Effect of Antiallergic Drugs on Interleukin 5-Induced Eosinophil Infiltration of Rat Airways

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Interleukin (IL)-5 is thought to play important roles in asthma and to be a potential therapeutic target. An intratracheal injection of murine recombinant IL-5 (3–30 μ g/animal) induced a dose-dependent increase in the number of eosinophils in the bronchoalveolar lavage fluid of Brown Norway (BN) rats 24 h after administration. Bovine serum albumin (30 μ g/animal), used as reference material, did not cause any change. The reaction was not observed in F344 rats. The increase in the number of eosinophils did not accompany bronchial hyperreactivity in BN or F344 rats. Prednisolone (3–10 mg/kg, i.p.) and emedastine (30 mg/kg, p.o.) reduced the increased number of eosinophils induced by the IL-5 challenge. These results suggest that IL-5 is a potent inducer of eosinophils in the airway of BN rats. Prednisolone and emedastine are effective against IL-5-induced eosinophilia.

Key words interleukin 5 (IL-5); eosinophil; Brown Norway rat; emedastine

Airway inflammation is an important component in the pathogenesis of bronchial asthma.²⁾ Histologically, eosinophils often predominate, and a large body of evidence points to activated eosinophils as key effector cells in mediating tissue damage.³⁾

Animal models have been valuable for the investigation of the underlying pathology of allergic pulmonary diseases. Rat models are becoming more useful as many immunological reagents including monoclonal antibodies to cell adhesion molecules and cytokines have become available.⁴⁾ Mishima *et al.* reported that the number of interleukin (IL)-5-expressing cells in bronchoalveolar lavage fluid (BALF) were increased in antigen-challenged rats and described the importance of IL-5 in asthma.⁴⁾ The Brown Norway (BN) rat model mimics human allergic asthma in several aspects.⁵⁾ This strain exhibits a T helper (Th)2-driven response to allergic sensitization⁶⁾ with high levels of allergen-specific IgE.^{7,8)} Following aeroallergen challenge of sensitized animals, early- and late-phase bronchoconstriction occurs,⁹⁾ associated with pulmonary inflammation and bronchial hyperreactivity (BHR) to methacholine.¹⁰⁾ F344 rats have been reported to have hyperreactive airways compared with other strains,¹¹⁾ but it has been reported that they did not develop airway inflammation upon allergic sensitization and allergen challenge.⁵⁾

In this study, we found that intratracheal challenge with murine recombinant (mr)IL-5 induced an increase in the number of inflammatory leukocytes in airways in BN rats without affecting bronchial response. We also investigated the effect of prednisolone and emedastine on the increased number of eosinophils induced by exogenous IL-5.

MATERIALS AND METHODS

Animals Female BN rats and F344 rats aged 6–8 weeks (Charles River Japan, Yokohama) were used. The animals were fed a standard laboratory diet and water *ad libitum* in an air-conditioned room at $22 \pm 2^\circ\text{C}$ and relative humidity of $60 \pm 15\%$. The experiments were carried out in accordance with the Guidelines for the Care and Use of Laboratory Ani-

mals of Gifu Pharmaceutical University.

Materials mrIL-5 was provided by the Suntory Institute for Biochemical Research (Osaka, Japan). Bovine serum albumin (BSA) (Sigma, St. Louis, MO, U.S.A.), Turk solution (Wako Pure Chemicals, Osaka, Japan), and sodium pentobarbital (Abbot Labs., Chicago, IL, U.S.A.) were purchased commercially. BSA and mrIL-5 were dissolved in sterile saline. Prednisolone acetate (Shionogi, Osaka, Japan) was suspended in saline containing 0.5% carboxymethylcellulose-Na. Emedastine (Kanebo, Osaka, Japan) was dissolved with 0.5% carboxymethylcellulose-Na.

