

Cysteinyl leukotriene-dependent interleukin-5 production leading to eosinophilia during late asthmatic response in guinea-pigs

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Summary

Background Allergic airway eosinophilia is suppressed by cysteinyl leukotriene (CysLT) receptor (CysLT₁ receptor) antagonists in several species including humans and guinea-pigs, suggesting that CysLTs are directly or indirectly involved in induction of the response.

Objective We examined the effect of CysLT antagonists (pranlukast and MCI-826) on antigen inhalation-induced eosinophilia in peripheral blood and lung, and on IL-5 activity in serum during late increase of airway resistance (late asthmatic response, LAR) in sensitized guinea-pigs.

Methods Guinea-pigs inhaled ovalbumin (OVA) + Al(OH)₃ and OVA mists alternately for sensitization and challenge, respectively, once every 2 weeks. At the fifth challenge, the effects of CysLT antagonists and an anti-IL-5 antibody (TRFK-5) on the occurrence of LAR, and blood and lung eosinophilia, which appeared at 5 h after challenge, were examined. The time-course of IL-5 activity in the serum after the challenge was evaluated by measuring *in vitro* 'eosinophil survival prolongation activity'. The influence of CysLT antagonists on IL-5 activity was assessed.

Results CysLT antagonists and TRFK-5 completely abolished blood and lung eosinophilia. LAR was suppressed by both MCI-826 and TRFK-5 by 40–50%. Sera obtained from sensitized, challenged animals 3 h and 4 h after challenge induced an obvious prolongation of eosinophil survival. The activity of the sera was completely neutralized by prior exposure to TRFK-5, suggesting that it reflected IL-5 activity. Increased IL-5 activity in the serum was inhibited by both pranlukast and MCI-826 by over 90%.

Conclusions CysLTs produced after antigen provocation sequentially induced IL-5 production from some immune component cells via CysLT₁ receptor activation. Thus, it is likely that CysLTs indirectly cause antigen-induced eosinophilia through IL-5 production.

Keywords asthma, cysteinyl leukotrienes, cysteinyl leukotriene antagonist, CysLT₁ receptor, eosinophils, guinea-pig, interleukin-5, late asthmatic response

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Introduction

The late asthmatic response (LAR) is one of the characteristic features of allergic bronchial asthma in addition to the early asthmatic response (EAR) and bronchial hyper-responsiveness. Suggested causes of LAR are related to lung eosinophilia [1,2]. The migration of eosinophils into the airway is thought to be due to the release of chemotactic factors such as IL-5 [3], eotaxin [4] and lipid mediators [5,6] from activated immunocomponent cells. Of lipid mediators formed in the allergic reaction, the cysteinyl leukotrienes (CysLTs), LTC₄, LTD₄ and LTE₄, may play a significant role in eosinophil infiltration into the airways of several species including humans [7] and guinea-pigs [8,9]. Underwood et al. [9] have indicated that a single exposure to LTD₄ at high doses causes CysLT₁ receptor-mediated airway eosinophilia in guinea-pigs and that an anti-IL-5 antibody

(TRFK-5) completely inhibited it, suggesting that the eosinophilia induced by LTD₄ inhalation depends on IL-5 production. In addition, antigen-induced eosinophilia in the lung tissue of sensitized guinea-pigs is suppressed by both CysLT₁ receptor antagonists [8,10] and TRFK-5 [11,12]. However, whether CysLTs produced following an antigen–antibody reaction sequentially induce IL-5 production leading to lung eosinophilia during LAR is not clear.

In contrast to the involvement of CysLTs in tissue eosinophilia [7–10], CysLT-induced blood eosinophilia has not been identified. IL-5 may be an important factor for the mobilization of eosinophils, probably from the bone marrow to the peripheral blood and for priming eosinophils to easily respond to other chemotactic factors such as chemokines that may migrate eosinophils to local tissues [13].

On the other hand, a chronic experimental asthmatic model showing not only LAR and lung eosinophilia but also blood eosinophilia, which are suggested to be elicited upon repeated exposures to antigen [14], is indispensable for analysing these phenomena. We established a repeated antigen inhalation-induced asthmatic model using guinea-pigs, in which LAR

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appears after a latent period of repetitive sensitization and challenge with antigen [15]. In addition, we reported that eosinophilia in the peripheral blood and the airway preceded or coincided with the LAR, which occurred 5–7 h after challenge [15,16]. Blood eosinophilia transiently appeared 3–5 h after antigen challenge [16], and at 5 h eosinophils had accumulated in the lung tissue [17] but not in the airway cavity [16]. However, a few hours later, eosinophils started to migrate into the airway cavity, followed by sustained accumulation up to day 3 [16]. Thus, antigen provocation first triggers eosinophil recruitment into the blood from putative tissues of the cellular pool or from bone marrow as found in guinea-pigs [13]. Eosinophils then migrate to the lung and finally infiltrate the airway cavity. That eosinophilia precedes or coincides with LAR suggests that eosinophils infiltrating the lung are involved in the induction of LAR. However, detailed mechanisms leading to blood eosinophilia and subsequent lung eosinophilia are not clear.

The present study first evaluated the possible role of CysLTs in the eosinophilia at LAR in our model using the CysLT₁ receptor antagonists, pranlukast [18,19] and MCI-826 [20]. Secondly, the involvement of IL-5 in not only eosinophilia but also LAR was assessed in the sensitized guinea pig using TRFK-5. Finally, assuming that blood eosinophilia is induced by IL-5 that was sequentially produced by CysLTs following antigen provocation, time-course changes in circulatory IL-5 activity after challenge were observed, and the effect of CysLT antagonists on the increase of IL-5 activity was evaluated.

