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Letter to the Editor

Effect of LTRA on IP-10-induced eosinophil adhesion to ICAM-1

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Dear Editor,

Cysteinyl leukotrienes (cysLTs), such as LTC₄, LTD₄, and LTE₄, can augment the accumulation of eosinophils in the tissues of asthmatic airways. We previously reported that LTD₄ up-regulates the expression of β 2 integrins on human eosinophils *in vitro* and increases eosinophil adhesion.¹ We also reported that LTD₄ directly induces transendothelial migration, superoxide anion generation, and the degranulation of eosinophils.² Administration of leukotriene receptor antagonist (LTRA) reduces the number of eosinophils in sputum of asthmatics, which supports the role of cysLTs in the accumulation of eosinophils in airways. Moreover, recent evidence suggests that cysLTs are increased in the airways during virus-induced asthma.³ As the numbers of eosinophils and neutrophils increase in asthmatic airways during virus infection, cysLTs may be involved in the virus-induced enhancement of eosinophilic inflammation. Therefore, LTRA may provide a useful strategy for controlling virus-induced eosinophilic airway inflammation and asthma exacerbation.

Interferon- γ -inducible protein of 10 kDa (IP-10) is involved in the virus-induced exacerbation of asthma. For example, rhinovirus (RV) infection induces bronchial epithelial cells to produce IP-10 *in vitro* and *in vivo*, and serum IP-10 concentrations are specifically increased in RV-induced asthma exacerbation.⁴ The increased levels of IP-10 correlate with disease severity during RV-induced exacerbation. IP-10 binds to CXCR3 expressed on eosinophils. We reported that IP-10 up-regulates eosinophil functions such as adhesion, the generation of superoxide anion, degranulation, and cytokine production.⁵ These findings suggest that IP-10 may also be involved in the development of eosinophilic inflammation in asthma during viral infection.

Intercellular adhesion molecule 1 (ICAM-1) is an adhesion molecule that plays an important role in the recruitment of inflammatory cells and is a cellular receptor for most RV-A and all RV-B species of RV. Furthermore, RV infection increases ICAM-1 expression on epithelial cells,⁶ and adhesion to ICAM-1 can activate eosinophil functions. In this study, to evaluate the relevance of

LTRA for virus-induced eosinophilic inflammation, we examined whether montelukast, the most commonly prescribed LTRA, modifies the IP-10-induced eosinophil adhesion to ICAM-1.

Eosinophils were isolated from the peripheral blood of non-atopic healthy donors and by negative selection using immunomagnetic beads as described previously.^{5,7} The study procedures were approved by the Ethics Committee of Saitama Medical University Hospital, and informed consent was obtained from the donors before sample collection. Eosinophils (100 μ l of medium with 10⁵ cells/ml) were pre-incubated with either montelukast or control medium for 15 min. The eosinophils were then incubated with LTD₄ (100 nM) or IP-10 (100 nM) in ICAM-1-coated plates for 20 min. Eosinophil adhesion to ICAM-1 was assessed based on the residual eosinophil peroxidase (EPO) activity of adherent eosinophils.^{5,7} For determination of cysLT production, eosinophils (10⁶ cells/ml) were incubated with or without IP-10 in the presence of ICAM-1 for 180 min, and the cysLT concentrations in the supernatant were measured by ELISA (Cayman). Results were compared using a one-way factorial analysis of variance with Tukey's test for multiple comparisons or a paired t-test for analysis of the differences between two groups.

