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Dear Editor

Increased Serum Levels of Soluble IL-18 Receptor Complex in Patients with Allergic Asthma

The inflammatory process in allergic asthma is initiated by T-helper 2 (Th2) $CD4^+$ cells, which produce a repertoire of cytokines including IL-4, IL-5, IL-9 and IL-13. These Th2 cytokines play a critical role in IgE production, airway eosinophilia, and goblet cell hyperplasia. Many previous studies have shown that activated $CD4^+$ T cells, producing Th2 cytokines, are increased in the airways of patients with mild asthma.[1] In contrast, IFN-γ-producing Th1 cells are thought to prevent asthma disease activity, although in some experimental models, Th1 cells have not suppressed Th2 cell-mediated AHR and pulmonary inflammation.[1]

IL-18 is known to play an important role in Th1/Tc1 polarization, and promoting the production of Th2 cytokines (e.g. IL-4, IL-5, IL-9, and IL-13) by T cells, NK cells, basophils, and mast cells. Recent studies have reported that IL-18 plays a key role in the pathogenesis of pulmonary inflammatory diseases including interstitial lung diseases and chronic obstructive pulmonary disease (COPD).[2] The IL-18 receptor (IL-18R) complex is composed of the IL-18 Rα and IL-18Rβ chains. IL-18Rα (IL-1R5/IL-1Rrp1) is the extracellular signaling domain,[3] whereas IL-18Rβ (IL-R7/accessory protein-like [AcPL]/IL-18R accessory protein [AP]) is an adaptor molecule[4] in the complex. We have reported the presence of a soluble IL-18Rα complex in serum that exhibits antagonistic activity *in vitro*, and contains a diametric IL-18 protein and the soluble form of the IL-18Rβ chain. In addition, the serum levels of soluble IL-18Rα complex in rheumatoid arthritis (RA) and adult-onset Still's disease are significantly higher than those in healthy controls.[5] However the roles of soluble IL-18R complex in allergic asthma remain unclear. Therefore, in this study we measured the serum levels of soluble IL-18R complex in patients with allergic asthma.

We obtained serum samples from 19 age-matched subjects with allergic asthma, 14 allergic nonasthmatic subjects, and 14 healthy controls (Table 1). All subjects underwent a methacholine inhalation challenge and skin prick tests using a panel of 16 environmental allergens, as reported previously.[6] Ninety-eight COPD patients and 36 age-matched smokers and 51 age-matched nonsmokers were enrolled at Kurume University Hospital (Kurume, Fukuoka, Japan). Soluble IL-18R complex was measured using a method we had employed previously.[5] The limit of sensitivity of this ELISA system was <5 ng/mL. The serum level of IgE was measured using commercially available ELISA kits (Minneapolis, MN, USA). Results are expressed as means ± standard error of the mean (SEM). Nonparametric tests (Kruskal-Wallis and Mann-Whitney U-tests) were used to compare differences between the groups. Correlations were analyzed by simple regression. The level of statistical significance was set at $p < 0.05$. The SAS 9.1.3 software package, Japanese edition (SAS Institute, Cary, NC, USA), was used for statistical analysis.

The serum levels of the IL-18Rα protein complex in the 19 subjects with allergic asthma, 14 allergic nonasthmatic subjects and 14 healthy controls were 32.9

Table 1 Characteristics of the study subjects

	Healthy controls	Allergic nonasthmatics	Allergic asthmatics
Patients *n*	14	14	19
Age (yr)	25.4 ± 1.1	25.6 ± 1.9	35.4 ± 4.7
Males (*n*)	5	6	10
Females (*n*)	9	8	9
FEV_1 % pred	99.4 ± 2.5	97.0 ± 4.2	94.6 ± 4.0
Methacholine PC_{20} mg/mL	>32	>32	0.94 (0.33)**
Total IgE IU/mL	20.7 ± 5.4	131.2 ± 31.3	214.1 ± 56.9*
Systemic steroids	0	0	0
ICS	0	0	7
$Beta_2$-agonist	0	0	7
Theophylline	0	0	0
LTRA	0	0	5
No drug treatment	14	14	12

Data are presented as *n*, mean ± SEM or mean (geometric SEM), unless otherwise stated. FEV_1, forced expiratory volume in 1 s; % pred, % predicted; PC_{20}, provocative concentration causing a 20% decrease in FEV_1; Ig, immunoglobulin; ICS, Inhaled Corticosteroids; LTRA, Leuko Triene Receptor Antagonist. *$p < 0.05$ versus healthy controls; **$p < 0.01$ versus healthy controls and allergic nonasthmatics.

