AAT Polymorphisms in Intron 20 of *NOS1* Confer Vulnerability to Mite-Induced Allergic Rhinitis in Chinese Patients

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Palabras clave: Rinitis inducida por ácaros. Polimorfismo *NOS1* STR. Riesgo de modulación.

Allergic rhinitis (AR) is caused by hereditary and environmental factors and the interaction between them [1]. Asthma is frequently a comorbid condition of AR [2]. Both disorders are generally atopic diseases, with similar pathophysiology, immunopathology, and treatment [3], and are thus components of airway disease. Fewer data are available on the genetic origin of AR than on that of asthma. However, gene–disease association studies of asthma may provide insight into the genetic etiologies of AR. In a linkage mapping study, Ober et al [4] reported an asthma-susceptible locus at the distal region of chromosome 12. This chromosomal location encompasses several genes, one of which, *NOS1* (12q14-24.2), encodes a neural isoform of nitric oxide synthase (NOS) and is considered an asthma susceptibility gene. Among the NOS enzymes, the only association reported to date is the positive association between NOS1 and atopic asthma [5]. Interestingly, in animal models, knockdown *NOS1* expression led directly to lower exhaled NO production, and gene-deficient mice were less sensitive to methacholine, an NO inducer [6]. In contrast, a high exhaled NO concentration was frequently detected in a large number of patients with asthma and was considered a hallmark of asthma symptoms [7]. The small tandem repeat polymorphisms (STRs) of *NOS1*, particularly the exon 29 CA and intron20 AAT polymorphisms, are also thought to be involved in modulating vulnerability to asthma [5]. Consequently, *NOS1* STR polymorphisms may confer susceptibility to asthma.We also suspected that these polymorphisms could modulate the personal risk of suffering from AR. Therefore, we investigated the intron 20 ATT polymorphism because of its association with AR in Chinese patients.

Our sample comprised 66 patients with AR (36 male and 30 female; mean [SD] age, 26.59 [15.19] years) recruited from the Department of Otolaryngology of Kaohsiung Armed Forces General Hospital, Kaohsiung, Taiwan from July 2007 to June 2008. All patients were Han Chinese, had been diagnosed with mite-induced AR, and met the inclusion criteria described by Gorski et al [8]. Serum total immunoglobulin (Ig) E levels were abnormal (>100 kU_A/L), and allergen-specific IgE level was 0.35 kU_A/L (*Dermatophagoides pteronyssinus*–specific and *Dermatophagoides farinae*–specific IgE). The control group comprised 198 unrelated individuals (86 male and 112 female; mean age, 44.61 [13.97] years) originally recruited for a community health screening program and with no family history of AR. There were significant differences in age between cases and controls (t=8.88, *P*<.001). Written informed consent was obtained from all participants, and the study protocol was approved by the Institutional Review Board of Kaohsiung Armed Forces General Hospital.

As expected, we found significant differences between the prevalence of genotypes 12/12 and 13/13 between cases and controls (12-repeat, *P*=.001; 13-repeat, *P*<.001), even after a Bonferroni correction (12-repeat, *P*=.008; odds ratio [OR], 3.111 [95% confidence interval (CI), 1.612-6.005]; 13-repeat, *P*=.007; OR, 11.415 [95% CI, 2.733-47.438]). Moreover, the prevalence of the 12-repeat allele was greater in cases than in controls, possibly because of a higher risk of AR in our population (power, 0.90). The logistic regression analysis (Table) revealed that both the genotype L/L (≥12-repeat-allele) and the long-form allele were independently associated with the vulnerability of AR after adjusting for age and gender (genotype L/L, *P*=.012; OR, 4.00 [95% CI, 1.36-11.73]; long-form allele, *P*=.003; OR, 4.39 95% CI, 1.63-11.78]). Moreover, age interacted with the long-form allele and synergistically conferred a risk of AR (*P*=.044; OR, 1.07 [95% CI, 1.00-1.15]). This result indicated that genotype L/L and long-form allele carriers may have an increased risk of developing AR. Elderly people with the long-form allele may have an even higher risk of developing AR later in life than other patients. This finding also indicates that the association between the long-form allele in intron 20 of *NOS1* and AR might be age-dependent, since the long-form allele lost statistical significance (*P*=.420) when age was included in the analysis. Our findings on the role of AAT repeats in intron 20 associated with asthma and/or NO production was consistent with the findings of previous studies. Grasemann et al [9] suggested that the increased concentration of exhaled NO in asthmatic patients is closely related to the number of AAT repeats. The 12-repeat and 13-repeat alleles were more frequent in patients with AR than in controls, indicating that these variants may confer vulnerability to AR. This finding was conceptually consistent with the idea of long-form variants (>12 repeats) being associated with asthmatic disorders. In fact, long-form variants of intron 20 AAT linked to decreased airway NO production are thought to be due to relatively poor *NOS1* activity [10]. Thus, we suspect that the long-form allele could lead to reduced NO production, protect against mite exposure, and, subsequently, increase the risk of AR.

Although we suggest that the AAT polymorphisms in intron

Table. Multivariate Logistic Regression of Factors Associated With Vulnerability to AR After Controlling for Age and Gender

	ß	SE	Wald	df	*P*	Exp(B)	95% CI	
							Lower	Upper
Genotype L/L[a]	1.39	0.55	6.37	1	.012	4.00	1.36	11.73
Age	–0.10	0.02	39.37	1	<.001	0.91	0.88	0.94
Gender	–0.26	0.36	0.55	1	.46	0.77	0.38	1.54
Constant	2.74	0.74	13.57	1	<.001	15.42		
Long-form allele[b]	1.48	0.50	8.61	1	.003	4.39	1.63	11.78
Age	–0.10	0.02	39.96	1	<.001	0.91	0.35	1.44
Gender	–034	0.36	0.89	1	.345	0.71	0.88	0.93
Constant	2.92	0.75	15.13	1	<.001	18.46		
Age	–0.12	0.02	36.52	1	<.001	0.89	0.86	0.92
Long-form allele	–1.04	1.29	0.65	1	.420	0.35	0.03	4.44
Age by long-form allele	0.07	0.04	4.06	1	.044	1.07	1.00	1.15
Constant	-0.99	0.16	38.20	1	<.001	0.37		

Abbreviation: CI, confidence interval.
[a]Genotype L/L, homozygous for ≥12-repeat allele.
[b]Long-form allele, ≥12-repeat allele.

20 of *NOS1* may confer vulnerability to AR, our findings must be interpreted in the light of the limitations of our study. Since the number of AR patients was relatively small, current findings on the association between the AAT polymorphisms in intron 20 of NOS1 and AR should be interpreted with caution and further evaluated in a larger-scale study. In addition, possible environmental confounding factors, such as allergen exposure, climate, and traffic pollution, were not analyzed. The precise role of *NOS1* STR variants in relation to NOS1 activity, mRNA diversity, and vulnerability to AR should be investigated.

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Glatiramer Acetate Anaphylaxis: Detection of Antibodies and Basophil Activation Test

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Immunomodulatory therapies, including interferon beta and glatiramer acetate (GA: Copaxone, Cop1, Sanofi-Aventis, Barcelona, Spain), are first-line options for the treatment of relapsing-remitting multiple sclerosis (RRMS). GA is a mixture of synthetic peptides composed of 4 amino acids (L-alanine, L-glutamic acid, L-lysine, and L-tyrosine), with an average molecular mass of 4.7 kDa to 11.0 kDa. Daily subcutaneous injection of GA reduces the relapse rate of patients with RRMS by more than 50% and slows the development of disability [1].

