
Immediate hypersensitivity to human recombinant granulocyte-macrophage colony-stimulating factor associated with a positive prick skin test reaction

Renata J M Engler, MD* and Raymond B Weiss, MD†

Background: Recombinant human granulocyte-macrophage colony-stimulating factor (rhu GM-CSF), also known as sargramostim, is used to accelerate myeloid recovery following bone marrow transplantation or cytotoxic chemotherapy. "Anaphylactic" reactions to sargramostim have been reported on a limited basis and are poorly characterized.

Objective: It is the purpose of this report to describe an adverse reaction to sargramostim treatment involving palmar itching, urticaria, angioedema, and throat tightness and to demonstrate the utility of prick skin testing to determine type I sensitization.

Methods: Prick skin testing with 100 and 250 $\mu\text{g/mL}$ sargramostim and 300 $\mu\text{g/mL}$ rhu G-CSF (filgrastim) was performed in the patient and four control subjects.

Results: The patient experienced an immediate wheal and flare reaction with both concentrations of sargramostim while the control subjects demonstrated no reaction. There was also no reaction with filgrastim (rhu G-CSF) in either group and the patient subsequently tolerated filgrastim therapy.

Conclusion: Prick skin testing with rhu GM-CSF and rhu G-CSF may be useful to demonstrate type I sensitization. Additional studies are needed to determine the incidence and prevalence of skin test reactions in larger numbers of patients with cytokine therapy exposure.

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INTRODUCTION

Recombinant human (rhu) granulocyte-macrophage colony-stimulating factor (GM-CSF) is approved for acceleration of myeloid recovery follow-

ing bone marrow transplantation or high dose cytotoxic chemotherapy. It is also used to treat neutropenia in such diverse settings as radiation therapy, neonatal septicemia, human immunodeficiency virus (HIV) infection, burns, surgery, and glycogen storage disease-1b.¹⁻⁵ The native protein is a 23-kD glycoprotein, produced by T-lymphocytes, fibroblasts (including keratinocytes), macrophages and endothelial cells, and has numerous stimulatory functions to include those outlined in Table 1.¹⁻⁸ As part of the four α -helix family of cytokines, this colony stimulating factor promotes growth and differentiation of all cell lines, particularly granulocytes and mononuclear phagocytes. In addition, GM-CSF has the ability to induce the expression and release of cytokines

and arachidonic acid (enhancing the production of leukotrienes).

Marketed since 1991, sargramostim (LEUKINE, Immunex Corporation, Seattle, WA 98101) is a GM-CSF produced by recombinant DNA technology in a yeast (*S. cerevisiae*) expression system. The amino acid sequence is identical to the natural human GM-CSF except for a substitution at position 23; the carbohydrate moiety and molecular mass differ from the native protein.^{1,4} Sargramostim is formulated as a sterile, white, preservative-free lyophilized powder containing either 250 or 500 μg of sargramostim, mannitol, sucrose, and tromethamine.

"Anaphylactic" reactions to sargramostim have been reported to the Food and Drug Administration (FDA) on a limited basis and are poorly characterized. Experience with skin testing has not been described. We report a patient with cancer receiving sargramostim (GM-CSF), with cyclings of high dose chemotherapy, who developed progressive immediate hypersensitivity reactions to self-administered subcutaneous doses. This reaction was associated with an immediate wheal and flare skin test reaction suggestive of IgE-mediated hypersensitivity. We also show a lack of cross-reactivity in this patient to recombinant human granulocyte colony-stimulating factor (rhu G-CSF or filgrastim).

CASE REPORT

We report the case of a 27-year-old woman with metastatic schwannoma who developed a systemic immediate hypersensitivity reaction to sargramostim after 5 months of tolerating monthly treatment cycles. In conjunc-

* Allergy-Immunology Service and † Hematology-Oncology Service, Department of Medicine, Walter Reed Army Medical Center, Washington, DC.

