Successful Desensitization to Idursulfase in a Patient with Type II Mucopolysaccharidosis (Hunter syndrome)

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Hunter syndrome or type II mucopolysaccharidosis is a rare lysosomal storage disease of X-linked recessive inheritance. It is characterized by a lack of the enzyme iduronate 2 sulfatase (I2S), which leads to the accumulation of glycosaminoglycans in many cells and tissues, resulting in myocardiopathy, airway obstruction, skeletal deformities, and severe and progressive neurologic disturbances. Those affected often die in the second decade of life [1].

Until the appearance of enzyme replacement therapy, there were few therapeutic options that had a significative impact on the natural course of the disease. Nowadays, however, it is possible to compensate for the enzymatic loss with idursulfase, a purified form of I2S produced using DNA recombinant technology in a continuous human cell line [2]. However, the administration of idursulfase is associated with infusion-related hypersensitivity reactions in a high percentage of patients [3].

We report the case of a 10-year-old boy diagnosed with Hunter syndrome at the age of 8 years. He had bilateral neurosensorial hypoacousia, aortic valvular stenosis without hemodynamic alterations, chronic obstruction of the upper airway, restrictive lung disease, umbilical hernia, and a history of recurrent seizures. Since he had only received support therapy, weekly replacement therapy with intravenous idursulfase was initiated.

The first 5 sessions were well tolerated, but during the sixth session, the patient developed generalized urticaria 1 hour after beginning therapy. Idursulfase infusion had been programmed for a total of 3 hours in accordance with the manufacturer's recommendations, ruling out an inadequate infusion rate as the cause of the reaction. On detection of urticaria, the infusion was stopped and the reaction was successfully treated with hydrocortisone and clemastine. Four weeks later, we carried out an allergy study with skin prick tests (2 mg/mL) and intradermal skin tests (0.02, 0.2 and 2 mg/mL), but all the results were negative.

Although there was no evidence of type I hypersensitivity, we decided to perform a short desensitization protocol (Table), which was well tolerated. The patient has since received 16 weekly administrations of idursulfase using this protocol and has experienced no further episodes of urticaria or other signs of hypersensitivity. There have also been clinical improvements in the disease.

Enzyme replacement therapy with idursulfase is the best treatment for type II mucopolysaccharidosis (Hunter syndrome). Patients who have received this therapy have shown significant functional improvement compared to those who have received placebo after a year [4]. Although the use of idursulfase has been associated with several adverse effects

Table. Proposed 8-hour Desensitization Protocol for Idursulfase[a]

Dilution	Concentration	Preparation	Infusion Rate	Duration
1	1/1000 (0.000012 mg/mL)	10 mL of dilution #2 in 90 mL of NS	1st hour: 10 mL/hr 2nd hour: 90 mL/hr	2 hr
2	1/100 (0.00012 mg/mL)	10 mL of dilution #3 in 90 mL of NS	1st hour: 10 mL/hr 2nd hour: 90 mL/hr	2 hr
3	1/10 (0.0012 mg/mL)	10 mL of dilution #4 in 90 mL of NS	1st hour: 10 mL/hr 2nd hour: 90 mL/hr	2 hr
4	Basal (0.012 mg/mL)	12 mg (6 mL) of idursulfase in 94 mL of NS	1st hour: 10 mL/hr 2nd hour: 90 mL/hr	2 hr

[a]The patient should receive 0.5 mg/kg weekly. Each ampule contains 6 mg of idursulfase diluted in 3 mL of normal saline (NS). The dilutions shown here were calculated according to the patient's body weight (24 kg) and prepared in progressive steps from dilution #4. Thirty minutes before initiating the protocol, we administered 1 mg of intravenous clemastine. To prepare individual protocols, the dose should be calculated according to specific body weight. The total volume of dilution #4 should be 100 mL.

(eg, headache, nasopharyngitis, abdominal pain, arthralgias, infusion site swelling, dyspepsia, anxiety, and chest wall pain), infusion-related reactions (IRRs) are the most relevant as they have a direct impact on ongoing and subsequent infusions. Most IRRs are hypersensitivity reactions and many could be classified as anaphylactic or anaphylactoid. The immediate treatment is to stop the infusion.

