Late-onset X-linked Chronic Granulomatous Disease in a Female Carrier: Therapeutic Role of Interferon-γ and Hematopoietic Stem Cell Transplantation

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Research Article

Keywords: chronic granulomatous disease, primary immunodeficiency, interferon-γ, hematopoietic stem cell transplantation, X chromosome inactivation

Posted Date: March 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-344043/v1

Abstract

X-linked Chronic Granulomatous Disease (CGD) is a rare inherited immunodeficiency characterized by early life-threatening infections from bacteria and fungi in male children. Female carriers of X-linked CGD usually do not develop any manifestations of the disease, yet in rare cases they may present with CGD-related manifestations due to skewed X chromosome inactivation, even in advanced age. Here, we report the case of a 49-year-old woman with no history of previous frequent or severe infections, who presented acutely with life-threatening bilateral pneumonia caused by *Nocardia asteroides* and was eventually diagnosed with late-onset X-linked CGD due to skewed X chromosome inactivation in white blood cells. Treatment with interferon-γ as a rescue therapy resulted in normalization of the intensity of the oxidative burst in the residual positive cells and resolution of the infection, which was otherwise resistant to conventional treatments. After discharge, however, recurrent severe pulmonary infections despite prophylactic treatments as well as appearance of granulomatous colitis led to considering definitive treatment. Hematopoietic stem cell transplantation from unaffected HLA-identical brother using a non-myeloablative conditioning protocol with intravenous busulfan followed by high-dose peripheral blood stem cell graft and post-transplant cyclophosphamide was successfully performed. After three years of follow-up, white blood cell chimerism remained stable with about 60% donor cells in the myeloid lineage, with no further infections and no recurrence of inflammatory bowel disease.

Introduction

Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency (~1 in 250.000 live births) [1-4] characterized by a defective NADPH oxidase complex, leading to an impaired respiratory burst and ineffective killing of pathogens by phagocytes (neutrophils, monocytes, and macrophages). CGD patients are especially vulnerable to infections caused by fungi or catalase-positive bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Nocardia asteroides,* and *Burkholderia cepacia* [1–4]. Moreover, patients can also present with clinical manifestations related to hyperinflammatory responses particularly in the lungs and gastrointestinal tract, where it can closely resemble Crohn's disease, as well as granuloma formation in various organs [5–9].

The NADPH oxidase complex subunits are encoded by five genes (*CYBB, CYBA, NFC1, NFC2,* and *NFC4*) and pathogenic mutations in any of these can cause CGD, albeit with variable phenotypes [1,2,10]. The most common form of CGD is caused by hemizygous mutations in the *CYBB* gene, encoding the $gp91^{phox}$ subunit, whereas the remaining cases are due to autosomal recessive mutations in one of the other four genes. X-linked CGD usually manifests in male children at an early age, while female carriers of a *CYBB* mutation generally do not show any signs of the disease. However, there are reports of females with a CGD phenotype due to progressive skewing of X-chromosome inactivation in leukocytes [11–18]. Diagnosis and treatment of these patients may be difficult. Here, we report the clinical and therapeutic course of a woman with late-onset clinical problems of CGD due to extremely skewed X chromosome lyonization, who was ultimately cured by hematopoietic stem cell transplantation (HSCT) by a matched sibling peripheral blood stem cell (PBSC) transplant after non-myeloablative conditioning.

Methods

Dihydrorhodamine assay. Stimulation of peripheral blood sample with phorbol 12-myristate 13-acetate (PMA) induces the production of reactive oxygen species (ROS) by the NADPH oxidase enzyme activity in granulocytes. The release of ROS was revealed using dihydrorhodamine 123 (DHR123), conversion to fluorescent rhodamine 123. The fluorescence signal was read by means of a cytometer, MACSQuant Analyzer 10, and analyzed with FlowLogic software.

X-chromosome inactivation (XCI). Human androgen receptor (HUMARA) assay was used to determine the percentage of X-chromosome inactivation (XCI). A hypervariable CAG short tandem repeat and several methylation-sensitive restriction sites (such as HpaII) permits the discrimination between the paternally and maternally derived X chromosome and the selectively digestion of the active unmethylated allele. Using a subsequent polymerase chain reaction (PCR), it is possible to distinguish the active (not amplified) from the inactive alleles. The area below each peak were used to calculate the percentage of X inactivation. Usually, a random XCI occurs in each female during embryogenesis and is estimated about 50%. In case of significant deviation from this ratio, non-random or skewed X-chromosome inactivation condition occurred. The percentage of inactivation is considered unbalanced for values greater than 80% [19,20].

