

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Association between human IL-10 gene polymorphisms and serum IL-10 level in patients with food allergy

Tuo-Kang Chen, Jyh-Hong Lee, Hsin-Hui Yu, Yao-Hsu Yang, Li-Chieh Wang, Yu-Tsan Lin, Bor-Luen Chiang*

Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan, ROC

Received 3 July 2011; received in revised form 23 August 2011; accepted 1 November 2011

KEYWORDS

interleukin-10;
food allergy;
polymorphism;
gene

Background/Purpose: Single nucleotide polymorphisms (SNPs) of the interleukin-10 (IL-10) gene are associated with allergic diseases in different populations. This study aimed to determine the distribution of two SNPs at $-1082A/G$ and $-592A/C$ (rs1800896 and rs1800872, respectively) in the IL-10 gene promoter of Taiwanese food allergy (FA) patients, and also to compare the serum IL-10 levels between patients with (FA) and controls.

Methods: Thirty-seven patients with FA and 52 controls were enrolled, and their peripheral blood was collected for IL-10 SNP genotyping and the corresponding serum IL-10 level of each genotype.

Results: The thirty-seven FA patients had positive food-specific IgE (≥ 0.75 kU/L) to more than one food, and the most frequent allergens were shrimp and crab (56.8% and 35.1%, respectively). The genotype distributions in the FA patients compared to the control group were AA and AG at $-1082A/G$ (86.5% and 13.5% vs. 86.6% and 13.4%, respectively), and AA, AC, and CC at $-592A/C$ (45.9%, 43.3% and 10.8% vs. 38.5%, 48.1% and 13.4%, respectively). Serum IL-10 levels were significantly lower in the FA group than in the control group ($p = 0.0187$), and the IL-10 level of $-592A/C$ of genotype AA was significantly lower than that of the other genotypes (AC+CC) ($p = 0.007$). Patients with AA/AA haplotype homozygotes (10 of 24) had significantly lower serum IL-10 levels than those with other haplotypes.

Conclusions: The two SNPs at $-1082A/G$ and $-592A/C$ of IL-10 were associated with FA in our Taiwanese population, and FA patients with the genotype AA/AA haplotype homozygotes had lower serum IL-10 levels. This suggests that IL-10 might play a critical role in the pathogenesis

* Corresponding author. Department of Pediatrics, National Taiwan University Hospital, 8 Chung-Shan South Road, Taipei 10002, Taiwan, ROC.

E-mail address: gicmbor@ntu.edu.tw (B.-L. Chiang).

of FA. We suggest that it may be practicable to evaluate the serum IL-10 levels of FA patients and to predict the possibility of FA if genotypes and haplotypes are checked regularly.
Copyright © 2012, Elsevier Taiwan LLC & Formosan Medical Association. All rights reserved.

Introduction

Adverse food reactions include any abnormal reaction resulting from food ingestion that is due to certain intolerances (non-allergic food hypersensitivities) or food allergy (FA). Food allergies are adverse immunologic reactions caused by IgE- or non-IgE-mediated immune mechanisms.^{1,2} Current clinical classifications of FA diseases are mainly based on the presence, or absence, of food-specific IgE antibodies. IgE-mediated FAs are mostly characterized by an immediate-type anaphylactic reaction, which is a Type I reaction and by far the most studied type of food-related disease. Review articles by Madsen³ showed that the reported prevalence of FA varies from 3.24% to 34.9%, the differences being due to reporting lifetime prevalence compared to point prevalence. A meta-analysis study⁴ showed that the prevalence of self-reported FA is very high compared with objective measures, with the variation in self-reported prevalence ranging from 3% to 35% for any food. The pathogenesis of FA is incompletely understood and no fully effective treatment is available to cure or actively prevent it. The current study focused on IgE-mediated FA in a Taiwanese population.

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that can down-regulate cellular immunity and allergic inflammation. Several important polymorphic sites in the IL-10 gene have been reported, including three in the promoter region (-1082 A/G, -819 T/C, -592 A/C) that control transcription and may influence the transcription of IL-10 messenger RNA and IL-10 expression *in vitro*.⁵ A case-control association study⁶ suggested that the IL-10 -1082A/G gene polymorphism is related to FA susceptibility in atopic Japanese children. However, the functional relationship of IL-10 gene polymorphisms in FA has not been investigated in detail.

