

Inhaled α 1-Antitrypsin Administered to Treat Pneumatocele in Autosomal Dominant Hyperimmunoglobulin E Syndrome

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Members of the serine protease, matrix metalloproteinase, and cysteinyl protease families have been found to be associated with lung inflammation and airway extracellular matrix [1]. The serine protease neutrophil elastase induces protease and tissue destruction in the lung [1]. Protease-antiprotease imbalance in humans has been described in chronic destructive lung diseases such as cystic fibrosis and α -1 antitrypsin deficiency [2,3]. Aerosolized α -1 antitrypsin therapy has been administered to restore the protease-antiprotease imbalance and inhibit destructive lung inflammation [4,5].

Autosomal dominant hyperimmunoglobulin E syndrome (AD-HIES) causes significant lung destruction and pneumatoceles owing to aberrant inflammatory responses without the classic findings of inflammation (warmth, redness, and fever) [6]. Pneumatocele is a common and particularly problematic complication of AD-HIES. However, the only effective approach for the management of progressive lung destruction involves antibiotic prophylaxis and therapy.

While susceptibility to recurrent infections due in part to

decreased subtype 17 helper T-cell formation [7] contributes to lung tissue damage, intrinsic susceptibility to exaggerated inflammation and tissue destruction may also be at work. The signal transducer and activator of transcription 3 gene (*STAT3*) is the target of the heterozygous loss of function mutation in this disorder and has been shown to protect against different forms of lung injury, including hyperoxia, in part by suppressing expression of metalloproteases [8]. Therefore, we hypothesized that *STAT3* deficiency leads to dysregulated protease expression, which may in turn contribute to pneumatocele formation.

Here, we report our experience with inhaled α -1 antitrypsin applied to treat a girl with AD-HIES and progressive formation of bilateral pneumatoceles despite appropriate administration of antibiotic prophylaxis, intravenous immunoglobulin, and subcutaneous interferon gamma.

A 9-year-old Turkish girl presented with recurrent pneumonia that had begun at 9 months of age. As a newborn, she developed erythroderma and skin abscesses. Physical examination revealed eczema, high palate, coarse facial features, double-row teeth, and scoliosis. Her National Institute of Health score was 78, which is highly suggestive of AD-HIES (>40) [6]. The initial laboratory evaluation revealed mild eosinophilia (550/mm³) and anemia. Immunological analyses disclosed normal serum immunoglobulin (Ig) levels (IgA, 44.3 mg/dL; IgG, 1912 mg/dL; IgM, 150 mg/dL), except for increased IgE (14 000 IU/mL), and normal values for CD3⁺, CD4⁺, CD8⁺ T cells and CD19⁺ and CD20⁺ B cells. Computed tomography of the lung revealed 2 large pneumatoceles (longest diameter, 53 mm in the right lung and 43 mm in left lung) (Figure). Sequencing studies confirmed the presence of a heterozygous missense mutation (g.58854G>A; c.1145g>A, R382Q) in the DNA-binding domain of the *STAT3* gene, thus confirming the diagnosis of AD-HIES. Treatment included inhaled corticosteroids (fluticasone propionate 2x500 μ g/d since age 6 years), intravenous immunoglobulin (1 dose of 0.8 g/kg every 3 weeks for 1 year followed by 1 dose of 1 g/kg every 2 weeks for the last 2 years of follow-up) and

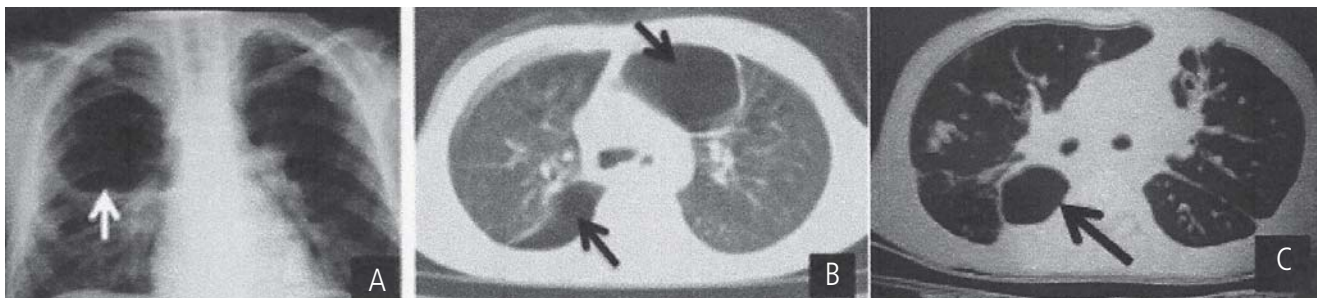


Figure. A, Posteroanterior chest x-ray showing a round hyperlucent area in the right lung (arrow). B, Computed tomography scan of pneumatoceles before treatment. C, Computed tomography scan of pneumatoceles after treatment.

