

Effect of Genetic Variation of IL-13 on Airway Remodeling in Bronchial Asthma

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ABSTRACT

Background: IL-13 is a major stimulator of inflammation and tissue remodeling at sites of Th2 inflammation, and common single-nucleotide polymorphisms in *IL13* are associated with allergic phenotypes in several ethnically diverse populations. In particular, IL13Q110 is the non-conservative replacement of a positively charged arginine (R) with a neutral glutamine (Q) at position 110 of IL-13, and as we already know, individuals homozygous for glutamine (Q110/Q110) are strongly associated with asthma and IgE. IL13Q110 has been demonstrated to show that increased allergic inflammation depended on the enhanced IL-13-mediated Th2 effector function. Therefore, we investigated whether Q110/Q110 accelerated the decline in pulmonary function and development of airway remodeling of asthmatic patients in the general population.

Methods: A total 336 asthmatic subjects living in Japan were recruited, genotyped, and had a pulmonary function test performed on them. To analyze airway inflammation and remodeling, bronchial lavage fluid (BLF) and endobronchial biopsy specimens were examined.

Results: Forced expiratory volume in one second (FEV1), %predicted, forced expiratory volume/forced vital capacity ratio, and forced expiratory flow 25-75%, % predicted were significantly decreased in Q110/Q110 compared to R110/R110, and the decline in FEV1 was increased significantly in Q110/Q110 compared to R110/R110. The concentration of IL-13, IL-23, IL-11, GM-CSF, hyaluronic acid, and CCL8 in BLF were increased in Q110/Q110 compared to R110/R110 and the thickness of the subepithelial layer was thicker.

Conclusions: Our study demonstrates that Q110/Q110 increases, at least in part, allergic inflammation and the propensity for airway remodeling, thus resulting in low lung function.

KEY WORDS

bronchial asthma, bronchial biopsy, bronchial lavage fluid, IL-13, polymorphisms

INTRODUCTION

The histopathologic features of bronchial asthma are associated with airway remodeling as consequences of hypertrophy/hyperplasia of the airway smooth muscles and of subepithelial basement membrane thickening.¹ The latter response is most prominent in the lamina reticularis² and results from interstitial collagen and proteoglycan deposition.³ The formation of new blood vessels may also play an important role both in the development of bronchial asthma and in the physiologic repair process.⁴ Although initially de-

scribed in postmortem studies, these structural alterations can be observed even in mild and newly diagnosed cases of asthma.^{5,6} To date, many studies have focused on the mechanisms underlying the acute and presentation of bronchial asthma, and the factors responsible for the more chronic structural changes within the airway have been defined. IL-13 is a pleiotropic 12-kDa product of a gene on chromosome 5 at q31 that is produced in large quantities by stimulated Th2 cells. IL-13 contributes as the major effector of Th2 inflammation and tissue remodeling.⁷ In accordance with these observations, IL-13 dysregu-

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Received 25 August 2010. Accepted for publication 29 October 2010.

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lation has been documented, and IL-13 has been implicated in the pathogenesis of a variety of diseases characterized by inflammation and tissue remodeling, including asthma, idiopathic pulmonary fibrosis,⁸ and chronic obstructive pulmonary disease (COPD).⁹ Several genome-wide searches for quantitative traits underlying IL-13 have been performed. For example, IgE levels and allergic inflammation is associated with IL13-1112C > T in some populations¹⁰ (functional studies support a regulatory role for the -1111 variant, which enhances IL13 promoter activity in CD4 positive lymphocytes¹¹) and IL13-1512A > C in others.¹² We have found a variant of the *IL13* gene (rs20541), in which arginine residue at amino acid 110 (R110) is substituted with glutamine (Q110).¹³ This variant was associated with bronchial asthma in both Japanese and British populations. The variant showed a lower affinity with the IL-13 receptor $\alpha 2$ chain (IL13R $\alpha 2$), which is a decoy receptor that antagonizes inflammatory, remodeling, and physiologic responses, causing less clearance.¹⁴ The variant also demonstrated an enhanced stability in plasma level.¹⁴ These data lead us to hypothesize that IL13 Q110 contributes to airway remodeling by driving inflammation and fibrosis production. As the pulmonary function of Q110 homozygote in the asthmatic has not been established, we first examined the airway limitation manifested by a low force expiratory volume in 1s (FEV1) adjusting for covariates, such as age, gender, height, atopy, and a history of smoking of asthmatic patients, and performed comparisons among Q110/Q110, Q110/R110, and R110/R110. Subsequent bronchial specimens' examinations provided insights into the mechanisms of airway remodeling of asthmatic patients with IL13 Q110 homozygote.

