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Case Report

Effect of filgrastim (recombinant human granulocyte colony stimulating factor) on IgE responses in human asthma: A case study

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ABSTRACT

Rationale: The role of peripheral blood progenitor cell mobilization on Immunoglobulin E (IgE) responses has not been studied.**Methods:** Distributions of blood lymphocytes (CD4+, CD8+, CD8+CD60+, CD19+, CD23+, CD16/56+, CD25, CD45RA+, CD45RO+, CD34+), and levels of serum immunoglobulins (IgM, IgG, IgA, IgE) were studied in an allergic asthmatic serum IgE+ (181 IU/mL) adult (m/45 y/o) donor undergoing routine stem cell mobilization protocol (American Society of Hematology) before (day-30), during (day 4), and after (1 wk post last dose) filgrastim (subcutaneous, 480 mcg, 2qd) treatment (flow cytometry, nephelometry, UniCAP Total IgE Fluoro enzyme immunoassay).**Results:** On day 4 of filgrastim treatment, numbers of CD8+CD60+T cells and CD23+ blood cells dramatically increased (98% and 240% respectively) compared with pre treatment. In contrast on day 4 of treatment, serum IgE levels decreased (>50%) compared with pre treatment. CD8+CD60+T cells and CD23+ blood cells and serum IgE levels approached pre-treatment levels at 1 week post treatment.**Conclusions:** Filgrastim treatment transiently increases numbers of CD8+CD60+T and CD23+ expressing cells, which are known to regulate human IgE responses, while also transiently suppressing ongoing IgE responses. These results suggest that filgrastim affects IgE related responses, and may be useful in modulating allergic responses.

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Abbreviations: Ig, immunoglobulin; Th, T helper; rhG-CSF, recombinant human granulocyte colony-stimulating factor; mAbs, monoclonal antibodies; Ab, antibody; EDTA, ethylenediaminetetraacetic; PBSC, peripheral blood stem cells; WBC, white blood cell.

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1. Introduction

It is well known that filgrastim [recombinant human granulocyte colony-stimulating factor (rhG-CSF)], is administered to related and unrelated normal donors to mobilize peripheral blood progenitor cells for the purpose of donation [1,2]. Filgrastim controls neutrophil production within the bone marrow by stimulating the

proliferation and differentiation of myeloid progenitor and mature cells [3,4]. G-CSF has also been used as a secondary prophylaxis when used with full dose myelotoxic chemotherapy following a previous cycle with febrile-neutropenia in solid tumors [5].

In other studies, the use of filgrastim following chemotherapy appeared to be beneficial in the management of neutropenia associated complications [5,6]. In patients with lymphoma, filgrastim, as well as pegfilgrastim administration post transplant reduced the time to neutrophil recovery, and aided in neutrophil engraftment [7,8].

In addition to its role in bone marrow haemopoiesis, the role of filgrastim in humoral (Th2/Immunoglobulin E (IgE) receptor mediated activation responses) or cell-mediated (Th1) responses has been less well described; the immunological consequences and alterations to immune function in healthy or allergic/asthmatic donors given G-CSF to mobilize PBPC for collection by leukapheresis need to be assessed.

The aim of the current study was to investigate the clinical and immunological responses in an asthmatic volunteer blood donor who received short-term treatment with filgrastim. We found that filgrastim treatment transiently increases the numbers of CD8+CD60+T cells, which is known to regulate human IgE responses [9], while also transiently suppressing ongoing IgE responses. These results suggest that filgrastim affects IgE related responses and might be useful in modulating allergic responses.

2. Materials and methods

2.1. Patient history

A 45 year old allergic asthmatic, serum IgE+ (181 IU/mL) man presented to the Asthma Center of Excellence (SUNY Downstate Medical Center, Brooklyn, NY) for routine follow-up on a previous asthma exacerbation. Patient mentioned that the following week, he would begin a 5 day course of NEUPOGEN® (filgrastim; Amgen Manufacturing, Limited, Thousand Oaks, CA) (subcutaneous, 480 mcg, 2qd), which is used to mobilize hematopoietic stem cells into peripheral blood for successful allogeneic peripheral blood stem cells (PBSC) transplantation [10]. He was found to be a full match (6/6 allele loci; Mount Sinai Medical Center, New York, NY) to donate PBSC to his ailing 60 y/o brother who was diagnosed with multiple myeloma, and in need of a PBSC transplant. The apheresis target was determined by the dose requested by the transplantation center, performed via peripheral lines.

