

Clinical characteristics of oral tolerance induction of IgE-mediated and non-IgE-mediated food allergy using interferon gamma

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ABSTRACT

Food allergies are classified as IgE-mediated food allergies (IFAs) and non-IgE-mediated food allergies (NFAs). Recently, oral immunotherapy (OIT) has been found to be successful for treating both IFA and NFA, especially using interferon (IFN) gamma. This study was designed to clarify the clinical characteristics of IFA and NFA and compare the therapeutic characteristics of OIT using subcutaneously administered IFN-gamma for both types of food allergy. In this study, 148 patients were categorized into the IFA and NFA group following food challenge, skin-prick test and food-specific IgE tests. The patients were then treated using protocols specific for IFA and NFA using subcutaneous IFN-gamma injection as a randomized controlled trial. The principle of complete allergy resolution at prior dose in the case of IFA was also evaluated. Only the patients with IFA and NFA treated with OIT using IFN-gamma achieved tolerance successfully. Tolerance was achieved from low-dose range in IFA and in high-dose range for NFA. Complete tolerance was not obtained without achieving complete allergy resolution at each dose of the allergen before increasing the dosage in IFA. Both IFA and NFA can be successfully treated with OIT using IFN-gamma but show different clinical and therapeutic characteristics. IFN-gamma is necessary for the tolerance induction but not for tolerance maintenance. Additional study for the mechanisms of tolerance induction by IFN-gamma is needed.

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Key words: Food allergy, high dose, IFN-gamma, IgE-mediated food allergy, low dose, non-IgE-mediated food allergy, oral immunotherapy, tolerance, tolerance induction

Food allergies are classified as IgE-mediated food allergy (IFA), non-IgE-mediated food allergy (NFA), and mixed-type allergy.¹ IFA is easily diagnosed via its characteristic clinical symptoms and IgE-related laboratory results. However, NFA is underdiagnosed and undertreated due to a lack of proper laboratory tests.² NFA frequently involves the gastrointestinal system, skin, and respiratory system. Recently, late eczematous reactions were found to be

typical and diagnostic symptoms of NFA.³ In the past, avoidance of the offending foods was considered the only therapeutic modality for both IFA and NFA; thus, differentiation between IFA and NFA was not necessary.

Recent studies have shown that oral immunotherapy (OIT) is successful for the treatment of IFA.^{4,5} OIT using interferon (IFN) γ has been found to be effective not only for managing human NFA,⁶ but also for treating IFA.⁷ However, the treatment protocols differ according to the type of food allergy. Thus, it is necessary to classify food allergies as IFA or NFA for therapeutic purposes. In this study, we compared the therapeutic characteristics of OIT using subcutaneously administered IFN- γ for IFA and NFA, especially with regard to the principle of complete allergy resolution in IFA and the dose relationship with the type of food allergy.

PATIENTS AND METHODS

Subjects and Study Design

A total of 148 patients (mean age, 10.3 ± 8.5 years; M/F ratio, 75:73) were enrolled in this study who visited the Department of Allergy and Clinical Immunology at the Seoul Allergy Clinic (Seoul, Korea) be-

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Table 1 Patient profile and study design

	Complete Res	Incomplete Res	IFN- γ Only	Food Only	None
IFA					
<i>n</i>	10	6	5	3	7
M/F	9:1	4:2	3:2	1:2	4:3
Mean age (yr)	4.8 $\pm$ 3.5	3.8 $\pm$ 1.7	5.6 $\pm$ 1.1	7.1 $\pm$ 2.1	3.4 $\pm$ 0.8
NFA					
<i>n</i>	—	37	10	10	20
M/F	—	18:19	3:7	8:2	7:13
Mean age (yr)	—	11.9 $\pm$ 1.1	10.6 $\pm$ 1.7	10.8 $\pm$ 1.9	11.4 $\pm$ 2.5
Neg control		<i>n</i> = 40; M/F = 18:22; mean age = 12.1 $\pm$ 10.4			

IFA = IgE-mediated food allergy; NFA = non-IgE-mediated food allergy; IFN = interferon; Complete Res = complete resolution at prior dose; Incomplete Res = incomplete resolution at prior dose; Neg control = no allergy to the tested foods.

tween January 2007 and July 2009. Thirty-one patients with IFA, 77 patients with NFA, and 40 subjects without IFA or NFA were selected for the randomized clinical trial (Table 1). Six other patients with IFA to wheat and one with IFA to soybeans were treated with OIT using IFN- γ . Parents and patients signed consent forms and this study was approved by the Ethical Committee of the Seoul Allergy and Immunology Association, Seoul, Korea.

