JLB

Article

High MafB expression following burn augments monocyte commitment and inhibits DC differentiation in hemopoietic progenitors

Kirstin Howell,[*,†] Joseph Posluszny,[†,‡] Li K. He,[†,§] Andrea Szilagyi,[†] John Halerz,[†] Richard L. Gamelli,[†,§] Ravi Shankar,[†,§] and Kuzhali Muthu[†,§,1]*

*Department of Surgery, Mount Sinai Medical Center, Chicago, Illinois, USA; †Burn and Shock Trauma Institute and §Department of Surgery, Loyola University Medical Center, Maywood, Illinois, USA; and ‡Department of Surgery, Case Western Reserve University, Cleveland, Ohio, USA

RECEIVED JULY 6, 2011; REVISED AUGUST 16, 2011; ACCEPTED SEPTEMBER 6, 2011. DOI: 10.1189/jlb.0711338

ABSTRACT

We have previously shown that perturbed bone marrow progenitor development promotes hyporesponsive monocytes following experimental burn sepsis. Clinical and experimental sepsis is associated with monocyte deactivation and depletion of mDCs. Decrease in circulating DCs is reported in burn patients who develop sepsis. In our 15% TBSA scald burn model, we demonstrate a significant reduction in the circulating MHC-II$^+$ population and mDCs (Gr1^{neg}CD11b$^+$CD11c$^+$) with a corresponding decrease in bone marrow MHC-II$^+$ cells and mDCs for up to 14 days following burn. We explored the underlying mechanism(s) that regulate bone marrow development of monocytes and DCs following burn injury. We found a robust bone marrow response with a significant increase in multipotential HSCs (LSK) and bipotential GMPs following burn injury. GMPs from burn mice exhibit a significant reduction in GATA-1, which is essential for DC development, but express high levels of MafB and M-CSFRs, both associated with monocyte production. GMPs obtained from burn mice differentiated 1.7 times more into Mϕ and 1.6-fold less into DCs compared with sham. Monocytes and DCs expressed 50% less MHC-II in burn versus sham. Increased monocyte commitment in burn GMPs was a result of high MafB and M-CSFR expressions. Transient silencing of MafB (siRNA) in GMP-derived monocytes from burn mice partially restored DC differentiation deficits and increased GATA-1 expression. We provide evidence that high MafB following burn plays an inhibitory role in monocyte-derived DC differentiation by regulating M-CSFR and GATA-1 expressions. *J. Leukoc. Biol.* 91: 69–81; 2012.

Introduction

Monocytes and DCs are potent APCs, critical in host defense, bridging the innate and adaptive immune responses. Critically injured burn patients suffer from immune-cell dysfunction, predisposing them to nosocomial infections, sepsis, and multiorgan failure [1–3]. Trauma and sepsis are often associated with a long-lasting, functional deactivation of monocytes [4] and a significant reduction in DCs [5–8], characteristic of immune suppression. Increasing the number of DCs by intrapulmonary transplantation in experimental sepsis [9] or by prophylactic treatment with appropriate growth factors in a burn model [10] improves sepsis-induced immune suppression and survival, suggesting the importance of DC preservation as a therapeutic strategy. Depletion of DCs and not Mϕ is reported in burn patients with sepsis [5, 8]. Although the origin of DCs is well described, the etiology of DC depletion following burn injury is not known.

Although the majority of DCs in mice expresses high levels of CD11c and MHC-II, at least two distinct lymphoid and myeloid developmental pathways have been identified. It was believed that DCs that express CD8a arise from lymphoid-committed progenitors [11–13], and those that express CD11b arise from myeloid-committed precursors [14, 15]. However, as evidenced in recent review articles, a common DC progenitor can give rise to lymphoid and nonlymphoid DCs [16]. Nonetheless, DCs derived from monocytes originate from myeloid-committed precursors [17] and can therefore be referred to as

Abbreviations: Flt3L=fetal liver tyrosine kinase 3 ligand, GATA-1=GATA binding factor 1, GMP=granulocyte monocyte progenitor, HSC=hematopoietic stem cell, Lin=lineage, LK=lineage-negative,cKit-positive, LSK=lineage-negative,stem cell antigen-1-positive,cKit-positive, Mϕ=macrophage, MafB=V-maf musculoaponeurotic fibrosacroma oncogene homolog, M-CSFR=monocyte-colony stimulating factor receptor, mDC=myeloid DC, MFI=mean fluorescence intensity, PBD=postburn day, Sca1=stem cell antigen 1, SCF=stem cell factor, siRNA=small interfering RNA, TBSA=total body surface area

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

1. Correspondence: Loyola University Medical Center, 2160 S. 1st Ave., Room 4251, Bldg. 110, Maywood, IL 60153, USA. E-mail: kmuthu@lumc.edu

mDCs. Emerging studies from our own laboratory indicate that myeloid commitment shifts toward monocytopoiesis [18] and that perturbed bone marrow monocyte development not only promotes hyporesponsive monocytes but also has impaired DC differentiation potential in experimental burn sepsis [7]. Granulocytes, Mϕ, and DCs arise from common bone marrow precursors [16, 19, 20]. Congruent finding by Geissmann and coworkers [21], advocating a common, clonogenic bone marrow progenitor specific for Mϕ and DCs, leads us to propose that a traumatic injury such as burns could reprogram bone marrow progenitors toward monocyte production and not DC development, causing depletion of DCs.

Lineage commitment and differentiation of bone marrow progenitors toward monocytes and DCs is tightly orchestrated by specific transcription factors and essential cytokines [22]. In this context, the transcription factor basic leucine zipper domain protein MafB is an inducer of monocytic differentiation [23], and the zinc finger protein GATA-1 is essential during DC development [24]. Although much is known about the differentiation program of precursors in normal physiology, the regulation of DCs in burn pathology remains elusive. Studying the differentiation path of the myeloid-committed bone marrow progenitors toward monocytes and DCs will provide insight into the regulatory mechanisms dictating DC depletion following burn injury.

Here, we have tested the premise that burn injury alters the differentiation potential of the myeloid-committed GMPs toward monocytes and away from DCs through transcriptional regulation. Our results show that burn injury augments monocyte differentiation through up-regulation of MafB and M-CSFRs and thereby, suppresses their responsiveness to GM-CSF and IL-4, inhibiting DC differentiation. Transient silencing of MafB mRNA in GMP-derived monocytes allows for the transdifferentiation of monocytes into DCs, which was blocked by burn injury. This could be possible through down-regulation of M-CSFR or up-regulation of GATA-1 expression or both. These results provide evidence for transcription factor-induced hematopoietic reprogramming as one of the underlying mechanisms leading to aberrations in the immune-cell constitution in burn pathology.

MATERIALS AND METHODS

Animals and burn injury

Approval from the Loyola University Medical Center's Institutional Animal Care and Use Committee (Maywood, IL, USA) was obtained for all experimental protocols. Six-week-old $B_6D_2F_1$ male mice (The Jackson Laboratory, Bar Harbor, ME, USA) were allowed to acclimatize to their environment with 12-h light/dark cycles for 1 week prior to experiment start. Food and water were provided ad libitum. All mice were anesthetized using i.p. ketamine (100 mg/kg) and xylazine (2.5 mg/kg) injection and inhaled 5% isoflurane. The dorsal fur was shaved, and animals were randomized into burn and sham groups. Burn animals received a 15% scald burn to their dorsum via 8 second immersion in a 100°C water bath, and tepid water was used for the sham animals. All animals were resuscitated with 2 ml i.p. normal saline.

Cell isolation

Mice were killed via inhaled CO_2 overdose following burn injury. Blood was collected by cardiac puncture. Bilateral femurs were harvested, and total bone marrow was eluted into McCoy's medium (Invitrogen, Carlsbad, CA, USA). Total bone marrow-nucleated cells were counted using a Neubauer hemocytometer subsequent to the lysis of RBCs with 3% acetic acid.

Hematopoietic progenitors were isolated from total bone marrow cells using FACS. Briefly, total bone marrow cells were labeled with biotin-conjugated Lin-specific primary antibodies: anti-CD86, anti-CDllc, anti-Ter119, anti-CD19, anti-B220, anti-CD11b, anti-CD90, anti-CD8a, anti-Gr1, and anti-CD3e (BD Biosciences, San Diego, CA, USA). MACS MicroBead (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany)-conjugated antibiotin secondary antibody was added, and the cells were then separated via LS columns (Miltenyi Biotec GmbH), according to the manufacturer's instructions. The Lin^{neg} cells were collected and labeled with PerCP-Cy5.5-conjugated anti-IL-7R, Pacific Blue-conjugated anti-CD34, PE-Cy7-conjugated anti-FcγR (eBioscience, San Diego, CA, USA), allophycocyanin-conjugated anti-cKit, and PE-conjugated anti-Sca1 (BD Biosciences). The LSK compartment comprising of long-term, short-term, and self-renewal repopulating potential was gated as IL-$7R^{neg}Sca1^{hi}$, $cKit^{hi}$, and the bipotential GMP population was gated as IL-$7R^{neg}$, $Sca1^{neg}$, $cKit^{hi}$, $CD34^{+}$, and $Fc\gamma R^{hi}$.

Ex vivo culture of GMPs into monocytes and DCs (GMP-Mϕ-DC)

GMPs were first differentiated into monocytes/Mϕ and then into DCs to obtain mDCs. Sorted GMPs (5×10^4) were cultured in Costar low-attachment culture plates (Sigma-Aldrich, St. Louis, MO, USA) with IMDM (Invitrogen), supplemented with 20% FBS (Gemini Bio-Products, West Sacramento, CA, USA), 2% penicillin/streptomycin (Invitrogen), and 0.1% fungizone (Invitrogen); enriched with growth factors IL-3 (20 ng/ml; Invitrogen), SCF (10 ng/ml; Stemcell Technologies, Vancouver, BC, Canada), Flt3L (20 ng/ml), and M-CSF (50 ng/ml; R&D Systems, Minneapolis, MN, USA); and maintained at 37°C with 5% CO_2 for differentiation and proliferation into monocytes. An aliquot from the cultures was analyzed for monocyte phenotype, and the rest was washed and supplemented with GM-CSF (50 ng/ml; R&D Systems) and IL-4 (20 ng/ml; Invitrogen) from Days 6 to 10 to promote DC differentiation. LPS (200 ng/ml; BD Biosciences) was added the last 48 h of culture to allow DC maturation.

