

IgE-mediated anaphylaxis and allergic reactions to idursulfase in patients with Hunter syndrome

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Abstract

Background: Enzyme replacement therapy (ERT) with recombinant human idursulfase is effective for the treatment of Hunter syndrome, mucopolysaccharidosis (MPS) type II. However, various adverse events can occur by the infusion of idursulfase. The purpose was to evaluate the occurrence of infusion-related allergic reactions, including anaphylaxis, to idursulfase in patients with MPS II receiving ERT and to elucidate its possible mechanism.

Methods: A total of 34 patients with MPS II were enrolled to receive ERT with Elaprase[®] at a dose of 0.5 mg/kg intravenously once a week. Information regarding the symptoms, frequency, and timing of anaphylaxis during treatment was analyzed. Presence of anti-idursulfase IgE antibody was assessed by skin prick test (SPT) and enzyme-linked immunosorbent assay (ELISA). Western blotting was performed to confirm the reaction between idursulfase and specific IgE.

Results: Three patients (8.8%) showed anaphylaxis by infusion of idursulfase. No deaths occurred during the study. Anti-idursulfase IgE antibody was detected by SPT and ELISA. Immunoblotting with patients' sera and Elaprase[®] showed a single band of specific IgE binding to the protein around 70 kD, and idursulfase did not display amino acid sequence homology to known allergens. SPT with idursulfase demonstrated positive results in all patients with anaphylaxis. However, we failed to reveal any risk factors for the development of infusion-related immediate-type allergic reactions.

Conclusions: Anaphylaxis related to infusion of idursulfase is mediated by anti-idursulfase IgE antibody, which might be produced by de novo synthesis. SPT is useful in predicting the occurrence of anti-idursulfase IgE-mediated anaphylaxis during infusion.

Mucopolysaccharidosis (MPS) type II, also known as Hunter syndrome, is a rare lysosomal storage disorder in which glycosaminoglycans accumulate within tissues and affect multiple organs. The incidence of the disease is 0.30–0.71 per 100 000 live births (1) and is inherited in an X-linked recessive pattern. The cause is a deficiency of the lysosomal enzyme iduronate-2-sulfatase, which leads to chronic and progressive symptoms such as skeletal deformities, neurologic impairments, airway obstruction, and cardiomyopathy. In some patients with a severe form of MPS II, death usually occurs in the teenage years. Enzyme replacement therapy (ERT) with intravenous infusion of idursulfase, a purified form of recombinant

human iduronate-2-sulfatase, is available for the treatment of MPS II and has been used in clinical practice. It is important to initiate ERT as early as possible before irreversible changes develop in many organs (2).

Recombinant human idursulfase (Elaprase[®]; Shire Human Genetic Therapies, Inc., Cambridge, MA, USA), a glycoprotein composed of 525 amino acids (3), was approved around the world for the treatment of MPS II. It is a purified form of iduronate-2-sulfatase produced in a human cell line via recombinant DNA technology. In a randomized, double-blind, placebo-controlled trial of ERT with idursulfase for 53 weeks in 96 patients with MPS II,

adverse effects (AEs) were found at least 9% more frequently in idursulfase-treated patients than in placebo-treated patients (4). A Japanese study of 10 patients with MPS II also reported that 50% of patients showed infusion-related reactions (5). These previous studies reported severe reactions including urticaria, vasovagal syncope, bronchospasm, and obstructive airway disease (5–7). Interestingly, IgG anti-idursulfase antibodies were detected in about 50% of patients who received ERT with idursulfase (4, 5). However, no associations were found between the presence of antibodies and AEs. In those studies, no anti-idursulfase IgE antibodies were detected (4–6, 8).

Recently, we experienced a patient with MPS II who showed urticaria, facial edema, wheezing, dyspnea, and hypotension after infusion of idursulfase, although there has been no report of anaphylaxis according to the literature (4–7). It is important to determine the predictive and risk factors pertaining to the development of IgE-mediated reactions, because ERT is the only imperative treatment in these patients. Therefore, we performed complete enumeration study to evaluate the occurrence of infusion-related allergic reactions including anaphylaxis to idursulfase in patients with MPS II and to elucidate its possible mechanism.