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Table 1. Human GM-CSF: Cytokine Effects In Vivo and In Vitro (summarized from references 1–8):

Cell Type	Growth/Differentiation	Activation	Effector Actions
Granulocytes	Progenitor cell	↑ adhesion molecules, FcR 30%	↑ phagocytosis
Monocytes, macrophages	Progenitor cell	↑ surface HLA-DR	↑ phagocytosis ↑ APC function chemoattractant
Langerhans'	↑ cell viability Progenitor cell	↑ surface HLA-DR	↑ APC function
Dendritic cell	Progenitor cell	↑ HLA-DR expression	↑ APC function
Platelets	Progenitor cell, weak		
Red blood	Progenitor cell, weak		

* APC = antigen presenting cell.

tion with high dose cyclophosphamide (3 g/m²), GM-CSF rescue (5 µg/kg) was self-administered (subcutaneously) on days 3 to 10 of each monthly cycle. The patient had no atopic history nor any past drug allergies.

After several months of asymptomatic cycles, the patient developed erythematous reactions at the injection sites. After 7 cycles, she developed palmar itching about one and one-half hours after the injection. After two additional injections (with similar pruritus), the fourth dose of GM-CSF was associated with palmar itching, followed by urticaria, angioedema (generalized) and throat tightness. The reaction lasted one and one-half to two hours and resolved prior to arrival at the emergency room. There was no objective documentation of wheezing, hypotension, or laryngeal edema. Prick skin testing with 100 and 250 µg/mL of sargramostim resulted in an immediate wheal and flare reaction (no delayed erythema or induration). Table 2 summarizes the results of skin testing with sargramostim in the patient and

four control subjects (no prior exposure to rhu-GM-CSF) who demonstrated no reactions. The patient subsequently tolerated rhu G-CSF (filgrastim), a structurally distinct colony-stimulating factor, without adverse reactions locally or systemically. Prick skin testing to filgrastim at 300 µg/mL was nonreactive.

DISCUSSION

Reports of specific immunologic sensitization to rhu GM-CSF have described IgG antibodies binding to unprotected glycosylation sites on the recombinant proteins, whether derived from a yeast or bacterial (*E. coli*) transfected system.⁹ As a glycoprotein with potential differences in tertiary structure from the native cytokine, rhu GM-CSF can predictably cause immunologic sensitization based on experience with other therapeutic biologic agents.^{10–13} Immediate hypersensitivity reactions with an IgE basis are also a predictable (although rare) event among all patients receiving a medication. Although our case experience

does not prove an IgE-mediated mechanism, an immediate hypersensitivity skin test reaction by prick method without evidence of an irritant or non-specific skin response in normal controls strongly suggests this mechanism is present. Skin testing with either GM-CSF or G-CSF has never been reported in the medical literature. In addition, the manufacturer has no reports of patients with immediate hypersensitivity reactions having been skin tested. The demonstration of a specific skin test reaction to GM-CSF in our patient suggests that this may be a useful in vivo test for determining type I sensitization. Additional studies, however, are needed to determine the incidence and prevalence of skin test reactions in larger number of patients with cytokine therapy exposure. The development of in vitro assays for recombinant cytokine specific IgE may also be useful in characterizing adverse reactions.

Although rashes are relatively common with GM-CSF, as are local reactions at injection sites, it is important

Table 2. Prick Skin Testing with GM-CSF and G-CSF in a Patient with a Systemic Allergic Reaction to GM-CSF versus 4 Unexposed Controls: Mean Diameters of Immediate Prick Skin Test Reactions: MM of Wheal (W) by Erythema (E)

Prick Skin Testing Reagents	Patient W × E	Control #1 W × E	Control #2 W × E	Control #3 W × E	Control #4 W × E
Histamine 0.275 mg/mL	8 × 19	8 × 12.5	7 × 22.5	6.5 × 26	9 × 23
Saline/HSA*	0	0	0	0	0
GM-CSF†					
100	5 × 10	0	0	0	0
250	7.5 × 24	0	0	0	0
G-CSF‡ 300	0	0	0	0	0

* HSA: human serum albumin in saline diluent for skin testing.

† GM-CSF: granulocyte-macrophage colony-stimulating factor in µg/mL.

‡ G-CSF: granulocyte colony-stimulating factor in µg/mL.

that discrimination of maculopapular, large locals versus generalized urticaria and angioedema be made by the clinician since the risk of a life threatening anaphylactic reaction may increase in the setting of any generalized urticarial reaction. In addition, desensitization, as performed for penicillin, may be feasible if the benefit/risk ratio merits it. In our patient, an alternative "cytokine" was available and felt to be clinically beneficial, so there was no indication for pursuing this approach.