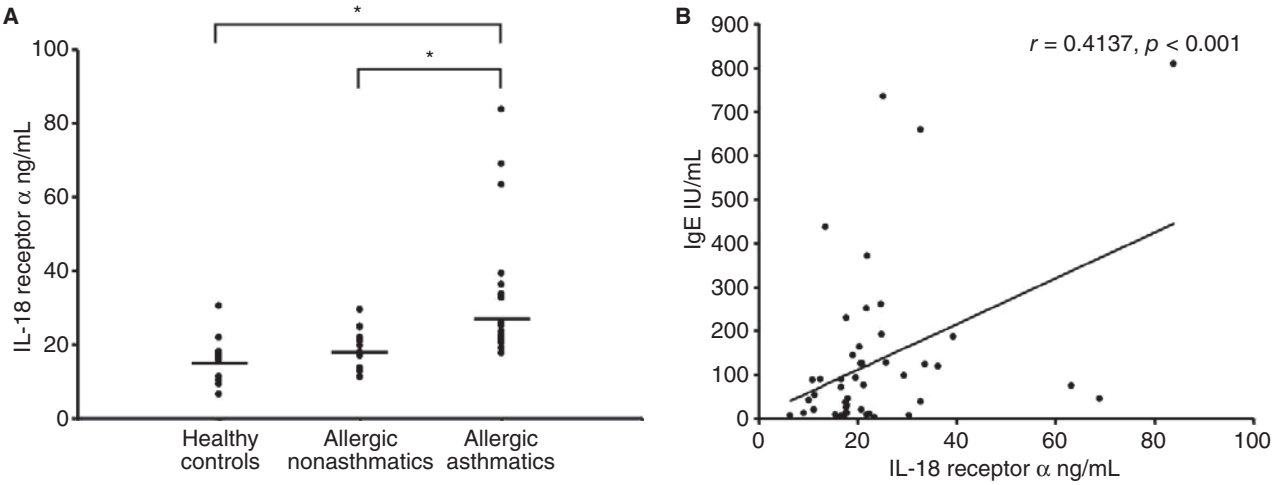

Fig. 1 (**A**) Serum levels of interleukin (IL)-18 receptor α complex in healthy controls (n = 15), allergic nonasthmatics (n = 14) and allergic asthmatics (n = 19). *p < 0.01. (**B**) Correlation between serum levels of interleukin (IL)-18 receptor α complex and IgE in subjects overall (n = 47). p < 0.001, R = 0.4137.

+ 4.3, 18.8 + 1.5, and 16.1 + 1.6 ng/mL, respectively. The serum levels of the IL-18Rα complex in the allergic asthmatics were significantly (p < 0.01) higher than those in the allergic non-asthmatic subjects and healthy controls. In contrast, the serum levels of the IL-18Rα complex in the allergic non-asthmatic subjects were not significantly higher than those in healthy controls (Fig. 1A). Moreover, the serum levels of IL-18Rα complex were positively and significantly correlated with the serum levels of IgE in the subjects overall (n = 47) (p < 0.001, R = 0.4137) (Fig. 1 B). However, there was no significant association between the serum levels of IL-18Rα complex and IL-18 protein (data not shown). Moreover, there was no correlation between the serum levels of IL-18Rα complex and pulmonary function (FEV1) or airway hyperresponsiveness (Methacholine PC_{20} mg/mL) (data not shown). Using receiver operating characteristic (ROC) curve analysis, we evaluated whether the serum levels of the IL-18Rα complex could discriminate patients with allergic asthma from allergic nonasthmatics and healthy controls. The area under the ROC curve for the serum level of the IL-18Rα complex was 0.863. At a cut-off point of 19.0 ng/ml, the specificity was 0.679 and the sensitivity was 0.895 for detection of allergic asthma, suggesting that serum levels of soluble IL-18Rα complex may discriminate such patients. Further studies will be needed to verify this issue. Next, we examined the serum level of the sIL-18R complex in 98 COPD patients and 36 age-matched smokers and 51 nonsmokers. The mean age of COPD, smokers, and nonsmokers was 66.5 ± 9.4, 61.2 ± 2.4, and 62.1 ± 2.3 years, respectively. There was no significant difference of age among 3 groups. The serum levels of sIL-18R complex were 42.1 ± 8.2, 53.1 ± 17.5, and 58.6 ± 11.8 ng/mL, respectively. Interestingly, the serum levels of sIL-18R complex in nonsmokers were significantly (p < 0.05) higher than smokers and COPD patients but there was no significant difference between COPD patients and smokers. In this study, we could not compare the serum levels of sIL-18R complex between allergic asthmatics and COPD patients, because the mean age of allergic asthma patients (35.4 ± 4.7 years) was significantly (p < 0.05) lower than that of COPD patients (66.5 ± 9.4 years). Further studies are needed to clarify the reasons why serum levels of sIL-18R complex are decreased in COPD patients.

