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TRANSLATIONAL AND CLINICAL RESEARCH

The Impact of Integrin *β2* on Granulocyte/Macrophage Progenitor Proliferation

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Key Words. FcεRIα • GATA2 • Granulocyte/macrophage progenitors • Integrin β2 • Proliferation

ABSTRACT

Previously, we reported that although the HSPC frequency in bone marrow cells (BMC) was comparable between β2−/− and β2+/+ mice, transplantation of β2−/− BMC into lethally irradiated CD45.1 recipient resulted in more myeloid cell production than β2+/+ BMC. The objective of this study is to address if integrin β2 deficiency skews granulocyte/macrophage progenitor (GMP) proliferation. FACS analysis demonstrated that GMP frequency and cell number were higher and megakaryocyte/erythrocyte progenitor frequency and cell number were lower in β2−/− mice than β2+/+ mice. However, the common myeloid progenitors (CMP) frequency and cell number were similar between the two groups. The increased GMP number was due to GMP proliferation as evidenced by the percentage of BrdU-incorporating GMP. Whole genome transcriptome analysis identified increased FcεRIα expression in β2−/− CMP compared to β2+/+ CMP. FcεRIα expression on β2−/− GMP was detected increased in β2−/− mice by qRT-PCR and FACS. Although transplantation of FcεRIα^{hi} GMP or FcεRIα^{lo} GMP into lethally irradiated CD45.1 recipient resulted in comparable myeloid cell production, transplantation of β2 deficient FcεRIα^{hi} GMP generated more myeloid cells than β2+/+ FcεRIα^{hi} GMP. GATA2 expression was increased in β2−/− GMP. Using a luciferase reporter assay, we demonstrated that mutation of the GATA2 binding site in the FcεRIα promoter region diminished FcεRIα transcription. In vitro, the addition of IgE, the ligand of FcεRIα, promoted GMP expansion, which was abrogated by inhibition of JNK phosphorylation. Integrin β2 deficiency promoted GMP proliferation and myeloid cell production, which was mediated via FcεRIα/IgE-induced JNK phosphorylation in GMP. STEM CELLS *2019;37:430–440*

SIGNIFICANCE STATEMENT

Hematopoietic stem cells activation and sustained granulocyte/macrophage progenitor (GMP) proliferation both contribute to myeloid lineage production. This study demonstrated a novel function for the GATA2/FceRIa/pJNK axis in GMP proliferation. In the absence of integrin ß2, GATA2 expression was increased which could transcriptionally activate its target gene, FceRIa. Binding IgE to FceRIa induced JNK phosphorylation leading to GMP expansion. Ultimately, GMP become constitutively activated for proliferation, leading to myelocytosis.

INTRODUCTION

Hematopoietic stem cells (HSC) are the only cell source that generates all types of blood cells in one's life. On the way to blood cell production, HSC will differentiate into common lymphoid progenitors and common myeloid progenitors (CMP). CMP further give rise to megakaryocyte/erythrocyte (MEP) and granulocyte/macrophage progenitors (GMP) [1]. Each of these steps is tightly controlled to generate the appropriate number of terminally differentiated cells that could carry out physical function for immune defense, oxygen transportation, and coagulation.

Myeloid progenitors express a series of adhesion receptors including CD44, integrin β1, and CD41 which enable them to adhere to bone marrow (BM) stromal cells [2, 3]. Interestingly, blocking of CD44 using anti-CD44 antibody not only reduced hematopoietic cell adhesion to hyaluronate but also abrogated myelopoiesis, indicating the regulatory role of adhesion receptors in hematopoietic progenitor cell proliferation and/or differentiation [2]. In line with these findings, we previously identified integrin β2 expression in hematopoietic stem/progenitor cells (HSPCs) which regulated cell adhesion and migration toward intracellular adhesion molecule-1. Although the HSPC frequency in BM cells (BMC) was comparable in integrin-*β2* knockout (*β2*−/−) mice compared with wild-type mice, transplantation of integrin *β2*−/− BMC into lethally irradiated

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CD45.1 recipient resulted in greater white blood cell production compared with transplantation of integrin $\beta2$+/+ BMC [4]. In unpublished data, we found that the GMP frequency in total BMCs was higher in integrin $\beta2$−/− mice than their $\beta2$+/+ littermate controls. We thus postulated that integrin $\beta2$ deficiency could skew myeloid progenitor proliferation, aside from affecting cell adhesion in BM niche.

To test the hypothesis, we performed genome wide transcriptome studies using microarrays on FACS-sorted CMPs isolated from integrin $\beta2$−/− and integrin $\beta2$+/+ mice. We identified that the expression of the Fc epsilon receptor I (FcεRIα), a high affinity receptor for IgE, was increased in $\beta2$−/− GMP compared with $\beta2$+/+ GMPs. Here, we also describe how the FcεRIα/IgE axis regulated GMP proliferation.

Materials and Methods

Wild-type C57BL/6J (CD45.2, H-2 kb) mice, B.6SJL-PTPRCA (CD45.1), and integrin β_2−/− with C57BL/6J background were used at the age of 8–12 weeks. To assess the impact of $\beta2$ deficiency on myeloid lineage production, competitive BM transplantation was performed using total BMC, CMP, FcεRIα^{hi} GMP, or FcεRIα^{lo} GMP isolated from $\beta2$+/+ and $\beta2$−/− mice. Microarray analysis was carried out in $\beta2$+/+ and $\beta2$−/− CMP. Detailed methods are shown in Supporting Information data.

Results

Integrin $\beta2$ Deficiency Associated with GMP Proliferation

We previously reported leukocytosis in integrin $\beta2$−/− mice [4]. Consistent with the previous study [4], the numbers of granulocytes, monocytes, and lymphocytes were all dramatically increased in the PB of $\beta2$−/− mice (Fig. 1A–1C). The absolute number of granulocytes was 2.6-fold higher in BM of $\beta2$−/− mice compared with $\beta2$+/+ mice ($7.6 \pm 1.4 \times 10^6$ per mouse vs. $20.0 \pm 1.7 \times 10^6$ per mouse, $p = .0002$, $n = 6$ for each group) (Fig. 1D). Although monocyte number in BM was 28% lower in $\beta2$−/− mice than $\beta2$+/+ mice, it did not reach statistical significance ($12.7 \pm 1.9 \times 10^6$ per mouse vs. $9.2 \pm 4.2 \times 10^6$ per mouse, $p = .54$, $n = 6$–9 for each group) (Fig. 1D). Although the numbers of HSPCs and CMP did not differ between two groups, GMP frequency and number were higher while MEP frequency and number were lower in $\beta2$−/− mice than $\beta2$+/+ mice (Fig. 1E). Representative Fluorescence activated cell sorting (FACS) analysis of CMP, GMP and MEP are shown in Supporting Information Figure S1.

To dissect whether increased GMP number in BMC was due to enhanced proliferation, BrdU was injected intraperitoneally into mice. FACS analysis illustrated that the percentage of BrdU+ CMP among CMP was similar between the two groups (30.5% ± 10.5% vs. 31.5% ± 7.4%, $p = .85$). By contrast, BrdU-incorporating GMP was 26.4% among $\beta2$+/+ GMP but increased to 43.2% in $\beta2$−/− GMP ($p = .022$), indicating enhanced GMP proliferation in $\beta2$−/− mice (Fig. 1F, 1G).

As GMP are heterogenous fractions, CFU assay was performed to define GMP subfractions. After 10 days of methylcellulose culture, the number of CFU-G, CFU-M, and CFU-GM were all higher in $\beta2$−/− mice compared with $\beta2$+/+ controls (CFU-G: 10.5 ± 3.6 vs. 30.3 ± 17.4 per mouse, $p = .004$; CFU-M: 7.0 ± 1.8 vs. 10.9 ± 4.5 per mouse, $p = .029$; CFU-GM: 8.0 ± 2.1 vs. 10.8 ± 1.5 per mouse, $p = .006$) (Fig. 1H).