Although montelukast (1–10 μ M) alone had no effect on spontaneous eosinophil adhesion (Fig. 1a), it suppressed LTD₄ (100 nM)-induced eosinophil adhesion to ICAM-1 (Fig. 1b); both of these results were consistent with previous reports.⁸ We then examined the effect of LTRA on IP-10-induced eosinophil adhesion to ICAM-1. As we previously reported,⁵ IP-10 (100 nM) enhanced the adhesiveness of eosinophils to ICAM-1 (Fig. 2a). Montelukast (1–10 μ M) significantly suppressed IP-10-induced eosinophil adhesion to ICAM-1 (Fig. 2a; IP-10 (100 nM) alone 21.6% $\pm$ 0.5%, montelukast (1 μ M) 14.3% $\pm$ 0.4%, P < 0.05, montelukast (10 μ M) 11.1% $\pm$ 0.3%, P < 0.01). Finally, we examined whether IP-10 up-regulates production of cysLT from eosinophils. IP-10 induced cysLT production from eosinophils in the presence of ICAM-1 (Fig. 2b; control 101.9 $\pm$ 9.9 pg/ml, IP-10 (100 nM) 171.1 $\pm$ 16.8 pg/ml, P < 0.05). Similar results were obtained after 20-min incubation, although their concentrations were much lower than those after 180-min incubation (data not shown).

Recent evidence suggested that LTRA may be useful for the treatment of virus-induced asthma exacerbation. Bisgaard *et al.*

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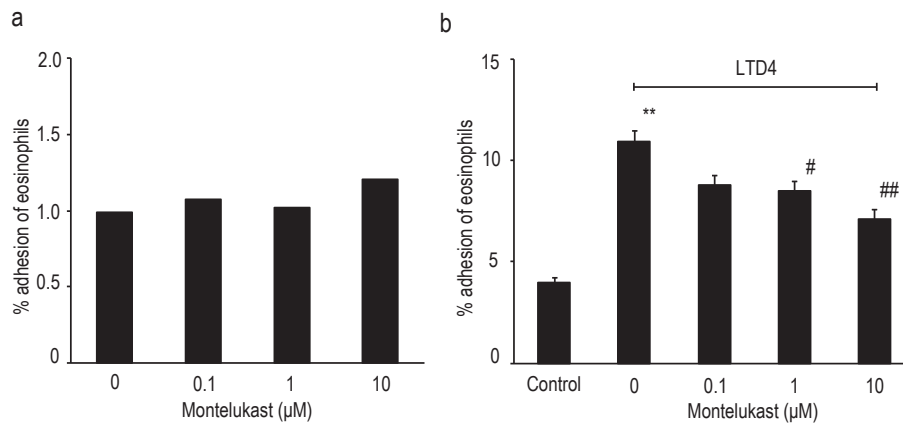


Fig. 1. Effects of LTRA on spontaneous or LTD₄-induced adhesion of eosinophils. **(a)** Effect of LTRA on spontaneous eosinophil adhesiveness to ICAM-1. Eosinophils (100 μ l of medium with 10⁵ cells/ml) were incubated with or without montelukast (0.1 μ M/1 μ M/10 μ M) at 37 °C for 15 min. The adhesiveness of eosinophils to ICAM-1 was assessed using residual EPO assays. Data are shown as means $\pm$ standard error of the mean (SEM) of eight experiments using cells from different donors. **(b)** Effect of LTRA on eosinophil adhesiveness to ICAM-1 enhanced by LTD₄. Eosinophils were pre-incubated with montelukast (0.1 μ M/1 μ M/10 μ M) for 15 min, and then stimulated with LTD₄ (100 nM). The adhesiveness of eosinophils to ICAM-1 was assessed using residual EPO assays. Data are shown as means $\pm$ SEM of eight experiments using cells from different donors. ***P* < 0.01 vs. eosinophil adhesion without LTD₄ (control). #*P* < 0.05 and ##*P* < 0.01 vs. eosinophil adhesion enhanced by LTD₄ without montelukast.