Administration of mrIL-5 The administration of mrIL-5 was carried out according to modified methods previously described.¹²⁾ In brief, the animals were anesthetized by an injection of sodium pentobarbital (50 mg/kg, i.p.). After the animals were sedated and restrained, the trachea was then surgically exposed and mrIL-5, BSA, or sterile saline (0.1 ml) was injected into the trachea with a 27-G needle. The wound was closed with sterile stitches. The lungs were lavaged 8, 24, and 48 h after the mrIL-5 challenge. To study the dose dependency, BHR, and the effect of drugs, BALF was collected 24 h after the mrIL-5 challenge.

Bronchoalveolar Lavage Study To assess mrIL-5-induced airway inflammation, we studied the accumulation of inflammatory cells in BALF according to methods previously reported.¹³⁾ Briefly, at various times after mrIL-5 challenge, animals were killed with an intraperitoneal injection of urethane (2 g/kg). The trachea was cannulated and the air lumen washed 4 times with 2.5 ml of Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline containing 0.1% BSA and 0.05 mM EDTA-2Na, and this procedure was repeated twice (total volume of 5 ml, recovery $>80\%$). BALF from each animal was collected in a plastic tube, cooled on ice, and centrifuged ($150 \times g$) at 4°C for 10 min. Cell pellets were resuspended in the same medium (1 ml). BALF was stained with Turk solution, and the number of nucleated cells was counted in a Burk-Türk chamber. A differential count was made on a smear prepared with a cytocentrifuge (Cytospin II; Shandon Scientific, Cheshire, U.K.) and stained with Diff-Quick solution, (based on standard morphologic criteria) on at least 500

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Table 1. Dose-Response for mrIL-5-Induced Changes in Bronchial Leukocyte Populations in BN Rats

Dose	Total cells	Leukocyte population ($\times 10^3$ cells/BALF)			
		Macrophages	Eosinophils	Neutrophils	Lymphocytes
BSA 30 μ g	780 $\pm$ 171	750 $\pm$ 171	12 $\pm$ 4	5 $\pm$ 3	13 $\pm$ 5
mrIL-5 3 μ g	896 $\pm$ 224	837 $\pm$ 162	30 $\pm$ 14	15 $\pm$ 6	14 $\pm$ 5
10 μ g	935 $\pm$ 235	857 $\pm$ 235	46 $\pm$ 10*	11 $\pm$ 3	21 $\pm$ 11
30 μ g	877 $\pm$ 64	791 $\pm$ 83	51 $\pm$ 13*	16 $\pm$ 5	19 $\pm$ 8

Results are expressed as mean $\pm$ S.E.M. ($\times 10^3$ cells/BALF) of total leukocytes and each leukocyte type recovered from BALF in 5 animals 24 h after the intratracheal injection of BSA or mrIL-5. * $p < 0.05$, significantly different from control.

Table 2. Time-Course for mrIL-5-Induced Changes in Bronchial Leukocyte Populations in BN Rats

Time (h)	Total cells	Leukocyte populations ($\times 10^3$ cells/BALF)			
		Macrophages	Eosinophils	Neutrophils	Lymphocytes
0	614 $\pm$ 135	594 $\pm$ 297	10 $\pm$ 8	2 $\pm$ 2	8 $\pm$ 3
8	946 $\pm$ 240	891 $\pm$ 257	28 $\pm$ 14	10 $\pm$ 8	17 $\pm$ 10
24	1177 $\pm$ 356	1086 $\pm$ 343	59 $\pm$ 12*	11 $\pm$ 10	21 $\pm$ 8
48	942 $\pm$ 311	914 $\pm$ 320	18 $\pm$ 8	8 $\pm$ 5	2 $\pm$ 4

Results are expressed as mean $\pm$ S.E.M. ($\times 10^3$ cells/BALF) of total leukocytes and each leukocyte type recovered from BALF in 5 animals before and 8, 24, and 48 h after the intratracheal injection of mrIL-5 (10 μ g). * $p < 0.05$.

cells (magnification $\times 400$).