Phenotype analyses

Total bone marrow cells (10^6) and peripheral blood (100 μl) were incubated with antibodies for anti-Gr1-PerCP-Cy5.5, anti-CD11b-PE-Cy7 (BD Biosciences), anti-F4/80-Pacific Blue, anti-CD11c-allophycocyanin, and anti-MHC-II-PE (eBioscience) for 20 min at 4°C. Bone marrow cells were washed with PBS (Invitrogen) and stored in 1% paraformaldehyde (Sigma-Aldrich) until flow cytometry analysis. RBCs were lysed, rinsed, and fixed using the Easy-Lyse whole blood erythrocyte lysing kit (Leinco Technologies, St. Louis, MO, USA) and analyzed by FACS. Blood and bone marrow MHC-II^{+} population was gated on histograms, and $CD11b^{+}$ $Gr1^{neg}CD11c^{+}$ cells were identified as mDCs. Approximately 5×10^5 cells from ex vivo GMP-derived monocytes and DCs were incubated with anti-F4/80, CD11b, and CD11c, as mentioned earlier. Only the viable cells were included based on the Fixable Dead Cell stain kit (Molecular Probes, Invitrogen, Eugene, OR, USA) for further analysis. $CD11c^{-}F4/80^{+}$ cells were identified as monocytes, and $CD11b^{+}CD11c^{+}$ cells were identified as mDCs from monocyte and DC differentiation cultures, respectively. Details of phenotype gating on GMP-derived Mϕ and GMP-derived DCs are shown in Supplemental Fig. 1A and 1B, respectively.

Intracellular transcription factors

GMPs (5×10^4) isolated from sham and burn mice were fixed and permeabilized using Cytofix/Cytoperm Plus (BD Biosciences) for 20 min. Nonspecific binding of primary antibodies was prevented by using 1% BSA before treating with rabbit primary mAb anti-PU.1 (Cell Signaling Technology, Beverly, MA, USA) or anti-MafB (Abcam, Cambridge, UK) for 24 h or anti-GATA-1 (Cell Signaling Technology) or anti-CEBP-α (Cell Signaling Technology) for 48 h. After rinsing with perm wash (BD Biosciences), cells were treated for 2 h with secondary FITC-conjugated antirabbit antibodies (Abcam), rinsed, and resus-

pended in 1% paraformaldehyde. An aliquot was cytospun for confocal imaging, and the rest was used for flow cytometry.

Confocal microscopy

Two methods were used to prepare cells for confocal microscopy. Following intracellular staining of GMPs for the transcription factors (FACS), an aliquot was cytospun onto microscopic slides, or the GMPs were cultured in adherent chamber slides for DC differentiation and incubated with antibodies specific for DC phenotype and preserved using Vectashield H-1500 mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA) until analyzed with a Zeiss LSM 510 laser-scanning microscope (Carl Zeiss MicroImaging GmbH, Germany). C-Apochromat 40× 1.20 water immersion was used for viewing and acquired the image using Zeiss LSM 510, version 4.2, SP1 software.

siRNA silencing

MafB expression was silenced in isolated GMPs (5×10^4) from burn mice and GMP-derived monocyte populations (10^6) prior to DC differentiation on Day 6 using the siRNA technique. Briefly, cells were rinsed with Opti-MEM (Invitrogen) to remove any residual FBS and then transfected with MafB siRNA (40 pmoles/10^6 cells; Santa Cruz Biotechnology, Santa Cruz, CA, USA) by the addition of X-tremeGENE siRNA transfection reagent (Roche Diagnostics, Mannheim, Germany) following kit instructions. After incubating for 4 h, DC differentiation cocktail was added as described previously for DC development and maturation. A control transfection with scrambled, nonspecific siRNA did not promote gene silencing as determined by protein transcription after 72 h.

Statistical analyses

All experiments were done with four to six animals/group and repeated two to three times, and the results are expressed as mean ± SEM. For comparison between groups, multivariate analysis was conducted using ANOVA statistics, followed by Tukey's post-hoc test for significance. Statistical significance was set at a two-tailed $P < 0.05$. Statistical comparison between two groups was done by Student's t test on unpaired data with equal variance using the KaleidaGraph program (Synergy Software, Reading, PA, USA).

RESULTS

MHC-II$^+$ cells and mDCs are down-regulated by burn injury

Down-regulation of human leukocyte antigen DR (equivalent of MHC-II in mouse) is a prognostic indicator for susceptibility to secondary infections in burn patients and predicts mortality in septic shock [25–27]. Therefore, we first evaluated the status of MHC-II$^+$ expression in blood and total bone marrow cells in our 15% scald burn model. Briefly, $B_6D_2F_1$ mice were randomly distributed into sham and burn groups before subjecting to scald burn, as detailed in Materials and Methods. Blood and total bone marrow were harvested from mice on PBD 5, 7, and 14, incubated with antibodies for MHC-II, and analyzed by flow cytometry. Four sham mice were included in the study at each time-point of harvest after burn injury as control. Scatter plots in **Fig. 1A** illustrate the percentage of MHC-II$^+$ cells in whole blood and total bone marrow cells, respectively. The percentage of MHC-II$^+$ cells in blood and bone marrow compartments was reduced significantly over the course of burn as compared with sham. However, the numbers were not significantly different between the burn groups on

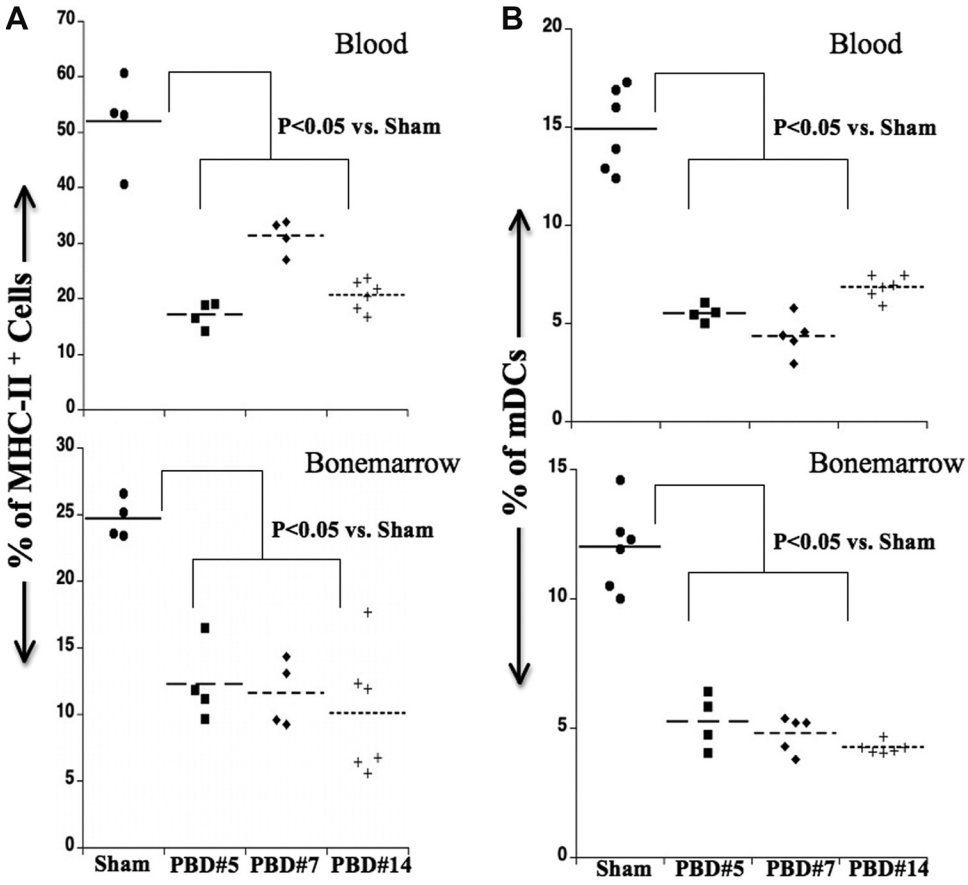

Figure 1. MHC-II$^+$ cells and mDCs are reduced in response to burn injury. Mice were subjected to sham burn or 15% TBSA burn. Blood and total bone marrow cells were collected from sham and burn mice on Days 5, 7, and 14 after burn injury and analyzed for MHC-II expression and mDC distribution by flow cytometry. Detailed mDC gating is given in Supplemental Fig. 2. Scatter plot representing the percentage of (A) MHC-II$^+$ cells and (B) mDCs in blood and bone marrow is decreased on PBD 5, 7, and 14 compared with the average of sham values obtained at each time-point. All experiments were repeated twice with at least four animals in each group.

PBD 5, 7, and 14. Therefore, the 15% TBSA scald burn model holds true with respect to burn-induced global suppression starting on PBD 5, as determined by MHC-II expression in blood and bone marrow cells.

Next, to establish that our 15% TBSA scald burn model induced changes in the peripheral blood mDC fraction, similar to clinical observations [5], we surveyed the temporal distribution of mDCs in response to burn on PBD 5, 7, and 14. In mice, the $CD11b^+Gr1^{neg}CD11c^+$ phenotype is characteristic of mDCs [28, 29], which originate from the bone marrow and populate blood, spleen, and other lymphoid organs. Therefore, any changes in mDCs, which are reflected in peripheral blood, should also be seen in the bone marrow compartment. Blood and total bone marrow were harvested from sham and burn mice on PBD 5, 7, and 14 and incubated with antibodies for CD11b, CD11c, and Gr1 for analysis by flow cytometry. $CD11b^+$ cells were first selected on a histogram and then gated on CD11c (*x*-axis) and Gr1 (*y*-axis) expressions. The percentage of $Gr1^{neg}CD11c^+$ cells present in the $CD11b^+$ fraction of whole blood is represented in the boxed area as mDCs. The majority of the mDC fraction was negative for F4/80 staining (Supplemental Fig. 2). Similar gating strategies were followed for total bone marrow cells. Respective results from blood and bone marrow are given as scatter plots in Fig. 1B. We observed a significant reduction in mDCs in peripheral circulation and in the bone marrow compartment on PBD 5, 7, and 14, as compared with sham, with no significant changes between the different PBDs.

The mean values from four to six different samples are shown in the scatter plots, and all experiments were repeated twice. Results from sham mice harvested at every time-point were steady, and therefore, the averages were taken for sham values in Fig. 1A and B. The results clearly indicate a temporal down-regulation of $MHC\text{-}II^+$ cells, as well as a reduction in mDCs following burn injury. Moreover, changes in $MHC\text{-}II^+$ cells and mDCs, as reflected in blood, also prevail in the bone marrow at all time-points (PBD 5, 7, and 14) tested, emphasizing the dysregulation of MHC expression and mDC production after burn injury. Overall, the scald burn model establishes similarity in blood and bone marrow over reduced levels of $MHC\text{-}II^+$ cells and mDCs, providing a strong platform to study bone marrow development of mDCs following a burn injury.

