HHS Public Access

Author manuscript

Inflammation. Author manuscript; available in PMC 2024 January 30.

Published in final edited form as:

Inflammation. 2021 February ; 44(1): 270–277. doi:10.1007/s10753-020-01330-w.

Low Plasma Gelsolin Concentrations in Chronic Granulomatous Disease

John Audley[1], **Emily F. Gliniewicz**[1], **Kol A. Zarember**[1], **Hanna S. Hong**[1], **Gal Wald**[1], **Douglas B. Kuhns**[2], **Elizabeth Kang**[1], **Harry L. Malech**[1], **Anthony F. Suffredini**[3], **Robert J. Noveck**[4], **Mark J. Dinubile**[5], **Susan L. Levinson**[5], **Thomas P. Stossel**[5], **John I. Gallin**[1,6]

[1]Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

[2]Neutrophil Monitoring Laboratory, Frederick National Laboratory for Cancer Research, Frederick, MD, USA

[3]Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD, USA

[4]Duke Clinical Research Unit, Duke University School of Medicine, Durham, NC, USA

[5]BioAegis Therapeutics, Inc., North Brunswick, NJ, USA

Abstract

Plasma gelsolin (pGSN) is the secreted isoform of an intracellular actin remodeling protein found in high concentrations in human plasma. Clinical studies demonstrate reduced pGSN concentrations in several disease states, including severe trauma, burns, and sepsis. Markedly decreased pGSN concentrations in these conditions precede and predict adverse clinical outcomes. In this study, we measured pGSN in patients with chronic granulomatous disease (CGD), a primary immunodeficiency characterized by recurrent infections and dysregulated inflammation. pGSN was quantified using a sandwich ELISA in plasma from healthy volunteers, clinically stable CGD patients, and X-linked CGD carriers and in sera from 12 CGD patients undergoing bone marrow transplantation. pGSN was also quantified in healthy volunteers challenged with intravenous endotoxin. pGSN concentrations were lower in CGD patients without active infection or systemic inflammation compared with healthy control subjects. In CGD patients undergoing bone marrow transplantation, pGSN concentrations increased significantly following successful transplant. X-linked carriers of CGD had normal pGSN. Despite reduction of pGSN in CGD patients, we did not detect significant changes in pGSN over 24 h following challenge of healthy volunteers with intravenous endotoxin (4 ng/kg) that elicited a febrile response. We describe, for the first time, significantly lower pGSN in clinically stable patients with CGD compared with age-

[6]To whom correspondence should be addressed at Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. jig@nih.gov.
Thomas P. Stossel is deceased.

Conflict of Interest. Susan Levinson and Mark DiNubile are employees of and own stock in BioAegis Therapeutics, Inc. Other authors declare that they have no conflicts of interest.

Ethical Approval. All subjects participated following informed consent and were enrolled in IRB-approved clinical protocols in the NIH Clinical Center in accordance with 45 CFR 46.

and sex-matched healthy volunteers. Low pGSN levels in CGD patients significantly increased following bone marrow transplantation. X-linked carriers of CGD had normal pGSN. In healthy volunteers challenged with intravenous endotoxin, pGSN is not an acute phase reactant.

Keywords

gelsolin; CGD; inflammation; endotoxin

INTRODUCTION

Gelsolin is an intracellular actin remodeling protein involved in the regulation of cellular architecture, phagocytosis, and apoptosis. Factors influencing the actin-binding properties of cytoplasmic gelsolin include cytosolic pH, lysophosphatidic acid, Ca^{2+}, and phosphatidylinositol 4,5-bisphosphate [1, 2]. There also exists an extracellular isoform of gelsolin secreted into plasma called plasma gelsolin (pGSN). These two isoforms are encoded by the same gene with pGSN resulting from a splice variant adding a 24 residue N-terminal extension with no recognized molecular motifs and the presence of a disulfide bond in the main coding sequence that enhances extracellular stability [2]. Gelsolin is ubiquitously expressed in a vast array of tissues; however, skeletal, cardiac, and smooth muscle are the main sources of pGSN in the bloodstream [3, 4]. The regulation and physiological effects of circulating pGSN on human health are active areas of investigation.

Until recently, research on pGSN function has been focused on its role in the extracellular actin scavenger system that clears circulating actin leaked from damaged cells [5–7]. Through the sequestering of pro-inflammatory actin, pGSN is thought to help localize inflammation to sites of infection and dampen systemic inflammatory responses. pGSN is also capable of binding other bioactive ligands including lysophosphatidic acid, sphingosine 1-phosphate, fibronectin and platelet activating factor, as well as cell wall constituents of Gram-negative and Gram-positive bacteria [8–10]. These findings suggest that pGSN may act as a broad-spectrum anti-inflammatory buffer. As such, profound and prolonged pGSN depletion may result in excessive, and possibly deleterious, promulgation of inflammatory signals.