Measurement of Bronchial Responsiveness Measurement of bronchial responsiveness to acetylcholine (ACh) at 24 h after the IL-5 challenge was carried out according to methods previously described.¹³⁾ Briefly, rats were anesthetized with urethane (1 g/kg, i.p.) and the jugular vein was cannulated for intravenous injection of ACh. Rats were injected with succinylcholine chloride (1.2 mg/kg, i.v.) to suppress spontaneous respiration and were ventilated with a rodent ventilator (New England Medical Instruments, Medway, MA, U.S.A.) at 60 strokes/min, at a stroke volume of 1 ml/100 g body weight. Bronchoconstriction was measured according to the overflow method using a bronchospasm transducer (Ugo Basile 7020, Milan, Italy) connected to the tracheal cannula. To evaluate bronchial responsiveness to ACh, changes in respiratory overflow volume were measured using increasing doses of ACh. The increase in respiratory overflow volume induced by ACh was represented as a percentage of the maximal overflow volume (100%) obtained by clamping the tracheal cannula. The PD₅₀ values (provocative dose that would induce a 50% increase in overflow volume) were calculated by linear regression.

Drug Administration Prednisolone (1–10 mg/kg, i.p.) or emedastine (3–30 mg/kg, p.o.) was administered 1 h before mrIL-5 challenge.

Statistical Analysis All data are represented as mean $\pm$ S.E.M. and were analyzed by Student's *t*-test or Dunnett's multiple-range test.

RESULTS

mrIL-5-Induced Leukocyte Infiltration in BALF Twenty-four hours after intratracheal injection of mrIL-5 (3–30 μ g/animal), the number of eosinophils in the BALF increased in a dose-dependent manner, but no significant change in the number of macrophages, neutrophils, and lymphocytes occurred (Table 1). In contrast, BSA did not affect

Fig. 1. Numbers of Eosinophils in BALF from BN Rats and That from F344 Rats Challenged with Saline, BSA, or mrIL-5

BALF was recovered 24 h after the challenge. Each value represents the mean $\pm$ S.E.M. of 4–6 animals. * $p < 0.05$, significantly different from other groups.

the number of macrophages, eosinophils, neutrophils, or lymphocytes (Table 1). In the time-course experiments, the number of eosinophils increased 24 h after the challenge with mrIL-5 (10 μ g). The number of macrophages, neutrophils, and lymphocytes revealed a tendency to increase, but these effects were not significant (Table 2).

Effect of mrIL-5 in BN Rats and F344 Rats To examine whether the effect of mrIL-5 depended on the rat strain, we compared the reaction induced by mrIL-5 in BN rats and F344 rats. As shown in Fig. 1, the number of eosinophils was increased in BN rats when they were challenged with mrIL-5. In contrast, the number of eosinophils in F344 rats was not increased by mrIL-5.

Effects of mrIL-5 Administration on BHR in BN Rats and F344 Rats To elucidate the role of IL-5 in BHR, we measured ACh-induced bronchoconstriction in BN rats and F344 rats challenged with mrIL-5. Administration of ACh (3.9–250 μ g/kg, i.v.) induced an increase in respiratory resistance in dose-dependent manner. The dose-response

Fig. 2. Effects of Prednisolone and Emedastine on Eosinophil Accumulation in BALF from BN Rats Challenged with mrIL-5

Prednisolone (i.p.) or emedastine (p.o.) was administered 1 h before IL-5 challenge. Each value represents the mean $\pm$ S.E.M. of 7–8 animals. * $p < 0.05$, ** $p < 0.01$ compared to control.

curves were not affected by the challenge with $10 \mu\text{g}$ of mrIL-5 or BSA in BN rats or F344 rats. The PD_{50} values of each agent in BN rats and F344 rats ranged from 145 ± 19 to $166 \pm 22 \mu\text{g/kg}$.

Effects of Prednisolone and Emedastine on the Number of Eosinophils in BALF As shown in Fig. 2, prednisolone (3 and 10 mg/kg , i.p.) significantly inhibited the increase in eosinophils induced by intratracheal injection of mrIL-5 ($10 \mu\text{g}$). Emedastine (3– 30 mg/kg , p.o.) also reduced the mrIL-5-induced increase in eosinophils in a dose-dependent manner.