Materials and methods

Animals

Allergic asthmatic models were generated and eosinophils were collected from 5-week-old (300–400 g) and 15–20-week-old (700–900 g) male Hartley guinea-pigs (Japan SLC, Hamamatsu, Japan), respectively. The animals were housed in an air-conditioned room for at least 2 weeks after purchase at a temperature of $23 \pm 1^\circ\text{C}$ and $60 \pm 10\%$ humidity, illuminated from 08.00 to 20.00 h, fed with a standard laboratory diet and given water ad libitum. Experimental protocols and the animal care methods were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Reagents

Reagents and their sources were as follows: pranlukast hemihydrate (supplied by Dr H. Ohno of Ono Pharmaceutical Co., Osaka, Japan); MCI-826 (supplied by Dr K. Takahashi of Mitsubishi Kasei, Tokyo, Japan); purified rat anti-mouse/human IL-5 antibody (TRFK-5) and purified rat IgG₁, κ isotype standard (R3–34) (Pharmingen, San Diego, CA, USA); recombinant murine (rm) IL-5 (Genzyme, Cambridge, MN, USA); ovalbumin (OVA), Triton X-100®, methylcellulose, trypan blue, bovine serum albumin (BSA), 2-mercaptoethanol (2-ME) and H₂O₂ (Wako Pure Chemicals, Osaka, Japan); mepyramine maleate (Sigma Chemicals, St Louis, MO, USA); penicillin G potassium (Banyu Pharmaceutical Co., Tokyo, Japan); streptomycin sulphate (Meiji Confectionery Co., Tokyo, Japan); heparin sodium (Takeda Chemical Industries, Osaka, Japan); sodium pentobarbital (Abbott Laboratories, North Chicago, IL, USA); 3,3',5,5'-tetramethylbenzidine (Dohjindo Laboratories, Kumamoto, Japan); KBr (Merck, Darmstadt,

Germany); Sephadex G-200 (Superfine) and Percoll (Pharmacia, Uppsala, Sweden); RPMI-1640 medium and fetal bovine serum (FBS, Life Technologies, Grand Island, NY, USA); sucrose and Turk's stain solution (Nacalai Tesque, Kyoto, Japan); Diff-Quik® solution (International Reagents, Kobe, Japan).

Pranlukast and MCI-826 were suspended and dissolved in 0.5% methylcellulose.

OVA + Al(OH)₃ for sensitization was prepared as reported [15,21]. OVA solution for challenge was prepared at a concentration of 16 mg/mL.

Sensitization and challenge with antigen

Sensitization and challenge by inhalation of mists of OVA + Al(OH)₃ and OVA, respectively, were performed as described [15]. In brief, conscious guinea-pigs were sensitized by forced inhalation of OVA + Al(OH)₃ mists at a dose of 15 µg OVA/750 µg Al(OH)₃/animal/time, once every 2 weeks for the first 4 weeks. The animals were then forced to inhale OVA (10 µg OVA/animal/time) and the OVA + Al(OH)₃ mists alternately for challenge and sensitization, respectively, once every 2 weeks until the fifth challenge (week 20) (sensitized–challenged group). Two negative control groups were: (i) guinea-pigs that had been sensitized and challenged as described above, were forced to inhale physiological saline instead of OVA at the time corresponding to the fifth challenge (sensitized–non-challenged group); (ii) guinea-pigs that had been treated with physiological saline mist instead of antigens once every 2 weeks were treated with physiological saline on the same experimental day as the sensitized–non-challenged group (non-sensitized–non-challenged group).

In preliminary experiments over 60% of the sensitized guinea-pigs died immediately (within 20 min) following the first challenge, and the second challenge induced severe anaphylactic symptoms including laboured breathing and cyanosis in most of the animals. Therefore, all animals were administered mepyramine prior to the first to third challenges. However, as very few animals exhibited such severe reactions at the fourth challenge, the fourth and the fifth provocations were performed without mepyramine.

As reported elsewhere [15], at the fourth to tenth challenges, increase of airway resistance (R_{aw}) was biphasically observed in an almost reproducible fashion: transient increase of R_{aw} was induced 10 min after challenge followed by restoration of the elevation to the level before challenge at 2–3 h. At 5–7 h after challenge, R_{aw} of challenged guinea-pigs increased again in a relatively persistent manner.

Administration of drugs or anti-IL-5 antibody

Pranlukast (30 mg/kg), MCI-826 (3 mg/kg) or vehicle (0.5% methylcellulose) was administered orally 1 h before the fifth challenge at a volume of 2 mL/kg. TRFK-5 (50 µg/kg) or R3–34 (50 µg/kg) was administered intravenously 10 min before the challenge at a volume of 2 mL/kg.

Blood drawing for counting leucocytes

Five hours after the fifth OVA inhalation challenge, peripheral blood was drawn in the presence of a 100 U/mL final concentration of heparin from the abdominal vein of each of the sensitized–challenged guinea-pigs under pentobarbital anaesthesia (40 mg/kg, i.p.). Blood samples from the sensitized–

non-challenged and the non-sensitized–non-challenged groups were also drawn 5 h after saline inhalation in the same manner. The total leucocyte number was determined by staining with Turk's solution. To determine the differentiated leucocyte number, the blood specimen was centrifuged on a cell settling chamber (Neuro Probe, Cabin John, MD, USA) at 50 g for 30 s at 4 °C after hypotonic treatment, then settled leucocytes were stained with Diff-Quik® solution, followed by the microscopic counting of at least 500 cells.