Material and methods

Study population

A total of 34 male patients with MPS II participated in this study. The diagnosis of MPS II was made by clinical findings and abnormal excretion of urinary glycosaminoglycan. Genetic tests as well as enzyme assays using leukocyte pellets were used to confirm the diagnosis. Clinical severity of MPS II was divided into severe and attenuated forms according to the neurological involvement including cognitive impairment and hyperactivity (9). The mean age at the start of ERT was 12 years (range 3–38 years). Parents were asked to respond to a questionnaire regarding basic demographic information and allergy history of patients and their parents. A history of allergic diseases was defined as atopic dermatitis, allergic rhinitis, or asthma confirmed by a physician.

All patients received intravenous infusions of recombinant human idursulfase (Shire Human Genetic Therapies, Inc.) once a week at a dose of 0.5 mg/kg. The infusion rate started at 0.04 mg/kg/h for the first 15 min and was increased to 0.08 mg/kg/h for the next 15 min. If the infusion was tolerated, the rate was increased up to 0.135 mg/kg/h until the full volume was administered.

The diagnosis of anaphylaxis was based on the criteria developed in the working group of the National Institute of Allergy and Infectious Disease and Food Allergy and Anaphylaxis Network (10). Information regarding the symptoms, frequency, and timing of anaphylaxis during treatment was collected. Sensitization to common allergens such as house dust mites, tree pollens, grass pollens, weed pollens, cats, and dogs was determined by ImmunoCAP (Thermo Fisher Scientific Inc., Waltham, MA, USA), using the cutoff value of 0.35 kU/l. Blood samples from 33 patients were obtained,

and their sera were frozen at -80°C until use. One patient with anaphylaxis (patient no. 3) refused to provide blood for further studies.

This study was approved by the institutional review board at Samsung Medical Center, and informed consent was obtained from all parents.

Skin prick test (SPT) and intradermal test (IDT)

Skin prick test (SPT) was performed on the volar aspect of the forearm using a disposable 25-gauge needle to identify the presence of specific IgE antibody to Elaprase[®] with a concentration of 2 $\mu\text{g/ml}$. Histamine was used as a positive control and normal saline as a negative control. Fifteen minutes later, the largest diameter and its perpendicular diameter of the wheals were measured. Both diameters were recorded, summed, and divided by 2. A positive response of SPT was regarded when the calculated value was ≥ 3 mm larger than the negative control. Intradermal test (IDT) was carried out in patients showing allergic reactions and negative results in SPT. IDT involved injection of 0.02 ml at a concentration of 0.002 $\mu\text{g/ml}$. IDT was regarded positive if erythema developed and the wheal size was over the outline of the original bleb (11–13). These tests were performed at least 6 weeks after an anaphylactic reaction.

Electrophoresis and Western blotting

Idursulfase (Shire Human Genetic Therapies, Inc.) was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (14). Detailed methods are described in the supporting information.

Enzyme-linked immunosorbent assay (ELISA) and ELISA inhibition assay

These are described in detail in the supporting information.

Bioinformatics

To determine whether the amino acid sequence of idursulfase has significant homology to any known allergens, a search was performed using Allergenic Protein Sequence Searches in AllergenOnline, the Food Allergy Research & Resource Program allergen protein database. (www.allergenonline.org).

Statistical analysis

Data were analyzed using spss for Windows (version 20.0; Chicago, IL, USA). The Fisher's exact test was applied to examine the association between the occurrence of allergic reactions and categorical variables such as clinical severity of MPS II, past medical history of allergic diseases, allergic diseases of the parents, total IgE, sensitization to common inhalant allergens, and idursulfase-specific IgE. Detection of idursulfase-specific IgE by ELISA was defined as absorbance more than two standard deviations (SD) from the mean in

seven healthy, nonatopic individuals who did not have MPS and had never received IDS infusion. Differences in the total IgE levels and total eosinophil counts between patients with and without allergic reactions to Elaprase® were also analyzed by Mann–Whitney U-tests. The levels of total IgE were analyzed on a logarithmic scale. A *P* value <0.05 was considered significant.