Results

Here, we describe the case of a 49-year-old woman admitted to the Pneumology department because of severe respiratory failure due to bilateral pneumonia. Her medical history was characterized by discoid lupus and Raynaud's phenomenon, which had appeared three years before. Notably, she had no history of frequent, severe, or unusual infections. After unsuccessful treatment with broad-spectrum antibiotics, analysis of bronchoalveolar lavage fluid demonstrated *Nocardia asteroides* infection. Despite targeted intravenous antibiotics, she developed acute respiratory distress syndrome and bilateral pneumothorax, requiring intensive care unit admission for invasive ventilation and ultimately extracorporeal membrane oxygenation. Blood tests, lymphocytes subpopulations, and interferon-γ (IFNγ) levels were normal, nevertheless, DHR123 assay showed superoxide anion production only in a small portion of her neutrophils; indeed, only 6.68% of the patient's neutrophils showed were oxidase activity (healthy controls: 96-100% of neutrophils display oxidase activity by DHR assay). Moreover, the fluorescence intensity in positive cells was lower than expected (Fig. 1), suggesting a quantitative defect in superoxide anion production. Genetic analysis revealed a heterozygous mutation on *CYBB* gene (c.[1661_1662insT]:[=],p.[Glu555*]). X-chromosome inactivation assay performed in her peripheral white blood cells showed a skewed pattern, with a ratio of 97.8:2.2%, supporting the conclusion that almost all of her blood cells had inactivated the X-chromosome without the mutated gene, thus confirming the functional diagnosis of X-linked recessive CGD despite her heterozygous carrier X-CGD genotype. After one week of extracorporeal respiratory support, her condition did not improve, therefore subcutaneous IFNγ was started (200 μg/3 times a week). Notably, this treatment did not influence the overall percentage of functional neutrophils at DHR123 assay, yet it led to a normalization of the fluorescence intensity in the remaining positive cells

(Fig. 2), and was soon followed by a significant improvement of pneumonia, allowing progressive reduction of respiratory support and ultimately weaning. After hospital discharge, she developed persistent diarrhea and rectal bleeding; colonoscopy showed diffuse colitis, later histologically confirmed to be granulomatous colitis, requiring treatment with oral corticosteroids. Despite anti-microbial prophylaxis and maintenance of IFNγ administration, in the following months she required multiple hospitalizations due to recurrent pulmonary infectious complications. In particular, she developed several lung infections due to *Klebsiella pneumoniae*, *Nocardia, asteroides* and *Pseudomonas aeruginosa* colonization, often with associated severe sepsis, ultimately requiring a resection of two pulmonary lobes of the right lung due to lung abscess with cavitation. Hematopoietic stem cell transplantation (HSCT) was recommended due to her overall worsening condition and recurrent infections. At the age of 51 years, the patient was transferred to the National Institutes of Health (NIH) in Bethesda, Maryland, USA, where she received a healthy matched sibling HSCT graft of mobilized PBSC after non-myeloablative conditioning with intravenous busulfan and alemtuzumab. For graft versus host disease (GVHD) prophylaxis she received post-transplant cyclophosphamide on Days 3 and 4 and then sirolimus for 6 months. Just prior to initiating transplant conditioning a DHR123 assay showed showed oxidase activity in 3.1% of circulating neutrophils. At 15 days after transplantation, 99% of the early-appearing circulating neutrophils were functional neutrophils on a DHR123 assay, and neutrophil engraftment to absolute neutrophil count >500/μL occurred on the 23rd day from HSCT. Soon after transplantation, the patient developed grade I acute skin GVHD, which was treated by topical steroids, and a self-limited asymptomatic reactivation of Cytomegalovirus and Epstein-Barr virus. No other infections or complications occurred in the follow-up. Currently, almost three- and one-half years after HSCT, her peripheral blood donor chimerism is stable (70% of lymphocytes and 60% of granulocytes donor derived), with 62% of the neutrophils displaying normal superoxide production capacity at DHR assay, and the patient maintains an overall good health, with resolution also of granulomatous colitis.

