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Expression of *gilt* acts as a Positive Regulator of Mouse Hematopoietic Progenitor Cells

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Abstract

Gamma interferon inducible lysosomal thiol reductase (GILT), is known to be involved in immunity, but its role in hematopoiesis has not been previously reported. Herein, we demonstrate using *gilt* knockout (−/−) mice that loss of *gilt* associates with decreased numbers and cycling status of femoral hematopoietic progenitor cells (CFU-GM, BFU-E, and CFU-GEMM) with more modest effects on splenic progenitor cells. Thus, GILT is associated with positive regulation of hematopoietic progenitor cells in mice, mainly in bone marrow.

Keywords

Gilt; Bone Marrow; Spleen; Hematopoietic Progenitor Cells; CFU-GM; BFU-E; CFU-GEMM

Introduction

Hematopoiesis and hematopoietic stem (HSC) and progenitor (HPC) cells are regulated by cytokines, chemokines, other growth modulating proteins, and induction of intracellular signaling, and gene regulation (1–3). While knowledge in HSC and HPC regulation is greatly increasing, we are far from knowing and understanding all of the positive and negative aspects of the regulation of these cells under normal conditions or disease states. Hence, the research is ongoing to identify factors and genes that control hematopoiesis. We became intrigued with Gilt as a possible influence on the regulation of hematopoietic progenitor (granulocyte-macrophage (CFU-GM), erythroid (BFU-E), and multiple potential (CFU-GEMM)) cells. Gilt (Gamma interferon inducible lysosomal thiol reductase) is an enzyme, also known by other names such as IFI30, IP30, lysosomal thiol reductase, and IFI30 lysosomal thiol reductase. Gilt has been associated with immunological regulation (4–8) and cancer (9–11). Its function is in major histocompatibility (MHC) class II restricted antigen processing and MHC class I restricted cross-presentation by reducing disulfide

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bonds of endocytosed proteins which facilitate their unfolding and optimal degradation (4, 8–10). Gilt's influence on peptide repertoires during antigen presentation alters the characteristics of immune responses and effects controlling tolerance. However all functions of Gilt are not known, and some other actions involve its activity as a host factor for certain bacteria, the maintenance of the levels of glutathione, and perhaps as secretion of enzymatically active gilt outside the cell, after secretion by cells such as activated macrophage (4, 12–16). First identified as an interferon-gamma-inducible 30KDa polypeptide, it is the only known lysosomal thiol reductase, with the precursor and mature forms of GILT having enzymatic activity. It has also been implicated in production of reactive oxygen species (ROS) and autophagy (14–16). We now report a role for GILT in regulation of hematopoiesis. Since ROS has been implicated in the regulation of HSC and HPC (17,18), we utilized *gilt* −/− mice to assess the effects of loss of *gilt* on nucleated cellularity, absolute numbers of CFU-GM, BFU-E, and CFU-GEMM in bone marrow and spleen of *gilt* −/− vs. control wildtype mice. We report that *gilt* loss is associated with decreased numbers and cycling status of HPC, suggesting a positive role for *gilt* in the regulation of HPC. We believe that this is the first evidence of a role for gilt in hematopoietic cell regulation.

Materials and Methods

Mice

Gilt −/− mice were supplied by Dr. Janice Blum, Indiana University School of Medicine (IUSM) and control wildtype (WT) mice from the animal core at the IUSM, both on a C57Bl/6 mouse strain background. The mice were 6–7 weeks old; with the WT mice being female, and the *gilt* −/− mice being half female and half male. Results of male and female *gilt* −/− mice were similar.