The major aim of the study was to determine the distribution of two single nucleotide polymorphisms (SNPs) at positions -1082 and -592 (rs1800896, rs1800872, respectively) in the IL-10 gene promoter of Taiwanese FA patients and controls. Serum IL-10 levels were measured and analyzed for the functional significance of each haplotype.

Materials and methods

Subjects' characteristics

From July 1, 2009 to May 31, 2011, 37 FA patients and 52 controls with no history of FA or systemic disease were enrolled from the National Taiwan University Hospital and Keelung Hospital. Their demographic characteristics are shown in Table 1. The patients were asked to participate if they had a history of an immediate reaction to a food. All

physician-diagnosed FA patients were sensitive to at least one of nine common food allergens (i.e., milk, egg white, egg yolk, shrimp, crab, soybean, codfish, mango, and peanut) using the Pharmacia CAP system (Uppsala, Sweden). The ethics committee of the National Taiwan University Hospital approved the study (reference no. 201008038R) and all participants provided written informed consent for blood sampling.

Definition of IgE-mediated FA for analyzing the correlation between IL-10 polymorphisms and FA

Boyce et al.⁷ reported guidelines for the diagnosis of FA. The term "allergic sensitization" is used to describe the presence of allergen-specific IgE to food allergens without having clinical symptoms on exposure to those foods. IgE-mediated FA requires both the presence of sensitization and the development of specific signs and symptoms on exposure to that food. In this study, a food-specific IgE level of 0.7 kU/L (class 2) or more to at least one of the nine aforementioned common food allergens was considered food sensitization. The patients all had clinical symptoms on exposure to the offending food, and the organ systems most commonly involved included the skin, gastrointestinal tract, and respiratory tract. Elimination diets were implemented for diagnostic and therapeutic purposes and the clinical manifestations of FA were followed-up. The diagnosis of FA was standardized in order to analyze the correlation between IL-10 gene SNPs at positions -1082 and -592 using the following criteria: (1)

Table 1 Characteristics of the study population.

	Food allergy (n = 37)	Controls (n = 52)
Sex, n (%)		
Male	23 (62.2)	20 (39.6)
Female	14 (37.8)	32 (60.4)
Age (years)		
Mean±SD	8.06 ± 6.25	17.85 ± 15.19
Range	1–32	1–59
Food allergen, n (%)		
Shrimp	19 (51.4)	N.A.
Crab	12 (32.4)	N.A.
Egg white	7 (18.9)	N.A.
Milk	7 (18.9)	N.A.
Egg yolk	6 (16.2)	N.A.
Peanut	3 (8.1)	N.A.
Codfish	3 (8.1)	N.A.
Mango	1 (2.7)	N.A.
Soy	1 (2.7)	N.A.

N.A. = not available.

physician-diagnosed FA by clinical manifestations to the offending food; (2) food-specific IgE levels ≥ 0.7 kU/L (class 2); and (3) improved clinical manifestations to elimination diets.

Molecular methods

Peripheral blood was collected from the patients and controls. Genomic DNA was extracted from EDTA-anticoagulated whole blood with a Genomic DNA Mini Kit (GeneAid, Agoura Hills, California, USA) according to the manufacturer's instructions.

Genotyping of IL-10 gene polymorphisms

The two SNP loci at positions -1082 A/G (rs1800896) and -592 A/C (rs1800872) in the promoter region of the IL-10 gene were determined with Custom TaqMan SNP genotyping assays (Applied Biosystems, Foster City, California, USA). The primer and probe sequences are available upon request. All assays were run in a total volume of $20 \mu\text{L}$ with 1 ng of genomic DNA, $0.5 \mu\text{L}$ of $40\times$ assay mix, and $10 \mu\text{L}$ $2\times$ TaqMan Genotyping Master Mix (Applied Biosystems). The polymerase chain reaction (PCR) programs were 95°C for 10 minutes, 92°C for 15 seconds, and 60°C for 1 minute for 40 cycles with the subsequent end point reading obtained on an ABI 7500HT Real-Time PCR System (Applied Biosystems).

Measurement of serum IL-10 levels by enzyme-linked immunosorbent assay

Serum samples were individually collected and centrifuged at 1500 rpm for 10 min. Serum IL-10 levels of the controls and FA patients were measured using enzyme-linked immunosorbent assay (ELISA) kits specifically designed for this cytokine (R&D Systems, Inc., Abingdon, UK).

