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Serum-free immunoglobulin E

A useful biomarker of atopy and type 2 asthma in adults with asthma

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ABSTRACT

Background: It has been known that a high serum total immunoglobulin E (IgE) level is a predisposing factor of allergic asthma; however, there are considerable limitations to apply it in clinical practice.
Objective: To determine the clinical significance of the serum-free IgE level in patients with adult asthma.
Methods: We measured free IgE levels using our homemade enzyme-linked immunosorbent assay by applying a novel IgE TRAP protein (GI innovation, Seoul, Republic of Korea) in sera of adults with asthma (n = 116) compared with healthy controls (n = 32); enzyme-linked immunosorbent assay inhibition test was performed to validate its binding specificity. Associations between asthma-related clinical and laboratory parameters were analyzed. The diagnostic value and cutoff point for detecting atopy and type 2 asthma were determined using receiver operating characteristic curve analysis.
Results: The serum-free IgE levels were significantly higher in adults with asthma than in healthy controls and were significantly associated with atopic status and type 2 asthma (all $P < .001$). In the receiver operating characteristic analysis, serum-free IgE had a significantly greater area under the curve (AUC) than serum total IgE for assessing asthma, especially type 2 asthma (AUC, 0.810 vs 0.743; $P = .006$ and AUC, 0.729 vs 0.572; $P < .001$). The optimal cutoff points for predicting atopy and type 2 asthma were 82.8 and 120.8 ng/mL, respectively.
Conclusion: It is suggested that a higher serum-free IgE level may be a useful biomarker of atopy and type 2 asthma in adults with asthma.

Introduction

Immunoglobulin E (IgE) is well known as a key mediator in the development and maintenance of allergic inflammation. It is known to have the following 2 principal receptors: the high-affinity receptor for IgE (FcεRI) is expressed on tissue mast cells, blood basophils, airway smooth muscle cells, and antigen-presenting cells.[1,2] Crosslinking of IgE-FcεRI complexes causes the release of inflammatory mediators and cytokines from IgE effector cells, which contributes to eosinophil recruitment into inflammatory tissue.[3,4] FcεRII (CD23), the low-affinity receptor for IgE, plays a central role in enhancing the activation and proliferation of allergen-specific T cells through the process called IgE-facilitated allergen presentation.[5,6] T-cell activation by CD23 increases the levels of type 2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13, which are responsible for the late phase of allergic inflammations.[5]

Despite the importance of IgE in allergic airway disease, currently available methods for the measurement of serum total IgE have limitations to apply in clinical practice owing to a wide overlap in the distribution of its concentrations between atopic and nonatopic individuals.[7,8] Elevated levels of serum total IgE are found in patients not only with allergic asthma but also with other conditions, such as parasitic infestations, inflammatory diseases, hematologic malignancies, and some primary immunodeficiency diseases, whereas low or normal values do not exclude the presence of allergic asthma.[8,9] Consequently, total IgE levels could not be considered an independent marker for the diagnosis of allergic asthma.[10] Furthermore, serum total IgE levels include both complex forms of IgE and free IgE.[1] In particular, total IgE levels are increased after omalizumab treatment in individuals with severe asthma owing to the detection of IgE-omalizumab complexes, which makes it impossible to monitor therapeutic

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responses and determine the appropriate dose of omalizumab.[11,12] Alternatively, serum-free IgE levels are decreased up to 95% after omalizumab treatment in patients with clinical responses, suggesting it could be used to determine therapeutic responses.[11-14]

To date, there has been no reliable method for the measurement of serum-free IgE. Its clinical and diagnostic value has not been investigated. The present study aimed (1) to establish a new technique for detecting serum-free IgE using a novel IgE_{TRAP} protein and (2) to analyze the associations between clinical and laboratory parameters to provide relevant cutoff points for representing atopy or the endotype of asthma.

Methods

Study Subjects

A total of 116 adult patients with a diagnosis of asthma based on clinical symptoms (such as cough, dyspnea, chest tightness, and wheezing), airway reversibility (an increase in forced expiratory volume in 1 second [FEV_1] of ≥12% and 200 mL from prebronchodilator use), and airway hyperresponsiveness (provocative concentration of methacholine that results in a 20% decrease in FEV_1 of ≤16mg/mL) were recruited from Ajou University Hospital in South Korea. All subjects had no history of any chronic or current acute disease. Patients with the use of biological agents were excluded from the study. Their demographic characteristics were collected, including age, sex, and history. A control group consisted of 32 healthy subjects with neither history of asthma nor other chronic diseases for comparative analyses. All subjects agreed to participate voluntarily in the study and provided written informed consent. This study was approved by the institutional review board of Ajou University Hospital (AJIRB-BMR-SMP-19-001; AJIRB-BMR-SUR-15-498).