Isolation of the lung to measure eosinophil peroxidase (EPO) activity in the homogenate

Following drawing blood, animals of the respective groups were exsanguinated from the abdominal aorta. The lungs were perfused with physiological saline via the pulmonary artery, then isolated. The right lower lobe was weighed and used to determine EPO activity. Procedures for measurements were as follows. The lobe was homogenized (4 °C, 12 000 r.p.m., 20 s × 2) in 10 volumes (v/w) of assay buffer consisting of 0.3 M sucrose in 50 mM acetate buffer (pH 5.4) and then again (4 °C, 8000 r.p.m., 20 s × 2) in the presence of 0.2% Triton X-100®. EPO activity in the homogenate was measured as described by Tagari et al. [22], who modified the method of Bozeman et al. [23]. A 50-μL aliquot of the 50-fold diluted homogenate was added to 200 μL of the assay buffer with or without 5.3 mM KBr in a polypropylene tube. After adding 100 μL of 3.9 mM 3,3',5,5'-tetramethylbenzidine containing 2.8 mM H₂O₂ and mixing well, the mixture was incubated for 5 min at 21 °C. The peroxidase reaction was terminated by adding 200 μL of 1 N H₂SO₄. After centrifugation (20 °C, 1700 g, 5 min), 200 μL of the supernatant was transferred into a microtitre plate (Maxisorp; Nunc, Roskilde, Denmark), and the optical density at 450 nm was measured. EPO activity is expressed as $\Delta OD \pm K_{Br}$. This method using bromide ions is specific for EPO [23].

Measurement of pulmonary function

As described [15], airway resistance (R_{aw}) was measured using a two-chambered, double-flow plethysmograph system (Pulmos-I; M.I.P.S., Osaka, Japan) according to the method of Pennock et al. [24] before, and at 10 min and 5 h after challenge without anaesthesia.

Measurement of 'eosinophil survival prolongation activity' (IL-5 activity) in serum

IL-5 activity in serum was indicated by prolonged eosinophil survival (hereafter referred as 'eosinophil survival prolongation activity'). To measure such prolongation activity, blood was drawn 1, 3 and 4 h after the fifth challenge from the abdominal vein of the sensitized–challenged guinea-pigs under pentobarbital anaesthesia. Blood samples from the sensitized–non-challenged and non-sensitized–non-challenged groups were also withdrawn.

In vitro eosinophil survival prolongation activity in the sera was measured using eosinophils that had been collected from bronchoalveolar lavage fluid (BALF) of Sephadex G-200-treated guinea-pigs. As described [25], 18 h after an intravenous administration of Sephadex G-200 (10 mg/kg), BAL was performed with Ca²⁺-free Tyrode's solution containing 0.1% BSA. The recovered cells were treated hypotonically and incubated in polystyrene flasks at 37 °C for 1 h, then suspended non-adherent cells were stratified on discontinuous (50, 60 and 70%) Percoll

layers. After centrifugation at 360 g for 30 min at 4 °C, eosinophils were recovered from the bottom of centrifuge tubes. Purity of eosinophils was over 97% as determined by staining with Diff-Quik®. The viability of the recovered cells was over 99% as determined by trypan blue dye exclusion test.

Purified eosinophils suspended in RPMI-1640 medium containing 10% FBS, 50 μM 2-ME, 200 U/mL penicillin and 200 μg/mL streptomycin (10^5 cells/100 μL/well) were incubated with 100 μL of serum or 2×10^{-14} – 2×10^{-10} g/mL rmIL-5 in a polystyrene 96-well microplate (Iwaki, Chiba, Japan) at 37 °C for 7 days in 5% CO₂ and 95% air. To neutralize the activity, serum and rmIL-5 were treated with TRFK-5 or the isotype matched control, R3-34, at 4 °C for 30 min before being added to the eosinophil suspension. After a 7-day incubation, cell viability was determined by trypan blue dye exclusion.

Statistical analysis

Statistical analysis was performed by the one-way analysis of variance. If significant difference was detected, the individual group difference was determined by Bonferroni's multiple test. A probability value (*P*) below 0.05 was considered statistically significant.

Results

Effect of CysLT antagonists on antigen-induced blood and lung eosinophilia, EAR and LAR

The effects of the specific antagonists against CysLTs, pranlukast and MCI-826, on blood and lung eosinophilia are shown in Table 1. The elevation of EPO activity in the lung homogenate was regarded as an indicator of tissue eosinophilia. Both pranlukast and MCI-826 abolished blood and lung eosinophilia. In addition, MCI-826 significantly suppressed both EAR (Fig. 1a) and LAR (Fig. 1b) to approximately 50% and 40%, respectively. Inhibitions of these obstructive responses by MCI-826 was to the same degree as that induced by pranlukast [17]. On the other hand, neither MCI-826 nor pranlukast affected the numbers of other leucocytes (monocytes, neutrophils and lymphocytes) in the blood of the sensitized–challenged group (data not shown).

Effect of anti-IL-5 antibody on antigen-induced blood and lung eosinophilia, EAR and LAR

The effects of an anti-IL-5, TRFK-5, on the blood and lung eosinophilia, are shown in Table 2. TRFK-5 decreased the number of blood eosinophils to a level lower than that of sensitized–non-challenged animals. Induction of eosinophilia in the lung tissue was also abolished by TRFK-5. On the other hand, the numbers of other leucocytes (monocytes, neutrophils and lymphocytes) in the blood in the sensitized–challenged group were not significantly influenced by the anti-IL-5 antibody (data not shown).

Although EAR was not affected by TRFK-5 (Fig. 2a), the antibody attenuated LAR by approximately 50% (Fig. 2b).

