Brief report

GATA3 is redundant for maintenance and self-renewal of hematopoietic stem cells

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GATA3 has been identified as a master regulator of T helper cells, as well as being important for early thymic progenitors and T-cell commitment. However, *Gata3* expression initiates already at the hematopoietic stem cell (HSC) level, implicating a potential role also in the regulation of HSCs. Herein we used a conditional *Gata3* knockout strategy in which *Gata3* expression was completely deleted from the earliest stage of embryonic hematopoietic development after emergence of HSCs from hemogenic endothelium. Through a detailed analysis of HSCs at the phenotypic and functional level, we demonstrate that steady-state levels of HSCs are normal in *Gata3*$^{fl/fl}$*Vav-Cre*$^{tg/+}$ mice. Moreover, through long-term primary and secondary transplantation experiments, we also unequivocally demonstrate that *Gata3* has a redundant role in post-transplantation HSC self-renewal. (*Blood*. 2011;118(5):1291-1293)

Introduction

GATA1, GATA2 and GATA3 are expressed at distinct stages in the hematopoietic hierarchy. GATA1 is required for erythropoiesis, megakaryocytes, and eosinophils,[1-3] GATA2 for primitive hematopoietic stem/progenitor cells,[4,5] and GATA3 for multiple stages of T-cell development.[6-8] However, *Gata3* is expressed already in hematopoietic stem cells (HSCs),[9] multipotent progenitors,[10] and early thymic progenitors.[11] Although the role of GATA3 has been carefully investigated in T-lymphopoiesis, its role in HSCs has been much less explored.[12] Recent studies showing that *Gata3* null fetal liver cells can normally generate short-lived myeloid cells 10 weeks after transplantation[8] are compatible with GATA3 having little or no role in HSC regulation. However, the HSC phenotype was not directly analyzed in either the fetal liver or the bone marrow (BM) of transplanted mice, nor was the defining stem cell property to self-renew after transplantation into secondary recipients.

Methods

Mice

Wild type (WT) transplantation recipients and competitor cells were on a pure C57BL/6 (CD45.1) background. *Gata3*$^{fl/fl}$, *Vav-iCre*, and *R26R-eYFP* mice have been described.[13-15] Animal experiments were approved by the local ethics committee at the University of Oxford and the United Kingdom Home Office.

Fluorescence-activated cell sorter analysis

BM cells were stained according to previously described protocols.[16] For specification of antibodies, see supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Gene expression analysis

Global gene expression analysis was performed using Mouse Genome 430 2.0 Arrays. *Gata3* deletion efficiency was evaluated with dynamic array Biomark Fluidigm analysis (supplemental Methods). *Gata3* was amplified and analyzed for gene expression using a dynamic array (Biomark Fluidigm) as previously described.[17] A total of 100 cells were sorted/well (2-4 wells/genotype). Data were analyzed using BioMark Real-Time PCR Analysis Software Version 2.0 (Fluidigm), and the ΔC_t method was applied.[18] *Hprt* TaqMan Gene Expression Assay ID Mm01337569_m1 was used.

Statistical analysis

Statistical significances were determined with 2-tailed Mann-Whitney test.

Results and discussion

We not only confirmed that *Gata3* mRNA is expressed in highly purified LSKFLT3$^-$CD48$^-$CD150$^+$ HSCs,[16] but that it is subsequently down-regulated in lymphoid-primed multipotent progenitors[19,20] in which thymic-seeding progenitors are thought to reside, before being up-regulated again in LIN$^-$CD25$^-$KIT^{hi}FLT3$^+$ early thymic progenitors[11] (Figure 1A). *GATA3*$^{fl/fl}$ mice[13] were crossed with *Vav-iCre* mice,[14] ensuring *Gata3* deletion shortly after the emergence of definitive HSCs from the hemogenic endothelium.[21] *Vav-iCre* mice crossed with *R26R-eYFP*$^{fl/fl}$ mice[15] demonstrated that *Vav-iCre* ensures recombination in virtually all ($> 99\%$) BM LSK cells (supplemental Figure 1). BM cellularities of control (*Gata3*$^{fl/fl}$*Vav-Cre*$^{+/+}$) and *Gata3*-deficient (*Gata3*$^{fl/fl}$*Vav-Cre*$^{tg/+}$) littermates were indistinguishable (Figure 1B), as were the frequencies of LSKCD150$^+$CD48$^-$ HSCs (Figure 1C-D), in which complete *Gata3* deletion was confirmed (Figure 1E). These findings