Data Collection

All clinical data were obtained by clinical investigators and stored in a database. The presence of atopy was determined by a positive skin prick test result (allergen/histamine ratio of wheal size ≥ 1) or elevated serum-specific IgE level (≥0.35 IU/mL) to at least 1 common aeroallergen (pollens, house dust mites, animal dander, or mold). Spirometry with bronchodilator and methacholine challenge test were conducted, and peripheral eosinophil counts and fraction of exhaled nitric oxide (FeNO) levels (NIOX; Aerocrine AB, Solna, Sweden) were obtained. The serum levels of total IgE were measured with the ImmunoCAP system (Thermo Fisher, Waltham, Massachusetts). Serum periostin levels were measured by using commercial enzyme-linked immunosorbent assay (ELISA) kits (Shino-Test Corporation, Tokyo, Japan), according to the manufacturers' instructions. Induced sputum samples were collected by inhalation of nebulized hypertonic saline solution and dispersed using dithiothreitol for sputum cell count assessment. Sputum eosinophils were counted as a percentage of total sputum leukocytes.

Definition of Type 2 Asthma and Asthma Control Status

Type 2 airway inflammation was defined on the basis of the Global Initiative for Asthma (GINA) guidelines.[15] Patients with type 2-high asthma were defined according to either blood eosinophil count greater than or equal to 150/μL and/or FeNO greater than or equal to 20 ppb and/or sputum eosinophils greater than or equal to 2%. In contrary, patients with type 2-low asthma were defined according to blood eosinophil count less than 150/μL and FeNO less than 20 ppb and sputum eosinophils less than 2%. Asthma control status was also assessed according to the GINA guidelines.[15] Patients with well-controlled status were classified as the controlled asthma group, whereas patients in partly controlled and uncontrolled status were classified as the uncontrolled asthma group. A cutoff point of 150 IU/mL was used to determine elevated serum total IgE.[16]

Preparation of Immunoglobulin E_{TRAP} Protein

The IgE_{TRAP} protein, developed by GI Innovation (Seoul, South Korea), comprises a human FcεRIα extracellular domain fused to a human IgG4 Fc domain (eFig 1A). The FcεRIα domain of IgE_{TRAP} has a high binding affinity for the Fc chain of IgE, which allows effective detection of circulating IgE.[1] This IgE_{TRAP}-Fc fusion protein was expressed in dihydrofolate reductase-deficient Chinese hamster ovary cells transfected with α-2, 6-sialyltransferase gene. The IgE_{TRAP} protein was purified using an Amsphere A3 column (JSR, Ibaraki, Japan) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing and nonreducing conditions and size exclusion high-performance liquid chromatography (eFig 1B and C).

Measurement of Free Immunoglobulin E in the Sera of the Study Subjects

Measurement of serum-free IgE was made using ELISA with applying a novel IgE_{TRAP} protein as previously described.[17] Briefly, IgE_{TRAP} protein was used to coat ELISA plates with 0.1 μg/well and incubated overnight at 4°C. After washing 3 times with 0.05% phosphate-buffered saline (PBS)-Tween, the remaining binding sites were blocked with 300 μL of blocking buffer (PBS containing 1% BSA) for 30 minutes. Diluted serum (1:10) from patients or control subjects was incubated for 1 hour at room temperature in IgE_{TRAP} protein-coated wells. After washing with 0.05% PBS-Tween, antihuman IgE antibody (1:2000; Thermo Fisher) was added to each well and incubated for 1 hour. The wells were washed 5 times with 0.05% PBS-Tween and incubated with HRP-conjugated antirabbit IgG secondary antibody (1:2000; Novusbio, Littleton, Colorado) for 1 hour, followed by incubation with TMB substrate (BD Biosciences, Franklin Lakes, New Jersey) for 10 minutes. The reaction was stopped by the addition of 2N H_2SO_4, and the absorbance values were read by using an ELISA reader (BioTek, Winooski, Vermont) at 450 nm. The absolute levels of free IgE in each serum were calculated from the standard curve incubated simultaneously. The cutoff point determined by the mean plus 2 times the SD of the absorbance values of control sera was set as the threshold for high free IgE vs low free IgE level; 1 IU/mL of serum total IgE is equivalent to 2.4 ng/mL of serum-free IgE.

Enzyme-Linked Immunosorbent Assay Inhibition Test

Competitive ELISA inhibition tests were carried out to determine the binding specificity of free IgE to the IgE_{TRAP} protein and to compare between those of omalizumab and antihuman IgE antibody (Thermo Fisher). The pooled sera (90 μL) from 2 patients with asthma with high free IgE levels were preincubated overnight at 4°C, with increasing amounts (0.1-10 μg/mL protein concentration) of IgE_{TRAP}, omalizumab (Xolair; Novartis, Basel, Switzerland), and antihuman IgE antibody. Each serum was incubated with the IgE_{TRAP} protein-coated microtiter plates, and the same steps were taken in the ELISA assay as previously described. The sera were preincubated with PBS, instead of inhibitors as control parameter. The percent inhibition was calculated as follows: 100 − (absorbance with inhibitor/absorbance without inhibitor) × 100.