METHODS

STUDY SUBJECTS

All study subjects were recruited from the Iwate Medical University Hospital. Patients with bronchial asthma diagnosed according to the American Thoracic Society (ATS) criteria, who had no other medical disorders, and were not current smokers, were considered for the study, which was approved by Iwate Medical University Hospital Ethics Committee. Prospective patients were then notified of our desire to include them in our study and asked if they would be willing to participate. Upon acceptance, the subjects were provided written informed consent according to the ethical protocols of our institution. Subjects were assessed for age, sex, duration of asthma, history of smoking, spirometry, and eosinophils count. DNA was isolated from lymphocytes using standard procedures. Subjects were genotyped using (rs20541) Q110/Q110, Q110/R110, and R110/R110 (7500 Fast Real-Time PCR System, Applied Biosystems, CA, USA) to evaluate the relationship of pulmonary function with airway remodeling.

SPIROMETRY AND METHACHOLINE CHALLENGE

To fulfill the ATS criteria for asthma, spirometry was performed and airway methacholine responsiveness was measured using an Astograph (Jupiter 21, CHEST, Tokyo, Japan), according to the method described by Takishima *et al.*¹⁵ The maximum FEV1 of as many as 5 technically acceptable blows was recorded, both at baseline and at the end of the follow-up, according to the ATS criteria for reproducibility.

ONE YEAR OF FOLLOW-UP AND COMPARISON OF FEV1

In this study, the decline in FEV1 was calculated by the differences between the results of baseline and those of the following year. Patients recorded details of asthma-related events, use of additional asthma medications, asthma symptoms, restrictions in daily activities, and sleep problems caused by asthma between scheduled hospital visits. Changes in concurrent medications, including inhaled or systemic corticosteroids, could be made if an investigator judged this necessary to achieve asthma control. Serious adverse events were collected from submitted reports and by questioning at the hospital.

FIBREOPTIC BRONCHOSCOPY AND SPECIMEN HANDLING

Bronchial lavage fluid (BLF) and bronchial biopsy were performed by inserting a flexible fiberoptic bronchoscope (Olympus; Olympus Optical Co., Ltd., Tokyo, Japan) under local anesthesia, as described elsewhere in detail.¹⁶ BLF was always done before the biopsy to prevent blood contamination. The BLF was carried out in one of the subsegmental bronchi of the left lingular division by the injection of two 20 ml aliquots of sterile phosphate-buffered saline (PBS) pre-warmed to 36.5°C and gently aspirated back into polypropylene tubes kept on ice. We obtained 20-25 ml of BLF from all subjects. Immediately after lavage, mucus was removed from the fluid by filtration through gauze, and fluid was then centrifuged at 200x g for 10 min at 4°C. The supernatant was decanted and stored at -80°C. The cell pellet was washed in PBS and a total cell count was performed using a disposable cell counting chamber (Cellometer, Nexcelom Bioscience LLC, MA, USA). The cells were then diluted with PBS to a concentration of 1×10^6 /ml. Cytocentrifuge slides were prepared and stained with a May-Grünwald-Giemsa, and a differential cell count was done in 300 cells. BLF cells were used for immunocytochemistry and the supernatant of BLF was stored at -80°C until use. Five biopsies were taken using forceps (Olympus; Olympus Optical Co., Ltd.) in the segmental bronchi of the right lobe and the bifurcations of right bronchi. Biopsied specimens were fixed in 4% paraformaldehyde and immersed in su-

crose, thereafter embedded in OCT compound. Five micrometer tissue sections were affixed to microscope slides and readied for histology. Hematoxylin and eosin (HE) staining was then carried out. The thickness of the subepithelial layer was measured on the biopsies stained by HE. All stained sections were coded and examined in a blinded manner at the end of the study. All available sections for each patient were evaluated. Biopsies were considered suitable for examination when there was at least 1.0 mm of subepithelial layer length with an intact epithelium. At least 3 suitable biopsy sections were examined per subject. The thickening of the subepithelial layer in individual biopsy specimens were measured in micrometers (μm), making measurements at 20 μm intervals along the whole length of the membrane, while taking only the adequately preserved and oriented area into account. There were then analyzed using the Olympus DP70 system (Olympus) and computer image analysis (Image-Pro Plus 4.1; Media Cybernetics, MD, USA). The intra-observer error was assessed by performing three separate counts of the same section on different occasions and was calculated to be less than 5%.