GA may cause side effects, usually injection site reactions [2]. About 10% of patients develop an immediate postinjection systemic reaction (IPISR), consisting of dizziness, flushing, confusion, chest pain, skin rash, hives, itching, dyspnea, and dysphagia. These events are self-limiting and generally resolve without treatment [3]. Sixty-six nonfatal anaphylactic reactions in about 80 000 treated patients have been reported worldwide [4]. Given the clinical similarity between IPISR and true anaphylaxis, testing for GA hypersensitivity may be of critical importance. We analyzed the performance of in vivo and in vitro allergy tests in the study of 3 patients with anaphylaxis to GA.

Patient 1

A 27-year-old woman was diagnosed with MS in April 2007, and GA was started in September 2007. One month later, less than 5 minutes after injection, she developed generalized itching, hives, facial angioedema, and dyspnea and almost lost consciousness.

Patient 2

A 29-year-old woman was diagnosed with MS in July 2003. She started GA 2 years later and was treated for 30 months. Treatment was stopped because of an immediate injection reaction consisting of facial angioedema, dyspnea, abdominal cramps, and vomiting.

Patient 3

A 28-year-old woman was diagnosed with MS in February 2008. Five months after starting GA injections, she reported an episode of itching and fainting 5 minutes after an injection.

All patients required treatment with epinephrine, dexchlorpheniramine, and methylprednisolone.

Patients underwent a workup for suspected anaphylactic reactions after injection of GA (Table). This included skin tests (prick and intradermal), determination of immunoglobulin (Ig) E and IgG antibodies to GA, and a basophil activation test (BAT) [5]. The results of an intradermal test with Copaxone at a dilution of 1/100 was positive in all 3 patients. In patient 1, the results of skin tests with common inhalant allergens were also positive. Mannitol (an excipient in Copaxone) produced negative skin test reactions in all 3 patients. The results of intradermal skin tests with GA were negative in 3 healthy controls.

Table. Results of In Vivo and In Vitro Tests With Glatiramer Acetate

	Skin Tests		Serum Antibodies		
	Skin Prick Test	Intradermal Test	IgE to GA	IgG to GA	BAT
Patient					
1	+	+	1/2	–	–
2	–	+	1/32	1/100	+
3	–	+	–	1/400	+

Abbreviations: BAT, basophil activation test; GA, glatiramer acetate; Ig, immunoglobulin

All patients showed either IgG or IgE antibodies to GA by enzyme-linked immunosorbent assay (ie, result above mean [+3 SD] optical density of 10 controls). In 1 patient with a high IgG titer and negative IgE, interference of IgG with IgE binding was excluded by protein G sepharose absorption prior to IgE testing.

The results of the BAT were positive in 2 patients (1 mg/mL). Only 1 of the 6 controls not treated with GA had a positive result in the BAT at 1 mg/mL. This control was an atopic patient who also had IgE and IgG to GA above the cutoff.

All 3 patients had positive skin test results with GA. In addition, in vitro findings were positive, with a variable frequency supporting an immediate hypersensitivity mechanism. All patients had antibodies to GA, either IgG (patients 2 and 3) or IgE (patients 1 and 2). Patient 1 had a low IgE titer, and negative IgG, probably because she had been on treatment for only 1 month when the reaction occurred. This finding is consistent with the observation that patients receiving GA show a peak IgG titer 3 months after starting the injections [4] and may indicate that IgE to GA can appear very early in therapy, even in the absence of IgG antibodies.

In previous studies, analysis of antibody isotypes showed that IgG1 antibodies predominate at 3 months, while IgG4 antibodies tend to peak at month 9 of treatment and may be the main subclass in patients receiving long-term therapy [4, 6]. This switch in subclass profile has been interpreted as a consequence of the changes in the T_H1/T_H2 balance in patients

treated with GA (ie, increased T_H2 activity). However, in most patients it does not imply an increased tendency towards the production of IgE antibodies. Other authors have found that GA can induce IgE antibodies and anaphylaxis [4], although no risk factors have been recognized to date. In our study, 1 atopic control showed low titers of IgE to GA and a positive BAT result. Given his lack of exposure to GA, this positivity could be related to the probable heterogeneity of antigenic determinants generated by the random nature of synthetic peptides contained in GA. A possible association between sensitivity to tetanus toxoid and GA should be ruled out.

In conclusion, skin tests seem to be the most useful diagnostic approach in patients with anaphylaxis by GA. In vitro tests can prove to be a useful additional method for diagnosis, by providing evidence of an IgE-mediated mechanism in some cases. Nevertheless, results should be interpreted with caution.

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Azathioprine-Induced Sweet's Syndrome

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Key words: Sweet's syndrome. Azathioprine. Polyangiitis. Drug-Induced. Hypersensitivity.

Palabras clave: Síndrome de Sweet. Azatioprina. Poliangeítis. Inducido por drogas. Hipersensibilidad.

Sweet's syndrome is an infrequent disorder characterized by abrupt onset of erythematous plaques and nodules associated with fever, neutrophilic leukocytosis, and dense dermal neutrophilic infiltrates on biopsy [1]. It has been described as idiopathic, inflammatory (infection or inflammatory/autoimmune disease), neoplastic (especially hematological), pregnancy-associated, and drug-induced [2].

Most cases of Sweet's syndrome are idiopathic, and very few are induced by drugs. The drugs typically implicated are granulocyte colony-stimulating factor and all-trans retinoic acid [2].

We report a case of Sweet's syndrome induced by azathioprine.

A 79-year-old man with hypertension, microscopic polyangiitis, and rapidly progressive glomerulonephritis presented with malaise, high fever, and nonpruritic edematous and erythematous plaques on the head, the neck, the upper back, and the palms in association with conjunctival hyperemia and edema. The symptoms had begun 36 hours after starting azathioprine treatment. The patient was admitted 4 days later with suspected sepsis and immunosuppression; azathioprine was discontinued and treatment initiated with corticosteroids and antibiotics. The symptoms improved within 7 days and the patient was discharged.

One month later, the patient once again developed fever, malaise, and the same skin eruption with conjunctivitis. Twelve hours previously he had started to take azathioprine as treatment for polyangiitis. The drug was withdrawn, and all signs and symptoms resolved after 2 days of corticosteroid treatment.

In both episodes the patient had neutrophilia (> 70%), an elevated sedimentation rate, and C-reactive protein levels. Exhaustive tests failed to detect any infections.

Histological examination of a skin biopsy specimen showed the presence of edema and intense polymorphonuclear leukocyte infiltration in the reticular dermis, without leukocytoclastic vasculitis or dermal necrosis. The findings suggested a diagnosis of Sweet's syndrome.

Allergy diagnostic tests (prick, intradermal, and patch tests) with azathioprine were all negative.

Azathioprine is a prodrug for mercaptopurine (analog of adenine and hypoxanthine) widely used as an adjunctive immunosuppressive and corticosteroid-sparing agent in patients receiving solid-organ transplants, in rheumatology, dermatology, and gastroenterology [3].

In our case, the clinical and histological features were compatible with a diagnosis of Sweet's syndrome. A reaction to azathioprine was suspected due to the temporal relationship between drug administration and onset of lesions and the resolution of signs and symptoms after withdrawal. Recurrence after an oral challenge confirmed the suspected diagnosis of azathioprine-induced Sweet's syndrome. In the second episode the symptoms resolved sooner, presumably due to a shorter exposure to the drug.

The first convincing case of azathioprine-induced Sweet's syndrome was reported by Stapleton in 2003. Since then, only 6 cases with a plausible link to the use of azathioprine have been described. In 2 of these, the causal relationship was not firmly established because an oral challenge was not performed and the underlying diseases (myasthenia gravis and Crohn's disease) might have been responsible for the reactions reported. The other 4 reports were associated with inflammatory bowel disease [4-7]. In all of these cases, azathioprine was strongly implicated as the causal agent as there was a well-defined temporal relationship, resolution of lesions after drug discontinuation, and a new eruption after the reintroduction of azathioprine. In none of the cases were allergy tests carried out.

