Effects of a New Antiallergic Drug, VUF-K-8788, on Infiltration of Lung Parenchyma by Eosinophils in Guinea Pigs and Eosinophil-Adhesion to Human Umbilical Vein Endothelial Cells (HUVEC)

Toshiaki TAKIZAWA,^{*,a,b} Chie WATANABE,^c Ikuo SAIKI,^c Yasushi WADA,^b Tsutomu TOHMA,^b and Hiroichi NAGAI^a

Department of Pharmacology, Gifu Pharmaceutical University,^a Gifu 502–8585, Japan, Tokyo Research Laboratories, Kowa Company Ltd.,^b Higashimurayama, Tokyo 189–0022, Japan, and Research Institute for Wakan-yaku, Toyama Medical and Pharmaceutical University,^c Toyama, Japan. Received March 29, 2001; accepted June 19, 2001

Airway inflammation and reversible airway obstruction are hallmarks of bronchial asthma. In this study, we investigated the effects of a new antiallergic drug, 7-[3-[4-(2-quinolinylmethyl)-1-piperazinyl]-propoxy]-2,3-dihydro-4H-1,4-benzothiazin-3-one (VUF-K-8788), on histopathological changes in lung parenchyma of guinea pigs during late-phase asthmatic reaction (LAR), and on eosinophil-adhesion to human umbilical vein endothelial cells (HUVEC).

Repeated exposure to ovalbumin of sensitized guinea pigs induced inflammatory phenomena such as hyperplasia of airway epithelial cells, perivascular edema and infiltration of lung parenchyma by eosinophils. VUF-K-8788 inhibited these histopathological phenomena at 10 mg/kg *p.o.* Moreover, the eosinophil-adherence to HUVEC was inhibited by VUF-K-8788 at the concentration of 10–30 μ M. In conclusion, this inhibitory effect of VUF-K-8788 on eosinophil-adherence might contribute to the prevention of LAR and infiltration by eosinophils in the experimental asthmatic model in guinea pigs.

Key words VUF-K-8788; asthma; eosinophil; human umbilical vein endothelial (HUVE) cell; guinea pig

Allergen provocation induces immediate- and late-phase reactions in allergic disorders, such as bronchial asthma,¹⁾ atopic dermatitis²⁾ or allergic rhinitis.³⁾ Pepys and Hutchcroft⁴⁾ reported that immediate- and late-phase asthmatic responses were observed 15–30 min and 6–12 h after allergen provocation, respectively. Hargreave *et al.*^{1,5)} and Cartier *et al.*⁶⁾ reported that late-phase asthmatic reactions (LAR) were observed up to 2 or 3 d after allergen provocation and were accompanied by airway hyperresponsiveness to a wide variety of stimuli including histamine and methacholine. The development of LAR is associated with the influx and activation of eosinophils.⁷⁾

A new model for biphasic asthmatic response in guinea pigs was reported by Takeda *et al.*⁸⁾ In this model, LAR was caused by repeated airway inflammation with eosinophilia and chemical mediator release including histamine, thromboxane A₂ and leukotriene D₄. VUF-K-8788, 7-[3-[4-(2-quinolinylmethyl)-1-piperazinyl]-propoxy]-2,3-dihydro-4H-1,4-benzothiazin-3-one, is a newly synthesized anti-allergic drug, which antagonized histamine H₁ receptor and inhibited the biphasic asthmatic reaction in guinea pigs.^{9,10)}

One of the mechanisms which is important in the process of infiltration by eosinophil involves the interaction of adhesion molecules on the surface of the circulating cells and the vascular endothelium.^{11,12)} Bochner *et al.* reported that eosinophils express functional counter-ligands for the inducible adhesion molecules on the endothelial surface including the vascular cell adhesion molecule-1 (VCAM-1, CD106) and the intercellular adhesion molecule-1 (ICAM-1, CD54).¹³⁾

In our previous experiment,^{9,10)} VUF-K-8788 (0.3–10 mg/kg) inhibited immediate- and late-phase allergic reactions, airway hyperresponsiveness and eosinophils in bronchoalveolar fluids, while terfenadine (100 mg/kg) inhibited only the immediate reaction. In this study, we investigated

the effect of VUF-K-8788 on histopathological changes in lung parenchyma in guinea pigs during LAR, eosinophil-adhesion to human umbilical vein endothelial cells (HUVEC), and expression of adhesion molecules on HUVEC.