PRELIMINARY STUDY USING MICROARRAY ANALYSIS

Microarray analysis was carried out to estimate what biomarkers increased in BLF from those homozygous for Q110 compared to those homozygous for R110.

CO-CULTURE OF STIMULATED AND IL-13-EXPRESSED PBMCs WITH FIBROBLASTS

Antibodies were immobilized on 6-well tissue culture plates by incubating 20 $\mu\text{g}/\text{ml}$ anti-CD3 (R&D Systems, Inc., MN, USA) and 10 $\mu\text{g}/\text{ml}$ anti-CD28 (R&D Systems, Inc.) in PBS overnight at 4°C. Excess antibodies were removed by washing the wells with PBS. PBMCs were randomly obtained from three asthma patients with Q110/Q110 and three asthma patients with wild type (wt) R110/R110. Heparinised venous blood was centrifuged on Histopaque 1077 (Sigma, Tokyo, Japan) at 600 g for 20 min at room temperature. The detailed protocol of PBMCs isolation has been reported elsewhere.¹⁷ PBMCs were plated at the bottom of the 6-well tissue culture plates to be stimulated by CD3 and CD28 at a density of 1×10^6 cells/well. Co-culture experiments were performed in 6-well plate ThinCert Cell Culture Inserts Systems (Greiner Bio-One, Frickenhausen, Germany). Human lung fibroblasts were purchased (Takara BIO Inc., Shiga, Japan) and maintained in fibroblast basal medium (FBM; Takara BIO Inc.) supplemented with 10% fetal calf serum. Fibroblasts (1×10^5) were seeded onto the inserts and incubated at 37°C with 5% CO₂ in the air until they were 80% confluent, and thereafter inserts with fibroblasts were put into the 6-

well tissue culture plates of CD3 and CD28- stimulated PBMCs' and incubated in FBM for 18 h. Fibroblasts, which were stimulated by IL-13 Q110/Q110 or IL-13 R110/R110 produced by PBMCs, were submitted to microarray analysis.

MICROARRAY

The fibroblasts' mRNA expressions were assessed by co-culture with or without stimulated PBMCs obtained from Q110/Q110 and R110/R110 individuals. RNA was extracted from cultured fibroblast by an miRNA Isolation Kit (Applied Biosystems) according to the procedure provided by the vender.

cRNA PREPARATION

A 500 ng of aliquot of total RNA was reverse transcribed into cDNA using the Quick Amp Linear Amplification Kit (Agilent Technologies, CA, USA). The synthesized cDNA was transcribed into cRNA and labelled with either cyanine 3-labelled nucleotide (Agilent Technologies). Labelled cRNA was purified with RNeasy Mini columns (Qiagen, CA, USA). The quality of each cRNA sample was verified by total yield and specificity calculated based on a NanoDrop ND-1000 spectrophotometer measurement (NanoDrop Technologies, DE, USA).

MICROARRAY HYBRIDIZATION

Labelled cRNAs with a specificity greater than 8 were used for hybridization using the *in situ* hybridization kit plus (Agilent Technologies). Arrays were incubated at 65°C for 17 h in Agilent's microarray hybridization chambers. After hybridization, arrays were washed according to the protocol (Agilent Technologies).

IMAGE PROCESSING

Slides were scanned immediately after washing on the Agilent DNA Microarray Scanner (G2505C) using the one color scan setting for 4×44 k array slides (Scan Area 61×21.6 mm, scan resolution of 5 μm , dye channel is set to Green and Green PMT is set to 100%).

The scanned images were analyzed with Feature Extraction Software 10.5.1.1 (Agilent Technologies) using the default parameters (protocol GE1-v5_10_Apr08 and Grid: 014850_D_F_20080627) to obtain background subtracted and spatially detrended Processed Signal intensities.