Colony Assays for HPC

Assays for numbers of BM and spleen CFU-GM, BFU-E, and CFU-GEMM and the cycling status of these HPC was as reported previously (17,19). Cells were suspended in a 1% methylcellulose culture system with FBS, and purified recombinant human erythropoietin (Epo, 1U/ml), mouse stem cell factor (SCF, 50ng/ml), mouse granulocyte macrophage colony stimulating factor (GM-CSF, 10ng/ml), mouse interleukin-3 (IL-3, 10ng/ml) and hemin (0.1mM). Cells were cultured in a humidified atmosphere, 5% CO_2 and lowered (5%) O_2 tension, for 7 days. Estimates of the cell cycling of the HPC utilized the thymidine kill assay in order to determine the percent of HPC in S-phase of the cell cycle (19). These culture conditions result in optimal colony formation of CFU-GM, BFU-E and CFU-GEMM. Absolute numbers these HPC were calculated from the BM and spleen nucleated cellularity.

Statistics

P values are calculated by 2-tailed student's t test for *gilt* −/− vs. WT HPC. P values less than or equal to <0.05 were considered significantly different.

Results

To assess a role for *gilt* expression on hematopoiesis, we utilized *gilt* −/− mice, and evaluated bone marrow (BM) and spleen nucleated cellularity and absolute numbers of granulocyte-macrophage (CFU-GM), erythroid (BFU-E), and multipotential (CFU-GEMM) progenitor cells and their cell cycling, the latter assessed by high specific activity tritiated thymidine kill, compared to control WT mice (Figure 1). While there was no significant difference in BM nucleated cellularity between WT and *gilt* −/− mice (Figure 1A), there were highly significant decreases in absolute numbers of CFU-GM, BFU-E, and CFU-GEMM per femur (Figure 1C) and in the cycling status of these progenitors (Figure 1E). While the nucleated cellularity of spleen cells was significantly decreased in *gilt* −/− mice (Figure 1B), the only significant decrease in spleen progenitors was for CFU-GEMM with non-significant trends to decreased numbers of splenic CFU-GM and BFU-E seen (Figure 1D). Splenic CFU-GM, BFU-E, and CFU-GEMM from WT mice are usually in a slow or non- cycling state, as we noted for the data in our study, and there were no significant differences in the cycling status of *gilt* −/− splenic CFU-GM, BFU-E and CFU-GEMM compared to WT controls (Figure 1F).

Thus, *gilt* −/− was associated with decreased BM progenitors and their cycling status with more modest effects on splenic progenitors. This suggests that *gilt* expression plays a role in the positive regulation of progenitors.

Discussion

Our studies have now implicated *gilt* expression as a positive regulator of CFU-GEM, BFU-E, and CFU-GEMM, as these HPC are decreased in *gilt* −/− vs. WT control mice. While our studies do not assess the mechanisms of gilt effects on numbers and cycling status of BM, and to a lesser extent splenic HPC, they do, perhaps for the fist time demonstrate a new and not previously known role for gilt on hematopoiesis at the level of HPC. Whether these hematopoietic effects of gilt are direct or indirect, or are in part regulated by ROS as noted for *gilt* −/− mice in other circumstances (14), and exactly how the effects are mediated are yet to be defined. They likely reflect the enzymatic actions of gilt, and its lysosomal thiol reductase activity, with its effects on ROS production possibly playing a role.

It is yet to be elucidated how gilt fits into the overall regulation of hematopoiesis as a direct intracellular or indirect extracellular mediator, at the level of the HPC, or on the microenvironmental niche that plays an intricate role in the regulation of hematopoiesis. Further studies on a role of gilt on HSC regulation, and greater mechanistic insight into gilt effects on HSC and HPC are warranted. How gilt functions in aging, where hematopoiesis is known to be defective (20), is of interest. Such studies might eventually lead to gilt/GILT modulation of hematopoiesis for clinical translation and benefit.