To the best of our knowledge, there are no reports of azathioprine-induced Sweet's syndrome associated with microscopic polyangiitis in the literature.

The pathogenesis of Sweet's syndrome is unknown. It has been defined as a type-III hypersensitivity phenomenon, although there is new evidence that suggests the involvement of other mechanisms, including elevated granulocyte colony-stimulating factor [8], associations with determined histocompatibility (Bw54)[9], and a possible role of antineutrophil cytoplasmic antibodies in the activation of neutrophils [10].

We emphasize the importance of evaluating azathioprine as a possible, though uncommon, cause of Sweet´s syndrome and of replacing it with a non-purine analog treatment if the involvement of azathioprine is confirmed.

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SCID-Like Phenotype Associated With an Inhibitory Autoreactive Immunoglobulin

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Key words: Severe combined immunodeficiency. Autoantibody. SCID-like phenotype. T-cell activation defect. Serum inhibitory factor.

Palabras clave: Inmunodeficiencia combinada grave. Autoanticuerpo. Fenotipo IDCG. Defecto de activación de linfocitos T. Factor inhibidor sérico.

Severe combined immunodeficiency (SCID) includes a number of distinct entities that share several clinical hallmarks such as life-threatening infections and intractable diarrhea [1], rapidly leading to growth failure and malnutrition. The clinical course is severe and patients usually die before the second year of life unless a stem cell transplantation is performed. SCID is currently classified into several groups based on the presence or absence of the major lymphocyte cellular components T, B or natural killer (NK) cells, with each group being suggestive of 1 or more genetic causes [1]. However, irrespectively of the individual genetic form, T cell–related functions are constantly abnormal, thus overall compromising a normal, productive

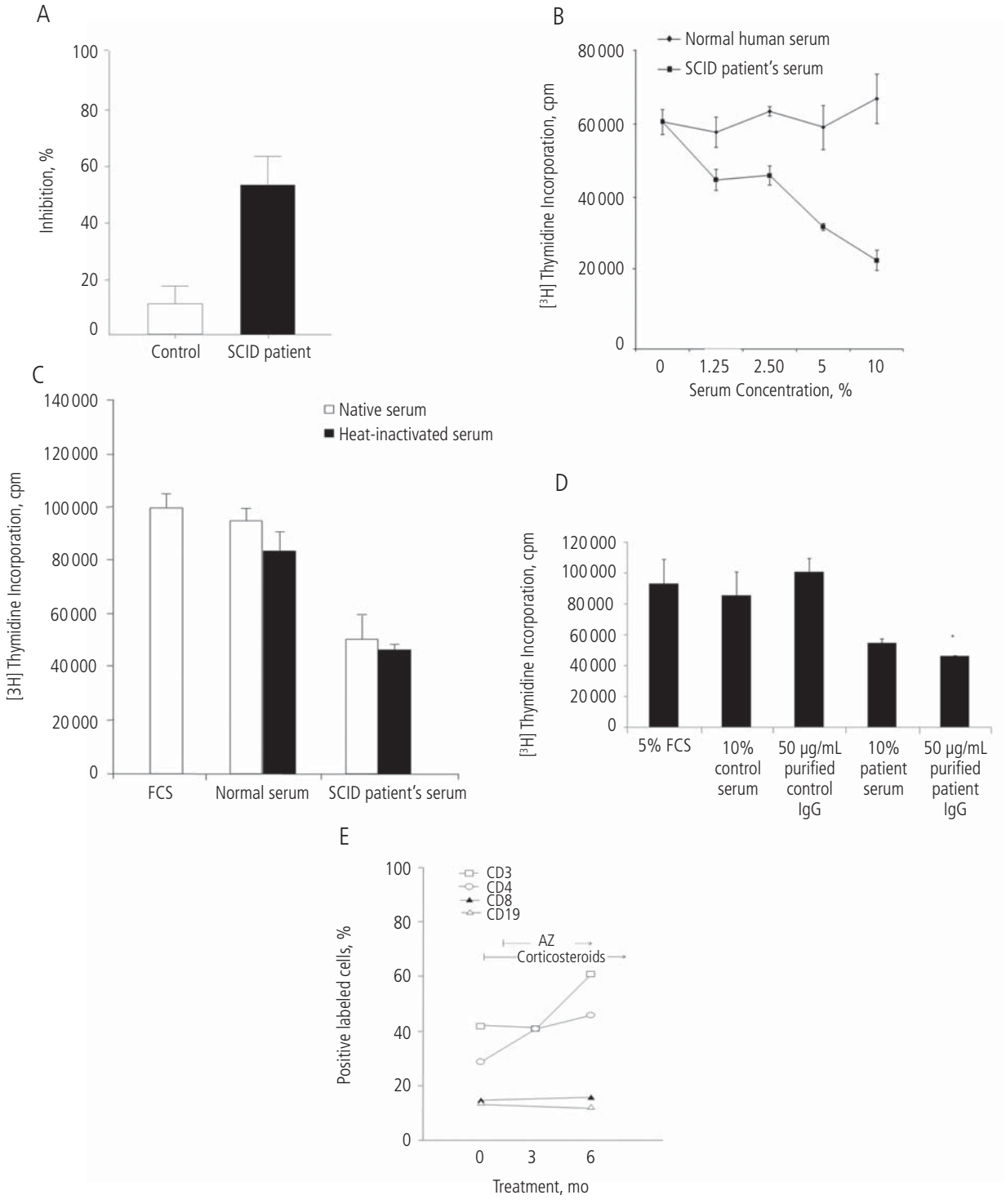

Figure. A, Inhibition (%) of normal peripheral mononuclear cell (PBMC) proliferation after stimulation with phytohemagglutinin (PHA, 8 µg/mL). B, [3H] thymidine incorporation by normal PBMCs stimulated with PHA, (8 µg/mL) and incubated with scalar concentrations (0%, 1.25%, 2.5%, 5%, and 10%) of normal human serum or serum from a patient with severe combined immunodeficiency (SCID). Each point represents the mean (SD), (n=3). C, Effect of native and heat-inactivated serum on proliferative response by normal PBMCs stimulated with PHA (8 µg/mL). Values are expressed as mean (SD) (n=3). D, Inhibition by patient's immunoglobulin (Ig) G of proliferative response by normal PBMCs stimulated with PHA (8 µg/mL). Negative control: fetal calf serum (FCS). Bars show means (SD). *Statistically significant difference ($P<.05$) compared to cultures containing purified control IgG fraction. E, Increase in the percentage of major lymphocyte subsets in patient during treatment with azathioprine (AZ) and corticosteroids. The horizontal lines indicate the period of immunosuppressive treatment with AZ (dotted line) and corticosteroids (solid line). Cpm indicates counts per minute.

immune response in all effector tasks [2]. Severe impairment of T-cell function, however, may also be acquired and induced by viruses, such as the human immunodeficiency virus (HIV) [3]. To date, no autoreactive anti-lymphocyte antibody capable of inducing a SCID-like phenotype has been described.