Results

Clinical data

A total of 34 patients with MPS II received ERT with idursulfase. Four patients showed urticaria/angioedema, and three patients manifested anaphylaxis during infusion (Table 1). The total duration of ERT was 154 ± 34 weeks (range, 13–180 weeks), and no deaths occurred during the study period. The first reaction was found at 67 ± 46 weeks (range, 3–109 weeks) after the first ERT with idursulfase.

Detection of anti-idursulfase IgE and IgG antibody

One patient refused to undergo SPT and IDT, and 4 of 33 patients reacted to the SPT. SPT with idursulfase demonstrated positive results in all patients with infusion-related anaphylaxis. Two patients showing urticaria and negative results in SPT revealed a positive reaction in IDT (Table 1). All 27 patients who did not show IgE-mediated AEs demonstrated negative reactions in SPT. The sensitivity and specificity rates of SPT were 66.7% and 100%. The positivity of SPT was related to IgE-mediated allergic reactions (*P* < 0.001) (Table 2).

The absorbance value of anti-idursulfase IgE antibody (mean $\pm$ SD) by ELISA was 0.022 ± 0.007 in seven healthy,

nonatopic controls. Anti-idursulfase IgE antibody level over the mean plus 2 SD in normal controls was detected in the sera from seven patients with allergic reactions (Fig. 1). This specific IgE binding was confirmed by ELISA inhibition test in two patients with anaphylaxis (patient no. 1 and 2) (Fig. 1). Idursulfase-specific IgG was also detected in these patients by ELISA (Fig. 2). ELISA for the detection of idursulfase-specific IgG was not performed in one patient with anaphylaxis (patient no. 3), because his mother refused blood sampling. The sensitivity and specificity rates of specific IgE detection by ELISA were 100% and 22.2%. No association was found between the detection of anti-idursulfase IgE by ELISA and allergic reactions (*P* = 0.398) (Table 2).

SDS-PAGE and immunoblotting

A thick and strong single band was shown between 70 and 80 kDa in SDS-PAGE (Fig. 3). Immunoblotting analysis was conducted to investigate IgE reactivity against idursulfase using the sera of two patients with anaphylaxis and a negative control. One patient (patient no. 1) clearly showed a reaction to a protein around 70 kDa. On the other hand, IgE binding was rarely seen in patient no. 2 (Fig. 3). It seemed to be the sensitivity problem of immunoblotting, because serum from the same patient showed positive idursulfase-specific IgE by ELISA and inhibition ELISA (Fig. 1).

Bioinformatics

When comparing the amino acid sequence of idursulfase with known allergens, no matches of >35% identity over successive 80 amino acid sequence were found. With regard to eight or more contiguous amino acids in common with any allergen sequence, no homology was detected.

Table 1 Clinical characteristics of patients who showed immediate-type adverse reactions to IDS

Patient	Age (years)	Severity	Total duration of ERT (weeks)	Symptoms of reaction after ERT	Time to reaction after ERT (min)	Dose to elicit reaction (mg)	Cycle number at the first reaction		IDS-specific IgE		
							Urticaria	Anaphylaxis	SPT	IDT	ELISA
1	10	Severe	168	Urticaria, respiratory distress	10	0.24	3	68	Positive	ND	Positive
2	13	Attenuated	162	Urticaria, respiratory distress	10	0.195	85	115	Positive	ND	Positive
3	15	Attenuated	165	Urticaria, respiratory distress, LOC	60	2.34	3	3	Positive	ND	Positive
4	12	Attenuated	166	Urticaria	40	0.68	74	–	Positive	ND	Positive
5	12	Attenuated	166	Urticaria	10	0.16	109	–	Negative	Positive	Positive
6	7	Severe	164	Urticaria	120	5.16	109	–	Negative	Positive	Positive
7	9	Attenuated	161	Urticaria	5	0.08	89	–	ND	ND	Positive