MATERIALS AND METHODS

Animals Male Hartley guinea pigs aged 4 weeks (body weight: 250–300 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). The animals were fed standard laboratory diet and water *ad libitum* in an air-conditioned room at 23 $\pm$ 3 $^{\circ}$ C and relative humidity of 55 $\pm$ 15%.

The experiments were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals of Gifu Pharmaceutical University.

Materials HUVEC (Seikagaku Kogyo Inc., Tokyo, Japan), HUVE cell culture medium (Nissui Seiyaku Inc., Tokyo, Japan), CD16 Ab (MG 38, mouse anti-human IgG₁, Nichirei, Tokyo, Japan), sheep anti-mouse IgG₁ coated beads (Dynal As, Oslo, Norway), bovine endothelial mitogen (Biomedical Technologies Inc., Stoughton, MA, U.S.A.), recombinant human TNF- α (Peptide Tech EC LTD, London, England), Diffquick Solution (Kokusai-Shiyaku Inc., Kobe, Japan), anti-human VCAM-1, anti-human CD54 (ICAM-1), and FITC-labeled rabbit anti-mouse IgG₁ conjugate (Endogen Inc., MA, U.S.A.) were purchased. VUF-K-8788 was synthesized in Kowa Tokyo Laboratories (Tokyo). Ovalbumin (OA) and other chemicals were purchased from SIGMA Inc. (MO, U.S.A.).

Induction of LAR and Histopathological Analysis Induction of LAR was carried out as previously described.¹⁰⁾ In brief, male Hartley guinea pigs were actively sensitized twice with OA (0.5 mg/body *i.p.* on day 1 and 1 mg/body *i.p.* on day 3). Actively sensitized guinea pigs were repeatedly exposed to aerosolized antigen. One-minute inhalations of 0.1,

* To whom correspondence should be addressed. e-mail: ttakizaw@kowa.co.jp

0.2, 0.4, 0.5, 1 and 1% OA solution were administered on days 23, 25, 28, 32, 37 and 39, respectively, using an ultrasonic nebulizer (TUR-3200, Nihon Kohden, Tokyo, Japan). VUF-K-8788 or terfenadine was administered orally 1 h before the inhalations. Four hours after the last inhalation, when LAR had been induced, the animals were sacrificed. The lungs including bronchi were dissected and were fixed in 10% neutral buffered formalin. Sections 3 μ m thick were stained with hematoxylin–eosin or Hansel's solution. The histopathological changes, such as hyperplasia of airway epithelium and perivascular edema, were scored as follows by the method of Blyth *et al.*¹⁴: 0: none, 100: occasional, 200: small, 300: moderate, 400: heavy, 500: massive. Each score was evaluated by comparing with typical histological standards which were taken in preliminary experiments. Eosinophils that infiltrated to lung parenchyma were counted by the method of Sagara *et al.*¹⁵ For cell counting, each slide glass was coded and read in a "blind" fashion. The regions were chosen at random in the sections.

Cell Adhesion Assay Cell Culture: HUVEC were cultured in HUVE cell culture medium at 37 °C in a 5% CO₂-incubator. HUVEC were subcultured every week and were used within the first 6 passages.

Eosinophil Purification: Blood from asthmatic volunteers (40 ml) was mixed with 10 ml of 40% dextrin–saline solution and kept at room temperature for 1 h. Supernatant was layered over lymphocyte separation medium and centrifuged at 20 °C, 200 $\times g$, for 7 min. Polymorphonuclear cells (PMN) were washed twice with PBS and resuspended in RPMI 1640 supplemented with 5% fetal bovine serum (FBS) and 4 mM EDTA. Immunomagnetic negative selection, using CD16 Ab and sheep anti-mouse IgG₁ coated beads, was executed to purify eosinophils from PMN. The purity of eosinophils was 80.6%.