Current recommendations for preventing IRRs include premedication with corticosteroids, acetaminophen, and antihistamines, and slowing of the infusion rate [5]. However, there are no specific recommendations on this matter (eg, drug doses, moment at which the drugs should be administered, or rate by which the infusion should be slowed). We only administered clemastine (1 mg according to the patient´s body weight) 30 minutes before the first infusion and this measure has proven sufficient. We performed an allergy study but found no evidence of immunoglobulin (Ig) E antibodies. This finding is consistent with information that has been published on the formation of IgG antibodies (~50% of cases) against idursulfase, but not with that published on IgE antibodies (both measured by enzyme-linked immunosorbent assay) [4]. Because IgE-mediated hypersensitivity could not be demonstrated, it is possible that premedication might not be necessary, but we cannot recommend excluding this step.

To our knowledge this is the first report showing the usefulness of a desensitization protocol for idursulfase. We propose its use in patients with Hunter syndrome who develop hypersensitivity reactions during replacement therapy with idursulfase.

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Molecular Characterization of Bruton's Tyrosine Kinase Deficiency in 12 Iranian Patients With Presumed X-Linked Agammaglobulinemia

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X-linked agammaglobulinemia (XLA) is a primary immunodeficiency disorder characterized by a lack of B cells, extremely reduced levels of all serum immunoglobulins, and recurrent bacterial infections [1]. The gene responsible for this disorder is called Bruton's tyrosine kinase (*BTK*) [2]. It has been shown that mutations at intron 1 and at the putative promoter of the *BTK* gene are functional and that transcriptional activity of the mutant *BTK* is significantly lower than in normal cases [3,4]. In this study, 12 male patients, from unrelated families, with presumed manifestations of Btk deficiency were enrolled. To screen for the Btk deficiency, the Western immunoblot assay was performed with appropriate antibodies. After genomic DNA extraction, all the exons and exon/intron boundaries of the *BTK* gene were screened by denaturing high-performance liquid chromatography followed by direct

sequencing. To screen regulatory regions, the *BTK* promoter and intron 1 regions were amplified using specific primers [5]. Complementary DNA (cDNA) analysis was performed in patients with nongenomic mutations. Indirect mutation analysis was performed in 1 patient (P12) and 12 of his relatives using polymerase chain reaction (PCR) amplification of the DXS7424 STR marker.

The protein expression and mutation analysis results are summarized in the Table. PCR amplification of DXS7424 led to this patient being ruled out as an XLA case.

Generally, it has been shown that some patients with a clinical history of antibody deficiency are affected by a condition of unknown etiology in which reduced *BTK* mRNA levels are not correlated with any mutations in *BTK* coding regions and may have some transcriptional regulatory defects [3,7].

In this study, we hypothesized that patients with no coding or transcriptional mutations might have deficiencies in certain regulatory regions such as the putative promoter region or the initial base pairs of *BTK* intron 1, as 2 *cis*-acting elements that control transcription have been discovered in intron 1. Alterations affecting Btk transcription and stability in these regions have also been reported [3,4]. Although these regions were not affected in our group, it is possible that patients with no coding, cDNA, or regulatory defects who have normal Btk expression (P1-3) might have some autosomal or other rare regulatory defects [7]. However, in patients with null Btk expression and no coding, cDNA, or regulatory mutations (P7-9), some deep intronic mutations are possible [8].

Five different mutations, 2 of which were novel, were found. Patient 4 had a c.1749 del T mutation, which results in a premature stop codon (F 583 fs X 586). It has also been shown that c.1749 del T results in the lack of *BTK* mRNA [9]. Absence of Btk expression in patient 4 confirmed that this was the case.

Patient 5 had a single substitution (c.763 C>T). This mutation leads to a premature stop codon at arginine 255. So far, at least 24 cases of this mutation have been reported (*BTK*base-a database of XLA-related mutations).