Consistently, when BMC were stained with anti-lineage, anti-CD117, anti-CD115, anti-CD135, anti-Ly6c, and anti-CD11b as described before [5], the percentages of monocyte–macrophage DC progenitors (MDP) and common monocyte progenitors (cMoP) were 5.9- and 4.3-fold greater in $\beta2$−/− mice than controls (%MDP: 0.02% ± 0.003% vs. 0.10% ± 0.02%, $p = .0002$; %cMoP: 0.17% ± 0.01% vs. 0.99% ± 0.14%, $p < .0001$; $n = 8$ for each group). Likewise, the absolute numbers of MDP and cMoP were 6.1- and 4.2-fold higher in $\beta2$−/− mice than wide type controls (#MDP: 16,123 ± 2,158 per mouse vs. 71,062 ± 10,914 per mouse, $p = .0002$; #cMoP: 115,741 ± 6,704 per mouse vs. 709,327 ± 101,200 per mouse, $p < .0001$; $n = 8$ for each group) (Fig. 1I) (Supporting Information Fig. S3). Nevertheless, the percentages and absolute numbers of Ly6c^{hi} monocytes and Ly6c^{lo} monocytes were lower in $\beta2$−/− mice than controls (%Ly6c^{hi} monocytes: 0.37% ± 0.03% vs. 0.27% ± 0.02%, $p = .007$; %Ly6c^{lo} monocytes: 0.08% ± 0.01% vs. 0.03% ± 0.01%, $p = .003$; #Ly6c^{hi} monocytes: 257,377 ± 19,161 per mouse vs. 193,233 ± 11,961 per mouse, $p = .013$; #Ly6c^{lo} monocytes: 51,932 ± 5,854 per mouse vs. 23,997 ± 5,629 per mouse, $p = .004$; $n = 8$ for each group) (Fig. 1I) (Supporting Information Fig. S4).

Cytokine Expression Profiles of $\beta2$+/+ and $\beta2$−/− Mice

IL-3, IL-6, Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), tumor necrosis factor-α (TNF-α), and S100A8/A9 have been shown to promote myeloid lineage production [6–12]. Quantified by ELISA, neither IL-3 nor IL-6 differed in PB and BM fluid of $\beta2$+/+ *and* $\beta2$−/− mice ($p > .15$ for all). Although PB S100A8 levels were higher in $\beta2$−/−mice, S100A9, TNF-α, and GM-CSF levels in the PB were similar in $\beta2$+/+ *and* $\beta2$−/−mice (Fig. 2A–2D). When normalized by total amount of protein, the levels of S100A8, S100A9, TNF-α, and GM-CSF in BM fluid were 1.1-, 1.3-, 1.4-, and 1.3-fold higher in $\beta2$−/− mice compared with $\beta2$+/+ mice (Fig. 2E–2H). It has previously been reported that binding of S100A8/S100A9 to RAGE elicited GMP proliferation [13]. When BMC were stained with antibodies against RAGE and GMP surface markers, the percentage of RAGE+ GMP did not differ between $\beta2$+/+ and $\beta2$−/− mice (6.14% ± 1.04% vs.4.46% ± 1.1.9%, $p = .16$).

$\beta2$ Deficiency Enhanced Myeloid Lineage Production

Next, we characterized the impact of $\beta2$ deficiency on myeloid lineage reconstitution. Equal amounts of integrin $\beta2$+/+ BMC or integrin $\beta2$−/− BMC were mixed with the same amount of CD45.1 BMC and injected into lethally irradiated CD45.1 recipient. By chimerism analysis, transplantation of integrin $\beta2$−/− BMC resulted in greater CD8+ T cells, B220+ B cells, and CD11b+ myeloid cell production after 16 weeks of transplantation (Fig. 3A).

To compare the in vivo differentiation potential, $\beta2$+/+ and $\beta2$−/− CMP were isolated by FACS sorting and injected into irradiated CD45.2 recipients. Chimerism analysis of the recipient BMC indicated that $\beta2$−/− CMP gave rise to significantly more GMP than $\beta2$+/+ CMP although MEP production was similar between two groups (ratio of CD45.2/CD45.1 for GMP production: 0.76 ± 0.07 vs. 1.10 ± 0.08, $p = .011$; ratio of CD45.2/CD45.1 for MEP production: 0.13 ± 0.04 vs. 0.19 ± 0.02, $p = .24$; $n = 5$–6) (Fig. 3B).