the antifungal agent itraconazole (5 mg/kg/d for 3 years). Antistaphylococcal prophylaxis was administered with trimethoprim-sulfamethoxazole (4 mg/kg/d) and cephalexin (12.5 mg/kg/d), which were interchanged every 3 months during follow-up. Nevertheless, the pneumatoceles became enlarged and the patient developed pneumothorax twice. Subcutaneous interferon gamma (100 µg/m²/dose, 3 times weekly) was initiated at 7.5 years of age. The patient's condition continued to deteriorate and she was frequently admitted to hospital with lower respiratory tract infections. In addition, persistent colonization by *Aspergillus fumigatus* and *Pseudomonas aeruginosa* was detected in sputum and treated with voriconazole (8 mg/kg), caspofungin (50 mg/m²), ceftazidime (100 mg/kg), ciprofloxacin (30 mg/kg), and amikacin (15 mg/kg). One of the pneumatoceles became so enlarged that it had to be surgically removed. As her disease worsened with numerous admissions, another approach was sought to control inflammation. Based on limited clinical experience and the anti-inflammatory effect of inhaled α-1 antitrypsin in destructive chronic lung disorders, current medication was supplemented with inhaled α-1 antitrypsin (2 doses of 250 mg/d). During the year the patient was receiving this regimen, the number of hospital admissions due to infections or pneumothorax decreased, as did the size of the pneumatocele on the right lobe of the lung (pretreatment, 53×32×50 mm; posttreatment, 44×22×26 mm) with no new pneumatocele formation (Figure). However, during the interim period, pulmonary function parameters continued to decrease (forced expiratory volume in the first second, from 73% to 56%; and forced vital capacity, from 69% to 63%), and the ground glass appearance of the lung parenchyma persisted. The most marked improvement for both the patient and her parents was in sputum drainage, which improved quality of life and reduced the number of hospital admissions. No side effects of therapy were recorded. The parents gave their written informed consent for inhaled α-1-antitrypsin therapy.

No specific treatment for AD-HIES is currently available; therefore, therapy is based on prevention of infection with prophylactic antimicrobial and antifungal drugs. High-dose intravenous immunoglobulin and subcutaneous interferon gamma showed clinically beneficial results, possibly owing to their anti-inflammatory and/or immunomodulating effects [9]. Despite these interventions, death from AD-HIES is mainly related to pulmonary infection by *Pseudomonas* or *Aspergillus* species in the presence of concomitant cystic lung disease [10]. Prevention of these infections and pneumatocele formation in patients with AD-HIES is challenging.

STAT3, which is defective in AD-HIES, is expressed in several cell types in the fetal and postnatal lung. When exposed to hyperoxia, the pulmonary epithelium of mice with *STAT3* deficiency is subject to excessive inflammation and airspace enlargement, in much the same way as the pneumatoceles that form following bacterial pneumonia in patients with HIES (7).

In patients with cystic fibrosis, inflammation-derived neutrophil elastase is thought to be the most important protease [2]. Therefore, attempts have been made to suppress this activity using the plasma-derived inhibitor, α-1-antitrypsin. This agent is reported to be safe and efficient in cystic fibrosis, with clinical improvement and suppression of inflammatory markers [4]. As

in cystic fibrosis, administration of α-1-antitrypsin in patients with HIES may slow down the progressive deterioration observed in the natural course of the disease.

In summary, we report the first pediatric case of AD-HIES treated with inhaled α-1-antitrypsin. Therapy for 1 year with this agent prevented hospital admissions due to lung infection and pneumothorax. Additionally, a significant reduction in pneumatocele size was observed compared with baseline, and no new pneumatoceles developed.

Inhaled α-1-antitrypsin may be a useful adjunct with a favorable safety profile in patients with AD-HIES. Duration of therapy and use for prevention of pneumatocele have yet to be explored.

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Conflicts of Interest

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Comparative Study of Bronchial Hyperresponsiveness to Methacholine and Mannitol in the Initial Diagnosis of Asthma

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Asthma is a heterogeneous syndrome characterized by variable airflow obstruction, airway inflammation, and bronchial hyperresponsiveness (BHR). These features seem to be interrelated [1]. Bronchial inhalation challenge (BIC) may be useful for establishing a definitive diagnosis of asthma in individuals with compatible symptoms and normal pulmonary function test results [2]. Methacholine challenge is the most commonly performed test. Because of its high sensitivity and negative predictive value, a negative result allows us to rule out current asthma with reasonable certainty [2]. However, recent data show that challenges with indirect stimuli (eg, mannitol) better reflect airway inflammation [3] and may be ideal for confirming BHR thanks to their high specificity. Airway inflammation is easily and rapidly assessed by measuring the fraction of exhaled nitric oxide (FE_{NO}) using the standardized procedure [4].

The objective of this study was to compare the diagnostic efficiency and tolerability of the BIC using both mannitol and methacholine as the stimulus in patients whose symptoms were consistent with asthma in daily clinical practice.

We recruited 28 consecutive patients (21 women; mean age, 32 years; range, 15-54 years) from among those reporting persistent asthma-like symptoms (shortness of breath, wheezing, cough, and/or chest tightness). The inclusion criteria

were current asthma symptoms, normal spirometry values, and a negative bronchodilator test result (after inhalation of 400 µg of salbutamol using a metered-dose inhaler). All patients underwent BIC with both methacholine and mannitol in a random fashion. Written informed consent was obtained. Asthma medication was withdrawn before the challenges, as described elsewhere [5].