Statistical analysis

Demographic data were compared between groups by using χ^2 analysis. Allele frequencies were estimated by direct gene counting, while the chi-square test was used to compare allele and genotype frequencies between patients and controls. Comparisons of IL-10 level and total IgE were undertaken using the Mann-Whitney U test or Kruskal-Wallis test. A p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (version 12.0, SPSS Inc., Chicago, IL, USA).

Results

Thirty-seven patients had positive food-specific IgE (≥ 0.7 kU/L) to more than one food. The foods most frequently related to allergy were shrimp and crab (51.4% and 32.4%, respectively), followed by egg white (18.9%), milk (18.9%), egg yolk (16.2%), peanut (8.1%), and codfish (8.1%). The food-specific IgE levels of each food allergen are listed in Fig. 1. Crab, shrimp, and egg white had higher IgE levels in this population.

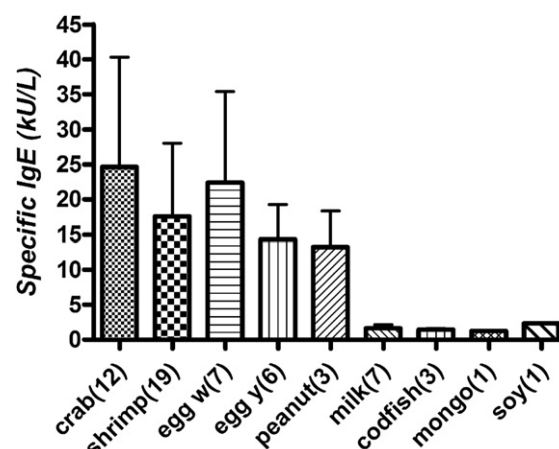


Figure 1 Mean specific IgE to each food allergen. Among the food allergy (FA) patients, a food-specific IgE level of ≥ 0.7 kU/L (class 2) to at least one of nine common food allergens was considered food sensitization. Crab, shrimp, and egg white had higher IgE levels in this population. Error bars represent the standard error of mean (SEM).

IL-10 gene polymorphism in FA patients and controls

The genotype distributions and allele frequencies of the two SNPs at positions -1082 A/G and -592 A/C of the IL-10 promoter gene were counted and summarized in Table 2. As expected, the distributions of individual IL-10 alleles followed the Hardy-Weinberg equilibrium. The most frequent genotypes in the FA patients were AA at position -1082 (86.5%) and AA at position -592 (45.9%). Haplotype frequencies in the FA patients and healthy controls are summarized in Table 3. There were three different estimated IL-10 $-1082/-592$ promoter genotypes in this population: AA, GC, and AC. Categorizing the IL-10 haplotypes into these three groups, i.e. AA/AA haplotype

Table 2 IL-10 genotype and allele frequencies in FA patients and controls.

	Food allergy (<i>n</i> = 37)	Controls (<i>n</i> = 52)	<i>p</i>
IL-10 −1082 A/G			
Genotype, <i>n</i> (%)			
A/A	32 (86.5)	45 (86.6)	0.994
A/G	5 (13.5)	7 (13.4)	
G/G	0 (0)	0 (0)	
Allele frequencies			
A	0.932	0.933	0.995
G	0.068	0.067	
IL-10 −592 A/C			
Genotype, <i>n</i> (%)			
A/A	17 (45.9)	20 (38.5)	0.770
C/A	16 (43.3)	25 (48.1)	
C/C	4 (10.8)	7 (13.4)	
Allele frequencies			
A	0.675	0.625	0.486
C	0.325	0.375	

Table 3 IL-10 haplotype in food allergy patients and controls.

	Food allergy (n = 37)	Controls (n = 52)	p
Genotype, n (%)			
AA/AA	17 (45.9)	20 (38.5)	
AA/–			
AA/GC	1 (2.7)	5 (9.6)	
AA/AC	15 (40.6)	20 (38.5)	
–/–			0.138
AC/GC	4 (10.8)	2 (3.8)	
AC/AC	0 (0)	5 (9.6)	
Haplotype frequencies			
AA	0.675	0.625	
AC	0.257	0.308	
GC	0.068	0.067	0.754

There were three groups: AA/AA haplotype homozygotes, AA/– haplotype heterozygotes and –/– haplotype homozygotes (“–” = non-AA haplotypes).

homozygotes, AA/– haplotype heterozygotes and –/– haplotype homozygotes (‘–’ meaning non-AA haplotypes), the most frequent in the study patients was AA at position –1082/–592 (67.5%), and the most frequent combined genotype was AA/AA haplotype homozygotes (45.9% in patients vs. 38.5% in controls). FA associated specific IgE was evaluated for any association with two polymorphisms of IL-10 gene (Table 4). There was a significant association of IL-10 –592 A/C polymorphism with the prevalence of serum specific IgE to egg white. FA patients carrying the homozygous AA genotype of IL-10 –592 A/C had a significantly higher prevalence of specific IgE to egg white than FA patients carrying CC or AC genotypes ($p = 0.019$).

