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Mobilization Characteristics and Strategies to Improve Hematopoietic Progenitor Cell Mobilization and Collection in Patients with Chronic Granulomatous Disease and Severe Combined Immunodeficiency

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Abstract

Background—G-CSF mobilized autologous hematopoietic progenitor cells (HPC) may be collected by apheresis of patients with chronic granulomatous disease (CGD) and severe combined immunodeficiency (SCID) for use in gene therapy trials. CD34+ cell mobilization has not been well characterized in such patients.

Study Design and Methods—We retrospectively evaluated CD34+ cell mobilization and collection in 73 consecutive CGD and SCID patients and in 99 age, weight and G-CSF dose-matched healthy allogeneic controls.

Results—In subjects aged <20 years, day 5 pre-apheresis circulating CD34+ counts were significantly lower in CGD and SCID than in controls; mean peak CD34+ cells 58, 64, and 87/uL, respectively, $p=0.01$. The SCIDs had lower CD34+ collection efficiency than CGDs and controls; mean efficiency 40%, 63% and 57%, respectively, $p=0.003$. In subjects >20 years, the CGDs had significantly lower CD34+ cell mobilization than controls; mean peak CD34+ cells 41 and 113/uL, respectively, $p<0.0001$. In a multivariate analysis, lower sedimentation rate (ESR) at mobilization was significantly correlated with better CD34+ cell mobilization, $p=0.007$. In SCIDs, CD34 collection efficiency was positively correlated with higher red cell indices (MCV: $R^2=0.77$; MCH: $R^2=0.94$; MCHC: $R^2=0.7$, $p<0.007$) but not hemoglobin.

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Conflicts of Interest: None

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Conclusions—CGD and SCID populations are characterized by significantly less robust CD34+ HPC mobilization than healthy controls. The presence of active inflammation/infection as suggested by an elevated ESR may negatively impact mobilization. Among SCIDs, markedly reduced CD34 collection efficiencies were related to iron deficiency, wherein decreased red cell size and density may impair apheresis cell separation mechanics.

Keywords

Hematopoietic progenitor cells; apheresis; mobilization; CGD; SCID; G-CSF; filgrastim; plerixafor; iron deficiency; MCV

Introduction

Primary immunodeficiency disorders such as chronic granulomatous disease (CGD) and severe combined immune deficiency (SCID) are genetic disorders of the innate and adaptive immune systems, respectively. Greater than 120 gene mutations have been identified which cause impairment in the differentiation and/or function of immune cells, resulting in clinical syndromes with varying degrees of severity.¹⁻³ The most severely affected patients present in childhood with recurrent life-threatening infections, inflammation, such as CGD-associated granulomas and colitis, and failure to thrive. Hematopoietic progenitor cell (HPC) transplantation from an HLA-matched sibling donor may be curative and is the treatment of choice in these conditions. Gene therapy may be an alternative therapy if the genetic defect has been identified.⁴⁻⁶

Since the discovery of their engraftment capability more than two decades ago, HPC's collected by apheresis of cytokine-stimulated donors have largely replaced bone marrow as the preferred source of stem cells for allogeneic and autologous transplantation^{6,7}. Granulocyte colony-stimulating-factor (G-CSF) mobilized HPC's have also been shown to be an efficient source of autologous cells for somatic cell gene therapy in CGD and SCID⁸⁻¹⁰. Compared to bone marrow aspiration, the relative ease of HPC collection by apheresis and larger CD34+ cell yields have made them the preferred cell source in gene therapy trials. Autologous HPC apheresis collections may then be enriched by immunoaffinity techniques and transduced with viral vectors carrying the missing genes to manufacture a clinically relevant dose of final product¹¹.

Factors influencing HPC mobilization and methods to maximize progenitor cell apheresis yield have been studied extensively in the HPC transplantation setting. Parameters shown to significantly affect mobilization in healthy allogeneic HPC apheresis donors include donor age, sex, race, and total G-CSF dose administered during mobilization¹². Higher G-CSF dose, larger volumes processed per leukapheresis procedure, and selection of donors based on demographic parameters that are associated with more robust CD34 mobilization may be used to increase CD34+ cell yields during apheresis¹². Donor characteristics may also affect the collection efficiency of HPC apheresis procedures. In an analysis of 793 consecutive healthy allogeneic donors undergoing HPC apheresis procedures, red blood cell microcytosis was noted to impair cell yields independent of pre-apheresis CD34+ cell counts¹³. Lastly, in the setting of autologous HPC apheresis collection, mobilization failure,

defined as the collection of less than 2×10^6 CD34+ cells/kg, commonly results from prior exposure to myelotoxic chemotherapy or radiation¹⁴⁻¹⁷. CD34+ cell mobilization responses may be improved in donors with limited marrow reserve by the addition of plerixafor to G-CSF during the mobilization process^{18,19}.

In patients with primary immunodeficiency disorders, chronic infections and/or inflammation can result in persistent leukocytosis. Inflammatory states have also been associated with increased endogenous G-CSF levels in mouse models²⁰. Paradoxically, Sekhsaria et al. reported significantly impaired HPC mobilization in 18 CGD and 2 adenosine deaminase (ADA)-deficient SCID patients (not receiving pegylated ADA) compared to normal healthy controls⁸. They also noted the lack of an association between peak mobilized CD34+ cell counts and baseline absolute neutrophil counts among their subjects. The goal of the current study was to more fully characterize the CD34+ mobilization response to G-CSF, and in some cases G-CSF plus plerixafor, in a larger cohort of patients with CGD and SCID, and to identify and possibly mitigate the causes for poor CD34+ mobilization and collection yields in this population. We hoped in this manner to develop strategies to enable the collection of at least 5×10^6 CD34+ cells/kg, which is the typical targeted starting material for patients undergoing autologous HPC mobilization in gene therapy trials²¹.