Statistical Analysis

The χ^2 test was used to examine differences in categorical variables, and the *t* test was applied to determine differences in

continuous variables for comparative analyses of the 2 groups. For variables with log-normal distributions, log-transformations were performed. Geometric means and asymmetric SDs were calculated from log_{10}-scaled mean and SD as follows: geometric mean = 10^ (mean); asymmetric upper SD = 10^ (mean + SD) − 10^ (mean); asymmetric lower SD = 10^ (mean − SD) − 10^ (mean). Correlations were analyzed with the Pearson correlation coefficient (*R*). Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic value and to compare utility between serum-free IgE and total IgE for assessing atopic status and type 2-high asthma. All statistical analyses were performed using IBM SPSS 25.0 (IBM Corp, Chicago, Illinois) and MedCalc version 15.5 (MedCalc, Mariakerke, Belgium) statistical software. Levels of significance were presented as *P* values less than .05, less than .01, or less than .001. Statistical significance was set at *P* less than .05.

Results

Clinical Characteristics of the Study Subjects

In total, 116 patients with asthma and 32 healthy control subjects were enrolled in this study. The clinical characteristics of the study subjects are presented in Table 1. Compared with healthy control subjects, patients with asthma did not differ with respect to mean age or sex proportion. However, as expected, patients with asthma had higher atopic status ($P = .03$), serum total IgE levels ($P < .001$), and blood eosinophil counts ($P < .001$) and had lower forced expiratory volume in 1 second (FEV_1) percent ($P < .001$) and FEV_1/forced vital capacity (FVC) ratios ($P < .001$) than did healthy controls.

Enzyme-Linked Immunosorbent Assay and Immunoglobulin E Enzyme-Linked Immunosorbent Assay Inhibition Test

Figure 1 reveals comparison of serum-free IgE levels (measured by ELISA) between patients with asthma and healthy controls. Significantly higher levels were noted in patients with asthma than in healthy controls ($P < .001$; Fig 1A). A close positive correlation was observed between log-transformed free IgE and total IgE levels ($r = 0.853$, $P < .001$; Fig 1B). There were no significant differences in the distribution of serum-free IgE and total IgE levels in patients with asthma and atopy (eFig 2A and B). When ELISA inhibition tests were performed in the sera of 2 patients with asthma with a high serum-free IgE level, significant inhibitions were noted with serial additions (0.1-10 μg/mL) of the IgE_{TRAP}, omalizumab, and antihuman IgE antibody in a dose-dependent manner, revealing greater inhibitions were noted by IgE_{TRAP} than by omalizumab or antihuman IgE antibody (Fig 2A and B). In addition, the reproducibility of the ELISA was found within 10%.

Associations of Serum-Free Immunoglobulin E Levels With Type 2 Inflammation Markers and Lung Function Parameters

Patients with asthma were classified into the high free IgE group (≥220.7 ng/mL, n = 58) and the low free IgE group (<220.7 ng/mL, n = 58); the cutoff point was determined from mean + 2SD of serum-free IgE for healthy controls (70.1 + 2 × 75.3). Comparisons of patient characteristics between the 2 groups are summarized in Table 1. Blood eosinophil counts ($P = .02$), serum periostin levels ($P = .01$), FeNO levels ($P = .03$), and serum total IgE levels ($P < .001$) were significantly higher in the high free IgE group than in the low free IgE group. FEV_1(%) values ($P = .02$) and FEV_1/FVC ratios ($P = .09$) were lower in the high free IgE group than in the low free IgE group. In all, 75 patients were able to produce induced sputum, comprising 42 patients in the high free IgE and 33 patients in the low free IgE groups. Sputum eosinophil counts were significantly higher in the