We report on a 3-year-old patient with a phenocopy of T_{low} B^+ NK^+ SCID. The patient was born at 42 weeks of gestation to healthy, unrelated parents. At 6 months of age the child was hospitalized because of chronic diarrhea, dystrophic features, and febrile seizures. At 8 months of age, the immunological evaluation revealed decreased immunoglobulin (Ig) G serum levels (<47 mg/dL) and normal IgA and IgM. Moreover, the patient had autoimmune hemolytic anemia. At the time of the study, lymphocytes were 3×10^9/L, with 34.5% of $CD3^+$ cells, 25.5% of $CD4^+$ cells, 15.3% of $CD8^+$ cells, 4% of $CD19^+$ cells, and 15% of $CD56^+CD3^-$ cells. Severe lymphocyte functional impairment, in the absence of infection by HIV or any other viruses, was noted. The proliferation assays using phytohemagglutinin (PHA, 8 µg/mL) was performed as previously described [4]. The patient's peripheral blood mononuclear cells (PBMCs) exhibited absent proliferative response to PHA (mean [SD] of 768 [61] counts per minute [cpm] vs 104 649 [21 743] cpm in controls). Over the 3-year follow-up, the patient's PBMC proliferative response ranged between 300 and 8095 cpm. To identify a potential inhibitory factor, the patient's serum was added to PBMCs from 5 healthy controls. A significantly higher inhibitory effect was noted in the patient's serum (53% [10%]) compared to that of the controls (12% [5%]) (Figure A). To define the potency of the inhibitory effect, scalar doses (0%, 1.25%, 2.5%, 5%, and 10%) of the patient's serum and the normal serum were used to produce a dose-response curve. A linear increase in inhibition was observed, with maximum inhibition being reached at the 10% concentration (23 729 [2701] cpm vs 67 050 [6638] cpm in the presence of the control serum) (Figure B). Serum heat inactivation did not abolish the inhibitory effect on the proliferative response to PHA in the control PBMCs, in that the inhibition was 47% (compared to 51% for native serum) (P>.05) (Figure C), thus ruling out a role of the complement in the phenomenon.

To evaluate whether the inhibitory effect in the patient's serum was attributable to an anti-lymphocyte autoantibody, affinity-purified IgG from both the patient's and the control serum was tested for the inhibitory property. Significant inhibition of the proliferative response of normal PHA-stimulated PBMCs was seen in the former but not in the latter case (46 204 [473] cpm vs 100 778 [8988] cpm; P<.05). The inhibition in the presence of the patient's IgG fraction was comparable to that observed with the patient's serum (P>.05) (Figure D).

Thereafter, a progressive decline in $CD4^+$ cells, resulting in a typical lymphocytopenic (0.5×10^9/L) T^- B^+ NK^+ form of SCID, was observed. A similar phenotype is generally due to an impairment in the T-cell differentiation process, resulting in a severe reduction in peripheral T-cell pool size associated with molecular alterations of genes implicated in T-cell ontogeny and function [5]. The prototype of severe T-cell defects, in which NK cells are also often compromised, is related to mutations of the *IL-2Rγ* gene. However, in our case, such gene alterations were ruled out. Two episodes of bronchopneumonia and an interstitial pneumopathy occurred despite intravenous IgG replacement therapy and antibiotic treatment. Autoreactive antibodies toward smooth muscle and red and white blood cells were detected. The patient also developed severely progressive active autoimmune hepatitis, which was diagnosed according to the scoring system established by the International Autoimmune Hepatitis group [6] and treated with azathioprine (1.5 mg/kg/d) and corticosteroids. Diarrhea, autoimmunity, and liver disorders are usually described in relation to T^+ oligoclonal B^-Omenn syndrome [7] and T_{low} B^+ $IL^-7R\alpha$ deficiency [8]. Even though these genetic defects were not ruled out, since at the time of evaluation this information was not available, the clinical and immunological features in our patient are quite different from those seen in these syndromes. At 4 years of age the patient died of disseminated interstitial pneumopathy while the search for an HLA-matched donor was still underway. During this period a paradoxical effect of immunosuppression on cell subsets was noted in that, as depicted in Figure E, there was an increase in $CD3^+$ cells from 42.0% to 60.9% and in $CD4^+$ cells from 28.0% to 46.3%. By contrast, $CD19^+$ and $CD8^+$ cells did not change significantly.

Our data indicate a direct role of the antibody as a negative regulator of T-cell function. However, it is also possible that the inhibitory autoantibody in our patient was the consequence of hyperimmune dysregulation rather than of the T-cell defect, whose genetic alteration still remains to be identified. A similar serum inhibitory effect has also been described in epilepsy, leading to moderate T-cell dysfunction associated with impairment of other immunological cell activities and a decrease in the C4 complement component [9]. However, this functional lymphocyte defect was not as severe as that reported in our patient. Although the functional defect observed in our case may theoretically be related to viral-induced anergy [10], no viral infection was documented and the functional defect was completely differently from that observed in viral-induced anergy.

In conclusion, we have documented a novel pathogenetic mechanism due to an inhibitory anti-lymphocytic autoantibody in a SCID-like phenotype, resulting in total T-cell activation deficiency associated with autoimmunity. This complex phenotype represents a phenocopy of the congenital forms of SCID.

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Bronchospasm Induced Selectively by Paracetamol

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Key words: Paracetamol. Bronchospasm. Neurogenic inflammation.

Palabras clave: Paracetamol. Broncoespamo. Inflamación neurogénica.

It is well known that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) can trigger bronchospasm in susceptible individuals as a result of inhibitory cyclooxygenase (COX) activity. Paracetamol (acetaminophen) is a weak inhibitor of this pathway, but in some patients with NSAID idiosyncrasy, high doses of paracetamol can also provoke bronchospasm.

Although in recent years several epidemiological studies have reported an increased risk of asthma in relation to paracetamol use [1,2], to our knowledge, no cases of selective bronchospasm induced by paracetamol have been published.

We report the case of a patient who developed bronchospasm after paracetamol intake.

A 19-year-old woman was referred to our hospital because she had experienced 8 to 10 episodes of dyspnea within 10 minutes of taking paracetamol over the previous 3 years; this had not occurred with any other NSAIDs (metamizole, ibuprofen). The patient also had a clinical history of rhinitis associated with contact with cats and bronchospasm after exercise.

Skin prick tests to aeroallergens showed positivity for *Alternaria alternata* and for *Olea europaea* pollen. Total immunoglobulin (Ig) E was 41 kU/L and specific IgE to *Alternaria alternata* and cat dander was 3.47 kU/L and 0.23 kU/L, respectively. Spirometry showed mild ventilatory alteration and the bronchodilator response was positive (>12% increase in forced expiratory response in the first second [FEV_1]).

With a diagnosis of rhinitis and asthma with *Alternaria alternata* sensitization, we performed a study with paracetamol with the patient's consent. The prick test (10 mg/mL) and intradermal test (1 mg/mL) to paracetamol were negative. An oral challenge test with paracetamol (100-250-500 mg) and acetylsalicylic acid (ASA) (500 mg) was carried out on different days. Spirometry was performed at baseline and 10 minutes after the intake of placebo and the above-mentioned drugs. The challenge was considered positive when there was a decrease of over 12% in FEV_1 compared to baseline. Salbutamol inhalation was used to evaluate bronchial reversibility after the paracetamol challenge.

Ten minutes after the administration of 500 mg of paracetamol the patient presented bronchospasm and a significant decrease in spirometric values, which returned to baseline after salbutamol inhalation. There were no other systemic or cutaneous symptoms. The administration of 500 mg of ASA did not induce any changes (Table).

To our knowledge this is the first report that confirms the induction of bronchospasm by paracetamol without the involvement of other NSAIDs.

The mechanism by which paracetamol induces bronchospasm in our patient is unclear.

NSAIDs have the ability to induce bronchospasm by inhibiting COX-1, and paracetamol is a weak inhibitor of this enzyme. The fact that high doses of ASA did not induce bronchospasm in the patient makes it highly unlikely that paracetamol acts in this pathway. We also failed to demonstrate specific IgE by means of skin tests.