Methods

Patient cases and matched healthy controls

We performed a retrospective case-control study involving 73 consecutive CGD (n=63) and SCID (n=10) subjects who underwent HPC collection by apheresis at our institution from April 1997 to October 2012. The control population consisted of healthy sibling or unrelated donors participating in institutional allogeneic transplant protocols during the same period, who were selected based on matching criteria for age, sex, weight, and total G-CSF dose received. All patients and normal volunteers signed written informed consent for mobilization and apheresis under Institutional Review Board (IRB)-approved protocols 94-I-0073, 07-I-0075, 00-CC-0165 and other NIH IRB-approved transplantation protocols. Baseline demographic data including age, sex, race, weight, and BMI were collected on all cases. Subjects were divided into pediatric (< 20 years) and adult (> 20 years) cohorts by age, based on prior observations that HPC mobilization responses differ in children versus adults³². A higher than usual cut-off for the definition of pediatric age was used due to the growth retardation and low weight of many of the subjects. The pediatric group consisted of CGD (n=38) and SCID (n=9) cases and a 1:1 ratio of healthy controls (n=47). The adult group included CGD (n=25) and SCID (n=1) cases and a 2:1 ratio of controls (n=52). The availability of a larger number of matched controls for the older age group allowed a 2:1 comparison; this was not possible in the younger age group.

Ancillary medications in immunodeficiency patients

Six weeks prior to HPC mobilization, administration of interferon-gamma (IFN-g) (1.5 mg/kg subcutaneously 3 times weekly) was discontinued in 56 CGD patients. Seven CGD patients continued to take INF-g during HPC mobilization and collection. As management

for CGD-associated colitis, oral prednisone was given to 12% of patients at therapeutic doses (5-10 mg daily) and to 42% at prophylactic doses (2.5-5 mg daily); 46% did not receive steroids during CD34+ cell mobilization. All patients received trimethoprim/sulfamethoxazole (160 mg/800 mg daily) and itraconazole (200 mg daily) prophylaxis. Data were collected on the presence of active infection and/or CGD-associated colitis at the time of collection by review of clinical, microbiologic, serologic, and radiographic records.

HPC mobilization and collection

Patients and healthy allogeneic donors were given subcutaneous injections of G-CSF (filgrastim, Neupogen®; Amgen, Thousand Oaks, CA) for 5 consecutive days at a dose of 10-16 mcg/kg/day, with the fifth dose given at least two hours prior to the start of the HPC apheresis procedure. The actual dose administered was obtained from a review of pharmacy and nursing records. Circulating CD34+ cell counts were assessed by flow cytometry on day 4, on day 5 pre-apheresis, on day 5 post-apheresis, and pre- and post-apheresis on all subsequent days in which apheresis was performed. Methods used to stain, gate, and quantitate CD34 positive cells did not change substantially over the course of the study. Flow cytometry instrumentation upgrades occurred only after successful validation of counting equivalency between device versions (FACScan and FACSCanto, Becton-Dickinson). In a subset of patients (n=14) whose day 4 CD34+ cell count was below 20 cells/ μ L, a single dose of plerixafor (Mozobil, Genzyme, Cambridge, MA) 240 mcg/kg was given 10 hours prior to the start of the apheresis procedure.

HPC were collected by leukapheresis on day 5 of G-CSF. The pre-apheresis CD34+ count was used to determine the volume to be processed during the procedure based on recipient weight and targeted CD34+ cell dose. Three to eight blood volumes (3 to 30 liters) were processed per procedure, with the goal of collecting the entire targeted dose in a single procedure²². Leukapheresis was performed with use of either a CS-3000 Plus instrument (Fenwal Division, Baxter, Deerfield, IL) from April 1997 through December 2008 or a COBE Spectra Apheresis device (Terumo BCT, Lakewood, CO) from January 2009 to October 2012. In all three cohorts (CGD, SCID, controls) approximately half the subjects had collections performed with the CS3000 and half with the Spectra. Prophylactic intravenous calcium infusions were administered as previously described²³. Central venous catheters were placed if peripheral access was insufficient to support apheresis. Collection efficiencies were calculated using the formula²²:

$$\frac{\text{CD34+content in product}}{(\text{Mean of pre - and post - apheresis CD34+counts}) \times (\text{Volume processed})}$$

Laboratory data

Complete blood counts were measured at baseline before G-CSF administration (pre-G-CSF), and before and after HPC collection (post-G-CSF, pre- and post-apheresis). In the patient group, data were collected on erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fibrinogen within a week of collection, as indicators of active inflammation and infection. Serum ferritin and red cell indices were analyzed as indicators

of iron deficiency. To determine whether red cell microcytosis was due to thalassemia trait or iron deficiency, a Combined Cell Index was calculated using the formula²⁴:

$$\frac{\text{Red Cell Distribution Width} \times 10^4}{(\text{Mean Corpuscular Hemoglobin}) \times (\text{Mean Corpuscular Volume})}$$

The higher the Combined Cell Index, the greater the likelihood of iron deficiency anemia. Iron replete blood donors have a mean Index of about 50, and iron deficient donors a significantly higher mean of about 70.¹⁹

Statistical analysis

The total mononuclear cell count (MNC) was calculated as the sum of lymphocyte and monocyte counts reported on the CBC differential. Summary statistics were calculated for all numerical results. Significance tests for comparisons between 2 groups were conducted with two-tailed, non-paired t tests. Values between more than 2 groups were compared using analysis of variance. Multivariate analyses were performed using stepwise forward logistic regression, based on parameters of significance in univariate analysis, using a commercial statistics program (JMP, Version 7 SAS Institute Inc., Cary, NC). Proportions between 2 groups were compared using a two-tailed Fisher exact test, and comparisons of proportions between multiple groups were made using chi-square analysis. Results are provided as the mean $\pm$ SD, unless otherwise stated. Only first collections of first mobilization cycles were included in the data analysis. Descriptive data were provided for repeat collections and mobilizations. The single adult SCID patient was included in the overall analysis but excluded from the age-group (>20 years) analysis.