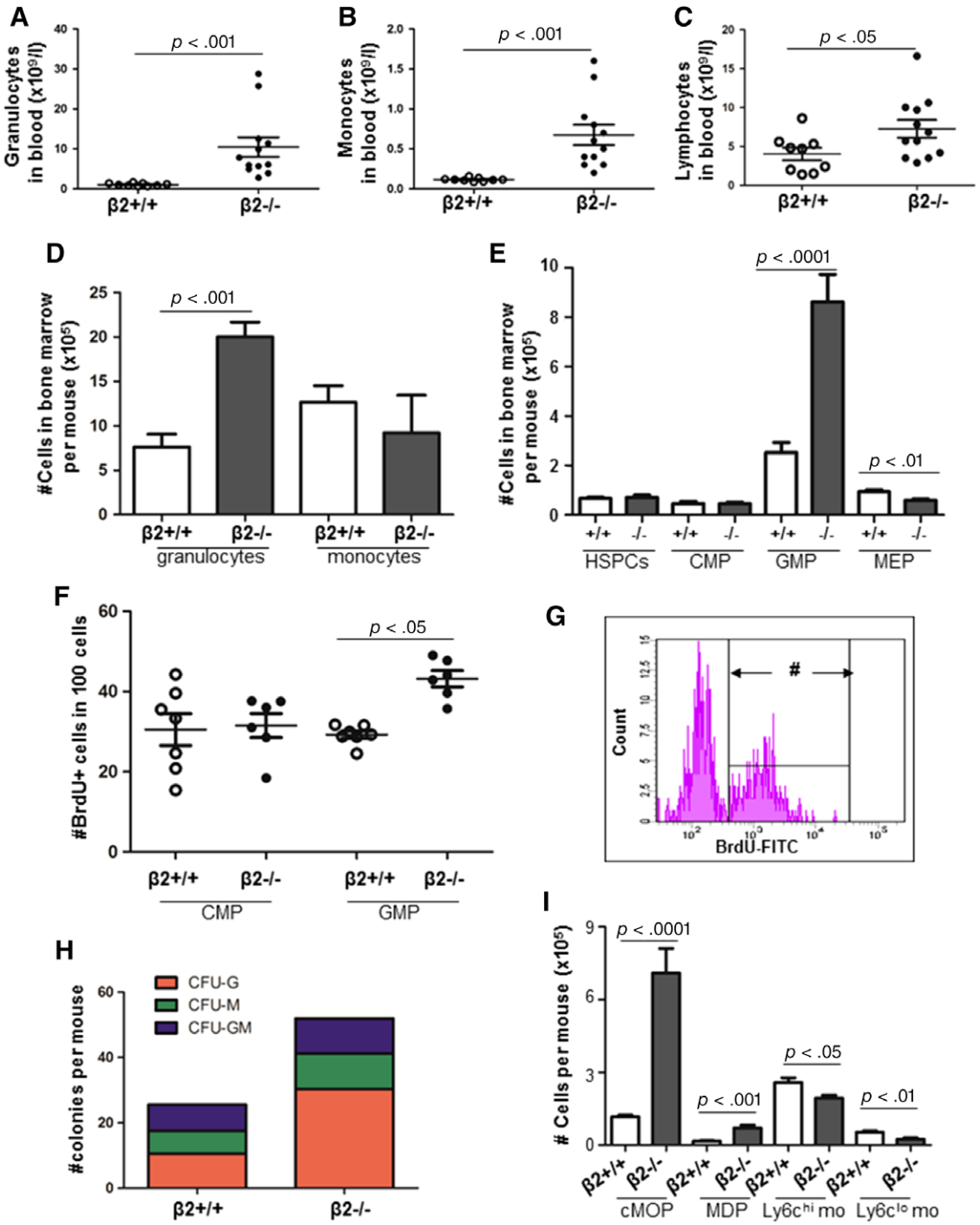

Figure 1. GMP proliferation in integrin *β2*−/− mice. The absolute number of granulocyte **(A)**, monocyte count **(B)**, and lymphocyte **(C)** in the PB of integrin *β2*−/− and wild-type mice. *n* = 9–12. **(D):** Bone marrow cells (BMC) were stained with anti-CD11b and anti-Gr-1. The numbers of GR-1$^+$ granulocytes and CD11b$^+$ monocytes were shown. *n* = 7 for each group. **(E):** BMC were stained with lineage cocktail, anti-Sca-1, anti-cKit, anti-CD16/32, and anti-CD34. The numbers of HSPCs, CMP, GMP, and MEP in BMC were obtained. *n* = 11–13. **(F):** BMCs were permeabilized and stained with surface markers together with BrdU-FITC. BrdU-incorporating CMP and GMP were analyzed by FACS. The percentage of BrdU-incorporating CMP or GMP within CMP or GMP population was shown. *n* = 6–7. **(G):** Representative BrdU+ cells when gated on lineage$^{-/low}$Sca-1$^-$cKit$^+$CD34$^+$CD16/32$^+$ cells, that is, GMP. # denoted BrdU+ cells. **(H):** Colony-forming unit assay using 1 × 10^4 BMC of *β2*+/+ and *β2*−/− mice. Ten days after plating, myeloid colonies were counted under light microscope. *n* = 8–9. **(I):** BMC were stained with anti-lineage, anti-cKit, anti-CD135, anti-CD115, anti-Ly6C, and anti-CD11b. GMP subpopulations were analyzed by FACS. cMoP: CD117$^+$CD115$^+$CD135$^+$Ly6C$^+$CD11b$^-$lineage$^{-/low}$; MDP: CD117$^+$CD115$^+$CD135$^+$Ly6C$^-$CD11b$^-$lineage$^{-/low}$; Ly6C^{hi} monocytes: CD117$^-$CD115$^+$CD135$^-$Ly6C^{hi}lineage−/low; and Ly6C^{lo} monocytes: CD117$^-$CD115$^+$CD135$^-$Ly6C^{lo}lineage$^{-/low}$. *n* = 8 for each group. Abbreviations: BrdU, 5-bromo-2-deoxyuridine; cMoP, common monocyte progenitors; CMP, common myeloid progenitors; FACS, Fluorescence Activated Cell Sorting; FITC, Fluorescein isothiocyanate; GMP, granulocyte/macrophage progenitor; HSPCs, hematopoietic stem/progenitor cells; MDP, monocyte–macrophage DC progenitors; MEP, megakaryocyte/erythrocyte progenitor; PB, peripheral blood.

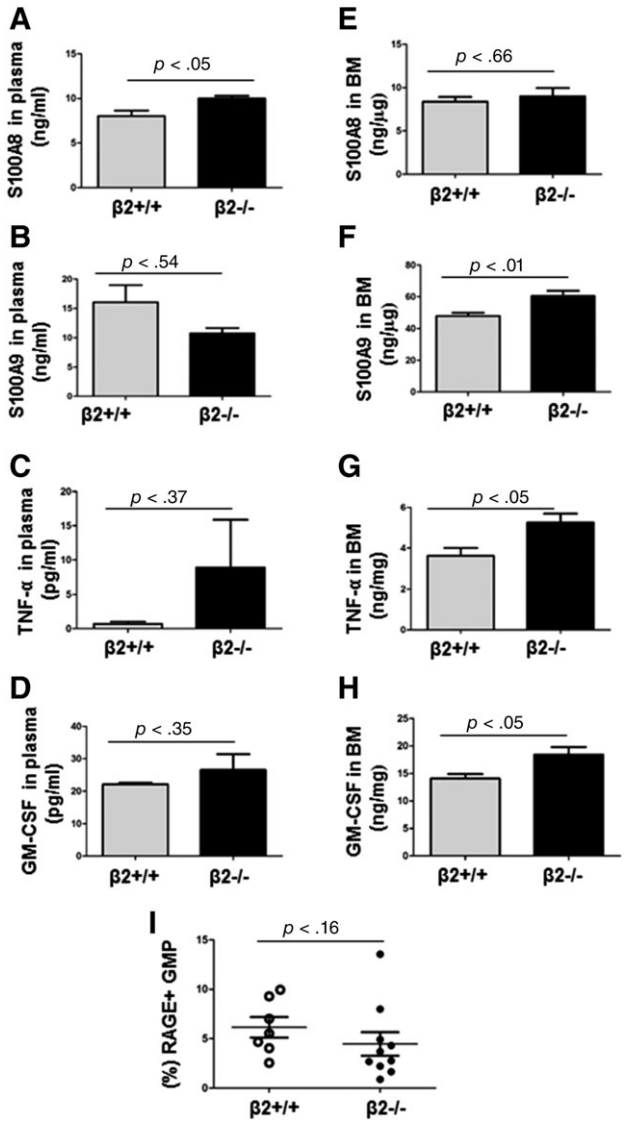

Figure 2. Cytokine expression profiles in $\beta 2$+/+ and $\beta 2$−/− mice. **(A–D):** The levels of S100A8, S100A9, TNF-α, and GM-CSF in plasma. n = 4–16. **(E–H):** The levels of S100A8, S100A9, TNF-α, and GM-CSF in BM fluid after being normalized by amount of proteins. n = 7–14. **(I):** Bone marrow cells were stained with antibody against RAGE together with surface markers of GMP. The percentage of RAGE positive GMP cells was analyzed by FACS. n = 7–10. Abbreviations: BM, bone marrow; CSF, colony stimulating factor; FACS, Fluorescence Activated Cell Sorting; GM, granulocyte-macrophagea; GMP, granulocyte/macrophage progenitor; RAGE, receptor for advanced glycation endproducts; TNF-α, tumor necrosis factor-α.

Increased FcεRIα Expression in GMP By Microarray Analysis

To further dissect the mechanism underlying the increased GMP proliferation in $\beta 2$−/− mice, CMP were sorted by FACS and whole genome transcriptome profiles were obtained by microarray analysis. Differentially expressed genes were filtered out by gene-set analysis. Pathways enriched in $\beta 2$−/− CMP as compared with $\beta 2$+/+ CMP were assessed by KEGG pathway analysis (Fig. 3C). By qPCR, the different expression levels of FcεRIα were confirmed in CMP as well as GMP (Fig. 3D and 3E).