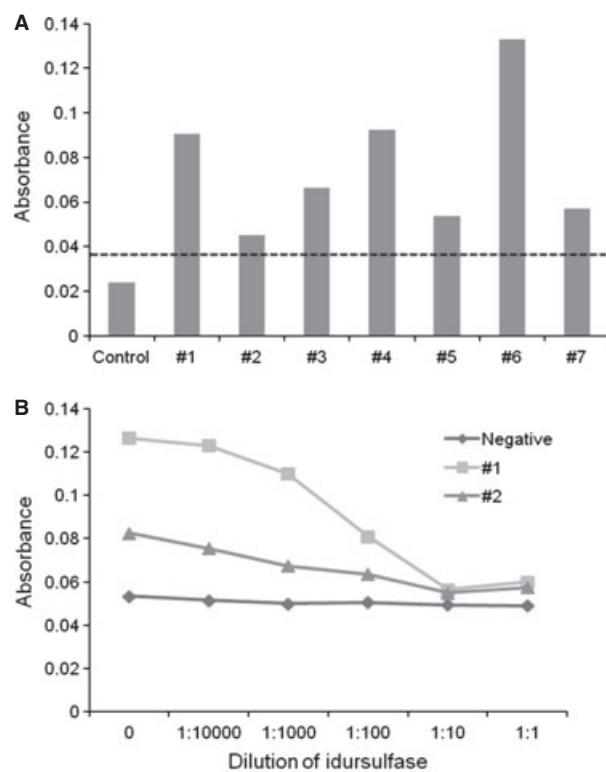
ERT, enzyme replacement therapy; IDS, idursulfase; SPT, skin prick test; IDT, intradermal test; ELISA, enzyme-linked immunosorbent assay; LOC, loss of consciousness; ND, not determined.

Table 2 Predictive factors for IgE-mediated adverse reactions to idursulfase

	IgE-mediated adverse reaction (+) (<i>n</i> = 7)	IgE-mediated adverse reaction (–) (<i>n</i> = 27)	<i>P</i> value	Sensitivity	Specificity
IDS-specific IgE by skin prick test*(%)					
Positive	4 (66.7)	0 (0.0)	<0.001	66.7	100.0
Negative	2 (33.3)	27 (100.0)			
IDS-specific IgE by ELISA (%)					
Positive	7 (100.0)	21 (77.8)	0.398	100.0	22.2
Negative	0 (0.0)	6 (22.2)			

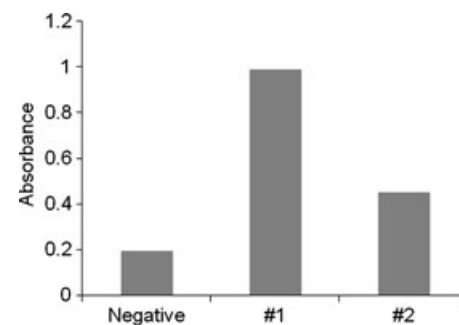
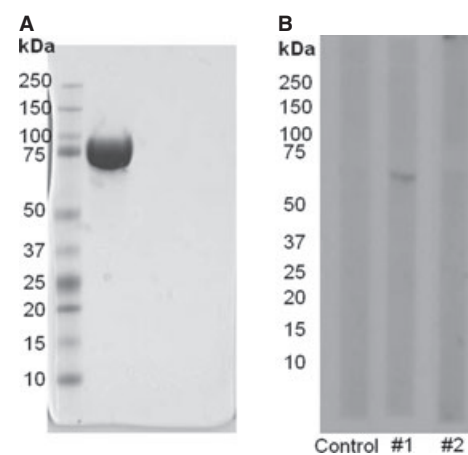
IDS, idursulfase; ELISA, enzyme-linked immunosorbent assay.