Results

Subject demographics and CD34+ cell yield

Demographic characteristics for CGD and SCID subjects are shown in Table 1. The patients were predominantly Caucasian (CGD=78%; SCID=100%) and male (CGD=95%; SCID=100%). SCID were significantly younger (13 vs. 19 years, $p<0.05$) and weighed less than the CGD patients (33 vs. 46 kg, $p<0.04$). When analyzed by age groups, the CGD, SCID and control subjects under 20 years were similar in mean age, BMI and total G-CSF dose administered (these parameters were matched *a priori*). There were significantly more non-Caucasians and females among the controls. Laboratory data at baseline, including platelet and MNC counts, were similar in the 3 groups, with the exception of hemoglobin and ferritin levels (Table 2). The cases had lower hemoglobin levels than controls and ferritin levels were markedly lower among SCID than CGD subjects (20 vs. 124 mcg/L, $p<0.04$). Ferritin data were unavailable in controls. The mean peak circulating CD34+ cell count just prior to apheresis was significantly lower among cases than controls (58, 64 and 87 cells/uL in CGD, SCID and controls, respectively, $p=0.01$). In SCID but not CGD subjects, CD34+ cell apheresis collection efficiencies were significantly lower than in healthy controls (63, 40 and 57% in CGD, SCID and controls, respectively, $p=0.003$) (Figure 1).

In the adult cohort (>20 years old), CGD and control subjects were similar in age, sex and total G-CSF dose received (matched *a priori*) (Table 3). The two subject groups differed by race (more Caucasians in the CGD group) and weight (CGD subjects weighed less). Platelet counts were similar in the 2 groups. Hemoglobin and MNC counts were significantly lower in CGD than control subjects. Mean peak circulating pre-apheresis CD34+ cell counts were significantly lower among the CGD than control subjects (41 vs. 113 cells/uL, $p<0.001$). CD34+ apheresis collection efficiency was similar in the two groups (67 vs. 58%, $p=0.08$) (Figure 1).

CD34+ cell counts and G-CSF dose

In both the CGD and SCID patients, a higher G-CSF (filgrastim) dose was associated with a trend toward better mobilization (mean peak CD34+ cell count at G-CSF 10 mcg/kg = 40/uL and at G-CSF 16 mcg/kg = 63/uL, $p=0.07$), independent of age (Tables 4 and 5). Among controls, however, a higher G-CSF dose was associated with a significantly increased CD34+ cell mobilization only in adults (age > 20) (Table 5). Within each age category, when stratified by G-CSF dose, cases mobilized more poorly than controls at both 10 mcg/kg and 16 mcg/kg. As pre-apheresis circulating CD34+ cell count is the strongest predictor of CD34+ cell yield, the CD34+ cell yield per liter processed was also significantly lower among cases than controls. Four patients who received a G-CSF dose of 5 mcg/kg were excluded from this analysis and the 14 patients who received G-CSF plus plerixafor were analyzed separately. No major differences in adverse effect profile or severity were seen in the patients receiving G-CSF 10 vs. 16 mcg/kg/day.

CD34+ cell counts and plerixafor

Mean day 4 circulating CD34+ cell counts in the patients who received G-CSF plus plerixafor were significantly lower than day 4 counts in patients receiving G-CSF alone (13 ± 5 vs. 60 ± 45 cells/uL, $p=0.001$). Day 5 counts increased 2 to 11-fold in patients who received supplemental plerixafor, but did not change significantly in the G-CSF only group, such that day 5 counts were similar in both groups (72 ± 38 vs. 77 ± 47 cells/uL, $p<0.1$) (Figure 2). Grade 1-2 adverse effects due to stimulating agent were not routinely recorded; however, no grade 3-4 effects occurred with use of either G-CSF alone or in combination with plerixafor.³³

Analysis of parameters affecting CD34+ cell mobilization

In univariate regression analysis of immunodeficiency subjects who received only G-CSF (no plerixafor), higher CD34+ cell counts on day 4 and day 5 of mobilization were strongly correlated with greater subject weight, higher baseline platelet count, lower ESR at time of mobilization, higher total dose of G-CSF received and higher dose per kg. In the control population, higher peak CD34+ counts were similarly associated with greater weight, higher baseline platelets, and higher G-CSF dose, but in addition, male sex and higher baseline MNC count were also significant contributors. In multivariate regression analysis, only a higher baseline platelet count and lower ESR at time of mobilization remained significantly correlated with CD34+ cell mobilization in the immunodeficiency cohort, $p=0.01$ and 0.007 , respectively; total G-CSF dose no longer made a significant contribution. In contrast, in

multivariate analysis of the control group, male sex, higher baseline platelets and MNCs and higher total G-CSF dose remained significantly correlated with mobilization efficacy. Mean ESR was significantly higher among subjects with active infection and/or CGD-associated colitis compared to those without these events (37 vs 17 mm/hr, $p<0.001$) (Figure 3). Additionally, patients receiving higher G-CSF (16 mcg/kg) doses demonstrated significantly better peak mobilization compared to those receiving lower G-CSF (10 mcg/kg) doses within the low ESR (≤ 25 mm/hr) group (mean peak CD34+ count at G-CSF 10 mcg/kg = 46/uL and at G-CSF 16 mcg/kg = 79/uL, $p=0.05$). This difference by dose was not evident among those with elevated ESR (>25 mm/hr) (mean peak CD34+ count at G-CSF 10 mcg/kg = 33/uL and at G-CSF 16 mcg/kg = 43/uL, $p=0.55$). Although 22%, 26% and 37% of patients had clinical evidence of active CGD-associated colitis, fungal and bacterial infections, respectively, no correlation could be established between these clinical diagnoses and peak CD34+ cell counts. Other markers of inflammation such as CRP and fibrinogen were excluded from the statistical model due to insufficient data points. Interferon use in the peri-collection period among 7 CGD subjects, steroid use and antibiotic administration were poorly correlated with mobilization in the initial univariate analysis and were excluded from the final multivariate model.