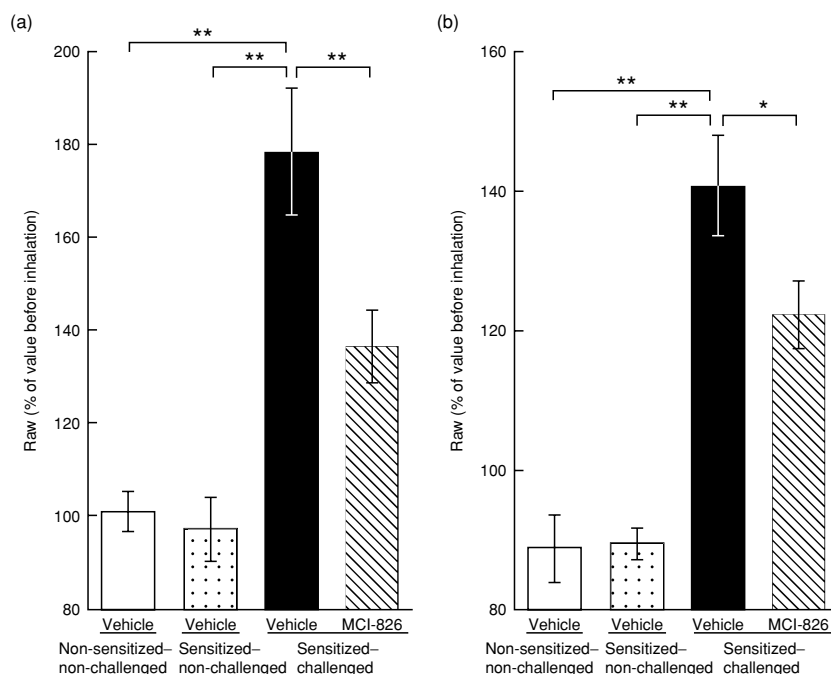
Time-course of 'eosinophil survival prolongation activity' (IL-5 activity) in the serum after antigen challenge

Figure 3(a) shows the time-course of eosinophil survival prolongation activity in the serum after the fifth antigen inhalation

Table 1. Effect of cysteinyl leukotriene antagonists (pranlukast and MCI-826) on antigen-induced blood and lung eosinophilia in the sensitized guinea-pig

Group	Number of eosinophils in blood ($\times 10^4$ cells/mL)	EPO activity in lung homogenate ($\Delta OD \pm \kappa_{BR}/\text{mg tissue}$)
Non-sensitized–non-challenged		
Vehicle	16.6 ± 3.8	0.50 ± 0.11
Sensitized–non-challenged		
Vehicle	16.1 ± 2.2	0.89 ± 0.22
Sensitized–challenged		
Vehicle	$38.9 \pm 5.9^{**}$	$2.21 \pm 0.55^*$
Pranlukast	$10.8 \pm 1.3^{\dagger\dagger}$	$0.92 \pm 0.18^{\dagger}$
MCI-826	$11.6 \pm 1.1^{\dagger\dagger}$	$0.80 \pm 0.19^{\dagger}$

Pranlukast (30 mg/kg), MCI-826 (3 mg/kg) or vehicle (0.5% methylcellulose) was administered orally 1 h before antigen inhalation challenge. Values are means $\pm$ SE of 13 or 14 animals. $^*P < 0.05$ and $^{**}P < 0.01$ vs. sensitized–non-challenged + vehicle, $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$ vs. sensitized–challenged + vehicle.

**Fig. 1.** Effect of cysteinyl leukotriene antagonist (MCI-826) on antigen-induced early (EAR, a) and late (LAR, b) asthmatic response in sensitized guinea-pigs. MCI-826 (3 mg/kg) or vehicle (0.5% methylcellulose) was administered orally 1 h before antigen inhalation challenge. Airway resistance (R_{aw}) was measured 10 min (EAR) and 5 h (LAR) after challenge. Each column represents mean $\pm$ SE of five or eight animals. Significant difference ($^*P < 0.05$ and $^{**}P < 0.01$).

challenge. The viability of eosinophils exposed to RPMI-1640 medium containing 10% FBS for 7 days was approximately 10%. Sera from the non-sensitized–non-challenged and the sensitized–non-challenged (at 0 h) guinea-pigs did not affect viability on day 7. However, serum harvested from sensitized–challenged animals 1 h after challenge slightly elevated viability of eosinophils. Furthermore, sera from sensitized–challenged guinea-pigs at 3 h and 4 h significantly prolonged eosinophil survival.

Figure 3(b) shows that the eosinophil survival prolongation activity of the serum at 3 h was neutralized by 10^{-7} g/mL TRFK-5, indicating that the activity of the serum totally reflects IL-5 activity. The neutralizing effect of TRFK-5 was concentration-dependent in the range of 10^{-9} – 10^{-7} g/mL (data not shown). Yet, an isotype matched control antibody (rat IgG1), R3-34, induced no inhibition of eosinophil survival prolongation activity at 10^{-7} g/mL. On the other hand, rmIL-5 also concentration-dependently prolonged eosinophil survival at

2×10^{-14} – 2×10^{-10} g/mL (data not shown). The activity of rmIL-5 at 2×10^{-11} g/mL was also neutralized by 10^{-7} g/mL TRFK-5 but not by R3-34 (Fig. 3b).

Effect of CysLT antagonists (pranlukast and MCI-826) on antigen-induced elevation of IL-5 activity in the serum

Figure 4 shows the effect of pranlukast and MCI-826 on the elevated IL-5 activity in the serum of sensitized guinea-pigs 3 h after antigen inhalation challenge. Both pranlukast and MCI-826 significantly inhibited the increased IL-5 activity by over 90% ($P < 0.001$).

