

Hypersensitivity reactions to *Escherichia coli*-derived polyethylene glycolated-asparaginase associated with subsequent immediate skin test reactivity to *E. coli*-derived granulocyte colony-stimulating factor

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L-asparaginase is a cancer chemotherapeutic agent derived from *Escherichia coli*. It is effective in treating acute lymphoblastic leukemia (ALL) and other related diseases, but it has been associated with hypersensitivity reactions in from 6% to 43% of patients studied.¹ Such reactions can occur after one dose of the drug, but most occur after 2 weeks of daily or thrice weekly therapy.¹ The highest prevalence has been observed when L-asparaginase was given as a single agent or when given in higher doses (6000 IU/m²).¹ Mechanisms proposed for L-asparaginase reactions include trace endotoxin contamination, IgE-mediated hypersensitivity, IgG or IgM antibody-mediated sensitivity, and complement-mediated reactions.²

Use of intramuscular preparations of L-asparaginase has been associated with a decrease in severe hypersensitivity reactions without loss of efficacy.³ Polyethylene glycolated (PEG)-asparaginase is a further improvement to this because it has a sustained release from tissues and is longer acting. There have been reports of delayed hypersensitivity reactions to intramuscular L-asparaginase³; however, there have been no previously reported cases of anaphylaxis to PEG-asparaginase.

Granulocyte colony-stimulating factor (G-CSF) (Filgrastim) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Sargramostim) are hematopoietic growth factors used in treating myelosuppression following cancer chemotherapy. They are structurally distinct, and are derived from different recombinant expression systems: G-CSF from *E. coli* and GM-CSF from *Saccharomyces cerevisiae*. Both have been associated with hypersensitivity reactions^{4,5}; however, they are not believed to be cross-reactive. Because both PEG-asparaginase and G-CSF are produced in *E. coli*, there may be a theoretic concern regarding cross-sensitization in patients who have had life-threatening reactions to one of these agents.

Abbreviations used

ALL:	Acute lymphoblastic leukemia
G-CSF:	Granulocyte colony-stimulating factor
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
PEG:	Polyethylene glycolated

We present two cases of anaphylaxis to PEG-asparaginase and discuss our experience with skin testing these patients with G-CSF and GM-CSF.

CASE REPORTS

Patient 1

Patient 1 is an 11-year-old white male with ALL and central nervous system relapse admitted for chemotherapy. On hospital day 1, he received intravenous vincristine and methotrexate, intrathecal cytosine arabinoside, methotrexate, and hydrocortisone, and was started on a 7-day course of dexamethasone. He was on no other daily medications. On hospital day 3, he received 2500 U/m² intramuscular PEG-asparaginase, and approximately 2 hours afterward experienced pruritus, generalized urticaria, dyspnea, and hypotension.

He was treated with intravenous epinephrine, diphenhydramine, cimetidine, and hydrocortisone acutely and transferred to the pediatric intensive care unit. There he required continuous intravenous phenylephrine drip for 12 hours. Despite treatment, he continued to have intermittent flares of pruritus and urticaria for the next 48 to 72 hours. Serum tryptase (radioimmunoassay at Specialty Laboratories, Inc., Santa Monica, Calif.), drawn 2 hours after the initial reaction, was 7.1 U/L (normal, < 0.5 U/L).

Patient 2

Patient 2 is a 7-year-old white male with ALL and central nervous system relapse who was admitted for chemotherapy. On hospital day 1, he received intravenous vincristine and methotrexate, intrathecal cytosine arabinoside, methotrexate, and hydrocortisone, and was started on a 7-day course of dexamethasone. He was taking no other daily medications. On hospital day 3, he

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TABLE I. Skin test results for Patient 1, Patient 2, and control subjects (mm wheal/mm flare)

	Patient 1	Patient 2	Control subject 1	Control subject 2	Control subject 3
Histamine					
Skin prick test	8/30	5/28	4/12	5/9	4/10
Intradermal test	23/65	14/42	8/15	11/35	10/22
Saline					
Skin prick test	2/6	2/5	0/0	0/0	2/2
Intradermal test	5/6	2/3	3/3	3/5	3/3
G-CSF (300 µg/ml)					
Skin prick test	2/6	0/2	0/0	0/2	2/2
Intradermal test	13/24*	0/9†	0/5	3/3	5/5
GM-CSF (100 µg/ml)					
Skin prick test	0/0	0/4	0/4	0/0	2/2
Intradermal test	6/6	2/9	2/2	0/3	3/3
GM-CSF (250 µg/ml)					
Skin prick test	0/0	0/9	0/5	0/0	3/3
Intradermal test	6/7	2/5	2/5	3/3	3/5

*Positive skin test response (wheal 8 mm greater than that produced by saline control with surrounding flare).

†Patient had diffuse urticarial eruption 6 to 8 hours after skin testing with G-CSF.

received 2500 U/m² intramuscular PEG-asparaginase, and approximately 2 hours later experienced abdominal cramps, flushing, urticarial rash, mild wheezing, and a drop in room air oxygen saturation to 91%.

He was treated with subcutaneous epinephrine, diphenhydramine, ranitidine, hydrocortisone, and low-flow oxygen with resolution of the acute symptoms. Despite treatment, he continued to have intermittent recurrence of the abdominal cramps, flushing, and urticaria for approximately 40 hours after the initial reaction. Serum tryptase was not obtained.

According to their respective protocols, both patients were to receive G-CSF for myelosuppression after their chemotherapy. Because of the concern of cross-sensitization between two *E. coli*-derived agents, consultation for skin testing to G-CSF was requested before administration of any hematopoietic growth factor.