Apart from studies on pGSN functionality *in vitro*, clinical studies have demonstrated low pGSN concentrations in a variety of medical conditions, including major trauma, burns, and septic shock. In these conditions, a precipitous drop in circulating pGSN has been associated with adverse medical outcomes including changes in lung permeability and death [11–18]. In patients admitted to the intensive care unit because of trauma, pGSN concentrations at presentation were about 2 standard deviations below the mean of the control group and were associated with prolonged mechanical ventilation, development of acute respiratory distress syndrome, and an increased risk of mortality [11]. In burn patients, lower pGSN was associated with larger burn sizes and correlated with development of multiple organ dysfunction syndrome and enhanced burn- and sepsis-associated mortality [12, 13]. Several studies in preclinical animal models have demonstrated that repletion with recombinant

human (rhu)-pGSN can significantly improve survival, suggesting that pGSN administration may be a viable therapeutic intervention [19–21].

In this study, we measured pGSN in patients with CGD, a primary immunodeficiency caused by mutations in genes encoding one of the five subunits of the phagocyte-derived NADPH oxidase (NOX2), including $p22^{phox}$, $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ (autosomal CGD), or $gp91^{phox}$ (X-linked CGD). In patients with CGD, production of superoxide anion and other reactive oxygen species (ROS) by phagocytic cells and B lymphocytes is impaired, resulting in granulomatous inflammation and recurrent infections [22]. Although NOX2 function is deficient in all CGD patients, residual NOX2 activity ranges from 0.1 to 27.0% of normal. Survival of patients with CGD is positively associated with residual ROS production as a continuous variable, independent of genotype [23]. X-linked CGD carriers with variable degrees of X chromosome inactivation (lyonization) also exhibit a spectrum of ratios of abnormal to normal cells with intact NOX2 function [24]. We assessed pGSN in healthy volunteers, CGD patients ($gp91^{phox}$ and $p47^{phox}$), X-linked CGD carriers, and CGD patients undergoing bone marrow transplantation. Additionally, pGSN was quantified in serial plasma samples from healthy volunteers following challenge with intravenous endotoxin.

METHODS

Research Subjects

Plasma was obtained following informed consent from patients ($n = 19$ for $p47^{phox}$ CGD, $n = 22$ for $gp91^{phox}$ CGD, and $n = 41$ for X-linked CGD carriers) and healthy volunteers ($n = 54$) enrolled in a NIH Institutional Review Board (IRB)–approved protocol (10-I-0029) conducted at the NIH Clinical Center. At the time of study, the CGD patients were clinically stable with no evidence of active infection or active inflammation such as inflammatory bowel disease. Except for studies of bone marrow transplantation described below, subjects were free of clinically apparent infections or clinically active granulomatous processes, such as active inflammatory bowel disease although clinically stable CGD patients routinely have evidence of chronic inflammation with increased C-reactive protein [25]. Patients were over 18 years of age with either a clinical diagnosis of CGD, characterized by $gp91^{phox}$ or $p47^{phox}$ deficiency, or identified as X-linked CGD carriers [24]. Of 41 CGD patients in this study, 1 was taking prophylactic interferon gamma (IFN-γ), 16 were taking prophylactic itraconazole, 31 were taking prophylactic Bactrim, and 12 were receiving corticosteroids. Additional studies of CGD subjects were performed on preand post-transplant serum samples from patients enrolled after informed consent in a study of bone marrow transplantation to treat CGD [25].

Clinical center reference endotoxin (GMP endotoxin prepared from *E. coli* O113 by List Biological Laboratories, Inc., Campbell, CA) was administered after informed consent to healthy subjects enrolled in an IRB-approved protocol (Pro00070829 at Duke University) [26]. Results from the highest dose of a dose escalation trial (4 ng/kg IV) are reported along with the pGSN levels quantified from plasma collected at the indicated times post-administration of endotoxin.

Quantification of pGSN

Blood collected after informed consent was either allowed to clot for serum preparation or anticoagulated with heparin for the preparation of plasma then stored at − 80 °C until analysis. pGSN was quantified on thawed specimens using a custom sandwich ELISA developed by BioAegis Therapeutics, Inc. A 96-well Nunc-Immuno Microwell plate (Thermo Scientific) was incubated overnight with a monoclonal antibody specific to the N-terminal region of plasma gelsolin (BioAegis Therapeutics, Inc.) at a concentration of 2 μg/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6. Following overnight incubation, the plate was washed with PBST (phosphate buffer saline with 0.05% Tween-20, Sigma) and blocked with 3% BSA (bovine serum albumin, Sigma) in PBS for 1 h. Patient samples or recombinant human plasma gelsolin standard (rhu-pGSN, BioAegis Therapeutics, Inc.) were diluted 1:1000 in assay buffer consisting of 0.4 μg/ml anti-gelsolin antibody (Sigma, Clone 2C4) and HRP-conjugated anti-mouse IgG (Thermo Scientific) in PBST. One hundred microliters of diluted patient samples and the rhu-pGSN standard were added to the plate and incubated for 30 min at room temperature. The plate was washed 5 times with PBST and the amount of bound HRP-conjugated anti-mouse IgG antibody was detected by addition of TMB color reagent (Invitrogen). After approximately 2 min, 0.2 N H_2SO_4 was added to stop the reaction and the absorbance was measured at 450 nm with 600-nm correction using a multimode plate reader (Varioskan™ Lux, Thermo Scientific). pGSN concentrations were calculated from the rhu-pGSN standard curve after 4-parameter curve fitting using GraphPad Prism (version 8.2.0).