Cell Adhesion Assay: The subcultured HUVEC were collected and resuspended in D-MEM/F12 medium supplemented with 10% FBS, bovine endothelial mitogen 100 μ g/ml and recombinant human TNF- α . They were seeded on 96-well plates at 1×10^5 cells in 100 μ l of medium, and preincubated in a CO₂-incubator. After 1 h incubation, VUF-K-8788 or terfenadine was added to confluent HUVEC in 100 μ l of HUVE cell medium. Following 24 h incubation, eosinophils (1×10^5 cells) were added to HUVEC and co-incubated for 30 min. The un-adhered eosinophils were then washed out with PBS, and the eosinophils adhering to HUVEC were stained with Diffquick Solution and counted under a microscope at a magnification of $\times 400$.

Flowcytometry Subcultured HUVEC were seeded on 6-well plates at 1×10^5 cells in 2 ml of HUVEC medium and cultured for 3 d. HUVEC monolayers were rinsed with 1 ml of warmed PBS, and TNF- α and test compounds in 2 ml of D-MEM/F12 medium containing 10% FBS and 100 μ g/ml endothelial mitogen were added. After 24 h incubation, HUVEC were treated with 20% trypsin–EDTA and collected. Single cell suspensions were centrifuged (2000 $\times g$, 30 s) and resuspended in PBS.

Cells were incubated in microtubes at 4 °C, first with the primary mAbs (anti-human VCAM-1 or anti-human CD54, 30 min) and next with the FITC-labeled rabbit anti-mouse IgG₁ conjugate (30 min). Cells were washed twice by centrifugation after each incubation. The final suspension was

made in 500 μ l of medium containing propidium iodide (2.5 μ g/ml). Irrelevant isotype-matched primary Abs served as control. Cell samples were analyzed on FACSscan (Becton Dickinson Inc., CA, U.S.A.).

Cytotoxicity for HUVEC and Eosinophils To investigate the cytotoxicity of the test compounds for HUVEC and eosinophils, the crystal violet staining assay and WST-1 cell counting Kit (Dojindo, Kumamoto, Japan) were applied, respectively.¹⁶ In brief, HUVEC were preincubated in HUVE cell medium for 2 h at 37 °C in an atmosphere of 5% CO₂ and 95% air. VUF-K-8788 or terfenadine was added to the medium and the cells were incubated for 24 h. After fixation with glutaraldehyde, the wells were washed with water. The HUVEC remaining in the wells were stained with 0.5% crystal violet and washed with water. Stained crystal violet was extracted with 30% acetic acid and quantified by spectrophotometry ($\lambda = 590$ nm).

Eosinophils were preincubated in RPMI 1640 medium containing 10% FBS for 2 h at 37 °C in an atmosphere of 5% CO₂ and 95% air. VUF-K-8788 or terfenadine was added to the medium and the cells were incubated for 24 h. After addition of WST-1 solution, eosinophils were incubated for 4 h. The absorbance of the cell suspension was measured by spectrophotometry ($\lambda = 450$ nm).

Relative cell numbers in control and drug-treated wells were compared based on the absorbance.

Statistics Student's *t*-test or Mann–Whitney's U-test was used to analyze significance of the difference between groups. Differences were accepted as statistically significant at *p*-values <0.05.

RESULTS

Effects on Hyperplasia and Edema Repeated inhalation of OA to actively sensitized guinea pigs induced hyperplasia of airway epithelium, perivascular edema and infiltration of bronchial parenchyma and airways by eosinophils (Figs. 1b and 3b). No obvious change was observed in normal animals (Figs. 1a and 3a). Effects of VUF-K-8788 on the hyperplasia and the edema were investigated by morphometric method. VUF-K-8788 (10 mg/kg, Figs. 1c and 2) and terfenadine (100 mg/kg, Figs. 1d and 2) inhibited the hyperplasia of airway epithelium and perivascular edema significantly.