Association of IL-10 –1082/–592 genotypes with IL-10 production

To determine whether IL-10 SNPs were related to the IL-10 cytokine phenotype in serum, 24 FA patients and 30 normal controls were randomly selected and their corresponding serum IL-10 levels examined and compared (Fig. 2). The mean (standard error of mean) serum IL-10 level (pg/mg)

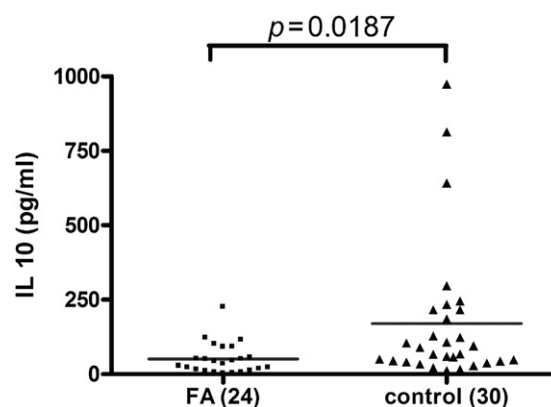


Figure 2 Serum IL-10 levels were compared between food allergy patients and controls. Twenty-four food allergy (FA) patients and thirty normal controls were randomly selected and their serum IL-10 levels examined. The mean serum IL-10 level (pg/mg) was significantly lower in the FA group than in the control group (51.59 vs. 170.1).

was significantly lower in the FA group [51.59 (10.59)] than in the control group [170.1 (42.75)] ($p = 0.0187$). Moreover, the IL-10 level at position –592 of genotype AA was significantly lower than that of the other genotype (AC+CC) in the FA group ($p = 0.007$, Fig. 3). Patients with AA/AA haplotype homozygotes (10 of 24) had significantly lower serum IL-10 levels than the other haplotypes, including the AA/– haplotype heterozygotes (AA/GC, AA/AC) and –/– haplotype homozygotes (AC/GC) ($p = 0.018$; Fig. 4).

Correlation between clinical symptoms, food-specific IgE, and IL-10 SNPs

All patients had developed clinical symptoms in response to the offending foods, and the organ systems most commonly involved included the skin, gastrointestinal tract and respiratory tract. There was no correlation between IL-10 SNPs and these clinical symptoms (Table 5). Food-specific IgE levels were evaluated for any association with two polymorphisms of the IL-10 gene. There was no significant correlation between food-specific IgE level and IL-10 SNPs (data not shown).

Table 4 Comparison of the food allergens and the genotypes of food allergy at IL-10 –1082 A/G and –592 A/C.

	–1082 A/G			–592 A/C		
	AA	AG	p	AA	AC or CC	p
Serum total IgE (IU/mL) ^a	797.4 ± 739.8	249 ± 96.2	0.344	811.4 ± 740.9	686.3 ± 732.7	0.507
Specific IgE to shrimp ^b (positive/total)	17/32 (53.1%)	2/5 (40%)	0.585	8/17 (47%)	11/20 (55%)	0.630
Specific IgE to crab ^b (positive/total)	11/32 (34.3%)	1/5 (20%)	0.523	5/17 (29.4%)	7/20 (35%)	0.717
Specific IgE to milk ^b (positive/total)	6/32 (18.8%)	1/5 (20%)	0.947	2/17 (11.8%)	5/20 (25%)	0.306
Specific IgE to egg white ^b (positive/total)	7/32 (21.9%)	0/5 (0)	0.245	6/17 (35.3%)	1/20 (5%)	0.019*
Specific IgE to egg yolk ^b (positive/total)	5/32 (15.6%)	1/5 (20%)	0.805	4/17 (23.5%)	2/20 (10%)	0.266

Total IgE value was presented as means ± SD.

*A value $p < 0.05$ was considered to be statistically significant.

^a Mann-Whitney U test.