Quantitative Analysis of Superoxide Generation

Residual superoxide production by polymorphonuclear (PMN) cells of patients with CGD was quantified using superoxide dismutase-inhibitable ferricytochrome *C* reduction as previously described [27].

Determination of Degree of X Chromosome Lyonization in CGD Carriers

NADPH oxidase activity of individual cells in X-linked CGD carriers was measured by flow cytometry of dihydrorhodamine 123 (DHR) staining as previously described [27]. Percent DHR-positive cells were used to determine degree of lyonization.

Statistical Methods

The data are expressed as mean ± standard deviation and specific statistical analyses are described in the “Results”.

RESULTS

pGSN is Lower in CGD

To avoid the potential influence of active inflammatory processes, clinically well patients with CGD were analyzed. C-reactive protein was elevated in these patients (8.9 ± 10.1 mg/l for 41 CGD subjects *vs.* 2.4 ± 3.8 mg/l for 25 healthy subjects, $p \leq 0.001$). In healthy volunteers ($n = 54$), there was no difference in pGSN between males and females (53.7 ± 15.3 μg/ml for male volunteers *vs.* 52.4 ± 16.7 μg/ml for female volunteers, $p > 0.9999$).

No statistically significant correlation between age and pGSN was observed, although there was a trend toward higher concentrations in older subjects (Spearman rho = 0.2474, p = 0.0611). CGD patients had significantly lower pGSN concentrations compared with healthy volunteers (52.5 ± 16.3 μg/ml for healthy volunteers *vs.* 33.2 ± 8.3 μg/ml for gp91^{phox}-deficient CGD (n = 22) (p <0.0001) and 40.1 ± 10.1 μg/ml for p47^{phox}-deficient CGD (n = 19, p = 0.0076) (Fig. 1)). There was no significant difference in pGSN concentrations between gp91^{phox} and p47^{phox} CGD genotypes (p = 0.4883) (Fig. 1). As with the control group, no significant correlation was detected for pGSN and age in the CGD cohort across an age range of 18 to 48 (Spearman rho = 0.1342, p = 0.4027). There was an insufficient number of p47^{phox} CGD patients to assess differences between males (n = 9) and females (n = 10). Patients with CGD frequently receive itraconazole, Bactrim or interferon prophylaxis, and corticosteroids for treatment of granulomatous processes. Of the 41 CGD patients, neither itraconazole nor Bactrim prophylaxis had any effect on pGSN (35.2 ± 7.1 μg/ml for 16 patients on itraconazole, 35.97 μg/ml ± 9.86 for 31 patients on Bactrim *vs.* 37.7 ± 11.3 μg/ml for patients on neither agent, p > 0.24 for each comparison). Only one CGD patient was on IFN-γ with a pGSN level of 30.54 μg/ml for that patient. Twelve patients were receiving corticosteroids at the time of study and pGSN from those patients was no different from 29 patients not receiving corticosteroids (32.96 ± 7.32 μg/ml *vs.* 38.48 ± 9.98 μg/ml, p = 0.115).

Among CGD patients, the amount of residual superoxide production has been correlated with favorable clinical outcomes [23]. CGD patients with p47^{phox} mutations generally have greater residual neutrophil superoxide production than those with gp91^{phox} mutations. Therefore, we investigated whether there was a correlation between residual superoxide production by neutrophils and pGSN levels for the CGD patients in gp91^{phox} and p47^{phox} patients in our study and found a significant correlation (Spearman rho = 0.4273, p = 0.0053) (Fig. 2a). In contrast, pGSN concentrations in carriers of X-linked CGD (n = 41) were statistically indistinguishable from those of healthy volunteers (52.5 ± 16.3 μg/ml for healthy volunteers *vs.* 53.4 ± 15.1 μg/ml for X-linked CGD carriers, p > 0.9999) (Fig. 1). pGSN did not correlate to the degree of lyonization of the X chromosome as measured by neutrophil DHR reduction (Spearman rho = 0.2479, p = 0.1449) (Fig. 2b).