Statistical analyses were performed by using the Wilcoxon signed-rank test and Mann-Whitney *U* test with Bonferroni corrections for individual groups using the SigmaPlot software (Systat Software, Inc., San Jose, CA). Values of *p* < 0.05 were considered significant for all comparisons.

Food Challenges

An elimination diet was used before food challenges. For NFA, the elimination diet comprised seven foods (milk, eggs, soybeans, wheat, beef, pork, and chicken) and for food(s) to which the patients showed a history of allergy including peanuts as described previously.⁸

Open food challenges (OFCs) were also conducted for IFA when patients showed food-specific IgE levels of >10 kU/L for milk and 5 kU/L for eggs⁹ and/or had a "convincing" history of a severe allergic reaction to other foods in addition to milk and eggs (Table 2).⁷ Confirmatory OFC tests were also performed according to the OFC protocol for NFA just after finishing OIT using IFN- γ to confirm the acquisition of tolerance to the allergenic foods. The patients were followed up for 2 years after the study initiation and open challenge tests were repeated to evaluate the maintenance of tolerance for the allergenic food(s).

Double-blinded placebo-controlled food challenge was performed for diagnosing NFA to milk and eggs as described previously.¹⁰ Freeze-dried flour of eggs and skim milk powder were added to a vehicle of mixed cereal flour for double-blinded placebo-con-

trolled food challenge. The patients consumed the food twice a day, at breakfast and dinner. Two challenges, separated by 5 days, were performed; one comprised only the placebo whereas the other contained the suspected food antigen in the vehicle. OFCs were performed for beef, pork, chicken, and other foods that were suspected as causes of NFA according to the protocol described previously (Table 2).

Clinical Severity Scoring System of IFA and NFA

The clinical severity of IFA was evaluated using the modified Clark's scoring system.¹¹ The symptoms and signs were classified into four categories: (1) acute allergic skin manifestations such as urticaria or angioedema; (2) respiratory symptoms such as dyspnea, wheezing, or cyanosis; (3) circulatory symptoms and signs of hypotension or palpitation; and/or (4) gastrointestinal or other symptoms. The patient responses in each category were assigned 5 points. Moreover, 10 points and 5 points were added for onset time of <15 minutes and >15 minutes, respectively. The clinical severity scores for IFA were in the range 10–30 points.

The clinical severity of NFA was evaluated by using the Scoring Atopic Dermatitis Index, which is used worldwide to assess the severity of atopic eczema.¹² After the food challenges, the patients with NFA exhibited new developments and/or exacerbation of the symptoms of atopic dermatitis. Therefore, the scoring system for atopic dermatitis was used in this study to evaluate NFA.

Diagnosis of NFA and IFA

Patients showing an increase in the severity score of >20% in the case of clinical severity scores of >50 or 10 points in the case of clinical severity scores of $\leq$ 50 points were diagnosed with NFA. Apparently, new skin lesions or itching were regarded as positive reactions and included in the diagnosis of NFA.