Factors affecting CD34+ cell collection efficiency

Multivariate regression analysis of all subjects showed no effect of complete blood count parameters, including red cell indices and ESR, on CD34+ cell collection efficiency. Procedural parameters including device type, peripheral vs central venous access, inlet flow rate, and volume processed were similarly not correlated with collection efficiency. When the analysis was limited to SCID subjects, however, univariate regression demonstrated that higher red cell indices correlated strongly with better collection efficiencies (MCV: $R^2=0.77$, $p=0.004$; MCH: $R^2=0.94$, $p<0.001$; MCHC $R^2=0.7$, $p<0.007$) but not with hemoglobin. Consequently, a lower Combined Cell Index was correlated with significantly higher collection efficiencies ($R^2=0.8$; $p<0.002$). This association was not observed in CGD subjects (MCV: $R^2=0.005$, RBC: $R^2<0.001$, RDW: $R^2=0.04$, Combined Cell Index: $R^2=0.04$, $p>0.2$ for all four indices) (Figures 4 and 5). As noted above, mean ferritin was 20 vs 124 mcg/L in SCID vs CGD patients, respectively. Among control subjects with normal hemoglobin levels (ferritin not measured), red cell indices were moderately correlated with collection efficiency, with correlation coefficients similar to those previously reported¹³.

Multiple day collections

Thirty-three of 73 patients underwent repeat, consecutive collections; none received plerixafor. Mean pre-apheresis CD34+ cell count on the second day of apheresis, after an additional dose of G-CSF on day 6, was 17% lower than on day 5. In 4 patients who underwent a third consecutive HPC collection, mean pre-procedure CD34+ counts decreased another 35% (to 18.5 cells/uL) prior to the third procedure.

Discussion

We demonstrate, in the largest population of such subjects studied to date, that patients with CGD and SCID undergoing HPC collection for gene therapy trials are characterized by

significantly less robust CD34+ cell mobilization than healthy age- and weight-matched controls. In the CGD population, a 27 and 37% decrease in peak CD34 response to conventional doses of filgrastim was seen in the less than and greater than age 20 cohorts, respectively, compared with age-matched controls. In the SCID patients, a 21% decrease in peak CD34 response compared to controls was seen. Our control groups demonstrated a more vigorous peak CD34+ cell mobilization response, 81 cells/uL, than that previously reported in healthy donors,¹² possibly because of the younger age of the allogeneic donors included in this retrospective case-control study. Our patient population consisted largely of children and young adults with lower than average body mass indices due to chronic illness. We attempted to eliminate the possible confounding effects of age, BMI, sex, and G-CSF dose by selecting age, weight, sex, and G-CSF-dose matched controls. Our study also provides novel information on correlates of donor and procedural parameters that influence CD34+ cell mobilization responses in patients with CGD and SCID. Higher baseline platelet count, perhaps reflecting more healthy marrow reserve and common pathways of thrombopoiesis and HPC production^{12,25}, was strongly correlated with better CD34 mobilization, whereas higher ESR was associated with poor mobilization responses. In contrast to the findings in the control group, increased weight and BMI, and higher total G-CSF dose were not associated with better CD34 mobilization. Our data suggest that the confounding effect of systemic inflammation, as reflected in an elevated ESR, may have obscured the relationship between weight and total G-CSF dose, and CD34+ cell mobilization response.

The presence of active infection/inflammation at collection, as suggested by an elevated ESR, appeared to be a unique contributor to decreased CD34+ cell mobilization among CGD and SCID subjects, despite similar levels of G-CSF-associated leukocytosis in both patient and control groups. Studies of the role of inflammatory markers such as TNF- α , IFN- γ , IL-2, IL-6, and endogenous G-CSF in stem cell mobilization have been evaluated mainly in the setting of bone marrow failure syndromes such as aplastic anemia and yielded mixed results^{26,27}. Primary immune deficiency disorders may represent an informative setting in which to study pathways involved in cytokine-mediated marrow suppression. From a clinical perspective, collection yields may be improved if patients undergo mobilization after maximum control of active infections, when possible. As shown in our study, use of a higher G-CSF dose (16 rather than 10 mcg/kg) in the setting of persistent infection is only of modest benefit. It is possible that endogenous increases in G-CSF levels under these conditions may result in tachyphylaxis and inadequate response to exogenous G-CSF²⁰. Alternately, efficacy of G-CSF may be blunted by other pro-inflammatory cytokines such as TNF- α and IFN- γ . Exogenous administration of interferon-gamma did not appear to influence CD34+ cell mobilization in our study, although it has previously been reported to impair mobilization^{27,28}.

Preemptive administration of plerixafor in addition to filgrastim, based on circulating CD34+ count measurements obtained the day prior to apheresis, appears to be an effective approach to mitigating low CD34 mobilization responses in patients with active infections. Plerixafor is approved for use as an adjunct to filgrastim in HPC mobilization in patients with multiple myeloma and lymphoma and is associated with engraftment pace and durability similar to that seen following filgrastim mobilization alone, despite gene-

expression profiling differences in cells mobilized with filgrastim vs plerixafor alone^{29,30}. The effect of plerixafor on gene transduction efficiencies, engraftment and transgene expression has not been fully characterized. Initial investigations suggest that progenitor cells collected with use of plerixafor have unique transduction and gene expression characteristics, especially with use of newer lentiviral vector protocols³¹.