Donor subject did not receive systemic corticosteroids, but did receive daily treatment with a controller medication. Subject was skin prick positive to mixed ragweed antigen (Ag) (tall and short) (Center Laboratories), and standardized mite Ags (*Dermatophagoides farinae*, Miles Laboratory). Donor was without history of autoimmune disease or malignant illness.

In order to further study the immune/inflammatory response to the short – term course of filgrastim, written informed consent was obtained, and blood and serum samples were drawn at various time points [pre (1 week

prior, during (day 4) and post (1 wk) treatment]. The protocol was approved by the SUNY Downstate Medical Center Institutional Review Board, and the procedures followed were in accordance with institutional guidelines involving human subjects.

2.2. Filgrastim description

Filgrastim is a man-made form of human granulocyte colony-stimulating factor (G-CSF) with a specific activity of $1.0 \pm 0.6 \times 10^8$, available as a prefilled syringe containing 480 mcg filgrastim at a fill volume of 0.8 mL.

2.3. Immunoglobulin determination

Blood was collected and immunoglobulin (Ig) levels (IgM, IgG, IgA, IgE) were detected in serum. All serum Ig determinations were carried out in the Clinical Diagnostic Laboratory at SUNY Downstate Medical Center (nephelometry, UniCAP Total IgE fluoroenzymeimmunoassay, Pharmacia & Upjohn Diagnostics, Kalamazoo, MD) which was performed according to manufacturer's recommendation.

2.4. Flow cytometry

For flow cytometry studies, blood was collected into ethylenediaminetetraacetic (EDTA) Monoject tubes (Sherwood Medical) and retained for up to 2 h at room temperature.

2.5. Antibodies

Mouse anti-human monoclonal antibodies (mAbs) directly conjugated to fluorescein isothiocyanate (FITC) (IgG₁ anti-CD45RA, CD25, CD23; IgM anti-CD60); phycoerythrin (PE) (IgG₁ anti-CD8, CD34; IgG_{2a} anti-CD45RO), Simultest (FITC/PE-conjugated) reagents (CD3/CD4, CD3/CD8, CD3/CD19), and appropriately matched isotype control monoclonal antibodies (FITC-conjugated IgG₁, PE-conjugated IgG₁ and IgG_{2a}, Simultest control γ_1/γ_{2a} , FITC-conjugated IgM). All antibodies were purchased from BD Biosciences, San Jose, CA, except IgM anti-CD60 which was purchased from Ancell, Bayport, Minn, and IgG₁ anti-CD34, which was purchased from BD Pharmingen (San Diego, CA); all were used according to manufacturers' recommendation.

2.6. Assay

For labeling studies, conjugated antibodies (10 mcl) directed against 1–3 markers, were added to blood (100 mcl) in 12 × 75 mm (5 ml) tubes (Fisher Scientific, Springfield, NJ) and incubated for 10 min at room temperature, after which erythrocytes were lysed with whole blood lysing reagent (Immunoprep, Beckman Coulter, Hialeah, FL), and the cells counted. Lymphocyte distributions were determined with a Coulter Epics XL/MCL Flow Cytometer with System II software (Coulter), and CytoComp (Coulter) QC Windows (Flow Cytometry Systems, San Juan, Puerto Rico) used to ensure consistent instrument settings. Absolute lymphocyte numbers were calculated from the total

lymphocytes. Data are expressed as total lymphocytes per cubic millimeter (mm^3) or mean percentage (%) of positive cells.

3. Results

3.1. Adverse side effects of filgrastim treatment

Side effects were assessed using a written questionnaire, inquiring about any adverse effects, pain, or need for pain control. In general, filgrastim treatment was well tolerated by our patient. However, towards the final days of treatment, symptoms of bone pain (lumbar spine and pelvic regions; legs and chest), headache, and malaise were reported; these are recognized side effects reported by the manufacturer and others [11]. Severity of pain was not formally assessed, and pain medication was not required. At the time of follow up post transplantation, all side-effects were resolved.