Effect of Intravenous Endotoxin on pGSN in Healthy Volunteers

Given previous reports as well as the observed lower concentrations of pGSN measured in patients with CGD, we hypothesized that pGSN may be a negative acute phase reactant or biomarker of chronic inflammation. To assess this possibility, we measured pGSN in healthy volunteers receiving experimental intravenous endotoxin to induce an acute phase response. As expected, intravenous endotoxin (4 ng/kg) resulted in a transient febrile response with temperature peaking at 2½ h and returning to baseline by 24 h (Fig. 3). The peak temperature response was preceded by a peak in TNFα at 1–1½ h. Despite the rapid induction of the acute phase response, we did not detect significant changes in the concentration of circulating pGSN over 24 h of observation.

pGSN Following Bone Marrow Transplantation in CGD

Bone marrow transplantation in CGD can correct the hematopoietic defect resulting in a decrease in rates of infection [25]. pGSN concentrations were measured in sera from twelve patients with CGD before and 100 days to 1.5 years after bone marrow transplantation (Fig. 4). Of the 12 CGD patients assessed, 6 patients had serious culture-proven active infections pre-transplantation, 1 had a probable infection of the lung based on clinical course and pulmonary infiltrates, 2 had no infections but had active colitis, and the remaining 3 had no infection pretransplantation. Among the 6 patients with pre-transplant infections, 3 patients had *Nocardia* of the lungs or bones, and 1 patient had *Aspergillus nidulans* of the lung. The two patients who died had *Pyrenochaeta romeroi* or *Phellinus igniarius* infection 27 and 91 days post-transplantation and had the lowest pGSN levels both pre- and post-transplantation. Patients with active infection pre-transplantation tended to have lower pGSN concentrations than those without recognized infections, although the limited data did not reach significance ($p = 0.2677$, Mann-Whitney U test) (Fig. 4). pGSN increased in each of the 10 post-transplant patients who survived (36.1 ± 11.0 μg/ml for pre-transplant *vs.* 53.5 ± 9.20 μg/ml for post-transplant, $p = 0.001$). The concentrations of pGSN in the sera from 10 CGD patients post-transplant did not differ significantly from healthy volunteer sera ($p = 0.1645$, Mann-Whitney U test).

DISCUSSION

Here, we report for the first time that patients with CGD have lower circulating pGSN concentrations than healthy volunteers. CGD is a disorder associated with potentially lethal infections, requiring prophylactic antimicrobials to prevent those infections, as well as a disease of episodic and chronic inflammation [22]. Lower pGSN concentrations correlated with residual NADPH oxidase activity in neutrophils of patients with CGD; however, there was no correlation between pGSN and the degree of lyonization in the X-linked CGD carriers. The correlation of pGSN with residual superoxide in CGD but not carriers suggests reduced pGSN is not directly due to low ROS in CGD but reflects chronic infection or inflammation characteristic of this disease.

In our study of healthy volunteers, pGSN did not change following intravenous endotoxin, although temperature and TNFα increased dramatically as expected. Our observations with endotoxin in human volunteers using a small intravenous dose of endotoxin (4 ng/kg) contrast with studies in a mouse model where after intraperitoneal challenge with a lethal dose of endotoxin (25 mg/kg), pGSN dropped to less than 50% of normal within 6 h and remained depressed for more than 24 h [19].

In addition to the cohort of clinically stable patients with CGD, we also measured pGSN in patients with CGD who experienced severe complications requiring bone marrow transplantation. All patients who survived greater than 3 months post-transplant had significantly increased pGSN concentrations that were statistically indistinguishable from normal subjects. The association of low pGSN in infected patients with CGD before bone marrow transplantation and high mortality post-transplantation is intriguing and suggests that a future study of rhu-pGSN administration in this difficult clinical setting may be beneficial.

Funding

This research was supported in part by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases, and in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. 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 US government.