Hematopoietic progenitors are increased following burn

Bone marrow progenitors have the ability to reconstitute the blood cells on demand. Burn injury certainly increases the demand for immune cells for surveillance and immune responses. Therefore, to rule out bone marrow failure as the cause of DC depletion, we looked at the constitution of bone marrow hematopoietic progenitors upstream of monocyte and common DC progenitors [30] on PBD 0, 2, 5, and 7 following burn injury. For these experiments, total bone marrow cells were depleted of Lin-committed cells by negative selection using magnetic beads and sort-purified by flow cytometry to characterize the progenitor populations [31]. Briefly, total bone marrow cells were processed to obtain the Lin^{neg} fraction by magnetic isolation. $IL\text{-}7R^+$ cells, which were gated out from the Lin^{neg} population, were selected for $cKit^+$ expression (LK; $Lin^{neg}IL\text{-}7R^{neg}cKit^+$). The LKs were again gated for $Fc\gamma R^{hi}$ and $CD34^+$ expression and sorted as GMPs already committed to myeloid lineage. Similarly, LSKs ($Lin^{neg}IL\text{-}7R^{neg}Scal^+cKit^+$), which contain short- and long-term repopulating, multipotent progenitors, were sorted from the Lin^{neg} fraction based on $Scal^+cKit^+$ expressions. **Fig. 2A** represents the Lin^{neg} cells isolated by magnetic columns (MACS) gated for $IL\text{-}7R^{neg}$ fraction. Fig. 2B and 2C illustrates the gating strategies for LSK and GMP fractions representing sham and burn. The bar graphs in Fig. 2D and E represent the mean and SEM of four different samples. Accordingly, we observed a significant increase in GMPs on PBD 5 and 7. Moreover, burn injury also augmented the LSK population with a multi-Lin potential that is upstream of GMPs as early as PBD 2, which increased progressively on PBD 5 and 7, indicating a continuous bone marrow turnover.

GMPs give rise to myeloid cells; similarly, monocytes and mDCs, which express MHC-II, are myeloid in origin. To delineate that the reduction in mDCs and $MHC\text{-}II^+$ cells following burn is not a result of defects in the proliferation of bone marrow progenitors, we evaluated the myelopoietic proliferation over the course of scald burn. Bone marrow myeloid fraction was followed for up to 21 days after burn injury. We observed a significant increase in the absolute number and percentage of $CD11b^+$ (myeloid marker) $CD45^+$ (pan hematopoietic marker) bone marrow cells starting on PBD 5, which remained high until PBD 21, reaching a peak on PBD 7 [32], suggesting a robust bone marrow proliferative response to burn injury.

Taken together, results indicate that burn injury results in a continuous reconstitution of the bone marrow myeloid population with a concomitant increase in progenitors committed for monocytic/granulocytic lineage and the multipotent stem cells that feed the myeloid population. Therefore, bone marrow failure or lack of bone marrow precursors is not the reason for mDC depletion in burn injury.

Burn injury augments monocyte-specific MafB and M-CSFR expressions in bone marrow progenitors

Although burn injury augmented bone marrow production of GMPs with the potential to give rise to granulocytes and monocytes (precursors of mDCs), it is plausible that their Lin commitment is skewed to favor one Lin over another. The Lin commitment, proliferation, and differentiation of hematopoietic progenitors are tightly regulated by transcription factors. Therefore, it is important to determine the expression of transcription factors that play a pivotal role in granulocyte/monocyte/DC lineage commitment of GMPs. The present study focused on myeloid-specific PU.1, granulocyte-specific CEBP-α, monocyte/Mϕ-specific MafB, and DC-specific GATA-1 transcription factors. Traditionally, the expression of these intracellular proteins is determined by Western blots or EMSA. Given that GMPs are only 5–10% of the Lin-negative bone marrow population, the yield/femur is extremely small and restricts such analyses, requiring an unreasonable number of animals to be killed. Alternately, we treated 5×10^4 GMPs from sham and burn mice with primary antibodies to the target transcription factors and identified the positive cells by flow cytometry using FITC-conjugated secondary antibodies. The

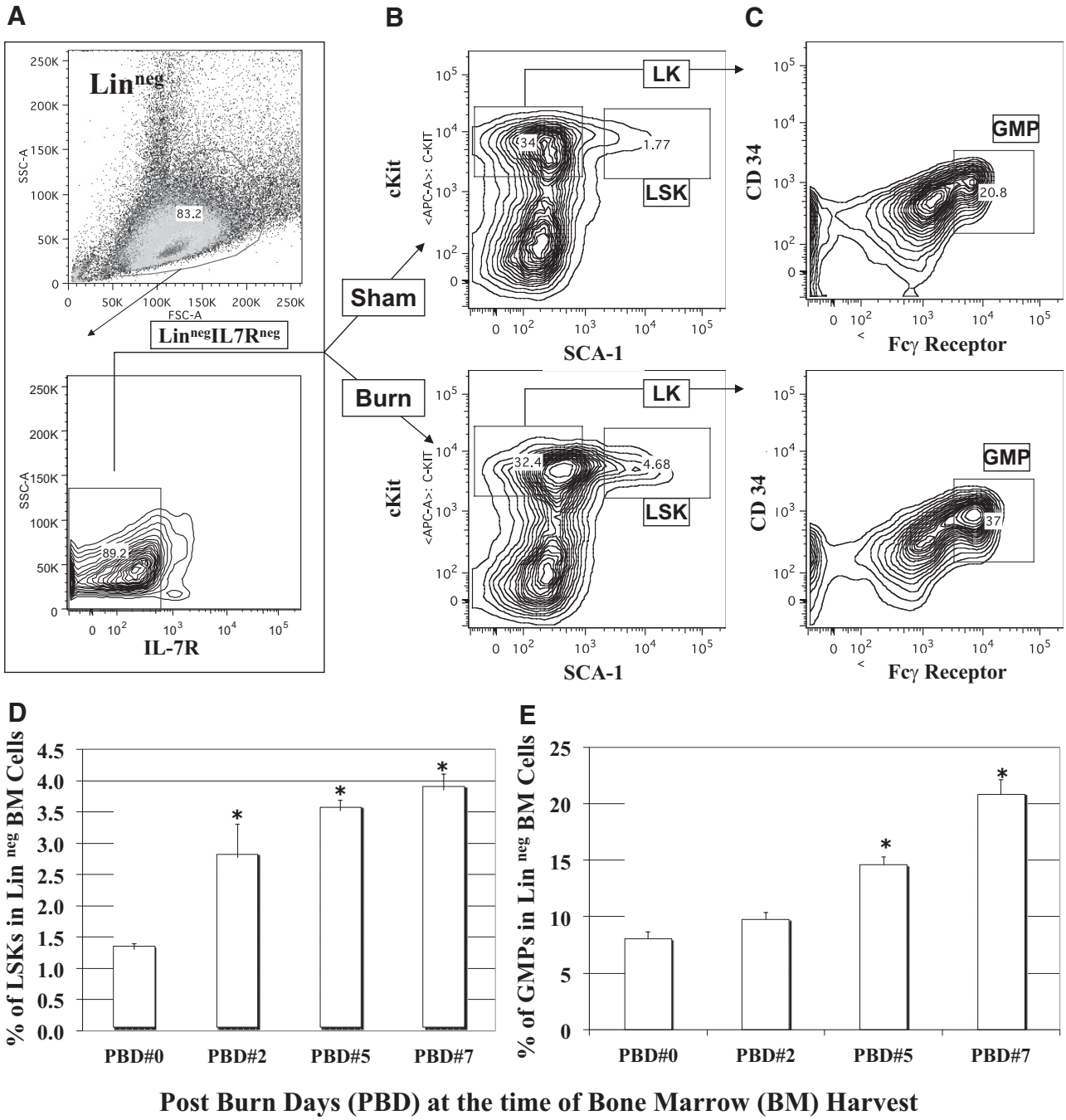

Figure 2. Burn injury augments bone marrow response. Total bone marrow cells were collected from sham and burn mice on Day 0 and on PBD 2, 5, and 7 and analyzed for hematopoietic progenitor populations by flow cytometry. (A) Lin-positive cells were depleted by negative selection of total bone marrow cells using antibody cocktail and MicroBead on magnetic columns. Lin^{neg} cells were further subjected to five-color fluorescence sorting to obtain the IL-$7R^{neg}$ fraction. Gating strategies for obtaining (B) LSK cells (IL-$7R^{neg}Sca1^{+}cKit^{+}$) and (C) GMPs (IL-$7R^{neg}Sca1^{neg}cKit^{+}Fc\gamma R^{hi}CD34^{+}$) from sham and burn. Percentage of LSKs and GMPs that constitute the bone marrow (BM) following burn is represented as bar graphs in D and E, respectively. All experiments were repeated three times with at least four animals in each group (mean±SEM; $n=4$; $^{*}P<0.05$ vs. sham). SSC-A, Side-scatter-area; FSC-A, forward-scatter-area; APC-A, allophycocyanin-area.

relative expression between groups was determined by the MFI and expressed as percentage change over sham; the results were visually verified by confocal microscopy. As we saw a maximum induction in GMPs on Day 7 after burn injury, we chose PBD 7 to study the transcriptional changes following burn. Representative histograms of GMPs from sham (red) and burn (blue) animals expressing intracellular PU.1, CEBP-α, MafB, and GATA-1 transcription factors and cell surface CD115 (M-CSFRs) are dis-