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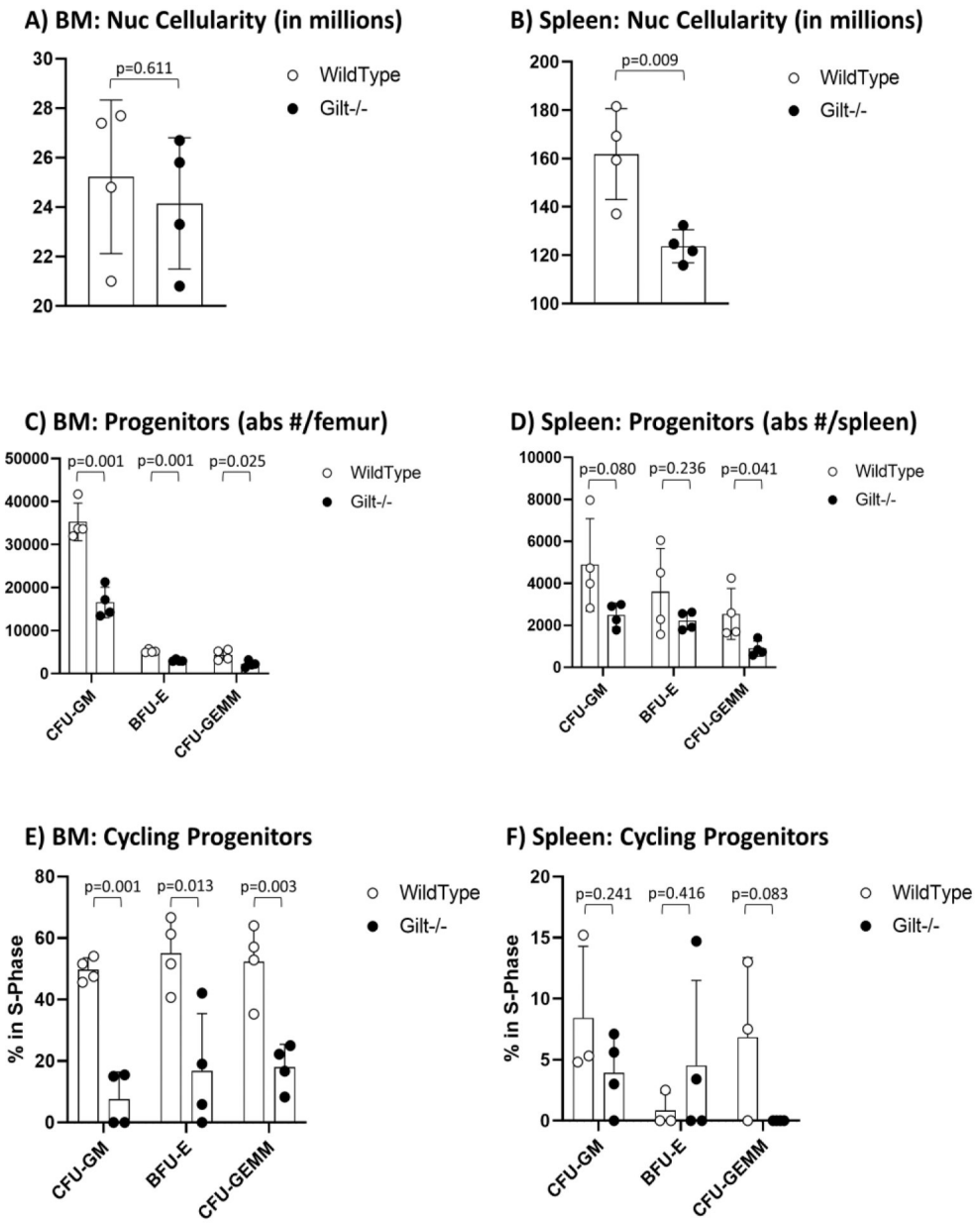

Figure 1.

Effects of loss of *gilt*, using gilt −/− mice on nucleated cellularity of BM and spleen (A,B), BM progenitors (C), spleen progenitors (D), and cycling status of BM (E) and spleen (F) progenitors. A 2-tailed t test was used to assess significance of significant differences between *gilt* −/− vs. wildtype control cells with a P value of at least <0.05 considered statistically significant.