

A Novel *CD40* Ligand Mutation in a Patient With Pneumonia, Neutropenia, and Hyperimmunoglobulin M Phenotype

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Immunoglobulin (Ig) class switch recombination deficiencies, also known as hyper immunoglobulin M (HIGM) syndromes, are a group of primary immunodeficiency diseases in which normal or elevated serum levels of IgM with concomitant low levels of other Ig classes lead to a variety of manifestations, in particular recurrent infections in the respiratory and gastrointestinal tracts [1-3]. The most common variant of the disease is X-linked HIGM syndrome (XHIM, OMIM#308230), which is caused by mutations in the gene encoding the CD40 ligand (*CD40LG*, OMIM*300386) and leads to failure to express CD40 ligand on T cells. CD40-CD40 ligand interaction is necessary for switching of IgM-producing B lymphocytes to express other Ig classes such as IgG, IgA, and IgE [2]. More than 130 unique mutations in the *CD40LG* gene have been described to date, and approximately half are located in exon 5.

We describe a male infant with recurrent respiratory tract infection and diarrhea for whom immunological screening tests suggested an HIGM phenotype. Subsequent identification of a novel *CD40LG* mutation confirmed the diagnosis.

A 16-month-old boy was referred to the Immunodeficiency Clinic at the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Teheran, Iran because of recurrent upper respiratory tract infections, otitis media, longstanding diarrhea, failure to thrive, and an 8-month history of anorexia. The boy was born by cesarean delivery, during which he experienced some degree of asphyxia due to

nuchal cord. His birth weight was 3.6 kg, and the parents were nonconsanguineous. The infant was the first offspring, and the history of other family members for chronic or immunologic diseases was unremarkable.

The patient had been admitted to a local hospital in northern Iran with pneumonia 1 month before the referral. A chest x-ray revealed diffuse patchy micronodular infiltrates in both lungs. High-resolution computed tomography of the lungs showed diffuse patchy alveolar infiltrates with a fine nodular appearance and no plural effusion, findings that were compatible with severe infections, including that caused by *Pneumocystis jiroveci*. A complete blood cell count revealed leukocytosis with marked eosinophilia (white cells, 35 000/mm³; lymphocytes, 63%; neutrophils, 14%; and eosinophils, 23%). The patient was treated successfully with trimethoprim-sulfamethoxazole and azithromycin.

On his visit to the clinic, the patient was afebrile, pale, and cachectic. No oral thrush or nonhealing ulcers were visible. A palpable submandibular lymph node (2 × 1 cm) was observed. The appearance of both tonsils was normal. Crepitations were heard during lung auscultation. A second complete blood count disclosed leukocytosis, marked eosinophilia plus neutropenia (white cells, 21 000/mm³; lymphocytes, 68.7%; neutrophils, 2.1%; eosinophils, 23%). Serum Ig determination revealed elevated IgM and decreased IgG and IgA levels (IgG, 76 mg/dL; IgM, 217 mg/dL; IgA, 6 mg/dL; IgE, 3 IU).

The decreased levels of IgG and IgA, normal to increased levels of IgM, male gender, and neutropenia raised suspicion of XHIM. To detect the underlying genetic defect, the patient's genomic DNA was analyzed. Amplification and sequencing of the coding region of the *CD40L* gene using polymerase chain reaction revealed a novel missense mutation (11192 G>A) in exon 5, which resulted from a replacement of glycine by aspartate at codon 252 (G252D). This mutation is considered to be potentially damaging (<http://genetics.bwh.harvard.edu/pph/>) (Figure).

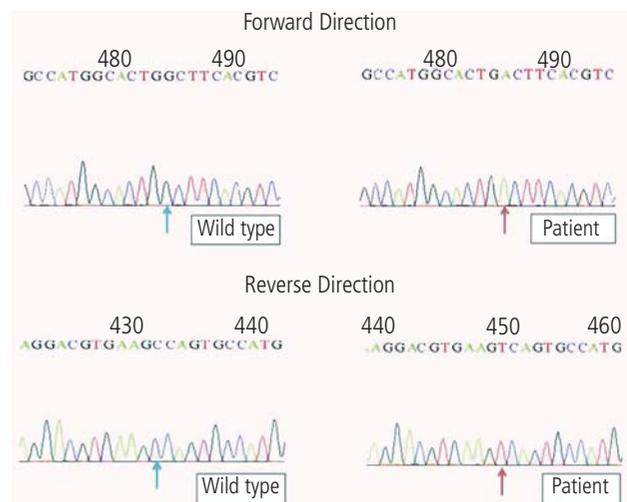


Figure. A novel g: 11192 G>A mutation at the coding region of exon 5 that resulted in the replacement of glycine by aspartate at codon 252 (G252D).

Once the diagnosis of XHIM was confirmed, monthly intravenous immunoglobulin (IVIg) replacement therapy (400 mg/kg) was prescribed. Serum IgG level subsequently increased (IgG, 503 mg/dL; IgM, 57 mg/dL; IgA, 7 mg/dL; IgE, 0 IU), as did the neutrophil count (white cells, 9640/mm³; lymphocytes, 75%; neutrophils, 10.8%; and eosinophils, 9.8%). The neutrophil count was raised without granulocyte colony-stimulating factor, but did not reach more than 1000 cells/ μ L during a 3-month follow-up; however, the patient did not experience any infections or diarrhea during this period. He is currently under close follow-up, which includes monitoring of Ig and neutrophil levels combined with IVIg and antibiotic prophylaxis.

We report a typical presentation of the XHIM phenotype, although the patient had experienced recurrent respiratory and gastrointestinal complications since early infancy. As more than half of all patients with XHIM present some manifestations during their first year of life [3,4], unnecessary laboratory tests are performed and futile therapeutic regimens prescribed during the period between the first manifestation and diagnosis. Therefore, a review of the natural course of such patients could provide physicians with appropriate diagnostic information.

Neutropenia is a common hematological finding in XHIM [5-7]—it affects about 60% of patients—and may be transient or chronic [8]. Although treatment with recombinant granulocyte colony-stimulating factor can increase the neutrophil count [9], IVIg alone can also resolve neutropenia in XHIM [10]. In our patient, the severe neutropenia improved to moderate levels after only 3 monthly IVIg sessions. Therefore, at least 6 months of follow-up may be required, and, if the absolute neutrophil count is not increased, granulocyte colony-stimulating factor should be considered. Hematopoietic stem cell transplantation has the potential to cure the disease; however, this approach requires careful monitoring of liver function and regular screening for *Cryptosporidium* infection.

We detected a novel mutation in exon 5 of *CD40LG* (located at Xq26.3) that is not present in current disease registries. As most *CD40LG* mutations identified to date are located in exon 5, which plays an important role in CD40L–CD40 interactions, screening for primary mutations in this region could prove cost-effective [3].

Diagnosis is confirmed based on an appropriate history and raised awareness of potential immunodeficiency disease, especially in patients with recurrent episodes and treatment-refractory infections. Given that XHIM is the most common form of HIGM, it should be included in the differential diagnosis of any boy with recurrent infections and hypogammaglobulinemia, especially in cases with concomitant neutropenia and normal or increased serum levels of IgM.

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Changes in BAFF/APRIL Levels in a 2-Year-Old Girl with Kawasaki Disease Refractory to Intravenous Immunoglobulin Therapy

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Palabras clave: APRIL. BAFF. IgA. Inmunoglobulina intravenosa. Enfermedad de Kawasaki.

Kawasaki disease (KD) is an acute, self-limited vasculitis of unknown etiology. Although high-dose intravenous immunoglobulin (IVIG) is the standard treatment for KD, the precise mechanism of action of the disease remains unknown. Tumor necrosis factor (TNF) superfamily ligand members related to B-cell activation, such as BAFF (B cell-activating factor) and APRIL (a proliferation-inducing ligand), are now thought to be involved in the pathogenesis of KD. BAFF is involved in the survival and maturation of B cells, and APRIL stimulates B- and T-cell proliferation and triggers humoral immune responses. In our previous study on KD [1], we found BAFF levels to be elevated before but reduced after treatment with IVIG, while APRIL levels vary inversely to BAFF levels. BAFF, APRIL, or both can participate in the development of KD and can be modulated by treatment with IVIG. However, changes in BAFF/APRIL levels in patients with KD refractory to IVIG remain uncharacterized.

A girl aged 2 years and 11 months was admitted to our hospital with fever and diarrhea (3 days), strawberry tongue (2 days), and conjunctival injection and skin rashes on the day of admission. She had had refractory KD 8 months previously and was treated unsuccessfully with 2 cycles of IVIG. She also received methylprednisolone pulse therapy combined with ulinastatin, which ameliorated her symptoms.

Physical examination during the current admission revealed high fever (40.4°C), bilateral conjunctival injection, strawberry tongue, erythema of the palms, and polymorphous exanthema on the trunk. Laboratory findings revealed an elevated white blood cell count (20 200/μL) with left shift. The erythrocyte sedimentation rate (ESR) was 67 mm/h and the C-reactive protein (CRP) level was 15.43 mg/dL. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were 265 IU/mL and 492 IU/mL, respectively. KD was suspected, and treatment with oral flurbiprofen was started. The following day (day 4), the 4 principal diagnostic criteria for KD were fulfilled, and IVIG (1 g/kg/day for 2 days) was started. On day 6, she received ulinastatin for fever, which persisted on day 8. CRP and ESR increased to 30.75 mg/dL and 130 mm/h, respectively. Chest radiography and echocardiography suggested cardiac depression. A second cycle of IVIG combined with methylprednisolone pulse therapy (30 mg/kg for 3 days)