We then validated FcεRIα protein expression on CMP and GMP by FACS. Although FcεRIα expression at the mRNA differed between $\beta 2$−/− CMP and $\beta 2$+/+ CMP, the FcεRIα^{hi} CMP subpopulation in total BMC was very small and did not differ between the groups (Fig. 4A) (FcεRIα^{hi} CMP%: 0.012% ± 0.001% vs. 0.014% ± 0.001%; p = .35; #FcεRIα^{hi} CMP: 8,582.0 ± 760.9 cells per mouse vs. 9,223 ± 792.9 cells per mouse, p = .56; n = 12–13). By contrast, FcεRIα^{hi} GMP frequency and absolute number were 3.1- and 3.2-fold increase in $\beta 2$−/− mice compared with wide type controls (FcεRIα^{hi} GMP%: 0.04% ± 0.01% vs. 0.11% ± 0.02%, p < .0001; #FcεRIα^{hi} GMP: 2.5 × 10^4 ± 0.5 × 104 per mouse vs. 8.1 × 10^4 ± 1.1 × 10^4 per mouse, p < .0001; n = 12–13) (Fig. 4A). Measurement of mean fluorescent intensity (MFI) further confirmed FcεRIα expression levels in CMP and GMP of $\beta 2$+/+ and $\beta 2$−/− mice (MFI for CMP: 134.0 ± 9.7 vs. 162.1 ± 20.1, p = .23; MFI for GMP: 248.7 ± 16.0 vs. 330.6 ± 20.2, p = .008).

The frequency and absolute number of FcεRIα^{lo} CMP was similar between the two groups (FcεRIα^{lo} CMP%: 0.3% ± 0.1% vs. 0.3% ± 0.2%, p = .37; #FcεRIα^{lo} CMP: 2.2 ± 0.2 × 10^5 per mouse vs. 1.9 ± 0.3 × 10^5 per mouse, p = .44, n = 12–13) (Fig. 4B). However, the frequency and absolute numbers of FcεRIα^{lo} GMP were 1.9- and 2.3-fold increase in $\beta 2$−/− mice compared with wide type controls (FcεRIα^{lo} GMP%: 1.2% ± 0.3% vs. 2.4% ± 0.4%, p < .0001; #FcεRIα^{lo} GMP: 3.9 ± 0.4 × 10^8 per mouse vs. 8.6 ± 1.0 × 10^8 per mouse, p < .0001, n = 12–13) (Fig. 4C). Thus, there was a discrepancy between mRNA identified by microarray analysis and protein levels defined by FACS. This could be partially explained by the contamination of CMP with GMP during FACS sorting, or different timing of mRNA and protein production during CMP differentiating into GMP. FACS analysis of FcεRIα expression on CMP and GMP of $\beta 2$+/+ and $\beta 2$−/− mice is shown in Supporting Information Figure S2.

In Vivo Differentiation Potential of FcεRIα-Expressing GMP

Because FcεRIα-expressing GMP were also defined as mast progenitors [14, 15], we examined histamine levels in both mice. The levels of histamine in plasma and BM fluid were also increased in $\beta 2$−/− mice when compared to their littermate controls (in PB: 84.2 ± 4.79 ng/ml vs. 95.3 ± 2.68 ng/ml, p = .039, n = 15–20; in BM: 0.99 ± 0.19 ng/mg vs. 2.28 ± 0.28 ng/mg, p = .003, n = 9–14). By FACS analysis, CD45+Fcer1a+cKit+ mast cell frequency in PB and BMC were comparable between two groups (PB: 0.22% ± 0.03% vs. 0.17% ± 0.04%, p = .36; BMC: 0.22% ± 0.03% vs. 0.32% ± 0.03%, p = .10; n = 7 for each group). Nevertheless, mast cell number was increased in PB and BM of $\beta 2$−/− mice compared with wide type controls (PB: 1.2 ± 0.2 × 10^7/l vs. 3.2 ± 0.8 × 10^7/l, p = .03; BM: 1.5 ± 0.2 × 10^5 per mouse vs. 2.3 ± 0.2 × 10^5 per mouse, p = .08; n = 7 for each group). Thus, the elevated mast cells contributed to the increased histamine levels in $\beta 2$−/− mice.

When we performed competitive BM transplantation using FcεRIα^{hi} GMP and FcεRIα^{lo} GMP, both types of GMP yielded comparable production of granulocytes, monocytes, and cKit+ FcεRIα+CD45 + mast cells (Fig. 4D–4F). But when FcεRIα^{hi} GMP isolated from $\beta 2$+/+ and $\beta 2$−/− mice were transplanted into irradiated recipients, $\beta 2$ deficient FcεRIα^{hi} GMP generated more granulocytes and monocytes but reduced mast cells than $\beta 2$+/+FcεRIα^{hi} GMP (Fig. 4G–4I). However, transplantation of $\beta 2$+/+FcεRIα^{lo} GMP and $\beta 2$−/−FcεRIα^{lo} GMP resulted in similar granulocyte and monocyte production (Fig. 4J, 4K). Mast cell