In addition to poor mobilization, we noted markedly reduced CD34+ cell collection efficiencies in SCID but not in CGD subjects. We have previously noted that low MCV values due to iron deficiency can markedly impair the efficacy of mononuclear cell collection by apheresis even in subjects who mobilize well. Variability in red cell size and density, as evidenced by MCV and MCH values, can affect optimal optical interpretation and automated sampling of the white blood cell layer by the apheresis device. The SCID subjects in our study had significantly lower hemoglobin and MCV levels than healthy controls, had profoundly low ferritin levels, and had an elevated Combined Cell Index in a range characteristic of iron deficiency in blood donors.²⁴ They had iron deficiency anemia, most likely due to frequent phlebotomies to get samples for diagnostic testing, gastrointestinal blood losses due to chronic diarrhea, poor nutrition with inability to compensate for these losses through dietary means, and inadequate parenteral iron supplementation. In contrast, the mean ferritin level in the CGD group was elevated, likely due to chronic inflammation, making it more difficult to confirm the diagnosis of iron deficiency by laboratory parameters alone. Based on individual and combined red cell indices, the CGD group had a high prevalence of iron deficiency as well. It is likely that vigorous, timely parenteral iron replacement in SCID and CGD subjects with microcytosis due to iron deficiency, and closer attention to technical adjustments in sampling the white cell layer during leukapheresis, would significantly improve apheresis collection efficiencies. We have seen this in our practice, particularly in the CGD cohort, which was characterized by normal CD34+ cell collection efficiency as a whole.

The availability of a pre-apheresis circulating CD34+ cell count result within a few hours of starting the HPC apheresis procedure allowed staff to adjust the volume processed according to the count and the targeted collection dose. Despite use of larger volumes processed in patients with poor CD34 mobilization responses (as many as 8 blood volumes per procedure), nearly 50% of patients who received filgrastim alone required more than one procedure to achieve the targeted number of CD34+ cells, 5 million per kg, with which to start *in vitro* gene therapy manipulations. In those patients who underwent multiple consecutive daily collections, pre-apheresis CD34+ counts decreased with each collection, despite additional filgrastim doses. The majority of patients achieved targeted cell dose collections after the second procedure.

In summary, cytokine-assisted mobilization of CD34+ cells from the marrow into the blood in patients with primary immunodeficiency disorders is significantly impaired compared with that in healthy allogeneic donors. Selective marrow suppression by inflammatory signals or possibly diminished marrow CD34+ cell reserve due to stem cell utilization for repair in these chronic conditions may be responsible for the poor mobilization responses. Tachyphylaxis to exogenous cytokine stimulation due to increased endogenous G-CSF secretion during active inflammation may further impair the mobilization response. A low

baseline platelet count, high erythrocyte sedimentation rate, and the presence of microcytosis due to iron deficiency can be used to predict those subjects at greatest risk of poor mobilization. The two most effective strategies to mitigate poor mobilization and inefficient apheresis collection in this population include prospective correction of iron deficiency and careful monitoring of day-4 pre-apheresis CD34+ cell counts to determine which patients would benefit from supplemental plerixafor. Strategies used in other situations, such as a higher filgrastim dose, were only modestly effective in this population. Last, vigorous prospective control of infection/inflammatory states, which act as marrow stressors, may improve mobilization.

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References

1. Buckley RH. The multiple causes of human SCID. *J Clin Invest.* 2004; 114:1409–11. [PubMed: 15545990]
2. Roos D, Kuhns DB, Maddalena A, Roesler J, Lopez JA, Ariga T, Avcin T, de Boer M, Bustamante J, Condino-Neto A, Di Matteo G, He J, Hill HR, Holland SM, Kannengiesser C, Koker MY, Kondratenko I, van Leeuwen K, Malech HL, Marodi L, Nunoi H, Stasia MJ, Ventura AM, Witwer CT, Wolach B, Gallin JI. Hematologically important mutations: X-linked chronic granulomatous disease (third update). *Blood Cells Mol Dis.* 2010; 45:246–65. [PubMed: 20729109]
3. Segal BH, Veys P, Malech H, Cowan MJ. Chronic granulomatous disease: lessons from a rare disorder. *Biol Blood Marrow Transplant.* 2011; 17:S123–31. [PubMed: 21195301]
4. Rivat C, Santilli G, Gaspar HB, Thrasher AJ. Gene therapy for primary immunodeficiencies. *Hum Gene Ther.* 2012; 23:668–75. [PubMed: 22691036]
5. Cavazzana-Calvo M, Fischer A, Hacein-Bey-Abina S, Aiuti A. Gene therapy for primary immunodeficiencies: Part 1. *Curr Opin Immunol.* 2012; 24:580–4. [PubMed: 22981681]
6. Neben S, Marcus K, Mauch P. Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor. *Blood.* 1993; 81:1960–7. [PubMed: 7681707]
7. Motabi IH, DiPersio JF. Advances in stem cell mobilization. *Blood Rev.* 2012; 26:267–78. [PubMed: 23068307]
8. Sekhsaria S, Fleisher TA, Vowells S, Brown M, Miller J, Gordon I, Blaese RM, Dunbar CE, Leitman S, Malech HL. Granulocyte colony-stimulating factor recruitment of CD34+ progenitors to peripheral blood: impaired mobilization in chronic granulomatous disease and adenosine deaminase--deficient severe combined immunodeficiency disease patients. *Blood.* 1996; 88:1104–12. [PubMed: 8704221]
9. Cassel A, Cottler-Fox M, Doren S, Dunbar CE. Retroviral-mediated gene transfer into CD34-enriched human peripheral blood stem cells. *Exp Hematol.* 1993; 21:585–91. [PubMed: 7681785]
10. Li F, Linton GF, Sekhsaria S, Whiting-Theobald N, Katkin JP, Gallin JI, Malech HL. CD34+ peripheral blood progenitors as a target for genetic correction of the two flavocytochrome b558 defective forms of chronic granulomatous disease. *Blood.* 1994; 84:53–8. [PubMed: 7517218]