^b Chi-square test.

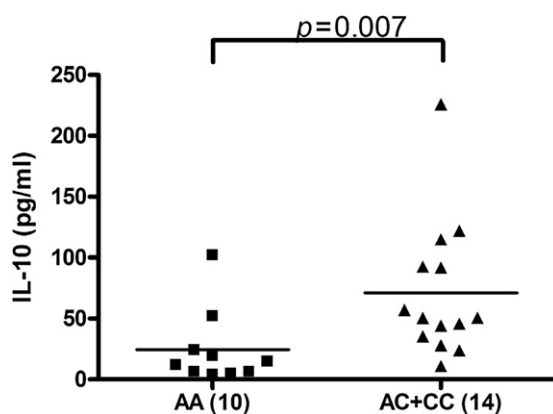


Figure 3 Comparison of serum IL-10 levels at IL-10 –592 A/C. The IL-10 level at IL-10 –592 A/C of genotype AA was significantly lower than that of the other genotype (AC+CC) in the food allergy group.

Discussion

IL-10 is a potent anti-inflammatory cytokine produced mainly by monocytes and lymphocytes that can down-regulate cellular immunity and allergic inflammation.^{5,8} It can be produced by other cell types, including macrophages, B cells, mast cells, and eosinophils. It inhibits both Th1 and Th2 T helper cell populations and interferes with IgE synthesis and eosinophil survival.⁹ In addition, IL-10 secreted by Type 1 regulatory (Tr1) cells skews antibody production from IgE towards the non-inflammatory isotypes IgG4 and IgA. Tr1 cells, also known as inducible T_{reg} (iT_{reg}) cells, are defined by their ability to produce high levels of IL-10. While natural T_{reg} cells are thought to primarily govern peripheral tolerance to self-antigens, iT_{reg} cells are responsible for food allergens.¹⁰ The balance between allergen-specific Tr1 cells and Th2 cells appears to be decisive in the development of allergy. Allergen-specific T cell suppression by IL-10, a known

suppressive cytokine of T cell proliferation and cytokine production, is essential in peripheral tolerance to food allergens.¹¹ FA is thought to result from a defect in oral tolerance, and it is likely that defects in T_{reg} cell activity contribute to the development of FA.

The IL-10 gene encoding is located on human chromosome 1q31-1q32, and is composed of five exons and four introns. Several important polymorphic sites in the IL-10 gene have been reported, including three in the promoter region (–1082 A/G, –819 T/C, and –592 A/C) that control transcription and influence the transcription of IL-10 messenger RNA and IL-10 expression *in vitro*.⁵ In patients with atopic dermatitis (AD), one study⁹ of the –1082 SNP in the IL-10 promoter region failed to demonstrate any association with AD. However, a more recent study¹² showed that the IL-10 promoter haplotype IL10-ht 2 (–1082A, –819C, –592C, +117 T) may be associated with reduced serum IgE levels in a gene dose-dependent manner. IL-10 promoter polymorphisms have also been attributed to increased IgE production in asthmatic patients.¹³

In FA, Negoro et al. found a significant association between IL-10 C-627A (rs1800872), the lower-producer genotype AA, with the severity of both FA and atopic eczema in 220 Japanese allergic children.¹⁴ They also noted that genotype AA was significantly associated with higher IgE levels in serum. A case-control association study⁶ suggested that the IL-10 –1082 A/G gene polymorphism is related to FA susceptibility in atopic Japanese children. However, the functional relationship of IL-10 gene polymorphisms in FA have not been investigated clearly.

The present study analyzed the IL-10 gene promoter region polymorphisms at positions –592 and –1082 (rs1800872 and rs1800896, respectively) in Taiwanese FA patients. The two IL-10 SNP genotype frequencies studied in the controls are similar to those observed in Chinese and Japanese populations, who are ethnically close to Taiwanese.^{6,14,15} The frequencies of the G allele and GG genotype of the IL-10 A–1082 G polymorphism in Caucasians are higher,^{8,16} whereas the GG genotype is not found in Taiwanese.

To investigate the functional difference of the IL-10 promoter gene polymorphisms, serum IL-10 levels of the FA patients and controls were measured, and the results showed that IL-10 serum levels were significantly lower in the FA patients. IL-10 levels at position –1082 were not significantly different among the FA patients with genotypes AA and AG, with mean levels of 50.95 pg/mL and 52.37 pg/mL, respectively. In contrast, IL-10 levels at position –592 were significantly different among the FA patients ($p < 0.05$). The mean IL-10 level of genotype AA was 24.48 pg/mL, while the other genotype (AC+CC) was 70.96 pg/mL.