Table 2 Dose protocols for open food challenge (OFC) and tolerance induction

	Milk	Eggs	Wheat	Soybeans	Beef	Chicken	Pork
Dose protocols of the OFC for IFA (for body weight of 15 kg)							
Starting dose	2	1	2	1			
2nd	4	2	4	2			
3rd	6	3	6	3			
4th	10	5	10	5			
Dose protocols of OFC for NFA (for body weight of 30 kg)							
Dose	200	50	100	100	200	120	120
Dose protocols of IFN- γ -induced tolerance induction for IFA (for body weight of 15 kg)							
Starting dose	1	1	2	2			
Increment dose		1	2	2			
1-10	1						
10-30	2						
30-60	3						
60-100	4						
Increment counts	40	25	25	25			
Target dose	100	25	50	50			
Dose protocols of IFN- γ -induced tolerance induction for NFA (for body weight of 30 kg)							
30-kg Dose							
1st	25	6	12	12	25	15	15
2nd	50	12	25	25	50	30	30
3rd	75	18	37	37	75	45	45
4th	100	25	50	50	100	60	60
5th	150	37	75	75	150	90	90
6th	200	50	100	100	200	120	120
7th	300	75	150	150	300	180	180
Unit	ml	g	g	g	g	g	g

IFA = IgE-mediated food allergy; NFA = non-IgE-mediated food allergy.

IFA was diagnosed by its characteristic symptoms and signs including urticaria/skin rash, angioedema, respiratory symptoms such as dyspnea and/or cough, cardiovascular symptoms and signs such as hypotension, and/or other typical symptoms.¹¹

OIT using IFN- γ for IFA and NFA

Recombinant IFN- γ (Intermax Gamma, LGCI, Seoul, Korea) with a specific activity of 2×10^6 IU (50 μ g) was administered by subcutaneous injection at a dosage of 3×10^6 IU/m², according to the body surface area, as previously reported,⁷ and the patients were instructed to take oral acetaminophen (10 mg/kg, maximum dose of 600 mg) twice, at 1 and 4 hours after the injection, to reduce the possibility of side effects. On receiving an IFN- γ injection, a scheduled dose of food was provided after 10–20 minutes.

Food intake was gradually increased from the starting dose to the target dose in the case of IFA (Table 2). For patients showing a positive reaction to the allergen, the dose was not increased, but the same dose was administered in the next step. This step was repeated until the patient no longer showed allergy provocation,

and then, the dosage was increased as indicated in the schedule. Treatment was performed once a day, nearly every day except on holidays and Sundays. For verification of the principle of complete allergy resolution in the treatment of IFA, the OIT protocol was altered such that the food dosage was increased when the patients showed weakened allergic reactions, even without complete resolution of the allergic reaction at the prior dosage.

The standard protocol for treating NFA with OIT consisted of a 7-day course. OIT was started with higher initial doses of food than those used in the OIT for IFA treatment. Food intake was gradually increased according to the protocol described in Table 2, regardless of allergy provocation at the prior dose.

Blood Tests and Skin-Prick Tests (SPTs)

All patients underwent blood tests, including complete blood cell counts with differential counts for the eosinophil fraction, serum total IgE levels, and specific IgE levels for the allergenic foods. These tests were conducted before and after tolerance induction. Food-specific IgE levels were measured by using the UniCap

Table 3 Diagnostic characteristics of IgE-mediated food allergy (IFA) and non-IgE-mediated food allergy (NFA)

	IFA	NFA
Symptoms		
Typical skin lesions	Urticaria*	Ecematous Lesion#
Cardiovascular	Possible	None
Respiratory	Possible	None
Onset time		
Onset time	Immediate or less than 15 minutes	Mostly after 4 hours
Max onset time	Less than 1 hour	Even after several days
Presentation as atopic dermatitis	None	Always
Symptom variation		
By food kinds	Uniform	Variable
By different patients with same foods	Uniform	Variable
History		
Emergency state	Frequent	Little
History of allergy provocation	Clear	Frequently unclear
Recognition by patient or parents	Well-known	Frequently unknown or unclear
Dose		
Provocation range	All range	High dose range
Minimal dose	Very low	High dose
Lab findings		
Skin-prick test	Equal to or +++<	Weak or negative (<+++)
Food-specific IgE	Very high	Low or negative (<+++)
*Fig. 1 A.		
#Fig. 1 B.		

(Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) method.

SPTs were performed on the patients' left forearms by using commercial allergen extracts (Bencard, Brentford, England). Histamine hydrochloride (1 mg/mL, Bencard) was used as a positive control. Physiological saline, distilled water, and glycerol were used as negative controls. The reactions were read after 15 minutes and classified as negative (0, no reaction) or positive (+, reaction greater than the control reaction but smaller than one-half the size of the histamine reaction; ++, reaction one-half the size of the histamine reaction; +++, reaction equal to the size of the histamine reaction; and +++++, reaction twice the size of the histamine reaction). The minimum size of a positive reaction was 3 mm, and the ratios of the wheal sizes of the allergen to that of histamine were also calculated.