11. Aiuti A, Roncarolo MG. Ten years of gene therapy for primary immune deficiencies. *Hematology Am Soc Hematol Educ Program*. 2009;682–9. [PubMed: 20008254]
12. Vasu S, Leitman SF, Tisdale JF, Hsieh MM, Childs RW, Barrett AJ, Fowler DH, Bishop MR, Kang EM, Malech HL, Dunbar CE, Khoo HM, Wesley R, Yau YY, Bolan CD. Donor demographic and laboratory predictors of allogeneic peripheral blood stem cell mobilization in an ethnically diverse population. *Blood*. 2008; 112:2092–100. [PubMed: 18523146]
13. Wang TF, Chen SH, Yang SH, Su YC, Chu SC, Li DK. Poor harvest of peripheral blood stem cell in donors with microcytic red blood cells. *Transfusion*. 2013; 53:91–5. [PubMed: 22738220]
14. Chow S, Lazo-Langner A, Ormond G, Howson-Jan K, Xenocostas A. Predictors of unsuccessful mobilization with granulocyte colony-stimulating factor alone in patients undergoing autologous hematopoietic stem cell transplantation. *J Clin Apher*. 2013
15. Kuittinen T, Nousiainen T, Halonen P, Mahlamäki E, Jantunen E. Prediction of mobilisation failure in patients with non-Hodgkin's lymphoma. *Bone Marrow Transplant*. 2004; 33:907–12. [PubMed: 15034543]
16. Popat U, Saliba R, Thandi R, Hosing C, Qazilbash M, Anderlini P, Shpall E, McMannis J, Korbling M, Alousi A, Andersson B, Nieto Y, Kebriaei P, Khouri I, de Lima M, Weber D, Thomas S, Wang M, Jones R, Champlin R, Giral S. Impairment of filgrastim-induced stem cell mobilization after prior lenalidomide in patients with multiple myeloma. *Biol Blood Marrow Transplant*. 2009; 15:718–23. [PubMed: 19450756]
17. Tournilhac O, Cazin B, Lepretre S, Divine M, Maloum K, Delmer A, Grosbois B, Feugier P, Maloisel F, Villard F, Villemagne B, Bastit D, Belhadj K, Azar N, Michallet M, Manhes G, Travade P. Impact of frontline fludarabine and cyclophosphamide combined treatment on peripheral blood stem cell mobilization in B-cell chronic lymphocytic leukemia. *Blood*. 2004; 103:363–5. [PubMed: 12969985]
18. Flomenberg N, Devine SM, Dipersio JF, Liesveld JL, McCarty JM, Rowley SD, Vesole DH, Badel K, Calandra G. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood*. 2005; 106:1867–74. [PubMed: 15890685]
19. Jantunen E, Lemoli RM. Preemptive use of plerixafor in difficult-to-mobilize patients: an emerging concept. *Transfusion*. 2012; 52:906–14. [PubMed: 21981351]
20. Quinton LJ, Nelson S, Boe DM, Zhang P, Zhong Q, Kolls JK, Bagby GJ. The granulocyte colony-stimulating factor response after intrapulmonary and systemic bacterial challenges. *J Infect Dis*. 2002; 185:1476–82. [PubMed: 11992284]
21. Kang EM, Malech HL. Gene therapy for chronic granulomatous disease. *Methods Enzymol*. 2012; 507:125–54. [PubMed: 22365772]
22. Bolan CD, Carter CS, Wesley RA, Yau YY, Barrett AJ, Childs RW, Read EJ, Leitman SF. Prospective evaluation of cell kinetics, yields and donor experiences during a single large-volume apheresis versus two smaller volume consecutive day collections of allogeneic peripheral blood stem cells. *Br J Haematol*. 2003; 120:801–7. [PubMed: 12614213]
23. Bolan CD, Cecco SA, Wesley RA, Horne M, Yau YY, Remaley AT, Childs RW, Barrett AJ, Rehak NN, Leitman SF. Controlled study of citrate effects and response to i.v. calcium administration during allogeneic peripheral blood progenitor cell donation. *Transfusion*. 2002; 42:935–46. [PubMed: 12375668]
24. Boulton F. Evidence-based criteria for the care and selection of blood donors, with some comments on the relationship to blood supply, and emphasis on the management of donation-induced iron depletion. *Transfus Med*. 2008; 18:13–27. [PubMed: 18279189]
25. Ott MG, Merget-Millitzer H, Ottmann OG, Martin H, Bruggenolte N, Bialek H, Seger R, Hossle JP, Hoelzer D, Grez M. Mobilization and transduction of CD34(+) peripheral blood stem cells in patients with X-linked chronic granulomatous disease. *J Hematother Stem Cell Res*. 2002; 11:683–94. [PubMed: 12201957]
26. King KY, Goodell MA. Inflammatory modulation of HSCs: viewing the HSC as a foundation for the immune response. *Nat Rev Immunol*. 2011; 11:685–92. [PubMed: 21904387]
27. Maciejewski J, Salleri C, Anderson S, Young NS. Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates

- cytokine-mediated hematopoietic suppression in vitro. *Blood*. 1995; 85:3183–90. [PubMed: 7538820]
28. Sato T, Selleri C, Anderson S, Young NS, Maciejewski JP. Expression and modulation of cellular receptors for interferon-gamma, tumour necrosis factor, and Fas on human bone marrow CD34+ cells. *Br J Haematol*. 1997; 97:356–65. [PubMed: 9163602]
 29. Jantunen E, Kvalheim G. Mobilization strategies in hard-to-mobilize patients with lymphoid malignancies. *Eur J Haematol*. 2010; 85:463–71. [PubMed: 20738393]
 30. Donahue RE, Jin P, Bonifacino AC, Metzger ME, Ren J, Wang E, Stroncek DF. Plerixafor (AMD3100) and granulocyte colony-stimulating factor (G-CSF) mobilize different CD34+ cell populations based on global gene and microRNA expression signatures. *Blood*. 2009; 114:2530–41. [PubMed: 19602709]
 31. Uchida N, Bonifacino A, Krouse AE, Metzger ME, Csako G, Lee-Stroka A, Fasano RM, Leitman SF, Mattapallil JJ, Hsieh MM, Tisdale JF, Donahue RE. Accelerated lymphocyte reconstitution and long-term recovery after transplantation of lentiviral-transduced rhesus CD34+ cells mobilized by G-CSF and plerixafor. *Exp Hematol*. 2011; 39:795–805. [PubMed: 21549175]
 32. Fowler CJ, Yau YY, Bolan CD, Leitman SF. Analysis of donor demographic and laboratory parameters affecting CD34+ cell mobilization in healthy allogeneic pediatric peripheral blood stem cell (PBSC) donors. *Blood*. 2010; 116(21):2259A.
 33. [Accessed April 17, 2014] Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. USDHHS/NIH/NCI. http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf Published: May 28, 2009 (v4.03: June 14, 2010)