Effects on Eosinophils Infiltration by eosinophils was evaluated at 4 h after OA challenge (Figs. 3 and 4). Infiltrating eosinophil counts were 0.9 ± 0.4 cells/mm² in normal animals and 16.5 ± 4.4 cells/mm² in control animals (Figs. 3a and 3b). VUF-K-8788 obviously inhibited the infiltration by eosinophils (Figs. 3c and 4), while terfenadine failed to do so (Figs. 3d and 4). There was a significant difference between the effects of VUF-K-8788 and terfenadine on this infiltration.

Eosinophil-Adhesion to HUVEC To investigate the mechanism by which VUF-K-8788 inhibits infiltration of lung parenchyma and airways by eosinophils, we tested whether VUF-K-8788 was capable of inhibiting the eosinophil adhesion to TNF- α stimulated HUVEC. To unstimulated HUVEC, 48 cells/high power field (h.p.f., $\times 400$) of eosinophils adhered; after the stimulation with 5 ng/ml TNF- α , 95 cells/h.p.f. of eosinophils adhered. VUF-K-8788

Fig. 1. Hematoxylin and Eosin Staining of Lung Sections from Guinea Pigs

The animals were actively sensitized with OA, and made to inhale OA 6 times. Four hours after the 6th inhalation, lung parenchymas were dissected. The specimens were sectioned and stained with hematoxylin and eosin. A, normal; B, control; C, VUF-K-8788-treated; D, terfenadine-treated. Bar=200 μ m.

Fig. 2. Effect of VUF-K-8788 (VUF) and Terfenadine (Ter) on Hyperplasia of Epithelium and Perivascular Edema

Each column and point represent mean and individual score, respectively. * $p < 0.05$, ** $p < 0.01$. $n = 6$.

inhibited the adhesion of eosinophils to stimulated HUVEC down to the unstimulated level depending on the concentration from 3 to 30 μ M, although terfenadine (100 μ M) failed to do so (Fig. 5).

Expression of VCAM-1 and ICAM-1 The expression of VCAM-1 and ICAM-1 on HUVEC stimulated with TNF- α was also investigated. The mean fluorescence intensity (MFI) of VCAM-1 on unstimulated HUVEC was 82. MFI of VCAM-1 on HUVEC stimulated with TNF- α increased to 138 (Table 1).

VUF-K-8788 (1–30 μ M) and terfenadine (100 μ M) did not inhibit the expression of VCAM-1. Dimethylfumarate, as a positive compound, significantly inhibited the expression of VCAM-1.

HUVEC stimulated with TNF- α also expressed ICAM-1. VUF-K-8788 did not affect the expression of ICAM-1, whereas terfenadine and dimethylfumarate inhibited its expression significantly.

Cytotoxicity of the Compounds The cytotoxicity of VUF-K-8788 and terfenadine was examined. As shown in

Fig. 3. Hansel's Staining of Lung Sections from Guinea Pigs

The specimens from the animals in Fig. 1 were stained with Hansel's solution. A, normal; B, control; C, VUF-K-8788-treated; D, terfenadine-treated. Note that eosinophils (arrows) infiltrated to lung parenchyma and airway in control animals, which decreased in VUF-K-8788-treated animals. Bar=20 μ m.

Fig. 4. Effect of VUF-K-8788 (VUF) and Terfenadine (Ter) on Infiltration by Eosinophils

The eosinophils infiltrating lungs were counted. Each value represents mean $\pm$ S.E.M. * p <0.05, ** p <0.01. n =6.

Fig. 6A, VUF-K-8788 did not show cytotoxicity for HUVEC at the concentration of 1–30 μ M; terfenadine (100 μ M) showed little cytotoxicity. Examination of the cytotoxicity to eosinophils showed no cytotoxicity by either VUF-K-8788 or terfenadine.