REFERENCES

1. Sun HQ, Yamamoto M, Mejillano M, and Yin HL. 1999. Gelsolin, a multifunctional actin regulatory protein. J Biol Chem 274: 33179–33182. 10.1074/jbc.274.47.33179. [PubMed: 10559185]
2. Yin HL, Kwiatkowski DJ, Mole JE, and Cole FS. 1984. Structure and biosynthesis of cytoplasmic and secreted variants of gelsolin. J Biol Chem 259: 5271–5723. [PubMed: 6325429]
3. Pellieux C, Desgeorges A, Pigeon CH, Chambaz C, Yin H, Hayoz D, and Silacci P. 2003. Cap G, a gelsolin family protein modulating protective effects of unidirectional shear stress. J Biol Chem 278: 29136–29144. 10.1074/jbc.M300598200. [PubMed: 12754261]
4. Candiano G, Bruschi M, Pedemonte N, Caci E, Liberatori S, Bini L, Pellegrini C, Viganò M, O'Connor BJ, Lee TH, et al. 2005. Gelsolin secretion in interleukin-4-treated bronchial epithelia and in asthmatic airways. Am J Respir Crit Care Med 172: 1090–1096. 10.1164/rccm.200409-1185OC. [PubMed: 16100010]
5. Lee WM, and Galbraith RM. 1992. The extracellular actin-scavenger system and actin toxicity. N Engl J Med 326: 1335–1341. [PubMed: 1314333]
6. Cooke NE, and Haddad JG. 1989. Vitamin D binding protein (Gc-globulin). Endocr Rev 10: 294–307. [PubMed: 2476303]
7. Lind SE, Smith DB, Janmey PA, and Stossel TP. 1986. Role of plasma gelsolin and the vitamin D-binding protein in clearing actin from the circulation. J Clin Invest 78: 736–742. [PubMed: 3018044]
8. Osborn TM, Dahlgren C, Hartwig JH, and Stossel TP. 2007. Modifications of cellular responses to lysophosphatidic acid and platelet-activating factor by plasma gelsolin. Am J Phys Cell Phys 292 (4): 1323–1330.
9. Bucki R, Kulakowska A, Byfield FJ, Zendzian-Piotrowska M, Baranowski M, Marzec M, Winer JP, Ciccarelli NJ, Górski J, Drozdowski W, Bittman R, and Janmey PA. 2010. Plasma gelsolin modulates cellular response to sphingosine 1-phosphate. Am J Phys Cell Phys 299: 1516–1523.
10. Lind SE, and Janmey PA. 1984. Human plasma gelsolin binds to fibronectin. J Biol Chem 259: 13262–13266. [PubMed: 6092370]
11. Lee PF, Drager LR, Stossel TP, Moore FD, and Rogers SO. 2006. Relationship of plasma gelsolin levels in outcomes of critically ill surgical patients. Ann Surg 243: 399–403. [PubMed: 16495706]
12. Xianhui L, Pinglian L, Xiaojuan W, Wei C, Yong Y, Feng R, Peng S, and Gang X. 2014. The association between plasma gelsolin level and prognosis of burn patients. Burns 40: 1552–1555. 10.1016/j.burns.2014.02.020. [PubMed: 24690274]
13. Huang LF, Yao YM, Li JF, Dong N, Liu C, Yu Y, He LX, and Sheng ZY. 2011. Reduction of plasma gelsolin levels correlates with development of multiple organ dysfunction syndrome and fatal outcome in burn patients. PLoS ONE 6: e25748. 10.1371/journal.pone.0025748. [PubMed: 22069445]
14. Suhler E, Lin W, Yin HL, and Lee WN. 1997. Decreased plasma gelsolin concentration in acute liver failure, myocardial infarction, septic shock, and myonecrosis. Crit Care Med 25: 594–598. [PubMed: 9142022]
15. Mounzer KC, Moncure M, Smith YR, and DiNubile MJ. 1990. Relationship of admission plasma gelsolin levels to clinical outcomes in patients after major trauma. Am J Respir Crit Care Med 160: 1673–1681.
16. Dahl B, Schiodt FV, Ott P, Gvozdenovic R, Yin HL, and Lee WM. 1999. Plasma gelsolin is reduced in trauma patients. Shock 12: 102–104. [PubMed: 10446889]

17. Lind SE, Smith DB, Janmey PA, and Stossel TP. 1988. Depression of gelsolin levels and detection of gelsolin-actin complexes in plasma of patients with acute lung injury. Am Rev Respir Dis 138: 429–434. [PubMed: 2848430]

18. Ito H, Kanbe H, Kimura Y, Nakamura H, Hayash E, Kishimoto T, Kishimoto S, and Yamamoto H. 1992. Depression of plasma gelsolin during acute liver injury. Gastroenterology 102: 1686–1692. [PubMed: 1314752]

19. Lee PS, Waxman AB, Cotich KL, Chung SW, Perrella MA, and Stossel TP. 2007. Plasma gelsolin is a marker and therapeutic agent in animal sepsis. Crit Care Med 35 (3): 849–855. [PubMed: 17205019]

20. Rothenbach PA, Dahl B, Schwartz JJ, O'Keefe GE, Yamamoto M, Lee WM, Horton JW, Yin HL, and Turnage RH. 2004. Recombinant plasma gelsolin infusion attenuates burn-induced pulmonary microvascular dysfunction. J Appl Physiol 96: 25–31. [PubMed: 12730154]

21. Zhang QH, Chen Q, Kang JR, Liu C, Dong N, Zhu XM, Sheng ZY, and Yao YM. 2011. Treatment with gelsolin reduces brain inflammation and apoptotic signaling in mice following thermal injury. J Neuroinflammation 8: 118. [PubMed: 21936896]

22. Segal BH, Leto TL, Gallin JI, Malech HL, and Holland SM. 2000. Genetic, biochemical, and clinical features of chronic granulomatous disease. Medicine (Baltimore) 79 (3): 170–200. [PubMed: 10844936]

23. Kuhns DB, Alvord WG, Heller T, et al. 2010. Residual NADPH oxidase and survival in chronic granulomatous disease. N Engl J Med 363 (27): 2600–2610. 10.1056/NEJMoa1007097. [PubMed: 21190454]

24. Sibley CT, Estwick T, Zavodni A, Huang CY, Kwan AC, Soule BP, Long Priel DA, Remaley AT, Rudman Spergel AK, Turkbey EB, Kuhns DB, Holland SM, Malech HL, Zarember KA, Bluemke DA, and Gallin JI. 2014. Assessment of atherosclerosis in chronic granulomatous disease. Circulation 130 (23): 2031–2039. [PubMed: 25239440]

25. Parta M, Kelly C, Kwatemaa N, Theobald N, Hilligoss D, Kuhns DB, Zerbe C, Holland SM, Malech S, and Kang E. 2017. Allogeneic reduced-intensity hematopoietic stem cell transplantation for chronic granulomatous disease: a single-center prospective trial. J Clin Immunol 37: 548–558. [PubMed: 28752258]

26. Suffredini AF, and Noveck RJ. 2014. Human endotoxin administration as an experimental model in drug development. Clin Pharmacol Ther 96: 418–422. 10.1038/clpt.2014.146. [PubMed: 25236665]

27. Kuhns DB, Priel DAL, Chu J, and Zarember KA. 2015. Isolation and functional analysis of human neutrophils. Curr Protoc Immunol 111: 7.23.1–7.23.16. 10.1002/0471142735.im0723s111.