It would be useful if future studies of cytokines would include at least prick skin testing prior to the onset of therapy and following a treatment course in order to define the value of a negative or positive skin test in the setting of an immediate generalized reaction. It is feasible, that like the story for Hymenoptera venom hypersensitivity, large local reactors may have positive skin tests without an increased risk of anaphylaxis.

It is noteworthy that in a recent report of 81 patients with advanced breast cancer who received GM-CSF (*E. coli* derived), nine patients (11%) developed anaphylactoid reactions (hypotension, dyspnea, flushing, and facial swelling) after tolerating several cycles of therapy. Seven were unable to tolerate further treatment; unfortunately, the clinical course of the other two was not described.¹⁴ Another report briefly mentions three cases of

anaphylactoid reactions causing cessation of therapy in a series of 44 patients (7%) with "poor risk" testicular carcinoma.¹⁵

G-CSF, also effective for accelerated bone marrow recovery following high dose chemotherapy, is structurally distinct from GM-CSF and its commercially available recombinant form has no amino acid substitutions (in contrast to GM-CSF). Although carbohydrate side chains are different in the recombinant cytokines from the native forms, it may be that GM-CSF is more sensitizing for some patients due to the amino acid substitution rather than side chain differences. It is noteworthy that fillers such as mannitol are present in both the G-CSF and GM-CSF preparations and could in rare instances cause sensitization as well.¹⁶⁻¹⁷ Since our patient tolerated rhu G-CSF, mannitol sensitivity was not an issue. There is at least one report of acute anaphylaxis occurring ten minutes after the third dose of subcutaneous filgrastim, but no skin testing data were included and the setting was complicated by multiple drug administrations.¹⁸

A recent report of local cutaneous side effects associated with rhu GM-CSF therapy in 11 breast cancer patients suggests that this phenomenon was due to local immune stimulation.¹⁹ We have seen one such patient with progressively worsening localized (at

the injection sites) reactions with indurated, erythematous, macular, itching and burning lesions (see color photograph of local injection sites: Figure 1). There were no associated systemic symptoms and prick skin testing was entirely negative in contrast to our index case (reported here). Non-urticarial rashes may become desquamating as reported by Horn et al in patients receiving intravenous GM-CSF.²⁰ In two of three cases, keratinocyte expression of HLA-DR and ICAM-1 with neutrophilic infiltration of maculopapular lesions was demonstrated and may be due to the direct immune activating properties of the growth factor. Cytokine-specific IgG assays have not been reported in association with these types of reactions.

Improved reporting and classification of adverse reactions to biologics such as GM-CSF are needed in the future in order to determine the nature of the reactions and to define those reactions that present a potentially life-threatening risk of anaphylaxis. There are currently no in vitro assays for cytokine specific IgE but this might be developed in the near future utilizing skin test positive and negative sera.

REFERENCES

1. Metcalf D. The colony-stimulating factors: discovery, development, and clinical applications. *Cancer* 1990;65: 2185-95.
2. Hermann F, Schulz G, Wieser M, et al. Effect of granulocyte-macrophage colony-stimulating factor on neutropenia and related morbidity induced by myelotoxic chemotherapy. *Am J Med* 1990;88:619-24.
3. Vadhan-Raj S, Broxmeyer HE, Hittelman WN, et al. Abrogating chemotherapy-induced myelosuppression by recombinant granulocyte-macrophage colony-stimulating factor in patients with sarcoma: protection at the progenitor cell level. *J Clin Oncol* 1992; 10:1266-77.
4. Thompson JA, Lee DJ, Kidd P, et al. Subcutaneous granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome: toxicity, pharmacokinetics, and hematologic effects. *J Clin Oncol* 1989;7: 629-37.



Figure 1. Left side of panel: patient's abdomen demonstrating large local reactions to GM-CSF subcutaneous injections. Proceeding counterclockwise from the top left corner, local reactions reflect sequential subcutaneous injections of GM-CSF. Right side of panel: patient's left lateral thigh demonstrating a 10 by 8 cm local reaction to GM-CSF demonstrating progressive worsening of delayed local indurated reactions (after abdominal sites shown at left). All local reactions were aborted with antihistamine pretreatment and topical steroid application immediately after subsequent injections.