In patient 10, 3 AGG nucleotides were deleted from the coding sequence. AGG generally encodes the arginine amino acid. However, in this case, the in-frame deletion affected 2 consecutive glycines. Interestingly, the first glycine was still present in the context of the altered protein, but its codon had been changed from GGA to GGT. The result of this change is a silent mutation of glycine 302 followed by the deletion of the second glycine 303.

Direct mutation analysis was impossible in patient 12 because he needed immediate medical care. We therefore performed indirect mutation analysis using the DXS7424 STR marker. It has been shown that there is complete linkage between the DXS7424 marker and the *BTK* gene [6]. PCR amplification of the DXS7424 marker in patient 12 and in 12 of his relatives (parents, grandparents, sister, maternal uncles and aunts) indicated that he should not be considered an XLA case because the position of the appropriate band was similar to that of his 2 healthy uncles and 4 maternal aunts. In conclusion, indirect mutation analysis may be considered an alternative method for providing fast answers in certain situations such as emergencies or in diseases with more than one causative gene or with genes containing numerous coding sequences.

Table. Molecular Characteristics of Iranian Patients With X-Linked Agammaglobulinemia

Patient No.	BtK Expression	PC	Nucleotide Change	cDNA Analysis	Protein Alteration	5´-UTR Analysis
1	Normal	Yes	NMF	NMF	IP	NMF
2	Normal	Yes	NMF	NMF	IP	NMF
3	Normal	Yes	NMF	NMF	IP	NMF
4	Null	No	c.1749 delT[a]	NA	F583fs X586	NA
5	Null	No	c.763 C>T[a]	NA	R255X	NA
6	Very low	No	c.240+2T>C[a]	NA	SAD	NA
7	Very Iow	No	NMF	NMF	IP	NMF
8	Null	No	NMF	NMF	IP	NMF
9	Null	No	NMF	NMF	IP	NMF
10	Null	No	c.906-908 delAGG[b]	NA	del G 303	NA
11	Null	No	c.777-2delA[b]	NA	SAD	NA
12	Normal	No	IMA	NA	NA	NA

Abbreviations: cDNA, complementary DNA; NMF, no mutation found; NA, not analyzed; IMA, indirect mutation analysis; SAD; splice acceptor defect; IP, intact protein; PC, parental consanguinity; UTR, untranslated region.
[a]Recurrent mutation.
[b]Novel mutation.

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Usefulness of Lymphocyte Activation Test in Atorvastatin Hypersensitivity

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Palabras clave: Hipersensibilidad. Atorvastatina. Test de activación linfocitaria. Simvastatina.

Atorvastatin is a synthetic 3-hydroxy-3-methyglutaril coenzyme A (HMG-CoA) reductase widely used in the treatment of hypercholesterolemia [1]. It is a safe, well-tolerated drug [2] and only a few cases of atorvastatin hypersensitivity have been reported. These have consisted of chronic urticaria [3], angioedema and eosinophilia [4] and the DRESS (drug reaction with eosinophilia and systemic symptoms) syndrome [5]. The lymphocyte activation test (LAT) has been used to detect sensitization of T cells to drugs in peripheral blood samples from drug-allergic patients; it tests CD69 expression on $CD4^+$ lymphocytes ex vivo [6]. We report the case of a systemic hypersensitivity reaction to atorvastatin confirmed by the LAT and describe how this test helped us to find a therapeutic alternative.

We describe the case of a 40-year-old man with a previous history of advanced chronic kidney disease requiring hemodialysis. Three days after treatment initiation with atorvastatin 80 mg due to hypercholesterolemia, the patient reported an episode involving lip, eyelid, and larynx angioedema, dyspnea, fever, and hidrorrhea. The symptoms resolved spontaneously 48 hours after interrupting medication.