Serum levels of IL-18 are higher in asthmatic subjects when compared to healthy controls. In addition, significantly higher serum IL-18 levels have been reported in patients with acute severe asthma.[1] Polymorphism of the IL-18 gene has been associated with asthma severity, the rs5744247 variant reflecting both higher transcriptional activity and higher serum IL-18 levels.[7] In addition, the IL-18R gene (on 2q21) has been identified as a candidate gene associated with increased susceptibility to asthma in children,[8] and polymorphisms of the gene are related to allergic asthma and airway hyper-responsiveness (AHR).[9] Recently, we reported that IL-18Rα protein was expressed in the airway epithelium of patients with allergic asthma.[6] Soluble IL-18Rα complex exhibits antagonistic activity *in vitro*.[5] IL-18 can act as a cofactor for Th2 cell development and IgE production.[10] IL-18 may increase the level of IgE in sera. Therefore, increased levels of soluble IL-18Rα complex in serum may also exert an antagonistic effect *in vivo* and play an important role in the inflammatory process in allergic asthma.

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REFERENCES

1. Kawayama T, Okamoto M, Imaoka H, Kato S, Young HA, Hoshino T. Interleukin-18 in pulmonary inflammatory diseases. *J Interferon Cytokine Res* 2012;**32**:443-9.
2. Imaoka H, Hoshino T, Takei S *et al*. Interleukin-18 production and pulmonary function in COPD. *Eur Respir J* 2008;**31**:287-97.
3. Torigoe K, Ushio S, Okura T *et al*. Purification and characterization of the human interleukin-18 receptor. *J Biol Chem* 1997;**272**:25737-42.
4. Sims JE. IL-1 and IL-18 receptors, and their extended family. *Curr Opin Immunol* 2002;**14**:117-22.
5. Takei S, Hoshino T, Matsunaga K *et al*. Soluble interleukin-18 receptor complex is a novel biomarker in rheumatoid arthritis. *Arthritis Res Ther* 2011;**13**:R52.
6. Imaoka H, Gauvreau GM, Watson RM *et al*. Interleukin-18 and interleukin-18 receptor-alpha expression in allergic asthma. *Eur Respir J* 2011;**38**:981-3.
7. Harada M, Obara K, Hirota T *et al*. A functional polymorphism in IL-18 is associated with severity of bronchial asthma. *Am J Respir Crit Care Med* 2009;**180**:1048-55.
8. Wu H, Romieu I, Shi M *et al*. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. *J Allergy Clin Immunol* 2010;**125**: 321-7.e313.
9. Zhu G, Whyte MK, Vestbo J *et al*. Interleukin 18 receptor 1 gene polymorphisms are associated with asthma. *Eur J Hum Genet* 2008;**16**:1083-90.
10. Hoshino T, Yagita H, Ortaldo JR, Wiltrout RH, Young HA. In vivo administration of IL-18 can induce IgE production through Th2 cytokine induction and up-regulation of CD40 ligand (CD154) expression on CD4+ T cells. *Eur J Immunol* 2000;**30**:1998-2006.