RESULTS

Food Allergy Profile

The patients were classified into the IFA and NFA groups on the basis on the characteristic symptoms (Table 3). IFA and NFA showed clearly different skin manifestations; IFA showed typical urticaria (Fig. 1 A) and/or angioedema whereas NFA showed ecematous

reactions (Fig 1 B). Immediate (<15 minutes) symptoms and signs developed in the case of IFA, whereas the symptoms and signs were delayed by at least 4 hours to days after the food challenges in the case of NFA. Milk, eggs, soybeans, and wheat were the main causes of IFA and NFA, whereas meats (beef, pork, and chicken) caused only NFA in this study (Table 4).

OIT Using IFN- γ for IFA and Principle of Complete Allergy Resolution

Ten patients with IFA who were treated with IFN- γ -induced specific oral tolerance induction achieved successful tolerance (changes in clinical severity scores by food challenge from 15.6 ± 3.7 to 0 ± 0 points, $p < 0.001$). Five patients who received only IFN- γ failed to obtain tolerance to the allergenic foods (changes in clinical severity scores by food challenge from 14.4 ± 2.5 to 14.4 ± 2.2 points, $p > 0.05$). Three subjects dropped out at the early stage of food challenges without IFN- γ because of the severe allergenic reactions (changes in clinical severity scores by food challenge from 14.7 ± 1.9 to 14.8 ± 2.0 points, $p > 0.05$). Six patients who received IFN- γ -induced specific oral tolerance induction for IFA with incomplete adherence to the principle of complete allergy resolution showed

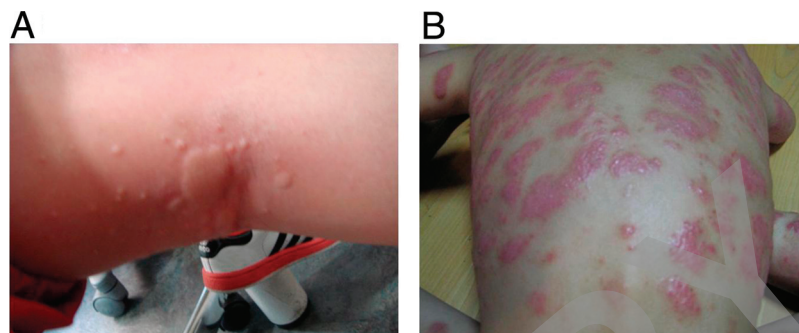


Figure 1. Representative skin manifestations. (A) Urticaria for IgE-mediated food allergy (IFA); (B) Eczematous skin lesions for non-IgE-mediated food allergy (NFA).

Table 4 Characteristics of the causative foods in IgE-mediated food allergy (IFA) and non-IgE-mediated food allergy (NFA)

Food	IFA	NFA	Neg	Total
Milk	14	32	119	165
Eggs	17	44	111	172
Soybeans	1	35	124	160
Wheat	7	37	89	133
Beef	—	34	114	148
Pork	—	35	102	137
Chicken	—	42	86	128
Total	39	259	745	1043

Neg = negative in the food challenge.

weakening of allergy provocation (changes in clinical severity scores by food challenge from 17.0 ± 4.2 to 11.4 ± 5.7 points, $p > 0.05$; Fig 2 A). However, they did not obtain complete tolerance and still displayed weakened allergy provocation and one patient failed to obtain tolerance. The control group of seven patients showed no significant changes (changes in clinical severity scores by food challenge from 16.0 ± 2.3 to 16.0 ± 2.3 points, $p > 0.05$). OIT using IFN- γ for IFA to wheat and soybeans was also successful.