Table 1
Clinical Characteristics of the Study Subjects

Characteristic	Patients with asthma			Healthy controls (n = 32)	*P* value	
	Overall (n = 116)	High free IgE (n = 58)	Low free IgE (n = 58)		Patients with asthma vs controls	High vs low free IgE
Age (y)	50.0 ± 15.0	49.1 ± 15.8	51.0 ± 14.3	45.0 ± 10.5	.16	.49
Female (%)	79 (68.1)	40 (69.0)	39 (67.2)	20 (62.5)	.55	.84
Atopy (%)	71 (61.2)	44 (75.9)	27 (46.6)	13 (40.6)	.03	<.001
Uncontrolled asthma status (%)	43 (37.1)	29 (50.0)	14 (24.1)	ND	ND	.004
Serum total IgE (IU/mL)[a]	173.7 + 432.6/−124.0	453.0 + 319.5/−187.4	66.6 + 114.3/−42.1	51.1 + 130.3/−36.7	<.001	<.001
Blood eosinophil count (/μL)[a]	238.6 + 358.4/−143.2	287.7 + 354.1/−158.7	197.2 + 334.5/−124.0	118.4 + 145.0/−65.2	<.001	.02
Serum periostin (ng/mL)	79.6 ± 29.4	86.6 ± 32.9	72.6 ± 23.7	71.8 ± 22.9	.27	.01
Sputum eosinophil (%)[a]	26.1 + 68.6/−18.9	31.4 + 69.9/−15.8	20.9 + 64.9/−15.8	ND	ND	.02
FeNO (ppb)[a]	22.4 + 34.7/−13.6	27.1 + 45.2/−16.9	18.8 + 25.5/−10.8	20.2 + 14.7/−8.5	.46	.03
FEV_1 (% predicted)	91.6 ± 15.8	88.2 ± 16.1	95.0 ± 15.1	107.3 ± 11.8	<.001	.02
FEV_1/FVC (%)	83.0 ± 8.3	81.7 ± 8.8	84.3 ± 7.6	87.3 ± 3.8	<.001	.09
PC_{20} level (mg/mL)[a]	2.7 + 10.2/−2.1	2.3 + 7.6/−1.8	3.1 + 13.4/−2.5	ND	ND	.37
Serum-free IgE (ng/mL)[a]	175.2 + 493.1/−129.3	513.1 + 310.7/−193.5	59.8 + 106.2/−38.3	32.5 + 104.5/−24.8	<.001	ND

Abbreviations: IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ND, no data; PC_{20}, provocative concentration of methacholine that resulted in a 20% decrease in the FEV_1.
Patients with asthma were classified into the high free IgE group (≥220.7 ng/mL) and low free IgE group (<220.7 ng/mL); the cutoff point was determined by the mean plus or minus 2-fold SD of the levels of healthy controls.
[a]Geometric mean plus or minus asymmetric SD. Geometric means and asymmetric SDs were estimated from the log_{10}-scaled means and SDs. *P* values were calculated from log-transformed data.

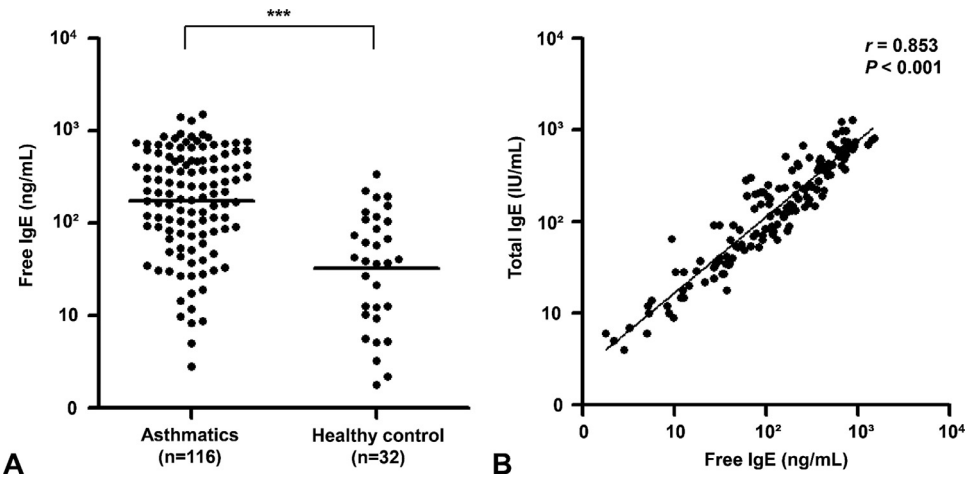

Figure 1. Free IgE levels in serum. A, Comparisons of serum-free IgE levels in adult patients with asthma and healthy control subjects. B, Correlation analysis between serum-free and total IgE levels. Horizontal bar indicates geometric mean value. The single asterisk represents $P < .05$; double asterisk represents $P < .01$; triple asterisk represents $P < .001$. IgE, immunoglobulin E.