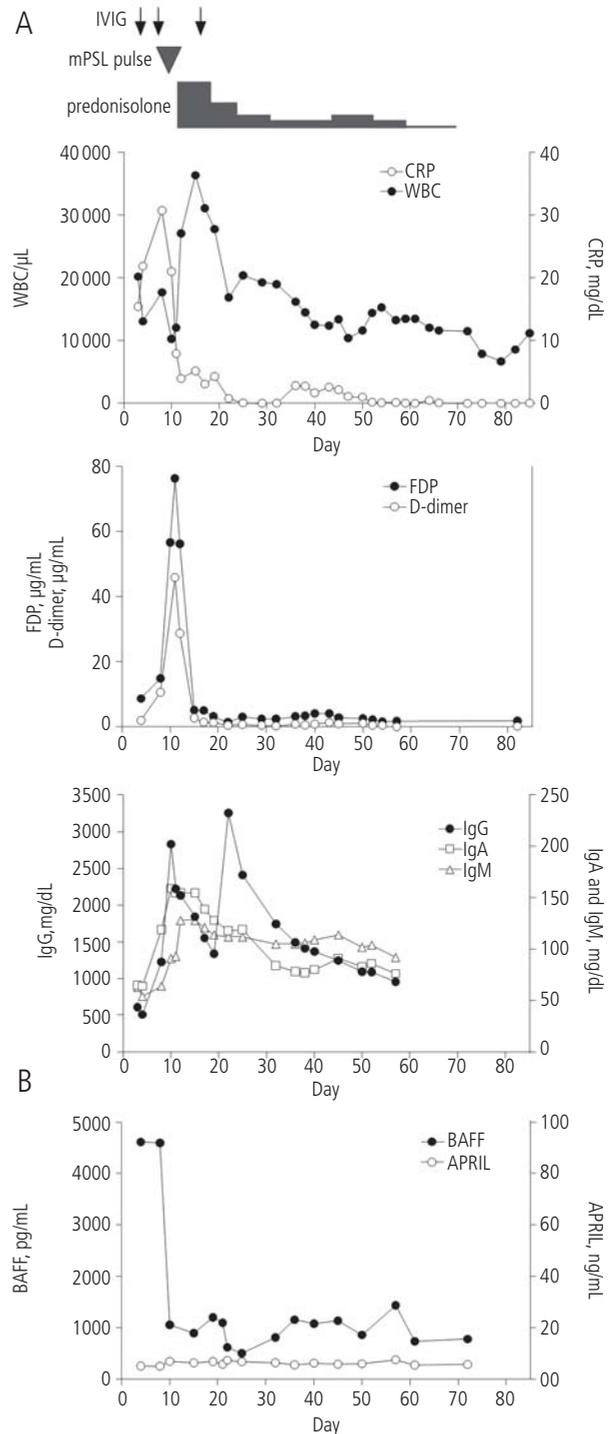


Figure. Changes in white blood cell counts and in levels of coagulation/fibrinolysis markers, immunoglobulins, and BAFF/APRIL. A, The treatment regimen, including 3 cycles of IVIG, methylprednisolone pulse therapy, and tapering oral prednisolone. B, Elevated BAFF levels fell rapidly after IVIG therapy. In contrast, APRIL levels remained very low, even after IVIG therapy. IVIG indicates intravenous immunoglobulin; mPSL, methylprednisolone; WBC, white blood cells; CRP, C-reactive protein; BAFF, B cell-activating factor; Ig, immunoglobulin; APRIL, a proliferation-inducing ligand.

followed by oral prednisolone was begun. On day 11, cardiac depression and hepatic dysfunction were ameliorated, and flurbiprofen was replaced with acetylsalicylic acid. However, as CRP levels remained positive (3-5 mg/dL) and fever was occasionally high, a third infusion of IVIG was given on day 17. CRP was negative on day 25. The dosage of oral prednisolone was tapered and the drug was stopped on day 78. The patient was discharged on day 85.

Changes in the patient's BAFF/APRIL levels are shown in the Figure. The levels of BAFF were high (4 614 pg/mL on day 4, immediately before the first round of IVIG) and remained so on day 8, immediately before the second round of IVIG (4597 pg/mL), but fell to 1059 pg/mL on day 10. In contrast, the levels of APRIL were low at 5.104 ng/mL on day 4 and did not increase, even after repeated IVIG and methylprednisolone pulse therapy. APRIL levels did not increase over the course of the patient's treatment, in contrast to our findings in KD patients who responded to IVIG [1].

Vasculitis in patients with KD is believed to stem from endothelial cell damage by activated T cells during the acute phase. Polyclonal autoreactive T-cell activation by bacterial superantigens may lead to the production of autoantibodies against blood vascular components [2]. B cells are now thought to play an important role in the production of pathogenic autoantibodies in KD, as is the case for most autoimmune diseases. In fact, polyclonal B-cell activation is well documented in acute KD, with immunoglobulin (Ig) A, IgM, and IgG levels peaking at different points after disease onset. Our previous study revealed that IgA and IgM levels rise immediately after administration of high-dose IVIG, although the differences were not statistically significant [1]. Elevated BAFF levels diminish after IVIG, while APRIL levels are inversely related to those of BAFF. Furthermore, BAFF levels were high before IVIG and decreased significantly thereafter, whereas APRIL levels that were within the normal range rose significantly after IVIG. In addition, a significant inverse correlation between BAFF and APRIL levels is observed in KD patients. Considering that BAFF levels are elevated in patients with autoimmune diseases [3] and that BAFF is involved in B-cell antibody production [4], elevated levels of BAFF in KD patients may result in the production of autoantibodies through B-cell activation.

Infiltration of vessel lesions by IgA plasma cells and the oligoclonal IgA response observed in KD suggest the importance of IgA in pathogenesis. The presence of the etiologic agent can induce an unusual antigen-driven immune response [5]. IgA can be induced independently of T cells through stimulation by BAFF and APRIL [6], and APRIL can promote IgA class switching [7]. In the present case, IVIG was associated with a rapid 2-fold increase in IgA levels comparable to that seen in IVIG-responsive patients. However, as an elevation in APRIL levels was not observed after treatment with IVIG, the elevated BAFF levels in the acute phase might independently account for the rise in IgA titers. Patients carrying a homozygous deletion in the BAFF receptor gene were observed to have lower IgG and IgM levels but normal IgA concentrations [8], suggesting that a BAFF-BAFF receptor disorder does not necessarily lead to depression of IgA levels. The rapid decrease in BAFF levels after treatment with IVIG might not be associated with alterations in IgA levels.

The significance of an elevation in APRIL levels after IVIG remains unclear. Since APRIL levels did not increase after

IVIG in the present case, a reduction in BAFF levels might not be sufficient for a successful outcome after IVIG, and an increase in APRIL might be essential. Although a recent study showed that both BAFF and APRIL have both proinflammatory and anti-inflammatory activity [9], APRIL has also been shown to regulate inflammation [10]. Taken together, APRIL might modulate autoimmune vasculitis in patients with KD.

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Allergen Stimulation Induces Simultaneous Production of Type 2 Helper T cells and Regulatory Cytokines in Patients With Pollen Allergy

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Key words: Pollen allergy. Foxp3. IL-4. Treg. TGF- β .

Palabras clave: Polinosis. Foxp3. IL-4. Treg. TGF- β .

Deficient immune tolerance and an inappropriate type 2 helper T cell (T_H2) response are considered to cause type I allergy in predisposed individuals [1]. Interleukin (IL) 4 plays a key role in differentiation of T_H towards the T_H2 phenotype and production of immunoglobulin (Ig) E. Accordingly, overproduction of T_H2 cytokines at the site of allergic inflammation and defective production of interferon (IFN) γ by $CD4^+$ T cells have been recorded in atopic patients [2]. Although central tolerance is the major mechanism involved in establishing the T-cell repertoire, the immune system has developed mechanisms that mediate tolerance in the peripheral lymphoid organs, thus providing the necessary safety mechanisms to prevent anomalous immune responses. Regulatory T cells (Treg) suppress allergen-induced specific T-cell activation, the action of effector cells involved in allergic inflammation (eg, mast cells, basophils, and eosinophils), and production of immunoglobulin (Ig) E [3]. Tregs are characterized by their ability to produce IL-10 and transforming growth factor (TGF) β [4]. A defining feature of Tregs is the expression of the transcription factor forkhead box p3 (Foxp3) [5].

We studied $CD4^+$ T-cell responses after in vitro stimulation with olive and grass pollen extracts in samples from allergic and nonatopic individuals. To this end, we obtained heparinized peripheral blood from 31 patients who were allergic to olive pollen, grasses, or both (grass 6, olive 3, and both 22) and 18 healthy donors with no history of atopy. Participants were matched in mean (SD) age (34.3 [8.7] vs 27.3 [10.3] years) and sex (male 50% vs 51.6%). Allergic patients were selected based on their clinical history, a positive skin prick test result, and a positive IgE determination for the recombinant allergens Phl p 1, Phl p 5, and Ole e 1. Blood was drawn outside the pollen season (November-December). Peripheral blood mononuclear cells (PBMC: $\times 10^6$ cells/mL) were stimulated with pollen extracts containing 10 μ g/mL of Phl p 5 and 10 μ g/mL of Ole e 1 or culture medium only at 37°C for 72 hours. Except in the case of Foxp3 determination, brefeldin A (50 ng/mL) was added during the last 6 hours of culture to accumulate

synthesized proteins inside the endoplasmic reticulum, and phorbol 12-myristate 13-acetate (50 ng/mL) + ionomycin (500 ng/mL) was added to express the imprinted cytokine pattern. Staining for CD4 and intracellular staining for IL-4, IFN- γ , IL-10, and TGF- β was performed using flow cytometry. Staining for Foxp3 was performed using fixation and permeabilization buffers provided with the FOXP3 kit (BD Biosciences) following the manufacturer's instructions. Tregs were identified using simultaneous

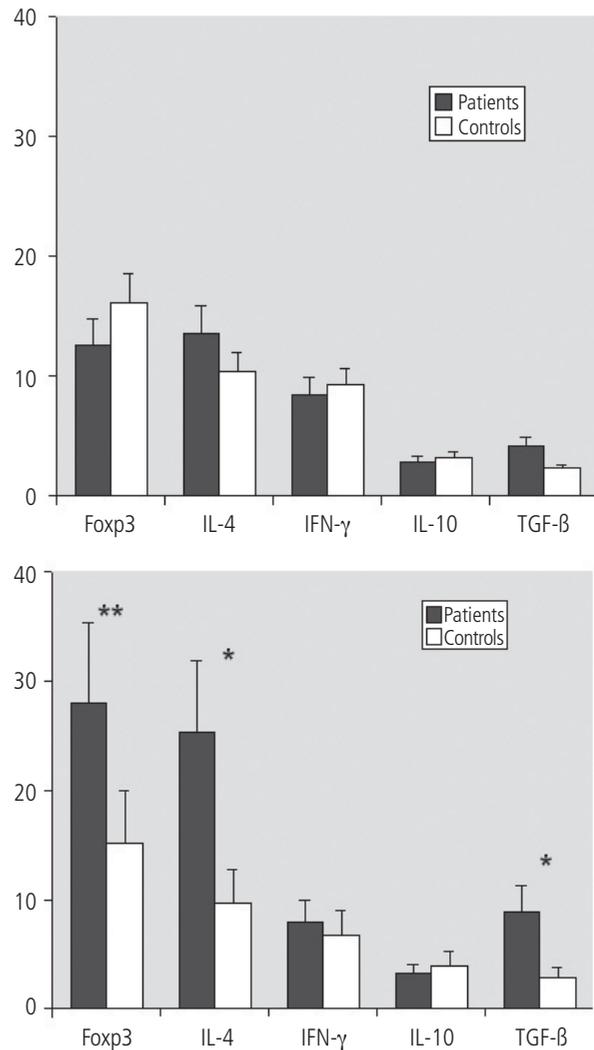


Figure. Percentage of cytokine-producing cells and Foxp3⁺ expression on gated $CD4^+$ cells from pollen-allergic patients and healthy controls. A, Before allergen stimulation. B, After stimulation with Phl p 5 and Ole e 1 in vitro. Peripheral blood mononuclear cells were cultured in both instances for 72 hours and studied using flow cytometry. The results are expressed as the mean of the percentage of gated $CD4^+$ cells with the 95% confidence interval. The black bars represent allergic patients (n=31), and the white bars healthy controls (n=18). * $P < .0001$, ** $P = .002$. Foxp3 indicates forkhead box p3; IL, interleukin; IFN, interferon; TGF, transforming growth factor.

staining for CD4⁺ and Foxp3⁺. Given the small size of the samples, a nonparametric test (Mann-Whitney) was applied to assess the statistical significance of differences between the groups.