OIT Using IFN- γ for NFA

All of the patients who showed late eczematous reactions were treated by using the NFA-specific OIT protocol. All patients became tolerant to the previous offending foods (changes in clinical severity scores by food challenge from 15.9 ± 3.6 to 1.8 ± 1.0 points, $p < 0.001$), whereas none of the other controls acquired tolerance. After the food challenges, the clinical severity scores changed from 17.4 ± 4.4 to 15.5 ± 5.0 points ($p > 0.05$) in the IFN- γ -only group, from 14.6 ± 3.6 to 14.1 ± 3.0 points ($p > 0.05$) in the food-only group, and from 15.9 ± 3.7 to 15.4 ± 4.3 points ($p > 0.05$) in the untreated group (Fig. 2 B).

Laboratory Characteristics of IFA and NFA

The mean allergen-to-histamine wheal-size ratio for milk and eggs on SPT was significantly higher in the

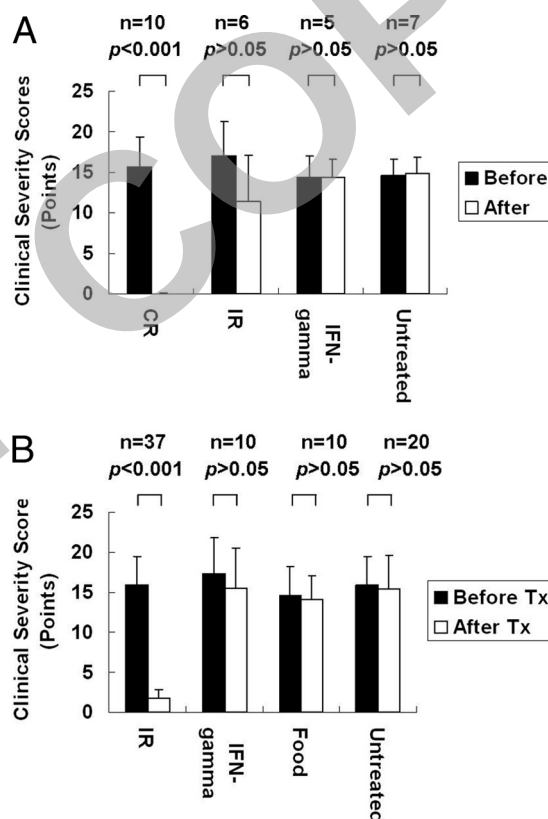


Figure 2. Therapeutic effects of interferon (IFN) γ -induced specific oral tolerance induction. The bars indicate changes in the clinical severity scores after open food challenges (OFCs). (A) Results of tolerance induction for IgE-mediated food allergy (IFA) with verification of the principle of complete allergy resolution, (B) Results of tolerance induction for non-IgE-mediated food allergy (NFA). CR, group with complete allergy resolution at prior dose; IR, group without complete allergy resolution at prior dose; IFN- γ , group that was treated with only IFN- γ ; untreated, group treated neither IFN- γ nor food; Food, group treated with only the offending foods; Before, before tolerance induction; After, after tolerance induction.

patients with IFA (2.5 ± 0.8 ; $n = 31$) than in those with NFA (0.5 ± 0.2 ; $n = 62$) or in the nonallergic controls (0.4 ± 0.2 ; $n = 40$; $p < 0.001$; Fig. 3). There was no significant difference between the wheal-size ratios of the NFA group and those of the nonallergic subjects

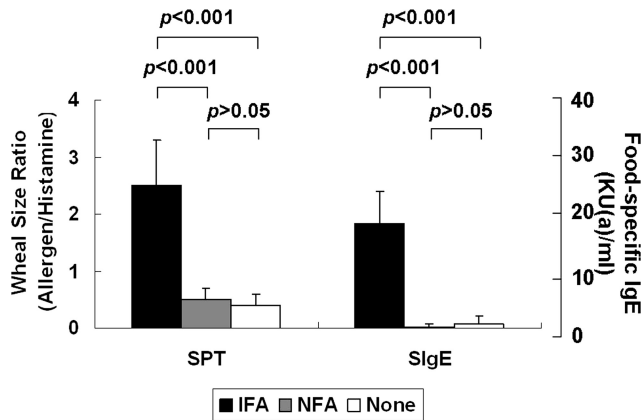


Figure 3. Laboratory characteristics of IgE-mediated food allergy (IFA) and non-IgE-mediated food allergy (NFA). Mean allergen-to-histamine wheal-size ratio according to the skin-prick test (SPT) and mean food-specific IgE levels. SIgE, food-specific IgE.