Discussion

CysLT antagonists and anti-IL-5 antibody completely inhibited antigen-induced lung eosinophilia, and this finding was in

Table 2. Effect of an anti-IL-5 monoclonal antibody (TRFK-5) on antigen-induced blood and lung eosinophilia in the sensitized guinea-pig

Group	Number of eosinophils in blood ($\times 10^4$ cells/mL)	EPO activity in lung homogenate ($\Delta OD \pm \kappa_{BR}/\text{mg tissue}$)
Sensitized–non-challenged		
R3–34	11.8 ± 2.7	0.91 ± 0.20
Sensitized–challenged		
R3–34	$23.2 \pm 3.0^*$	$1.95 \pm 0.38^*$
TRFK-5	$1.8 \pm 0.5^{\dagger\dagger\dagger}$	$0.87 \pm 0.22^{\dagger}$

TRFK-5 (50 $\mu\text{g/kg}$) or R3–34 (50 $\mu\text{g/kg}$) was administered intravenously 10 min before antigen inhalation challenge. Values are means $\pm$ SE of five or eight animals. $^*P < 0.05$ vs. sensitized–non-challenged + R3–34, $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ vs. sensitized–challenged + R3–34.

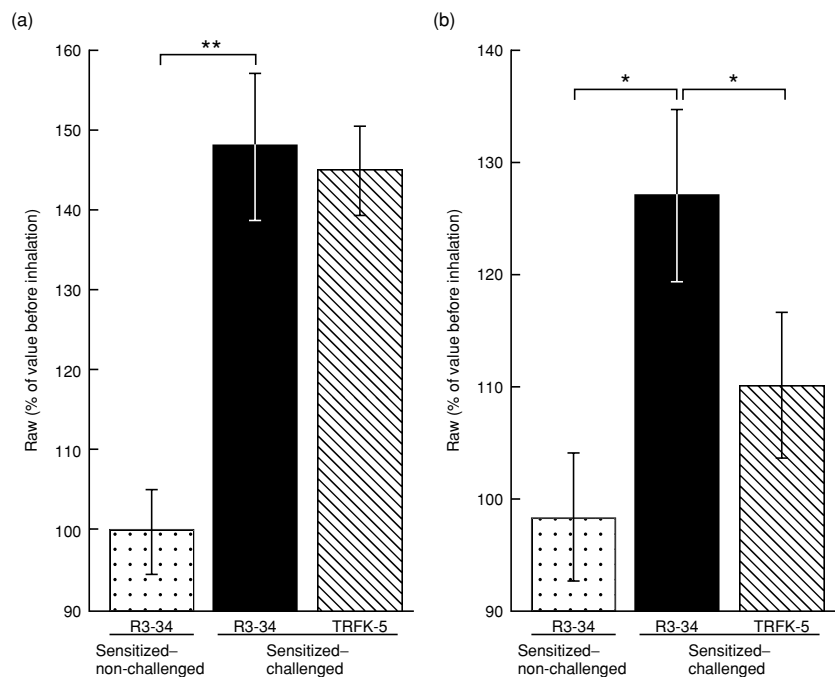


Fig. 2. Effect of anti-IL-5 monoclonal antibody (TRFK-5) on antigen-induced early (EAR, a) and late (LAR, b) asthmatic response in sensitized guinea-pigs. TRFK-5 (50 $\mu\text{g/kg}$) or rat IgG1 (R3–34, 50 $\mu\text{g/kg}$) was administered intravenously 10 min before antigen inhalation challenge. Airway resistance (R_{aw}) was measured 10 min (EAR) and 5 h (LAR) after challenge. Each column represents mean $\pm$ SE of 13 or 14 animals. Significant difference ($^*P < 0.05$ and $^{**}P < 0.01$).

agreement with those in other reports [8,10–12]. In addition, these agents completely suppressed the blood eosinophilia in our asthmatic model. These results suggest that eosinophilia in the lung and the circulation almost entirely depend on CysLTs and IL-5. To assess whether CysLTs are involved in the production of IL-5, we evaluated the effect of CysLT antagonists on the increase in serum IL-5 activity. Both specific CysLT₁ receptor antagonists, pranlukast and MCI-826, almost completely abolished elevated serum IL-5 activity. These findings indicate that CysLTs produced after the antigen–antibody reaction sequentially induced IL-5 production from immunocomponent cells via CysLT₁ receptor activation.

Guinea-pig lung mast cells can release large amounts of CysLTs, probably within 30 min of antigen provocation [26]. In the present study, the serum IL-5 activity was increased at 3 h after the challenge and blood eosinophilia peaked at 5 h after the challenge [16]. In addition, the intravenous administration of human recombinant IL-5 produced blood eosinophilia that peaked 1 h later in guinea-pigs [13,27]. These findings suggest that CysLTs produced at the EAR are responsible for IL-5

production. However, the cellular source of IL-5 is unclear. Lynch et al. [28] found that normal human peripheral blood leucocytes have surface CysLT₁ receptors. More recently, it was reported that a very small percentage of human CD4⁺ and CD8⁺ T cells from normal donors expresses CysLT₁ receptor on their surface [29]. In addition, it is well known that human CD4⁺ T cells are capable of producing IL-5 [30]. Therefore, cellular source of IL-5 in response to CysLTs might be T cells, although CysLT₁ receptors on guinea-pig T cell surface have not been identified yet. Figueroa et al. [29] speculated that CysLT₁ receptor expression may be up-regulated in lymphocytes from asthmatic subjects. The repeated sensitization/challenge with antigen in our model might also up-regulate the receptor expression on guinea-pig lymphocytes.