Discussion

Our case report highlights several peculiar aspects of late-onset X-linked CGD in female patients, underscoring the need for high clinical suspicion, but also the possibility of curative HSCT even in patients over 50 years of age. In our case, an immunodeficiency was suspected due to the occurrence of a severe and unusual infection in a woman with normal white blood cell counts and normal serum immunoglobulins without a significant previous infection history. She did have a history of discoid lupus, a finding frequently reported in female carriers of X-linked CGD [21], yet this association may go unnoticed. After secondary causes of immunodeficiency were ruled out, CGD was suspected and eventually confirmed by the finding of abnormal results at DHR testing. The presence of two functional populations of neutrophils, together with the detection of a recessive mutation in the *CYBB* gene confirmed the diagnosis of X-linked recessive CGD in a woman with skewed X chromosome lyonization. It is already known that female carriers can present with clinical manifestations later in life due to the aging of their hematopoietic cell lineage and to progressive skewing of X chromosome inactivation [11–18]. The factors leading to skewing of X chromosome inactivation in some female carriers are not completely

known. X chromosome inactivation is usually a random event, yet the presence of micro-deletions or other kinds of deleterious damages on one of the two X chromosomes may lead to its preferential inactivation, leading to a skewed X inactivation [22–25]. Moreover, differential DNA methylation has been described in hematopoietic cell lineage with aging, possibly resulting in greater functional skewing [26–29]. These processes may ultimately result in the selection of mutated alleles in other genes, as in the case of X-linked CGD female carriers, and thus to clinical disease when the number of functional neutrophils falls under a critical level [11–18].

Current treatment of CGD is based on prevention of severe infections, prompt and intensive antibiotic therapy, and proper treatment of granulomas and inflammatory complications. In our patient, the first episode of life-threatening pneumonia resolved only after rescue treatment with IFNγ was started. IFNγ enhances phagocyte activity in healthy subjects through NADPH oxidase and inducible isoform of Nitric Oxide Synthase (iNOS) transcription [30]. Even in CGD patients, IFNγ increases the oxidative burst in defective neutrophils both in vitro and in vivo, although though the mechanism is not fully understood [31–34]. Although IFNγ role and effectiveness for infection prophylaxis and treatment in CGD patients is still debated [35–37], our experience may suggest that it could have a greater role in heterozygous females as compared to male patients, as it could potentiate oxidase activity of the residual part of neutrophils expressing the wild type allele [38]. In fact, DHR testing in our patient showed a decreased oxidative burst also in the residual oxidase-positive neutrophils, possibly due to the concomitant sepsis, which can induce immunoparalysis of both innate and adaptive immunity [39–41]. Indeed, immunoparalysis may be a critical issue in subjects starting with already compromised immune functions.

HSCT remains the only definitive treatment for X-linked CGD to date, with a higher success rate in younger patients. Older patients, on the other hand, are more likely to develop transplant complications and have higher transplant-related mortality due to active infections, inflammation, and organ damage at the time of transplantation [42-44]. Gene therapy is also under development, with promising outcomes recently, but availability is still limited [45]. Our patient underwent HSCT because of her worsening condition despite standard prophylactic treatments (antibiotics, anti-fungal, steroids, and IFNγ). She was considered at high risk for transplant-related morbidity and mortality due to her age and the presence of active infectious and inflammatory complications; therefore, a non-myeloablative conditioning regimen followed by high dose PBSC graft was used. Reduced-intensity or reduced-toxicity regimens with low-dose busulfan (AUC 45-65 mg/L*h) or treosulfan have been shown to be associated with a higher over-survival (>90%) and event-free-survival (>85%) compared to myeloablative regimens, [46,47] but can lead to mixed chimerism as a long-term outcome as occurred in this patient. Her donor neutrophil chimerism remains stable at 60% which appears to be sufficient to maintain her current state of good health. The NIH regimen incorporates low dose busulfan (30-40mg/L*h) and alemtuzumab (1 mg/kg) for patients with related donors and even lower dose busulfan (median AUC of 20-30mg/L*h) combined with low dose total body irradiation (TBI) and alemtuzumab followed by sirolimus as single agent GvHD prophylaxis [48]. For this patient, enrolled on a new protocol, she also received post-transplant cyclophosphamide along with a high dose of unmanipulated PBSC (10 million CD34 per kg) to ensure

better engraftment but prevent GVHD. This HSCT protocol then resulted in stable donor chimerism with minimal transplant morbidity despite the patient's risk factors and age. With follow up of more than three years, this suggests that transplantation using this regimen is viable even in older patients with high risk factors.

