Increased total serum IgE levels in patients with asthma and promoter polymorphisms at CTLA4 and FCER1B

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Background: Increasing evidence indicates that total serum IgE levels are largely determined by genetic factors, and we recently established that the –109C/T promoter polymorphism at FCER1B is a genetic factor that affects total serum IgE levels. The gene encoding cytotoxic T lymphocyte antigen 4 (CTLA4) is another candidate factor in high IgE responsiveness, because B7-CD28/CTLA4 interaction can promote the differentiation and development of the T_H2 lymphocyte subset.
Objective: We intended to determine whether CTLA4 is associated with increased levels of total serum IgE or with the development of asthma or atopy.
Methods: We performed a case-control study involving 339 patients with asthma and 305 healthy control subjects, of whom 226 of the patients with asthma and 219 of the healthy control subjects had previously been genotyped for the –109C/T promoter polymorphism at FCER1B. In the current study, we genotyped 2 polymorphisms in the CTLA4 gene, one involving the promoter (–318C/T) and the other involving exon 1 (+49A/G), in addition to the FCER1B promoter polymorphism.
Results: Patients with asthma who were homozygous for the –318C allele at the CTLA4 promoter region had higher levels of total serum IgE than patients with asthma carrying the –318T allele (P = .00470). The analysis of –318C/T (at CTLA4) and –109C/T (at FCER1B) promoter polymorphisms showed a significant correlation between the combined genotypes and increased levels of total IgE in patients with asthma (P = .000014). In contrast, no correlation between total serum IgE levels and –318C/T or +49A/G genotypes was detected in 305 healthy control subjects. There was no evidence indicating an association between a putative allele for asthma or atopy and alleles at any of the CTLA4 polymorphic loci.
Conclusion: Our findings suggest that promoter polymorphisms of both CTLA4 and FCER1B are genetic factors that influence total serum IgE levels in patients with asthma. This supports the theory that variance in total serum IgE levels in patients with asthma is determined by mutations in multiple genes, each of which has a relatively small effect on the phenotype. (J Allergy Clin Immunol 2001;108:74-9.)

Key words: *Total serum IgE, IgE levels, asthma, atopy, CTLA4, FCER1B, promoter polymorphism*

T cells play a central role in atopy and asthma through the action of T_H2-type cytokines generated in response to allergens. Accumulating evidence supports the notion that costimulatory molecules play an important role in regulating the proliferation and activation of T cells in the immune response and that a key pathway of costimulation is interaction between CD28/CTLA4 on T cells and B7-1/B7-2 on antigen-presenting cells. Previous reports have emphasized the important roles of this pathway in regulation of the magnitude of the immune response and T_H1/T_H2 development.[1,2] Furthermore, the immune responses driven by T_H1 and T_H2 cells are counterregulated or suppressed by a third T cell type, T_H3/Tr1,[3] and the production of TGF-β and IL-10 depends on B7s-CTLA4 interaction on the stimulated self-MHC-reactive T_H3 cells.[4]

Murine models of allergic asthma have demonstrated that CTLA4-Ig completely inhibits the upregulation of IgE, airway eosinophilia, and hyperresponsiveness,[5,6] possibly influencing the development of T_H1/T_H2 subsets. Allergen-induced proliferation and IL-5 production by the PBMCs of patients with atopic asthma were also inhibited by CTLA4-Ig and anti–B7-2 mAbs.[7] Accordingly, although the precise biochemical mechanism by which both CD28 and CTLA4 function remains controversial, the balance of CD28- and CTLA4-derived signals profoundly alters the outcome of T-cell activation and might play an important role in the pathogenesis of the heightened IgE responsiveness seen in atopic diseases.

In addition, the CTLA4 gene lies on chromosome 2q33; recent genome-wide analysis conducted by the Collaborative Study on the Genetics of Asthma provided evidence for linkage of this area with asthma in Hispanics.[8] Accordingly, CTLA4 might influence IgE phenotypes, including increased total serum IgE levels, atopy, and asthma.

We performed a case-control study using 339 patients with asthma and 305 healthy control subjects (all Japanese) to determine whether 2 polymorphisms in the gene encoding CTLA4 are associated with increased levels of

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Abbreviations used
CTLA4: Cytotoxic T lymphocyte antigen 4
FCER1B: Chain of the high-affinity receptor of IgE
Mch: Methacholine

total serum IgE or with the development of asthma or atopy. Because recent evidence indicates that the –109C/T polymorphism at the FCER1B promoter region affects total serum IgE levels in patients with asthma,[9] we also examined the combined effect of CTLA4 polymorphisms and the FCER1B promoter polymorphism on total serum IgE levels.