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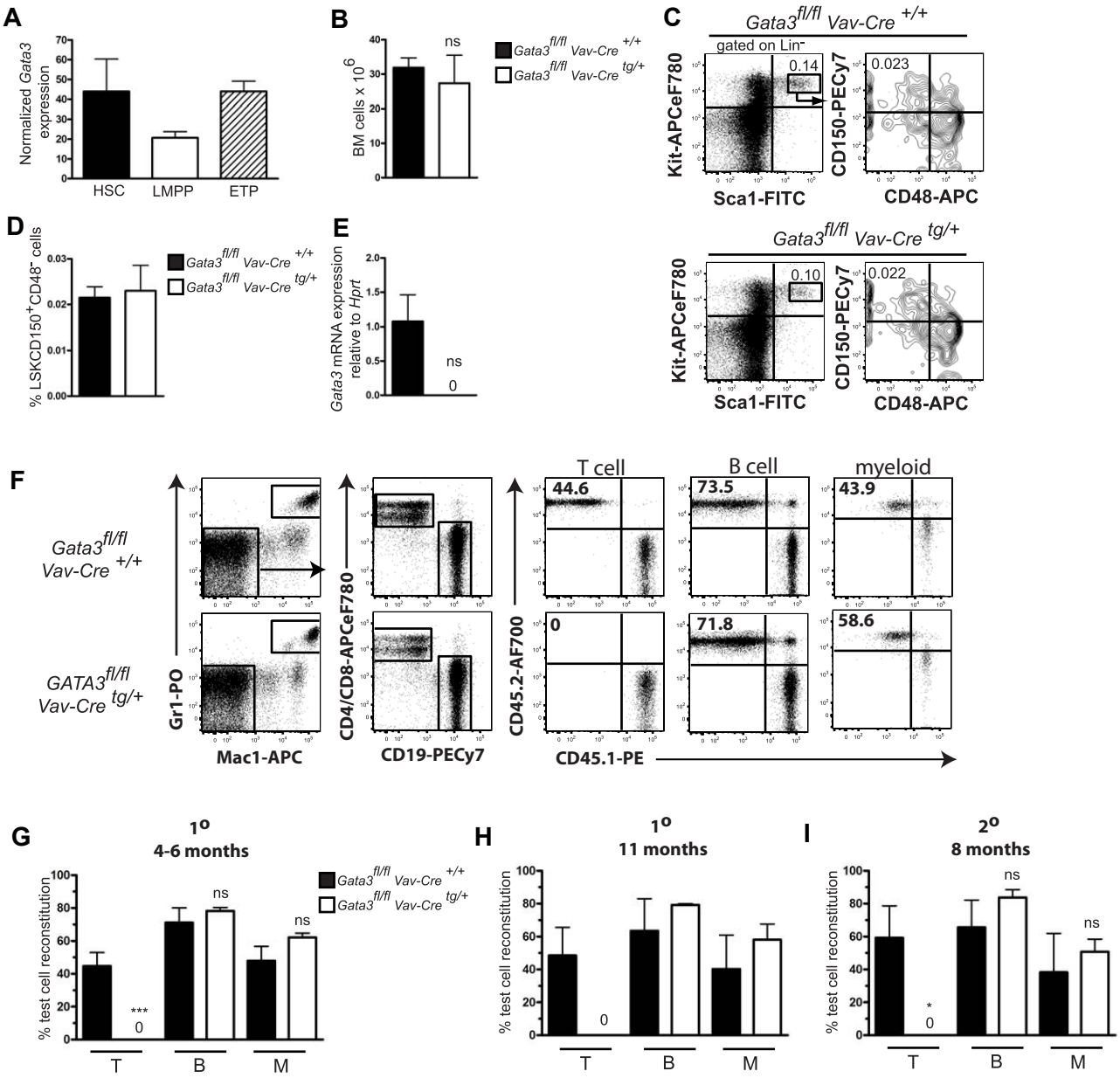