This case demonstrates a number of points. First of all, it underscores an emerging cornerstone of clinical immunology, i.e. that even a single severe or unusual infection should raise the suspicion of a primary immune deficiency disorder, even in previously healthy adults. Secondly, IFNγ may have a role as a rescue target therapy in severe infections, especially in females with X-linked CGD, as these patients still have a fraction of normal neutrophils. Lastly, HSCT with non-myeloablative conditioning with low dose busulfan and alemtuzumab, combined with an increased stem cell dose and post-transplant cyclophosphamide may represent a curative option even in high-risk patients at an advanced age.

Abbreviations

AUC: Area Under the Curve

CGD: Chronic Granulomatous Disease

DHR: Dihydrorhodamine assay

GVHD: Graft Versus Host Disease

HSCT: Hematopoietic Stem Cell Transplantation

HUMARA: Human androgen receptor

IFNγ: Interferon-gamma

iNOS: inducible Nitric Oxide Synthase

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NIH: National Institutes of Health

PBSC: Peripheral Blood Stem Cells

PCR: Polymerase Chain Reaction

PMA: Phorbol 12-Myristate 13-Acetate

ROS: reactive oxygen species

RTE: Recent Thymic Emigrants

TBI: Total Body Irradiation

XCI: X-Chromosome Inactivation

Declarations

Funding:

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E and Contract No. 75N910D00024, Task Order No. 75N91019F00131. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Conflicts of interest/Competing interests:

The authors declare that they have no conflict of interest.

Availability of data and material:

Not applicable

Code availability:

Not applicable

Authors' contributions:

All authors contributed to the study conception and design. The first draft of the manuscript was written by MT and EMK. FS, BR, PC, CK, MP, EMK, AT, HLM, and MC cared for the patient. Material preparation, clinical data collection and analysis were performed by EV, SN, MP and AT. LDN, AT, HLM and MC reviewed and improved the final draft. All authors read and approved the final manuscript.

Consent to publish:

The patient’s consent to publish was obtained.