In the absence of pollen extracts, no significant differences were detected between healthy controls and patients for any of the parameters studied (Figure, A). Allergen-stimulated CD4⁺ cells from atopic patients produced more IL-4 than those of healthy controls. In contrast, no differences were detected in the production of IFN- γ (Figure, B). The mean IL-4/IFN- γ ratio was significantly higher in patients than in healthy controls (15.9 [1.3] vs 4.6 [0.8]; $P=0.01$). These findings are consistent with the predominance of the T_H2 effector response previously described in patients with allergy [6].

Surprisingly, production of TGF- β and expression of Foxp3 on CD4⁺ cells were higher in allergic patients, while production of IL-10 was similar to that of controls (Figure, B). Tregs can develop in the absence of IL-10 through a mechanism mediated by TGF- β [7], and allergen-specific T cells showed modest production of IL-10 in allergic individuals [6]. Only mature Tregs and Tr1 cells have a considerable capacity to produce IL-10 [7].

In our study, the percentage of Foxp3 expression could be exaggerated. The increased percentage of CD4⁺Foxp3⁺ cells could also be due to a sharp decline in the percentage of naïve CD4⁺ cells upon allergen stimulation. We used percentages since we did not have the absolute counts of CD4⁺Foxp3⁺ lymphocytes. Nevertheless, synthesis of the regulatory cytokine TGF- β was significant in CD4⁺ cells.

Our results indicate that T cells from pollen-allergic patients can present regulatory features after stimulation with specific allergens. Allergy-promoting T_H2 effector and regulatory responses are not mutually exclusive. Tregs from atopic donors have been shown to reduce the ability to suppress the proliferation of effector T cells after allergen exposure [8]. In contrast to in vitro observations, regulatory cytokines are not released appropriately in vivo, although the reasons for this observation are not clear. The effectiveness of regulatory activity in allergic patients can be influenced by many factors, including adequate doses of allergen, site of localization, and appropriate presentation of antigen by dendritic cells. Our results demonstrate that it is possible to restore regulatory function to avoid an inappropriate immune response to allergens. Immunotherapy could promote appropriate development of the regulatory activity of the immune system to control the immune response to allergens.

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The authors declare that they have no conflicts of interest.

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DRESS Syndrome Involving 2 Unrelated Substances: Imipenem and Iodinated Contrast Media

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Palabras clave: Síndrome de hipersensibilidad a fármacos. DRESS. Imipenem. Medios de contraste iodados. Hipersensibilidad retardada.

Drug rash with eosinophilia and systemic symptoms (DRESS) is an uncommon life-threatening syndrome that appears 1 to 6 weeks after intake of the eliciting drug. Initially described with aromatic antiepileptic drugs [1], DRESS syndrome can be induced by several agents [2]. We report a case of DRESS syndrome in a patient receiving imipenem and ioversol.

An 83-year-old woman was treated with imipenem and amikacin for acute pancreatitis. Four days later, she underwent computed tomography with the contrast medium ioversol. Several hours after the scan, she developed a maculopapular

eruption with intensely pruriginous erythematous-violaceous lesions on her neck, back, and limbs. She was treated with parenteral dexchlorpheniramine. During the following days, she developed generalized edema and scaling (Figure, A), and imipenem and amikacin were withdrawn. Histopathology revealed a spongiform pattern and inflammatory changes with lymphocytic and eosinophilic infiltration (Figure, B). Laboratory studies disclosed 15.4×10^3 leukocytes/ μL (22% eosinophils) and an abnormal liver profile that she did not previously have. Systemic corticosteroids were prescribed and laboratory parameters returned to normal values.

Four weeks later, the results of patch tests with β -lactams (penicilloyl-polylysine, minor determinant mixture, benzylpenicillin, amoxicillin, cefuroxime, and imipenem) and amikacin were negative at 48 and 96 hours. The immediate reading in skin prick and intradermal tests with the same β -lactam derivatives was negative. However, 48 hours after the intradermal test with imipenem, the patient developed erythema and induration that lasted 6 days (Figure, C). The results of this test were negative in 10 controls. Patch tests with radiological contrast media showed positive reactions to iohexol (++) and ioversol (++) at 48 and 96 hours (Figure, D). The results of skin tests and an intramuscular single-blind placebo-controlled challenge test with amikacin were negative.

The pathogenesis of DRESS is not completely understood. Proposed mechanisms include genetic deficiency resulting in the accumulation of toxic drug metabolites, virus–drug interactions, and drug-specific T cell-mediated reactions [3]. In our patient, the delayed reaction to intradermal tests with β -lactams and positive patch test results with iodinated contrast media suggest a delayed hypersensitivity mechanism.

DRESS occurs most rapidly in previously sensitized patients, even within a day of readministration [4]. In our patient, symptoms began several days after starting treatment with imipenem and a few hours after administration of ioversol. She had received ioversol and imipenem several times before.

An episode of DRESS can also elicit massive nonspecific activation of the immune system and decrease the level of tolerance to drugs [3]. Therefore, the patient could have reacted to ioversol and become sensitized to imipenem during the episode.

In conclusion, based on the scoring system of Kardaun et al [5], we present a case of DRESS syndrome in a patient cosensitized to 2 chemically and antigenically unrelated substances: imipenem and ioversol. Although we could not rule out either substance as being responsible for the syndrome, a reaction to one could have predisposed the patient to a reaction to the other. To our knowledge, this is the first reported case of DRESS syndrome involving these 2 substances

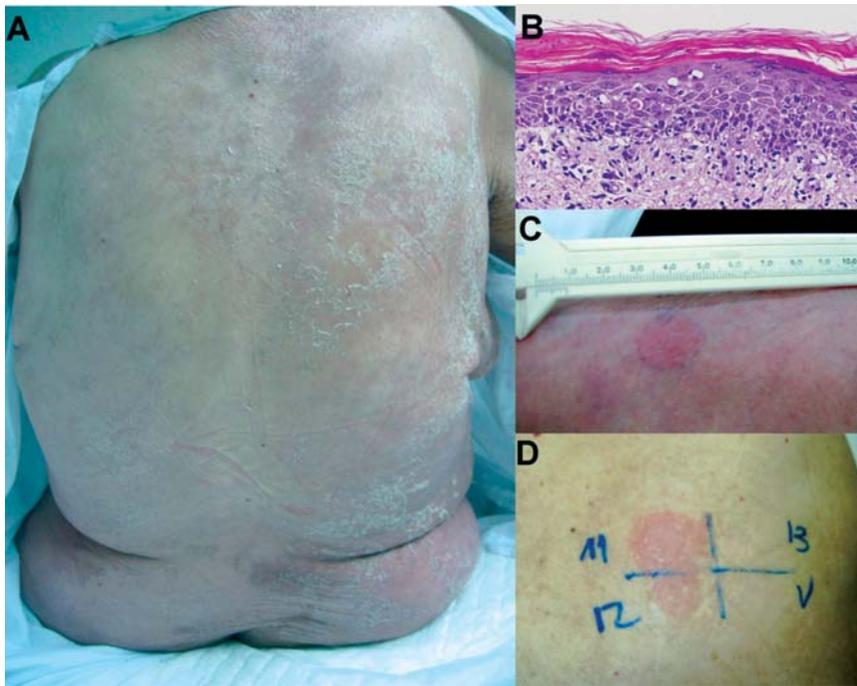


Figure. A, Erythematous-violaceous plaques (desquamative phase). B, Skin biopsy. C, intradermal test with imipenem (48 hours). D, Patch tests with iohexol and ioversol (48 hours). V indicates negative control (petrolatum).

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Has Sensitization to Cockroach Allergens Changed During the Last 17 Years in the Urban Atopic Population Living In Naples (Southern Italy)?

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Key words: Allergic rhinitis. Allergic sensitization. Bronchial asthma. Cockroach allergy. Hypersensitivity.

Palabras clave: Rinitis alérgica. Sensibilización alérgica. Asma bronquial. Alergia a cucaracha. Hipersensibilidad.

Cockroach is an important cause of allergic sensitization worldwide; however, few data are available on cockroach allergy in Italy. The only Italian multicenter study on this allergy showed the mean prevalence of sensitization to cockroach in adults to be 13% [1], although values of 4.58% and a peak of 20% have been recorded, respectively, in the Naples area [2] and among immigrants in Northern Italy [3]. Prevalence is lower in children, ranging from 0.45% to 12.7% [4-6]. With the exception of 2 recent studies [3,6], all the main studies [1,2,4,5] were performed several years ago.

The objective of our study was to assess the prevalence and clinical characteristics of allergic sensitization to cockroach in a population living in the city of Naples and the surrounding area in Southern Italy and to compare the results with those we recorded in 1993 [2].

We consecutively evaluated 414 individuals (mean age, 32.2 years; range, 8 to 74 years) in our allergy service from January 1, 2010 to July 31, 2010 for respiratory symptoms of a suspected immunoglobulin (Ig) E-mediated etiology.

A specially designed case report form was completed during the screening consultation with each patient. The data recorded were as follows: demographic data, type and duration of respiratory symptoms, pet ownership, possible exposure to cockroach allergens (assessed using predictors such as presence of cockroach in the dwelling and poor housing conditions), results of the skin prick tests (SPTs), and the results of specific IgE evaluation for cockroach. Respiratory allergy was diagnosed according to international guidelines [7,8].