Figure 1. Phenotypic and functional characterization of HSCs in *Gata3*$^{fl/fl}$ *Vav-Cre*$^{tg/+}$ mice. (A) *Gata3* mRNA expression in 1-week-old WT LSKFLT3$^-$CD150$^+$CD48$^-$ HSCs, LSKFLT3^{hi}IL7R$^-$ lymphoid-primed multipotent progenitors, and Lin$^-$CD25$^-$KIT$^+$FLT3$^+$ early thymic progenitors. Data are expressed as mean (SEM) normalized Robust Multi-Array Averages (RMA) expression ("Gene expression analysis"). n = 3 experiments. (B) BM cellularity in 1-week-old *Gata3*$^{fl/fl}$*Vav-Cre*$^{+/+}$ and *Gata3*$^{fl/fl}$*Vav-Cre*$^{tg/+}$ mice. Data are mean (SD); n = 3 or 4 mice/genotype. ns indicates not significant. (C) Fluorescence-activated cell sorter profiles from representative mouse showing gating strategy for LSKCD150$^+$CD48$^-$ cells in 1-week-old mice. Numbers indicate percentage of total BM cells within the indicated gate. n = 3 or 4 mice/genotype. (D) Bar graphs represent mean (SD) frequency of LSKCD150$^+$CD48$^-$ cells. n = 3 or 4 mice/genotype. ns indicates not significant. (E) Bar graphs show mean (SEM) *Gata3* mRNA expression levels (relative to *Hprt*) in LSKCD150$^+$CD48$^-$ HSCs isolated from 1- to 2-week-old *Gata3*$^{fl/fl}$ *Vav-Cre*$^{+/+}$ or *Gata3*$^{fl/fl}$ *Vav-Cre*$^{tg/+}$ mice. (F-I) A total of 0.5 to 2 million BM cells from 1- to 2-week-old *Gata3*$^{fl/fl}$ *Vav-Cre*$^{+/+}$ or *Gata3*$^{fl/fl}$ *Vav-Cre*$^{tg/+}$ mice (CD45.2) were transplanted into lethally irradiated (900 cGy) WT (CD45.1) recipients together with a similar dose of competitor WT (CD45.1) adult BM cells. (F) Representative fluorescence-activated cell sorter analysis of peripheral blood 4 months after transplantation. Numbers indicate percentage of indicated gate within reconstituted T (CD4$^+$/CD8$^+$), B (CD19$^+$), and myeloid (Gr1$^+$Mac1$^+$) cells. n = 3 to 6 mice/group. (G-H) Mean (SEM) percentage of test cell (CD45.2$^+$) reconstitution within the T, B, and myeloid blood cell lineages, respectively, 4 to 6 (G) or 11 (H) months after transplantation. (G) n = 6 to 9 mice/group. (H) n = 2 or 3 mice/group. (I) Six months after primary transplantation, one-half femur equivalent from each primary recipient was transplanted into one secondary WT (CD45.1) recipient. Secondary recipients were analyzed 8 months later. Data show mean (SEM) percentage of test cell reconstitution within T, B, and myeloid reconstituted cells, respectively. N = 3 to 6 mice/group. 1° indicates primary reconstitution; and 2°, secondary reconstitution. *$P < .05$. ***$P < .001$. ns indicates not significant.

suggest that *Gata3* null HSCs expand normally after their emergence in the embryo and remain unaffected in postnatal BM.

BM cells from *Gata3*$^{fl/fl}$*Vav-Cre*$^{+/+}$ or *Gata3*$^{fl/fl}$*Vav-Cre*$^{tg/+}$ mice were transplanted in competition with WT BM cells into lethally irradiated recipients. As evidence of the complete recombination induced by *Vav-Cre*, *Gata3* null BM cells failed to contribute to T-cell reconstitution, whereas B-cell (CD19$^+$) and myeloid (Gr1$^+$Mac1$^+$) reconstitution was unaffected 4 to 6 months (Figure 1F-G) and 11 months (Figure 1H) after transplantation. The myeloid and B-cell lineages remained reconstituted to a similar degree in *Gata3*-deleted as nondeleted BM cells, as late as 8 months after transplantation into secondary recipients (Figure 1I), demonstrating a dispensable role for GATA3 in HSC self-renewal.

Vav-Cre-induced recombination in the present studies ensured highly efficient hematopoietic recombination[14] while allowing viable *Gata3* null embryos to develop despite *Gata3* deletion

occurring already shortly after emergence of HSCs in the early embryo. This approach, combined with state-of-the-art HSC phenotypical analysis, and long-term HSC reconstitution assessment, including secondary transplantations, unequivocally establishes that GATA3 is dispensable for HSC regulation at multiple levels, including steady-state maintenance and self-renewal, as well as the expansion that takes place during development and after transplantation.[22]

GATA2 and GATA3 can partially rescue the *Gata1*-deficient erythroid phenotype.[23,24] As GATA2 has been demonstrated to play a key role in HSC regulation,[5] it remains plausible that a role of GATA3 in HSC regulation might be redundant to that of GATA2, and that this could be revealed in *Gata2*-deficient HSCs.

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Authorship

Contribution: S.E.W.J. and N.B.-V. designed and conceptualized the overall research, analyzed the data, and wrote the manuscript; N.B.-V. performed the transplantations and phenotypic fluorescence-activated cell sorter analysis; S.D. performed gene expression analysis; S.D., P.S.W., and T.B.-J. performed FACS analysis of stem/progenitors and peripheral blood; and S.L. performed affymetrix analysis.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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