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Figures

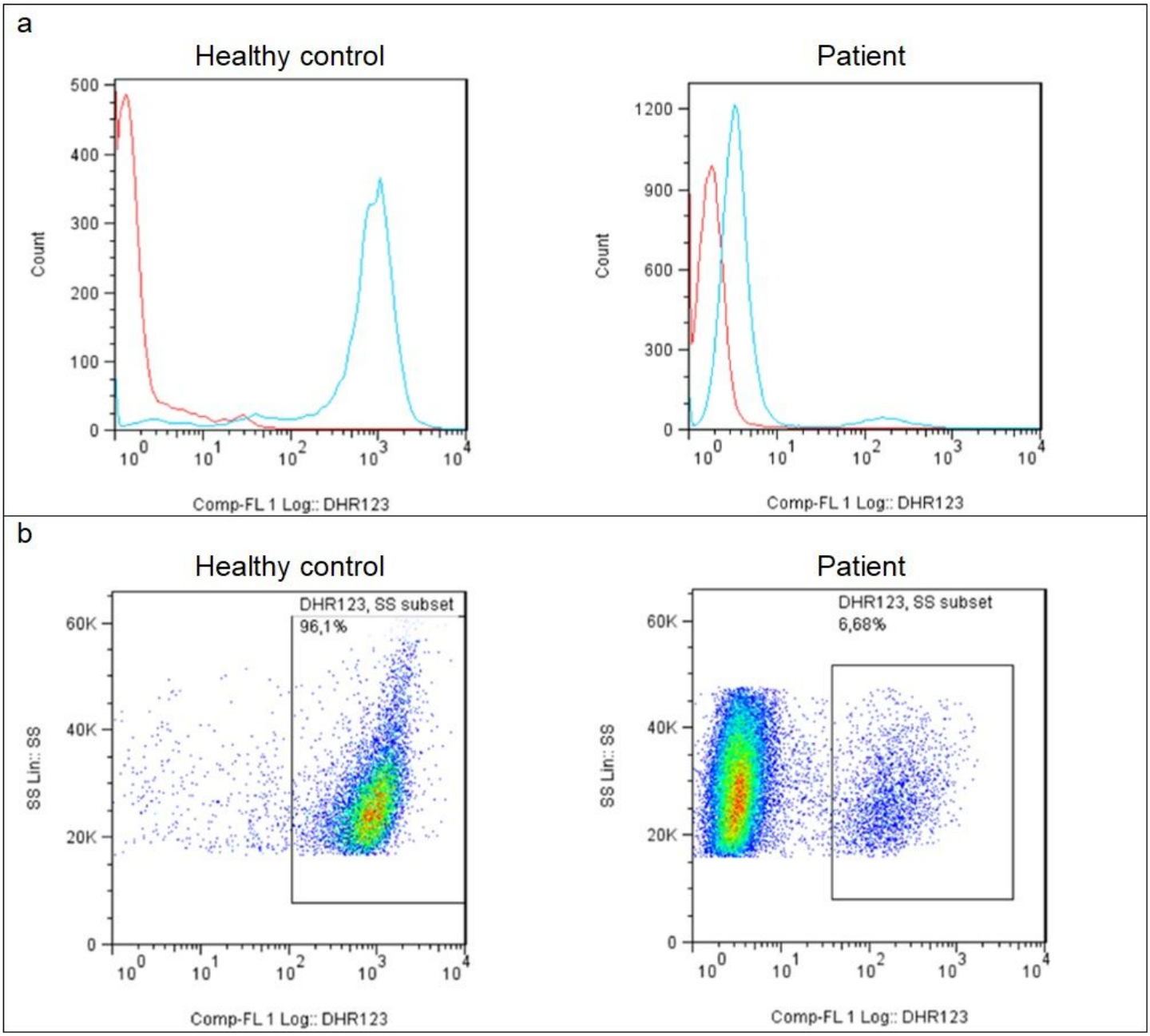

Figure 1

Dihydrorhodamine assay (DHR). Histogram graphs in Panel A show a low superoxidase production by the patient's neutrophils rather than control after PMA treatment (blue line); red line represents the untreated samples. Dot plot graphs in Panel B just to better display the low percentage of functional neutrophils (6.68%).

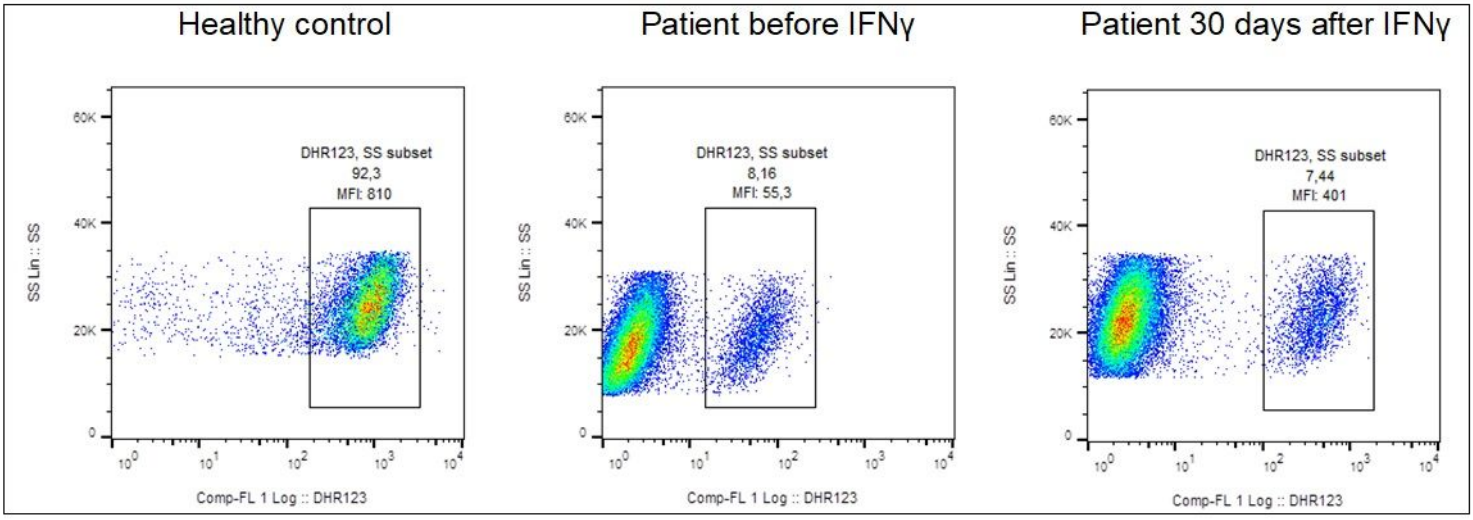

Figure 2

Dihydrorhodamine assay (DHR) before and after IFNγ treatment. Comparison of the fluorescence intensity of rhodamine 123 in functional neutrophils before and after IFNγ administration allows to observe an increase of median fluorescence intensity (MFI) values after the treatment (55,3 vs 401) without a corresponding rise in percentage of cells with oxidase activity.