The standard allergen panel used for the SPTs (ALK-Abelló Group) included *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, *Alternaria alternata*, *Cladosporium herbarum*, cat and dog dander, *Parietaria*, grass mix, *Artemisia vulgaris*, *Olea europaea*, *Betula pendula*, *Cupressus sempervirens*, and *Corylus avellana*. These

Table. Characteristics of Patients Sensitized to Cockroach Allergens

Demographic Data	Year 1993		Year 2010
No. of patients	19		15
Sex, male/female	13/6		7/8
Age, y	4-68		8-74
Mean age, y	24.1		27.2
Years with disease	7.8		9.4
Clinical Manifestations			
Rhinitis only	4		9
Asthma only	–		1
Rhinitis + asthma	15		5
Symptoms		Symptoms	
Perennial	14	Intermittent	3
Seasonal (autumn and spring)	5	Persistent	12
Associated sensitization		Associated sensitization	
<i>Dermatophagoides pteronyssinus</i>	17	<i>Dermatophagoides pteronyssinus</i>	15
Gramineae	4	Gramineae	4
<i>Parietaria</i>	4	<i>Parietaria</i>	4
<i>Artemisia vulgaris</i>	2	<i>Artemisia vulgaris</i>	4
<i>Olea europaea</i>	1	<i>Olea europaea</i>	2
<i>Alternaria alternata</i>	0	<i>Alternaria alternata</i>	2
Dog dander	0	Dog dander	2
Cat dander	0	Cat dander	5

Results of Diagnostic Tests

Skin prick test with allergenic extract	No. of patients			No. of patients		
+		1			9	
++		11			4	
+++		7			2	
++++		0			0	
Total		19			15	
Radioallergosorbent test	<i>Blattella germanica</i>	<i>Blattella orientalis</i>	<i>Periplaneta americana</i>	<i>Blattella germanica</i>	<i>Blattella orientalis</i>	<i>Periplaneta americana</i>
–	16	12	12	11	10	11
+	3	4	5	4	3	3
++	0	3	2	0	2	1
+++	0	0	0	0	0	0
++++	0	0	0	0	0	0
Total	19	19	19	15	15	15

allergens cover most of the causative agents of respiratory allergy in Italy. We also used allergenic extracts of cockroach (mix) (ALK-Abelló). Positive controls (10 mg/mL histamine dihydrochloride) and negative controls (saline solution in glycerine-phenol solution) were also used. SPTs were carried out and interpreted according to international guidelines [9].

Approximately 4 mL of serum was collected from each cockroach-sensitized patient and stored at –20°C. Specific IgE for *Blattella germanica*, *Blattella orientalis*, and *Periplaneta*

americana were determined using the CAP FEIA system (Thermo Fisher Diagnostics).

The number of patients, recruitment period, and diagnostic procedures were the same as in the previous study [2]. Only the symptom classification criteria were different, as these have changed during the last 17 years.

Positive SPT results to cockroach allergens were recorded in 15 of the 414 consecutive patients (3.62%). None of the patients were monosensitized. The characteristics of the

individuals examined in 1993 and in 2010 are compared in the Table. Only 2 of the 15 patients reported indoor conditions considered predictors for the presence of cockroach allergens. In these individuals, cutaneous and serological sensitization was more intense: in the remaining patients, the response to SPT and IgE determination was less intense (specific IgE was negative in 10 out of 15 individuals). Since all cockroach-sensitized patients had positive SPT results for other common allergens (mites, pollens, molds, and pets), we could not quantify the role of sensitization to cockroach in eliciting symptoms. Dust mites are the most common sensitizing allergens in all cockroach-allergic individuals; all other common allergens are associated with polysensitization.

Because monoclonal antibody-based methods for measuring the amount of cockroach allergen in the dust of indoor environments are not available in Italy, we have no information about levels of indoor exposure to these allergens. However, information provided by the patient on the presence of cockroach at home and predictors of cockroach infestation (eg, poor housing conditions) could allow us to hypothesize that individuals are exposed to cockroach allergen in indoor environments [10].

The results of our study suggest that the prevalence of allergic sensitization to cockroach allergens is low in an urban population in the Naples area (3.62%) and that this value is also lower than that (4.58%) reported 17 years ago (95%CI, 0.32-1.25; $t=3.445$; $P=.002$) [2]. This finding is probably due to the rarely reported presence (only 2 cases) of environmental conditions commonly considered high-risk for the presence of cockroach allergens [10], since most of the patients referred to our allergy service do not live in low-income areas of the city. These limitations are similar to those found in the previous study. As a consequence, we cannot exclude that a survey carried out in low-income districts of Naples and the surrounding area could reveal a higher prevalence of sensitization to cockroach.

Cosensitization to mite allergens in cockroach-sensitized individuals is a common feature, as shown in the previous report. Since cockroach and dust mites usually share the same indoor environments and some allergens, these mechanisms could account for such a high prevalence of cosensitization.

In conclusion, we detected a low prevalence of allergic sensitization to cockroach in populations living in an urban area of Naples and confirmed the low frequency found some years ago. However, we suggest that atopic individuals, especially those who are highly sensitized to mite allergens or those living in low-income areas, should be evaluated using SPT and serum specific IgE to cockroach allergens to rule out allergic sensitization. We are planning further studies to examine allergic individuals living in low-income districts of the Naples area in order to verify a possible increase in the rate of allergic sensitization to cockroach.

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Perphenazine as a Cause of Mother-to-Daughter Contact Dermatitis and Photocontact Dermatitis

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Key words: Allergic contact dermatitis. Perphenazine. Photoallergic dermatitis. Caregiver

Palabras clave: Dermatitis alérgica de contacto. Perfenazina. Dermatitis fotoalérgica. Cuidador.

A 56-year-old housewife was referred to our clinic with a 3-month history of eczema on both eyelids, mainly on the left one. She was left-handed, but was not able to relate her dermatitis to any trigger. Her condition had not improved after administration of various topical corticosteroids and withdrawal of cosmetics. She had no history of systemic illness and was not receiving treatment (topical or parenteral).

The standardized patch test procedure was performed with the TrueTest series (ALK-Abelló) supplemented with diallyl disulphide and topical corticosteroids. Readings were recorded on day 2 (D2) and day 4 (D4) using the International Contact Dermatitis Research Group scoring system. A positive response (++) was obtained with diallyl disulphide on D2 and D4. We then recommended her to avoid contact with Liliaceae (garlic, onion, and leek). However, the patient observed only a slight improvement. In springtime, she experienced considerable worsening with

acute eczema on photoexposed areas. Therefore, we performed photopatch testing according to the recommendations of the Spanish Photobiology Group of the Spanish Academy of Dermatology and Venereology. Positive responses (++) were observed on D2 and D4 with chlorpromazine in the photostimulation series. When questioned again about possible contact with neuroleptic drugs, the patient reported that she was administering orodispersible perphenazine tablets twice a day to her mother. Perphenazine 2% pet was applied in duplicate under occlusion. A positive response (++) was observed 24 hours later (D1) with both applications. One was exposed to radiation (5 J/cm²) from UV-A fluorescent tubes, and an intensely positive response (+++, blister) was observed on D3 (Figure). Four control patients had a negative response. The patient was diagnosed with contact allergy and photoallergic dermatitis to perphenazine. Her condition improved markedly (disappearance of the eczema) once she stopped handling her mother's tablets.

Perphenazine belongs to group 3 of the phenothiazine antipsychotics, which are common photoallergens [1]. Cross-reactivity between some of these drugs has been described previously [2]. We consider that our patient was primarily sensitized to perphenazine after handling the tablets and that the positive response to chlorpromazine was an incidental finding resulting from a cross-reactivity mechanism. However, it is generally agreed that major tranquilizers should be omitted from photopatch testing [3,4]. In the present case, chlorpromazine was key to finding the cause of the dermatitis, because we did not initially suspect perphenazine.

Dermatitis and photocontact dermatitis have been reported in health care professionals or caregivers handling drugs [5]. We propose the term "caregiver's dermatitis" to designate this type of dermatitis.

Correct diagnosis of contact and photocontact dermatitis should be based on an accurate clinical history with information on medicines or other substances used by persons with whom the patient comes into regular contact [6]. Given the ease with

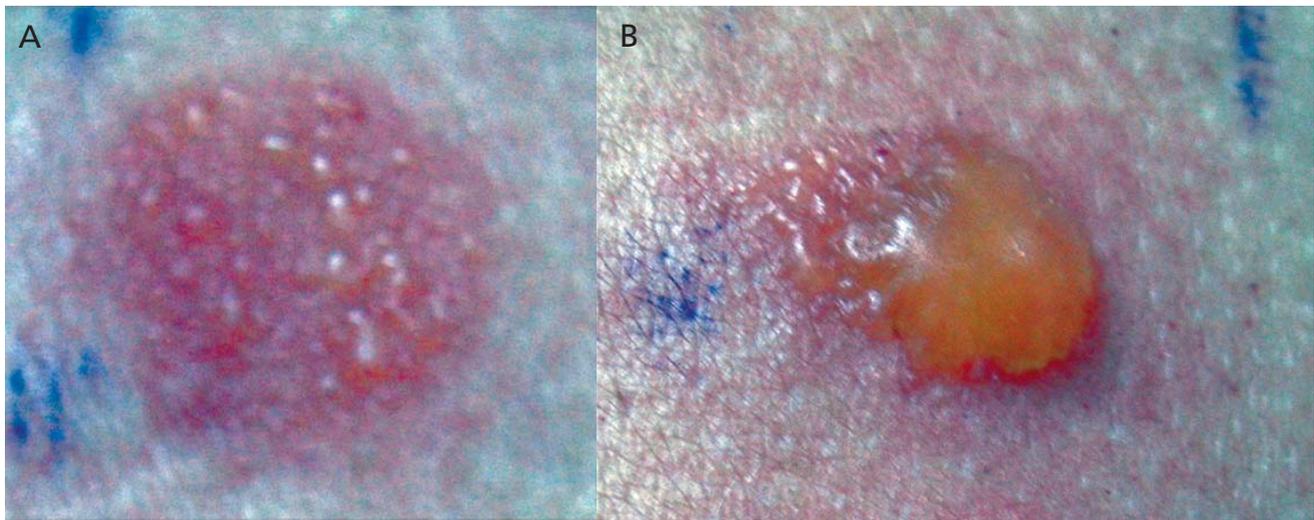


Figure. Delayed response to perphenazine in both series. A, Without photostimulation. B, With photostimulation.

which phenothiazines can produce photoallergy, it could be necessary to warn the relatives of patients with dementia taking these agents to use gloves when handling the pills.