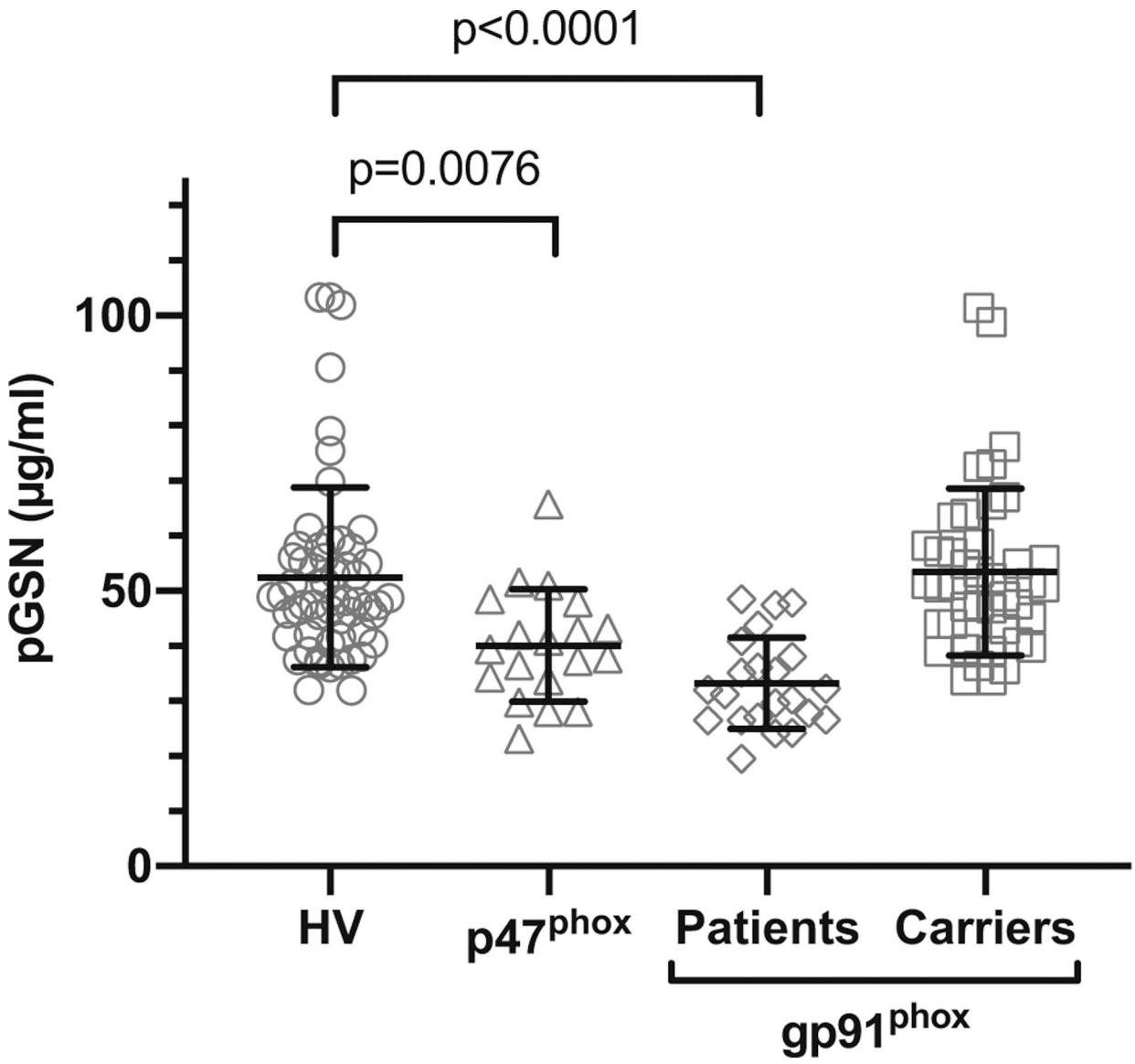

Fig. 1.

Plasma gelsolin levels in stable patients with CGD and carriers of X-linked CGD. Each point represents the mean of 4 independent ELISA measurements in each subject (n = 19 for p47^{phox} CGD, n = 22 for gp91^{phox} CGD, n = 41 for X-linked CGD carriers, and n = 54 for healthy volunteers). Bars denote mean ± SD of each patient population. Statistical significance was tested with a Kruskal-Wallis test with Dunn's correction for multiple comparisons.