3.2. Distributions of blood lymphocyte subpopulations

Distributions of blood lymphocytes (CD4+, CD8+, CD8+CD60+, CD19+, CD23+, CD34, CD45RA+, CD45RO+) were studied in peripheral blood. Pre-treatment, distributions of blood lymphocytes were within normal ranges (Tables 1 and 2). During treatment, distributions of all blood lymphocytes increased, with the greatest increases observed with CD8+CD60+T cells (98%) and CD23+ cells (240%). Effective CD34+ cell mobilization, for PBSC harvest, was also observed (4.0%, 114 mm^3 compared with <1.0%, 12 mm^3 pre-treatment). One week post treatment, all blood lymphocytes returned to pre-treatment levels (Tables 1 and 2), except the CD8+CD60+T cells which remained elevated (11%) (Table 1).

3.3. Serum immunoglobulins

3.3.1. Total IgM, IgG, IgA, and IgE

Pre-treatment, serum IgM, IgG, and IgA levels were within the normal range while serum IgE levels were elevated (181 IU/ml) (Table 3). During treatment, serum IgM and IgA levels slightly decreased (15% and 13%, respectively), while serum IgE levels demonstrated a robust decrease (>50%). However, 1 week post treatment, IgE levels approached pre-treatment levels and all other serum Igs were within the normal range. (Table 3) Serum IgG levels increased slightly during treatment and post treatment, but were within normal range (Table 3).

Table 2

Distributions of lymphocyte subpopulations (activation and memory markers) in peripheral blood of allergic asthmatic donor before, during, and after administration of filgrastim.

Time	CD25+		CD45 RA+		CD45RO+		CD34	
	(%)	mm^3	(%)	mm^3	(%)	mm^3	(%)	mm^3
Pre	5.0	104	33	676	68	1395	<1.0	12
Day 4	5.0	142	41	1208	69	2050	4.0	114
Post	5.0	85	35	554	68	1087	<1.0	14

4. Discussion

The present studies describe the immune response to filgrastim in an asthmatic volunteer donor before, during, and after filgrastim treatment for the purpose of PBSC mobilization. We found that filgrastim treatment transiently increases the numbers of CD8+CD60+T cells, known to regulate human IgE responses [9], while also transiently suppressing ongoing IgE responses.

These findings are consistent and in agreement with recent studies of Mueller et al. [11], who reported a long-term longitudinal study of healthy stem cell donor volunteers, which included standard biochemical/haematological tests and T-cell phenotyping [11]. These authors demonstrated completely normalized white blood cells (WBCs) at the time of the first follow-up visit (1 year post-treatment), and no change of WBCs over the 5 year-time of follow-up observation [11]. In addition, there was no evidence of altered helper/killer T-cell ratios at any time during or after G-CSF, and no indication of increased susceptibility to infections and no advent of autoimmune disease [11]. Thus, the authors concluded that there were no clinically overt adverse effects on immune function [11]. However distributions of other leukocyte or lymphocyte subpopulations were not studied (i.e. CD23+ cells or CD8+CD60+T cells) and immunoglobulin levels were not evaluated. In contrast, studies of Tassi et al. [12] reported that G-CSF-treated healthy allogeneic donors had evidence of prolonged suppression of humoral immune function (neutrophil reduction and lymphocytopenia) [12]. Further, the studies of Amariglio et al. found that the administration of G-CSF is associated with transient DNA and gene expression modifications in the lymphocytes of normal mobilized donors [13].

The effect of G-CSF on lymphocyte numbers in humans with cancer, after receiving chemotherapy has been documented [14,15]. Studies of Morstyn et al. reported that before chemotherapy (melphalan hydrochloride), the immediate effect of G-CSF was a transient depression of

Table 1

Distributions of lymphocyte subpopulations in peripheral blood of allergic asthmatic donor before, during, and after administration of filgrastim.

Time	CD3+CD4+		CD3+CD8+		CD8+CD60+		CD16+56+		CD19+		CD23+	
	(%)	mm^3	(%)	mm^3	(%)	mm^3	(%)	mm^3	(%)	mm^3	(%)	mm^3
Pre	46	950	31	639	8.0	160	4.0	77	11	233	2.0	42
Day 4	44	1294	33	976	11	317	4.0	104	11	320	5.0	143
Post	47	756	33	531	11	168	5.0	76	11	168	3.0	54

Table 3

Immunoglobulin levels in serum of allergic asthmatic donor before, during, and after administration of filgrastim.

Time	Immunoglobulin			
	IgM (mg/dl)	IgG (mg/dl)	IgA (mg/dl)	IgE (IU/ml)
Pre	54	725	322	181*
Day 4	46 [§]	736	281	87
Post	53	771	318	134*

Quest diagnostics reference range for healthy adult serum: IgM: 47–367 mg/dl; IgG: 648–2045 mg/dl; IgA: 55–375 mg/dl; IgE: 20–100 IU/mL.