NORMALIZATION AND STATISTICAL ANALYSIS

The microarray data were analyzed by using Agilent Genespring GX 11. The signal intensity of each gene was firstly normalized with the total intensity of all genes from the microarray, and then the normalized signal of each treatment was compared with the mock-treatment to determine the relative fold changes of gene expression. The threshold level for

Table 1 Subject demographic characteristics

Domain/IL-13 genotype	R110/R110 (n = 127)	Q110/R110 (n = 154)	Q110/Q110 (n = 55)	Statistical results MANOVA, ANOVA, or χ^2
Age, yr	42.7 (15.3)	49.3 (18.9)	45.1 (12.7)	NS
Sex, n (%)	63 men (51)	71 men (46)	26 men (59)	NS
Pack-years of smoking	5.2 (2.2)	4.1 (1.8)	5.5 (2.1)	NS
Eosinophil count, μl^{-1}	248.7 (73)	211.0 (98)	263.0 (102)	NS
Pulmonary function test				MANOVA < 0.01
FEV1, %predicted	98.4 (23.1)	92.7 (20.5)	65.3 (12.3)	0.03
FEV1/FVC	76.2 (14.9)	75.0 (11.0)	62.2 (15.9)	0.04
FEF25-75%, %predicted	73.0 (20.3)	59.1 (21.2)	35.7 (18)	0.03
Decline in FEV1 (ml/yr)	27.0 (3.1)	29.0 (2.2)	33.4 (11.9)	0.04

Data are presented as mean $\pm$ (standard deviation). NS = Not significant ($p > 0.05$). p values are for analyses of variance (ANOVA) unless otherwise specified. Multivariate analyses of variance (MANOVA) were performed to analyze the predictive factors for the impairment of lung function test parameters and were used as dependent variables, while age, sex, and tobacco were used as covariates.

ANOVA results reported only within outcome domains with significant MANOVA result.

Post hoc comparisons reported only for variables with significant ANOVA result.

FEV1, forced expiratory volume in one second; FEV, forced expiratory volume; FVC, forced vital capacity; FEF25-75%, forced expiratory flow 25-75%.

up- or down-regulation of gene expression was the level of changes ≥ 2 -fold.

CYTOKINE CONCENTRATION OF BLF SUPERNATANT

Various cytokines concentrations in BLF supernatant were analyzed when there were significant differences in the mRNA level of fibroblasts in the co-culture system between Q110/Q110 and R110/R110.

SAMPLE SIZE

The BLF and biopsy study sample size was calculated based on the results of previous studies, assessing the concentration of cytokines and hyaluronic acid, and the thickening of the subepithelial layer of asthmatic patients. However, other studies have examined the concentration of mediators using condensed bronchoalveolar lavage fluid obtained from the patients with bronchial asthma, which is a method different from our procedures. Subepithelial layer thickness was measured by Vignola *et al.*¹⁸ and our group² previously, and was not mentioned nor classified in the genetic variant of IL13 homozygous for Q110 and R110. Vignola *et al.* reported a 11 μm subepithelial layer thickness within severe asthmatic patients, and we reported a 8.1 μm subepithelial layer thickness with mild asthmatic patients. Therefore, the expected difference between mild and severe asthma patients' subepithelial layer thickness was 2.9 μm . Results from these studies indicated that 16 patients had to be included to show a significant difference ($\alpha = 0.05$, power = 80%). Accounting for a maximum of a 10% dropout rate and for 80% of patients potentially non-compliant with bronchoscopic examination from our previous experiments,^{4,16,19-21} a minimum of 160

patients had to be recruited.

STATISTICAL ANALYSIS

All data are expressed as the mean $\pm$ SD. Multivariate analyses of variance (MANOVA) were performed to analyze the predictive factors for the impairment of lung function parameters. In this model, pulmonary function test parameters (%FEV1, FEV/FVC, %FEF25-75%, and decline in FEV1) were used as dependent variables, while age, sex, history of tobacco use, and the group of subjects served as covariates. Comparisons of pulmonary function and thickening of subepithelial layer among the three groups were performed using the one-way ANOVA. JMP version 8.0.2 (SAS Institute Japan, Tokyo, Japan) was the software used for statistical analysis.