*One patient refused to undergo skin prick test.

**Figure 1** Idursulfase (IDS)-specific IgE in patients who showed immediate-type adverse reactions to IDS. (A) ELISA data for IDS-specific IgE from patient no. 1 to 7; (B) Inhibition ELISA data for IDS-specific IgE from patient no. 1 and 2 who showed anaphylaxis to IDS. Dotted lines indicate two standard deviations from the mean in seven healthy, nonatopic individuals who did not have MPS and had never received IDS infusion.

Risk factors related to allergic reactions

The median values for serum total IgE and total eosinophil counts were 130.0 kU/l (range, 26.2–385.0) and 190.0/mm³ (range, 0–321) in the patients with allergic reactions and 55.7 kU/l (range, 5.9–1,228.0) and 77.7/mm³ (range, 0–670.7) in the patients without allergic reactions. No dif-

**Figure 2** IDS-specific IgG in patient no. 1 and 2 who showed anaphylaxis to IDS.**Figure 3** Electrophoretic maps of idursulfase (A) and immunoblotting of idursulfase with sera from two patients (no. 1, no. 2) and a negative control (B).

ference was found in logarithmic total IgE and eosinophil counts ($P = 0.335$ and 0.177) between two groups. In addition, there were no differences in the past history of allergic diseases, parental allergic diseases, severity of MPS II, total IgE levels, and sensitization to common inhalant allergens

Table 3 Risk factors for the development of IgE-mediated adverse reactions to idursulfase

	IgE-mediated adverse reaction (+) (<i>n</i> = 7)	IgE-mediated adverse reaction (–) (<i>n</i> = 27)	<i>P</i> value
Past history of allergic diseases (%)			
Yes	2 (28.6)	14 (51.9)	0.405
No	5 (71.4)	13 (48.1)	
Paternal allergic diseases (%)			
Yes	0 (0)	4 (14.8)	0.559
No	7 (100)	23 (85.2)	
Maternal allergic diseases (%)			
Yes	1 (14.3)	3 (11.1)	1.000
No	6 (85.7)	24 (88.9)	
Disease severity (%)			
Severe form	5 (71.4)	10 (37.0)	0.199
Attenuated form	2 (28.6)	17 (63.0)	
Total IgE (%)			
<100	3 (42.9)	18 (66.7)	0.387
≥ 100	4 (57.1)	9 (33.3)	
Sensitization to inhalant allergen (%)			
Yes	5 (71.4)	12 (44.4)	0.398
No	2 (28.6)	15 (55.6)	

between patients with and without allergic reactions (Table 3).

Discussion

Anaphylaxis and urticaria are typical symptoms of IgE-mediated immediate-type hypersensitivity (15). In our study, 3 of 34 patients (8.8%) demonstrated recurrent anaphylactic reactions during infusion of idursulfase, while urticaria was shown in four patients (11.8%). In our patients with immediate-type allergic reactions during infusion, we identified specific IgE antibody against Elaprase® by SPT, ELISA, and inhibition ELISA. To our knowledge, this is the first report of the presence of specific IgE antibodies against Elaprase® in relation to AEs. We also found anti-idursulfase IgG antibodies in these patients with anaphylaxis. However, it is more plausible that urticaria or anaphylaxis, typical immediate-type allergic symptoms, is mediated by anti-idursulfase IgE antibody, because previous studies failed to demonstrate the associations between the presence of these specific IgG antibodies and AEs (4, 5). Moreover, specific IgG antibodies are more likely to involve in type 2 or 3 hypersensitivity rather than type 1 hypersensitivity (15, 16). Therefore, these AEs by infusion of idursulfase in our patients can be categorized as type β, which is mediated by specific IgE, according to the proposed classification of side

effects of biological agents (16). It is not surprising that IgE-mediated anaphylaxis has developed during infusion of idursulfase, because specific IgE antibodies have been reported in patients receiving infusion of the other biologic agents such as OKT3 monoclonal antibody in transplant recipients (17), laronidase for MPS I (18), and alglucerase for Gaucher disease (19).