5. Levine JD, Allan JD, Tessitor JH, et al. Granulocyte-macrophage colony stimulating factor ameliorates the neutropenia induced by azidothymidine in AIDS/ARC patients. *Proc Am Soc Clin Oncol* 1989;8:1(#3).
6. Weisbart RH, Gasson JC, Golde DW. Colony-stimulating factors and host defense. *Ann Intern Med* 1989;110:297-303.
7. Lopez AF, Williamson J, Gamble JP, et al. Recombinant human granulocyte-macrophage colony-stimulating factor stimulates in vitro mature neutrophils and eosinophil functions, surface receptor expression and survival. *J Clin Invest* 1986;78:1220-8.
8. Arnaout MA, Wang EA, Clark SC, Sieff CA. Human recombinant granulocyte-macrophage colony-stimulating factor increases cell-to-cell adhesion and surface expression of adhesion-promoting surface glycoproteins on mature granulocytes. *J Clin Invest* 1986;78:597-601.
9. Gribben JG, Devereaux S, Thomas NSB, et al. Development of antibodies to unprotected glycosylation sites on recombinant human GM-CSF. *Lancet* 1990;335:434-7.
10. Moonen P, Mermod JJ, Ernst JF, et al. Increased biological activity of deglycosolated recombinant human granulocyte-macrophage colony-stimulating factor produced by yeast or animal cells. *Proc Natl Acad Sci USA* 1987;84:4428-31.
11. Dorr RT. Clinical properties of yeast-derived versus *Escherichia coli*-derived granulocyte-macrophage colony-stimulating factor. *Clin Therapeutics* 1993;15:19-29.
12. Antonelli G, Currenti M, Turriziani G, Dianzani F. Neutralizing antibodies to interferon- α : Relative frequency in patients treated different interferon preparations. *J Infect Dis* 1991;16:882-5.
13. Spiegel RJ, Spicehandler JR, Jacobs SI, Oden EM. Low incidence of serum neutralizing factors in patients receiving recombinant alfa-2b interferon (Intron A). *Am J Med* 1986;80:223-8.
14. O'Shaughnessy JA, Denicoff AM, Venzon DJ, et al. A dose intensity study of FLAC (5-fluorouracil, leucovorin, doxorubicin, cyclophosphamide) chemotherapy and *Escherichia coli*-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) in advanced breast cancer patients. *Ann Oncology* 1994;5:709-16.
15. Bokemeyer C, Schmoll HJ, Metzner B, et al. Comparison of 5 vs 10 mcg/kg per day of GM-CSF following dose-intensified chemotherapy with cisplatin, etoposide, and ifosfamide in patients with advanced testicular cancer. *Ann Hematol* 1993;67(2):75-9.
16. Ackland SP, Hillcoat BL. Immediate hypersensitivity to mannitol: a potential cause of apparent hypersensitivity to cisplatin. *Cancer Treatment Rep* 1985;5:562-3.
17. Spaeth GL, Spaeth EB, Spaeth PG, Lucier AC. Anaphylactic reaction to mannitol. *Arch Ophthalmol* 1967;78:583-4.
18. Jaiyesimi I, Giralt SS, Wood J. Subcutaneous granulocyte colony-stimulating factor and acute anaphylaxis. *N Engl J Med* 1991;325:587. (Reply by Amgen included)
19. Locker GJ, Simonitsch I, Mader RM, et al. Cutaneous effects in breast cancer patients treated with cytostatic polychemotherapy and rh GM-CSF: Immune phenomena or drug toxicity? *Breast Cancer Res Treatment* 1995;34:213-9.
20. Horn TD, Burke PJ, Karp JE, Hood AF. Intravenous administration of recombinant human granulocyte-macrophage colony-stimulating factor causes a cutaneous eruption. *Arch Dermatol* 1991;127:49-52.

Request for reprints should be addressed to:
 Renata J M Engler, MD
 Director, Clinical & Laboratory Immunology
 Training Program
 % Allergy-Immunology Service
 Walter Reed Army Medical Center
 Washington, DC 20307-5001