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Toxic Epidermal Necrolysis Successfully Treated With Infiximab

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Key words: Dermatitis. Necrólisis epidérmica tóxica. Factor de necrosis tumoral alfa (FNT-alfa). Infiximab.

Palabras clave: Dermatitis. Toxic epidermal necrolysis. Tumor necrosis factor alpha (TNF α). Infiximab.

Toxic epidermal necrolysis (TEN) is a life-threatening drug-induced cutaneous reaction characterized by widespread apoptosis and detachment of the epidermis. The main causes of death are secondary infections resulting from epidermal damage and multiorgan failure [1]. The severity of illness score (SCORTEN) [2] analyzes 7 independent risk factors and predicts mortality. Older age, significant comorbidity, and greater skin involvement correlate with a worse prognosis [2].

The pathogenesis of TEN is unknown. Several studies suggest that tumor necrosis factor (TNF) α released from inflammatory cells and activated keratinocytes in the epidermis plays an important role in the immune response by inducing direct cytotoxicity and apoptosis [3]. Therefore, selective TNF blockade with infliximab has proven useful by leading to a quick recovery from lesions in patients with TEN [4-8]. Moreover, analysis of tissue samples before infliximab therapy reveals strong immunoreactivity for TNF- α in keratinocytes, inflammatory cells infiltrating the epidermis, and perivascular inflammatory cells in the dermis. This immunoreactivity is significantly decreased 24 hours after therapy [7].

Below, we report on 4 patients with TEN who were successfully treated with infliximab.

Patient 1 was a 76-year-old man who received furosemide for leg edema (40 mg daily). Two days later, he presented generalized erythema and developed sheet-like loss of epidermis with conjunctival involvement. The changes were observed on 100% of the patient's body surface. A diagnosis of TEN was made (SCORTEN, 2). Support therapy was started, and a single dose of 300 mg of infliximab was administered on day 2 of admission. The patient was also prescribed daptomycin for bacteremia caused by methicillin-resistant *Staphylococcus aureus*. Moderate eosinophilia (3300/ μ L) was observed after the second day. Both skin lesions and laboratory findings quickly returned to normal values, and, by the ninth day, more than 95% of the damaged skin was covered by a new epidermis (Figure).

Patient 2 was a 51-year-old woman who developed diffuse rash with epidermal sloughing involving 100% of her body surface after 1 day of treatment with intravenous ceftriaxone (2 g) for urinary tract infection. A diagnosis of TEN was

made (SCORTEN, 4). Intravenous methylprednisolone pulse therapy was administered, but the patient developed scaling erythroderma with skin detachment and mucosal involvement. Given the extent of the lesions, we administered a single dose of 300 mg of infliximab (second day of hospitalization and day 7 after the onset of symptoms). Disease progression ceased, no new erythematous lesions formed, and the patient had fully recovered after 7 days. Ocular involvement consisted of loss of eyelid skin with adhesions. The patient underwent symblepharon lysis, lamellar keratoplasty, amniotic membrane transplantation, and tarsorrhaphy. She subsequently developed bacteremia caused by extended-spectrum β -lactamase-producing *Escherichia coli* that responded well to doripenem. On day 24, an acute pulmonary thromboembolism developed, and anticoagulant therapy was started before discharge.

Patient 3 was a 17-year-old woman who received carbamazepine 200 mg twice daily for recently diagnosed frontal lobe epilepsy. Seven days later, she developed macular exanthema on the trunk that progressed rapidly to her limbs and affected about 50% of her body surface. The findings were consistent with TEN (SCORTEN, 3). She had received intravenous immunoglobulin (2 g/kg/day for 1 day) at another center. Carbamazepine was stopped, and a single 300-mg dose of infliximab was administered (second day of admission and day 8 after the onset of symptoms). Within 24 hours, blister formation stopped, and her general condition gradually improved. The patient developed bacteremia due to extended-spectrum β -lactamase-producing *Klebsiella pneumoniae*, which responded well to meropenem. On day 16 the patient's condition was good, and the blisters and erythema had completely disappeared.

Patient 4 was a 20-year-old woman with HIV-1 infection who started antiretroviral therapy with nevirapine (200 mg/d), zidovudine, and lamivudine. Fourteen days later, she presented with generalized exanthema that quickly progressed to detachment of the epidermis affecting 100% of the body surface and oral mucosal involvement that was consistent with TEN (SCORTEN, 2). She also developed toxic hepatitis. Antiretroviral drugs were withdrawn and, on the day following admission, a single 300-mg dose of infliximab was administered. Within a few days, the skin lesions and hepatitis had improved. She recovered completely after 7 days.

A gold standard therapeutic approach to TEN has not been established. The most important elements of treatment are prompt discontinuation of the culprit drug and initiation of support therapy. Treatment with high-dose systemic corticosteroids is controversial, and the effectiveness of therapies such as plasmapheresis and intravenous immunoglobulin are not yet proven.



Figure. A 76-year-old man with toxic epidermal necrolysis induced by furosemide. On day 9, the patient's condition was good, and more than 95% of the damaged skin was covered by new epidermis. He was discharged from hospital after 16 days. The photographs are from the day of the infusion (above) and 12 days later (below).

We report successful treatment with infliximab in 4 patients with TEN and extensive skin detachment. Disease progression was observed despite cessation of the culprit drug. Therefore, a single-dose of infliximab (300 mg) was administered, and progression of erythema and blister formation stopped in all 4 patients. The culprit drugs were consistent with those reported in the literature, except for furosemide, which has rarely been associated with TEN [9].

Consistent with previous reports [4-8], the time between onset of symptoms and administration of infliximab was 4, 7, 8, and 2 days (for furosemide, ceftriaxone, carbamazepine, and nevirapine, respectively). However, infliximab was administered on the first or second day of admission. The mortality rate was 0%, and after 5 to 14 days, re-epithelialization was almost complete. The complications we observed (eg, infections, pulmonary thromboembolism, toxic hepatitis, and eosinophilia) were consistent with those reported elsewhere [10]. TNF- α plays a critical role in defense against infection, and 3 of our patients developed bacteremia. However, none of the 3 developed severe sepsis or septic shock. Response to antibiotic therapy was good.

The rapid resolution of skin and mucosal lesions in our 4 patients after therapy with infliximab opens new perspectives in the treatment of TEN and supports a possible pathogenic role of TNF- α in tissue damage. Large-scale studies are needed before infliximab can be recommended as treatment for TEN.

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Observational Study of the Safety of a Cluster Schedule for Subcutaneous Immunotherapy in a Pediatric Population

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Controlled studies have demonstrated the safety of specific immunotherapy (SIT) with purified mite extracts for treating asthma and allergic rhinitis [1]. In recent years both the efficacy and safety of SIT have improved, thanks to patient selection, standardization, and chemical modification of allergens. Modification of allergens reduces their capacity to bind immunoglobulin (Ig) E, decreasing their allergenicity but maintaining their immunogenicity. It also allows the administration of higher allergen doses, with maintenance doses usually being reached after 6 injections in 5 weeks [2-3]. Cluster schedules may further shorten this uposing period, representing an advantage for both patients and practitioners. The aim of the present study was to evaluate: 1) the overall safety of a cluster schedule in a pediatric population using a mite allergoid, quantifying the number and severity of adverse reactions and 2) the safety of the same schedule in a subgroup of patients in whom SIT had been initiated before the age of 5 years.

This was an observational, retrospective study. The sample comprised 200 patients (115 male; mean age, 9.3 years) diagnosed with asthma and/or allergic rhinitis. All patients had sensitization to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* demonstrated by a prick test and/or specific IgE. The patients included in the study received information on the cluster schedule, which was approved by the hospital's clinical research ethics committee.

PURETHAL Mites (HAL Allergy BV), a mite extract mixture chemically modified with glutaraldehyde and adsorbed onto aluminum hydroxide was used at a concentration of 20 000 AUeq/mL (Der p1, 8 µg/mL and Der p2, 30 µg/mL). The maintenance dose of 0.5 mL was reached on the first day. It was divided into 2 doses of 0.2 mL and 0.3 mL administered 30 minutes apart, followed by an observation period of 1 hour. Patients then received a conventional monthly maintenance dose. The adverse reactions to SIT were recorded and classified as local and/or systemic.

Table. Total Systemic Reactions

Patient	Age of SIT initiation, y	Asthma	WAO-SC Classification	Reaction ^a	Modification of Schedule
1	5	Yes	I	Urticaria with angioedema	No
2	14	Yes	I	Generalized erythema and pruritus	No
3	8	Yes	I	Rhinitis	No
4	8	Yes	I	Rhinitis	No
5	13	Yes	I	Rhinitis	No
6	8	Yes	I	Conjunctivitis	No
7	5	Yes	II	Wheezing	No
8	12	Yes	II	Generalized pruritus and wheezing	Yes
9	3	Yes	II	Urticaria and wheezing	Yes

Abbreviation: WAO-SC, World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.

^aEighty percent of the systemic reactions occurred at a dose of 0.3 mL.

The 200 patients who were administered this cluster schedule received a total of 409 doses of SIT before the maintenance dose was reached. No patients discontinued the therapy. The overall incidence of systemic reactions was 4.5%, and the frequency per dose administered was 2.2% (Table). Sixty-six per cent of the systemic reactions (corresponding to 1.5% of the total doses administered) were grade I according to the World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System [4] and 33% were grade II (0.7% of the total doses administered). No grade III, IV, or V systematic reactions were observed. Thirty-four children experienced local reactions. Only in 4 cases (corresponding to 1% of the total doses administered) was the size of the reaction larger than 5 cm.

Twenty-one children under the age of 5 years were included. Four of these children experienced local adverse reactions. The size of the local reaction was greater than 5 cm in only 2 cases, representing 4.5% of the 45 doses administered in this subgroup. After the second dose of the cluster schedule 1 child under 5 years of age presented a systemic reaction (grade II), representing a frequency per dose administered of 2.2% in this subgroup of patients. The reaction resolved within 15 minutes with the administration of a single dose of intramuscular adrenalin and nebulization with salbutamol. Subsequently the patient presented good tolerance to a conventional schedule with oral antihistamine premedication.

In the literature few reports have focused on the use of cluster schedules in the pediatric population, especially in children under the age of 5 years [5-9]. The present study shows that the cluster schedule used in the induction phase was safe and well tolerated in a large pediatric population. Our results are in agreement with those reported by the only study published to date of cluster schedules with purified mite extracts in a pediatric population [6] and by other studies that included a proportion of pediatric patients and used cluster schedules with different types of allergen extracts [7-8]. These findings support the results published by Cox et al [10], who concluded that cluster schedule SIT constitutes an alternative to conventional approaches and does not increase the risk

of adverse reactions. No control group using a conventional up dosing scheme was included. However, in an adult study with the same product, the incidence of local and systemic reactions was similar or even slightly higher [3]. The fact that we used SIT with a high allergen concentration in a population comprising 77% of asthmatic children should be borne in mind in the interpretation of our results. We also stress that we only evaluated the induction phase of the SIT, when the risk of adverse systemic reactions is greater.