The allergy study consisted of prick tests (pure substance, 1/10, and 1/100 dilution) and patch tests (pure substance, 10%, 5%) with atorvastatin 80 mg and simvastatin 40 mg, which were performed as previously described [7,8]. All the results were negative, as were the same tests performed in a control group consisting of patients with good tolerance of atorvastatin or simvastatin. The LAT was performed with both drugs using a modification of the method described by Beeler et al [9], who tested 3 different concentrations of the drug (100, 10, and 1 mg/mL). Data were expressed as normalized mean fluorescence intensity, which was calculated by multiplying the percentage of $CD4^+/CD69^+$ lymphocytes by the mean fluorescence intensity of $CD69^+$ lymphocytes. Results were expressed as a stimulation index (SI). An SI of over 2 was considered a sign of sensitization [9]. The LAT was positive for atorvastatin and negative for simvastatin in our patient (Figure). Both test results were negative in the LAT control group. The risk and benefits were discussed with the patient, informed consent was obtained, and a single-blind, placebo-

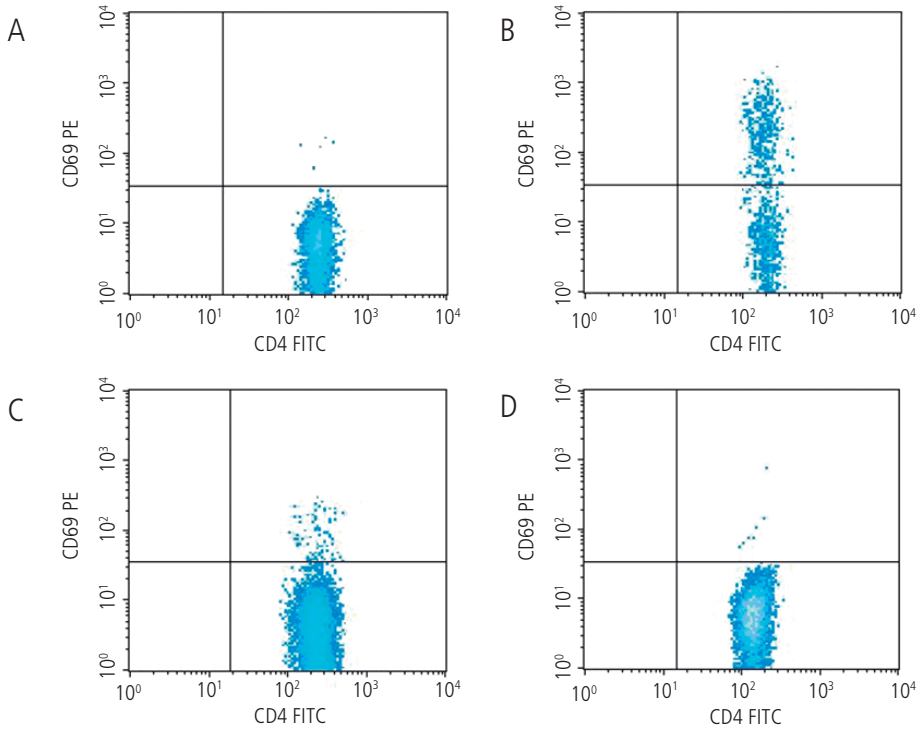

Figure. CD69 expression on $CD4^+$ lymphocytes was measured by flow cytometry. A, Negative control; B, Positive control; C, Atorvastatin 100 mg/mL; D, Simvastatin 100 mg/mL. PE, indicates phycoerythin; FITC, fluorescein isothiocynate.

controlled oral challenge with simvastatin was carried out, with no immediate or delayed reactions observed. We also performed a single-blind placebo-controlled oral challenge with atorvastatin (5 mg and 10 mg at 2-hour intervals on the first day; 20 and 40 mg at 2-hour intervals on the second day, and 40 and 40 mg at 2-hour intervals on the third day). Two hours after a 20-mg dose, the patient developed nasal blockage and lip and periorbital angioedema, which required treatment with parenteral methylprednisolone and antihistamines. To date, the patient has taken simvastatin 40 mg with good tolerance.

In our case, the LAT supported the diagnosis of atorvastatin hypersensitivity (accelerated reaction) and helped us to design an oral tolerance test with an alternative statin (simvastatin). To the best of our knowledge, this is the first case reported in which the LAT has been useful in helping to choose a therapeutic alternative for a patient with atorvastatin hypersensitivity.