DISCUSSION

Airway inflammation has emerged as an important contributor to the pathological mechanisms of bronchial asthma. Of the effector cells in asthma, eosinophils have possibly the most important and potentially pivotal role in generating airway inflammation.²⁾ IL-5 has been particularly interesting in the pathophysiology of asthma as it is associated with eosinophil inflammation.¹⁴⁾ IL-5 is produced by Th2 cells, and there is evidence for increasing expression of IL-5 by T cells in asthmatic patients.¹⁵⁾ Endobronchial allergen challenge resulted in mRNA expression of IL-5 in eosinophils, and an increase in IL-5 concentrations have also been reported in sera from both atopic and nonatopic patients with chronic asthma.¹⁶⁾ A monoclonal antibody to IL-5 inhibited eosinophil infiltration into the airways of animals sensitized and challenged with allergen.^{17,18)}

Direct effects of IL-5 on pulmonary eosinophils have also been investigated. Intratracheal administration of mrIL-5 in guinea pigs increased the number of eosinophils 24 h after

the challenge.¹²⁾ A similar effect of IL-5 was reported in asthmatic patients. Topical application of human recombinant IL-5 induced eosinophilia and activation of eosinophils in asthmatic patients.¹⁹⁾

In the present study, we demonstrated that mrIL-5 increased the number of eosinophils in BALF from BN rats. The eosinophilia was BN rat-specific, as it was not induced in F344 rats. It was not accompanied by BHR. Prednisolone and emedastine inhibited the eosinophilia in dose-dependent manner.

First, we investigated the characteristics of changes in bronchial leukocyte populations after mrIL-5 challenge in BN rats. Intratracheal challenge with mrIL-5 (3– $30 \mu\text{g}$) increased the number of eosinophils in dose-dependent manner, although no significant change in other cells occurred. We also found that the number of eosinophils increased most 24 h after the mrIL-5 challenge. Therefore we chose the time point of 24 h after the mrIL-5 challenge and the dose of $10 \mu\text{g}$.

The increase in the number of eosinophils in BN rats was compared with that in sex- and age-matched F344 rats. No reaction was observed in F344 rats, and thus it is specific to BN rats. It has been reported that BN rats produce high IgE levels after sensitization and develop a late-phase asthmatic response and show increased BHR after antigen challenge.²⁰⁾ Thus BN rats seem to represent a model equivalent to human atopic asthma.⁶⁾ In the view of responsiveness to IL-5 as well as antigen, BN rats mimic atopic asthmatic patients, who respond to IL-5 challenge with infiltration by eosinophils.¹⁹⁾

IL-5 has various effects on eosinophils: stimulation of growth; differentiation and cytokine synthesis; upregulation

of adhesion molecules (e.g., CD11b) and induction of adherence; and priming for degranulation.²¹⁾ An investigation in IL-5-deficient mice demonstrated that this cytokine provides an essential signal for the induction of eosinophilia which was observed during allergic pulmonary inflammation.²²⁾ A report by Egan *et al.* supported that finding.²³⁾ Intratracheal administration of monoclonal anti-IL-5 antibody, Sch55700, abolished pulmonary eosinophilia induced by human IL-5 in guinea pigs.²³⁾ Mould *et al.* showed that pulmonary-selective expression of IL-5 by gene transfer to naive mice induced a pronounced and selective pulmonary eosinophilia that occurred independently of induction of blood eosinophilia.²⁴⁾ The observations confirmed that IL-5 elicits a chemotactic signal (directed migration to the airways) for eosinophils *in vivo*. In our study, topical application of mrIL-5 increased the number of eosinophils in BN rats, it consistent with previous reports.

In spite of significant eosinophilia in BN rats, there were few eosinophils in BALF from F344 rats treated with mrIL-5. Mikus *et al.* demonstrated that natural killer cells and CD8⁺T cells from BN rats had a reduced capacity to secrete interferon (IFN)- γ in response to IL-12 compared with F344 rats.²⁵⁾ Inasmuch as IL-12 is known to play a critical role in generation of Th1-type immunoresponses, the decreased ability of NK cells and CD8⁺T cells to secrete IFN- γ in BN rats could lead to a cytokine imbalance and a shift toward a Th2-type response. The dominance of the Th2-type response would contribute to the high responsiveness of eosinophils in BN rats. On the other hand, the Th1-type response results in low responsiveness in F344 rats.