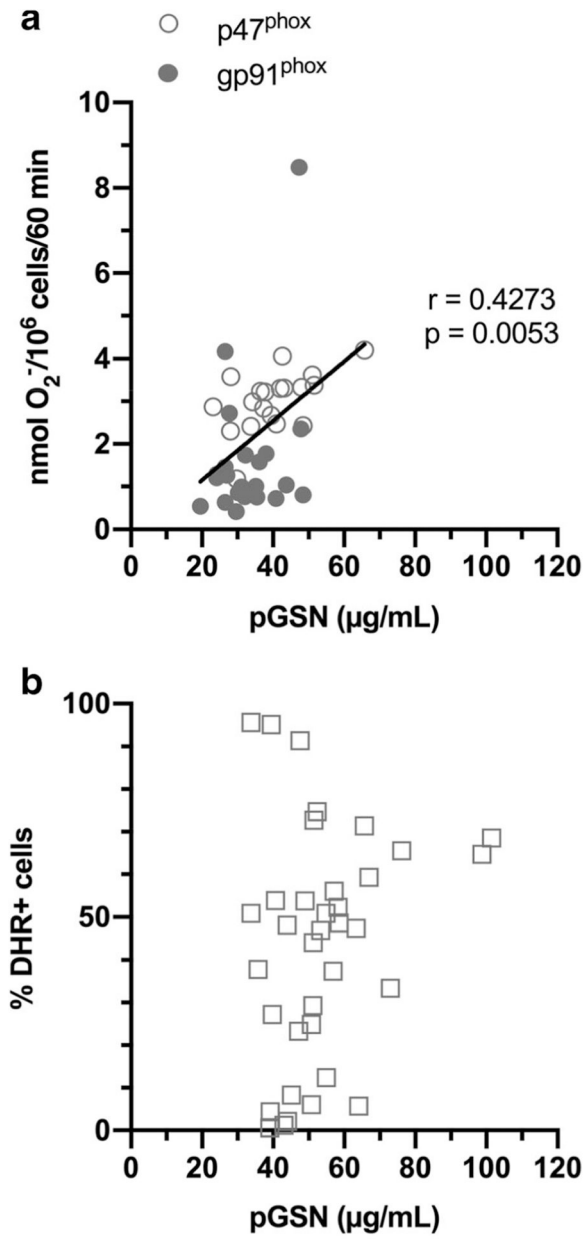

Fig. 2.

Spearman correlation analysis of pGSN concentrations and (**a**) CGD residual reactive oxygen species production or (**b**) lyonization of X-linked carriers. **a** Residual reactive oxygen species production was quantified by ferricytochrome *C* reduction for each patient and plotted against the corresponding pGSN value. **b** Lyonization in X-linked CGD carriers was quantified by dihydrorhodamine (DHR) oxidation and plotted against the corresponding pGSN value. A Spearman correlation analysis was performed for both populations and a line of best fit was only plotted for significant correlations.

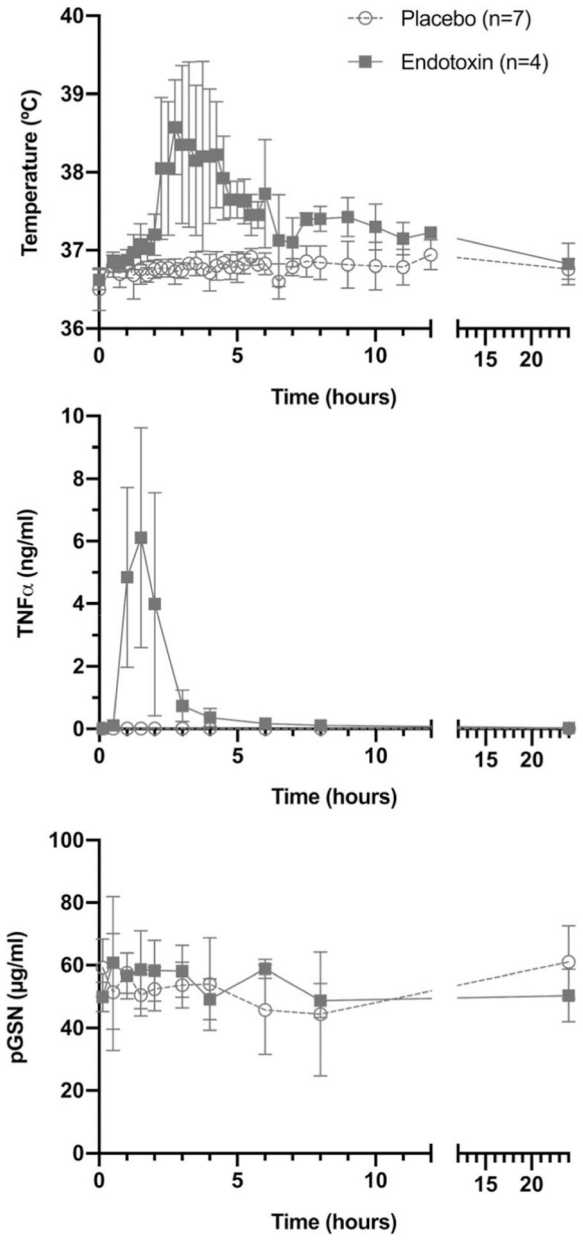

Fig. 3.

pGSN levels in healthy volunteers after intravenous endotoxin. After intravenous administration of endotoxin (4 ng/kg), plasma was collected from volunteers at the indicated time points. Temperature (top panel), TNFα (middle panel), and pGSN (bottom panel) are expressed as means ± SD.