In conclusion, our results show that the cluster schedule described is safe and well tolerated in the pediatric population, even in children under the age of 5 years, providing that adequate selection criteria are applied and that the schedule is administered in a controlled fashion at a pediatric immunotherapy unit. This approach significantly shortens the time needed to reach the maintenance dose.

Conflicts of Interest

MJ Cruz and JD Boot are employees of Hal Allergy, Leiden, The Netherlands. The rest of authors have no conflicts of interest to disclose

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Novel Mutation of *IL2RG* Gene in a Korean Boy With X-linked Severe Combined Immunodeficiency

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Palabras clave: XSCID. Receptor IL-2. Mutación. Sibilancias.

Severe combined immunodeficiency (SCID) represents a group of rare, sometimes fatal, genetic disorders in which the adaptive immune system is impaired. X-linked SCID (X-SCID) occurs in approximately 50% of patients with SCID and is immunologically characterized by markedly diminished numbers of T cells and natural killer (NK) cells and normal or slightly increased numbers of dysfunctional B cells (TB*⁺NK-SCID) [1]. Affected patients have a profound deficiency of both cellular and humoral immunities [2,3]. X-SCID is mapped to the Xq13.1 locus and is caused by mutations in the *IL2RG* gene encoding the interleukin 2 receptor (IL-2R) γ chain (common γ chain), which is also associated with cytokine receptors for IL-4, IL-7, IL-9, IL-15, and IL-21 [4]. Therefore, cytokine signaling through the common γ chain is impaired in patients with X-SCID, leading to the impaired development of T and NK cells [4]. Various types of mutation in the *IL2RG* gene have been described in patients with X-SCID. We describe the case of a 5-month-old Korean male with X-SCID who had a novel mutation in the *IL2RG* gene.

A 5-month-old male infant with recurrent bronchiolitis was referred to Chungnam National University Hospital. His weight was 6.3kg (fifth percentile) and his height was 63.9 cm (25th percentile). The patient was born at 39 weeks of gestation after a normal pregnancy and delivery; however, his birth weight was 2.2 kg and there had been intrauterine growth retardation. There was no history of consanguinity in the family. His family history disclosed that 2 maternal uncles had died of infection during the first year of life. From the age of 3 months, the patient had frequently visited and had been admitted to a primary hospital because of recurrent bronchiolitis.

Physical examination on admission showed an emaciated infant with an otherwise normal appearance. The patient had undergone regular scheduled vaccinations until the age of 2 months (BCG, diphtheria-tetanus-acellular pertussis, polio-first shot, hepatitis B virus-second shot), but had not been vaccinated thereafter. He had a BCG scar, but no lymphadenopathy, organomegaly, or skin rash. Rales and expiratory wheezing were positive on auscultation. Human respiratory syncytial virus A antigen was detected in the tracheal aspirated fluid. Computed tomography of the chest revealed bronchial wall thickening with no apparent thymus. The blood counts on admission included white blood cells of 6630/ μ L (49.3% neutrophils and 35.6% lymphocytes), hemoglobin of 13.1 g/dL, and platelets of 384 000/ μ L. The patient had absolute lymphopenia (2360/uL).

Further investigation demonstrated hypogammaglobulinemia (immunoglobulin [Ig] G, 109mg/dL; IgM, 10 mg/dL; IgA, 1mg/dL, and IgE, 0.1 IU/mL) with marked reduction of T cells and NK cells and an increased percentage of B cells (94.3%). Proliferative responses of peripheral blood cells to mitogens were markedly decreased. These results implied TB⁺NK SCID. On the basis of the family history, recurrent infections, absence of thymus, failure to thrive, and deficiency of cellular and humoral immunity, X-SCID was suspected.

Blood samples were obtained from the patient and his mother following informed consent. Flow cytometry demonstrated deficient expression of the IL-2R γ chain (CD132) on CD20⁺ B cells in the patient, indicating IL-2R γ chain deficiency or X-SCID (Figure). The 8 exons and surrounding genomic sequences of the *IL2RG* gene were amplified from genomic DNA as previously described and directly sequenced [5]. A single base insertion at exon 6 (854insG), which resulted in a frameshift mutation (Thr286AspfsX1), was detected in the patient. His clinical course was complicated by recurrent regurgitation, diarrhea, acute suppurative otitis media, and persistent bronchiolitis. At the age of 8 months, he underwent cord blood stem cell transplantation without conditioning at Seoul National University Hospital. The posttransplant course was complicated by grade 1 graft versus host disease of the skin, and the disease responded to prednisolone. However, the patient died of treatment-related complications 1 month after transplantation.

We identified a novel mutation of the *IL2RG* gene in a Korean infant with X-SCID. The patient showed classical clinical features and laboratory data, such as absolute lymphopenia, low percentages of T cells and NK cells, and an increased number of B cells. He was found to have a single nucleotide (guanine) insertion at position 854+1 (854insG) within exon 6, resulting in a frameshift amino acid change. No mutations have been identified at position 854 in the *IL2RG* gene, although many different mutations involving other nucleotide positions in exon 6 have been confirmed. This may be the second genetically

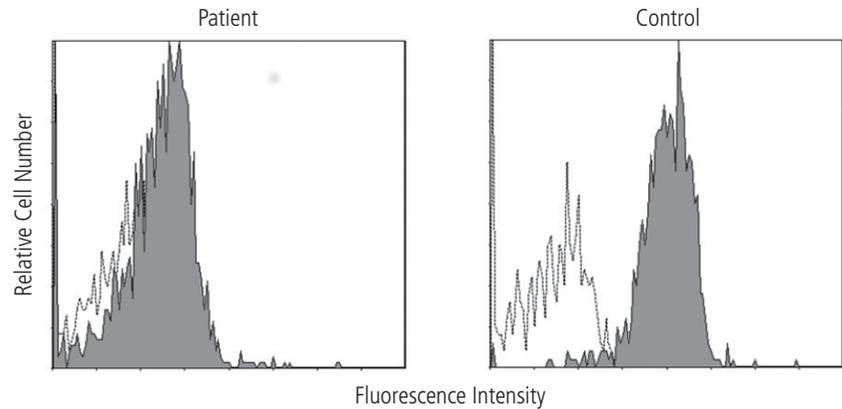


Figure. Interleukin 2 receptor γ (CD132) expression in CD20⁺ B cells from the patient and a healthy control. Cells were incubated with phycoerythrin-conjugated anti-CD132 monoclonal antibody (clone: TUGh4) and fluorescein isothiocyanate-conjugated anti-CD20 monoclonal antibody, and were analyzed using a flow cytometer. The dotted lines represent the negative control stained with isotype control antibody. The filled areas represent CD132-specific staining.

identified case of X-SCID in Korea. The first patient showed a single point mutation (C690T) with a missense mutation (R226C), which has been previously reported in many ethnic backgrounds[6]. A total of 344 mutation entries, comprising 198 unique molecular events, are now present in the X-linked SCID mutation database (*IL2RG*base) (<http://research.nhgri.nih.gov/scid/>). Many types of mutation have been reported throughout all 8 exons of the *IL2RG* gene.

In conclusion, we have reported a novel mutation of the *IL2RG* gene in a patient with X-SCID. A diagnosis of SCID should be suspected in patients with persistent infection and absolute lymphopenia in early infancy, as occurred in our patient. We believe we might have reported the second case of X-SCID and the third case of SCID including IL-7R α chain deficiency in Korea [7]. There may be more Korean patients with SCID. Further studies of X-SCID in this country are needed to clarify the differences observed in mutations and disease between different ethnic groups. Neonatal screening of the measurement of the T cell receptor excision circle is useful for detecting patients with SCID and severe T lymphopenia[8]. This method has been applied in some states in the United States, and it may be applicable in Korea.

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Food Allergy to Caper (*Capparis spinosa*)

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Palabras clave: *Capparis spinosa*. Alcaparra. Alergia a alimentos. Mostaza. Pro-heveina. Hev b 6.

Capparis spinosa is a bush that belongs to the Capparaceae family, member of the Brassicales order, as well as the mustard family (Brassicaceae), whose ability to induce allergic symptoms has been well documented, with several allergens described [1,2]. *C spinosa* is typical of the Mediterranean region. Its buds and fruits are eaten pickled, as a snack, seasoning, or as part of sauces. Moreover, members of the Capparaceae family have numerous medical applications thanks to their antimicrobial, antioxidative, anti-inflammatory, immunomodulatory, and antiviral properties [3-5]. All the species of this family contain isothiocyanate (mustard oil), and are therefore expected to irritate the skin [6]. There have been reports of contact dermatitis due to *C spinosa* [7], but to the best of our knowledge, the present study is the first to report allergy following the ingestion of caper fruit.

We describe a 24-year-old man who reported allergic symptoms after the ingestion of shellfish paella and caper fruit. He presented at the emergency department of Complejo Hospitalario in Jaén, Spain, with redness, angioedema of the face and hands, and aphonia. The patient was treated with methylprednisolone and dexchlorpheniramine. After several hours under observation, he was discharged with antihistamine and oral corticosteroid treatment.

The patient had previously been diagnosed as allergic to *Olea europaea* and grasses, and experienced rhinoconjunctivitis and moderate, persistent asthma in the spring. However, he had not previously presented allergic reactions to food.

The patient was skin-prick tested with a battery of standardized aeroallergens including grasses, *Salsola kali*, *Chenopodium album*, *Cupressus arizonica*, *Parietaria judaica*, *Ambrosia elatior*, *O europaea*, and *Platanus hybrida*, mites (*Dermatophagoides*), epithelia (cat and dog), mold (*Alternaria alternata*), *Anisakis simplex*, and latex (Bial-Aristegui). The results were positive for *Lolium perenne* (wheal diameter, 8 mm), *Cynodon dactylon* (4 mm), and *O europaea* (9 mm), and negative in all other cases. We also performed prick to prick tests with mustard, clam, shrimp, squid, caper bud, and caper fruit.

The results were negative for clam, shrimp, and squid, and positive for caper fruit, caper bud, and mustard (wheal diameters of 7, 8, and 5 mm, respectively), so analysis with these extracts was undertaken. The patient had never experienced problems after the ingestion of mustard.

A serum sample was obtained after receiving oral consent.