Figure 1.

Panels A and B show CD34+ cell mobilization (CD34+ cell count immediately prior to apheresis) in the CGD and SCID patients and matched controls by age groups ≤ 20 and >20 years, respectively. Patients in both age groups had significantly lower mobilization than controls. Panels C and D show CD34+ cell collection efficiencies among patients and matched controls stratified by the two age groups, respectively. In the ≤ 20 age group, SCID patients had significantly lower apheresis collection efficiencies than CGD patients or controls. Rectangular plots show the middle 50% of patients (25th to 75th percentiles and median). Diamond plots show the mean $\pm$ 95% confidence intervals.

Figure 2. Effect of adding plerixafor to G-CSF on CD34+ cell counts

(A) Day 4 CD34+ cell counts were significantly lower in patients receiving plerixafor plus G-CSF compared to those receiving G-CSF alone ($p<0.0001$). (B) Day 5 (pre-apheresis) CD34+ counts increased significantly in the plerixafor plus G-CSF group ($p<0.0001$) and were now similar to the counts in the group receiving G-CSF alone.

Figure 3. Correlation of erythrocyte sedimentation rate (ESR) with the presence of infection and/or inflammation (A) and CD34+ cell counts (B)

Subjects with active infection and/or CGD-associated colitis had significantly higher mean ESR compared to those without these events (38 vs 17 mm/hr, $p=0.0007$). ESR in turn was inversely related to mean peak pre-apheresis circulating CD34+ cell counts ($p=0.03$).

Figure 4. Effect of iron deficiency on CD34+ cell collection efficiency in SCID subjects
 Panels A-D show that red cell indices (x-axes) on the day of collection, including MCV, MCH, and MCHC, and the Combined Cell Index, all correlated significantly with CD34+ cell collection efficiency (y-axis) among SCID patients, as described in the text. Combined Cell Index = $RDW \times 10^4 / (MCH \times MCV)$. The higher the Combined Cell Index, the greater the likelihood of iron deficiency anemia.

Figure 5. Lack of effect of iron deficiency on collection efficiency in CGD subjects

Panels A-B show the absence of any correlation between CD34+ cell collection efficiency (y-axis) and red cell parameters (x-axes), including MCV and the Combined Cell Index in CGD patients. The same was true for other red cell indices including RDW, MCH and MCHC.

Table 1
Patient demographics

Characteristic [*]	CGD	SCID [†]	p-value
N	63	10	----
Male, n (%)	60 (95)	10 (100)	0.48
Age (years)	19 ± 10	13 ± 6	0.02
Race, Caucasian %	78	100	0.74
Weight (kg)	46 ± 20	33 ± 12	0.01
Height (M)	1.49 ± 0.3	1.36 ± 0.2	0.11
BMI	20 ± 5	18 ± 2	0.05
Peak CD34+ count per uL	51 ± 37	66 ± 55	0.43
CD34+ × 10 ⁶ yield/liter processed	22 ± 15	17 ± 22	0.53

^{*} Only first collections of first mobilization cycles were included in the data analysis. Descriptive data were provided for repeat collections and mobilizations. The single adult SCID patient >20 years was included in the overall analysis.

[†] Nine patients had x-linked SCID (SCID-X1) and one had RAG1-SCID.

Table 2
Patient and control demographic and laboratory data (age 20 years)

Characteristic*	CGD	SCID	Controls	p-value
N	38	9	47	----
Male, %	97	100	66	<0.001
Age (years)	11.7 ± 5.4	11.7 ± 5.2	11.5 ± 4.6	0.97
Race, Caucasian %	74	100	43	0.02
Weight (kg)	34.7 ± 15.6	31.3 ± 11.2	40.2 ± 18.2	0.18
Height (M)	1.38 ± 0.3	1.32 ± 0.2	1.45 ± 0.2	0.25
BMI	17.5 ± 3.0	18.5 ± 1.8	17.9 ± 3.3	0.87
% requiring central venous catheters	95	100	66	0.001
Volume processed (L)	11.5 ± 5.8	12.7 ± 5.7	12.6 ± 5.5	0.68
Baseline hemoglobin (g/dL)	11.9 ± 1.6	12.4 ± 1.3	13.7 ± 1.7	<0.0001
Baseline RBC ($\times 10^6$ /mcL)	4.53 ± 0.6	4.91 ± 0.4	4.83 ± 0.6	0.05
Baseline MCV (fL)	79.9 ± 6.8	77.0 ± 8.8	84.1 ± 6.0	0.002
Baseline RDW (CV %)	15.4 ± 2.5	15.0 ± 2.2	12.6 ± 2.1	<0.0001
Baseline platelet count ($\times 10^9$ /L)	295 ± 97	273 ± 137	299 ± 80	0.7
Baseline MNC count ($\times 10^9$ /L)	2.43 ± 0.9	2.59 ± 1.3	2.91 ± 0.7	0.06
Total G-CSF dose (mcg)	413 ± 192	412 ± 218	430 ± 207	0.92
Additional plerixafor, n	7	1	0	
Peak circulating CD34+ count/uL	58.0 ± 37	64.3 ± 58	87 ± 46	0.01
CD34+ $\times 10^6$ yield/liter processed	24.3 ± 15.6	16 ± 22.8	34.4 ± 19.9	0.006
CD34+ collection efficiency (%)	63.2 ± 12	39.8 ± 24	56.9 ± 15	0.003
Ferritin [†] mcg/L)	124 ± 226	20 ± 27	--	0.04

* Cases and controls matched by age and weight in a 1:1 ratio.