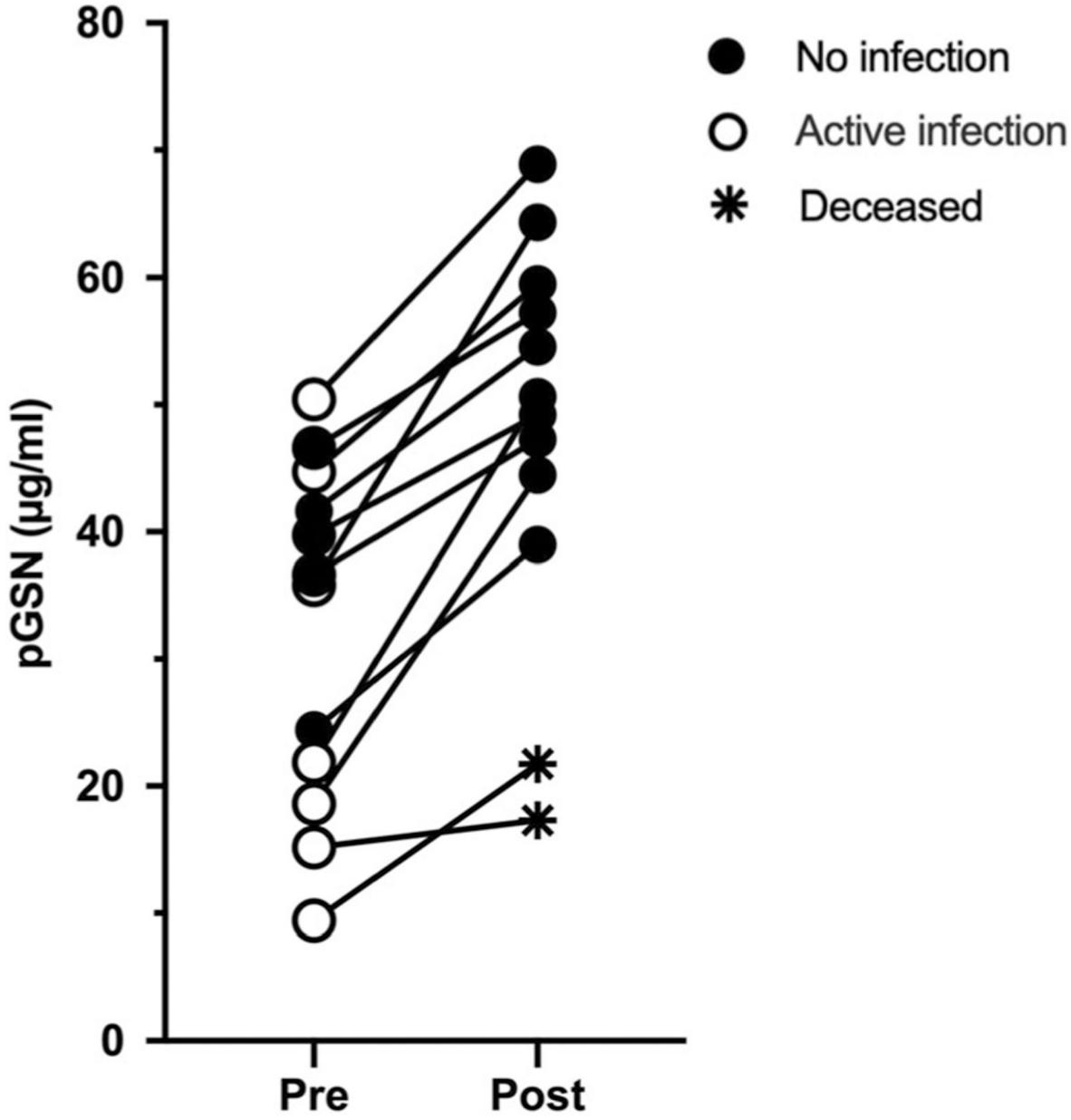

Fig. 4.

pGSN levels in pre- and post-bone marrow transplant CGD serum. Each point represents the mean of 4 independent ELISA measurements in each subject. Patient clinical status is indicated. All post-transplantation samples were collected after 3 months post-transplant except for the deceased patients who died earlier (see text). Statistical significance of pre- and post-transplantation pGSN was tested with a Wilcoxon matched-pairs signed rank test and samples from the deceased patients were excluded from statistical analysis. CGD, chronic granulomatous disease; hsCRP, high sensitivity C-reactive protein; IFN-γ, interferon gamma; pGSN, plasma gelsolin.