On the other hand, LTD₄ may behave as a potent chemoattractant *in vitro* for human eosinophils at physiologically relevant concentrations as low as 10^{-10} M [31]. We used an *in vitro* chemotaxis assay to examine whether or not LTD₄ directly causes guinea-pig eosinophil migration. Only high concentrations (10^{-7} and 10^{-6} M) of LTD₄ induced

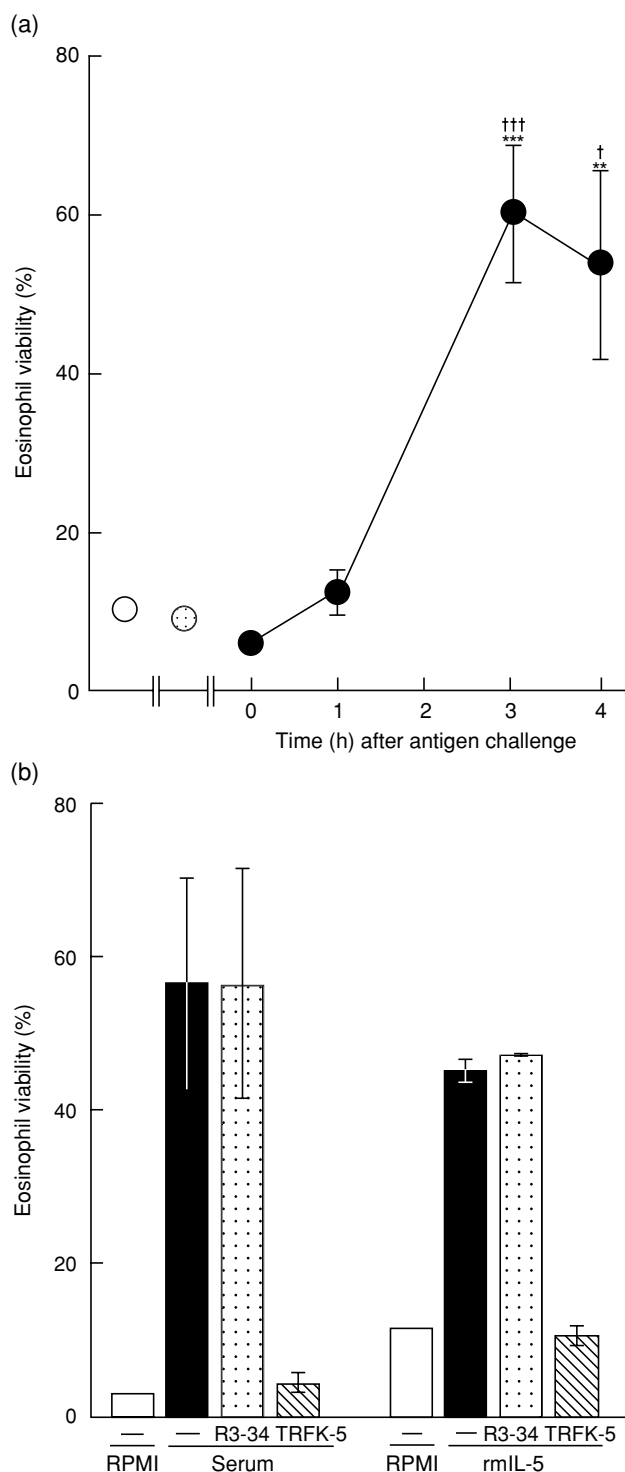


Fig. 3. Time-course of eosinophil survival prolongation activity in serum of sensitized guinea-pigs after antigen inhalation challenge (a), and neutralizing effect of anti-IL-5 monoclonal antibody (TRFK-5) on activities of serum and recombinant murine (rm) IL-5 (2×10^{-11} g/mL) (b). Sera obtained from sensitized (●) and non-sensitized guinea-pigs (○), and RPMI-1640 medium containing 10% FBS (○) were applied to *in vitro* assay of survival of eosinophils harvested from Sephadex G-200-treated guinea pigs. Serum obtained at 3 h after challenge and 2×10^{-11} g/mL of rmIL-5 were incubated with 10^{-7} g/mL of TRFK-5 or R3-34 for 30 min at 4 °C prior to survival assay. Each point and column represents mean $\pm$ SE of three to five animals (a) and three experiments (b), respectively. ** and ***: $P < 0.01$ and 0.001 (vs. sensitized at 0 h). † and †††: $P < 0.05$ and 0.001 (vs. non-sensitized).

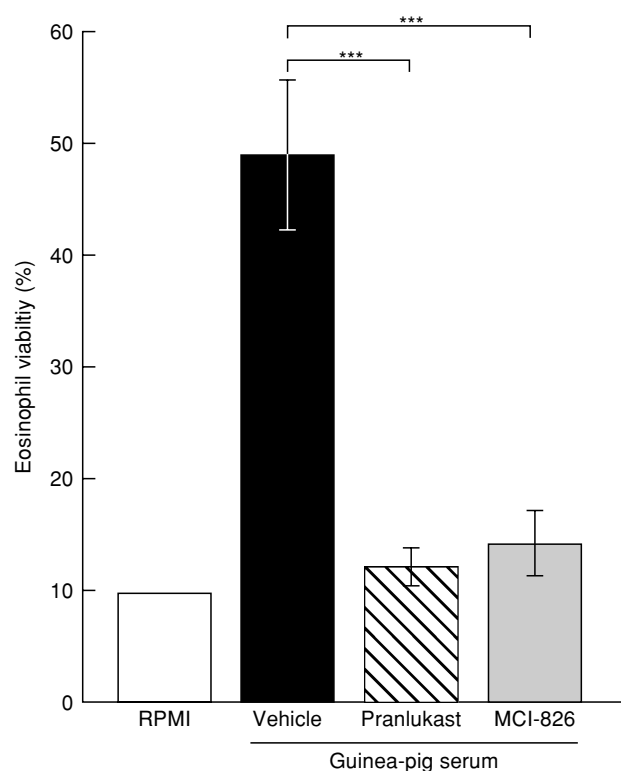


Fig. 4. Effect of cysteinyl leukotriene antagonists (pranlukast and MCI-826) on increase in IL-5 activity in serum obtained from sensitized guinea-pigs 3 h after antigen inhalation challenge. Pranlukast (30 mg/kg), MCI-826 (3 mg/kg) or vehicle was administered 1 h before antigen challenge. Sera obtained from sensitized-challenged guinea-pigs treated with vehicle, pranlukast and MCI-826 were applied to *in vitro* assay of eosinophil survival. Eosinophils were harvested from Sephadex G-200-treated guinea-pigs. Each column represents mean $\pm$ SE of five or six animals. Significant difference (*** $P < 0.001$).

concentration-dependent mobilization of the eosinophils (data not shown), indicating a species difference between humans and guinea-pigs in eosinophil responsiveness to LTD₄. CysLT-driven eosinophilia in guinea-pigs is mainly due to indirect mechanisms involving other chemotactic factors such as IL-5.