We found specific IgE binding between patient's sera and Elaprase® and concluded that anti-idursulfase IgE antibody exists in those sera. Although this reactivity might be caused by the transfer of a cross-reactive protein from Elaprase® and it binds with the already existing specific IgE from patient's sera, this possibility seems to be low, because process- or product-related impurities are consistently eliminated through the purification process and the final product is reported to be >99.9% pure (20). We also demonstrated that only a single band was shown at the same size in SDS-PAGE and immunoblotting. In addition, we could not find any amino acid sequence similarity between idursulfase and known allergens (21). It is presumed that anti-idursulfase IgE antibody was newly produced after exposure of a new protein, which does not exist in patients with MPS II. This idea is more plausible because in our patients, anaphylaxis did not occur at the first dose but developed after several times of infusion.

A possible reason why IgE has never been detected in previous studies is the difference in the duration of ERT. Sensitization to a specific allergen requires repeated exposure for a certain period of time. The duration of exposure in our study was approximately 3 years compared with 1–2 years in previous studies (4–7). With regard to the two patients with anaphylaxis in the present study, it took 16 months and 27 months to show the first episode of anaphylactic reactions, except for one case (patient no. 3) that took 3 weeks. Unfortunately, we do not know the exact timing of seroconversion, because SPT and ELISA were performed after the development of anaphylaxis. Another reason might be the sensitivity of the test to detect anti-idursulfase IgE antibody.

Our results indicate that life-threatening anaphylaxis can develop and be mediated by anti-idursulfase IgE antibody during ERT in patients with MPS II. Close observation is required for these patients due to the possible occurrence of anaphylaxis while idursulfase is administered. We attempted to figure out the patients more prone to develop anti-idursulfase IgE antibodies and subsequent anaphylaxis. Unfortunately, we failed to reveal any risk factors in terms of past history or family history of allergic diseases, sensitization to common allergens, disease severity, and so on. Instead, we found that SPT or IDT with idursulfase was positive in all patients who showed anaphylaxis. It suggests that SPT before infusion of idursulfase could be used as a screening test of allergic reactions, although positive SPT does not always predict anaphylaxis. Of note, subsequent infusion can be managed with a slower rate of administration for the purpose of preventing anaphylaxis in patients with MPS II who need continuous ERT.

When drug allergy is suspected, skin tests can be performed with biological agent as well as beta-lactams (15, 22,

23). Although SPT and IDT are known to be most sensitive methods for the diagnosis of immediate-type allergic reactions to beta-lactams, skin tests for biological agents have been performed in few patients with a history of infusion-related reactions (22–24). Nevertheless, our study showed that SPT can be used to predict allergic reactions with an acceptable sensitivity and a high specificity. Further clinical experiences will present more accurate data regarding the usefulness of skin tests for the prediction of biological agent-related hypersensitivity.

In conclusion, anaphylaxis can occur by infusion of idursulfase in 8.8% of children with MPS II. This immediate-type hypersensitivity is mediated by anti-idursulfase IgE antibody, which might be produced by de novo synthesis. SPT might be useful in predicting the development of anti-idursulfase IgE-mediated anaphylaxis during infusion.

Authors' contributions

The present work was carried out in collaboration between all authors. Jihyun Kim, Kangmo Ahn, and Dong-Kyu Jin defined the study theme. Jihyun Kim, Youngshin Han, Kangmo Ahn, and Dong-Kyu Jin designed the methods. Mi-Ran Park, Do-Soo Kim, Se-Hyun Maeng, Sung-Yoon Cho, and Dong-Kyu Jin enrolled patients and conducted follow-up

reviews. Jeong-Ok Lee and Youngshin Han carried out the laboratory experiments. Jihyun Kim, Youngshin Han, Mi-Ran Park, Do-Soo Kim, Se-Hyun Maeng, and Sung-Yoon Cho entered and analyzed the data. Jihyun Kim and Kangmo Ahn interpreted the results and wrote the manuscript. All authors have contributed to, seen and approved the manuscript.

