- Namovic M. T., Walsh R. E., Goodfellow C., Harris R. R., Carter G. W., Bell R. L., *Eur. J. Pharmacol.*, **315**, 81–88 (1996).
- Shahabuddin S., Ponath P., Schleimer R. P., *J. Immunol.*, **164**, 3847–3854 (2000).
- Shinagawa K., Trifileff A., Anderson G. P., *Int. Arch. Allergy Immunol.*, **130**, 150–157 (2003).
- Kleemann R., Deschl U., Dietmann G., Wilhelms O. H., Juchem R., Rebel W., Hartig F., *Exp. Toxicol. Pathol.*, **48**, 233–241 (1996).
- Conroy D. M., Sirois P., *Inflammation*, **23**, 437–448 (1999).
- Maghni K., Nantel F., Lanoue C., Cloutier S., Cristol J. P., Cadieux A., Sirois P., *Inflammation*, **28**, 195–206 (2004).
- Bernstein D. I., Ploysongsang Y., Mittman R. J., Piyamahunt A., Bernstein I. L., *Chest*, **101**, 437–441 (1992).
- Belvisi M. G., Bundschuh D. S., Stoeck M., Wicks S., Underwood S., Battram C. H., Haddad El-B., Webber S. E., Foster M. L., *J. Pharmacol. Exp. Ther.*, **314**, 568–574 (2005).
- Birrell M., Hele D., McCluskie K., Webber S., Foster M., Belvisi M. G., *Eur. Respir. J.*, **16**, 947–950 (2000).
- Finnses F., Lyberg T., Christensen G., Skjongsberg O. H., *Respir. Med.*, **96**, 901–906 (2002).
- Lee E., Robertson T., Smith J., Kilfeather S., *Am. J. Respir. Crit. Care Med.*, **161**, 1881–1886 (2000).
- Saito K., Nagata M., Kikuchi I., Sakamoto Y., *Ann. Allergy Asthma Immunol.*, **93**, 594–600 (2004).
- Ohshima N., Nagase H., Koshino T., Miyamasu M., Yamaguchi M., Hirai K., Yamamoto K., Fujisawa T., Nakagawa N., Kishikawa K., Morita Y., *Int. Arch. Allergy Immunol.*, **129**, 67–75 (2002).
- Zhang Y. J., Zhang L., Wang S. B., Shen H. H., Wei E. Q., *Acta Pharmacol. Sin.*, **25**, 1341–1346 (2004).
- Ying S., Meng Q., Zeibecoglou K., Robinson D. S., Macfarlane A., Humbert M., Kay A. B., *J. Immunol.*, **163**, 6321–6329 (1999).
- Ying S., Robinson D. S., Meng Q., Rottman J., Kennedy R., Ringler D.

- J., Mackay C. R., Daugherty B. L., Springer M. S., Durham S. R., Williams T. J., Kay A. B., *Eur. J. Immunol.*, **27**, 3507—3516 (1997).
- 36) Kawano T., Matsuse H., Kondo Y., Machida I., Sacki S., Tomari S., Mitsuta K., Obase Y., Fukushima C., Shimoda T., Kohno S., *J. Allergy Clin. Immunol.*, **112**, 369—374 (2003).
 - 37) Maeba S., Ichiyama T., Ueno Y., Makata H., Matsubara T., Furukawa S., *Ann. Allergy Asthma Immunol.*, **94**, 670—674 (2005).
 - 38) Wu Y., Zhou C., Tao J., Li S., *Can. J. Physiol. Pharmacol.*, **84**, 531—537 (2006).
 - 39) Nabe T., Yamashita K., Miura M., Kawai T., Kohno S., *Clin. Exp. Allergy*, **32**, 633—640 (2002).
 - 40) Eum S. Y., Maghni K., Hamid Q., Campbell H., Eidelman D. H., Martin J. G., *Am. J. Respir. Cell Mol. Biol.*, **28**, 25—32 (2003).
 - 41) Humbles A. A., Conroy D. M., Marleau S., Rankin S. M., Palframan R. T., Proudfoot A. E., Wells T. N., Jeffery P. K., Griffiths-Johnson D. A., Williams T. J., Jose P. J., *J. Exp. Med.*, **186**, 601—612 (1997).
 - 42) Mould A. W., Ramsay A. J., Matthaeci K. I., Young I. G., Rothenberg M. E., Foster P. S., *J. Immunol.*, **164**, 2142—2150 (2000).
 - 43) Moritani C., Ishioka S., Haruta Y., Kambe M., Yamakido M., *Chest*, **113**, 452—458 (1998).
 - 44) Pavord I. D., Ward R., Woltmann G., Wardlaw A. J., Sheller J. R., Dworski R., *Am. J. Respir. Crit. Care Med.*, **160**, 1905—1909 (1999).
 - 45) O'Shaughnessy K. M., Wellings R., Gillies B., Fuller R. W., *Am. Rev. Respir. Dis.*, **147**, 1472—1476 (1993).
 - 46) Myou S., Fujimura M., Leff A. R., *Allergol. Int.*, **53**, 211—217 (2004).
 - 47) Kagoshima M., Tomomatsu N., Iwahisa Y., Yamaguchi S., Kawakami Y., Terasawa M., *Pharmacology*, **54**, 261—270 (1997).
 - 48) Arakida Y., Ohga K., Okada Y., Morio H., Suwa K., Yokota M., Yamada T., *Eur. J. Pharmacol.*, **403**, 169—179 (2000).