These results suggest that the IL-10 –592 SNP influences IL-10 expression, while the IL-10 –1082 SNP has no impact on serum IL-10 level. Examining the combined effect of these two SNPs on the IL-10 promoter, the AC/AC haplotype homozygote was only detected in the control group. The AA/AA haplotype homozygotes had significantly decreased serum levels of IL-10 ($p = 0.018$).

IL-10 and the transforming growth factor TGF- β are thought to play important roles in maintaining intestinal homeostasis and oral tolerance induction, as illustrated by the finding that TGF- β 1 is involved in inducing T-regulatory

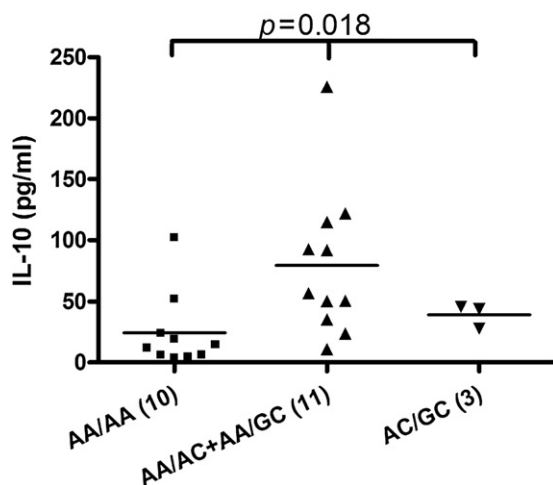


Figure 4 Comparison of serum IL-10 levels of each haplotype. Patients with AA/AA haplotype homozygotes had significantly lower serum IL-10 levels than the other haplotypes, including the AA/– haplotype heterozygotes (AA/GC, AA/AC) and –/– haplotype homozygotes (AC/GC). IL-10 levels were compared using the Kruskal Wallis test.

Table 5 Comparison of clinical symptoms to offending food allergens and the genotypes of food allergy at IL-10 –1082A/G and –592A/C.

Clinical symptoms	–1082A/G			–592A/C		
	AA	AG	<i>p</i>	AA	AC or CC	<i>p</i>
Respiratory tract	8/32 (25%)	2/5 (40%)	0.597	4/17 (23.5%)	6/20 (30%)	0.871
Skin	22/32 (68.8%)	4/5 (80%)	1.000	12/17 (70.6%)	14/20 (70%)	1.000
Gastrointestinal tract	13/32 (40.6%)	1/5 (20%)	0.630	6/17 (35.3%)	8/20 (40%)	0.801

The chi-square test was used to compare clinical symptoms between these two IL-10 SNPs.

cells expressing Foxp3, which in turn produce IL-10.^{17,18} Recently, two independent groups developed genetically modified mouse strains in which IL-10-producing cells could be “tracked” *in vivo* by fluorescence. These studies^{19,20} show that intestinal lymphocytes were strongly committed to IL-10 production, particularly in Peyer’s patches, the lamina propria, and the epithelial compartment. IL-10 could be measured in these intestinal T lymphocytes either under normal conditions or after stimulation, thereby emphasizing again the important regulatory function of T cell-derived IL-10 in controlling inflammatory responses to the normal intestinal flora. Scott-Taylor et al²¹ reported that children with FA also had reduced numbers of TGF- β -producing T cells in their intestinal mucosa and IL-10-producing T cells in the circulation. Moreover, the proportions of T cells of food-sensitized patients spontaneously secreting IL-10 without antigen stimulation were lower than non-atopic controls, a finding consistent with the present study.