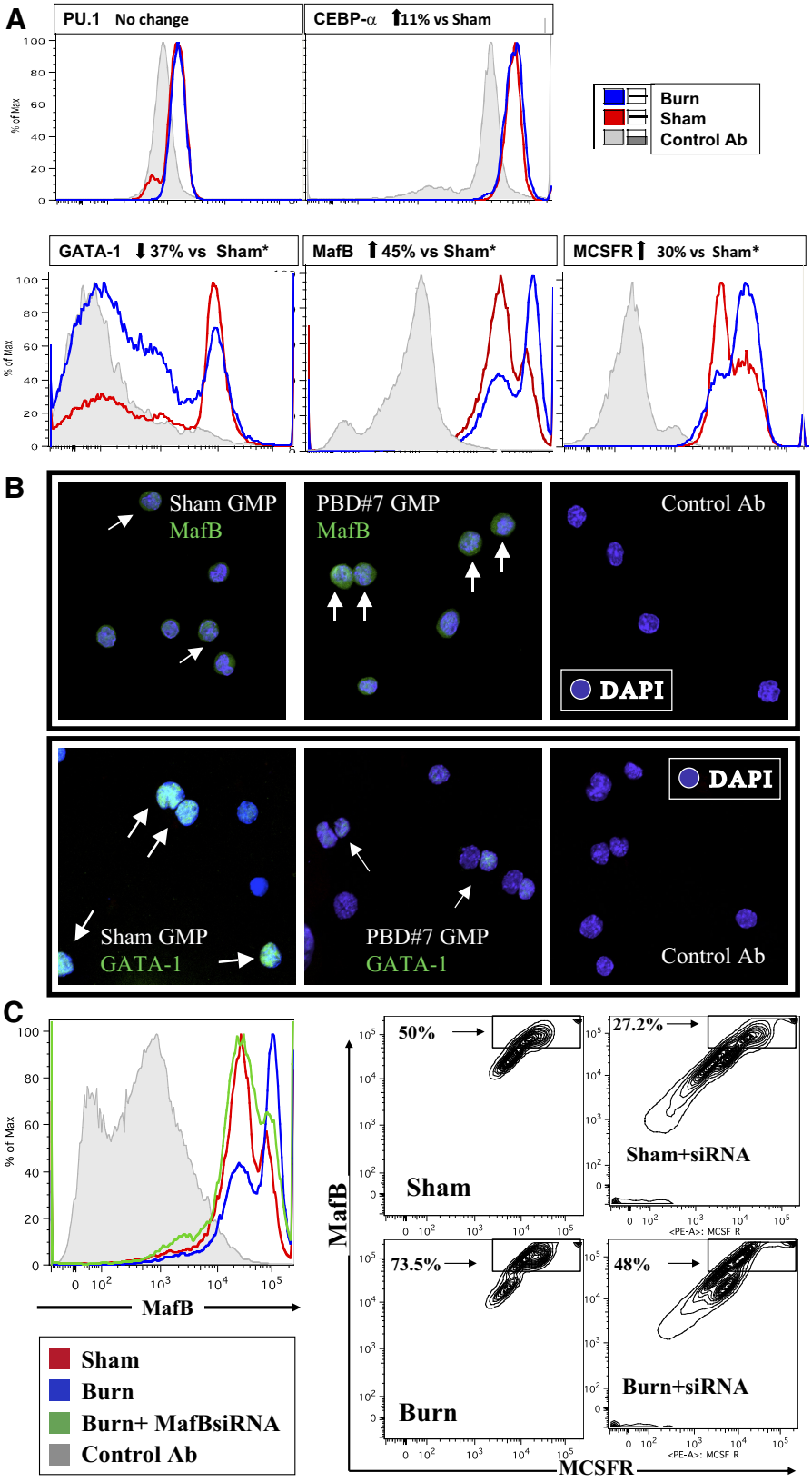

Figure 3. (A) Changes in relative expression of transcription factors. Sorted GMPs from sham and burn mice (PBD 7) were stained for surface expression with anti-M-CSFR antibody or for intracellular expression with anti PU.1, CEBP-α, MafB, and GATA-1 antibodies and analyzed by flow cytometry. Superimposed histograms show transcription factor expressions in sham (red) and burn (blue). The MFI in sham was taken as 100% for each transcription factor measured. There is no change in PU.1 expression, minimal increase in CEBP-α, large increase in MafB, and significant reduction in GATA-1 expressions following burn. M-CSFRs are increased in GMPs following burn injury. All experiments were repeated two times ($n=3$; $^*P<0.05$ vs. sham). (B) Confocal images display MafB and GATA-1 expressions (green) in GMPs, obtained from sham and burn (PBD 7) mice. High expression (thick arrow stems) of MafB and low expression of GATA-1 (thin arrow stems) are more prevalent following burn injury. (C) Gene-targeted MafB silencing. MafB expression was silenced in GMPs from burn mice using targeted siRNA or a scrambled, nonspecific control before placing in culture for monocyte differentiation. The superimposed histogram demonstrates (70–80%) reduction in MafB expression in burn GMPs following siRNA transfection. In the contour plots, compared with sham, a significant percentage of GMPs from burn mice expresses M-SCFR and MafB, which is attenuated by MafB siRNA transfection. All experiments were repeated three times. Three to four animals were pooled, and at least three different samples were analyzed in each group.

played in **Fig. 3A**. We noticed a steady level of PU.1 in the myeloid-committed progenitors, which is not surprising, as medium levels of PU.1 are associated with myeloid commitment of multipotent progenitors. In terms of granulocyte- versus monocyte-restricted transcription factors, only a marginal increase in CEBP-α but a significant (45%) increase in MafB following burn injury was observed, suggesting a preference toward monocyte commitment in burn GMPs. This theory is strengthened further by a 30% increase in M-CSFRs, signifying potential responsiveness to the cytokine M-CSF by GMPs following burn [33]. In contrast, the DC-specific GATA-1 expression [24, 34] was reduced by 30% in GMPs as a result of burn. The confocal images in Fig. 3B demonstrate GMPs from sham and burn mice, where green represents the intracellular transcription factors MafB or GATA-1 expressions, and blue represents the nucleus (DAPI). Thick arrow stems point to high expression and thin ones to low expression. The specificity of primary antibodies used is confirmed by lack of green fluorescence in GMPs treated with FITC-conjugated secondary antibody only (control antibody).

Targeted silencing of MafB gene in GMPs from burn mice down-regulates M-CSFRs

To determine the role of MafB on M-CSFR expression, GMPs from burn (PBD 7) mice were subjected to targeted disruption of MafB transcription by adding MafB siRNA. Forty-eight hours later, we checked the transfection efficiency by measuring the MFI of MafB. We also evaluated M-CSFR expressions in GMPs, with and without MafB silencing by flow cytometry. As presented in the superimposed histogram in Fig. 3C, MafB transcription was reduced significantly with a transfection efficiency of 70–80% in burn GMPs within 48–60 h of transfection [sham (red)=21,298±2235; burn (blue)=39,321±1890; burn+siRNA (green)=22,753±4329]. Moreover, burn injury augmented the percentage of GMPs (70–75%), expressing MafB and M-CSFR, whereas MafB silencing brought down the dual-expressing fraction to sham (45–50%) levels. Similarly, MafB siRNA in sham GMPs significantly reduced M-CSFR-expressing cells (25–30%), establishing a role for MafB in M-CSFR expression following burn injury (contour plots in Fig. 3C).

These results strongly indicate that GMPs from burn-injured mice have the potential for monocyte differentiation, as evidenced by enhanced MafB and M-CSFR expressions. It becomes apparent that burn injury skews the lineage commitment of bone marrow progenitors to produce more monocytes—the precursors of mDCs—but are they capable of generating abundant mDCs?

Burn injury promotes monocyte commitment and impairs DC differentiation in GMPs

To address the question of whether the myeloid precursors resulting after burn injury can produce mDCs, we studied the monocyte/Mϕ versus DC differentiation profile of GMPs, which are shown to differentiate into monocyte/Mϕ ex vivo in the presence of M-CSF-rich cocktail [18, 31], and these monocytes can then be differentiated into DCs with GM-CSF and IL-4 [35]. The differentiation into monocyte/Mϕ was initiated by placing the GMPs, obtained from sham and PBD 7 mice, in M-CSF-rich cocktail, whereas the morphology was verified periodically using a phase-contrast microscope. At the end of monocyte differentiation (Day 6), an aliquot was taken to evaluate the phenotype of differentiated cells using monocyte-specific markers by flow cytometry. Detailed gating strategy for GMP-derived Mϕ is given in Supplemental Fig. 2A. The rest of GMP-derived monocyte cultures was washed and placed in GM-CSF + IL-4-rich cocktail for DC differentiation from Days 6 to 12 and analyzed for the presence of DC-specific antigens, as detailed in Materials and Methods. Detailed gating strategy for GMP-derived DCs is given in Supplemental Fig. 2B. The results from monocyte cultures are represented as histograms in **Fig. 4A**. As predicted, GMPs from burn mice in monocyte cultures gave rise to a high percentage of $F480^+$ monocytes in comparison with sham (burn: 70±2% vs. sham: 43±3.5%; $P<0.05$). Similarly, ~80% of the monocyte cultures from burn GMPs expressed M-CSFR compared with 60% in sham. On the other hand, MHC-II-expressing cells were reduced significantly in monocyte cultures following burn injury (burn: 26±0.4% vs. sham: 46±2%; $P<0.05$). In contrast to monocyte cultures, the percentage of $CD11c^+$ cells was reduced significantly in burn versus sham DC cultures (burn: 26.57±1% vs. sham: 42.8+0.6%; MFI of CD11c expression, burn: 820±34 vs. sham: 1704±100; $P<0.05$). MHC-II molecules are concerned with the processing of extracellular pathogens and presentation of the antigens to $CD4^+$ T cells and are therefore essential for monocytes and DCs to perform as potent APCs. Here, we found that MHC-II expressions in $CD11c^+$ cells from DC cultures were reduced by 50% following burn in comparison with sham (burn: 21.5±0.4% vs. sham: 40.5+1.9%; MFI of MHC-II expression, burn: 5283±421 vs. sham: 12,024±1245; $P<0.05$). Results from DC cultures are represented in Fig. 4B. GMP-derived DCs exhibited the characteristic veil-like morphology, as shown in the confocal images.

Taken together, our results indicate that burn injury alters the lineage commitment of bone marrow GMPs toward monocyte differentiation and away from DCs. Interestingly, GMP-derived monocyte and DC populations from burn mice exhibited a reduction in MHC-II expression, characteristic of a suppressive phenotype.