METHODS

Study participants

Patients with bronchial asthma (n = 339) were recruited from the pulmonary clinic at the First Department of Medicine, Hokkaido University Hospital. The patients with asthma included in the study had experienced recurrent episodes of at least 2 of 3 symptoms (cough, wheeze, dyspnea) that are associated with a demonstrated reversible airflow limitation (15% variability in FEV_1 or peak expiratory flow rate, either spontaneously or with an inhaled short-acting β_2-agonist), increased airway responsiveness to methacholine (Mch), or both. Airway responsiveness was measured through use of an Astograph (Chest, Tokyo, Japan) in patients with asthma whose FEV_1 was greater than or equal to 1.5 L or 60% of the predicted value. Briefly, respiratory resistance was measured by the forced oscillation method (3 Hz) during continuous inhalation of Mch in incrementally increased concentrations (from 0.049 to 25 mg/mL), each concentration of Mch being inhaled for a period of 1 minute, until respiratory resistance reached twice the baseline value.[10] Airway hyperresponsiveness was evaluated as the minimum cumulative dose of Mch that induced an increase in respiratory resistance.[11] Healthy control subjects (n = 305) who had requested annual physical examinations were included only if they had no history of allergic diseases, including bronchial asthma, allergic rhinitis, and atopic dermatitis. The current study included 226 asthmatic subjects and 219 healthy controls who had previously been genotyped for the –109C/T FCER1B promoter polymorphism.[9]

Total serum IgE levels (IU/mL) and specific IgE responses to 10 common inhaled allergens, including *Dermatophagoides farinae*, grass pollens, animal dander, and molds, were determined. A specific IgE Ab level (IgE CAP RAST) greater than or equal to 0.35 UA/mL was considered positive. We defined atopy as a positive RAST score (≥ 0.35 UA/mL) to at least one of the 10 common allergens. All participants (n = 644) were Japanese, and each gave written informed consent for enrollment in the study and for all associated procedures. The Ethics Committee of the School of Medicine, Hokkaido University, approved the study.

DNA genotyping

Workers who were unaware of the clinical status of the individual participants performed genotyping. The –318C/T and +49A/G polymorphisms in the CTLA4 gene were genotyped as previously described.[12,13] Briefly, a –318C/T polymorphism in the CTLA4 promoter was amplified through use of the primers 5′-AAA TGA ATT GGA CTG ATG GT-3′ (sense) and 5′-TTA CGA GAA AGG AAG CCG TG-3′ (antisense); this was followed by digestion with the restriction enzyme MseI (New England Bio Labs, Inc, Beverly, Mass). A +49A/G polymorphism in exon 1 of the CTLA4 gene was amplified through use of the primers 5′-AAG GCT CAG CTG AAC CTG GT-3′ (sense) and 5′-CTG CTG AAA CAA ATG AAA CCC-3′ (antisense); this was followed by digestion with the restriction enzyme BstEII (New England Bio Labs, Inc).

In the present study, the 113 asthmatic subjects who had not previously been genotyped for the –109C/T polymorphism at the FCER1B gene were genotyped according to a previously described method.[9]

Statistical analysis

Statistical analysis was performed through use of the SYSTAT program (SPSS Inc, Chicago, Ill). The χ^2 test was used to check for significant departures from Hardy-Weinberg equilibrium. To assess the CTLA4 polymorphisms as a risk factor for asthma or atopy, odds ratios (with 95% CIs) were calculated for asthma and atopy through use of a logistic regression model adjusted for sex (female or male), age (as a continuous variable), and smoking status (never smoked, smoked formerly, or smokes currently).

We then compared total serum IgE levels to assess correlation with genotypes of the CTLA4 polymorphisms (separate comparisons for patients with asthma and healthy control subjects). Total serum IgE levels were log-transformed to normalize the distribution. We compared total serum IgE levels through use of ANOVA models that were adjusted for sex, age, and smoking status as possible confounding variables. Age at onset of asthma (as a continuous variable) was also controlled for in the analysis of patients with asthma, because in our previous study the observed effects of the –109C/T promoter polymorphisms (at the FCER1B gene) on total serum IgE levels were greatest when age at disease onset was taken into account.[9] Because the +49A/T polymorphism did not affect total serum IgE levels in patients with asthma or healthy controls, it was not studied further.