RESULTS

CHARACTERISTICS OF STUDY POPULATION

The characteristics of patients are presented in Table 1. There were 336 asthmatic patients in the study group. R110 homozygote occupied 38%, heterozygote occupied 46%, and Q110 homozygote occupied 16% of the total recruited asthmatic patients. There were no differences in age, sex, history of smoking, or eosinophils count status among the three groups, however, FEV1, FEV1/FVC, %FEF25-75%, and a decline in FEV1/year were significant differences among three groups. There are significant decreases of %FEV1, FEV1/FVC, and %FEF25-75% in Q110/Q110 compared to R110/R110 (Table 1). There is also a significant increase of decline in FEV1 in Q110/Q110 compared to R110/R110 (Table 1). No patients in either group discontinued participation during the 1-year follow-up.

Table 2 Summary of IL-13 Q110/Q110-regulated genes identified by microarray analysis

Gene name	Gene accession number	Expression ratio
IL23A	NM_016584	93.336
IL11	NM_000641	32.284
CR618615	CR618615	29.078
IL24	NM_006850	28.190
THC2640944	THC2640944	27.431
CSF2	NM_000758	18.526
HAS1	NM_001523	17.756
PTGS2	NM_000963	15.075
CSF3	NM_000759	13.536
FLJ33590	NM_173821	12.448
RHCG	NM_016321	12.315
CCL8	NM_005623	9.130
C15orf48	NM_032413	7.711
ENST00000303979	ENST00000303979	5.677
A_24_P928031	A_24_P928031	4.131

EFFECTS OF GENETIC VARIANT OF IL-13 ON FIBROBLAST GENE EXPRESSION PROFILES AS MEASURED WITH THE cDNA MICROARRAY

The lower pulmonary function that was found in asthmatic patients with a genetic variant of IL13Q110/Q110 suggested that the airway strongly responded to this cytokine, and then proceeded to fibrosis. To test this idea, we seeded PBMCs derived from three of each of the Q110/Q110 or R110/R110 patients with asthma onto the well to detect their interaction with fibroblasts, which were seeded onto inserts. Some of the mRNA expression of fibroblasts by co-cultivation with PBMCs were increased when the PBMCs were obtained from Q110/Q110 patients with asthma. The summarized results for important genes are shown in Table 2. These data were the average of each of the three patients. IL-23A mRNA expression increased in response to CD3 and CD28-stimulated PBMCs derived from asthmatic patients with Q110 compared to R110 (93.336-fold). The mRNA expression of IL-11, IL-24, CSF2/GM-CSF, HAS1, PTGS2, CSF3/G-CSF, CCL8/MCP-2 were also increased compared to R110.

DETECTION OF IL-13, IL-23, IL-11, GM-CSF, HYALURONIC ACID, AND CCL8 IN BLF

To confirm whether Q110/Q110 individuals expressed increased protein levels, which were indicated in the microarray data, we performed a bronchoscopy and collected BLF from the three groups, R110/R110, Q110/R110, and Q110/Q110. Sixteen asthmatic patients with Q110/Q110, fifteen patients with Q110/R110, and eighteen patients with R110/R110 were accepted for the BLF and bronchial biopsy study. A chi-square test was used to compare all the

gene assessed patients and the bronchoscope treated patients in pulmonary function, but we found no differences between the two groups. Bronchoscopic examination with BLF collection was performed with safety and was well tolerated in all subjects. All indicated data above were determined by a commercially available ELISA test. IL-13 levels in BLF were assessed by the SRL laboratories (Tokyo, Japan). Because of the different sensitivities of Q110 and R110 to this assay, the values of Q110 and R110 obtained by the kit were adjusted to the basis of known proteins as a standard. IL-13, IL-23, IL-11, CSF-2/GM-CSF, hyaluronic acid, and CCL8/MCP-2 are significantly increased in Q110/Q110 compared to R110/R110 individuals (Table 3), while no significant difference was found for G-CSF and IL-24 between asthmatic patients with Q110/Q110 and R110/R110 (data not shown).

THICKNESS OF SUBEPITHELIAL LAYER

Subsequently, we investigated the differences of thickness in the subepithelial layer of the bronchial mucosa (Table 3). The subepithelial layer was significantly thicker in the biopsies taken from the asthmatic subjects with Q110/Q110 compared to R110/R110 individuals (Table 3, Fig. 1). Thus, airway remodeling in Q110/Q110 had increased progression compared to that in R110/R110 subjects.