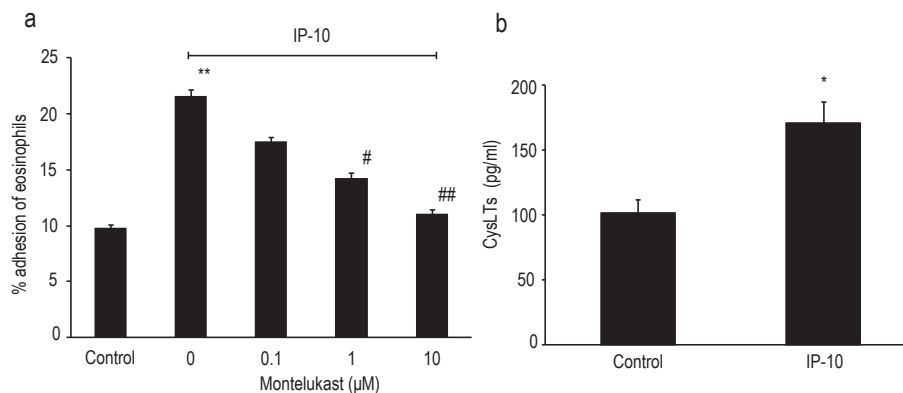


Fig. 2. Effect of LTRA on IP-10-induced adhesion of eosinophils. **(a)** Effect of LTRA on eosinophil adhesiveness to ICAM-1 enhanced by IP-10. Eosinophils were pre-incubated with montelukast (0.1 μ M/1 μ M/10 μ M) for 15 min, and then stimulated with IP-10 (100 nM). The adhesiveness of the eosinophils to ICAM-1 was assessed using residual EPO assays. Data are shown as means $\pm$ SEM of eight experiments using cells from different donors. ***P* < 0.01 vs. eosinophil adhesion without IP-10 (control). #*P* < 0.05 and ##*P* < 0.01 vs. eosinophil adhesion enhanced by IP-10 without montelukast. **(b)** Effect of IP-10 on the production of cysLTs from eosinophils. Eosinophils (10⁶ cells/ml) were incubated with or without IP-10 (100 nM) in the presence of ICAM-1 for 180 min. Then, the cysLT concentrations in the supernatant were measured by ELISA. **P* < 0.05 vs. the control (without IP-10).

reported that montelukast could suppress asthma symptoms of respiratory syncytial virus bronchiolitis.⁹ Furthermore, they also reported that montelukast suppressed the frequency of virus-induced asthma exacerbation.¹⁰ In this study, we found that montelukast suppressed the eosinophil adhesion induced by the virus-infection-related proteins IP-10 and ICAM-1 (Fig. 2a), supporting the usefulness of LTRA treatment for virus-induced airway inflammation and asthma exacerbation.

The mechanisms underlying the suppression of IP-10-induced eosinophil adhesion by LTRA were not fully clarified in this study. We found that IP-10 induced cysLT production from eosinophils (Fig. 2b). As such, cysLTs produced from IP-10-stimulated eosinophils may play roles in the increased eosinophil adhesion induced by IP-10 in an autocrine fashion. In other words, IP-10-induced eosinophil adhesion may be partly due to the actions of the cysLTs produced by IP-10. We previously reported that LTRA partially suppresses IL-5-induced eosinophil adhesion.⁸ As IL-5 and ICAM-1 can up-regulate the generation of cysLTs by eosinophils, we speculate that IL-5-induced eosinophil adhesion may also be partly due to the actions of cysLTs produced by IL-5. Taken together, if generation of cysLTs is induced, LTRA may

suppress eosinophil adhesion in a non-specific manner irrespective of stimulants.

One limitation in interpreting the findings of our study is that there is little information on montelukast concentrations in plasma or airways. After oral administration of 10 mg of montelukast, the maximum plasma concentration of montelukast reaches approximately 865 nM (526 ng/ml) according to the Japanese drug package insert; this value is almost the same as the concentration used in this study. Therefore, it is possible to postulate that adhesion of peripheral blood eosinophils to endothelium can be affected by clinical use of montelukast.

In conclusion, LTRA suppressed eosinophil adhesion to ICAM-1 when eosinophils are stimulated with IP-10. As IP-10 and ICAM-1 are involved in virus infection and virus-induced asthma exacerbation, our results suggest that administration of LTRA could be an effective strategy for treating asthma exacerbation.

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Conflict of interest

MN received honoraria from MSD, Torii Pharmaceutical and AstraZeneca. The rest of the authors have no conflict of interest.

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