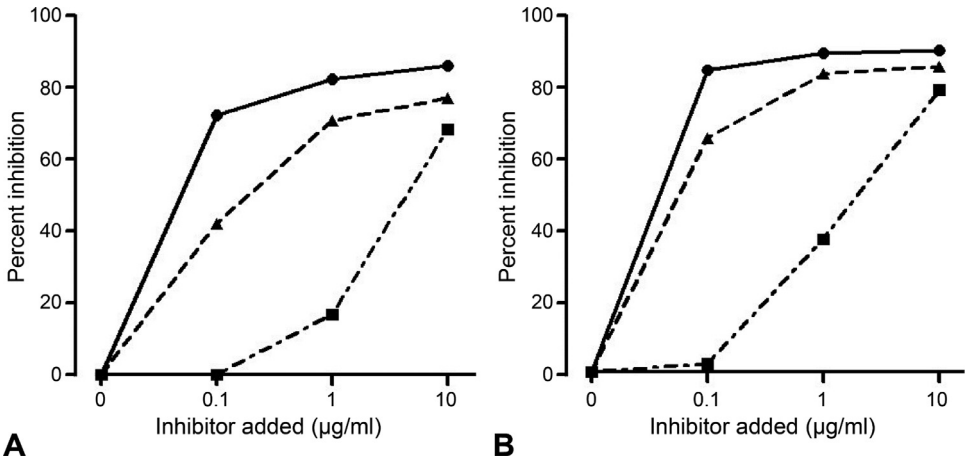

Figure 2. Results of competitive ELISA inhibition tests with serial additions of IgE_{TRAP} (shaded circle), antihuman IgE antibody (shaded triangle), and omalizumab (shaded square) in a dose-dependent manner. A and B, The ELISA inhibition tests are presented from serum of 2 patients with high free IgE levels. ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E.

high free IgE group than in the low free IgE group ($P = .02$). No significant difference in provocative concentration of methacholine causing a 20% decrease in FEV_1 was found between the 2 groups ($P = .37$). In contrary, serum total IgE was not associated with these clinical and laboratory parameters (eTable 1 in the Online Repository).

Comparisons of Serum-Free Immunoglobulin E Levels Between the 2 Subgroups According to Type 2 Inflammation Markers

Subjects with asthma were classified into 2 subgroups each according to blood eosinophil count, sputum eosinophil count, and FeNO level: high (≥150/μL) and low (<150/μL) blood eosinophil subgroups, high (≥2%) and low (<2%) sputum eosinophil subgroups, and high (≥20 ppb) and low (<20 ppb) FeNO subgroups (Fig 3A-C). The cutoff points for determining high and low subgroups were defined according to the GINA guidelines. The serum-free IgE levels were significantly higher in subgroups with high levels of individual biomarkers than those with low levels of individual biomarkers (blood eosinophil count, $P = .004$; sputum eosinophil, $P = .003$; FeNO, $P = .02$). When subjects with asthma were divided into the type 2-high and type 2-low groups, as prescribed previously, the type 2-high group had significantly higher levels of serum-free IgE than the type 2-low asthma group ($P < .001$; Fig 3D).

Comparisons of Serum-Free Immunoglobulin E Levels According to Asthma Control Status and Atopy

When patients with asthma were classified into the controlled and uncontrolled asthma groups according to the GINA-defined asthma control status, the uncontrolled group had significantly higher levels of serum-free IgE than the controlled group ($P < .001$; Fig 3E). When subjects were classified into the atopic and nonatopic groups according to results of skin prick test and/or allergen-specific IgE levels, the atopic group had significantly higher serum-free IgE levels than the nonatopic group in both patients with asthma and healthy control subjects ($P < .001$; Fig 3F). There was no significant difference between patients with nonatopic asthma and healthy controls with atopy ($P = .68$).

Diagnostic Value of Serum-Free Immunoglobulin E in Predicting Atopy and Type 2 High Asthma

Receiver operating characteristic curves were used to evaluate the potential value of serum-free IgE and to determine the cutoff points for identifying atopic status and type 2 high asthma. The diagnostic value of serum-free IgE was compared with serum total IgE, a typically used traditional serologic marker. In the ROC analyses of detecting type 2-high asthma, the AUC of serum-free IgE was 0.729 with 71.3%