While a neurogenic mechanism has been implicated as responsible for airway inflammation [3], some authors, based on experimental studies, have more recently suggested that the activation of specific receptors expressed on sensorial neurons could induce inflammation in the airways. In this

Table. Spirometric Values of Oral Challenge Test with Paracetamol and Acetylsalicylic Acid (ASA)

	Paracetamol (500 mg)				ASA (500 mg)	
	Baseline (% of predicted)[a]	Placebo (% var)[b]	Paracetamol (% var)[b]	After salbutamol inhalation (% var)[c]	Baseline (% var)[a]	ASA (% var)[b]
FVC	2.99 (72)	2.95 (0)	2.60 (-13)	2.91 (11)	3.10 (74)	3.10 (0)
FEV_1	2.70 (76)	2.53 (-5)	2.10 (-28)	2.53 (18)	2.72 (76)	2.77 (2)
FEV_1/FVC	90.37 (104)	85.85 (-4)	80.86 (-10)	86.91 (7)	87.90 (101)	89.36 (2)
FEF 25%-75%	3.38 (81)	2.91 (-14)	1.97 (-71)	3.03 (42)	3.30 (78)	3.27 (0)

Abbreviations: FEF, forced expiratory flow; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; var, variability.
[a]% of variability with respect to reference values.
[b]%of variability with respect to baseline values.
[c]%of variability with respect to values obtained in paracetamol challenge.

respect, TRPA1, a member of the transient receptor potential cation channel family, could be the main neuronal mediator in the induction of asthma [4]. TRPA1 is activated by reactive substances and also by N-acetyl-p-benzoquinoneimine (NAPQI), an acetaminophen metabolite.

Studies performed on mice have shown that administration of acetaminophen (at a single or repeated dose) quickly produces detectable levels of NAPQ1 in the lungs, and also increases neutrophil infiltration, myeloperoxidase activity, and cytokine and chemokine levels in the airway [5].

In conclusion, we have reported a case of a patient with bronchospasm induced by paracetamol, but not by other NSAIDs. We propose the implication of a neurogenic mechanism activated by a metabolite of this drug.

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Oral Desensitization to Egg Enables CD4+FoxP3+ Cells to Expand in Egg-Stimulated Cells

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Key words: Egg allergy. Food allergy. Oral desensitization. Treg. FoxP3.

Palabras clave: Alergia al huevo. Alergia alimentaria. Desensibilización oral. Treg. FoxP3.

The only current treatment option in food allergy is strict allergen avoidance with appropriate pharmacological treatment in the event of accidental ingestion. Specific desensitization in food allergy can be induced by controlled oral administration of the offending food. The initial daily doses are very low and are gradually increased until an amount equivalent to a usually relevant daily intake is achieved; this must be followed by a daily maintenance dose.

Regulatory T cells (Tregs), which are a subset of $CD4^+$ cells with immunoregulatory properties, have been described as a

key component in the induction and maintenance of immune tolerance to allergens [1].The phenotypic and functional characterization of Tregs has been a focus of intense study. It is now well established that forkhead box (Fox) P3 acts as a master switch transcription factor for Treg development and function [2]. FoxP3 expression closely correlates with $CD4^+CD25^{high}$ cells in humans [3]. The induction and maintenance of peripheral tolerance depend on the generation of Tregs, which can be affected by specific circumstances and microenvironments. It has been shown that increased numbers of $FoxP3^+$ $CD25^+$ Tregs in immunotherapy correlate with clinical efficacy [4]

We recently reported an oral rush egg desensitization protocol in which desensitization was performed using pasteurised raw egg white mixed with a food product well tolerated by the patient (yoghurt, milkshake) [5]. The protocol for the oral administration of pasteurized egg is described in this previous report. Desensitization was considered complete when tolerance was achieved for 8 mL of raw egg white (1 egg=30 mL of egg white) and a whole cooked egg. Patients who achieved tolerance to a whole egg continued with daily ingestion of 1 egg for 3 months, followed by 1 egg every 48 hours for a further 3 months. The criteria for including patients in the protocol were an age of over 5 years, a history suggestive of immediate allergy to egg, and immunoglobulin (Ig) E–mediated egg allergy demonstrated by a positive skin prick test (SPT) to egg and/or the detection of serum specific IgE (sIgE) to egg white.

Here we report the results of the impact of oral desensitization on $CD4^+$ $FoxP3^+$ cell numbers, and $CD4^+FoxP3^+$ cell expansion in egg white–stimulated cells. Twenty patients included in the desensitization protocol and 9 controls not included and matched at baseline for clinical characteristics (age, sex, sIgE, sIgG4, and SPT) were recruited. Peripheral blood mononuclear cells were stimulated with 1% of pasteurised raw egg white or cultured in medium alone for 48 hours. Determinations were made prior to starting the desensitization protocol, at 3 weeks, and for some patients (n=9) at 12 weeks. For ethical reasons the control group could not be studied at 12 weeks as they were included in the desensitization protocol. $CD4^+FoxP3^+$ cells were assessed by flow cytometry. Intracellular staining for FoxP3 was performed using phycoerythrin-conjugated antibody, and fixation and permeabilization buffers provided with the FoxP3 kit (BD Biosciences, San Diego, California, USA).

Eighteen patients (90%) achieved desensitization to egg. Unlike controls, children undergoing the desensitization protocol showed significantly increased $CD4^+FoxP3^+$ expansion in egg white–stimulated cells at 3 weeks. Cell expansion secondary to in vitro egg stimulation persisted at 12 weeks, though it did not differ significantly from the results obtained at 3 weeks (Figure A). Desensitization seems to enable the expansion of $FoxP3^+$ cells in food allergy as confirmed in a report of peanut allergy desensitization where peripheral $FoxP3^+$ T cells increased in peanut-stimulated cells [6]. All these data suggest that oral desensitization has a powerful effect on the expansion of $CD4^+FoxP3^+$ cells. It is interesting to note that just before starting the desensitization protocol, egg white–stimulated cells multiplied fivefold the number of

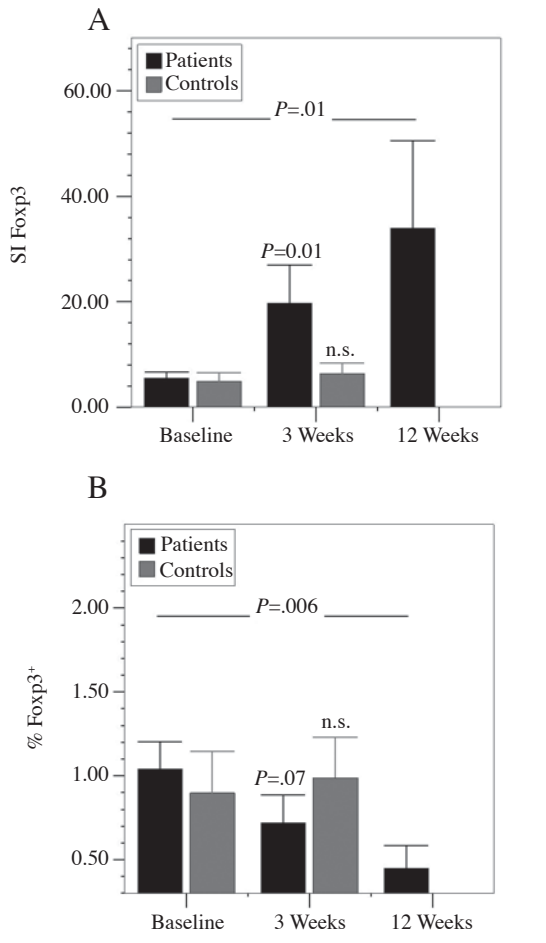

Figure. Effect of oral egg desensitization protocol on the increment of $CD4^+FoxP3^+$ cells in egg white–stimulated cells. The effect of stimulation is expressed as the ratio between egg white–stimulated cells and their respective controls without stimulation (SI FoxP3). The results are expressed as the mean with 95% confidence interval (CI). The black bars represent the children included in the oral desensitization protocol (n=20), and the gray bars the controls not included in the protocol (n=9). B, Effect of oral egg desensitization protocol on the peripheral decline in $CD4^+FoxP3^+$ cells. The results are expressed as the mean (with 95% CI) of the percentage of $CD4^+FoxP3^+$ cells in unstimulated cells. The black bars represent the children included in the oral desensitization protocol (n=20), and the gray bars the patients not included in the protocol (n=9). n.s. indicates nonsignificant.