Targeted silencing of the MafB gene in GMP-derived monocytes offsets the deficit in DC development caused by burn

If high expression of MafB and M-CSFRs in GMPs is promoting M-CSF-responsive monocyte differentiation and inhibiting GM-CSF-responsive DC differentiation, then silencing of MafB gene expression in GMP-derived monocytes following burn should offset the suppression in DC differentiation. To test the inhibitory role of MafB in DC differentiation, we used the method of targeted gene silencing. GMPs from burn (PBD 7) mice, placed in M-CSF-rich monocyte differentiation cocktail (for 6 days), were subject to targeted disruption of MafB transcription by adding MafB siRNA before placing in GM-CSF + IL-4-containing DC differentiation cocktail for 7 days. LPS was added during the last 72 h of culture to augment DC matura-

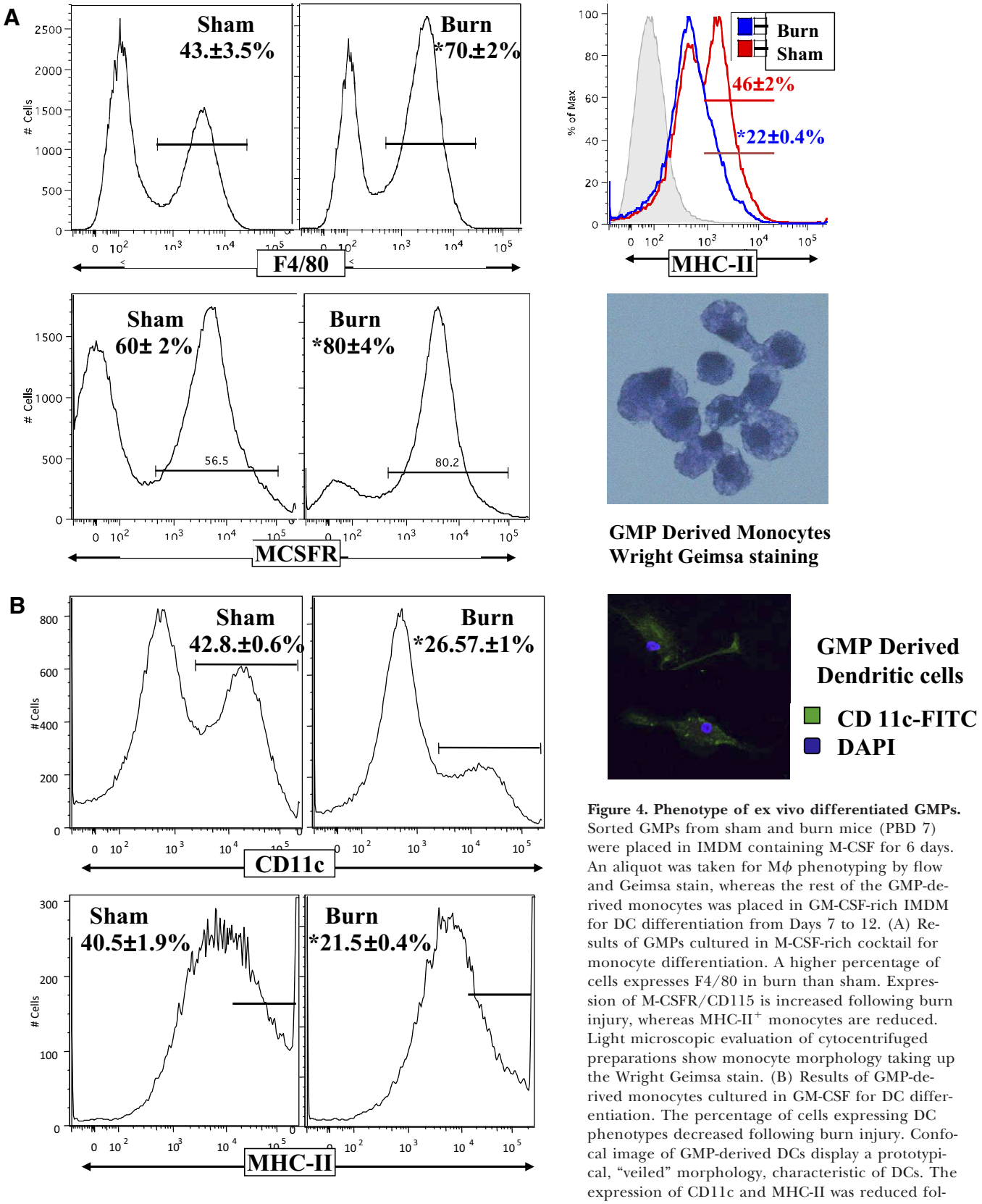

Figure 4. Phenotype of ex vivo differentiated GMPs. Sorted GMPs from sham and burn mice (PBD 7) were placed in IMDM containing M-CSF for 6 days. An aliquot was taken for Mϕ phenotyping by flow and Geimsa stain, whereas the rest of the GMP-derived monocytes was placed in GM-CSF-rich IMDM for DC differentiation from Days 7 to 12. (A) Results of GMPs cultured in M-CSF-rich cocktail for monocyte differentiation. A higher percentage of cells expresses F4/80 in burn than sham. Expression of M-CSFR/CD115 is increased following burn injury, whereas MHC-II$^+$ monocytes are reduced. Light microscopic evaluation of cytocentrifuged preparations show monocyte morphology taking up the Wright Geimsa stain. (B) Results of GMP-derived monocytes cultured in GM-CSF for DC differentiation. The percentage of cells expressing DC phenotypes decreased following burn injury. Confocal image of GMP-derived DCs display a prototypical, "veiled" morphology, characteristic of DCs. The expression of CD11c and MHC-II was reduced following burn compared with sham. All experiments were repeated three times with at least four animals in each group (mean±SEM; n=4; *P<0.05 vs. sham).

tion and was subjected to phenotype analysis by FACS, as explained in Materials and Methods.

The phenotype of GMP-derived DCs from sham and PBD 7 mice, without and with MafB transfection, is signified in contour plots (**Fig. 5A**). The boxed areas represent the dual expression of $CD11c^+$ (*y*-axis) DCs, also expressing $CD11b^+$, MHC-II^+, or GATA-1^+ (*x*-axes, respectively). The mean percentage ± SEM from four different samples is outlined in **Table 1**. The relative expressions of CD11c, MHC-II, and GATA-1 in GMP-derived DCs from sham (light black line), burn (dark black line), and burn + MafB silencing (gray line) are shown as superimposed histograms in Fig. 5B. Silencing of MafB in GMP-derived monocytes from burn mice significantly increased the percentages of $CD11b^+CD11c^+$ mDCs and MHC-II-expressing DCs, as well the MFI of CD11c expression, but failed to bring it up to sham levels. More importantly, MHC-II and GATA-1 expressions were increased by MafB suppression in burn DC cultures and were rescued to sham levels (Table 1). Results obtained from FACS analysis of liquid cultures were verified by differentiating the GMPs in multichambered culture slides and incubated with fluorescent DC markers. **Fig. 6** illustrates the confocal images of GMP-derived DCs expressing CD11c (green) and MHC-II (red). Nucleus is identified with DAPI (blue). The prototypical DC morphology is shown; additionally, the fluorescence of CD11c and MHC-II, which are reduced significantly following burn, was enhanced after MafB was silenced in GMP-derived monocytes.

Taken together, results from ex vivo cultures indicate that up-regulation of MafB in GMPs following burn promotes monocyte production, perhaps by increased responsiveness to M-CSF through M-CSFR up-regulation. However, the molecular mechanism dictating the suppressive phenotype of GMP-derived monocytes in burn mice remains obscure. Nonetheless, transient knockdown of MafB gene expression in GMP-derived monocytes from burn mice partially rescued MHC-II expression in mDCs, in addition to improving DC differentiation. These results signify that MafB plays a repressive role on GATA-1 transcription, which is essential during DC differentiation, and also regulates the function of monocytes and DCs, besides their development. Moreover, our results provide evidence for the inhibitory role of MafB on GATA-1 expression during DC development of monocytes. Alternately, MafB siRNA in GMPs from burn mice significantly reduced the percentage of F4/80-expressing cells in monocyte cultures, thereby inhibiting Mϕ differentiation (Supplemental Fig. 3), providing evidence for the role of MafB in augmenting monocyte differentiation.

Nonetheless, a significant reduction in M-CSFR expression with MafB siRNA in GMPs from sham mice (Fig. 3C) and enhanced DC differentiation when GMP-derived Mϕ from sham mice were subjected to MafB silencing (Supplemental Fig. 4) confirm that high MafB in GMPs, resulting from burn injury, has a predominant role in augmenting monocyte development and alternately, reducing the potential for DC development following burn injury.

DISCUSSION

Depletion of DCs can be attributed to sequestration, increased turnover, or impaired production of DCs. Here, we report that burn injury impairs the production of DCs by the aberrant differentiation potential of the bone marrow precursor population. The migratory trait of DCs allows for their recruitment to the site of injury in response to inflammatory signals for the capture of antigens and consequent migration to lymphoid and nonlymphoid organs for presentation of the processed antigen to trigger adaptive immunity [36–38]. DCs egress from the bone marrow and migrate to the site of burn injury through circulation. In the present burn model, we observed a significant reduction in circulating mDCs for up to 14 days following burn, reducing the chance for increased sequestration as the reason for DC depletion following burn. However, several studies also support the notion of increased apoptosis of DCs leading to immune suppression following trauma and sepsis [39–41], which was not a contributing factor in the present study. Nonetheless, unlike other lymphocytes, DC homeostasis is achieved by the differentiation of precursor cells rather than proliferation of existing differentiated cells. Using diphtheria toxin to deplete DCs in a transgenic mouse model, Hochweller et al. [42] have demonstrated a feedback regulation of DC generation by increased differentiation of pro-DCs. Therefore, any defect in the differentiation phenomenon could further extend the gap between production and injury-induced sequestration and apoptosis of the antigen-presenting DCs. The unique homeostatic control mechanism of DCs supports our present finding that although burn injury increases bone marrow constitution of myeloid progenitors, the production of DCs is inhibited by the inherent alteration in the differentiation potential of DC precursors.

In the present study, we report that burn injury augmented the bipotential GMPs in the bone marrow compartment. Moreover, these progenitors exhibited a phenotype of high MafB and M-CSFR dual expressions. In a similar study about experimental burn sepsis, using the technique of radioligand binding, Santangelo et al. [18] demonstrated an increase in M-CSFR expression in Lin-positive bone marrow monocyte progenitors expressing ER-MP12 and ER-MP20 markers, whereas the present study focused on a Lin-negative, myeloid-committed hematopoietic progenitor population with exclusive granulocytic monocytic bipotential [31]. M-$CSFR^{hi}$ expression discriminates between monocytic and granulocytic progenitors [33], and MafB induces monocytic differentiation in murine and human hematopoietic cells [23, 43]. Our subsequent analysis of GMP differentiation in a M-SCF-rich cocktail confirms that the lineage commitment is favored toward monocyte production following burn injury, in line with the previous findings that GMPs cultured in M-CSF give rise to monocyte colonies exclusively [44]. Here, we report that the potential of GMPs from burn mice toward M-CSF-responsive monocytic lineage commitment is regulated by high MafB, as MafB silencing resulted in the down-regulation of M-CSFRs. Moreover, up-regulation of the $F4/80^+$ population in GMP-derived monocyte cultures from burn mice is in line with the report that MafB is essential for F4/80 expression in Mϕ [45].