($p > 0.05$). The cutoff values for predicting IFA differed according to the food type. However, an allergen-to-histamine wheal-size ratio of ≥ 1.5 had a 90% positive predictive value for IFA. Most of the patients with an allergen-to-histamine wheal-size ratio > 2.0 had IFA.

The mean food-specific IgE levels for milk and eggs were also significantly higher in the patients with IFA (18.4 ± 5.6 kU(A)/mL; $n = 30$) than in those with NFA (0.3 ± 0.5 kU(A)/mL; $n = 58$) or in the nonallergic controls (0.7 ± 1.4 kU(A)/mL; $n = 40$; $p < 0.001$; Fig. 3). There was no significant difference between the mean food-specific IgE levels of the NFA group and those of the nonallergic subjects ($p > 0.05$). The cutoff IgE levels for the predicting IFA showed more variability according to the food type than the cutoff values of the SPTs. The cutoff IgE levels resulting in a 90% positive predictive value for IFA were 10.0 kU(A)/mL for milk, 5.0 kU(A)/mL for eggs, and 20 kU(A)/mL for wheat.

The SPT responses decreased significantly after treatment (from 2.0 ± 0.9 to 0.8 ± 0.4 ; $n = 10$; $p < 0.01$) in the IFA group but were unchanged (from 0.4 ± 0.3 to 0.3 ± 0.2 ; $n = 35$; $p > 0.05$) in the NFA group (Fig. 4). However, the change in the food-specific IgE levels was interesting; the levels increased significantly after tolerance induction (from 19.7 ± 8.5 to 35.3 ± 12.6 ; $n = 10$; $p < 0.01$) in the IFA group (Fig 4). These levels were unchanged in the NFA group after treatment (from 0.3 ± 0.4 to 0.7 ± 0.5 ; $n = 35$; $p > 0.05$).

Therapeutic Characteristics of OIT Using IFN- γ for IFA and NFA

OIT for IFA and NFA showed typical characteristics of their own (Table 5). The clinical severity score curve gradually decreased from the low- to the mid-dose range of allergen in the case of OIT using IFN- γ for IFA, and the symptoms weakened with the progress of treatment (Fig 5).

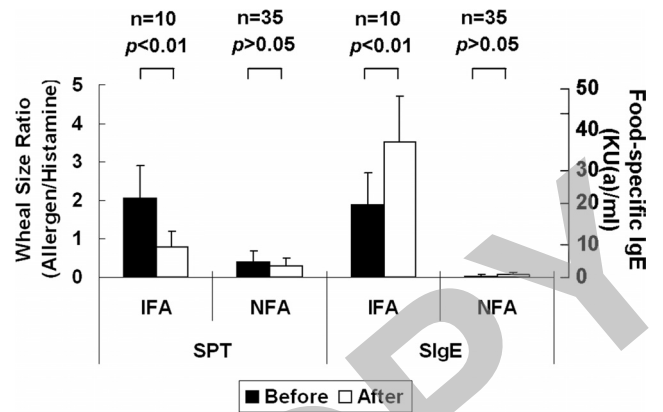


Figure 4. Change of the skin-prick test (SPT) reactions and change in the food-specific IgE levels on interferon (IFN) γ -induced specific oral tolerance induction for IgE-mediated food allergy (IFA) and non-IgE-mediated food allergy (NFA). SIgE, food-specific IgE.

In the case of OIT using IFN- γ for NFA, the curve was parabolic. Tolerance acquisition seemed to occur from the low-dose zone for IFA and in the high-dose zone for NFA. The initial allergenic dose for IFA was very low but was much higher for NFA (Table 5). The dose increment was very small in the low-dose range during the early stage of OIT using IFN- γ for IFA and was relatively large in the mid- to high-dose range.