Ours was a cross-sectional, crossover study. Patients were asked to attend 3 study visits within a 2-week period. At the first visit, a staff physician took a clinical history and performed a physical examination, and the patient underwent skin prick testing with common aeroallergens (ALK-Abelló), [4], spirometry, and a bronchodilator test. FE_{NO} was measured using a portable nitric oxide analyzer (NIOX MINO, Aerocrine). Demographic data were recorded, and patients were randomly assigned to undergo a BIC with either methacholine (13 patients) or mannitol (15 patients) first. At the second and third visits, BIC was performed with methacholine and mannitol, respectively.

Atopy was defined as the presence of ≥1 positive skin prick test result with common aeroallergens (wheal diameter ≥3 mm larger than that of the negative control).

Methacholine inhalation challenge was performed using a Spira nebulizer and an electronic dosimeter (Spira Elektro, Respiratory Care Center) following the 5-breath dosimeter method, as previously described [5].

BIC was performed with mannitol dry powder (Aridol/Osmohale, Pharmaxis Ltd) using a protocol described elsewhere [6]. The dose-response ratio (DRR) was calculated for all patients and for both challenges. This ratio was defined as the percentage fall in forced expiratory volume in 1 second (FEV₁) at the last dose administered divided by the total dose administered [7].

BHR was defined as the provocative concentration of AMP required to produce a 20% fall in FEV₁ (PC₂₀) ≤16 mg/mL of methacholine, which is equivalent to a DRR that produces a ≥20.9% fall in FEV₁/mg of methacholine, and/or the provocative dose of mannitol that produced a 15% fall in FEV₁ (PD15) ≤635 mg of mannitol, equivalent to a DRR that produces a ≥0.023% fall in FEV₁/mg of mannitol [5,6]. Salbutamol was administered (400 µg) after all the positive challenges, and patients were observed until complete recovery.

Severity of cough was assessed after both BICs on a scale of 0 to 4 as follows: 0 no cough at all; 1 occasional hems; 2 mild, isolated cough, without additional symptoms; 3 moderate, paroxysmal cough, without additional symptoms; 4 severe, strenuous cough accompanied by chest discomfort [8]. Up to 75% of the patients were atopic. Only 11% of the patients reported asthma symptoms alone, while 86% had both asthma and rhinitis symptoms and 25% had symptoms consistent with exercise-induced asthma.

The result of the methacholine BIC was positive in 9 patients (32%), with a PC₂₀ (expressed as geometric mean) of 2.50 mg/mL, which is equivalent to a DRR that produces a 3.22% fall in the FEV₁/mg of methacholine. The mannitol BIC was positive in 10 patients (35%), with a PD15 (expressed as geometric mean) of 2.87, which is equivalent to a DRR that produces a 2.11% fall in the FEV₁/mg of mannitol. The results of both techniques showed an agreement of 89.28%, with a κ index of 0.76.

Patients were classified into 2 different groups according to the results of the BIC: asthma patients (a positive BIC result with mannitol, methacholine, or both) and nonasthma patients (negative BIC results with both agents). A diagnosis of asthma was made in the 11 patients (39.3%) who had a positive result to at least 1 BIC. Of these patients, 8 had a positive result in both BICs and 3 patients in only 1 of them (2 to mannitol and 1 to methacholine). No significant linear correlations were found between mannitol PD₁₅ and methacholine PC₂₀ or between mannitol DRR and methacholine DRR ($P=.127$). The nonasthma group comprised 17 patients with negative results to both BICs (60.7%).

No statistically significant differences were observed between the 2 groups for age, sex, smoking, personal history of atopy, concomitant rhinitis, and sensitization to aeroallergens. However, we did find statistically significant differences between the 2 groups for family history of atopy, exercise-induced asthma, and mean FE_{NO} values (Table).

The methacholine BIC was better tolerated (mean cough severity score, 1.48 points) than the mannitol challenge, during which significantly more frequent coughing, wheezing, and chest tightness was recorded (3.81 points, $P<.01$).

Asthma was finally diagnosed in 39% of the asthma patients, who showed significantly higher FE_{NO} levels (49 vs 27 ppb, $P<.01$) than the nonasthma patients. This finding emphasizes the importance of clarifying the diagnosis of asthma in patients with high FE_{NO} (>30 ppb), as previously suggested [9] and as recommended in Spanish asthma guidelines [10].

In conclusion, we found that bronchial challenge with mannitol yielded similar results to the most widely used direct-acting stimulus, methacholine, in the initial diagnosis of asthma. However, the BIC with methacholine was better tolerated and had fewer side effects. Therefore, in daily practice, we consider that mannitol challenge would be useful for confirming asthma in centers that do not have the equipment required to perform bronchial methacholine challenge.