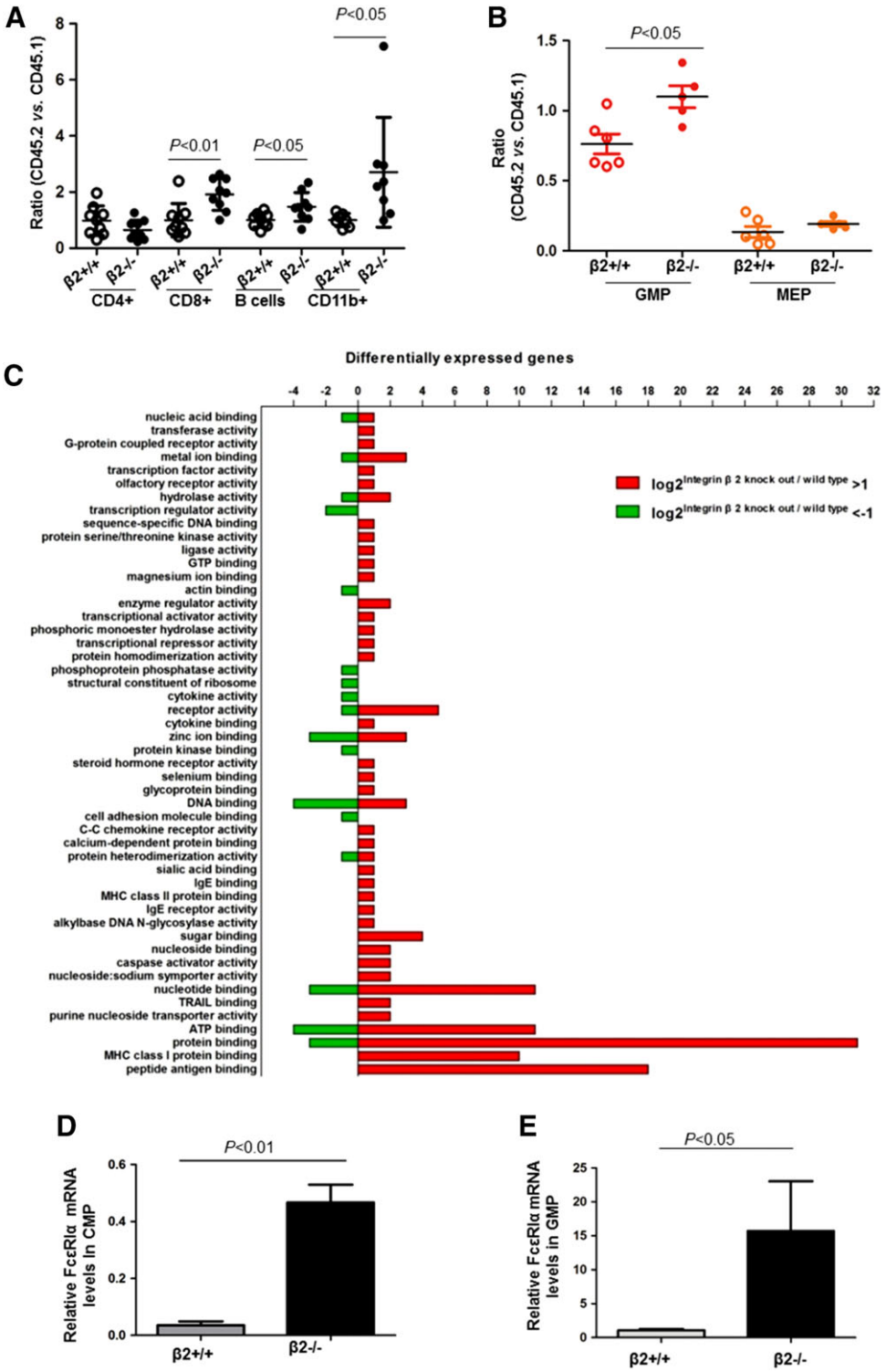

Figure 3. Microarray analysis. **(A):** Equal amount of integrin *β2*+/+ bone marrow cells (BMC) or integrin *β2*−/− BMC was mixed with 1 × 10^6 CD45.1 BMC and then injected into lethally irradiated CD45.1 recipient. Sixteen weeks after transplantation, blood cells were stained with anti-CD45.1, anti-CD45.2, and anti-CD11b for myeloid lineage production. Likewise, blood cells were stained with anti-CD45.1, anti-CD45.2 and anti-CD4, anti-CD8 and anti-B220 for chimerism analysis of lymphoid lineage production by FACS. *n* = 7–9. **(B):** *β2*+/+ and *β2*−/− CMP were sorted out by FACS and competitive transplantation was performed by injecting 1,000 CMP and 1 × 10^5 CD45.1 BMC to irradiated CD45.2 recipient. Twelve days after injection, recipient's BMC were stained with anti-CD45.1, anti-CD45.2, and GMP markers for chimerism analysis. *n* = 5–6. **(C):** CMP were sorted out by FACS and proceeded for microarray analysis. Pathways enriched in *β2*−/− CMP as compared with *β2*+/+ CMP were listed by KEGG pathway analysis. *n* = 3 for each. qRT-PCR analysis of FcεRIα in CMP **(D)** and GMP **(E)**. Gene expression was normalized to β-actin. *n* = 3–4. Abbreviations: CMP, common myeloid progenitors; FACS, fluorescence activated cell sorting; GMP, granulocyte/macrophage progenitor; MEP, megakaryocyte/erythrocyte progenitor.

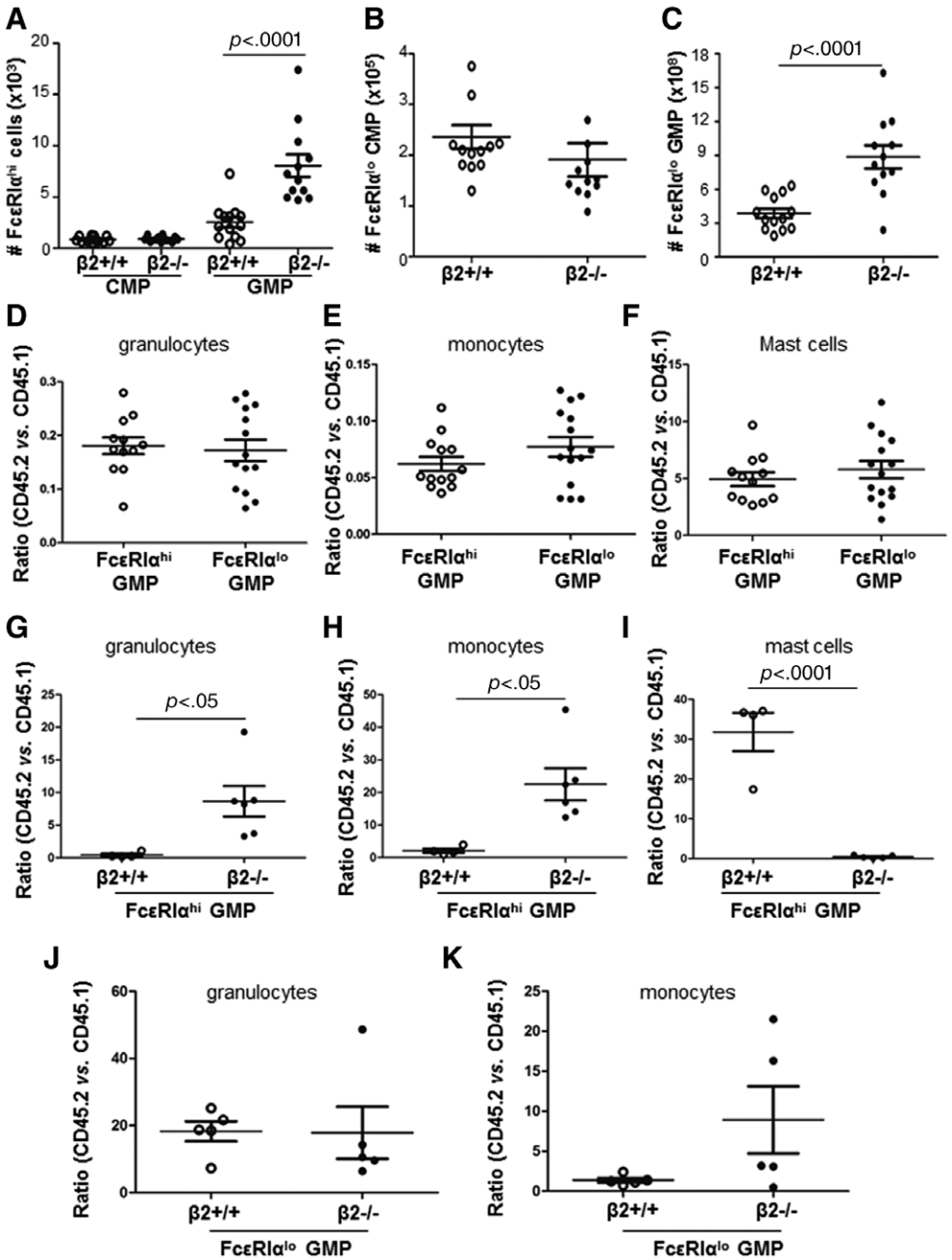

Figure 4. Characterization of FcεRIα^{hi} GMP in myeloid production. **(A):** The absolute numbers of FcεRIα^{hi} CMP and FcεRIα^{hi} GMP in *β2*+/+ and *β2*−/− mice. *n* = 12–13. **(B, C):** The absolute numbers of FcεRIα^{lo} CMP and FcεRIα^{lo} GMP in *β2*+/+ and *β2*−/− mice. *n* = 12–13. **(D–F):** Equal amount of FcεRIα^{hi} GMP or FcεRIα^{lo} GMP were mixed with 1 × 10^5 CD45.1 bone marrow cells (BMC) and then transplanted to lethally irradiated CD45.2 recipients. Twelve days after transplantation, blood cells were stained with anti-CD.1, anti-CD45.2, anti-CD11b, and anti-Gr-1 for chimerism analysis by FACS. *n* = 12–15. **(G-I):** Equal amount of FcεRIα^{hi} GMP from *β2*+/+ and *β2*−/− mice were mixed with 1 × 10^5 CD45.1 BMC and then transplanted to lethally irradiated CD45.2 recipients. Chimerism analysis was performed at 12 days after transplantation. *n* = 4–5. **(J, K):** Equal amount of FcεRIα^{lo} GMP from *β2*+/+ and *β2*−/− mice were mixed with 1 × 10^5 CD45.1 BMC and then transplanted to lethally irradiated CD45.2 recipients for chimerism analysis. *n* = 5 for each group. Abbreviations: CMP, common myeloid progenitors; FACS, fluorescence activated cell sorting; GMP, granulocyte/macrophage progenitor.

production was too low to be quantified by FACS in the recipients received with either *β2*+/+ FcεRIα^{lo} GMP or *β2*−/−FcεRIα^{lo} GMP (data not shown).