The intravenous injection of IL-5 significantly increased the number of eosinophils in the peripheral blood of guinea-pigs [13,27]. However, an intradermal injection of IL-5 did not produce tissue eosinophilia [13]. IL-5 (i.v.) potentiates the intradermal injection of eotaxin-induced cutaneous eosinophilia [13]. These findings indicate that although IL-5 directly induces blood eosinophilia by releasing cells from the bone marrow, the role of IL-5 in induction of tissue eosinophilia is indirect through priming the cells to easily mobilize from the circulation to the tissues. The present study found that CysLT antagonists suppressed not only blood eosinophilia that may be induced by IL-5, but also lung eosinophilia. Furthermore, the forced inhalation of LTD₄ induces airway eosinophilia [8–10]. Thus, CysLTs might produce other eosinophil chemotactic factors that cause eosinophils primed by IL-5 to migrate from the circulation to the lung.

In contrast to our view of the mechanisms of antigen-induced eosinophilia through the production of CysLTs and IL-5, another group postulated a different mechanism in rat asthmatic

models [32,33]. Naive recipients (Brown Norway rats) of CD4⁺ T cells isolated from the lymph nodes of OVA-sensitized donor rats were challenged by inhalation with OVA. The LAR was induced and the numbers of eosinophils and IL-5 mRNA positive cells in BALF increased, indicating that these responses were triggered through antigen presentation by airway dendritic cells or other antigen-presenting cells that activate OVA-specific CD4⁺ T cells [33]. In addition, as these responses were suppressed by pranlukast, the triggering events may induce CysLT production from unidentified lung cells followed by IL-5 production, probably from T cells [33]. Whether or not CD4⁺ T cell-dependent LAR and eosinophilia occur in guinea-pigs is unclear. However, although we found that EAR in guinea-pigs was apparently suppressed by the CysLT antagonist, the early increase in pulmonary resistance in actively sensitized Brown Norway rats following antigen inhalation was hardly attenuated by a CysLT antagonist [32]. It remains unclear whether the ineffectiveness resulted from the insufficient release of CysLTs immediately after the challenge or from the failure of the airway smooth muscle to contract in response to CysLTs as has been demonstrated in another strain of rats [34]. Species differences exist between guinea-pigs and rats in terms of CysLT production and induction of biological actions.

On the other hand, complete and partial inhibition of the respective eosinophilia and the LAR by CysLT antagonists and anti-IL-5 antibody suggest that eosinophils that infiltrated the lung are at least partly involved in the LAR. We demonstrated that the thromboxane (TX) A₂ antagonist, seratrodist, suppressed the LAR by approximately 40% [17], and that abundant TXA₂ was released from guinea-pig eosinophils in response to non-immunological stimuli [25,35,36]. Thus, migrated eosinophils may participate in the induction of the LAR, probably through TXA₂ production. In contrast to the effect of anti-IL-5 antibody on the LAR in our guinea-pig model, a humanized monoclonal antibody to IL-5 did not affect the LAR in a double-blind randomized placebo-controlled clinical trial, although the treatment strongly suppressed eosinophilia in the circulation and sputum [37]. This discrepancy means that the involvement of IL-5 and eosinophils in the LAR in guinea pigs and humans may be species specific.

The present results suggest that CysLTs, which may be produced by lung mast cells immediately after antigen-antibody reaction, sequentially induced IL-5 production from immune component cells via CysLT₁ receptor activation. Furthermore, eosinophils recruited into the lung through the CysLT- and IL-5-dependent mechanism are probably involved in the occurrence of the LAR.