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Conflicts of Interest

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Table. Comparison of Variables Between the 2 Groups^a

	Mannitol BIC	Methacholine BIC	P
Asthma patients	11 Patients (39%) 10 Positive PD ₁₅ , 2.87 (2.56-3.97) DRR, 2.11 (0.028-1.27) Mean BMI, 26.5 (18.1-38.1) Family history of atopy, 58.3% Exercise induced asthma, 27.3% FE _{NO} , 49.00 ppb (19-135)	9 Positive PC ₂₀ , 2.50 (0.25-13.29) DRR, 3.22 (0.19-19.2)	<.001 <.001 <.01 <.001
Nonasthma patients	17 Patients, 61% FE _{NO} , 27.23 (13-89) ppb Mean BMI, 23.1 (18.6-33.3) Family history of atopy, 45.5% Exercise-induced asthma, 25.0%		<.001 <.001 <.001 <.01

Abbreviations: BMI, body mass index; DRR, dose-response ratio; FE_{NO}, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; PC₂₀, concentration of methacholine that provoked a 20% fall in FEV₁; PD₁₅, provocative dose of mannitol that produced a 15% fall in FEV₁.

^aData are presented as mean (range).

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Anaphylactic Reactions Requiring Hospitalization Among Bedouin and Jewish Children in Southern Israel

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Southern Israel is inhabited by 2 different populations: Jews and Bedouins. The Jewish population's standard of living is comparable to that of developed countries, whereas the Bedouin population is in transition from a seminomadic lifestyle to permanent settlement and a standard of living comparable to that of a developing country. Since medical insurance in Israel is universal and free of charge, children of both populations have access to the same medical services. The Soroka University Medical Center is the only hospital for the entire region. All patients with an anaphylactic reaction requiring hospitalization (ARRH) are admitted to this hospital. The objective of the present study was to compare the epidemiology of ARRH in Bedouin and Jewish children in southern Israel.

The local ethics committee approved the study. We reviewed all cases of ARRH in children younger than 18 years from January 1, 2005 until July 31, 2010. Children hospitalized with more than 1 anaphylactic reaction were analyzed only once. The diagnosis of anaphylactic reaction was made after a review of the medical records and based on previously published criteria [1].

To calculate the incidence of anaphylactic reactions, we used data published by the Israeli Central Bureau of Statistics [2]. Differences between children of Jewish and Bedouin ethnicity were analyzed using the χ^2 test or Fisher exact test, as appropriate. Variations in yearly incidence were studied using linear regression analysis.

During the study period, 93 patients were hospitalized because of an anaphylactic reaction (40 Bedouin, 53 Jewish); 11 patients were hospitalized twice. Overall, 104 cases (42 Bedouin, 62 Jewish) were recorded. Anaphylactic reactions were more common in boys than in girls (66/93 [70.1%] vs 27/93 [29.08%], $P=.004$). This finding was significant in Bedouin children (31/40 [77.5%] vs 9/40 [22.5%], $P=.011$), while in Jewish children a nonsignificant trend was recorded (35/53 [66.0%] vs 18/53 [34.0%], $P=.096$).

The average incidence of anaphylactic reactions was similar in Bedouin and Jewish children (7.0 and 7.4 cases/100 000 children/year, respectively, $P=.828$). Linear regression analysis of the incidence of ARRH by ethnicity did not reveal significant trends for an increase or decrease in the incidence of ARRH during the study period ($P=.301$ for the Bedouin population and $P=.420$ for the Jewish population).

Foods were a more common cause of ARRH episodes in Jewish children than in Bedouin children (19/53 [35.8%] vs 2/40 [5.0%], $P<.001$). Specifically, milk allergy was more common in ARRH affecting Jewish children (11/53 [20.8%] vs 1/40 [2.5%], $P=.009$). More reactions due to hymenoptera stings were recorded in Bedouin children (27/40 [67.5%] vs 3/53 [5.7%], $P<.001$) (Table).

More Jewish children younger than 4 years were hospitalized because of ARRH than Bedouin children (20/53 [37.7%] vs 5/40 [12.5%], $P=.009$).

Dermatological and respiratory manifestations of ARRH were similar in both populations; however, more Bedouin children had gastrointestinal manifestations than Jewish children (10/40 [25.0%] vs 5/53 [9.4%], $P=.043$), and more hemodynamic manifestations (21/40 [52.5%] vs 13/53 [24.5%], $P=.006$).

No differences were recorded in the number of intensive care admissions for ARRH between Bedouin and Jewish children (7/62 [11.3%] vs 4/42 [9.5%], $P=.522$).

Biphasic ARRH was diagnosed in 2/104 (1.9%) cases.

Our data showed that the incidence of ARRH was similar in both populations; however, the main causes of ARRH in Bedouin children were hymenoptera stings, whereas food (specifically milk) was the most common cause of ARRH in Jewish children.