$CD4^+$ $FoxP3^+$ cells. This expansion did not occur in nonatopic individuals (data not shown).

Otherwise, the number of $CD4^+FoxP3^+$ cells in peripheral blood fell progressively during desensitization. In the absence of in vitro egg white stimulation, peripheral $CD4^+FoxP3^+$ cells showed a decreasing trend at 3 and 12 weeks, as shown in Figure B. The decline in peripheral $CD4^+FoxP3^+$ cells was not observed in the control group. It is surprising that a decrease in peripheral cells was seen at all. Egg-specific Tregs would account for a very small minority and it is difficult to understand how immunotherapy would cause an apparent decline in the whole $CD4^+$ $FoxP3^+$ population, which should comprise cells of different clonalities to different antigens.

However, a similar decline in $CD4^+FoxP3^+$ cells was recently reported in wasp venom immunotherapy, demonstrated by detection of massive Treg homing to lymph nodes by in vivo ultrasonography [7]. Desensitization promotes the circulation of Tregs from the periphery to lymph nodes.

Only 2 patients abandoned the oral rush desensitization protocol due to repeated reactions, and both of these showed normal $CD4^+FoxP3^+$ cell expansion. These findings suggest that tolerance depends on more than just the number of regulatory cells. Treg function in allergic patients could be affected by various genetic and environmental factors [8]. As Tregs are highly dependent on growth factors produced by effector cells, they may not be optimally stimulated and therefore may not function properly in vivo. We have previously reported that patients with marked effector function in terms of IgE levels or SPT results required a longer desensitization protocol [5].

In conclusion, providing very low and gradually increasing doses of allergen is critical to the expansion of Tregs, thus encouraging allergen desensitization. Oral desensitization enables allergen-induced $FoxP3^+$ cell expansion and seems to promote their movement from the periphery.

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Atopic Manifestations in Patients With Ulcerative Colitis: A Report From Chile

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Key words: Atopy. Asthma. Allergic disease. Chile. Ulcerative colitis.

Palabras clave: Atopia. Asma. Enfermedad alérgica. Chile. Colitis ulcerosa.

The pathogenesis of ulcerative colitis (UC) is a complex interplay between genetic and environmental compounds. Epidemiological studies have shown that chronic inflammatory diseases such as psoriasis and atopic manifestations cluster in patients with inflammatory bowel disease (IBD) [1]. A definitive biological explanation of why atopic diseases are associated with IBD has not yet emerged [2]. We report on a study in which we analyzed the association between atopic disorders, including asthma, and UC, in the central southern region of Chile.

We performed a hospital-based, case-control study involving 52 patients with UC and 174 controls in 2 public university hospitals in Chile (Valdivia and Temuco) in 2009/2010. The cases (age range, 6-45 years) comprised all patients with an established diagnosis of UC according to the International Classification of Diseases (ICD-10). Diagnoses were established by routine clinical practice, and all patients were attending specialist gastrointestinal clinics. Controls were

Table. Physician-Diagnosed Asthma, 12-Month Prevalence of Atopic Manifestations, and Skin Prick Test Sensitization in Patients With Ulcerative Colitis and Controls[a]

	Cases	Controls	OR (95% CI)[b]
Physician-diagnosed asthma, no. (%)	15.4 (8)	9.2 (16)	0.9 (0.8-1.1)
12-month prevalence of:			
Wheeze	5.9 (3)	8.1 (14)	0.7 (0.2-2.6)
Allergic rhinitis	11.5 (6)	10.3 (18)	1.2 (0.6-2.2)
Allergic rhinoconjunctivitis	36.5 (19)	29.3 (51)	1.4 (0.7-2.8)
Atopic dermatitis	19.2 (10)	16.7 (29)	1.4 (0.6-3.2)
Any positive skin prick test[c]	42.9 (21)	47.3 (69)	0.8 (0.4-1.6)
Weed	20.4 (10)	13.7 (20)	1.6 (0.7-3.8)
Pasture	22.5 (11)	12.3 (18)	1.9 (0.9-4.6)
Trees	8.2 (4)	7.5 (11)	0.9 (0.3-3.2)
Dermatophagoides pteronyssinus	30.6 (15)	37.7 (55)	0.7 (0.3-1.4)
Acarus siro	30.6 (15)	27.4 (40)	1.1 (0.5-2.3)
Alternaria tenuis	6.1 (3)	12.3 (18)	0.4 (0.1-1.5)
Cockroach	12.2 (6)	15.8 (23)	0.7 (0.3-1.9)
Cat hair % (No.)[a]	2 (1)	7.5 (11)	0.2 (0.003-1.8)
Dog hair % (No.)[a]	20.4 (10)	17.8 (26)	1.1 (0.5-2.5)

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Results are presented as number (%) of patients unless otherwise specified.
[b]Adjusted for age and sex.
[c]Skin prick tests were performed in 49 cases and 146 controls.

recruited from different departments at the same hospitals including dentistry (26%), neurology (eg, migraine patients) (21%), and gynecology (13%).

Cases and controls underwent a face-to-face-interview and skin prick testing (SPT). SPT was performed in 94% of cases (49/52) and in 83% of controls (146/174). Atopy was defined as sensitization to at least 1 of the following allergens: cat hair, dog hair, cockroach, house dust mites (*Dermatophagoides pteronyssinus, Acarus siro*), mold (*Alternaria tenuis*), trees, and local grass and weed mix (Laboratorio Leti, Madrid, Spain).

The study protocol was approved by the ethics committees at the Ludwig-Maximilians-University in Munich, Germany, the Medical Faculty of the Universidad Austral de Chile in Valdivia, Chile and the Medical Faculty of the Universidad de la Frontera in Temuco, Chile. All participants gave written informed consent.

χ^2 tests were used to compare cases and controls in terms of demographic characteristics, asthma, and atopic diseases. To determine the association between UC and atopic diseases (including asthma), unconditional logistic regression models were applied, with adjustments for age (<26 years/≥ 26 years) and sex (male/female). Results were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were performed using SAS 9.2. (SAS Institute Inc, Cary, North Carolina, USA).

The mean (SD) age of cases was slightly higher than that of controls (27.4 [8.3] vs 26.2 [8.2] years). The proportion of men in the 2 groups was 44% and 33%, respectively (P>.05) (data not shown). Allergic rhinitis was reported by 12% of cases and 10% of controls, allergic rhinoconjunctivitis by 37% of cases and 29% of controls, and symptoms of atopic dermatitis by 19% of cases and 17% of controls (P>.05).

More than 40% of the study participants were sensitized to at least 1 allergen, but no statistically significant differences were seen between cases and controls. The most common sensitizing agent was *D pternonyssinus* (31% in cases and 38% in controls) followed by dog hair (20% in cases, 18% in controls). Adjustment for age and sex did not change the results (Table).

Our results show that atopic manifestations are comparable between patients with UC and controls living in the central southern region of Chile. With a prevalence of 43% in cases and 47% in controls, atopic sensitization was generally high.

Our findings contrast with those of most studies that have examined the relationship between atopic diseases and IBD [2]. Furthermore, most of these studies have focused either on Crohn disease [2] or on isolated cases [3], and none have been carried out in Latin America.

In our series, patients with UC were more likely than controls to have atopic dermatitis, although the difference did not reach statistical significance. A large, prospective epidemiologic study from Japan, in contrast, reported a statistically significant association between atopic dermatitis and UC [4]. One explanation for this possible association could be the sharing of cytokine-related pathways considering that AD and UC are both characterized by skin barrier dysfunction [4]. Genome-wide association studies have failed to detect common loci for AD and UC, but atopy-related genes (eg *IL-4, IL-13*) have been linked to Crohn disease [5] and they might also be linked to UC.