Collectively, these data suggest that FcεRIα^{hi} GMP and FcεRIα^{lo} GMP have similar capacity in granulocyte and monocyte production. In the absence of *β2*, both FcεRIα^{hi} GMP and FcεRIα^{lo} GMP were increased. And *β2* deficient FcεRIα^{hi} GMP could differentiate into more granulocytes and monocytes compared with *β2*+/+ FcεRIα^{hi} GMP.

FcεRIα/IgE-Induced GMP Proliferation via JNK Phosphorylation

IgE is a well-known high affinity ligand of FcεRIα. By ELISA, we found that the levels of IgE in plasma and BM fluid were elevated

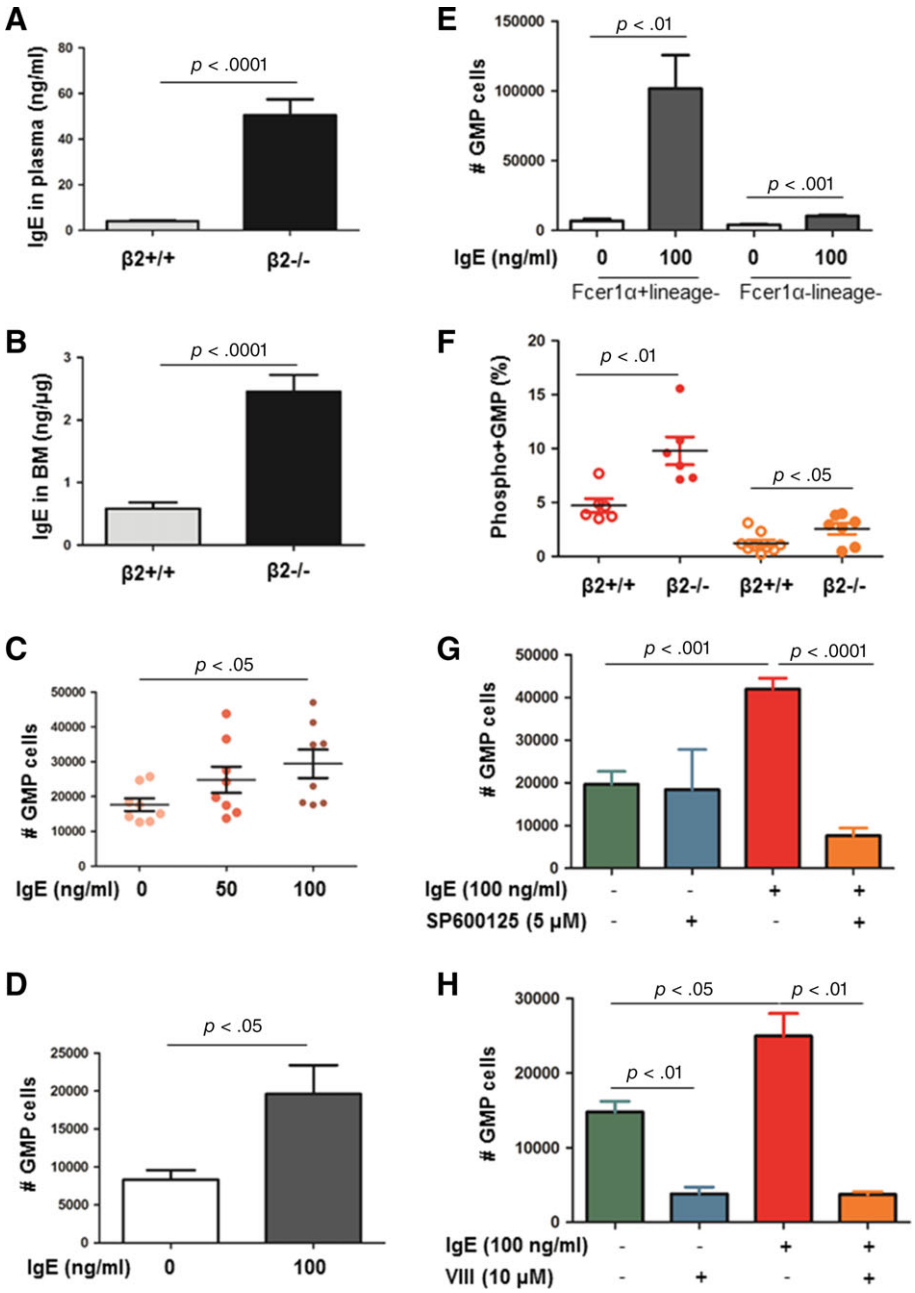

Figure 5. FcεRIα/IgE-induced GMP proliferation via JNK phosphorylation. **(A, B):** The levels of IgE in plasma and bone marrow detected by ELISA. n = 9–14. **(C–E):** Lineage$^{-/low}$ cells (C), FACS-sorted GMP (D), and FACS-sorted FcεRIα^{hi}lineage^{-} cells and FcεRIα^{-}lineage^{-} cells (E) were cultivated in vitro in the presence of IgE (0–100 ng/ml) for 5 days. Cells were harvested, numerated, and stained with GMP surface markers for FACS analysis. GMP number was computed by GMP proportion multiplied by cell number. n = 4–8. **(F):** Freshly isolated $\beta2$+/+ and $\beta2$−/− bone marrow cells were permeabilized and stained with anti-phosphoAkt (or phosphor-JNK) together with surface markers for GMP. pAkt+ GMP and pJNK+ GMP were quantified by FACS. n = 6–8. **(G, H):** Lineage$^{-/low}$ cells were cultivated with IgE in the presence or absence of pAkt inhibitor VIII (10 μM) or pJNK inhibitor SP600125 (5 μM). GMP number after cultivation was assessed as described above. n = 4–5. Abbreviations: BM, bone marrow; FACS, fluorescence activated cell sorting; GMP, granulocyte/macrophage progenitor.

in $\beta2$−/− mice (Fig. 5A, 5B). When lineage$^{-/low}$ or sorted GMP cells were allowed to differentiate in the presence or absence of IgE for 5 days in vitro, GMP number was increased by the addition of IgE (Fig. 5C, 5D). To dissect whether IgE acts through FcεRIα in GMP proliferation, FcεRIα^{hi} lineage$^{-/low}$, and FcεRIα^{lo} lineage$^{-/low}$ cells were sorted out by FACS and cultivated with or without 100 ng/ml IgE for 5 days. FACS analysis confirmed that IgE increased GMP number more dramatically in FcεRIα^{hi} lineage$^{-/low}$ than in FcεRIα^{lo} lineage$^{-/low}$ cells (Fig. 5E).

To investigate how IgE/FcεRIα induced GMP proliferation, fresh BMC of $\beta2$+/+ and $\beta2$−/− mice were permeabilized and stained with GMP markers together with antibody against phospho-ERK, phospho-Akt, or phosphor-JNK. FACS analysis did not reveal any difference in the percentage of pERK+ GMP between $\beta2$+/+ and $\beta2$−/− GMP cells (1.77% ± 0.43% vs. 2.91% ± 0.62%, p = .14). However, the percentages of pAkt+ GMP and pJNK+ GMP were both 2.1-fold higher in $\beta2$−/− GMP compared with $\beta2$+/+ GMP (Fig. 5F).