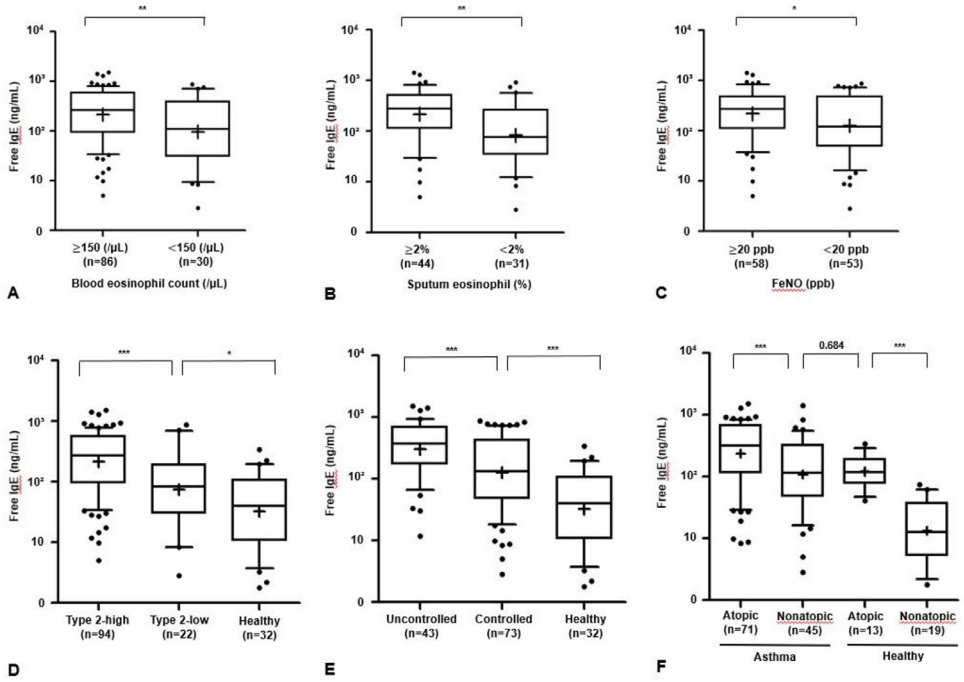

Figure 3. Comparisons of serum-free IgE levels according to (A) blood eosinophil counts, (B) sputum eosinophils, (C) FeNO levels, (D) type 2-high or low asthma, (E) asthma control status, and (F) atopic status. Box plots span data values between the first and third quartiles with the median line. Crosses represent the geometric mean values. I bars represent the 10 to 90 percentiles, and dots outliers. The single asterisk represents $P < .05$; the double asterisk represents $P < .01$; the triple asterisk represents $P < .001$. FeNO, fraction of exhaled nitric oxide; IgE, immunoglobulin E.

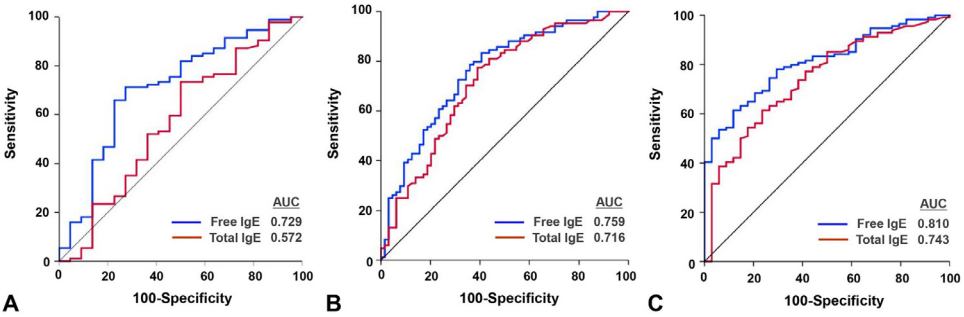

Figure 4. Diagnostic utility of serum-free and total IgE. A, ROC curves analyses for detecting type 2 asthma from patients with asthma. B, ROC curves analyses for predicting atopic status from all subjects. C, ROC curves analyses for assessing asthma from all subjects. AUC, area under the curve; ROC, receiver operating characteristic.

sensitivity and 72.7% specificity at a cutoff point of 120.8 ng/mL, and that of serum total IgE was 0.572 with 73.4% sensitivity and 50.0% specificity at a cutoff point of 91 IU/mL (Fig 4A). A pairwise comparison of ROC curves revealed that free IgE had a significantly greater AUC than total IgE ($P < .001$). In the ROC curves for predicting atopy status, the AUC of serum-free IgE is 0.759 with 83.3% sensitivity and 59.4% specificity at a cutoff point of 82.8 ng/mL, whereas that of serum total IgE is 0.720 with 77.4% sensitivity and 60.9% specificity at a cutoff point of 92.5 IU/mL (Fig 4B). The AUC of free IgE is higher than that of total IgE, albeit without a statistically significant difference ($P = .07$). In addition, the AUC of serum-free IgE for assessing asthma from healthy controls is 0.810 (68.4% sensitivity and 79.4% specificity at a cutoff point of 110.2 ng/mL), revealing free IgE has a significantly higher AUC compared with total IgE ($P = .006$; Fig 4C).

Discussion

Several studies performed in adult patients with asthma during omalizumab treatment have revealed that serum-free IgE could provide a useful guidance to select right patients and to monitor therapeutic responses.[11-14] To evaluate the clinical relevance of serum-free IgE in adults with asthma, we developed a new ELISA-based assay using a novel IgE_{TRAP} protein. Using this assay, we found that serum-free IgE level is a useful serologic marker for determining atopic status to differentiate between adults with asthma and healthy controls without atopy. The present study revealed significant associations between serum-free IgE level and type 2 inflammation markers (higher blood/sputum eosinophil counts and higher levels of serum periostin/FeNO/total IgE); patients with asthma with type 2 markers had higher free IgE level and lower lung function and uncontrolled status of asthma, suggesting circulating free IgE may affect the severity and control status of asthma. Taken together, high serum-free IgE may be a potential biomarker for the diagnosis of type 2-high asthma and atopic status in adults. In addition, serum-free IgE reveals better diagnostic performance compared with serum total IgE in assessing type 2 asthma and atopic status.

