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DEK Regulates Hematopoietic Stem Engraftment and Progenitor Cell Proliferation

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DEK is a biochemically distinct protein that is generally found in the nucleus, where it is vital to global heterochromatin integrity. However, DEK is also secreted by cells (eg, macrophages) and influences other adjacent cells (eg, acts as a chemoattractant for certain mature blood cells). We hypothesized that DEK may modulate functions of hematopoietic stem (HSCs) and progenitor (HPCs) cells. C57Bl/6 mice were used to demonstrate that absolute numbers and cycling status of HPCs (colony forming unit-granulocyte macrophage [CFU-GM], burst forming unit-erythroid [BFU-E], and colony forming unit-granulocyte erythroid macrophage megakaryocyte [CFU-GEMM]) in bone marrow (BM) and spleen were significantly enhanced in DEK −/− as compared with wild-type (WT) control mice. Moreover, purified recombinant DEK protein inhibited colony formation in vitro by CFU-GM, BFU-E, and CFU-GEMM from WT BM cells and human cord blood (CB) cells in a dose-dependent fashion, demonstrating that DEK plays a negative role in HPC proliferation in vitro and in vivo. Suppression was direct acting as determined by inhibition of proliferation of single isolated $CD34^+$ CB cells in vitro. In contrast, DEK −/− BM cells significantly demonstrated reduced long term competitive and secondary mouse repopulating HSC capacity compared with WT BM cells, demonstrating that DEK positively regulates engrafting capability of self-renewing HSCs. This demonstrates that DEK has potent effects on HSCs, HPCs, and hematopoiesis, information of biological and potential clinical interest.

Introduction

Hematopoiesis is regulated by cell-cell and cytokine-cell interactions on hematopoietic stem (HSCs) and progenitor (HPCs) cells [1]. Extracellular and intracellular players involved in this regulation continue to be identified, and understanding these factors is crucial to modulating hematopoiesis for clinical benefit.

In our continuing efforts to elucidate players involved in regulation of HSC and HPC growth [1], we focused on DEK, an abundant and unusual protein found in multicellular organisms [2]. DEK has 2 DNA binding modules and has some affinity for specific DNA sequences, but primarily recognizes and binds to superhelical and cruciform DNA and induces positive supercoiling. DEK manifests multiple cellular activities that include transcriptional repression and activation, mRNA processing, and chromatin architectural functions [2]. We recently demonstrated that DEK modulates global heterochromatin integrity in vivo [3]. Interestingly, DEK, an autoantigen in juvenile idiopathic arthritis (JIA), can leave the cell and act as a chemoattractant for $CD8^+$T cells and natural killer cells [4]. Its secretion from macrophages is modulated by casein kinase 2 and interleukin (IL)-8, while being inhibited by dexamethasone and cyclosporine A [5]. Further, DEK is present in synovial fluid and in immune complexes of patients with JIA, and the chemotactic activity of DEK suggest that DEK may contribute to joint inflammation [4]. DEK autoantigenicity is augmented by acetylation. DEK is an oncogene that is overexpressed in multiple different malignancies [6,7], and is involved in melanoma proliferation and chemoresistence [6,7], promotion of epithelial transformation in vitro and in vivo [8], and in the pathogenesis of breast cancer [9].

Being intrigued that a nuclear protein was able to be secreted by hematopoietic cells, and act on other hematopoietic cells, we hypothesized that DEK might play a role in HSC/HPC function and hematopoiesis. Towards this possibility, we utilized recombinant (r) DEK protein, and DEK −/− mice, to demonstrate that DEK is a positive regulator of long-term repopulating HSC proliferation and engraftment, and a negative regulator of HPC proliferation.

Materials and Methods

Recombinant human His-DEK

Recombinant human His-DEK (rhu DEK) was purified from insect cells essentially as described [10].