In line with our results, a German multicenter study found a significant association between allergic rhinitis and Crohn disease but not UC [6]. In another study which examined the relationship between IBD and chronic sinonasal disease, the prevalence of AD was significantly higher in patients with Crohn disease (53%) than in those with UC (32%) [7].

There is conflicting evidence regarding the association between asthma and UC. Some studies, for example, have used a cross-sectional design [1] and are therefore more prone to detection bias. In line with our results, a population-based case-control study of asthma and IBD did not find an association between the 2 conditions [8].

Forty-two percent of patients with UC in our study had SPT reactivity. This proportion is very similar to that reported by a Belgian study of 38 patients with UC [9]. Interestingly, in our study a slightly higher proportion of controls than cases had positive SPT results. This could be a chance finding based

on the fact that our study population was rather small. On the other hand, the fact that atopic sensitization was high in our study population possibly reflects the previously reported high prevalence of atopic sensitization in the general Chilean population [10].

One strength of our study is that we used SPT as an objective measurement of atopic sensitization. A comparison of those who underwent SPT and those who did not failed to reveal selection bias with respect to age and sex (data not shown). Our sample is relatively small compared to samples used in European studies [6], possibly explaining why our results did not reach statistical significance. We were unfortunately unable to recruit more participants as we contacted all eligible UC cases in the study area and most of them participated.

In conclusion, atopic manifestations were similar in patients with UC and controls in a Chilean case-control study. Further research is needed for this genetically different UC population.

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Specific Oral Tolerance Induction (SOTI) to Egg: Our Experience With 19 Children

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Key words: Egg allergy. Specific oral tolerance induction. Children.

Palabras clave: Alergia al huevo. Inducción específica de tolerancia oral. Niños.

Egg is the most common cause of food allergy in children [1]. The allergy tends to appear before the age of 2 and in 55% of cases, it disappears in the first 6 years of life [2,3]. Little information is available on the safety and efficacy of specific oral tolerance induction (SOTI) to egg in children. We present an egg SOTI protocol created based on previously published protocols, and report on its safety and efficacy.

This was a quasi-experimental, prospective, descriptive study. Male and female children (age, 3-14 years) with egg allergy were recruited from the pediatric allergy division at Hospital Universitario Dr. Peset Valencia, Spain between January 2008 and January 2010. The inclusion criteria were 1) a diagnosis of immunoglobulin (Ig) E–mediated allergy to egg, 2) a confirmed, reproducible diagnosis of clinical symptoms by

Table. Population Characteristics

Population (n=19)	Males=12 (63%)/Females=7 (36%)	
Age – At onset of symptoms – At start of protocol – Of children who started SOTI in the last semester of the study period	Mean (Range) 14 mo (5-48) 7 y (3-14) 5 y (3-6.7)	
Total IgE (kU/L) – At start of study – At end of study	Mean (Range) 463.5 (5.8-2247) 32 (3.7-1345)	
	Yes, No. (%)	No, No. (%)
Family history of atopy	6 (32%) 3 cases of first-degree relatives	13 (68%)
Personal history of atopic dermatitis	10 (53)	9 (47)
History of prematurity	0 (0)	19 (100)
Breastfed[a]	13 (68)	6 (32)
Allergy to other foods in addition to egg – Both fish and nuts – Milk – Fruit – Shellfish	10 (53) 5 (50) 4 (40) 3 (30) 2 (20)	9 (44) – – – –
Allergic respiratory disease: bronchial asthma or rhinitis	8 (42)	11 (58)
Determination of allergen-specific IgE (CAP Test) for inhalant allergens – Mites – Molds – Pollen – Epithelia of domestic animals	Positive, No. (%) 12 (62) 12 (62) 10 (53) 7 (37.5)	Negative, No. (%) 7 (38) 7 (38) 9 (47) 12 (62.5)
Clinical symptoms[b] – Urticaria – Vomiting – Angioedema – Generalized erythema – Mild anaphylaxis (25%) – Abdominal pain – Diarrhea	No. (%) 12 (62.5) 9 (50) 7 (37.5) 5 (25) 5 (25) 3 (15.8) 2 (12.5)	

Abbreviations: Ig, immunoglobulin; SOTI, specific oral tolerance induction.
[a]This variable was not related to the final result of desensitization. (Results were not statistically significant using the Fisher exact test, P=.088). The type of feeding followed (breast vs artificial) does not influence whether or not tolerance was achieved using the procedure (SOTI).
[b]The clinical manifestations (adverse reactions) to egg white protein were IgE-mediated immediate clinical reactions in most cases (onset of symptoms within 30 minutes of ingestion of egg).

an open oral-controlled provocation test, 3) written informed consent from the children's parents or legal guardians, 4) an adequate level of understanding and cooperation by patients and legal guardians, 5) an ability to maintain regular treatment and to attend all necessary visits to complete the treatment, and 6) access to clinical control or emergency care in the event of a reaction.

Patients were considered to have IgE-mediated egg allergy when the following criteria were met: 1) a clear history of immediate hypersensitivity reaction after egg ingestion; 2) a positive skin prick test (10 mg/mL) to egg and egg fractions (white [5mg/mL], yolk [5mg/mL], ovalbumin [1mg/mL], and ovomucoid [5mg/mL]), (Leti S.L. Laboratories, Madrid, Spain), performed according to the guidelines of the European

Academy of Allergy and Clinical Immunology [4]; and 3) a positive egg white challenge test.

The SOTI to egg protocol involved the administration of increasing amounts of egg at weekly intervals (regular administration of egg in progressive quantities from 0.5 mg to 30 g over 16 weeks), with the aim of inducing oral tolerance while protecting patients from a reaction caused by accidental ingestion. The first dose of each increase in quantity was always administered at the hospital. For the SOTI, we used Guillen pasteurized egg white, acquired in a local supermarket (Mercadona S.A.) (300 mL-containing 33.3 mL per egg white, which is the equivalent of approximately 34 mL of natural egg white). No ingestion of egg was allowed outside of the scheduled protocol. Oral antihistamine was not given to the patients during the up-dosing period. When an intercurrent illness occurred (common cold and/or fever) during the SOTI, the dose of egg was not increased, and the last dose was repeated. Adverse events were recorded during the study on a form that recorded all events, and the reactions were classified as local or systemic.

The ideal situation would have been to compare these results with ones from a control group. However, given the pioneering nature of this research, we did not have one. It was therefore decided to compare the percentage of patients who achieved tolerance with the expected rate of spontaneous remission in the general population. Data were analyzed using SPSS (version 17.0). Despite the small sample size and the fact that only nonparametric tests were used in the statistical inference, the Kolmogorov-Smirnov test was used to check the normality of distribution. We assumed a significance level of $P<.05$.

We demonstrated the efficacy and safety of the protocol (Table). Tolerance was achieved for a maximum dose of 30 g of egg after 16 weeks, after which the patients had 2 weekly meals with egg for several months followed by at least 1 meal with egg weekly. They were advised to eat at least half an egg, in whatever form, at each of these meals. In accordance with the null hypothesis, it was checked that the proportion of tolerance to egg achieved in our study (89.5%) was similar to the expected rate of spontaneous remission in the general population (55%) according to a review of the literature [2]. The result showed a significant difference ($P<.002$). We therefore rejected the null hypothesis at the specified CI (95%) and concluded that the SOTI procedure achieved a significantly greater tolerance to egg than that produced by simple spontaneous remission. Of the variables analyzed for statistically significant differences in terms of achievement of tolerance was age at onset of symptoms ($P<.027$), i.e., bothersome symptoms appeared at an earlier age in children who completed the protocol succesfully than in those who did not (2 /19).