Caper fruit and buds were purchased at a local market and extracts were manufactured using previously described methods (Laboratorios LETI, SL) [8]. Briefly, after homogenization and extraction in buffer solution (PBS/PVPP 0.01 M), the extracts were centrifuged and the supernatant collected and freeze-dried. The protein content was 203 and 54 µg protein/mg of freeze-dried material for caper fruit and buds, respectively, measured by the Lowry-Biuret method.

The protein profile of both extracts was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 15% T). Mustard extract was included as a control to evaluate possible cross-reactivity between these related families. The protein profile of both caper extracts was very similar, with bands at 43, 40, 36, 35, 26 and 10 kDa; the 26-kDa band was the most intense (Figure 1A). The 2 caper extracts differed in the intensity of the bands between 35 and 43 kDa, which was higher in caper buds. The mustard extract presented more bands (from 7 to 70 kDa). The 3 extracts shared the 25- and 26-kDa bands, although the most intense bands in mustard were 12 kDa and 7 kDa.

The presence of glycoproteins was evaluated with the Most of the caper bands (including the 26-kDa band) corresponded to glycosylated proteins and were different from those observed in mustard (data not shown).

Specific immunoglobulin (Ig) E was quantified using the ImmunoCAP system (Phadia). Caper extracts were biotinylated (Roche Diagnostics), and incubated with streptavidin ImmunoCAP. Commercial ImmunoCAPs were used for the determination of specific IgE to whole extracts of *L perenne*, *O europaea*, latex, *Sinapis alba*, some allergen components (rPhl p 1, rPhl p 5b, rPhl p 12, nOle e 1, and rHev b 6), and bromelain. Specific IgE determinations were negative in all cases except for *L perenne* (11.5 IU/mL), *O europaea* (11.5 IU/mL), rPhl p 1 (18 IU/mL), and nOle e 1 (0.58 IU/mL).

Even though the level of specific IgE to food allergens was very low (negative ImmunoCAP), several allergens were observed by Western blot analysis of caper and mustard in the solid phase (Figure 1B). Electrophoretically separated proteins were electro-transferred onto a nitrocellulose Immobilon-P membrane (Millipore). The membranes were incubated with serum diluted 1:2. Finally, peroxidase-labeled monoclonal mouse anti-human IgE (Ingenasa) was added and a chemiluminescent reaction

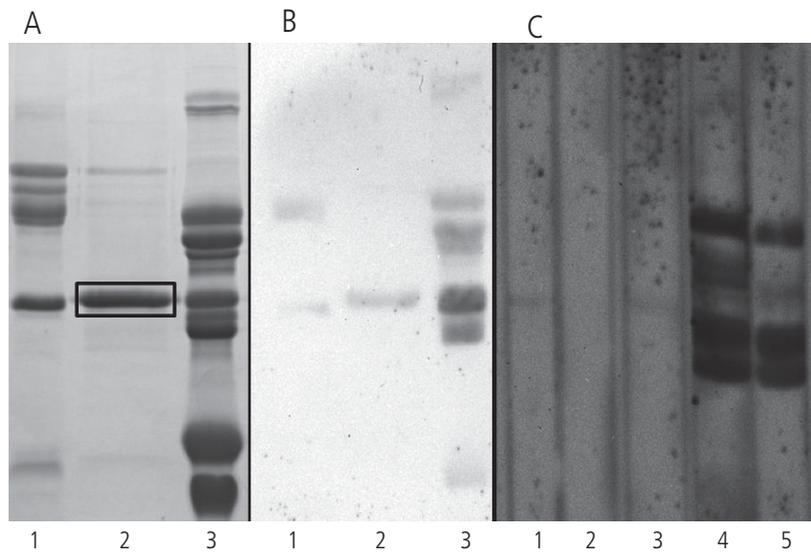


Figure 1. A, Sodium dodecyl sulfate polyacrylamide gel electrophoresis with Coomassie blue R-250 staining. B, Chemiluminescent Western blot (serum sample diluted at a ratio of 1:2). Sample distribution in A and B. Lane 1, caper bud (40 µg protein); Lane 2, caper fruit (40 µg protein); and Lane 3, mustard (40 µg protein). C, Western-blot inhibition. Lane 1, no inhibition; solid phase, caper bud (40 µg protein); Lane 2, solid phase, caper bud (40 µg protein) inhibited with caper fruit (400 µg protein); Lane 3, solid phase, caper bud (40 µg protein) inhibited with mustard (400 µg protein); Lane 4, no inhibition; solid phase, mustard (40 µg protein); Lane 5: solid phase, mustard inhibited with caper fruit (400 µg protein).

was observed using the Immun-Star WesternC kit (BioRad Laboratories). Western blot analysis showed that the serum sample had antibodies that recognized a 25-kDa band in the 3 extracts. Moreover, the sera recognized a 33-kDa band in the caper bud extract; mustard extract had the most intense bands, at 24, 26, and 30 to 35 kDa.

Immunoblot inhibition was also performed (Figure 1C). Caper fruit was capable of inhibiting caper bud because no bands were detected (Lane 2), indicating a high degree of cross-reactivity. Lane 4 shows the IgE-binding capacity of mustard extract. Finally, caper was able to partially inhibit the whole mustard extract (lane 5), demonstrating slight cross-reactivity between them, although the 26-kDa band, observed in the caper fruit extract, did not show total cross-reactivity with mustard (lack of inhibition in lane 3), although it was fully inhibited with caper bud (lane 2).

Only the 26-kDa band identified in caper fruits was sequenced, because the patient had only developed symptoms after the ingestion of this fruit. The sequences obtained covered 30% of the sequence and shared a 76% homology sequence with pro-hevein from latex (*Hevea brasiliensis*, Hev b 6.01, NCBI accession number ABW34946), which cleaves into hevein (Hev b 6.02) and a win-like protein upon latex coagulation. However, the patient had a negative skin prick test and negative specific IgE to both latex and Hev b 6. These results are consistent with previous reports that sensitization to Hev b 6 does not seem to be associated with other plant food allergies in latex-fruit syndrome [9].

To our knowledge, this is the first report of food allergy to caper. More cases should be reported to help determine the importance of the allergen identified.

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Early Diagnosis of an Allergic Reaction to Cisatracurium

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Key words: Allergy. Intravenous anaesthetics. Cisatracurium. Early diagnosis. Kounis syndrome.

Palabras clave: Anestésicos intravenosos. Cisatracurio. Diagnóstico temprano. Síndrome de Kounis.

The mortality of allergic reactions during anesthesia is estimated to be 6%, and between 1/10 000 and 1/1250 anesthetized patients die from such a reaction [1,2]. Given their quaternary ammonium structure, neuromuscular blocking agents (NMBAs) can behave as antigens. Consequently, they are responsible for more than two-thirds of anaphylactic or anaphylactoid reactions and for 80% of cases of anaphylactic shock during anesthesia [3]. The nondepolarizing muscle relaxant, cisatracurium, induces fewer allergic reactions than other NMBAs [4], such as succinylcholine, whose administration entails a higher risk than most of the other commonly used agents.

Cisatracurium belongs to the benzylisoquinoline group. Since it can be administered at a lower dosage than its predecessor atracurium, it releases less histamine and therefore produces fewer histamine-dependent hemodynamic changes and potentially fewer toxic metabolites.

Skin tests are the first step in the diagnosis of reactions induced by NMBAs, although they must be performed approximately 4 to 6 weeks after the allergic reaction [5]. Consequently, since identifying the causative drug could take some time—a key factor in patients with aggressive tumors or those who require emergency surgery—the skin test should be accompanied by a basophil activation test (BAT), which is considered useful for the diagnosis of NMBA-induced reactions [6].

A 59-year-old man received diazepam, fentanyl, and propofol during induction of general anesthesia for resection of obstructive rectal cancer. Immediately after administration of cisatracurium, the patient experienced supraventricular tachycardia, refractory and severe hypotension, and increased respiratory pressure. The electrocardiogram showed elevation of the ST segment. He was treated with vasoactive drugs (ephedrine) and plasma expanders (gelatin). Owing to the severity of the reaction, surgery was cancelled, and the patient was sent to the intensive care unit until his condition stabilized. Creatine phosphokinase-MB increased to 17.7 IU/L (reference value, <10 IU/mL). Therefore, cardiac catheterization was performed immediately, and echocardiogram findings were

Table. Results of the Basophil Activation Test With Neuromuscular Blocking Agents^a

Day 7 After Anaphylactic Shock												
	Baseline	Anti-IgE	Cisatracurium		Rocuronium							
			0.025	0.006	0.125	0.03						
BAT, % ^b	0	77.3	<i>37.1</i>	<i>17.9</i>	1.1	2						
Day 15 After Anaphylactic Shock												
	Baseline	Anti-IgE	Cisatracurium		Rocuronium							
			0.025	0.006	0.125	0.03						
BAT, % ^b	1.8	81	<i>19.6</i>	<i>39.6</i>	0.4	0.8						
Day 47 After Anaphylactic Shock												
	Baseline	Anti-IgE	Cisatracurium		Atracurium		Rocuronium		Vecuronium		Mivacurium	
			0.025	0.006	0.125	0.03	0.125	0.03	1.25	0.3	0.025	0.006
BAT, % ^b	2.7	30	<i>24.2</i>	<i>43.8</i>	1.6	<i>18.3</i>	0.8	1.6	3.8	2.1	1.5	2.8

Abbreviations: Ig, immunoglobulin; BAT, basophil activation test.

^aIncubation was with buffer (baseline), IgE, and 2 concentrations (mg/mL) for each neuromuscular blocking agent. A result was considered positive when the percentage of activated basophils was double the baseline percentage (always above 5% and marked in italic letters).

^bBAT (%) refers to the percentage of basophils activated during the test.

normal. Troponin levels were elevated (0.12 ng/mL 3 hours after the onset of the reaction; 0.22 ng/mL 10 hours later; reference value, <0.035 ng/mL). Serum tryptase levels reached 115 µg/L 2 hours after the reaction and then decreased to 5.48 µg/L after 36 hours. The initial presumptive diagnosis was cardiac ischemia. The tryptase results enabled us to confirm a diagnosis of anaphylactic shock (Kounis syndrome) [7]. As the patient required surgery as soon as possible for resection of the tumor, an alternative NMBA was sought.

A skin test and BAT were performed 1 week after the reaction. An intradermal test with cisatracurium (0.02 mg/mL) was positive (6 × 5 mm), and the results of the BAT were positive at both concentrations used (25 g/mL and 6 g/mL). Skin tests and BAT were negative for latex and the other drugs used.