The combined impact of the –318C/T polymorphism at the CTLA4 gene and the –109C/T polymorphism at the FCER1B gene was examined by comparing total serum IgE levels of different combined CTLA4/FCER1B genotypes. We also used a factorial ANOVA model to assess the genetic interaction between the CTLA4 and the FCER1B promoter polymorphisms by including an interaction term; this was defined as the –318C/T genotype multiplied by the –109C/T genotype. Because only 3 patients with asthma had the T/T genotype at the –318C/T polymorphism, C/T and T/T genotypes were combined into a single category for these statistical comparisons.

RESULTS

The clinical and genotypic data of the patients with asthma and healthy control subjects are summarized in Table I. Compared with the control subjects, the patients with asthma were significantly more likely to be atopic (χ^2, $P < .01$) and currently nonsmoking (χ^2, $P < .01$). Patients with asthma had higher levels of total serum IgE than healthy controls regardless of atopic status. The means of total serum IgE levels were 2.57 (SD, 0.56), 2.01 (SD, 0.56), 2.19 (SD, 0.53), and 1.58 (SD, 0.53) log IU/mL in atopic patients with asthma, nonatopic patients with asthma, atopic control subjects, and nonatopic control subjects, respectively. The mean age at onset of asthma was higher (unpaired t test, $P < .01$) in nonatopic patients with asthma (43.9 [SD, 19.8]) than in atopic patients with asthma (27.8 [16.9]). There was no difference in the mean FEV_1/forced vital capacity ratio between atopic (0.696 [SD, 0.139]) and nonatopic (0.664 [SD, 0.121]) patients with asthma.

TABLE I. Comparisons of demographic and genotypic data for 339 patients with asthma and 305 controls

Characteristics	Patients with bronchial asthma (n = 339)	Control subjects (n = 305)	*P* value*
Sex (M/F)	152/187	184/121	<.01
Mean (SD) age (y)	44.2 (16.39)	42.7 (11.35)	.25
Current smokers (%)	67 (19.8)	105 (34.4)	<.01
Mean (SD) total IgE (log IU/mL)	2.417 (0.626)	1.813 (0.611)	<.01
Atopy† (%)	269 (79.4)	115 (37.7)	<.01
CTLA4 polymorphisms‡			
–318C/T			
C/C	265 (78.2)	238 (78.0)	
C/T	71 (20.1)	65 (21.3)	
T/T	3 (1.7)	2 (0.7)	
+49A/G			
A/A	40 (11.8)	40 (13.1)	
A/G	178 (52.5)	140 (45.9)	
G/G	121 (35.7)	125 (41.0)	

*Calculated through use of an unpaired *t* test or the χ^2 test.
†Defined as positive RAST scores (> = 0.35 UA/mL) to at least one of 10 common allergens.
‡Allelic frequencies were 0.886 and 0.887 for –318C allele and 0.619 and 0.639 for +49G allele in asthmatic and in control groups, respectively.

The observed frequencies of genotypes at both CTLA4 polymorphisms for patients with asthma and healthy controls were in Hardy-Weinberg equilibrium (Table I). The numbers of patients with asthma who carried the T/T, T/C, and C/C genotypes at the –109C/T FCER1B polymorphism were 127, 178, and 34, respectively; the numbers of healthy control subjects who carried these genotypes were 104, 95, and 20, respectively. These genotypes also showed no evidence of departure from Hardy-Weinberg equilibrium in either the asthma group or the control group.

The evidence did not indicate an association between asthma or atopy and the CTLA4 polymorphic loci. The odds ratios for asthma of –318T carriers and +49G carriers were 1.04 (95% CI, 0.71, 1.53) and 1.16 (95% CI, 0.91, 1.49), respectively, and the odds ratios for atopy of –318T carriers and +49G carriers were 1.07 (95% CI, 0.71, 1.61) and 1.22 (95% CI, 0.94, 1.60), respectively. Total serum IgE levels differed between the –318C/T genotypes (P = .00470) in the 339 patients with asthma but not in the 305 healthy controls (Table II). In contrast, no difference was detected in total serum IgE levels between +49A/G genotypes, either in patients with asthma (P = .59) or in healthy controls (P = .68; Table II). Because we initially studied 2 markers (–318C/T and +49A/G polymorphisms) and 4 phenotypes (asthma, atopy, and separate comparisons of total serum IgE levels for patients with asthma and healthy controls), we multiplied our significance levels by $2 \times 4 = 8$. Through use of this stringent correction, an association between –318C/T polymorphism and total serum IgE levels in asthmatic subjects gave a P value (corrected) of less than .05.