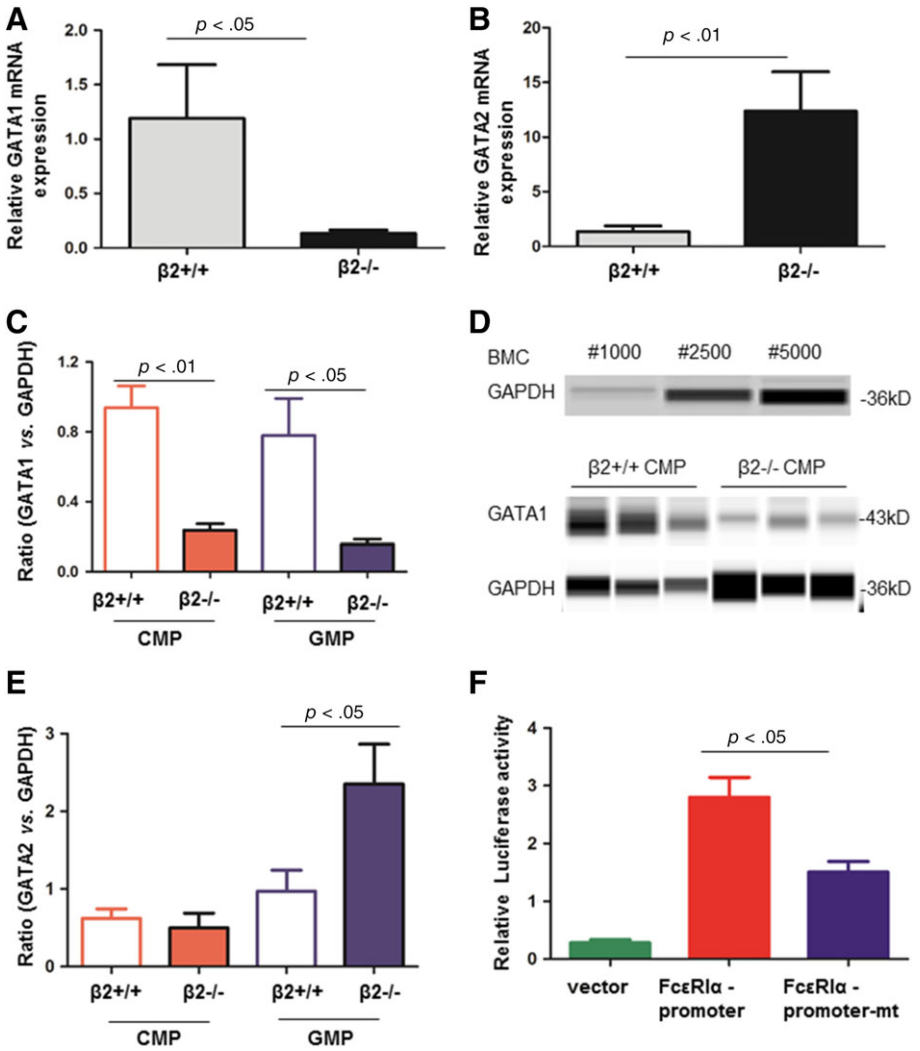

Figure 6. Transcriptional regulation of FcεRIα by GATA2. GMP were sorted out by FACS. After extracting RNA, qRT-PCR analysis the change of GATA1 **(A**, *n* = 3–5**)** and GATA2 **(B**, *n* = 6–9**)** in GMP cells. Gene expression was normalized to β-actin. **(C–E):** *β2*+/+ and *β2*−/− CMP and GMP were isolated by FACS sorting. They were lysed for protein extraction. After protein concentration determination, equal amount of proteins was separated by capillary western blot. GATA1, GATA2, and GAPDH expression were studied. Each sample was pooled from 2 to 4 mice. *n* = 3–6. **(F):** Dual luciferase reporter gene assay to analysis that whether GATA2 could be combined with FcεRIα. For FcεRIα promoter-mutant (mt) plasmid, the binding sequence of GATA2 on FcεRIα promoter region was mutated. Firefly luciferase activity was normalized to Renilla luciferase activity. *n* = 4. Abbreviations: BMC, bone marrow cells; CMP, common myeloid progenitors; FACS, fluorescence activated cell sorting; GMP, granulocyte/macrophage progenitor.

To further testify whether Akt or JNK phosphorylation was required for IgE-induced GMP proliferation, lineage$^{-/low}$ cells were stimulated with 100 ng/ml IgE in the presence or absence of Akt inhibitor VIII or JNK inhibitor SP600125 for 5 days. Inhibition of pJNK significantly abrogated the elevated GMP number induced by IgE without affecting GMP levels at basal condition. Nevertheless, inhibition of Akt phosphorylation reduced GMP number at both basal and IgE condition (Fig. 5G, 5H). Put together, these data hint that the elevation of GMP proliferation induced by IgE is mediated via phospho-JNK.

Regulation of FcεRIα Expression By GATA2

Previous data have shown that GATA1 and GATA2 are required for regulating FcεRIα mRNA and protein expression in mast cells [16]. We then tested if FcεRIα expression in GMP was also regulated by GATA2. *β2*+/+ and *β2*−/− GMP were sorted by FACS. qPCR data demonstrated that GATA2 was more highly expressed while GATA1 was less expressed in *β2*−/− GMP compared with *β2*+/+ GMP (Fig. 6A, 6B). When protein lysates of CMP or GMP were subjected for capillary western blot, GATA1 expression was reduced in both *β2*−/− CMP and *β2*−/− GMP compared with their correspondent controls (Fig. 6C, 6D). GATA2 expression was increased in *β2*−/− GMP but not in *β2*−/ CMP when compared with their correspondent controls (Fig. 6E).

To elucidate the transcriptional regulation of GATA2, HEK293 cells were co-transfected with a plasmid containing GATA2 cDNA and plasmid containing the FcεRIα promoter with luciferase as a reporter. Quantified by luciferase assay, FcεRIα promoter activity was increased. By contrast, when the binding sequences of GATA2 in the FcεRIα promoter region were mutated, transcription activity was dramatically decreased, suggesting direct regulation of GATA2 on FcεRIα (Fig. 6F).

Discussion

Maintenance of the blood system is a tightly controlled process which involves differentiation from HSC to progenitors and from progenitors to terminal differentiated blood cells [17]. In response to infection, HSC become activated to generate immune cells and myeloid cells to enhance the immune defense in the body. Once the acute infection is resolved, HSC returns to a quiescent status to retain stemness [18]. However, prolonged myelocytosis can be caused by sustained GMP proliferation even if HSC are quiescent [19, 20]. Compared with the vast number of studies exploring the regulation of HSC and HSPC biology, our knowledge of how myeloid progenitors are activated and differentiated is limited.

Integrin *β2* mediates cell adhesion to endothelial cells and stromal cells. Intriguingly, we demonstrated previously that *β2* deficient mice display increased GMP frequency and myelocytosis. Therefore, we initiated this study to examine the role of integrin *β2* in GMP proliferation. The key findings are summarized as follow: (a) *β2* deficiency increased GMP proliferation, leading to myeloid cell expansion; (b) *β2* deficiency enhanced GATA2 expression which could transcriptionally activate FcεRIα expression. Binding of IgE to its receptor, FcεRIα, potently induced GMP proliferation which was mediated via JNK phosphorylation; (c) transplantation of FcεRIα^{hi} and FcεRIα^{lo} GMP resulted in comparable granulocyte and monocyte production; and (d) *β2* deficient FcεRIα^{hi} GMP had greater capacity in giving rise to granulocytes and monocytes compared with wide type FcεRIα^{hi} GMP. Thus, GMP proliferation is delicately regulated by integrins on cell membrane and cytokines from the microenvironment [21, 22]. Our proposed model demonstrating how *β2* deficiency promoted GMP proliferation is presented in Figure 7.