DISCUSSION

Several new findings have emanated from this study. First, we have demonstrated asthmatic patients with genetic variants of IL-13Q110/Q110 are significantly associated with a reduced pulmonary function, as manifested by a low %FEV1, FEV1/FVC, %FEF25-75%, and a decline in FEV1 adjusting for covariates, such as age, sex, and history of smoking in Japanese patients. Secondly, an increased mRNA level of IL-23A, IL-11, IL-24, CSF2/GM-CSF, HAS-1, PTGS2, CSF3/G-CSF, and CCL8/MCP-2 in cultured fibroblasts were observed initially, and the protein level of IL-13, IL-23, IL-11, GM-CSF, hyaluronic acid, and CCL8/MCP-2 in BLF were shown. Finally, we have observed that the subepithelial layer was significantly thicker in the biopsy samples taken from the patients with Q110/Q110 compared to R110/R110 individuals.

A Q110 polymorphism in exon 4 has been shown to be associated with high total serum IgE levels,^{22,23} atopic dermatitis,²² and asthma¹³ in German,^{22,23} American,²³ British,¹³ and Japanese¹³ populations. In functional assays, Vladich *et al.*²⁴ have shown that IL-13 Q110 significantly increased signal transducer and activator of transcription-6 phosphorylation and CD23 expression in primary human monocytes compared to IL-13R110. Arima *et al.*¹⁴ have demonstrated that the affinity of Q110 and R110 to IL-13R α 1 was almost equal, whereas Q110 showed a slightly lower affinity with IL13R α 2, a decoy receptor, than with R110, and

Table 3 Clinical characteristics of bronchoscopic study population

Domain/IL-13 genotype	R110/R110 (n = 16)	Q110/R110 (n = 15)	Q110/Q110 (n = 18)	Statistical results ANOVA
Pulmonary function test				
FEV1, %predicted	94.3 (22.1)	90.7 (15.3)	66.4 (10.2)	0.02
FEV/FVC	78.2 (13.3)	77.2 (10.3)	64.0 (14.4)	0.02
FEF 25-75%, %predicted	71.8 (16.2)	62.0 (18.5)	33.8 (15.8)	0.01
Decline in FEV1 (ml/yr)	25.4 (2.6)	27.8 (3.0)	32.1 (11.3)	0.04
BLF concentration of mediators				
IL-13	9.8 (1.7)	11.5 (10.0)	19.3 (13.9)	0.02
IL-23	12.1 (5.6)	10.6 (3.3)	25.0 (11.4)	0.01
IL-11	8.7 (8.2)	11.1 (7.7)	22.6 (13.0)	0.02
GM-CSF	24.0 (7.4)	18.4 (14.3)	35.2 (12.6)	0.02
Hyaluronic acid	66.3 (51.5)	76.1 (69.2)	178.6 (102.9)	0.01
CCL8	11.4 (5.9)	18.0 (7.6)	24.6 (14.2)	0.02
Subepithelial layer thickness	6.4 (3.3)	8.7 (4.6)	11.6 (2.7)	0.04

Data are presented as mean $\pm$ (standard deviation). *p* values are for analyses of variance (ANOVA).

Post hoc comparisons reported only for variables with significant ANOVA result.

FEV1, forced expiratory volume in one second; FEV, forced expiratory volume; FVC, forced vital capacity; FEF25-75%, forced expiratory flow 25-75%.

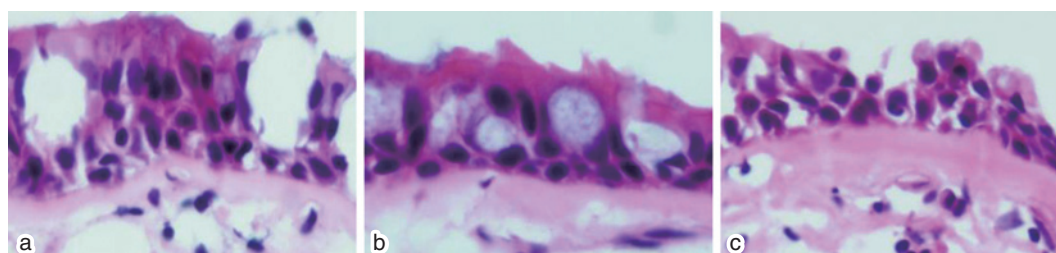


Fig. 1 Bronchial biopsies were obtained from asthmatic patients. Representative photomicrographs show bronchial mucosal biopsy specimens stained with hematoxylin and eosin ($\times 400$, HE staining).