Analysis of the CTLA4 and FCER1B promoter polymorphisms revealed a significant correlation between the combined genotypes and total serum IgE levels (overall P = .000014) in patients with asthma. The ANOVA model for this analysis yielded F ratios of 3.76 (P = .054), 17.02 (P = .0005), 0.007 (P = .93), and 0.711 (P = .39) for age, sex, smoking status, and age at onset of asthma, respectively. Patients who were homozygous for both the –318C allele at CTLA4 and the –109T allele at FCER1B had the highest levels of total serum IgE (Table II). Pairwise comparisons between the combined genotypes were conducted through use of the post hoc Bonferroni correction (Table II).

Moreover, factorial ANOVA suggested a multiplicative interaction between the CTLA4 and FCER1B promoter polymorphisms (F = 5.998, P = .014 for the interaction).

DISCUSSION

Our results suggest an association between –318C homozygosity at the CTLA4 promoter polymorphism and increased total serum IgE levels in patients with bronchial asthma. Three polymorphic regions within the CTLA4 gene have been found: an alanine/guanine substitution at position 49 in exon 1,[12] an $(AT)_n$ repeat polymorphism at a 3′ untranslated region of exon 3,[14] and a cytosine/thymine polymorphism at position –318 in a promoter region.[15] None of the polymorphisms have been definitely proven to be functional, and the mechanism by which the CTLA4 polymorphic loci or an unknown etiologic polymorphism influences total serum IgE levels remains unclear. The CTLA4 and FCER1B promoter polymorphisms could both be in linkage disequilibrium with causal alleles that are located in regions not investigated in our studies[9] and that are functionally involved in the increased IgE levels. However, the microsatellite repeat in the 3′ untranslated region could affect RNA stability,[16] and T-cell proliferative responses on exposure to anti-CD28 antibody (Ab) depend on the length of the $(AT)_n$ repeat.[17] Therefore, linkage disequilibrium with this potentially functional $(AT)_n$ repeat, which possibly influences T-cell reactivity via the B7-CD28/CTLA4 pathway, might explain the observed association between the –318C/T polymorphism and total serum IgE levels.

TABLE II. Association between promoter polymorphisms at CTLA4 and FCER1B and total serum IgE levels*

	Patients with asthma			Healthy control subjects		
	Log IgE	**SE**	**n**	**Log IgE**	**SE**	**n**
Genotypes at –318C/T CTLA4 promoter polymorphism†			339			305
C/C	2.475	0.0365	265	1.792	0.043	238
C/T or T/T	2.252	0.0691	74	1.866	0.081	67
Genotypes at +49A/G CTLA4 promoter polymorphism‡			339			305
A/A	2.493	0.093	40	1.833	0.101	40
A/G	2.402	0.044	178	1.767	0.056	140
G/G	2.455	0.056	121	1.834	0.058	125
Combined genotypes at –318C/T CTLA4 and –109C/T FCERIB§			339			219‖
C/C and T/T	2.689	0.063	91	1.734	0.068	80
C/C and [C/C or C/T]	2.363¶	0.046	174	1.659	0.069	85
[C/T or T/T] and T/T	2.207**	0.102	36	1.661	0.129	24
[C/T or T/T] and [C/C or C/T]	2.228‡‡	0.103	38	1.909	0.112	30

*Total serum IgE levels (IU/mL) were log-transformed to normalize the distribution and were compared by means of ANOVA adjusted for age, sex, and smoking status. T/T genotype at the –109C/T FCER1B polymorphism was associated with higher IgE levels in patients with asthma,[9] especially when age at onset of asthma was controlled for in addition to sex, age, and smoking status. Therefore, age at onset of asthma was also adjusted for when the analysis was limited to patients with asthma.

†–318C/T CTLA4 promoter polymorphism showed an association with total IgE levels in patients with asthma.
(F ratio = 8.11, $P = .00470$) but not in healthy controls (F ratio = 0.65, $P = .42$).

‡+49A/G CTLA4 promoter polymorphism was not associated with total IgE levels in patients with asthma (F ratio = 0.53, $P = .59$) or in healthy controls (F ratio = 0.38, $P = .68$).

§Combined genotypes showed a strong association with total IgE levels in patients with asthma (F ratio = 8.765, overall $P = .000014$) but not in healthy controls (F ratio = 1.29, overall $P = .28$).

‖A total of 219 healthy controls were genotyped for both –318C/T at CTLA4 and –109C/T at FCER1B.

¶$P = .00020$, **$P = .0039$, and ‡‡$P = .0043$ in comparison with patients with asthma with the [C/C and T/T] genotype (post hoc comparisons by Bonferroni adjustment).