Most of the untoward reactions during the treatment, such as fever, headache, myalgia, and abdominal pain, were suspected to be caused by IFN- γ (Table 6). However, these side effects were well controlled by oral acetaminophen. An interesting finding was that the side effects did not appear within six IFN- γ injections in most cases. They appeared in 48% (12/25) and in 32% (15/47) of the patients with IFA and NFA who received IFN- γ , respectively. Especially, 57% (12/21) of the patients with IFA who did not show any sign of NFA or atopic dermatitis showed adverse reactions caused by IFN- γ .

Eight of 10 patients with IFA and 35 of 37 NFA patients with NFA who received tolerance induction were followed up for 2 years (Table 7). Two of the eight patients in the IFA group and one patient in the NFA group requested dose limitation because of slight itching on consuming > 300 mL of milk at once. However, all of the patients consumed the allergenic foods without any inconvenience regardless of IFA or NFA. From these follow-up data, IFN- γ administration was no longer necessary once tolerance was obtained.

DISCUSSION

In this study IFA and NFA could be distinguished based on their characteristic symptoms and laboratory results of SPTs and food-specific IgE levels. OIT using subcutaneously injected IFN- γ was proved to be effective in treating both IFA and NFA (Fig. 2). In addition

Table 5 Clinical characteristics of oral immunotherapy using IFN- γ for IgE-mediated food allergy (IFA) and non-IgE-mediated food allergy (NFA)

	OIT Using IFN- γ		OIT without IFN- γ ^{5,6}	
	IFA	NFA	IFA	NFA
Severity curve shape	Semihyperbolic	Parabolic	N/A	No report
Tolerance acquisition range	Whole range from very tiny dose	High dose	N/A	No report
Tolerance acquisition pattern	Gradual step by step	Abrupt	N/A	No report
Severity peak	Whole range from very tiny dose	Late phase	N/A	No report
Initial dose	Extremely small	Very high	Extremely tiny	No report
Initial increment	Very small	High	Extremely small	No report
Late increment	Relatively high	High	Small	No report
Complete allergy resolution principle	Absolute	Unnecessary	Included	No report
Treatment period	Long (2–3 mo)	Short (7–10 days)	Very long (6–12 mo)	No report
Necessity of IFN- γ	Necessary	Absolute	Not used	No report

IFN = interferon; OIT = oral immunotherapy.

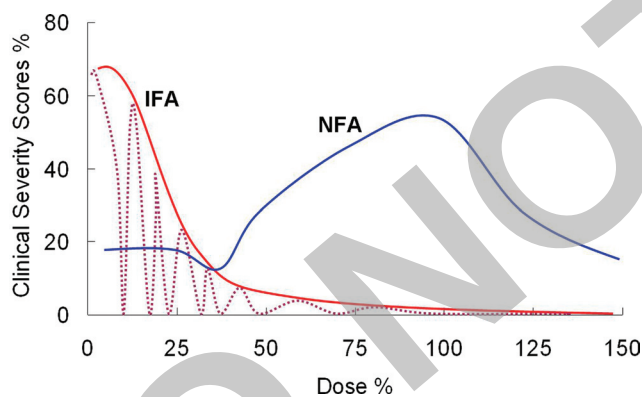


Figure 5. Schematic of the clinical course of interferon (IFN) γ -induced specific oral tolerance induction for IgE-mediated food allergy (IFA) and non-IgE-mediated food allergy (NFA). The dotted line indicates repetitive but decaying allergy provocation during the tolerance induction for IFA in the low-dose range. In the case of NFA, allergy provocation started to increase gradually at the high-dose range and stopped abruptly, resulting in gradual clinical improvement despite the increasing dose. Dose percent in the x-axis, 100% is a quantity of food that one takes at a meal.

to milk,⁷ OIT using IFN- γ was successful in the case of allergy to other foods.