DISCUSSION

Airway inflammation, reversible airway obstruction and airway hyperresponsiveness are the hallmarks of bronchial

Fig. 5. Effect of VUF-K-8788 on Adhesion of Eosinophils to HUVEC

HUVEC were seeded on 96-well plates at 1×10^5 cells in 100 μ l of HUVEC cell medium (D/F12 medium containing 20 ng/ml TNF- α , 10% FBS and 10 μ g/ml bovine endothelial mitogen). After 1 h incubation, VUF-K-8788 or terfenadine (Ter) was added to confluent HUVEC in 100 μ l of HUVEC cell medium. Following 24 h incubation, eosinophils (1×10^5) were added to HUVEC and co-incubated for 30 min. Cell adhesion was measured by counting the adhering eosinophils. Each value represents the mean $\pm$ S.E.M. of 5 experiments. ** p <0.01.

asthma.¹⁷⁾ In the development of bronchial asthma, airway inflammation, characterized by hyperplasia of the airway epithelium, perivascular edema and eosinophil infiltration have been implicated.¹⁸⁾

In this study, we investigated effects of VUF-K-8788 on

the histopathological changes in the lung parenchyma of guinea pigs during the LAR, and the eosinophil-adhesion to HUVEC to elucidate mechanisms by which VUF-K-8788 inhibited the LAR and the infiltration by eosinophils of the bronchoalveolar lavage fluid in guinea pigs.

Repeated exposures to OA of actively sensitized guinea pigs induced both immediate- and late-phase asthmatic reactions one minute and 4 h after the exposure of antigens, respectively.¹⁰⁾ The immediate-phase asthmatic reaction (IAR) was induced by histamine; the histamine antagonist terfenadine inhibited it. During the LAR, various inflammatory phenomena such as hyperplasia of airway epithelial cells, perivascular edema, and infiltration by inflammatory cells, especially eosinophils, were recognized in lung parenchymal specimens. The features were consistent with

Table 1. Effects of VUF-K-8788, Terfenadine and Dimethylfumarate on VCAM-1 and ICAM-1 Expression on HUVEC Stimulated with TNF- α

	TNF- α (20ng/ml)	Compound	Concentration	MFI (mean $\pm$ S.D.)
VCAM-1	—	—	—	82 $\pm$ 3
	+	—	—	138 $\pm$ 14
	+	VUF-K-8788	1 μ M	154 $\pm$ 14
	+		3 μ M	167 $\pm$ 8
	+		10 μ M	169 $\pm$ 15
	+		30 μ M	155 $\pm$ 13
	+	Terfenadine	100 μ M	144 $\pm$ 24
ICAM-1	+	Dimethylfumarate	100 μ M	92 $\pm$ 2**
	—	—	—	108 $\pm$ 5
	+	—	—	308 $\pm$ 15
	+	VUF-K-8788	1 μ M	324 $\pm$ 13
	+		3 μ M	342 $\pm$ 9
	+		10 μ M	334 $\pm$ 37
	+		30 μ M	348 $\pm$ 18
	+	Terfenadine	100 μ M	175 $\pm$ 32**
	+	Dimethylfumarate	100 μ M	194 $\pm$ 9**

HUVEC were seeded on 6-well plates at 1×10^5 cells in 2 ml of HUVE cell medium (MCDB 104 containing 5% FCS, 5 μ g/ml ECGs and 10 μ g/ml heparin), and cultured for 3 d. HUVE cell monolayers were rinsed with 1 ml of warmed PBS, and added with TNF- α (20 ng/ml) and each compound in 2 ml of DMEM/F12 medium containing 10% FBS and 100 μ g/ml endothelial mitogen. After 24 h incubation, HUVEC were collected with 20% trypsin-EDTA. VCAM-1 and ICAM-1 expression on HUVEC were analyzed by *FACSscan*. The data are represented as the mean $\pm$ S.D. of three experiments. ** $p < 0.01$. MFI: mean fluorescence intensity.

the results reported by Takeda *et al.*; the LAR was accompanied by eosinophilia, an increase of water content of whole lung, airway hyperresponsiveness and an increase in the amount of histamine, LTD₄ and TXB₂.⁸⁾ These results suggested that the histopathological changes were linked with the LAR followed by the airway hyperresponsiveness.