References

- De Monchy JGR, Kauffman HF, Venge P et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; 131:373–6.
- Rossi GA, Crimi E, Lantero S et al. Late-phase asthmatic reaction to inhaled allergen is associated with early recruitment of eosinophils in the airways. *Am Rev Respir Dis* 1991; 144:379–83.
- Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A. Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989; 19:701–5.
- Griffiths-Johnson DA, Collins PD, Rossi AG, Jose PJ, Williams TJ. The chemokine, eotaxin, activates guinea-pig eosinophils in vitro and causes their accumulation into the lung in vivo. *Biochem Biophys Res Commun* 1993; 197:1167–72.
- Nagy L, Lee TH, Goetzl EJ, Pickett WC, Kay AB. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin Exp Immunol* 1982; 47:541–7.
- Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest* 1986; 78:1701–6.
- Laitinen LA, Laitinen A, Haahtela T, Vikka V, Spur BW, Lee TH. Leukotriene E₄ and granulocytic infiltration into asthmatic airways. *Lancet* 1993; 341:989–90.
- Foster A, Chan CC. Peptide leukotriene involvement in pulmonary eosinophil migration upon antigen challenge in the actively sensitized guinea pigs. *Int Arch Allergy Appl Immunol* 1991; 96:279–84.
- Underwood DC, Osborn RR, Newsholme SJ, Torphy TJ, Hay DWP. Persistent airway eosinophilia after leukotriene (LT) D₄ administration in the guinea pig. *Am J Respir Crit Care Med* 1996; 154:850–7.
- Krell RD, Dehaas CJ, Lengel DJ, Kusner EJ, Williams JC, Buckner CK. Preclinical exploration of the potential antiinflammatory properties of the peptide leukotriene antagonist ICI204, 219 (Accolate). *Ann NY Acad Sci* 1994; 744:289–98.
- Chand N, Harrison JE, Rooney S et al. Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: a therapeutic approach. *Eur J Pharmacol* 1992; 211:121–3.
- Mausier PJ, Pitman A, Witt A et al. Inhibitory effect of the TRFK-5 anti-IL-5 antibody in a guinea pig model of asthma. *Am Rev Respir Dis* 1993; 148:1623–7.
- Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J Exp Med* 1995; 182:1169–74.
- Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977; 7:503–13.
- Nabe T, Shinoda N, Yamada M et al. Repeated antigen inhalation-induced reproducible early and late asthma in guinea pigs. *Jpn J Pharmacol* 1997; 75:65–75.
- Nabe T, Shinoda N, Yamashita K, Yamamura H, Kohno S. Leucocyte kinesis in blood, bronchoalveoli and nasal cavities during late asthmatic responses in guinea-pigs. *Eur Respir J* 1998; 11:636–42.
- Yamashita K, Nabe T, Tomioka H, Kohno S. Repeated antigen inhalations alter chemical mediators that cause asthmatic obstruction in guinea pigs. *Jpn J Pharmacol* 1999; 81:48–55.
- Nakagawa N, Obata T, Kobayashi T et al. In vivo pharmacological profile of ONO-1078: a potent, selective and orally active peptide leukotriene (LT) antagonist. *Jpn J Pharmacol* 1992; 60:217–25.
- Nabe T, Kohno K, Tanpo T et al. Inhibitory effect of ONO-1078 on specific binding of peptide leukotrienes to human lung crude membrane. *Prostaglandins, Leukotrienes Essential Fatty Acids* 1994; 51:163–71.
- Watanabe-Kohno S, Yamamura H, Nabe T, Horiba M, Ohata K. MCI-826 is a potent and selective antagonist of peptide leukotrienes (p-LTs) and has characteristics distinctive from those of FPL 55712. *Jpn J Pharmacol* 1992; 60:1–8.
- Nabe T, Shinoda N, Yamashita K, Yamada M, Yamamura H, Kohno S. Comparative studies on nebulizers for antigen inhalation in experimental asthma. *Allergol Intl* 1997; 46:261–7.
- Tagari P, Black C, Marshall S, Ford-Hutchinson AW. A rapid biochemical method for measuring antigen-induced pulmonary eosinophil migration in allergic guinea pigs. *J Immunol Methods* 1993; 163:49–58.

- 23 Bozeman PM, Learn DB, Thomas EL. Assay of the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. *J Immunol Methods* 1990; 126:125–33.
- 24 Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol* 1979; 46:399–406.
- 25 Nabe T, Yamamura H, Kohno S. Sephadex G-200-induced eosinophil infiltration into airways in non-sensitized and sensitized guinea pigs, and responsiveness of the cells to stimuli in vitro. *Jpn J Pharmacol* 1996; 70:337–45.
- 26 Doran O, Stahl J, Cook E, Buckner CK, Graziano M. Peptidoleukotriene (pLT) release from guinea pig lung mast cells. *Inflammation* 1994; 18:89–97.
- 27 Portanova JP, Christine LJ, Rangwala SH et al. Rapid and selective induction of blood eosinophilia in guinea pigs by recombinant human interleukin 5. *Cytokine* 1995; 7:775–83.
- 28 Lynch KR, O'Neill GP, Liu Q et al. Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature* 1999; 399:789–93.
- 29 Figueroa DJ, Breyer RM, Defoe SK et al. Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. *Am J Respir Crit Care Med* 2001; 163:226–33.
- 30 Bacchetta R, de Waal Malefijt R, Yssel H et al. Host-reactive CD4⁺ and CD8⁺ T cell clones isolated from a human chimera produce IL-5, IL-2, IFN- γ and granulocyte/macrophage-colony-stimulating factor but not IL-4. *J Immunol* 1990; 144:902–8.
- 31 Spada CS, Nieves AL, Krauss AH-P, Woodward DF. Comparison of leukotriene B₄ and D₄ effects on human eosinophil and neutrophil motility in vitro. *J Leukoc Biol* 1994; 55:183–91.
- 32 Ihaku D, Cameron L, Suzuki M, Molet S, Martin J, Hamid Q. Montelukast, a leukotriene receptor antagonist, inhibits the late airway response to antigen, airway eosinophilia, and IL-5-expressing cells in Brown Norway rats. *J Allergy Clin Immunol* 1999; 104:1147–54.
- 33 Hojo M, Suzuki M, Maghni K, Hamid Q, Powell WS, Martin JG. Role of cysteinyl leukotrienes in CD4⁺ T cell-driven late allergic airway responses. *J Pharmacol Exp Ther* 2000; 293:410–6.
- 34 Piper PJ, Samhoun MN. The mechanism of action of leukotriene C₄ and D₄ in guinea-pig isolated perfused lung and parenchymal strips of guinea pig, rabbit and rat. *Prostaglandins* 1981; 21:793–803.
- 35 Nabe T, Miura M, Kamiki T, Kohno S. Arachidonate 5-lipoxygenase and cyclooxygenase metabolites from guinea pig eosinophils and macrophages. *Jpn J Pharmacol* 2000; 83:261–4.
- 36 Giembycz MA, Kroegel C, Barnes PJ. Platelet activating factor stimulates cyclo-oxygenase activity in guinea pig eosinophils. Concerted biosynthesis of thromboxane A₂² and E-series prostaglandins. *J Immunol* 1990; 144:3489–97.
- 37 Leckie MJ, ten Brinke A, Khan J et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; 356:2144–8.