a) Asthmatic subject of IL13R110/R110. b) IL13Q110/R110. c) IL13Q110/Q110.

then that Q110 enhanced stability *in vitro* and *in vivo* compared to R110. Thus inflammation is amplified by the local responses of the epithelium, smooth muscle, fibroblasts, and macrophages through the IL13Q110 stability. In the present study, we demonstrated the increased IL-13, IL-23, IL-11, CSF-2/GM-CSF, hyaluronic acid, and CCL8/MCP-2 in BLF from IL13 Q110 homozygote, all of which are known to play essential roles in allergic inflammation and the generation of IL-13-induced airway remodeling. Previous studies showed the importance of IL-23 in the development of allergic airway inflammation.²⁵ IL-11 mRNA positive cells have previously been shown to have inverse correlations with the FEV1 values for the asthmatic patients.²⁶ Moreover, IL-13 transgenic mice studies have shown in full detail that IL-13 induced IL-11, IL-11 receptor α , hyaluronic acid, and MCP-2/CCL8 are found in their lungs.²⁷ Woolley *et al.*²⁸ have shown that allergen inhalation caused a significant increase in eosinophils and GM-CSF in bronchoalveolar lavage fluid in patients of asthma.

Thus, IL-13 dominated-tissue inflammation was preceded to the airway remodeling in asthmatic patients. Since a polymorphic variant of Q110/Q110 has been found with a minor allele frequency of approximately 20% in the Caucasian population,²³ it is likely that asthmatic patients with IL13Q110 were enrolled in pairs matched for pulmonary function regardless of being homozygous for Q110. The START study, which enrolled 7241 patients aged 5 to 66 years with recent-onset, mild persistent asthma to assess early intervention with the inhaled corticosteroid budesonide on long-term asthma control, demonstrated that early intervention with ICSs did not affect lung function.²⁹ The CAMP study treated children from 5 to 12 years of age with low-dose inhaled budesonide, nedocromil, or placebo for at least 4 years. The results from the CAMP study also did not demonstrate any difference in the postbronchodilator FEV1 between treatments.³⁰ These clinical asthmatic studies, which are inherently elusive, lead us to speculate that patients with IL-13Q110 population might influence

FEV1 against efficacy of ICS or nedocromil treatments.

In conclusion, we have demonstrated an important role for IL-13Q110 in airway remodeling using pulmonary function test and bronchial specimens. Additional investigations of therapeutic utility aimed at regulating IL-13Q110 in IL-13- and Th2-mediated milieu are warranted.

ACKNOWLEDGEMENTS

The present authors would like to acknowledge Y. Shibata, M. Shibana, and M. Niisato (Iwate Medical University School of Medicine, Iwate, Japan) for their help in performing the study. We would also like to thank K. Izuhara (Division of Medical Chemistry, Saga University Graduate School of Medicine, Saga, Japan) and T. Fujioka (Department of Urology, Iwate Medical University School of Medicine) for their helpful comments.