In order to select a nondepolarizing NMBA, skin tests and BAT were performed with rocuronium (aminosteroid), and the results of both were negative. Therefore, surgery was scheduled for 10 days after the reaction and rocuronium was used as an alternative NMBA with good tolerance.

Fifteen days after the reaction, the skin tests and BAT were repeated with all the drugs involved in the reaction, as well as with latex and rocuronium. Intradermal tests were positive to cisatracurium 0.02 mg/mL (6 × 4 mm) and negative to the rest of the drugs tested; the results of BAT were also positive only to cisatracurium. The study was completed 6 weeks after the reaction, and the NMBAs tested (skin test and BAT) were cisatracurium, atracurium, rocuronium, vecuronium, and mivacurium. The results of prick testing with cisatracurium and atracurium were positive (2 mg/mL); testing with the other NMBAs was negative. The results of the BAT were also positive for cisatracurium and atracurium (Table).

In our opinion, the association between the administration

of the drugs and the anaphylactic reaction, together with the results of the skin tests and the early BAT, supported our diagnosis. For ethical reasons, no challenge test was performed to confirm the diagnosis.

In the case we report, a diagnosis based on skin tests and BAT was reached as early as 1 week after the reaction. It is important to be able to make a rapid diagnosis, since in some cases, it is not possible to wait the 4 to 6 weeks recommended by guidelines to perform the allergy study. The results of intradermal tests were positive to cisatracurium in earlier studies; the results of prick tests were positive in later studies. Therefore, although the results of early studies can be used in emergency situations, they should be interpreted with caution and always confirmed in subsequent studies. Recent guidelines consider the possibility of studying the patient earlier, but taking into account only the positive results [4].

Even though the concentrations of the NMBAs used in the BAT were lower those reported by other authors [6], the result was always positive for cisatracurium and negative for rocuronium. Our patient tolerated rocuronium well, despite the high incidence of allergic reactions to rocuronium reported elsewhere [8].

Other authors have also reported a lack of cross-reactivity between benzylisoquinolines and aminosteroids. Ebo et al [6] observed a lack of cross-reactivity with cisatracurium in patients with rocuronium-induced perioperative anaphylaxis. In a similar case to ours, Dewachter et al [9] demonstrated tolerance to rocuronium after an anaphylactic reaction to cisatracurium.

We can conclude that an early allergy workup with drugs after an anaphylactic reaction (1 week) could be useful for rapid selection of alternative drugs. Furthermore, BAT could prove useful in the diagnosis of hypersensitivity to NMBAs. In the case we report, sensitivity was high when using BAT together with skin tests, even as early as 1 week after the reaction.

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Intravenous Immunoglobulin in Urticaria

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Key words: Chronic urticaria. Angioedema. Intravenous immunoglobulin. Infections.

Palabras clave: Urticaria crónica. Angioedema. Inmunoglobulina intravenosa. Infecciones.

Urticaria is a common skin disease with a lifetime prevalence of almost 20%. It is an epiphenomenon of various conditions, such as autoimmune diseases, viral infections, and allergies, and is often associated with angioedema (AE). Urticaria can be triggered by physical, pharmacological, and immunological factors or can occur with no apparent trigger factor, in which case it is known as idiopathic chronic urticaria (CU), which lasts <24 hours and recurs over a period of ≥ 6 weeks [1]. Management of urticaria involves identification and elimination of the underlying causes or eliciting triggers and administration of treatment to provide symptom relief. Treatment of the cause is not applicable in most cases of CU, although treatment of associated infectious and inflammatory processes could be helpful in selected cases. The treatment of choice is antihistamines; however, when these agents are ineffective, therapy can be challenging. Intravenous immunoglobulin (IVIG) has been used with variable success. We analyzed the clinical history of over 200 patients who presented at our outpatient clinic from January 1, 2000 to December 31, 2011 for assessment of CU, AE, or both before treatment with IVIG. We describe the clinical course of 6 patients who received IVIG to treat mild antibody deficiency with refractory CU, AE, or both.

Patient 1 was a 51-year-old man with a 2-year history of CU who required daily antihistamines. He also had a 10-year history of refractory, recurrent upper and lower respiratory tract infections. He was diagnosed with bronchiectasis and had reduced immunoglobulin (Ig) G and IgM and normal IgA levels.

Patient 2 was a 14-year-old boy with recurrent urticaria and AE, recurrent upper respiratory tract infections, and bronchitis. He had reduced IgG and slightly reduced IgM and IgA levels.

Patient 3 was a 65-year-old man with a 10-year history of CU, which persisted despite regular treatment with oral antihistamines. His medical history revealed recurrent upper respiratory tract infections. He had normal IgG levels with reduced IgG1 levels. Three years later, the severity of his symptoms intensified to the point that he required systemic corticosteroids.

Patient 4 was a 61-year-old man with a 5-year history of CU and AE. He had had pneumonia at 20 and 32 years of age. IgG and IgM levels were reduced, and IgA levels were normal.

Table. Results for the Cases Described

Patient No. (age, y)	IgA	IgM	IgG	IgG1	IgG2	IgG3	IgG4	Protective Antibody Responses	BAT/CD63 Upregulation ^a	Time to IVIG Response, Mo	Time to Recurrence After Stopping IVIG, Mo	Second Course of IVIG	Currently Receiving IVIG
1 (51)	0.98-4.5	0.76-1.48	8.54-12.6	4.90-11.4	1.50-6.40	0.20-1.10	0.08-1.40	Pneumococcal+ <i>Haemophilus</i> <i>influenza</i> B+	NE	10 (I/U)	No	No	No
2 (14)	0.93	0.55	6.9	NE	NE	NE	NE	Pneumococcal- <i>Haemophilus</i> <i>influenza</i> B+	NE	6 (I/U/A)	4 (I/U/A)	Yes	Yes
3 (65)	4.76	0.79	11.3	2.27	7.47	0.63	0.94	NE	Positive	1 (I/U)	10 (U)	Yes	No
4 (61)	1.79	0.66	4.69	2.89	1.47	0.17	0.16	Pneumococcal+ Tetanus+	NE	1 (I/U/A)	3 (U/A)	Yes	Yes
5 (65)	4.52	0.53	6.27	NE	NE	NE	NE	NE	NE	1 (I/A)	No	No	Yes
6 (42)	0.80	0.75	5.30	3.25	1.26	0.26	0.30	NE	Negative	5 (I/U/A)	No	No	No

Abbreviations: A, angioedema; I, infections; IVIG, intravenous immunoglobulin; NE, not evaluated; U, urticaria; +, protective; -, not protective.
^aBAT/CD63 upregulation on basophils.

Patient 5 was a 65-year-old woman with a history of recurrent AE. She had previously had multiple upper respiratory tract infections and bronchitis and had been diagnosed with bronchiectasis. She had reduced IgG and IgM level and normal IgA levels.

Patient 6 was a 42-year-old man with an 8-year history of CU and AE. He had a history of chronic obstructive pulmonary disease (GOLD stage II), had had 1 episode of pneumonia, and had reduced IgG and slightly reduced IgM and IgA levels.

We describe 5 patients with unspecified hypogammabulinemia and 1 patient with IgG subclass deficiency with CU, AE, or both (Table). All 6 patients reported an improvement in CU, AE, or both 1 to 10 months after starting monthly infusions of IVIG (0.2-0.5 g/kg). In 2 cases, CU and AE did not recur when IVIG was stopped. Three patients (patients 3, 4, and 5) had relapses of CU, AE, or both when IVIG was stopped, and 3 patients (patients 2, 4, and 5) are still receiving IVIG because of infectious problems. CU persisted in Patient 3 despite a second course of 16 monthly IVIG infusions. Finally, ciclosporin A was started (2×100 mg daily). The patient was lost to follow-up.

Our report provides data on the response to treatment of CU with low-dose IVIG. In autoimmune and inflammatory disorders, IVIG preparations are administered at doses of 1-3 g/kg. Since CU is thought to have an autoimmune basis in 30% to 40% of patients with the presence of IgG targeting the Fc receptor α subunit [2], high-dose IVIG (2 g/kg every 4-6 weeks) was tried in a small study of 6 patients with severe CU [3]. Remarkably, symptoms improved, thus enabling concomitant medication to be reduced after the first cycle. Another approach consisted of IVIG infusions of 0.4 g/kg/day for 5 days in patients with severe CU [4]; however, the effect of this treatment was less successful in another study [5]. In contrast, low-dose IVIG replacement therapy (0.2-0.5 g/kg) is recommended in primary immunodeficiency disorders such as common variable immunodeficiency (CVID). IVIG (0.15 g/kg every 4 weeks for a minimum of 6 months and a maximum of 51 months) proved to be an effective option in patients with severe CU refractory to conventional treatment in which an autoimmune mechanism was involved. Efficacy persisted for at least 12 months after treatment [6].

A review of the literature and our findings did not enable us to establish an association between response to therapy and disease duration with respect to trough levels of IgG in patients receiving low-dose IVIG. Furthermore, it is not clear how long therapy should be continued. A conceptual explanation of how low-dose IVIG

affects CU is challenging, and the anti-inflammatory effect of this approach in CU remains open to debate. It has been hypothesized that CU affecting patients with CVID might occur from infection-induced complement activation [2]. It seems reasonable to assume that potential trigger factors for recurrent infections had been eliminated in those patients who responded well to low-dose IVIG. We are unable to assess the extent to which our patients differed from other patients with CU. A detailed history revealed repeated respiratory infections, which led us to determine Ig levels. The association between CU and antibody deficiency has been addressed in CVID [7,8]. However, unspecified hypogammaglobulinemia occurs in association with a variety of clinical conditions or in circumstances where its relevance to the overall clinical picture is unclear [9]. Furthermore, the diagnosis and clinical relevance of IgG subclass deficiency is controversial, since low levels of 1 or more IgG subclasses can be found in 2% to 20% of healthy individuals [10]. We cannot prove any association between mild antibody deficiency and CU. Each could be an independent condition. In the absence of infection, Ig levels should not be routinely determined in patients with CU and AE. Low-dose IVIG can only be used as an alternative treatment of CU if triggers such as recurrent infections can be eliminated.

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ERRATUM

Hypereosinophilic Syndrome Associated With Regulatory T-cell Disruption as a Complication of Stem Cell Transplantation T Kersey-Barrett, A Glick, D Wald, H Meyerson, H Tcheurekdjian

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Page 454, Figure

The *P* values in the figure are incorrect: *P*=.83 should be *P*=.083 and *P*=.50 should be *P*=.05. The order of the ratios shown is also incorrect. It should be 4:1 followed by 1:1.