In a previous study, we reported that VUF-K-8788 inhibited antigen-induced IAR and LAR, airway hyperresponsiveness and the infiltration by eosinophils of bronchoalveolar lavage fluids in guinea pigs.¹⁰⁾ In the present study, we investigated the effects of VUF-K-8788 on the histopathological changes in the lung parenchyma of guinea pigs during the LAR. VUF-K-8788 inhibited the hyperplasia of airway epithelium, the perivascular edema and the infiltration by eosinophils. The effects of VUF-K-8788 observed in the histological study might contribute to the inhibition of the LAR and the airway hyperresponsiveness. On the contrary, terfenadine did not inhibit the eosinophilia although it inhibited the hyperplasia of airway epithelium and the perivascular edema significantly. In our previous report, terfenadine failed to inhibit the LAR.¹⁰⁾ This evidence would indicate the importance of eosinophils to evoke a LAR in the asthmatic model in guinea pigs. In fact, it was reported that the LAR was associated with an increased number of eosinophils in the bronchial mucosa in humans and mice.^{7,19)} Cieslewicz *et al.* also reported that inhibition of the influx of eosinophils was associated with the inhibition of LAR.¹⁹⁾

To study the mechanisms by which VUF-K-8788 suppressed the infiltration by eosinophils, we investigated the effect of VUF-K-8788 on eosinophil-adhesion with HUVEC. VUF-K-8788 inhibited eosinophil-adhesion to HUVEC without a cytotoxic effect at the concentration of 10–30 μ M. Eosinophils adhere to and migrate across the lung microvasculature to exert their effector functions in the lung parenchyma and the airways. This process is regulated by adhesion molecules including VCAM-1 and ICAM-1.²⁰⁾ In our investigation, VUF-K-8788 did not inhibit the expression of VCAM-1 or ICAM-1. It might antagonize the binding of adhesion molecules or inactivate eosinophils to adhere to endothelial cells, although the precise mechanisms were to be investigated.

Fig. 6. Cytotoxicity of VUF-K-8788, Terfenadine and Dimethylfumarate for HUVEC and Eosinophils

A: HUVEC were seeded on 96-well plates at 1×10^5 cells in 100 μ l of HUVE cell medium (D-MEM/F12 medium containing 10% FBS and 100 μ g/ml bovine endothelial mitogen). After 1 h incubation, VUF-K-8788, terfenadine or dimethylfumarate was added to confluent HUVEC in 100 μ l of HUVEC medium. Following 24 h incubation, cytotoxicity was evaluated by the method of crystal violet. B: Eosinophils were seeded on 96-well plates at 1×10^5 cells in 100 μ l of RPMI medium containing 10% FBS. After 1 h incubation, VUF-K-8788 or terfenadine was added to eosinophils in 100 μ l of HUVE cell medium. Following 24 h incubation, cytotoxicity was measured by WST-1 assay. Each value represents the mean $\pm$ S.E.M. of 5 experiments.

It is controversial which is more important, VCAM-1 or ICAM-1, in adhesion of eosinophils to endothelial cells. Akagi described that expression of ICAM-1 participated in the eosinophil recruitment, and that some antihistamines (*i.e.*, azelastine, oxatomide, levocavastine, terfenadine and loratadine) reduce ICAM-1 expression.²¹⁾ Yamamoto *et al.* reported that the VCAM-1/ α 4 integrin pathway is more important than the ICAM-1/ β 2 integrin pathway in adhesion of eosinophils to human pulmonary microvascular endothelial cells.²²⁾ In the present study, terfenadine did not inhibit the adhesion of eosinophils to HUVEC, although it suppressed the expression of ICAM-1. These results might support the latter hypothesis indirectly. Indeed, several studies on the eosinophilic inflammation in animals, in which blocking monoclonal antibodies were used, have demonstrated important roles of the interaction of VCAM-1 and VLA-4 during the eosinophil recruitment processes.^{15,23–25)}

In conclusion, the repeated exposure to OA of guinea pigs induced LAR accompanied by the inflammatory features of hyperplasia of airway epithelial cells, perivascular edema, and infiltration by inflammatory cells, especially eosinophils; VUF-K-8788 inhibited these histopathological phenomena. The mechanisms by which VUF-K-8788 inhibited LAR is at least attributable to suppression of the inflammatory phenomena and eosinophil adhesion to HUVEC in addition to the antihistaminic effect. Therefore, the new antiallergic drug, VUF-K-8788, should be useful in the treatment of asthmatic patients.