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Anaphylaxis Caused by *Crematogaster cutellaris* Sting in an Italian Child

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Ant stings can be responsible for severe, but rarely life-threatening, acute allergic reactions, mainly in ant-endemic areas. Ants belong to the Formicidae family (order Hymenoptera). The ants that have most frequently been reported to cause anaphylaxis are red imported fire ants (*Solenopsis invicta* and *Solenopsis richteri*) in the southeastern USA, jack jumpers (*Myrmecia pilosula*) and bull ants (*Myrmecia pyriformis*) in Australia, and *Pachycondyla chinensis* in the Far East [1]. There have, however, also been some reports of anaphylaxis caused by imported red fire in Europe [2,3] and reactions to less common species of *Solenopsis* and other species of ants [4,5].

We report the first case of anaphylaxis caused by a *Crematogaster scutellaris* sting in a 4-year-old child followed in our department for atopic dermatitis and multiple immunoglobulin (Ig) E–mediated food allergies (peanuts, nuts, shellfish, milk, and soy). The child had experienced severe anaphylactic reactions to shellfish and fish at the age of 13 and 24 months, respectively. One day in July, the child developed general malaise, pallor with cyanotic lips, sweating, hyporeactivity, hypotonia, and nausea a few minutes after being stung on his right big toe by an ant next to an anthill in his garden. The ant was captured by the child's father and subsequently identified as *C scutellaris* (acrobat ant) by an expert entomologist. The child had not eaten anything since breakfast 2 hours earlier. This meal had consisted of food he usually ate and tolerated.

His parents decided not to use the auto-injectable epinephrine pen they had been given and took their son to the closest emergency department. On arrival, 10 minutes later, the boy was still pale and weak. Physical examination revealed 2 red dots on the first and the second toes of his right foot, wheezing (SaO_2, 94%), hypotension (blood pressure, 65/40 mmHg; heart rate, 130 bpm), and lethargy. He was treated accordingly and his general conditions returned to normal within 3 hours.

On follow-up in our department, serum specific-IgE (Unicap Phadia, Uppsala, Sweden) proved positive for *S invicta* (0.27 kUA/L), *Apis mellifera* (1.06 kU/L), *Vespa crabro* (0.73 kU/L), *Dolichovespula maculata* (0.66 kU/L), and *Dolichovespula arenaria* (0.90 kU/L). We also tested *Vespula* spp (0.32 kU/L), *Polistes* spp (0.01 kU/L), and *Bombus terrestris* (0.37 k/UL); no specific test for *C scutellaris* was available. A component-based diagnosis was made using serum-specific IgEs (ImmunoCAP Phadia, Uppsala, Sweden) for available antigens: rApi m1 (phospholipase A2), rVes v5 (antigen 5), rPol d5 (antigen), which all proved negative (0.04 kU/L, 0.02 kU/L, and 0.01 kU/L, respectively). Serum-specific IgEs for nAna c2 (bromelain) were positive (0.59 kU/L). The full bodies of about 200 specimens of *C scutellaris* were homogenized in phosphate-buffered saline (PBS; 1:50 wt/vol). After extraction overnight at 4°C, they were centrifuged at 10 000 g for 30 minutes. The protein concentration of the supernatant was measured using the Lowry method (Bio-Rad, DC Protein Assay, cod. 1-800-424-6723). Subsequent immunoblotting showed strong specific reaction bands for *A mellifera* at 18 kD (phospholipase A2) and for *V crabro* at 23 kD (antigen 5) and 34 kD (phospholipase A1). Weaker bands were seen for *Vespula germanica* at 23 kD (antigen 5) and 34 kD (phospholipase A1) and for *Polistes* spp at 23 kD (antigen 5), 34 kD (phospholipase A1), and 42 kD (hyaluronidase). Finally, 2 pale reaction bands were observed for *C scutellaris* at 23 kD and 34 kD (Figure).

