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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.

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

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

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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 ethylenediaminotetraacetic (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) (IgG_1 anti-CD45RA, CD25, CD23; IgM anti-CD60); phycoerythrin (PE) (IgG_1 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 IgG_1, PE-conjugated IgG_1 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 IgG_1 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