REFERENCES

- 1) Hargreave F. E., Dolovich J., Robertson D. G., Kerigan A. T., *Can. Med. Assoc. J.*, **110**, 415–442 (1974).
- 2) Charlesworth E. N., Massey W. A., Kagey-Sobotka A., Norman P. S., Lichtenstein L. M., *J. Pharmacol. Exp. Ther.*, **262**, 964–970 (1992).
- 3) Naclerio R. M., Proud D., Togias A. G., Adkinson N. F., Jr., Meyers D. A., Kagey-Sobotka A., Plaut M., Norman P. S., Lichtenstein L. M., *N. Engl. J. Med.*, **313**, 65–70 (1985).
- 4) Pepys J., Hutchcroft B. J., *Am. Rev. Respir. Dis.*, **112**, 829–859 (1975).
- 5) Hargreave F. E., *J. Allergy Clin. Immunol.*, **83**, 525–527 (1989).
- 6) Cartier A., Thomson N. C., Frith P. A., Roberts R., Hargreave F. E., *J. Allergy Clin. Immunol.*, **70**, 170–177 (1982).
- 7) De Monchy J. G. R., Kauffman H. F., Venge P., *Am. Rev. Respir. Dis.*, **131**, 373–376 (1985).
- 8) Takeda H., Kogame A., Tanaka H., Nagai H., *Prostaglandins*, **54**, 805–820 (1997).
- 9) Takizawa T., Matsumoto J., Tohma T., Kanke T., Wada Y., Nagao M., Inagaki N., Nagai H., Zhang M. Q., Timmerman H., *Jpn. J. Pharmacol.*, **86**, 55–64 (2001).
- 10) Takizawa T., Yamada T., Takahashi Y., Tanaka H., Wada Y., Nagai H., *Pharmacology*, **59**, 127–134 (1999).
- 11) Springer T. A., *Nature (London)*, **346**, 425–434 (1990).
- 12) Springer T. A., *Cell*, **76**, 301–314 (1994).
- 13) Bochner B. S., Schleimer R. P., *J. Allergy Clin. Immunol.*, **94**, 427–438 (1994).
- 14) Blyth D. I., Pedrick M. S., Savage T. J., Hessel E. M., Fattah D., *Am. J. Respir. Cell Mol. Biol.*, **14**, 425–438 (1996).
- 15) Sagara H., Matsuda H., Wada N., Yagita H., Fukuda T., Okumura K., Makino S., Ra C., *Int. Arch. Allergy Immunol.*, **112**, 287–294 (1997).
- 16) Saito K., Oku T., Ata N., Miyashiro H., Hattori M., Saiki I., *Biol. Pharm. Bull.*, **20**, 345–348 (1997).
- 17) National Heart, Lung and Blood Institute. “Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention,” NHLBI/WHO workshop report. NHLBI publication No. 95-3659 (1995).
- 18) Redington A. E., Howarth P. H., *Thorax*, **52**, 310–312 (1997).
- 19) Cieslewicz G., Tomkinson A., Adler A., Duez C., Schwarze J., Takeda K., Larson K. A., Lee J. J., Irvin C. G., Gelfand E. W., *J. Clin. Invest.*, **104**, 301–308 (1999).
- 20) Seminario M. C., Gleich G. J., *Curr. Opin. Immunol.*, **6**, 860–864 (1994).
- 21) Akagi M., *Folia Pharmacol. Jpn.*, **111**, 217–222 (1998).
- 22) Yamamoto H., Sedgwick J. B., Busse W. W., *J. Immunol.*, **161**, 971–977 (1998).
- 23) Weg V. B., Williams T. J., Lobb R. R., Nourshargh S., *J. Exp. Med.*, **177**, 561–566 (1993).
- 24) Abraham W. M., Sielczak M. W., Ahmed A., Cortes A., Lau-redo I. T., Kim J., Pepinsky B., Benjamin C. D., Leone D. R., Lobb R. R., Weller P. F., *J. Clin. Invest.*, **93**, 776–787 (1994).
- 25) Sriramapo P., DiScipio R. G., Cobb R. R., Cybulsky M., Stachnick G., Castaneda D., Elices M., Broide D. H., *Blood*, **92**, 592–601 (2000).