For the general population, the term “food allergy” is widely used for toxic reactions (e.g. due to toxic components naturally occurring in some foodstuffs or present as contaminating agents), as well as for food intolerance that depends mainly on the metabolic characteristics of the patient (i.e. the ability to digest lactose). The major food allergens are water-soluble glycoproteins derived from animals and plants. These glycoproteins tend to remain stable during treatment with heat, acid, and proteases.²² Approximately 80% of the total protein content in egg white consists of four allergens, ovomucoid, ovalbumin, conalbumin, and lysozyme.²³ Ovalbumin, the major protein constituent of egg white, is a phosphorylated glycoprotein with unknown biological function. In one study, mice were completely tolerant to heated egg delivered by the oral route, suggesting that heating may influence the handling of egg allergens in the gastrointestinal tract in addition to altering the conformation of IgE-binding epitopes.²⁴ Food processing can alter the ability of food protein allergens to trigger reactions not only by interfering with their IgE binding but also by altering their degradation and absorption within the gastrointestinal tract. In this study, the prevalence of serum-specific IgE to egg white was significantly higher in FA patients with the homozygous AA genotype of IL-10 –592 A/C. This finding suggests that specific IgE to egg white in patients with FA and the AA genotype at position –592A/C might be involved in the pathogenesis of FA. There are also geographic differences in the prevalence of food allergies. There is a marked heterogeneity in FA prevalence that can be a result of differences in study design or methodology, or differences

between populations. One meta-analysis⁴ showed a very high prevalence of self-reported FA compared to objective measures, with variations ranging from 3% to 35% for any food.⁴ The prevalence for any food ranged from 1% to 10.8% based on food challenge, 2% to 5% based on symptomatic sensitization, 4% to 6% by IgE specific to food, and 7% to 17% based on skin prick tests.

Common clinical manifestations of food allergies include skin, gastrointestinal tract, and respiratory tract symptoms. Life-threatening anaphylaxis may also be manifested in some cases. A detailed clinical history is the most helpful diagnostic modality and remains essential for diagnosis. However, diagnosis based on history alone may not always be accurate. In 1976, May²⁵ introduced the use of double-blind, placebo-controlled oral food challenges (DBPCFC) to diagnose FA, a protocol now considered the gold standard for diagnosing FA. Unfortunately, this test is impractical in the busy setting of clinical practice, and the diagnosis of FA still relies on clinical history, physical examination, results of skin tests and food-specific IgE, and elimination diets. The DBPCFC requires several hours in hospital, results in discomfort when challenge results are positive, and places the patient at risk of severe anaphylactic reaction. Despite the excellent predictability of the DBPCFC, laboratory tests that can provide similar predictive values at less risk and expense will be extremely useful. Using the Pharmacia CAP System FEIA to quantify food-specific IgE antibody concentrations in patients previously diagnosed with FA, diagnostic decision points that were at least 95% predictive of clinical reactivity to egg, milk, peanut, and fish were established by Sampson.^{26,27} Those who show a serum egg white specific IgE level of ≥ 6 kU/L and a cow’s milk specific IgE level of ≥ 32 kU/L are considered to be allergic to eggs and cow’s milk, respectively, based on the diagnostic levels suggested by Sampson.

The ideal goal in FA management is to prevent its development. Dietary exclusion is the most effective measure for preventing allergic sensitization and anaphylactic responses in high-risk groups.²⁸ Novel immunotherapy by delivering specific or non-specific allergens via subcutaneous or sublingual routes have shown some degree of symptom alleviation in animal models and human studies.²⁹ Regulatory T cell subsets may suppress T cell activation and cytokine production, a mechanism for allergen injection immunotherapy since allergen-specific T cell anergy evoked by such therapy has been shown to depend on IL-10.⁹

The present study has some limitations. The diagnosis of FA in this study relied on clinical history, physical examination, results of food-specific IgE, and elimination diets. The DBPCFC is impractical in a busy clinical practice

setting, and patients hesitate due to safety concerns. Future studies will seek to enlarge sample sizes and increase SNP loci for analysis. In addition, the DBPCFC will be required to establish the diagnosis in future studies. Campos et al.⁶ suggested that the IL-10 -1082 A/G gene polymorphism is related to FA susceptibility in atopic Japanese children. In the current study, the number of FA patients may have been too small to detect polymorphisms. Since not all SNPs in the IL-10 gene have been investigated, we cannot conclude that other SNPs are not associated with FA among Taiwanese.

Conclusion

The development of FA was associated with IL-10 phenotype production and gene polymorphisms. There was an association between low serum concentrations of IL-10 and a higher risk of FA. There may also be a functional relationship between the IL-10 -592 A/C gene polymorphism and FA pathogenesis. The AA/AA haplotype homozygotes were associated with lower serum IL-10 levels, suggesting that IL-10 plays some immunologic role in the pathogenesis of FA. We suggest that it might be practical to evaluate serum IL-10 levels of FA patients and predict the possibility of FA if we check genotypes and haplotypes regularly.