SOTI was well tolerated and there were no life-threatening reactions. Seventeen patients (89.5%) completed the protocol with good tolerance. The remaining 2 patients had a local reaction (oral erythema) during the administration of 1 or several doses but these reactions subsequently remitted. One patient tolerated up to 30 g of egg; previous doses had triggered mild abdominal pain, which subsided in 20 minutes. Two (10.5%) of the 19 patients had to leave the study because of anaphylactic reactions at the initial doses, with total doses of 25 g and 200 mg in both cases. Both patients received intramuscular adrenaline, corticosteroids, oral antihistamines, and inhaled salbutamol and recovered quickly.

The children who reached partial tolerance were protected against potentially lethal allergic reactions due to accidental ingestion of small quantities of egg. SOTI is not devoid of severe adverse events, but the risk of a reaction due to inadvertent ingestion is certainly higher than the risk of a reaction during medically supervised desensitization. Our protocol proved to be efficacious and safe. It is therefore worthwhile weighing costs against benefits, especially in terms of patient health. It has been proven that the discomfort experienced by a patient during SOTI is minimal, as are the financial costs involved. Moreover, including egg in a patient's diet offers obvious advantages from a nutritional point of view. Furthermore, the risk to patients due to exposure to egg as a hidden allergen should be considered as it can trigger very serious reactions.

Finally, we believe that our protocol will make a significant contribution to helping improve the prognosis and quality of life of children with egg allergies.

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Severe Primary Antibody Deficiency Due to a Novel Mutation of μ Heavy Chain

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Key words: Agammaglobulinemia. Antibody deficiency. B cell development. Mutation. Immunodeficiency. Infection.

Palabras clave: Agammaglobulinemia. Deficiencia de anticuerpos. Desarrollo de linfocitos B. Mutación. Inmunodeficiencia. Infección.

Primary antibody deficiencies are the most common primary immunodeficiency diseases in children [1]. A subset of patients are affected by the loss of genes implicated in the maturation of B cells in the bone marrow. Early development of B cells is characterized by maturation of the B cell receptor (BCR) and its downstream signaling pathways. BCR maturation includes 2 checkpoints, namely a pro-B-cell stage ($CD34^+CD10^+CD19^+$) and a pre-B-cell stage ($CD34^-CD10^+CD19^+$). During the pro-B-cell stage, different components of BCR are expressed onto the surface of B cells to create appropriate conditions for the pre-BCR stage. Intact pre-BCR signaling leads to the rearrangement of light chains and the expression of BCR on naïve B cells [2]. B cells migrate to secondary lymphoid organs for further adaptive maturation and eventually plasma cell and memory B-cell formation.

Bruton tyrosine kinase (BTK) plays a crucial role in the pre-BCR signaling pathway. *BTK* mutations are responsible for X-linked agammaglobulinemia (XLA), which is the prototype of early B-cell maturation disorders [3]. Other known causes in humans are autosomal recessive mutations of pre-BCR components (μ-heavy chain [μHC], CD79a, CD79b, λ5) and BLNK, which is a scaffold protein in pro-BCR signaling [4].

Both XLA and recessive forms of agammaglobulinemia share 3 diagnostic criteria: profoundly decreased levels of serum globulins, recurrent bacterial infections, and absence of circulating B cells. However, clinical course may vary and therefore mutation analysis is needed to clearly identify etiology. Here, we present the case of a patient with impaired early B-cell maturation and severe clinical presentation in whom we found a novel homozygous mutation in the gene coding for μHC.

A 20-year old man was referred due to a life-long history of recurrent infections. He was the second child of consanguineous parents and had an older sister and a younger brother, both healthy. There were no signs of immunodeficiency in any other members of the family. The patient was fully immunized at birth, and no complications were observed.

He remained well until the age of 3 months, when he developed frequent pneumonia episodes, requiring several outpatient admissions, followed by recurrent arthritis with hospitalization every other year. Due to the destruction of joints, he had an unusual gait. At 10 years of age, he lost his hearing due to severe recurrent otitis media. Unfortunately, there was no information about the causative pathogens. We also noticed growth retardation (below the third percentile for his age group), unilateral inguinal hernia, delayed pubertal development, and small-for-age genitalia. Moreover the clubbing of his fingers indicated a prolonged respiratory disorder, confirmed by spirometry.

The levels of all immunoglobulin isotypes were below normal: immunoglobulin (Ig) G=25 mg/dL (normal range, 656-1350 mg/dL), IgM=4 mg/dL (normal range,120-320 mg/dL), IgA=absent (normal range, 86-320 mg/dL), IgE=1 IU/L (normal range, 0-46 IU/L). Immunophenotyping of peripheral blood lymphocytes showed extensive reduction of B cells (<1% of total lymphocytes) with a normal T-cell population. At 3 years of age, the patient was put on prophylactic antibiotics and intramuscular immunoglobulin, which was switched to intravenous immunoglobulin (IVIg) at the age of 5 years. Unfortunately, he died due to septicemia and disseminated intravascular coagulation at the age of 20 years.

Western blot analysis disclosed normal BTK expression. Given the consanguinity of the parents, we sequenced known genes previously shown to be associated with a similar phenotype (see above), and found a novel homozygous mutation in the gene coding for μHC. A frame shift insertion in the first exon (c.525-526 ins. C) led to a premature stop codon in the CH2 domain.

Disorders with impaired antibody production can be categorized according to early or late B-cell maturation. Defects affecting early B-cell development are associated with a reduced number of circulating B cells, whereas in later stages, B cells are not able to efficiently produce antibodies. While our understanding of early B-cell maturation is relatively solid, much remains to be learned about later stages. In fact, most patients with defective antibody production belong to this second category. Although mutated genes involved in somatic hypermutation, class switch recombination, and germinal center formation have been implicated in a substantial number of patients [5, 6], we do not have a clear explanation yet.

Patients with defective early B-cell maturation in the bone marrow are usually treated with life-long monthly IVIg infusions. Indeed, the introduction of agammaglobulinemia by Bruton in 1952 and its successful management [7] was followed by a series of observations indicating the relative benefits of parenteral immunoglobulin [8].

We have reported on a rather complicated case of antibody deficiency. The patient was clinically diagnosed at

3 years of age and subsequently received adequate treatment (400 mg/month IVIg) with conventional therapy. Despite this, however, he did not survive beyond his twenties. It is notable that the first manifestations were seen at 3 months, which is a relatively young age compared to the situation in XLA patients [3]. Given that early diagnosis of antibody deficiencies can dramatically improve treatment outcomes [9], the patient might have been a victim of delayed diagnosis. He also had recurrent bacterial arthritis, which is not a usual presentation of XLA [3]. Most patients with XLA reach a normal life span with IVIg and prophylactic antibiotics. It has been suggested that XLA is a leaky form of B-cell maturation block when compared to autosomal recessive forms of the phenotype [2]. Therefore, delayed diagnosis in recessive forms of agammaglobulinemia might have more dramatic consequences.

The patient also had delayed secondary sexual growth and developmental anomalies. To the best of our knowledge, pure B-cell deficiencies have not been described as a syndromic disorder. However, given the fact that the patient's parents were consanguineous, it might be possible that he was affected by other mutated genes. We cannot rule out such a putative coincidence.

In conclusion, we have described a novel mutation in the gene coding the μHC compartment of BCR. Our case report endorses the face that pro-B arrests show a more severe presentation than XLA. B-cell biology at its very early development stages has still as many obscure corners as in later stages.

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ERRATUM:

The title of the manuscript "High Prevalence of Asthma and Allergic Diseases in Children Aged 6 and 7 Years From the Canary Islands: The International Study of Asthma and Allergies in Childhood" published in Vol 19 nº 5 of JIACI should read as follows:

"High Prevalence of Asthma and Allergic Diseases in Children Aged 6 to 7 Years From the Canary Islands".
The running title should read "Allergy Prevalence in Canary Island Children"