Katz et al [3] recently reported a significantly lower

Table. Causes of Anaphylactic Reactions

	Bedouins (n=40) No. (%)	Jews (n=53) No. (%)	Total N=93	P Value
Food (all types)	2 (5.0%)	19 (35.8%)	21	<.001
Milk	1 (2.5%)	11 (20.8%)	12	.09
Medication	6 (15.0%)	15 (28.3%)	21	.129
Hymenoptera stings	27 (67.5%)	3 (5.7%)	30	<.001
Unknown	5 (12.5%)	10 (18.9%)	15	.408
Other ^a	0 (0%)	6 (11.3%)	6	.035

^a In Jewish children, the causes of ARRH due to other foods were attributed to peanut, egg, fish, nuts, strawberry, and banana (1 case each). In 2 Jewish children, no specific cause could be identified, as the children had ingested several foods. In Bedouin children, 1 case of ARRH due to food was attributed to fish.

incidence of milk allergy in Moslem Arab children (some of whom were of Bedouin ethnicity) than in Jewish children in central Israel. A lower incidence of milk allergy was recorded in children who consumed milk before 14 days of age. Significantly more Moslem Arab children consumed milk before the age of 14 days and, accordingly, only 3/66 children diagnosed as allergic to milk were Arabs [3]. If we hypothesize that the Bedouin population living in southern Israel have feeding habits similar to those of the Arab population in central Israel, the likely explanation for fewer ARRHS due to milk in Bedouin children is the early introduction of milk.

In a questionnaire study of 10 021 teenagers in Israel, Arab children reported significantly more stings and more allergic reactions of any kind, including anaphylactic reactions, than Jewish teenagers, with an odds ratio of 1.34 [4]. Given that Bedouin children in southern Israel live in open spaces, they are more likely to be exposed to hymenoptera stings; hence the higher rates of ARRHS caused by hymenoptera venom in this population.

More ARRHS occurred in Jewish children younger than 4 years. Milk allergy is more common in early life, although many cases resolve by the age of 3 years [5]. Since milk allergy is more common in Jewish children, ARRHS at an early age is an expected finding in this group.

Bedouin children had more gastrointestinal and hemodynamic manifestations. Most Bedouins live relatively far from the hospital. As the time from the anaphylactic reaction until arrival at hospital was probably longer in Bedouin children, the delay could explain the greater frequency of gastrointestinal and hemodynamic manifestations.

In this study, ARRHS was more common in boys. Anaphylaxis is known to be more common in boys before the age of 15 years [6]. Since our study included children younger than 18 years of age, we expected more boys to be affected.

The limitations of the present study are the retrospective collection of data and the fact that the causes of ARRHS were determined by analyzing medical records. We did not have the results of any additional allergy workups.

In conclusion, the incidence of ARRHS was similar in both populations; however, the most common cause of ARRHS in Bedouin children was hymenoptera stings. In Jewish children the most common cause was food, specifically milk and more often before age 4 years. The etiology of anaphylactic reactions can differ in populations residing in the same geographical area.

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Specific IgE to Honeybee Venom in Patients with Hypersensitivity to Asian Giant Honeybee (*Apis dorsata*)

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Key words: Api m 1. *Apis dorsata*. Giant honeybee. Bee allergy. Venom hypersensitivity.

Palabras clave: Api m 1. *Apis dorsata*. Abeja gigante. Alergia a abeja. Hipersensibilidad a veneno.

Apis dorsata, commonly known as Asian giant honeybee, is widely distributed over vast areas in south and southeast Asia and is found throughout Thailand. It plays an important role as the pollinator of plants and is a good source of high-quality honey and beeswax. *A dorsata*, however, is not used in the commercial honey industry [1]. Hence, *A dorsata* has received less attention in the literature. Research on *A dorsata* venom and its association with clinical hypersensitivity is limited.

In contrast, the European honeybee, *Apis mellifera*, plays an important role in the commercial honey industry, and its venom components have been extensively studied. The investigation of venom sensitization using serum specific immunoglobulin (Ig) E to bee venom (*A mellifera* venom extract) has proven to be highly sensitive and specific and might be comparable to venom skin testing [2]. Serum specific IgE has been used as an adjunct to the clinical history when selecting patients for venom immunotherapy [3]. Api m 1 is the major allergen of *A mellifera* venom. Specific IgE to recombinant Api m 1 (rApi m 1) has been shown to be a specific marker of bee venom sensitization and helpful in discriminating between true sensitization and cross-reactivity in patients with a double-positive specific IgE

result to conventional venom extracts [4,5]. Serum specific IgE to bee venom and rApi m1 is widely used in clinical practice owing to its availability in commercial kits. Cross-reactivity between different species of *Apis* venom has received little attention. Moreover, the benefit of using specific IgE to bee venom and Api m 1 in the detection of other types of sensitization to *Apis* remains unknown. The aim of this study was to determine the specific IgE level to bee venom and Api m 1 in patients with hypersensitivity to *A dorsata* venom. This study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University in Chiang Mai, Thailand.

On June 23, 2012, 76 novice Buddhist monks were attacked by swarms of honeybees while they were carrying out routine cleaning of a temple in Chiang Mai, Thailand. Of the 76 monks, 53 were sent to the Emergency Department at Chiang Mai University Hospital. Reactions were classified as large local reactions (LLR) and systemic reactions according to Mueller [6] in 50 patients, from whom blood was collected within 24 hours. The remaining 3 patients had mild reactions and did not undergo blood testing. Specific IgE to bee venom (*A mellifera* venom; i1) and rApi m 1 (i208) were measured using ImmunoCAP (Thermo Fisher Scientific) according to the manufacturer's instructions. A specific IgE level of ≥ 0.35 kU_A/L was considered positive.

Entomologists identified 3 large *A dorsata* nests on the eaves of the temple and confirmed that the body and stingers of culprit insects found on the patients' skin were from this species.

