Hyperinflammation in patients with chronic granulomatous disease leads to impairment of hematopoietic stem cell functions

Maren Weisser, PhD,[a] Uta M. Demel, MSc,[b,c] Stefan Stein, PhD,[a] Linping Chen-Wichmann, PhD,[a]* Fabien Touzot, MD, PhD,[d,e] Giorgia Santilli, PhD,[f] Stefanie Sujer, MSc,[b] Christian Brendel, PhD,[a]† Ulrich Siler, PhD,[g] Marina Cavazzana, MD, PhD,[d,e] Adrian J. Thrasher, MD, PhD,[f] Janine Reichenbach, MD,[g] Marieke A. G. Essers, PhD,[b,c] Joachim Schwäble, MD,[a,h]‡§ and Manuel Grez, PhD[a]§

Frankfurt and Heidelberg, Germany, Paris, France, London, United Kingdom, and Zurich, Switzerland

Background: Defects in phagocytic nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) function cause chronic granulomatous disease (CGD), a primary immunodeficiency characterized by dysfunctional microbicidal activity and chronic inflammation.

Objective: We sought to study the effect of chronic inflammation on the hematopoietic compartment in patients and mice with X-linked chronic granulomatous disease (X-CGD).

Methods: We used immunostaining and functional analyses to study the hematopoietic compartment in patients with CGD.

Results: An analysis of bone marrow cells from patients and mice with X-CGD revealed a dysregulated hematopoiesis characterized by increased numbers of hematopoietic progenitor cells (HPCs) at the expense of repopulating hematopoietic stem cells (HSCs). In patients with X-CGD, there was a clear reduction in the proportion of HSCs in bone marrow and peripheral blood, and they were also more rapidly exhausted after *in vitro* culture. In mice with X-CGD, increased cycling of HSCs, expansion of HPCs, and impaired long-term engraftment capacity were found to be associated with high concentrations of proinflammatory cytokines, including IL-1β. Treatment of wild-type mice with IL-1β induced enhanced cell-cycle entry of HSCs, expansion of HPCs, and defects in long-term engraftment, mimicking the effects observed in mice with X-CGD. Inhibition of cytokine signaling in mice with X-CGD reduced HPC numbers but had only minor effects on the repopulating ability of HSCs.

Conclusions: Persistent chronic inflammation in patients with CGD is associated with hematopoietic proliferative stress, leading to a decrease in the functional activity of HSCs. Our observations have clinical implications for the development of successful autologous cell therapy approaches. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Chronic granulomatous disease, hyperinflammation, hematopoietic stem cell, dysfunctional hematopoiesis, competitive repopulation assay, engraftment defect, cell cycle, IL-1β, anakinra, gene therapy*

Chronic granulomatous disease (CGD) is a rare inherited primary immunodeficiency characterized by defective antimicrobicidal activity of phagocytes, resulting in increased susceptibility to recurrent and life-threatening infections.[1-6] In addition, patients with CGD often display augmented inflammatory responses, even in the absence of infectious agents (sterile inflammation), leading to granuloma formation and inflammatory bowel disease.[7-10] Approximately two thirds of all patients with CGD have mutations within the X-linked *CYBB* gene (X-linked chronic granulomatous disease [X-CGD]), encoding the gp91^{phox} subunit of nicotinamide adenine dinucleotide phosphate oxidase 2. Mice with X-CGD faithfully reproduce the pathology observed in patients with X-CGD.[7,11]

From [a]the Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt; [b]Junior Research Group "Hematopoietic Stem Cells and Stress," German Cancer Research Center (DKFZ), INF280, Heidelberg; [c]the Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM), INF280, Heidelberg; [d]the Biotherapy Department, Necker Children's Hospital, Assistance Publique-Hôpitaux de Paris; [e]Paris Descartes–Sorbonne Paris Cité University, Imagine Institute, Paris; [f]the Section of Molecular and Cellular Immunology, UCL Institute of Child Health, London; [g]the Division of Immunology, University Children's Hospital, and Children's Research Centre Zürich; and [h]the Department of Medicine, Hematology/Oncology, Goethe University, Frankfurt.

*Linping Chen-Wichmann, PhD, is currently affiliated with Experimental Cell Therapy and Hematology, Department of Transfusion Medicine, Cell Therapy and Haemostaseology, Ludwig-Maximilians-Universität Hospital Munich, Munich, Germany.

†Christian Brendel, PhD, is currently affiliated with the Division of Hematology/Oncology, Boston Children's Hospital, Boston, Mass.

‡Joachim Schwäble, MD, is currently affiliated with the German Red Cross Blood Donor Service, Baden-Württemberg–Hessen, and the Institute for Transfusion Medicine and Immunohematology of Goethe University, Frankfurt, Germany.

§These authors contributed equally to this work.

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Corresponding author: Manuel Grez, PhD, Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Paul-Ehrlich-Str 42-44, 60596 Frankfurt, Germany. E-mail: grez@gsh.uni-frankfurt.de.