METHODS

After informed consent was obtained, skin prick testing was performed with undiluted G-CSF (filgrastim) at 300 µg/ml and with GM-CSF (sargramostim) at 100 µg/ml and 250 µg/ml by using a bifurcated needle. Intradermal skin testing was done with each drug by using 0.05 ml of the above concentrations. These test results were read after 20 minutes. Wheal/flare reactions were measured by averaging the sum of the greatest longitudinal and mid-point orthogonal axes. Skin testing to G-CSF and GM-CSF were performed on separate days approximately 2 weeks apart.

Positive controls for skin prick and intradermal testing were 1 mg/ml and 0.1 mg/ml histamine base, respectively. Negative control for both skin prick and intradermal testing was albumin saline. Three individuals with no prior exposure to these agents were used as negative control subjects. Skin test positivity was defined as a wheal diameter 5 mm greater than that produced by the negative control with surrounding flare. Skin testing in each patient occurred over 4 weeks after the initial systemic reaction.

RESULTS

Patient 1 had a positive intradermal skin test response to G-CSF at 300 µg/ml after a negative skin prick test response. Patient 2 had negative skin prick and intradermal skin test responses to G-CSF at 300 µg/ml, but he had generalized pruritus and urticaria 6 to 8 hours after skin testing. The urticaria involved the trunk and extremities, but it is unknown whether the patient had any wheal or flare at the skin test site. Both patients had negative test responses to GM-CSF. All negative control subjects had negative test responses to G-CSF and GM-CSF (Table I). Both patients subsequently received GM-CSF without any complications.

DISCUSSION

These are, to our knowledge, the first reported cases of anaphylaxis to *E. coli*-derived PEG-asparaginase. Although hypersensitivity to L-asparaginase has been well described, skin testing has proven to be of little diagnostic value because of the incidence of both false-positive and false-negative results.¹ The elevated serum tryptase in Patient 1 suggests a mast cell- (and possibly IgE-) mediated reaction. It is possible that the prolonged nature of these patients' reactions was caused by the sustained release activity of the PEG-asparaginase.

There have been cases described in which patients with reactions to *E. coli*-derived L-asparaginase were able to receive asparaginase derived from a parasitic bacterium, *Erwinia carotovora*, without any adverse events.^{1,3} The *Erwinia* asparaginase is immunologically distinct from *E. coli* asparaginase. This suggests a possible role for bacteria-specific polypeptides in asparaginase hypersensitivity reactions. It was this concern that prompted consultation for skin testing to G-CSF before its use in these patients.

Patient 1, despite nonreactive skin prick tests to

G-CSF, went on to have a clearly positive intradermal test response to G-CSF. The late reaction of Patient 2 was difficult to interpret. He had negative skin prick and intradermal test responses to G-CSF, but had diffuse urticaria 6 to 8 hours after the testing with no definite local reaction at the skin test site. This could have been consistent with an IgE- or possibly IgG-mediated late-phase reaction; however, he had no immediate wheal/flare response to either agent. Since we could neither confirm nor refute the relationship of the urticarial eruption to the skin testing, we offered to repeat the skin tests or administer a graded dose challenge with the G-CSF in our clinic. After discussion, the patient's parents declined rechallenge in any form. He subsequently received GM-CSF without complication.

Engler and Weiss⁴ were the first to report skin testing to GM-CSF in patients with suspected hypersensitivity to that growth factor. In their study they used both GM-CSF and G-CSF for skin testing, demonstrating the lack of cross-reactivity of the two agents. In our evaluation we used the same skin test concentrations used in their evaluation. Since our patients had not previously demonstrated hypersensitivity to G-CSF, we felt that we could safely attempt intradermal testing at the same concentrations. The dose used in skin testing (0.05 ml of 300 μ g/ml) was equivalent to a 1:35 dilution of their recommended dose of the G-CSF (10 μ g/kg). Had the patients demonstrated previous hypersensitivity to G-CSF, we would likely have used the protocol of Munoz et al,⁶ which begins with a 1:1000 dilution of 300 μ g/ml and titrates to full strength.

Our experience agrees with previous findings regarding the safety of skin testing with G-CSF and GM-CSF.^{4,6} Our results also demonstrate the lack of cross-reactivity between *E. coli*-derived G-CSF and yeast-derived GM-CSF, and support concern of cross-sensitivity between polypeptides produced in similar recombinant models. Hypersensitivity to mannitol is unlikely to have caused skin test positivity in our case because it is found in similar concentrations in both G-CSF and GM-CSF.

Because of the inaccuracy of asparaginase skin testing and the lack of published data on skin testing with G-CSF, we can not confirm the utility of skin testing to G-CSF in this situation. Though our experience does not

prove IgE-mediated sensitization, the presence of positive immediate skin test responses in the context of nonreactivity in the negative control subjects does support this mechanism. Further studies in patients with similar reactions to *E. coli*-derived asparaginase and in patients previously exposed to G-CSF would be needed to determine the positive and negative predictive value of this type of testing. Nevertheless, these two cases raise the possibility of immune cross-reactivity between *E. coli*-derived products.

We report the first presumed anaphylactic reactions to PEG-asparaginase, at least one of which demonstrates evidence of a mast-cell mediated mechanism. This patient also had immediate skin test reactivity to *E. coli*-derived G-CSF, suggesting possible sensitization to bacterial components.

We agree that patients with hypersensitivity to *E. coli*-derived PEG-asparaginase should avoid other products synthesized with a recombinant *E. coli* system.⁵ In situations that need clarification, skin testing to these agents may be safely performed. Larger studies will be needed to determine the predictive value of such testing in this and other situations in which it might be indicated. Development of in vitro assays for detecting cytokine-specific IgE would aid greatly in this effort.

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