It has been found that IgE plays a role in the regulation of FcεRI expression on mast cells, basophils, and antigen-presenting cells. Interestingly, omalizumab that binds to free IgE in the circulation markedly

reduced the density of FcεRI on mast cells, basophils, and dendritic cells, concomitantly with the reduction in serum-free IgE levels.[18,19] A recent study has suggested that CD23 on B cells is also up-regulated by serum IgE according to the rules that guide the expression of FcεRI.[6] Thus, it is reasonable to postulate that free IgE in the circulation could play a major role in controlling allergic immune responses. The present study demonstrated circulating free IgE levels using the ELISA technique with applying the newly developed hybrid protein, which was found to be a reliable and easy-to-perform method to detect serum-free IgE. In addition, the binding specificity and reproducibility thereof were validated. The results of free IgE were significantly associated with type 2 inflammatory markers including blood/sputum eosinophils, FeNO, total IgE, and baseline lung function and asthma control status in patients with asthma. IgE-activated basophils and mast cells lead to the synthesis and release of inflammatory mediators and cytokines, which initiate the late-phase allergic reaction with recruitment and activation of eosinophils. Persistent eosinophilic airway inflammation has been found to be associated with disease severity and airflow limitation. Eosinophils cause bronchial tissue damage by releasing proinflammatory cytokines, chemokines, and granule proteins as a consequence, leading to airway remodeling by persistent airway inflammation and repair.[20] It was found that treatment with omalizumab reduced not only blood/sputum eosinophils but also tissue eosinophils in bronchial biopsy.[21-24] Also, it has been found that omalizumab targeting circulating IgE improves asthma symptoms and reduces frequency of acute exacerbations in patients with asthma. It is conceivable that free IgE, as a key element of this vicious circle, not only maintains chronic eosinophilic inflammation in asthmatic airway but also leads to asthma exacerbation and uncontrolled status of asthma, resulting in airflow limitation and airway remodeling. Taken together, our findings suggest that free IgE could contribute to initiating and maintaining type 2 inflammatory cascades and persistent eosinophil activation in the asthmatic airway; therefore, free IgE is a major therapeutic target in patients with asthma, especially those with type 2 asthma.

IgE directly activates airway smooth muscle cells that express FcεRIs and CD23 on their surface and produce extracellular matrix (key factors involved in airway remodeling) leading to their proliferation and contraction.[21,25] Periostin, a matricellular protein, is released from airway epithelial cells and fibroblasts, which is up-regulated by IL-4/IL-13. Periostin has been reported as a biomarker for type 2 inflammation in asthma and could contribute to airway remodeling and fibrosis; therefore, it can be used to predict clinical efficacy after omalizumab treatment.[26] The omalizumab EXTRA study revealed better clinical responses to omalizumab treatment in the periositin-high group than in the periostin-low group.[27] In addition, omalizumab, which selectively binds to free IgE, has revealed clinical benefits in the reduction in airway wall thickness and improvement in lung function in patients with asthma with and without atopy.[22-24,28] The present study demonstrated that patients with high free IgE levels had high periostin levels but lower FEV_1 (%) and FEV_1/FVC values than those with lower free IgE levels. Taken together, serum-free IgE could contribute to airway remodeling and type 2 airway inflammation, contributing to lung function decline in individuals with asthma.

Several biologics targeting type 2 cytokines, such as IL-4, IL-5, and IL-13 including IgE, have recently emerged as promising treatment options for type 2 asthma. Although they have been found to reduce asthma exacerbation and corticosteroid use, not all patients have equal benefits from them because of heterogeneity of asthma.[29,30] Given the emergence of various new biologics, questions on the selection of right patients and right biologics remain to be answered. Our results suggest that serum-free IgE may be a useful serum biomarker for predicting and monitoring responses to T_H2-targeted biologics, especially anti-IgE antibody treatment, although further clinical trials are needed.

This study has 2 limitations. One is that this is a cross-sectional study. Allergen exposure leads to transient increases in serum-free IgE level as variable factors, such as pathogens and pollutants, can also influence asthma-related clinical/laboratory variables. However, we recruited all the study subjects who had maintained antiasthmatic medications (including inhaled corticosteroids and leukotriene modifiers) according to the GINA guideline by asthma specialists in a single tertiary asthma center; therefore, the data could reflect real-world clinical practice. The other is that although we confirmed the binding specificity and reproducibility of our ELISA method, it needs to be replicated in other cohorts of various allergic diseases. Further studies are also needed to validate the cutoff values in a completely distinct cohort. Despite these limitations, this is the first study to demonstrate serum-free IgE levels in association with type 2 inflammatory markers in adults with asthma.

In conclusion, high free IgE levels were detected in the sera of adults with asthma, especially in those with type 2 inflammatory markers. Its diagnostic value in predicting atopy and type 2 asthma was validated.