This study was funded by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

REFERENCES

- Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991;**87**:893-910.
- Hoshino M, Nakamura Y, Sim JJ. Expression of growth factors and remodelling of the airway wall in bronchial asthma. *Thorax* 1998;**53**:21-7.
- Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig M. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. *Am J Respir Crit Care Med* 1999;**160**:725-9.
- Hoshino M, Nakamura Y, Hamid QA. Gene expression of vascular endothelial growth factor and its receptors and angiogenesis in bronchial asthma. *J Allergy Clin Immunol* 2001;**107**:1034-8.
- Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;**1**:520-4.
- Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;**140**:1745-53.
- Elias JA, Lee CG, Zheng T, Ma B, Homer RJ, Zhu Z. New insights into the pathogenesis of asthma. *J Clin Invest* 2003;**111**:291-7.
- Belperio JA, Dy M, Burdick MD *et al*. Interaction of IL-13 and C10 in the pathogenesis of bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2002;**27**:419-27.
- van der Pouw Kraan TC, Küçükaycan M, Bakker AM *et al*. Chronic obstructive pulmonary disease is associated with the -1055 IL-13 promoter polymorphism. *Genes Immun* 2002;**3**:436-9.
- Hoffjan S, Ostrovskaja I, Nicolae D *et al*. Genetic variation in immunoregulatory pathways and atopic phenotypes in infancy. *J Allergy Clin Immunol* 2004;**113**:511-8.
- Cameron L, Webster RB, Strempel JM *et al*. Th2 cell-selective enhancement of human IL13 transcription by IL 13-1112C>T, a polymorphism associated with allergic inflammation. *J Immunol* 2006;**177**:8633-42.
- Maier LM, Howson JM, Walker N *et al*. Association of IL 13 with total IgE: evidence against an inverse association of atopy and diabetes. *J Allergy Clin Immunol* 2006;**117**:1306-13.
- Heinzmann A, Mao XQ, Akaiwa M *et al*. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet* 2000;**9**:549-59.
- Arima K, Umeshita-Suyama R, Sakata Y *et al*. Upregulation of IL-13 concentration in vivo by the IL13 variant associated with bronchial asthma. *J Allergy Clin Immunol* 2002;**109**:980-7.
- Takishima T, Hida W, Sasaki H, Suzuki S, Sasaki T. Direct-writing recorder of the dose-response curves of the airway to methacholine. Clinical application. *Chest* 1981;**80**:600-6.
- Nakamura Y, Hoshino M, Sim JJ, Ishii K, Hosaka K, Sakamoto T. Effect of the leukotriene receptor antagonist pranlukast on cellular infiltration in the bronchial mucosa of patients with asthma. *Thorax* 1998;**53**:835-41.
- Takahashi S, Nakamura Y, Nishijima T, Sakurai S, Inoue H. Essential roles of angiotensin II in vascular endothelial growth factor expression in sleep apnea syndrome. *Respir Med* 2005;**99**:1125-31.
- Vignola AM, Chanez P, Chiappara G *et al*. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997;**156**:591-9.
- Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinases-9 in asthmatic airway inflammation. *J Allergy Clin Immunol* 1998;**102**:783-8.
- Hoshino M, Nakamura Y, Sim JJ *et al*. Inhaled corticosteroid reduced lamina reticularis of the basement membrane by modulation of insulin-like growth factor (IGF)-I expression in bronchial asthma. *Clin Exp Allergy* 1998;**28**:568-77.
- Hoshino M, Aoike N, Takahashi M, Nakamura Y, Nakagawa T. Increased immunoreactivity of stromal cell-derived factor-1 and angiogenesis in asthma. *Eur Respir J* 2003;**21**:804-9.
- Liu X, Nickel R, Beyer K *et al*. An IL-13 coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German multicenter atopy study (MAS-90). *J Allergy Clin Immunol* 2000;**106**:167-70.
- Graves PE, Kabesch M, Halonen M *et al*. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;**105**:506-13.
- Vladich FD, Brazille SM, Stern D, Peck ML, Ghittoni R, Vercelli D. IL-13R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation. *J Clin Invest* 2005;**115**:747-54.
- Wakashin H, Hirose K, Maezawa Y *et al*. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 2008;**178**:1023-32.
- Minshall E, Chakir J, Laviolette M *et al*. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. *J Allergy Clin Immunol* 2000;**105**:232-8.
- Chen Q, Rabach L, Noble P *et al*. IL-11 receptor alpha in the pathogenesis of IL-13-induced inflammation and remodeling. *J Immunol* 2005;**174**:2305-13.
- Woolley KL, Adelroth E, Woolley MJ, Ellis R, Jordana M, O'Byrne PM. Effects of allergen challenge on eosinophils,

- eosinophil cationic protein, and granulocyte-macrophage colony-stimulating factor in mild asthma. *Am J Respir Crit Care Med* 1995;**151**:1915-24.
- 29.** Busse WW, Pedersen S, Pauwels RA *et al.* The Inhaled steroid treatment as regular therapy in early asthma (START) study 5-year follow-up: effectiveness of early intervention with budesonide in mild persistent asthma. *J Allergy Clin Immunol* 2008;**121**:1167-74.
- 30.** The Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med* 2000;**343**:1054-63.