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Abbreviations used
BM: Bone marrow
BrdU: 5-Bromo-2′-deoxyuridine
CFU: Colony-forming unit
CGD: Chronic granulomatous disease
CMP: Common myeloid progenitor
CRU: Competitive repopulating unit
eGFP: Enhanced green fluorescent protein
5-FU: 5-Fluorouracil
G-CSF: Granulocyte colony-stimulating factor
GMP: Granulocyte-monocyte progenitor
HPC: Hematopoietic progenitor cell
HSC: Hematopoietic stem cell
LSK: $Lin^-Sca\text{-}1^+c\text{-}Kit^+$
MEP: Megakaryocyte-erythroid progenitor
MNC: Mononuclear cell
MyD88: Myeloid differentiation response gene 88
PB: Peripheral blood
tBFP: Turbo blue fluorescent protein
TPO: Thrombopoietin
WT: Wild-type
X-CGD: X-linked chronic granulomatous disease

CGD can be cured by using allogeneic hematopoietic stem cell (HSC) transplantation, which has been particularly successful in patients with a fully HLA-matched donor in combination with reduced-intensity conditioning.[12,13] Despite the use of advanced HSC transplantation protocols, cases of low donor chimerism, graft-versus-host disease, and graft rejection have been observed.[14-16] Thus for those patients without suitable HSC donors and for those with critical health conditions, alternative treatment options beyond the standard of care are still required. The transplantation of autologous gene-modified cells is an alternative for the treatment of CGD and has been implemented predominantly in patients with X-CGD.[17-20] These clinical trials have provided evidence that gene therapy can offer significant clinical benefit to patients with CGD. However, most of these patients lacked significant long-term engraftment of transduced cells.[21,22] Although many factors might have influenced the engraftment potential of $CD34^+$ cells during cell processing, alterations in numbers, fitness, or both of HSCs in the CGD inflammatory background could also contribute to the engraftment deficit. Therefore we analyzed the influence of chronic inflammation on the HSC compartment in patients with X-CGD. We found a profound defect in HSC content, activity, or both in the bone marrow (BM) of both patients and mice with X-CGD.

The hematopoietic defects observed in mice with X-CGD were mainly mediated by IL-1β, and inhibition of IL-1β signaling suppressed hematopoietic progenitor cell (HPC) expansion in mice with X-CGD but did not reverse the functional defects in HSC activity.

Thus chronic inflammation in patients with CGD leads to a dysregulated hematopoietic homeostasis. Our findings might not be limited to CGD but could also be relevant to other pathologies with sustained chronic inflammation and autoinflammatory processes.[23-27]

METHODS

Patient material

BM and granulocyte colony-stimulating factor (G-CSF)–mobilized PBMCs were obtained from patients with X-CGD (BM: n = 3; median, 5.5 years old; range, 0.7-8 years old; peripheral blood [PB]: n = 4, median, 10 years old; range, 4-17 years old) and healthy control subjects (BM: n = 4; median, 11 years old; range, 3-14 years old; PB: n = 4; median, 29 years old; range, 25-37 years old) after informed consent and approval by the local ethics committee.

Mice

B6.129S6-*Cybb*^{tm1Din}/J (mice with X-CGD, CD45.2), C57BL/6J (wild-type [WT] mice, CD45.2), and B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ (CD45.1) mice were obtained from Charles River Laboratories (Sulzfeld, Germany). Health monitoring was conducted regularly by MFD Diagnostics (Wendelsheim, Germany), according to Federation for Laboratory Animal Science Associations guidelines. Animals with overt infections were not included in the study. Male littermates were used for experiments, unless stated otherwise. B6.Cg myeloid differentiation response gene 88 $(MyD88)^{tm1Aki}$ mice (MyD88-deficient mice)[28] were bred at the Animal Facility of the German Cancer Research Center. Animal experiments were approved by the regional council (Regierungspräsidium Darmstadt, Germany).

HSC assays and cytokine arrays

All assays, including HSC assays, cell-cycle analysis, cytokine stimulation experiments, lentiviral transductions, transplantation assays, competitive repopulation assays, cytokine arrays, and cell isolation, analysis, and sorting, were done according to standard protocols. Detailed methods are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analysis

Statistical significance was calculated by using unpaired 2-tailed *t* tests, 1-factor ANOVA with the Dunnett multiple comparison test, 2-factor ANOVA with Bonferroni posttests, and 3-factor ANOVA. Competitive repopulating unit (CRU) frequency was calculated with L-Calc Limiting Dilution Analysis Software (STEMSOFT, Version 1.1; STEMSOFT software, Vancouver, British Columbia, Canada). The overall test for differences in CRU frequencies between X-CGD and WT was performed with ELDA: Extreme Limiting Dilution Analysis (http://bioinf.wehi.edu.au/software/elda/index.html). Scatter plots indicate means ± SDs. Bar diagrams show means + SDs.

RESULTS

Increased numbers of HPCs in mice with X-CGD

We analyzed the hematopoietic compartment of mice with X-CGD and found significantly ($P < .05$-$.01$) increased levels of $Lin^-Sca\text{-}1^+c\text{-}Kit^+$ (LSK) cells, myeloid progenitors, common myeloid progenitors (CMPs), and granulocyte-monocyte progenitors (GMPs) in the BM, whereas the percentages of $Lin^-Sca\text{-}1^+c\text{-}Kit^+$ $CD150^+CD48^-$ (LSK SLAM), lineage-negative (Lin^-), and megakaryocyte-erythroid progenitor (MEP) cells were unchanged (Fig 1, *A-E*, and see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org, and data not shown). BM cells from mice with X-CGD generated higher numbers of colonies derived from GMPs than cells from WT mice (see Fig E1, *C*). Numbers of LSK cells and colony-forming units (CFUs) derived from the spleen and PB were higher in mice with X-CGD (Fig 1, *F* and *G*, and see Fig E1, *D*). Cell-cycle analysis revealed that the frequency of quiescent HSCs in BM from mice with X-CGD was significantly ($P < .01$) reduced