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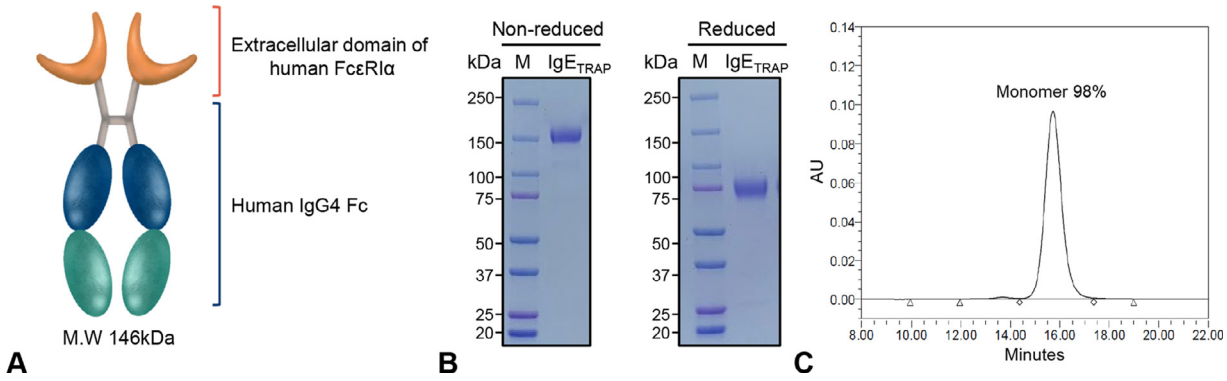
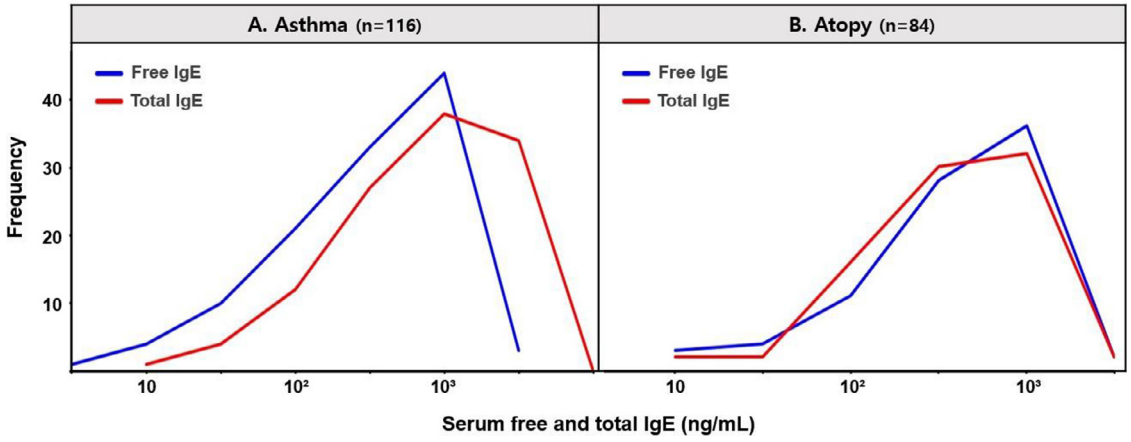


eFigure 1. Structural features of IgE_{TRAP}. A, Schematic diagram of the IgE_{TRAP}. B, SDS-PAGE analysis of IgE_{TRAP} under nonreducing and reducing conditions. C, Size exclusion high-performance liquid chromatography analysis of IgE_{TRAP}. IgE, immunoglobulin E; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.



eFigure 2. Distribution of serum-free IgE and total IgE levels in (A) patients with asthma and (B) patients with atopy. The serum IgE values are displayed on log-transformed scales. IgE, immunoglobulin E.

eTable 1
Comparison of Clinical Characteristics Between the High and Low Serum Total Immunoglobulin E Groups

Characteristic	Serum total IgE of ≥150 IU/mL(n = 70)	Serum total IgE of <150 IU/mL(n = 46)	*P* value
Age (y)	49.4 ± 15.4	50.9 ± 14.4	.60
Female (%)	48 (68.6)	31 (67.4)	.89
Atopy (%)	50 (71.4)	21 (45.7)	.005
Uncontrolled asthma status (%)	30 (42.9)	13 (28.3)	.11
Blood eosinophil count (/μL)	364.3 ± 269.0	275.8 ± 231.0	.07
Serum periostin (ng/mL)	84.7 ± 31.2	71.8 ± 24.8	.05
Sputum eosinophil (%)	32.6 ± 34.3	20.0 ± 30.3	.10
FeNO (ppb)	38.7 ± 33.4	26.9 ± 28.5	.06
FEV_1 (% predicted)	90.2 ± 16.6	93.7 ± 14.6	.24
FEV_1/FVC (%)	82.3 ± 9.0	83.9 ± 7.1	.29
PC_{20} level (mg/mL)	5.6 ± 7.8	8.8 ± 9.4	.09
Serum-free IgE (ng/mL)	496.8 ± 309.1	76.5 ± 60.2	<.001

Abbreviations: IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PC_{20}, provocative concentration of methacholine that resulted in a 20% decrease in the FEV_1.