* Lab value high.

§ Lab value low.

circulating neutrophils, followed by an increase of cells; however, G-CSF administration after melphalan treatment reduced the period of neutropenia [14]. Others have shown that the percentages and absolute numbers of large granular lymphocytes were increased in cancer patients who had received high dose antineoplastic chemotherapy followed by G-CSF treatment, compared with the control subjects [15]. However, in these studies specific lymphocyte subsets and immunoglobulin levels were not studied. In contrast, studies of Arat et al. reported that during collection of PBSC with continuous flow cell separators, high values of CD34+ cells were collected at the expense of high CD3+ lymphocytes, which may increase the risk of acute and chronic graft vs host disease after allogeneic hematopoietic cell transplantation [16].

In the present study, on day 4 of filgrastim treatment, we observed appropriate mobilization of CD34+ cell: however, numbers of CD8+CD60+T cells and CD23+ blood cells demonstrated the most dramatic increase (98% and 240% respectively) of all subsets studied. One week after treatment, numbers of CD23+ cells decreased to pre-treatment levels, however, CD8+CD60+T cells remained elevated. The ability of filgrastim to preferentially increase CD8+CD60+T cells and CD23+ blood cells is not well understood. It could be that G-CSF stimulates selective effects which are unique to CD60+T cells as previously described by Semnani et al., [17]. CD60 is a well described ganglioside antigen, GD3 [16]. Studies of Semnani [17] reported that the cell surface expression of the CD60 antigen+ cells were uniquely stimulated by a pathway which included co-stimulation with ICAM-1, LFA-3, GM-CSF (within the memory CD4+T cell population), and other cytokines, aspects of which may be similarly involved after G-CSF stimulation. However, in our study, CD4+CD60+T cells were not studied.

On day 4 of treatment, serum IgE levels decreased (>50%). However, one week after treatment, IgE levels approached pre-treatment levels. A possible explanation to our observation(s) might be that hematopoietic growth factors function as modulators of the immune mediator network [18]. Studies of Hartung et al. demonstrated that human volunteers treated with filgrastim had augmented proliferation of lymphocytes in response to OKT-3, phytohemagglutinin, or Daudi cells *ex vivo* [18]. However, 72 h after treatment, they reported suppression of the lymphocyte proliferative response [18]; during 12 days of treatment, NK cells increased significantly [18]. The authors further observed reduced levels of lipopolysaccharide-induced tumor necrosis factor- α (TNF- α), interferon

gamma (IFN- γ), and GM-CSF, thus suggesting that filgrastim is responsible for a shift from proinflammatory towards anti inflammatory cytokine responses [18]. However, the mechanism by which G-CSF can change lymphocyte expression and/or influence allergic/inflammatory responses is unknown.

Previous studies in our laboratory have demonstrated that other well known therapeutic agents have the ability to suppress IgE responses both *in vivo* and/or *in vitro* including green tea extract, minocycline, doxycycline, and substance P [19–23]. However, the mechanism(s) by which the above agents accomplish their effects are not well defined, but may include neural or endocrine pathways [22].

There are several limitations to our study, including: (1) the small study/sample size. Future larger scale studies are needed to confirm the findings of this pilot study. (2) The length of follow-up is limited; thus suggesting that brief exposure to filgrastim during the PBSC donation process might alter cellular immune responses or suppress total serum IgE levels. However, the strength of this study is that our results are highly relevant to addressing a possible relationship between short-term filgrastim treatment and its effect on cellular and humoral responses in human blood donors, as well as its possible anti-inflammatory/allergic effect.

Studies examining short-term effects of filgrastim administration in PBSC donors are scarce. The results from this study suggest that short-term treatment with filgrastim transiently increases numbers of IgE regulating CD23+ and CD8+CD60+T cells, while also transiently suppressing ongoing IgE responses, thus suggesting that filgrastim modulates IgE responses. These results also provide the prospect that filgrastim, at low concentrations, may be a potential therapy for use in modulating allergic responses. It could be that administration of filgrastim at a lower dose may provide appropriate allergy therapy while decreasing the probability of reported side effects. Further studies are warranted to further elucidate IgE and related immune responses to filgrastim treatment in healthy donors, which will help determine its plausibility as a therapeutic modality in allergic disease and contribute to favorable outcome.

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