This study provides an example stating of how GMP proliferation could cause exuberant myeloid cell expansion. We demonstrate that myelocytosis does not depend on HSC and HSPC activation but can result solely from myeloid progenitor proliferation. Nagareddy et al. reported that hyperglycemia stimulated GMP proliferation which was the core for monocytosis and neutrophilia in type 1 diabetes mellitus, even if HSC and HSPC frequency was not affected by the hyperglycemia in mice [13]. Mechanistically, hyperglycemia increased reactive oxygen species production and promoted S100A8 and S100A9 secretion from neutrophils in BM. Binding of S100A8 and S100A9 to RAGE on CMP triggered M-CSF production. In conjunction with GM-CSF, they induced CMP and GMP proliferation [13]. Here, we observed increased S100A9 levels in BM fluid of *β2−/−* mice but RAGE expression in GMP did not differ between the two groups.

Accumulative evidence has shown that IL-3, IL-6, GM-CSF, TNF-α, and S100A8/A9, by acting through their receptors on GMP, are able to promote myeloid cell production [6–12]. Except these conventional stimulators, our study verified that integrin *β2* could control GMP proliferation. Leukocyte adhesion deficiency (LAD) is a human disease caused by *β2* integrin deficiency [23] and mutations in the protein Kindlin-3 also causes a LAD (type III) syndrome [24]. It is well known that Talin and Kindlin bind to the cytoplasmic domains of integrin *β2* and mediate downstream signal transduction of the integrin upon ligand binding [25]. Recently, the role of Kindlin-3 in hematopoiesis was uncovered. Using Kindlin-3-deficient mice,

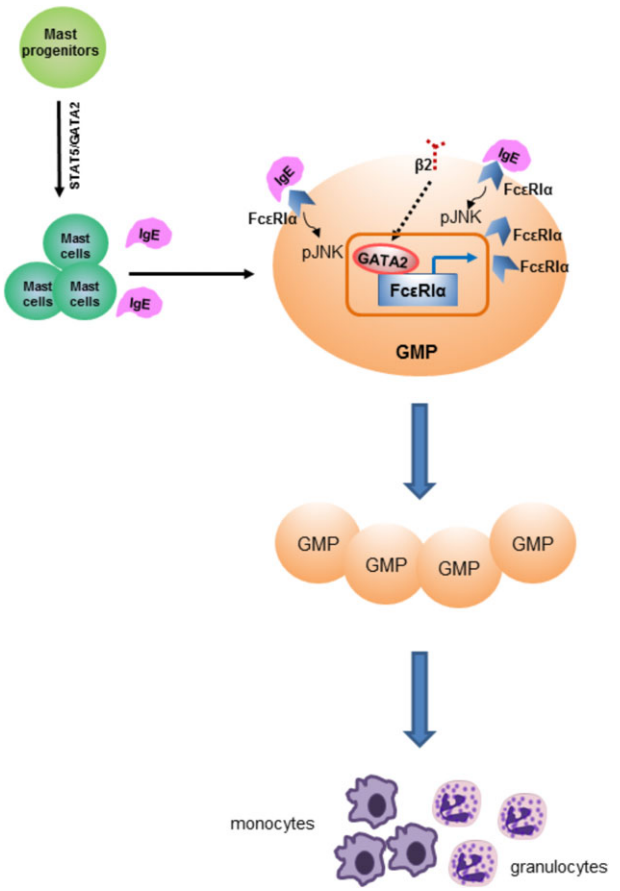

Figure 7. Proposed model. Integrin *β2* governs GMP proliferation under control. The deficiency of *β2* increases GATA2 expression in GMP, which could transcriptionally activate FcεRIα expression. Binding of IgE to FcεRIα promotes JNK phosphorylation for GMP proliferation. Ultimately, GMP become activated and constitutively remain in proliferative status. Abbreviation: GMP, granulocyte/macrophage progenitor.

they showed that Kindlin-3–deficient HSC were quiescence and remained in the BM but became hyperactivated and lost in the circulation when they were transplanted into irradiated recipients [26]. These observations together with our findings expand our understanding of integrin *β2* from cell adhesion to regulation of hematopoiesis and myelopoiesis.

GATA1 is required for the maturation of red blood cells, megakaryocytes, and mast cells [27] whereas GATA2 is a mediator of hematopoiesis, especially myeloid lineage differentiation [28]. Haploinsufficiency of GATA2 is implied in myelodysplastic syndrome and acute myeloid leukemia [29]. GATA1 and GATA2 have been identified transcription factors to FcεRI transcription in mast cells [16]. FcεRI consists of α-, β-, and γ-chains. Although transfection of small interfering RNAs (siRNAs) against GATA1 and GATA2 inhibit FcεRI expression in mast cell line, they regulate FcεR1 transcription in different manners. GATA2 siRNA suppresses both α and β transcript but GATA1 siRNA only suppresses the α transcript [16]. In our study, we observed increased mast cell number in blood and BM of *β2−/−* mice and increased GATA2 expression in *β2−/−* GMP, compared with wide type controls. In line with previous reports [15, 16], we confirmed that GATA2 could regulate FcεRIα transcription by luciferase reporter assay. These data indicate that GATA2

rather than GATA1 could be the driven force for mast cell production in $\beta 2-/-$ mice. In addition, in vitro, we demonstrated that the addition of IgE induced GMP expansion FcεRIα^{+}lineage$^{-/low}$ cells rather than FcεRIα^{-}lineage$^{-/low}$ cells. Although we do not know how $\beta 2$ deficiency increased GATA2 expression, transcriptional activation of the GATA2 target gene, FcεRIα, was the core for GMP proliferation in $\beta 2-/-$ mice.

The present study must be interpreted within the context of some potential limitations. FcεRIα^{hi} GMP are generally considered as mast progenitors [30]. First, we observed greater myeloid cell production in recipients transplanted with $\beta 2-/-$ BMC, which could be resulted from both impaired myeloid cell retention in BM niche and skewed GMP proliferation; second, we could only quantify FcεRIα^{+}CD45^{+}cKit^{+} mast cells to assess their production. Alternatively, histamine levels were elevated in the blood and BM fluid in $\beta 2-/-$ mice compared with their littermate controls. Third, we do not know how IgE levels were increased in the PB and BM fluid in $\beta 2-/-$ mice. Even though, a recent study reported that patients with type I LAD have increased risk of autoimmune complications [31]. And fourth, we do not know which partner of $\beta 2$ participated in GMP proliferation in the study. It is well known that integrin $\beta 2$ has four partners: CD11a, CD11b, CD11c, and CD11d. As reported in the literature, subjects with LAD disease were absence of CD18, CD11b, CD11c but CD11a expression and function was present [32–34]. Therefore, how $\beta 2$ integrins participate in immunity and which partner involves in skewed GMP proliferation need further investigation.

In conclusion, $\beta 2$ deficiency upregulated GATA2/FcεRIα expression in GMP. Binding of IgE to FcεRIα stimulated GMP proliferation which was abrogated by the inhibition of JNK phosphorylation. Therefore, GMP became sustained proliferation for enhanced myeloid cell production.

Conclusion

HSC activation and sustained GMP proliferation both contribute to myeloid lineage production. Herein, we demonstrated a novel function for the GATA2/ FcεRIα/pJNK axis in GMP proliferation. In the absence of integrin $\beta 2$, GATA2 expression was increased which could transcriptionally activate its target gene, FcεRIα. Binding IgE to FcεRIα induced JNK phosphorylation leading to GMP expansion. Ultimately, GMP become constitutively activated for proliferation, leading to myelocytosis.

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Author Contributions

Y.-M.F.: initiated, conducted the study, analyzed the data and drafted the manuscript; L.-J.Z., C.Y., S.S., X.-J.M., and D.Z.: performed the experiments; T.P.: generated CD18$^{-/-}$ mice and the littermates; C.M.V.: provided intellectual input and participated in manuscript preparation.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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