A genetic influence of the CTLA4 promoter polymorphism on total serum IgE levels was detected only in patients with asthma. CD28 is constitutively expressed on mature T cells and is upregulated on activation, whereas CTLA4 is expressed at very low levels on resting T cells and is induced after activation.[18] Furthermore, the effects of B7 costimulation via CD28 or CTLA4 depend on the strength of the signal delivered through the T-cell receptor and the activation state of T cells.[19] Given that the B7-CD28/CTLA4 pathway regulates T-cell activation and T_H2 development[20] via such a complex mechanism, genetic effects of CTLA4 might vary according to the environmental context of the airways of asthma patients; asthma is an inflammatory disorder of the airways that involves mast cells, eosinophils, macrophages, and T_H2-like lymphocytes. It also involves structural elements such as epithelial cells, smooth muscle cells, microvessels, and nerves. Activation and alteration of these components of asthmatic airway inflammation result in production of cytokines, growth factors, and mediators.[21] In fact, ex vivo allergen stimulation of bronchial biopsy tissue from atopic patients with asthma reportedly induces production of IL-4, IL-5, and IL-13, but this does not occur in tissue from atopic nonasthmatic subjects. In addition, production of these cytokines is reportedly inhibited by anti-CD80 or anti-CD86 antibodies, suggesting that the B7-CD28/CTLA4 pathway is essential for allergen-induced cytokine production in the airways of patients with asthma.[22]

An association between asthma and increased total serum IgE levels is well established (Table I).[23,24] However, the theory that high IgE responsiveness or atopy is a major risk factor for asthma has been challenged.[25,26] Lack of correlation between early exposure to allergens and the development of asthma raises the possibility that the relationship between asthma and sensitization to common allergens reflects the susceptibility of patients with asthma to augmented IgE responses to common environmental allergens rather than an increased risk of asthma after exposure to these allergens.[27] Our findings support this hypothesis, and the susceptibility of patients with asthma to higher IgE responsiveness might be partly attributable to genetic effects of the –318C/T polymorphism at CTLA4 and the –109C/T polymorphisms at FCER1B, which also only affect total serum IgE levels in patients with asthma.[9]

There was significant association between the combined CTLA4/FCER1B genotype and total serum IgE levels, and a multiplicative interaction between these 2 genes is also suggested by the results of this study. The immunogenetic mechanisms underlying the development of high IgE responsiveness seen in atopic diseases might include antigen (Ag)–specific and non–Ag-specific mechanisms.[28] The former mainly involves cognate T cell–B cell interaction and costimulatory signals, such as the B7-CD28/CTLA4 pathway.[29] The latter, noncognate regulation of IgE, involves basophils, mast cells, and other FcεRI+ cells.[30] Thus, although further genetic factors independent of CTLA4 and FCER1B are involved in cognate and noncognate immune responses, we postulate that the CTLA4 promoter polymorphism (or a susceptibility allele in linkage disequilibrium with it) primarily

influences cognate systems, whereas the FCER1B promoter polymorphism (or a susceptibility allele in linkage disequilibrium with it) mainly influences noncognate systems. Accordingly, the reason that levels of total serum IgE were highest in patients with asthma who were homozygous for both the –318C allele (at CTLA4) and the –109T allele (at FCER1B) appears to be that cognate and noncognate systems underlying high IgE responsiveness interact in many ways, and the overall production of IgE Ab by both cognate and noncognate pathways is reflected in the total serum IgE levels.[31]

Susceptibility loci for different immune diseases overlap,[32] suggesting that clinically distinct inflammatory immune diseases, including asthma and atopy, are controlled by a common set of susceptibility genes. In fact, positive linkage and association with CTLA4 gene polymorphisms have been identified in a broad spectrum of T cell–mediated immune diseases, such as primary biliary cirrhosis,[33] insulin-dependent diabetes mellitus,[12] multiple sclerosis,[34] and Grave's disease.[35] However, we did not find a significant association between asthma or atopy and the CTLA4 polymorphic loci, a result that is in agreement with the findings of previous reports. There is no evidence to support association or linkage between the CD28/CTLA4 region and asthma or atopy in German populations[36] or among the Hutterites.[37] Thus, the CTLA4 gene probably does not exert a major influence on the development of asthma or atopy.

In conclusion, our findings support the contention that the variance in total serum IgE levels seen in patients with asthma is determined by mutations in multiple genes, each of which has a relatively small effect on the phenotype. The identification of genetic factors such as polymorphisms in the CTLA4 and FCER1B genes, which predispose individuals to elevated total serum IgE levels, can provide a basis for a better understanding of the pathophysiologic nature of allergic diseases, including bronchial asthma.

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