All 50 patients were adolescent males (mean age, 14.5 years; range, 11.5-18.9 years). The reactions were classed as LLR in 16 cases (32%), grade I in 12 cases (24%), grade II in 10 cases (20%), grade III in 8 cases (16%), and grade IV in 4 cases (8%). Positive IgE to bee venom was detected in 46 patients (92%), and positive IgE to Api m 1 was detected in 24 patients (48%). All of the patients who tested positive to Api m 1 also had a positive result to bee venom. The concentration of specific IgE to bee venom (median, 3.55 kU_A/L; range, 0.03-49.8) was significantly higher than that of specific IgE to Api m 1 (median, 0.33 kU_A/L; range, 0-15.6; $P < .001$). The number of stings and laboratory results classified by the severity of the reactions are shown in the Table. The differences in specific IgE to bee venom and Api m 1

Table. Number of Stings and sIgE Level of Patients According to Severity

Severity ^a	LLR (n=16)	Grade I (n=12)	Grade II (n=10)	Grade III (n=8)	Grade IV (n=4)	P Value ^d
No. of stings ^b	7.5 (2-20)	10 (3-40)	18 (3-20)	20 (10-40)	15 (10-22)	.018
sIgE to BV, kU _A /L ^b	2.70 (0.04-23.50)	2.43 (1.18-45.5)	7.15 (0.03-49.8)	4.47 (0.12-22.20)	7.58 (4.52-18.30)	.065
BV-positive ^c	14 (87.5)	12(100)	9 (90)	7 (87.5)	4 (100)	
sIgE to Api m 1, kU _A /L ^b	0.41 (0-0.37)	0.16 (0.01-5.82)	0.75 (0-15.60)	0.245 (0.01-4.18)	2.17 (0.25-5.40)	.186
Api m 1-positive ^c	9 (56.25)	4 (33.33)	7 (70)	2 (25)	2 (50)	

Abbreviations: BV, bee venom; sIgE, specific immunoglobulin E; LLR, large local reactions.

^aAccording to Mueller [6].

^bMedian (range).

^cNo. (%); cutoff for positivity, ≥ 0.35 kU_A/L.

^dKruskal-Wallis test.

between severity groups did not reach statistical significance (bee venom, $P=.065$; Api m 1, $P=.186$). Only the number of stings was found to be significantly different ($P=.018$).

A dorsata venom allergen (Api d 1) belongs to the phospholipase A2 family. The sequence of its 134 amino acids was completely identified and showed 91% identity with Api m 1 [7]. Our study supports the existence of high cross-reactivity between both allergens. In addition, only half of the patients (48%) had specific IgE to Api m 1. This finding might confirm the high specificity of rApi m1 to *A mellifera* allergen. The level of specific IgE in our patients did not correlate with clinical severity. In accordance with recommendations, the clinician must interpret the laboratory results in conjunction with the clinical history [3].

Patients who experience systemic reactions to *A dorsata* might benefit from venom immunotherapy using commercial bee venom extract. However, a previous report in occupational bumblebee anaphylaxis demonstrated the failure of immunotherapy with bee venom, although protection was successful after the subsequent introduction of specific bumblebee venom immunotherapy [8,9]. Hence, further investigation into the usefulness of immunotherapy in *A dorsata* allergy is needed.

In conclusion, the present study revealed that most patients with *A dorsata* hypersensitivity responded to specific IgE to bee venom. Therefore, specific IgE to bee venom might prove very useful for identifying sensitization when venom immunotherapy is being considered. Further study is required to determine *A dorsata* allergen-specific epitopes and their clinical implications.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Successful Desensitization With Agalsidase Alfa in 2 Brothers With Fabry Disease

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Palabras clave: Agalsidasa alfa. Enfermedad de fabry. Desensibilización.

Fabry disease is the only X-linked sphingolipidosis caused by a deficiency of the lysosomal enzyme α -galactosidase A. This deficiency results in the progressive accumulation of globotriaosylceramide (Gb3) and related glycosphingolipids in vascular endothelial cells, smooth muscle cells, and ganglion cells, leading to ischemia and infarction especially in the kidney, heart, and brain. Renal and cardiovascular complications are the main cause of death in classic Fabry disease [1-3].

Disease-specific treatment with infusions of 2 similar products, agalsidase alpha and agalsidase beta, exists. Agalsidase alfa replacement therapy has been shown to be effective in reducing myocardial Gb3 content, heart rate, and urinary Gb3 excretion and in normalizing renal function. It has also been reported to have positive effects on hearing, sweating, pain, and gastrointestinal symptoms [4,5]. Finally, there is increasing evidence that enzyme replacement therapy can halt disease progression.

Agalsidase alfa therapy is generally well tolerated by patients with Fabry disease [6]. In a clinical trial, 8 of 14 patients who received this enzyme experienced mild reactions such as chills, facial flushing, nausea, and chest pain. The reactions occurred within an hour of infusion and reduced with antihistamines and low-dose corticosteroids. None of the patients stopped the therapy [7]. To date, no hypersensitivity reactions have been reported for agalsidase alfa. In this article, we describe 2 brothers who developed hypersensitivity reactions to this enzyme and who were later successfully desensitized.