[†] Ferritin data were unavailable in controls. Reference ranges: hemoglobin 13.7-17.5 (<age 12, 10.7-13.4) g/dL in males, 11.2-15.7 (<age 12, 10.6-13.2) g/dL in females; RBC 4.63-6.08 $\times 10^6$ /mcL in males, 3.93-5.22 $\times 10^6$ /mcL in females; MCV 79.0-92.2 fL; RDW 11.6-14.4%; platelet count 161-347 $\times 10^9$ /L in males, 173-369 $\times 10^9$ /L in females; ferritin 18-370 mcg/L in males, 9-120 mcg/L in females. None of the ranges changed substantially during the study period.

Table 3
Patient and control demographic and laboratory data (age > 20 years)

Characteristic *	CGD	Controls	p-value
N	25	50	-----
Male, %	92	94	0.74
Age (years)	29 ± 5.9	29 ± 6.8	0.88
Race, Caucasian %	84	70	0.04
Weight (kg)	63 ± 12	70 ± 9	0.02
Height (M)	1.69 ± 0.1	1.73 ± 0.1	0.08
BMI	22 ± 3	23 ± 2	0.08
% requiring central venous catheters	84	6	< 0.001
Volume processed (L)	18.7 ± 4.7	18.0 ± 4.8	0.56
Baseline hemoglobin (g/dL)	12.8 ± 2	15.0 ± 1	<0.0001
Baseline RBC (× 10 ⁶ /mcL)	4.53 ± 0.7	5.06 ± 0.4	0.0001
Baseline MCV (fL)	86.9 ± 6.4	87.1 ± 5.7	0.87
Baseline RDW (CV %)	13.9 ± 2.1	12.2 ± 1.1	<0.0001
Baseline platelets (× 10 ⁹ /L)	239 ± 118	237 ± 49	0.92
Baseline MNC count (× 10 ⁹ /L)	1.7 ± 0.8	2.5 ± 0.7	<0.001
Total G-CSF dose (mcg)	761 ± 266	837 ± 189	0.21
Additional plerixafor, n	4	0	
Peak circulating CD34+ count/uL	41 ± 34	113 ± 74	<0.001
CD34+ × 10 ⁶ yield/liter processed	18 ± 14	45 ± 27	<0.001
CD34+ efficiency (%)	67 ± 17	58 ± 16	0.08

* Only one SCID patient was >20 yrs and was not included in analysis; cases and controls were matched by age, weight and G-CSF dose (mcg/kg) in a 1:2 ratio. Reference ranges: hemoglobin 13.7-17.5 g/dL in males, 11.2-15.7 g/dL in females; RBC 4.63-6.08 × 10⁶/mcL in males, 3.93-5.22 × 10⁶/mcL in females; MCV 79.0-92.2 fL; RDW 11.6-14.4%; platelet count 161-347 × 10⁹/L in males, 173-369 × 10⁹/L in females; ferritin 18-370 mcg/L in males, 9-120 mcg/L in females. None of the ranges changed substantially during the study period.

Table 4
Effect of G-CSF dose on peak blood CD 34+ cell counts and CD 34+ apheresis yields
patients aged 20 years

Administered Drug & Dose *	CGD	SCID	Controls	p-value
G-CSF 10 mcg/kg				
N	17	5	31	
Weight (kg)	37±15	26 ± 8	40 ± 19	0.24
Total G-CSF dose (mcg)	366 ± 151	267 ± 81	396 ± 196	0.31
Peak CD34+ cell count per uL	49 ± 26	51 ± 31	93 ± 51	0.003
CD34+× 10 ⁶ yield/liter processed	22 ± 13	11 ± 9	36 ± 21	0.004
G-CSF 16 mcg/kg				
N	12	3	6	
Weight (kg)	31 ± 14	37 ± 15	39 ± 17	0.53
Total G-CSF dose (mcg)	491 ± 221	599 ± 243	597 ± 252	0.59
Peak CD34+ cell count per uL	55 ± 39	84 ± 102	70 ± 18	0.59
CD34+× 10 ⁶ yield/liter processed	23 ± 16	26 ± 41	25 ± 8	0.95

* Two patients who received a G-CSF dose of 5 mcg/kg were excluded from this analysis. 8 patients who received G-CSF plus plerixafor were analyzed separately.

Table 5
Effect of G-CSF dose on peak blood CD 34+ cell counts and CD 34+ apheresis yields in patients aged > 20 years

Administered drug & dose*	CGD	Controls	p-value
G-CSF 10 mcg/kg			
N	13	26	
Weight	64 ± 13	71 ± 7	0.098
Total G-CSF dose	648 ± 126	731 ± 95	0.05
Peak CD34+ cell count per uL	24 ± 20	86 ± 55	<0.0001
CD34+ × 10 ⁶ yield/liter processed	13 ± 12	36 ± 19	<0.0001
G-CSF 16 mcg/kg			
N	6	12	
Weight	68 ± 9	72 ± 9	0.45
Total G-CSF dose	1082 ± 128	1060 ± 182	0.77
Peak CD34+ cell count per uL	60 ± 51	171 ± 79	0.003
CD34+ × 10 ⁶ yield/liter processed	23 ± 18	62 ± 30	0.003

* Two patients who received 5 mcg/kg of G-CSF were excluded from analysis. 5 patients who received G-CSF plus plerixafor were analyzed separately.