In humans, $CD14^+$ monocytes have been shown to acquire the phenotype and function of DCs when cultured in GM-CSF and IL-4 [46]. Based on these studies, it is conceivable that given the increase in GMPs and high expression of MafB in our burn model, the differentiation of GMPs into monocytes and monocyte-derived DCs should be increased proportionately compared with sham. However, results from our ex vivo cultures of GMPs

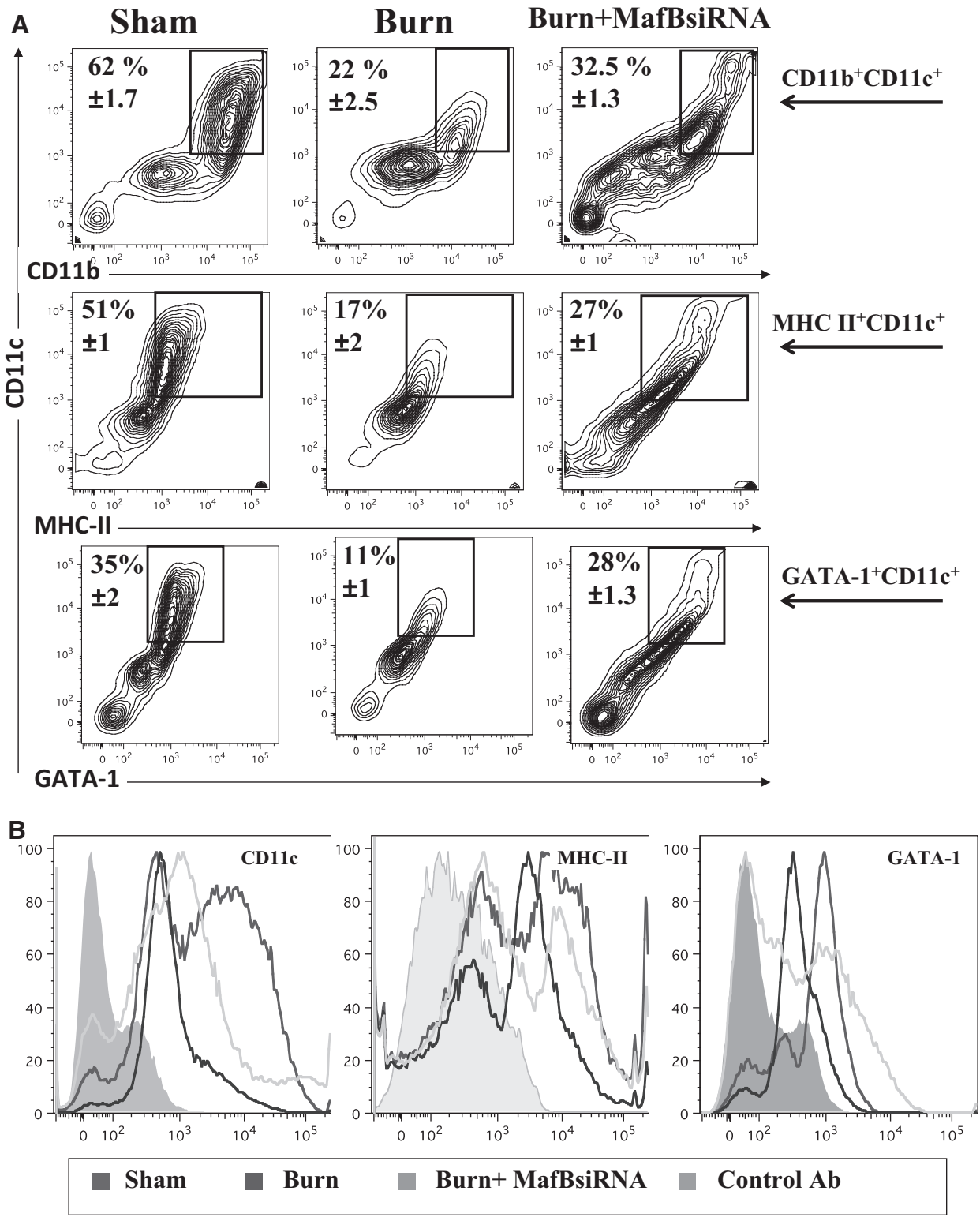

Figure 5. GMP-derived monocytes recover burn-induced loss in DC differentiation potential through MafB silencing. MafB expression was silenced in GMP-derived monocytes from burn mice using targeted siRNA or a scrambled, nonspecific control. (A) Contour plots of GMP-derived DCs from sham, burn, and burn + MafB siRNA. MafB silencing significantly increased the percentage of $CD11b^+CD11c^+$ mDCs and the percentage of MHC-II-expressing DCs. Increased GATA-1 expression is also seen following silencing. (B) The relative expressions of CD11c, MHC-II, and GATA-1 are represented as histograms superimposed from sham (light black line), burn (dark black line), burn + MafB silencing (gray line), and control antibody (gray). All experiments were repeated three times with at least four animals in each group (mean±SEM).

TABLE 1. Phenotype of GMP-Derived DCs with and without MafB Silencing

Phenotype	Sham	Burn	Burn + MafB siRNA
$CD11c^+CD11b^+$ myeloid DC	62 ± 1.7%	22 ± 2.5%[a]	32.5 ± 1.3%[b]
$CD11c^+$ DC expressing MHC-II^+ (%)	51 ± 1%	17 ± 2%[a]	27 ± 1%[b]
$CD11c^+$ DC expressing GATA-1^+ (%)	35 ± 2%	11 ± 1%[a]	28 ± 1.3%[b]
MFI of CD11c expression in GMP-derived DC	2336 ± 163	730 ± 13[a]	888 ± 16[b]
MFI of MHC-II expression in GMP-derived DC	861 ± 18	632 ± 13[a]	850 ± 56[b]
MFI of GATA-1 expression in GMP-derived DC	1086 ± 25	830 ± 29[a]	1731 ± 33[b]

[a]$P < 0.05$ versus sham; [b]$P < 0.05$ versus burn.

demonstrate that although there was robust M-CSF-responsive monocyte differentiation, there was an apparent inhibition in GM-CSF + IL-4-responsive DC differentiation following burn compared with sham. As evidenced in MacGreen transgenic mice, the majority of tissue DCs are M-$CSFR^+CD11c^{high}B220^{neg}$, indicating that they are myeloid in origin, and M-CSFR is essential during mDC differentiation [47]. Therefore, in our experimental model, we chose to silence MafB transcription subsequent to monocyte differentiation of GMPs and before initiating DC differentiation [in the sequential order of GMP–Mϕ (siRNA)–DC]. Interestingly, transient silencing of MafB transcription in GMP-derived monocytes from burn mice rectified DC differentiation defects acquired through burn injury, emphasizing the essential role of monocytes as DC precursors.

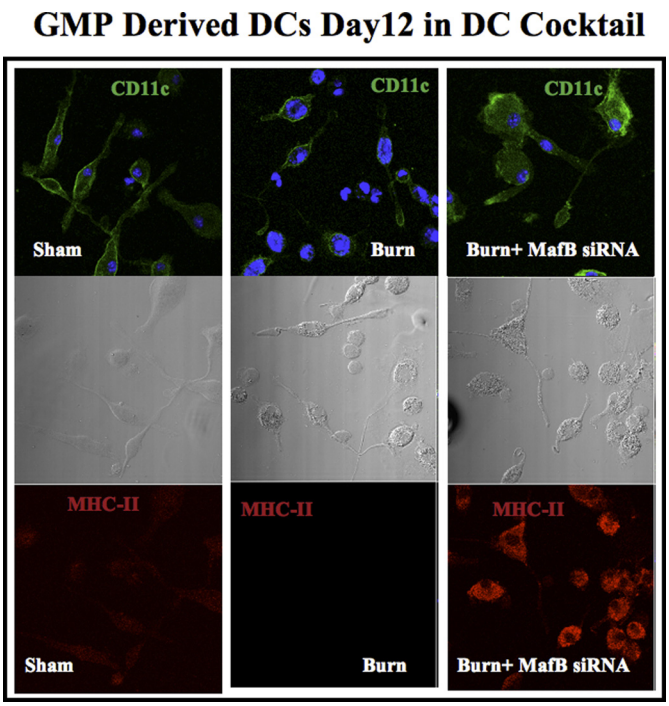

Figure 6. Confocal images. GMPs were differentiated into DCs in adherent multichamber slides before and after MafB siRNA transfection. The dendrites are positive for CD11c (green) and MHC-II (red) expression. Nucleus is stained with DAPI (blue). Decline in CD11c and MHC-II expression following burn injury is regained by transient silencing of MafB in GMP-derived monocytes before DC differentiation. Confocal images were obtained using Zeiss Axio Observer Z1 with a LSM 510 operating system. All experiments were repeated two times with at least three animals in each group.

Recent prospective, observational analyses of septic patients report a profound reduction in the number of mDCs and a significant increase in $CD14^{bright}$ monocytes. The same study acknowledges a long-lasting HLA-DR suppression on all monocyte and DC subsets in septic patients [4], in agreement with our current experimental finding of reduced MHC-II^+ expression, whereas another study reports a contradictory finding that the antigen-presenting function of DCs is not altered, but DC numbers are reduced in an experimental burn model [48]. First, the latter model examined the spleen that constitutes conventional lymphoid resident DCs, which do not migrate but acquire antigens from migratory DCs. Most migratory DCs arise from monocytes, and this explains why there may be a discrepancy between the two studies. Nonetheless, our findings that GMP-derived monocytes and DCs from burn mice have reduced MHC-II are comparable with the former report, which examined peripheral blood monocytes and DCs. The ex vivo GMP differentiation data combined with the blood and bone marrow distribution of mDCs imply that the in vivo decrease in the DC population with a suppressive phenotype following burn injury could be a result of a defect in the lineage commitment of bone marrow hematopoietic progenitors.