The first brother, aged 49 years, was diagnosed with Fabry disease 6 months previously and started on agalsidase alpha (Replagal) 11.5 mg every 15 days. He experienced urticarial lesions 2 to 3 hours after infusion during the last few courses. He reported no such lesions before this therapy. He was referred for desensitization to agalsidase alfa. He had no history of any other drug allergies or allergic disease. Laboratory tests revealed mild anemia (hemoglobin, 12.5 mg/mL) and renal failure (blood urea nitrogen [BUN], 30 mg/dL and creatinine, 2.69 mg/dL). As part of the allergy workup, he underwent skin prick tests (SPTs) and intradermal tests with agalsidase alfa (1:100, 1:10, and undiluted 14 mg/mL solutions), with negative results in all cases.

The second brother, aged 47 years, was referred to the allergy/immunology clinic following a hypersensitivity reaction to agalsidase alfa therapy. He had been on dialysis for 9 years before developing chronic renal failure and had received a kidney transplant 2 years earlier. He was diagnosed with Fabry disease 3 years ago and started on agalsidase alfa (Replagal) 0.2 mg/kg every other week. He received the treatment for 26 courses without problem. During the 27th course, he experienced dyspnea, chest tightness, and facial swelling with erythema and pruritus within minutes of the infusion. Laboratory tests revealed normal values, except for a slightly higher-than-normal BUN value (22 mg/dL). He

Table. Protocol for Intravenous Desensitization With Agalsidase Alfa

Step	Solution	Rate, mL/h	Time, min	Amount, mL	Dose, mg	Cumulative Dose, mg
1	1	2.5	15	0.625	0.00035	0.00035
2		5	15	1.25	0.0007	0.00105
3		10	15	2.5	0.0014	0.00245
4		20	15	5	0.0028	0.00525
5	2	5	15	1.25	0.0063	0.01155
6		10	15	2.5	0.0126	0.02415
7		20	15	5	0.0252	0.04935
8		40	15	10	0.0504	0.09975
9	3	10	15	2.5	0.126	0.22575
10		20	15	5	0.252	0.47775
11		40	15	10	0.504	0.98175
12		45	339	232.5	11.718	14

Target dose: 14 mg agalsidase alfa

Volume: 250 mL of normal saline solution (SS)

Solutions: Solution 3: 14 mg agalsidase alfa in 250 mL SS

Solution 2: 225 mL SS + 25 mL solution 3

Solution 1: 225 mL SS + 25 mL solution 2

Premedication: Cetirizine/ Montelukast/ acetylsalicylic acid (1 h before desensitization)

Total time: 504 minutes

underwent SPTs with agalsidase alfa at the same dilutions as those used in his brother. Both the SPTs and intradermal test were negative.

Both patients were desensitized with agalsidase alpha (Replagal) (Table). The same protocol was used in both cases as the brothers were scheduled to receive the same dose (14 mg). We used the 12-step, 3-bag protocol recommended by Castells [8]. Premedication with montelukast 10 mg, acetylsalicylic acid 325 mg, and cetirizine 10 mg was given 1 hour before starting the protocol. The starting dose was 0.00035 mg (1/40 000 of the therapeutic dose) of agalsidase alfa, and doses were doubled every 15 minutes. Both patients successfully completed the protocols in 504 minutes, with no adverse reactions observed. Therefore, the patients were scheduled to receive agalsidase alfa by desensitization with the same protocol. They are still receiving the drugs in this manner and furthermore the therapy stabilized a left ventricular mass in the heart of both patients.

We have reported a hypersensitivity reaction to agalsidase alfa in 2 brothers with Fabry disease. The most common adverse events with agalsidase alfa therapy are infusion-associated reactions, which are typically mild to moderate in severity, and include rigors, fever, nausea, vomiting, headache, tremor, dyspnea, somnolence, and chest pain [9]. Hypersensitivity reactions have been reported to be very rare. Although some immediate reactions such as anaphylactic shock have been reported, immunoglobulin (Ig) E-mediated immune mechanisms have rarely been shown with positive skin tests [10]. The negative SPT and intradermal test results with agalsidase alfa in both brothers suggests nonimmunological mast cell degranulation as the underlying mechanism in the 2 cases. However, we cannot claim that this immediate reaction was not due to an IgE-mediated mechanism as the specificity and sensitivity of skin testing with the culprit drug is not known. It is very interesting that the 2 patients were brothers, and while the immune response to agalsidase alfa might be genetically mediated, we cannot prove this.

We prepared a desensitization protocol for our patients as there was no treatment alternative due to the shortage of agalsidase beta. As no desensitization protocols had been published for this drug, we based our protocol on Castells' recommendations [8]. Both patients responded well to the desensitization procedure and safely completed the protocol.