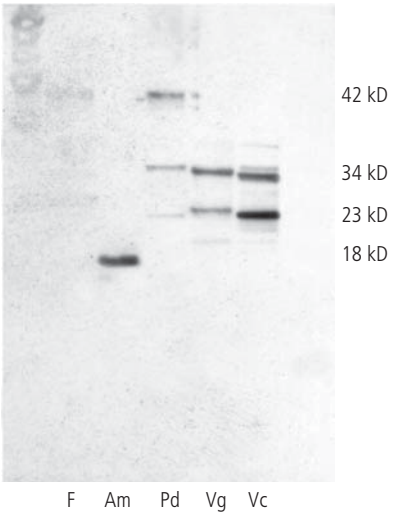

Figure. Immunoblotting with extract obtained from full bodies of *Crematogaster scutellaris*. F indicates *C scutellaris*; Am, Apis mellifera; Pd, Polistes dominulus; Vg, Vespula germanica; and Vc, Vespa crabro.

To our knowledge, this is the first case described of severe allergic reaction to *C scutellaris* and the first report of anaphylaxis caused by an ant sting in Italy.

C scutellaris is very common in Italy; like *S invicta* and *S richteri*, it belongs to the Myrmicinae subfamily. None of the allergenic molecule databases contain specific molecules for *Crematogaster* species. Ants of this genus use Dufour's gland secretion as a contact defensive venom through stinging. The constituents of the venom are a series of C_{23} long-chain derivatives characterized by a cross-conjugated dienone linked to a primary acetate, an aldehyde, or a carboxylic acid function [6].

Although high in vitro and in vivo cross-reactivity between different ant species [1] and between different genera of Hymenoptera has been observed, ant venoms are heterogeneous and many of their components may be specific

to each group [1]. The interpretation of our results is not simple as we did not obtain a completely clear immunoblot profile of *C scutellaris* by using the full-body extraction method. The existence of sensitization to wasp, bee, and red imported fire ant in our patient suggests the presence of cross-reacting molecules such as Sol i 1 (Phospholipase A1), Sol i 3 (Antigen 5), Sol i 2, and Sol i 4. The parents reported no previous stings by other hymenopteran species, but it is possible that the child had inadvertently become sensitized to *C scutellaris* after mild stings, as he used to play near the anthill in his garden. It is also possible that he might have developed specific IgE that cross-reacts with *S invicta* and other hymenopterans.

On the other hand, the presence of specific IgEs to Hymenoptera might have been caused by sensitization to tropomyosin, which has been described as a panallergen between food and insects (our patient was allergic to shellfish). Nevertheless, such in vitro cross-reactivity could not justify the in vivo reaction to the *C scutellaris* sting, as allergic reactions to tropomyosin occur following ingestion or, much more rarely, inhalation [7]. Also, sensitization to carbohydrate determinants (CCDs) might produce in vitro cross-reactivity between venoms of different Hymenoptera and between Hymenoptera allergens and food allergens. In particular, some proteins in the venoms of *A mellifera*, *V germanica*, *V Polistes*, and *V Bombus*, as well as phospholipase A_1B from *S invicta*, are glycosylated and IgE-binding to such antigens could be due to CCDs. Our child had anti-CCD IgEs (bromelain - nAna c2: 0.59 kU/L) and this could explain the presence of specific IgE to Hymenoptera. Nonetheless, it is improbable that such in vitro cross-reactivity could explain the clinical reaction to the ant sting in our patient as anti-CCD IgE has been seen to have no/low clinical relevance in patients who have experienced an allergic reaction to a Hymenoptera sting.

Furthermore, immunoblotting also showed reaction bands to nonglycosylated proteins, such as phospholipase A1 and antigen 5 of *V germanica* and *V crabro*, so it could be speculated that the IgEs were produced towards venom allergens and not towards CCDs. The fact that we did not detect serum-specific IgEs for rApi m1 (phospholipase A2), rVes v5 (antigen 5), or rPol d5 suggests that both the clinical reaction to ant stings and the cross-reactivity between *C scutellaris* venom and other Hymenoptera venoms could be due to a protein determinant other from those tested.

In conclusion, we wish to highlight the possible danger associated with *C scutellaris* bites. More information is needed on the mechanisms involved in this bite in humans, on the substances produced by this ant, and on the specific determinants of its venom.

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