HSC divisions and lineage commitments are dictated by the dynamic expressions of Lin-specific transcription factors, in concert with the instructive or permissive actions of cytokines. In the present study, we maintained a constant imperative cytokine environment in ex vivo cultures to eliminate any variability and to focus solely on transcriptional changes caused by burn injury. Although knocking down MafB in HSCs, which reside upstream of GMPs, showed a PU.1- and M-CSFR-dependent, competitive repopulation advantage, specifically in the myelomonocytic compartment [49], we failed to notice any change in PU.1 expression, in spite of high MafB expressions in GMPs following burn. Although this discrepancy cannot be explained in the present study, our results are in agreement with the finding of Bakri et al. [50], in that the constitutive MafB expression inhibited DC differentiation in Mϕ. Moreover, PU.1 is shown to regulate Flt3L-induced DC differentiation in early hematopoietic progenitors [51], and monocyte-derived DCs were not affected in $Flt3^{-/-}$ mice [52]. Additionally, Flt3L was not required for GMP-derived DC development in the present study, eliminating the significance of PU.1 in monocyte-derived DCs. Although MafB is essential for monocyte differentiation [23], a balance of MafB and PU.1 specifies alternative Mϕ or DC fate in myeloid-committed

progenitors [50]. How MafB inhibits DC differentiation, independent of PU.1, remains elusive.

Here, we report that a burn-endured deficit in GATA-1 expression can be augmented when MafB is silenced in monocytes, thereby increasing $CD11c^+GATA\text{-}1^+$ expression during DC differentiation. GATA-1 is integral for erythropoiesis [53, 54]; nonetheless, the essential role of GATA-1 in DC development and survival is also evidenced by studies where tamoxifen-induced ablation of GATA-1 expression decreased DC numbers in vivo and in vitro [24]. However, in the present study, GATA-1 expression was decreased significantly, whereas MafB was highly expressed in GMPs from burn mice, suggesting a reciprocal relationship between MafB and GATA-1 in DC depletion following burn injury. Several evidences address the presence of a negative regulatory interaction between hematopoietic transcription factors. More importantly, myeloid-specific transcription factor MafB represses ETS-1-responsive, erythroid-specific promoters, thereby affecting lineage commitment in erythroblasts [55]. Moreover, GATA-1 and vitamin D receptors exhibit reciprocal roles in human mDC differentiation [56], and in an independent study, vitamin D3 and MafB demonstrated synergistic functions during the monocyte differentiation of human $CD34^+$ hemopoietic progenitors [57]. These two independent studies are in support of our own observations that GATA-1 expression is increased following MafB suppression in GMPs after 48 h of transfection (Fig. 3B), and dual expression of CD11c and GATA-1 is enhanced in GMP-derived DCs following MafB siRNA transfection (Fig. 5). Taken together, these studies establish a previously undescribed, antagonistic role between MafB and GATA-1 in GMPs. These results lead to a compelling argument that increased MafB following burn dampens GATA-1 expression, thereby blunting the differentiation potential of DC precursors, resulting in a deficit in DC generation.

In an independent study, Chomarat et al. [58] demonstrates that $CD34^+$ progenitors and $CD14^+$ monocytes can be differentiated into DCs and that down-regulation of M-CSFR by TNF-α was critical during DC differentiation. In the present study, we noticed an increase in M-CSFR expression in GMPs and monocyte cultures following burn, leading to a speculation that M-CSFR may also play a significant role in inhibiting DC differentiation of bone marrow progenitors. Furthermore, CD115 (M-CSFR) up-regulation is indicated in monocytes obtained from trauma patients [6]. This concept is strengthened further by our observation that silencing MafB down-regulated M-CSFR expression in GMPs and simultaneously mitigated the deficit in DC production by GMP-derived monocytes, offsetting the consequence of burn injury.

In essence, we interrogated the underlying mechanism for burn-induced monocytopoiesis and the associated DC depletion following burn injury. Based on our experimental results, we conclude that bone marrow production of multipotent progenitors (LSK) and GMPs is increased in response to burn injury. Burn-induced up-regulation of MafB and M-CSFR skews the lineage commitment of GMPs toward monocyte production, whereas their DC differentiation is inhibited by increased M-CSFR or reduced GATA-1 or both. However, a suppressive phenotype is manifested in GMP-derived monocytes and DCs following burn injury. The inability to rescue all of the burn-induced DC defect by MafB suppression may exclude MafB as a single player in the DC deficit following burn or can be explained by the transient silencing (siRNA) of MafB against permanent shutdown of gene expression. Nevertheless, a significant increase in the MFI of CD11c, MHC-II, and GATA-1 expressions implies the predominant-negative role of MafB in the DC maturation process.

Further investigations about sequential Lin commitment of HSCs and progenitor cells are warranted to define burn-induced signals that initiate changes in transcription factor/s contributing to altered immune-cell distribution and function. Such studies can have broader implications, as blood monocytes are the reservoir for dominant APCs in response to live or dead bacteria in steady-state, and monocyte-derived DCs are strong in antigen-presenting function compared with Flt3L-expanded $CD8a^+$ and $CD8a^-$ DC equivalents [52].

ACKNOWLEDGMENTS

Funding from NIH grants R03 AI 079530-01 and RO1 GM 42577 and Dr. Ralph and Marion C. Falk Medical Research Trust supported this work. We thank Dr. Jiwang Zhang for his critical input and consultations during the preparation of this manuscript, Patricia Simms for her help with flow cytometric sorting of hemopoietic progenitors, and Linda Fox for her technical expertise in confocal imaging, respectively.

REFERENCES

1. Oberholzer, A., Oberholzer, C., Moldawer, L. L. (2001) Sepsis syndromes: understanding the role of innate and acquired immunity. *Shock* **16,** 83–96.
2. Munoz, C., Carlet, J., Fitting, C., Misset, B., Bleriot, J. P., Cavaillon, J. M. (1991) Dysregulation of in vitro cytokine production by monocytes during sepsis. *J. Clin. Invest.* **88,** 1747–1754.
3. Baue, A. E., Durham, R., Faist, E. (1998) Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): are we winning the battle? *Shock* **10,** 79–89.
4. Poehlmann, H., Schefold, J. C., Zuckermann-Becker, H., Volk, H. D., Meisel, C. (2009) Phenotype changes and impaired function of dendritic cell subsets in patients with sepsis: a prospective observational analysis. *Crit. Care* **13,** R119.
5. D'Arpa, N., Accardo-Palumbo, A., Amato, G., D'Amelio, L., Pileri, D., Cataldo, V., Mogavero, R., Lombardo, C., Napoli, B., Conte, F. (2009) Circulating dendritic cells following burn. *Burns* **35,** 513–518.
6. De, A. K., Laudanski, K., Miller-Graziano, C. L. (2003) Failure of monocytes of trauma patients to convert to immature dendritic cells is related to preferential macrophage-colony-stimulating factor-driven macrophage differentiation. *J. Immunol.* **170,** 6355–6362.
7. Muthu, K., He, L. K., Melstrom, K., Szilagyi, A., Gamelli, R. L., Shankar, R. (2008) Perturbed bone marrow monocyte development following burn injury and sepsis promote hyporesponsive monocytes. *J. Burn Care Res.* **29,** 12–21.
8. Hotchkiss, R. S., Tinsley, K. W., Swanson, P. E., Grayson, M. H., Osborne, D. F., Wagner, T. H., Cobb, J. P., Coopersmith, C., Karl, I. E. (2002) Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J. Immunol.* **168,** 2493–2500.
9. Benjamim, C. F., Lundy, S. K., Lukacs, N. W., Hogaboam, C. M., Kunkel, S. L. (2005) Reversal of long-term sepsis-induced immunosuppression by dendritic cells. *Blood* **105,** 3588–3595.
10. Bohannon, J., Cui, W., Cox, R., Przkora, R., Sherwood, E., Toliver-Kinsky, T. (2008) Prophylactic treatment with fms-like tyrosine kinase-3 ligand after burn injury enhances global immune responses to infection. *J. Immunol.* **180,** 3038–3048.
11. Vremec, D., Shortman, K. (1997) Dendritic cell subtypes in mouse lymphoid organs: cross-correlation of surface markers, changes with incubation, and differences among thymus, spleen, and lymph nodes. *J. Immunol.* **159,** 565–573.
12. Wu, L., Li, C. L., Shortman, K. (1996) Thymic dendritic cell precursors: relationship to the T lymphocyte lineage and phenotype of the dendritic cell progeny. *J. Exp. Med.* **184,** 903–911.
13. Ardavin, C., Wu, L., Li, C. L., Shortman, K. (1993) Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population. *Nature* **362,** 761–763.
14. Scheicher, C., Mehlig, M., Zecher, R., Reske, K. (1992) Dendritic cells from mouse bone marrow: in vitro differentiation using low doses of re-