Some chemokines (e.g., eotaxin) are also important factors in eosinophilia. Teran *et al.* investigated the effect of Th1- and Th2-type cytokines on the production of eotaxin by human fibroblasts.²⁶⁾ They observed that IL-4 preferentially stimulated lung fibroblasts to secrete eotaxin, whereas IFN- γ had negligible effect on the release of this chemokine. In BN rats, endogenous IL-4, a central member of Th2-type cytokines, might stimulate the production of eotaxin, which enhances eosinophilia, in cooperation with exogenous IL-5. On the contrary, endogenous IFN- γ , a central member of Th1-type cytokines, might suppress the production of eotaxin by inhibiting the production and/or effect of endogenous IL-4 and thus inhibit eosinophilia.²⁵⁾ From the viewpoint of the cooperation of cytokines and chemokines to regulate pulmonary eosinophilia, the precise mechanism by which IL-5 induces eosinophilia only in BN rats but not in F344 rats should be investigated in the future.

To elucidate the effect of IL-5 on BHR, we assessed ACh-induced bronchoconstriction. In our study, mrIL-5 did not induce BHR in BN or F344 rats. Clinical and experimental studies have demonstrated that antigen-specific CD4⁺Th2 cells and IL-5 play central roles in initiating and sustaining asthmatic responses by regulating the activation and recruitment of eosinophils.^{27,28)} The role of IL-5 in the induction of BHR has been reported,²²⁾ although a contradictory report exists.²⁹⁾ Regarding these observations, Drazen *et al.* proposed the BHR could be induced by two distinct mechanisms: one could be mediated by an IgE-mast cell pathway, whereas the other could be induced by an IL-5-eosinophil pathway.³⁰⁾ Thus the relative contribution of these mechanisms to the induction of allergen-induced airway inflamma-

tion and BHR could be different in each patient and with each experimental method. The present study on BHR suggested that IL-5 acts primarily on eosinophilia, and that additional factors, e.g., other cytokines, chemokines, and/or chemical mediators, would be necessary to evoke BHR.

Inhibiting the activity of IL-5 represents a possible therapeutic approach for the treatment of eosinophilic inflammatory disorders. There are some strategies for inhibiting IL-5 that include blocking the biosynthesis of IL-5 or IL-5 receptor, antagonizing the IL-5 receptor, neutralizing IL-5 with soluble receptors or high-affinity monoclonal antibodies, or inhibiting the signal transduction of IL-5.³¹⁾ Some monoclonal antibodies to IL-5, Sch 55700 and SB-240563, have been investigated in clinical trials.^{23,32)} Peptides and low molecular compounds that inhibit the effects of IL-5 have also been investigated in preclinical studies.^{33,34)} To determine the effects of the compounds on eosinophilia *in vivo*, it would be worthwhile to develop a new pharmacological model. In our eosinophilia model in BN rats, the number of eosinophils was decreased by prednisolone and emedastine. Emedastine is an antiallergic agent with an antihistamine effect. It has been reported that emedastine inhibits eosinophil migration elicited by platelet activating factor, eotaxin, RANTES, MCP-3, and antigen challenge.^{35–37)} The mechanism by which emedastine inhibits eosinophil migration was suggested to be the inhibition of activities of tyrosine kinases or protein kinases.³⁷⁾ Lyn, Syk and JAK2 tyrosine kinases are important for IL-5 signal transduction in eosinophils.²¹⁾ The mechanism by which emedastine inhibits the eosinophilia induced by mrIL-5 challenge would be attributed, at least in part, to the inhibition of tyrosine kinase activation.

In conclusion, mrIL-5 increased the number of eosinophils recruited to the lungs in BN rats without influencing to respiratory function. As prednisolone and emedastine inhibited the eosinophilia, this model would be useful to evaluate antiallergic drugs that inhibit the effect of IL-5 on eosinophils.

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