References

1. Sampson HA. Update on food allergy. *J Allergy Clin Immunol* 2004;113:805–19.
2. Bangash SA, Bahna SL. Pediatric food allergy update. *Curr Allergy Asthma Rep* 2005;5:437–44.
3. Madsen C. Prevalence of food allergy: an overview. *Proc Nutr Soc* 2005;64:413–7.
4. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007;120:638–46.
5. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997;24:1–8.
6. Campos Alberto EJ, Shimojo N, Suzuki Y, Mashimo Y, Arima T, Matsuura T, et al. IL-10 gene polymorphism, but not TGF- β 1 gene polymorphisms, is associated with food allergy in a Japanese population. *Pediatr Allergy Immunol* 2008;19:716–21.
7. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. *J Allergy Clin Immunol* 2010;126(6):1105–18.
8. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum* 1999;42:1101–8.
9. Koulis A, Robinson DS. The anti-inflammatory effects of interleukin-10 in allergic disease. *Clin Exp Allergy* 2000;30:747–50.
10. Vickery BP, Scurlock AM, Jones SM, Burks AW. Mechanisms of immune tolerance relevant to food allergy. *J Allergy Clin Immunol* 2011;127:576–84.
11. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor- β : the role of T regulatory cells. *Immunology* 2006;117:433–42.
12. Shin HD, Park BL, Kim LH, Kim JS, Kim JW. Interleukin-10 haplotype associated with total serum IgE in atopic dermatitis patients. *Allergy* 2005;60:1146–51.
13. Karjalainen J, Hulkkonen J, Nieminen MM, Huhtala H, Aromaa A, Klaukka T, et al. Interleukin-10 gene promoter region polymorphism is associated with eosinophil count and circulating immunoglobulin E in adult asthma. *Clin Exp Allergy* 2003;33:78–83.
14. Negoro T, Orihara K, Irahara T, Nishiyama H, Hagiwara K, Nishida R, et al. Influence of SNPs in cytokine-related genes on the severity of food allergy and atopic eczema in children. *Pediatr Allergy Immunol* 2006;17:583–90.
15. Ying B, Shi Y, Pan X, Song X, Huang Z, Niu Q, et al. Association of polymorphisms in the human IL-10 and IL-18 genes with rheumatoid arthritis. *Mol Biol Rep* 2011;38:379–85.
16. Belisle SE, Hamer DH, Leka LS, Dallal GE, Delgado-Lista J, Fine BC, et al. IL-2 and IL-10 gene polymorphisms are associated with respiratory tract infection and may modulate the effect of vitamin E on lower respiratory tract infections in elderly nursing home residents. *Am J Clin Nutr* 2010;92:106–14.
17. Weiner HL. The mucosal milieu creates tolerogenic dendritic cells and T(R)1 and T(H)3 regulatory cells. *Nat Immunol* 2001;2:671–2.
18. Faria AM, Weiner HL. Oral tolerance and TGF- β -producing cells. *Inflamm Allergy Drug Targets* 2006;5:179–90.
19. Kamanaka M, Kim ST, Wan YY, Sutterwala FS, Lara-Tejero M, Galán JE, et al. Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. *Immunity* 2006;25:941–52.
20. Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, et al. Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. *Nat Immunol* 2007;8:931–41.
21. Scott-Taylor TH, Hourihane JB, Harper J, Strobel S. Patterns of food allergen-specific cytokine production by T lymphocytes of children with multiple allergies. *Clin Exp Allergy* 2005;35:1473–80.
22. Lack G. Clinical practice. Food allergy. *N Engl J Med* 2008;359:1252–60.
23. Everberg H, Brostedt P, Oman H, Bohman S, Movérare R. Affinity purification of egg-white allergens for improved component-resolved diagnostics. *Int Arch Allergy Immunol* 2011;154:33–41.
24. Martos G, Lopez-Exposito I, Bencharitiwong R, Berin MC, Nowak-Węgrzyn A. Mechanisms underlying differential food allergy response to heated egg. *J Allergy Clin Immunol* 2011;127:990–7.
25. May CD. Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. *J Allergy Clin Immunol* 1976;58:500–15.
26. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444–51.
27. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;107:891–6.
28. Yu LC. The epithelial gatekeeper against food allergy. *Pediatr Neonatol* 2009;50:247–54.
29. Skripak JM, Sampson HA. Towards a cure for food allergy. *Curr Opin Immunol* 2008;20:690–6.