combinant granulocyte-macrophage colony-stimulating factor. *J. Immunol. Methods* **154,** 253–264.
15. Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., Steinman, R. M. (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* **176,** 1693–1702.
16. Liu, K., Nussenzweig, M. C. (2010) Origin and development of dendritic cells. *Immunol. Rev.* **234,** 45–54.
17. Steinman, R. M., Idoyaga, J. (2010) Features of the dendritic cell lineage. *Immunol. Rev.* **234,** 5–17.
18. Santangelo, S., Gamelli, R. L., Shankar, R. (2001) Myeloid commitment shifts toward monocytopoiesis after thermal injury and sepsis. *Ann. Surg.* **233,** 97–106.
19. Inaba, K., Inaba, M., Deguchi, M., Hagi, K., Yasumizu, R., Ikehara, S., Muramatsu, S., Steinman, R. M. (1993) Granulocytes, macrophages, and dendritic cells arise from a common major histocompatibility complex class II-negative progenitor in mouse bone marrow. *Proc. Natl. Acad. Sci. USA* **90,** 3038–3042.
20. Wu, L., Liu, Y. J. (2007) Development of dendritic-cell lineages. *Immunity* **26,** 741–750.
21. Fogg, D. K., Sibon, C., Miled, C., Jung, S., Aucouturier, P., Littman, D. R., Cumano, A., Geissmann, F. (2006) A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* **311,** 83–87.
22. Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., Ley, K. (2010) Development of monocytes, macrophages, and dendritic cells. *Science* **327,** 656–661.
23. Kelly, L. M., Englmeier, U., Lafon, I., Sieweke, M. H., Graf, T. (2000) MafB is an inducer of monocytic differentiation. *EMBO J.* **19,** 1987–1997.
24. Gutiérrez, L., Nikolic, T., van Dijk, T. B., Hammad, H., Vos, N., Willart, M., Grosveld, F., Philipsen, S., Lambrecht, B. N. (2007) GATA1 regulates dendritic-cell development and survival. *Blood* **110,** 1933–1941.
25. Lukaszewicz, A. C., Grienay, M., Resche-Rigon, M., Pirracchio, R., Faivre, V., Boval, B., Payen, D. (2009) Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. *Crit. Care Med.* **37,** 2746–2752.
26. Monneret, G., Lepape, A., Voirin, N., Bohe, J., Venet, F., Debard, A. L., Thizy, H., Bienvenu, J., Gueyffier, F., Vanhems, P. (2006) Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. *Intensive Care Med.* **32,** 1175–1183.
27. Venet, F., Tissot, S., Debard, A. L., Faudot, C., Crampe, C., Pachot, A., Ayala, A., Monneret, G. (2007) Decreased monocyte human leukocyte antigen-DR expression after severe burn injury: correlation with severity and secondary septic shock. *Crit. Care Med.* **35,** 1910–1917.
28. Pulendran, B., Smith, J. L., Caspary, G., Brasel, K., Pettit, D., Maraskovsky, E., Maliszewski, C. R. (1999) Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc. Natl. Acad. Sci. USA* **96,** 1036–1041.
29. Ferrero, I., Held, W., Wilson, A., Tacchini-Cottier, F., Radtke, F., MacDonald, H. R. (2002) Mouse CD11c(+) B220(+) Gr1(+) plasmacytoid dendritic cells develop independently of the T-cell lineage. *Blood* **100,** 2852–2857.
30. Auffray, C., Fogg, D. K., Narni-Mancinelli, E., Senechal, B., Trouillet, C., Saederup, N., Leemput, J., Bigot, K., Campisi, L., Abitbol, M., Molina, T., Charo, I., Hume, D. A., Cumano, A., Lauvau, G., Geissmann, F. (2009) CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. *J. Exp. Med.* **206,** 595–606.
31. Akashi, K., Traver, D., Miyamoto, T., Weissman, I. L. (2000) A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* **404,** 193–197.
32. Posluszny, J. A., Muthu, K., Kini, A. R., Szilagyi, A., He, L-K., Li, Y., Gamelli, R., Shankar, R. (2011) Burn injury dampens erythroid cell production through prioritizing bone marrow hematopoietic response. *J. Trauma*, In press.
33. Olweus, J., Thompson, P. A., Lund-Johansen, F. (1996) Granulocytic and monocytic differentiation of CD34hi cells is associated with distinct changes in the expression of the PU.1-regulated molecules, CD64 and macrophage colony-stimulating factor receptor. *Blood* **88,** 3741–3754.
34. Kozma, G. T., Martelli, F., Verrucci, M., Gutierrez, L., Migliaccio, G., Sanchez, M., Alfani, E., Philipsen, S., Migliaccio, A. R. (2010) Dynamic regulation of GATA1 expression during the maturation of conventional dendritic cells. *Exp. Hematol.* **38,** 489–503.e1.
35. Schreurs, M. W., Eggert, A. A., de Boer, A. J., Figdor, C. G., Adema, G. J. (1999) Generation and functional characterization of mouse monocyte-derived dendritic cells. *Eur. J. Immunol.* **29,** 2835–2841.
36. McWilliam, A. S., Napoli, S., Marsh, A. M., Pemper, F. L., Nelson, D. J., Pimm, C. L., Stumbles, P. A., Wells, T. N., Holt, P. G. (1996) Dendritic cells are recruited into the airway epithelium during the inflammatory response to a broad spectrum of stimuli. *J. Exp. Med.* **184,** 2429–2432.
37. McWilliam, A. S., Nelson, D., Thomas, J. A., Holt, P. G. (1994) Rapid dendritic cell recruitment is a hallmark of the acute inflammatory response at mucosal surfaces. *J. Exp. Med.* **179,** 1331–1336.
38. Austyn, J. M., Kupiec-Weglinski, J. W., Hankins, D. F., Morris, P. J. (1988) Migration patterns of dendritic cells in the mouse. Homing to T cell-dependent areas of spleen, and binding within marginal zone. *J. Exp. Med.* **167,** 646–651.
39. Tinsley, K. W., Grayson, M. H., Swanson, P. E., Drewry, A. M., Chang, K. C., Karl, I. E., Hotchkiss, R. S. (2003) Sepsis induces apoptosis and profound depletion of splenic interdigitating and follicular dendritic cells. *J. Immunol.* **171,** 909–914.
40. Gautier, E. L., Huby, T., Saint-Charles, F., Ouzilleau, B., Chapman, M. J., Lesnik, P. (2008) Enhanced dendritic cell survival attenuates lipopolysaccharide-induced immunosuppression and increases resistance to lethal endotoxic shock. *J. Immunol.* **180,** 6941–6946.
41. Efron, P. A., Martins, A., Minnich, D., Tinsley, K., Ungaro, R., Bahjat, F. R., Hotchkiss, R., Clare-Salzler, M., Moldawer, L. L. (2004) Characterization of the systemic loss of dendritic cells in murine lymph nodes during polymicrobial sepsis. *J. Immunol.* **173,** 3035–3043.
42. Hochweller, K., Miloud, T., Striegler, J., Naik, S., Hammerling, G. J., Garbi, N. (2009) Homeostasis of dendritic cells in lymphoid organs is controlled by regulation of their precursors via a feedback loop. *Blood* **114,** 4411–4421.
43. Gemelli, C., Montanari, M., Tenedini, E., Zanocco Marani, T., Vignudelli, T., Siena, M., Zini, R., Salati, S., Tagliafico, E., Manfredini, R., Grande, A., Ferrari, S. (2006) Virally mediated MafB transduction induces the monocyte commitment of human CD34+ hematopoietic stem/progenitor cells. *Cell Death Differ.* **13,** 1686–1696.
44. Rieger, M. A., Hoppe, P. S., Smejkal, B. M., Eitelhuber, A. C., Schroeder, T. (2009) Hematopoietic cytokines can instruct lineage choice. *Science* **325,** 217–218.
45. Moriguchi, T., Hamada, M., Morito, N., Terunuma, T., Hasegawa, K., Zhang, C., Yokomizo, T., Esaki, R., Kuroda, E., Yoh, K., Kudo, T., Nagata, M., Greaves, D. R., Engel, J. D., Yamamoto, T., Takahashi, S. (2006) MafB is essential for renal development and F4/80 expression in macrophages. *Mol. Cell. Biol.* **26,** 5715–5727.
46. Kiertscher, S. M., Roth, M. D. (1996) Human CD14+ leukocytes acquire the phenotype and function of antigen-presenting dendritic cells when cultured in GM-CSF and IL-4. *J. Leukoc. Biol.* **59,** 208–218.
47. MacDonald, K. P., Rowe, V., Bofinger, H. M., Thomas, R., Sasmono, T., Hume, D. A., Hill, G. R. (2005) The colony-stimulating factor 1 receptor is expressed on dendritic cells during differentiation and regulates their expansion. *J. Immunol.* **175,** 1399–1405.
48. Fujimi, S., Lapchak, P. H., Zang, Y., MacConmara, M. P., Maung, A. A., Delisle, A. J., Mannick, J. A., Lederer, J. A. (2009) Murine dendritic cell antigen-presenting cell function is not altered by burn injury. *J. Leukoc. Biol.* **85,** 862–870.
49. Sarrazin, S., Mossadegh-Keller, N., Fukao, T., Aziz, A., Mourcin, F., Vanhille, L., Kelly Modis, L., Kastner, P., Chan, S., Duprez, E., Otto, C., Sieweke, M. H. (2009) MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells. *Cell* **138,** 300–313.
50. Bakri, Y., Sarrazin, S., Mayer, U. P., Tillmanns, S., Nerlov, C., Boned, A., Sieweke, M. H. (2005) Balance of MafB and PU.1 specifies alternative macrophage or dendritic cell fate. *Blood* **105,** 2707–2716.
51. Carotta, S., Dakic, A., D'Amico, A., Pang, S. H., Greig, K. T., Nutt, S. L., Wu, L. (2010) The transcription factor PU.1 controls dendritic cell development and Flt3 cytokine receptor expression in a dose-dependent manner. *Immunity* **32,** 628–641.
52. Cheong, C., Matos, I., Choi, J. H., Dandamudi, D. B., Shrestha, E., Longhi, M. P., Jeffrey, K. L., Anthony, R. M., Kluger, C., Nchinda, G., Koh, H., Rodriguez, A., Idoyaga, J., Pack, M., Velinzon, K., Park, C. G., Steinman, R. M. (2010) Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* **143,** 416–429.
53. Ferreira, R., Ohneda, K., Yamamoto, M., Philipsen, S. (2005) GATA1 function, a paradigm for transcription factors in hematopoiesis. *Mol. Cell. Biol.* **25,** 1215–1227.
54. Welch, J. J., Watts, J. A., Vakoc, C. R., Yao, Y., Wang, H., Hardison, R. C., Blobel, G. A., Chodosh, L. A., Weiss, M. J. (2004) Global regulation of erythroid gene expression by transcription factor GATA-1. *Blood* **104,** 3136–3147.
55. Sieweke, M. H., Tekotte, H., Frampton, J., Graf, T. (1996) MafB is an interaction partner and repressor of Ets-1 that inhibits erythroid differentiation. *Cell* **85,** 49–60.
56. Göbel, F., Taschner, S., Jurkin, J., Konradi, S., Vaculik, C., Richter, S., Kneidinger, D., Muhlbacher, C., Bieglmayer, C., Elbe-Burger, A., Strobl, H. (2009) Reciprocal role of GATA-1 and vitamin D receptor in human myeloid dendritic cell differentiation. *Blood* **114,** 3813–3821.
57. Gemelli, C., Orlandi, C., Zanocco Marani, T., Martello, A., Vignudelli, T., Ferrari, F., Montanari, M., Parenti, S., Testa, A., Grande, A., Ferrari, S. (2008) The vitamin D3/Hox-A10 pathway supports MafB function during the monocyte differentiation of human CD34+ hemopoietic progenitors. *J. Immunol.* **181,** 5660–5672.
58. Chomarat, P., Dantin, C., Bennett, L., Banchereau, J., Palucka, A. K. (2003) TNF skews monocyte differentiation from macrophages to dendritic cells. *J. Immunol.* **171,** 2262–2269.

KEY WORDS:
mDC · GMP · M-CSF receptors · LSK · GATA-1 · bone marrow