Tolerance was achieved in the low-dose zone (low-dose tolerance) for IFA and in the high-dose zone (high-dose tolerance) for NFA. Similar results have been reported in animal studies. Low-dose oral tolerance suppresses antigen-specific Th2 cells and results in the suppression of antigen-specific IgE production,¹³ whereas high-dose oral tolerance prevents antigen-induced cellular immune responses, including eosino-

Table 6 Adverse reactions of IFN- γ after oral tolerance induction using subcutaneously injected IFN- γ

Side Effects	Pure IFA	IFA	NFA
Prevalence (%)	57%(12/21)	48%(12/25)	32%(15/47)
Symptoms and signs	IFA		NFA
Fever	12		15
Headache	4		4
Myalgia	2		1
Abdominal pain	1		1
Total	19		21

IFN = interferon; IFA = IgE-mediated food allergy; NFA = non-IgE-mediated food allergy.

phil recruitment, which are responsible for the delayed-type hypersensitivity of atopic dermatitis.¹⁴ Our study reproduced the results of previous animal studies concerning the relationship between the antigen-dose range and the type of food allergy for the first time in humans.

Achievement of complete resolution of allergy provocation at a given dose of allergen before increasing the dosage during OIT using IFN- γ was crucial for successful induction of tolerance for IFA (principle of complete allergy resolution). IL-4 is aberrantly produced during a food challenge, and food allergy is characterized by significantly higher spontaneous production of IL-4.¹⁵ The balance between exogenous IFN- γ and en-

Table 7 Follow-up results at 2 yr after the study initiation

Duration after Treatment	IFA				NFA			
	Total	F/Up	Incomplete	Recurrence	Total	F/Up	Incomplete	Recurrence
<6 mo	0	0	0	0	0	0	0	0
6–12 mo	1	1	0	0	3	3	0	0
12–18 mo	4	3	1	0	16	15	1	0
18–24 mo	5	4	1	0	18	17	0	0
Subtotal	10	8	2	0	37	35	1	0

IFA = IgE-mediated food allergy; NFA = non-IgE-mediated food allergy; F/Up, patients who were followed up.

ogenous Th2 cytokines including IL-4 and IL-5, which are produced during allergy provocation during the tolerance induction in a food challenge, seems to be important for tolerance induction to treat food allergy.

The key cytokines responsible for the induction of tolerance to specific allergens was not determined in this current study. IL-10 and transforming growth factor β affect T-regulatory cells and are suspected to be associated with allergy tolerance to food.^{16,17} Oral rIL-12 treatment, which is associated with increased IFN- γ production in mice,¹⁸ has therapeutic effects on peanut allergy. Tolerance induction for IFA to peanuts by herbal medicine is associated with the up-regulation of IFN- γ .¹⁹ Strong IL-10 and IFN- γ responses are associated with the development of tolerance.²⁰ Induction of IFN- γ expression by an oral antigen was possibly associated with systemic tolerance in NFA affecting the gastrointestinal tract in animal studies.²¹ Our study suggests that IFN- γ plays a significant role in tolerance induction for food allergy in humans. The remarkable finding is that IFN- γ was needed only during the induction of tolerance, which was maintained without further IFN- γ injection for both IFA and NFA.

The SPTs and food-specific IgE levels were useful for the diagnosis of IFA, as described previously.^{22,23} Milk, eggs, wheat, and soybeans caused both IFA and NFA, whereas IFA to beef, chicken, and pork was not observed in this study.

The limitation of this study is the difficulty in conducting a double-blind study because of the highly experimental nature of this therapy. However, this treatment modality is rapid and effective compared with the tolerance induction treatments by other investigators. More patients with IFA than with NFA showed adverse reaction attributable to IFN- γ in this study and this difference may suggest a difference in the immunopathological mechanisms of IFA and NFA, especially concerning Th1/Th2 imbalance. OIT using IFN- γ for NFA was very safe and effective.

In conclusion, OIT using subcutaneously administered IFN- γ was generally effective for IFA and NFA, although their protocols were quite different. IFN- γ

induced tolerance induction is recommended as a safe and effective treatment for atopic dermatitis in NFA. Low-dose tolerance can be achieved for IFA and high-dose tolerance can be obtained for NFA. IFN- γ seemed to be an important cytokine in the tolerance induction process for food allergy. These therapies are experimental still. Especially, OIT for IFA has very risky nature and should be performed very cautiously with the emergency preparation. Further investigation of the role of IFN- γ in tolerance induction is necessary.

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