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Conflict of interest

The authors declare that there are no potential conflict of interests with respect to the authorship and/or publication of this article.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Electrophoresis and Western blotting.

Data S2. Enzyme-linked immunosorbent assay (ELISA) and ELISA inhibition assay.

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Supporting information

Electrophoresis and Western blotting

The samples were then reduced by heating with 4 × SDS-PAGE gel loading buffer (LDS Sample Buffer, Invitrogen cat. # NP0007; mixed 1:10 with reducing agent, Invitrogen cat. # NP0004) and loaded into each well of the gel (4~12% Bis-Tris aminomethane Gel, NuPAGE Invitrogen, Carlsbad, USA). Precision plus protein standards (Bio-Rad, product # 161-0374) were used as molecular weight markers to estimate the protein size.

Proteins were transferred from the gel onto a polyvinylidene difluoride membrane (PVDF) using an iBlot™ Dry Blotting System (Invitrogen, Carlsbad, USA). The PVDF membrane was blocked with 2% non-fat dried milk (NFDM) for an hour at room temperature, after which it was incubated with the patients' sera overnight at room temperature. The membrane was then washed three times with PBST for 10 min each and incubated with Biotin labeled goat IgG-anti-human IgE (KPL, Maryland, USA) diluted at 1:4,000 in 2% NFDM for 1 hr at room temperature. Next, the membrane was washed with PBST three times as above, after which it was incubated with NeutrAvidin-HRP (Pierce Chemical Co., Rockford, IL, USA) for 30 min at room temperature. After three washes with PBST, the membrane was reacted with Amersham ECL reagent (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) for 2 min. The blotted membrane was then exposed to a high performance chemiluminescence film (GE Healthcare Limited, Buckinghamshire, UK), after which the film was developed.

Enzyme-linked immunosorbent assay (ELISA) and ELISA inhibition assay

Idursulfase (Elaprase®) was diluted in pH 9.6 carbonate coating buffer and incubated in microtiter plates overnight at 4°C to coat the wells. After washing with 0.05% PBST (PBS, pH 7.4, containing 0.05% Tween 20) and blocking with 2% BSA (Sigma A-21536) in 0.05%

PBST, serum samples were added to the wells. Sera from 7 healthy and non-atopic individuals who did not have MPS and had never received IDS infusion were used as negative controls. The wells were incubated for 2 hours at room temperature, and rinsed with 0.05% PBST. Next, the wells were incubated with a goat anti-human IgE-HRP (KPL, 074-1004) or goat anti-human IgG-HRP (Sigma, S-0170), which was diluted at 1:2,500 v/v in 0.05% PBST, for one hour at room temperature. After washing with PBST, 100 μ L of substrate tetramethylbenzidine (KPL) peroxidase was added, and absorbance $A_{595\text{nm}}$ was measured.

For the ELISA inhibition assay, coating buffer (0.1M carbonate/bicarbonate, pH 9.1) containing idursulfase at 50 μ g/mL was introduced into each well in the plate (Nunc-Maxisorp NO FDF). The plate was covered with plate-sealing tape and kept at 4°C overnight. The plate was washed with 0.05% PBST and blocked with 2% BSA in 0.05% PBST for one hour at room temperature. Sera from idursulfase-allergic subjects were pre-incubated with varying concentrations (0.00001-1.0 mg/ml) of idursulfase for 1 hr at 37°C. The inhibition samples were then added to the 0.05% PBST-washed plate and incubated for 1 hr at room temperature. Plates were washed and incubated with horseradish peroxidase-labeled goat anti-human IgE, developed with tetramethylbenzidine peroxidase substrate, and then read on a microplate reader at 595 nm.