In conclusion, we have reported infusion-associated reactions to agalsidase alfa in 2 brothers that suggest a possible genetic control over hypersensitivity reactions to this drug. The desensitization protocol worked well and can therefore be recommended for cases of immediate hypersensitivity reactions to agalsidase alfa.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Rechallenge in Pediatric Patients Diagnosed With Delayed Hypersensitivity to Penicillins

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β -Lactams are the most widely used drugs in children but the exact frequency of hypersensitivity reactions to these antibiotics is unknown. Studies based on skin and challenge tests show that allergy is confirmed in 12% to 60% of children with suspected hypersensitivity reactions to β -lactams [1]. Adverse reactions may be due to different mechanisms. Nonimmediate reactions occur more than 1 hour after drug administration, and most are T-cell mediated [2]. The most common manifestation is maculopapular rash. Conventional studies are of little value in the case of delayed reactions [1,2], and the oral challenge test remains the gold standard for confirming or ruling out β -lactam sensitization. The aim of our study was to reassess tolerance to β -lactams in children diagnosed with nonimmediate allergy to penicillins after at least 1 year of drug avoidance.

We studied 14 children with a history of maculopapular rash during treatment with penicillin. They had all been diagnosed with delayed hypersensitivity to penicillins by controlled oral challenge in the pediatric allergy department of Hospital Materno Infantil Gregorio Marañón. Responses to skin and challenge tests were studied after at least 1 year of avoidance. All the patients gave their written informed consent before the allergy workup.

The children underwent skin prick and intradermal tests

with known concentrations of the drug that had induced the initial hypersensitivity reaction [2]. The reagents used included amoxicillin (GSK) 20mg/mL, benzylpenicillin (Normon), penicilloyl-polylysine (PPL) 5×10^{-5} mmol/L (Diater), and minor determinant mixture (MDM) 2×10^{-2} mmol/L (Diater). Histamine 10mg/mL and sodium chloride (0.9%) were used as positive and negative controls, respectively. Immediate readings were taken after 15 minutes and late readings at 24 hours. A prick or intradermal test result was considered positive if the largest diameter of the wheal was at least 3 or 5 mm, respectively, as recommended by the European Academy of Allergology and Clinical Immunology guidelines [3]. All the tests were performed by the same experienced nurses. In the reassessment of patients, patch tests were not used as they have shown low yield in such cases [2]. Specific immunoglobulin (Ig) E against penicillin and amoxicillin was determined by fluorescence enzyme immunoassay (ImmunoCAP FEIA, Phadia). Because the variables were nonnormally distributed, differences were assessed by the Mann-Whitney U test. A *P* value of less than .05 was considered to be significant.

In patients with a negative skin test, an oral challenge was performed with the drug that had induced the initial allergic reaction. All patients were administered one-quarter of the total dose followed by the remainder of the dose. They were kept under observation for an hour after the last dose and continued the treatment at home for a week (therapeutic doses of the drug every 12 hours).

Skin tests and specific IgE were negative in all patients. A second challenge was performed at 4 to 6 weeks in patients with an initial negative challenge result to confirm that it was a true negative and not a false negative due to loss of immune memory [4].

At the time of the initial diagnosis, maculopapular rash was recorded in 10 patients (71%) and rash with joint involvement in 4 (29%). The drug most frequently involved was amoxicillin (9 patients, 64%), followed by amoxicillin-clavulanate (4 patients, 29%) and penicillin G (1 patient, 7%). These data are consistent with reports in the literature, in which amoxicillin, either alone or in combination with clavulanic acid, is the most common cause of drug-induced reactions in children [5].

Of the 14 patients, 8 were male and 6 were female. Rechallenge was positive in 7 of them (Group I) and negative in the other 7 (Group II). In the group analysis, age at onset of the reaction that prompted the allergy consultation and age

Table. Characteristics According to Positive or Negative Rechallenge Results^a

Patients With Positive Results		Patients With Negative Results	
*Age at time of reaction, y	3.25 (2.5-7.5)	*Age at time of reaction, y	1 (0.5-4)
**Age at time of first study, y	3.75 (2.41-7.5)	**Age at time of first study, y	1.6 (1-5.6)
***Age at time of second study, y	6 (4.41-14)	***Age at time of second study, y	3 (2-7)
Time to allergic reaction, d	6 (1-7)	Time to allergic reaction, d	7 (2-7)
Time to positive rechallenge result, d	6 (5-7)	Duration of treatment, d	7

P value between groups: **P*=.027, ***P*=.038, ****P*=.023.

^aData are presented as median (range) unless otherwise specified.

at the time of the rechallenge were lower in Group II than in Group I (Table). Significant differences were found between the groups for age at diagnosis ($P=.027$), age at the time of the first study ($P=.038$), and age at the time of the second study ($P=.023$).

These results suggest that, especially in the early years, allergy tests in patients with a history of reaction during treatment with penicillins may be positive as a result of latent viral infection or antibiotic interaction with other viruses, as has been described for Epstein-Barr virus and influenza virus [6,7]. Once the infection has cleared, patients are able to tolerate subsequent exposures to the drug concerned. At older ages, there is a greater likelihood that the reactions are due to true drug hypersensitivity.

The results of our study show that up to 50% of patients with delayed allergy to penicillins may tolerate subsequent treatment with the drug involved. For this reason, we believe it appropriate to repeat the allergy study, at least 1 year after avoidance, in patients under 2 years of age diagnosed with delayed allergy to penicillins. Home treatment should be maintained for at least a week, or in any case, for longer than the time it took for the delayed reaction to occur.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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