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Three days postinfection with a high-titer virus stock, HighFive cells were harvested and washed 3 times with phosphate-buffered saline prior to lysis with 2 mL of lysis buffer per 175-cm^2 flask (100 mM Tris-Cl (pH 7.5), 150 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 1% NP-40, 5 mM imidazole). The lysate was further treated with 1.3 M NaCl for 20 min at room temperature, cleared (100,000 *g*, 10 min), and diluted with lysis buffer to a final concentration of 700 mM NaCl. After incubation for 1 h at 4°C with Ni-nitrilotriacetic acid-agarose (Qiagen), the beads were washed 3 times with 10 volumes of buffer 1 [50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 50 mM imidazole], 3 times with 10 volumes of buffer 2 [50 mM Tris-Cl (pH 7.5), 300 mM NaCl, and 50 mM imidazole], and again with 10 volumes of buffer 1. Elution was performed with 50 mM Tris-Cl (pH 7.5)-150 mM NaCl-500 mM imidazole. Aliquots of recombinant protein were stored at −80°C.

DEK −/− mice

These mice (C57BI/6/129/SVEV) were obtained from Dr. Gerard Grosveld, St Jude Children's Hospital, Memphis, TN [8], and were generated at the University of Michigan to a C57Bl/6 background by back-breeding the −/− mice with WT C57BI/6 mice for 9 generations. Mice were housed in specific pathogen-free conditions at the Animal Maintenance Facility of the University of Michigan Medical Center and used at Indiana University for experiments at 10–13 weeks of age. The University of Michigan and Indiana University Committees on Use and Care of Animals reviewed and approved the animal protocols.

HPC assay

C57Bl/6 mouse bone marrow (BM) and spleen cells were plated respectively at 5×10^4 and 5×10^5 cells/mL in 1% methylcellulose culture medium in the presence of hemin 0.1 mM, 30% fetal bovine serum (FBS; Hyclone), and the following growth factors, unless otherwise noted: [1 U/mL rhu erythropoietin (EPO; Amgen), 50 ng/mL recombinant murine (rmu) stem cell factor (SCF; R&D Systems), and 5% vol/vol pokeweed mitogen mouse spleen cell conditioned medium (PWMSCM)] [11]. Colonies were scored after 7 days incubation at 5% $C0_2$ and lowered (5%) 0_2 in a humidified chamber and granulocyte macrophage (CFU-GM), erythroid (BFU-E), and multipotent (CFU-GEMM) progenitors distinguished. When other growth factor combinations were used for stimulation of CFU-GM-derived colonies, no hemin was added. Human cord blood (CB) cells were separated into a low density fraction, and plated at 5×10^4 cells/mL in 1% methylcellulose culture media with 30% FBS, and various rhu cytokines (EPO, 1 U/mL; IL-3, 10 ng/mL; granulocyte macrophage-colony stimulating factor (GM-CSF), 10 ng/mL; SCF, 50 ng/mL) as noted. rmu and rhu GM-CSF, rmu macrophage (M)-CSF, rmu, and rhu IL-3 and rmu and rhu SCF were from R&D Systems [11]. Before sorting, the CD34+ population of hu CB was enriched via MACS (Miltenyi Biotec). After enrichment via MACS, cells were stained with FITC-conjugated anti-CD34 antibodies (Miltenyi Biotec) until sorted by FACSAria (BD Biosciences Immunocytometry Systems). Single CD34+ cells were directly sorted into 1 well of a 96-well microtiter plate containing 100 μL methylcellulose (1%) culture medium, containing Iscove's modified Dulbecco's medium (IMDM), 30% FBS, SCF, IL-3, GM-CSF, and EPO [12].

HSC engrafting studies

All mice used were on a C57Bl/6 strain background. BM cells from DEK −/− or wild type (WT) control mice ($CD45.2^+$) served as donor cells and were used at a 1:1 (2.5×10^5 to 2.5×10^5) ratio with BoyJ congenic (CD45.1) competitor cells; they were intravenously transplanted together into primary F1 (dual CD45.1/CD45.2) mice that were given a lethal dose (950 cGy) of gamma irradiation [13]. For secondary transplants BM cells from primary mice engrafted for 6 months were transplanted at 5×10^5 cells into secondary lethally irradiated F1 mice in a noncompetitive transplant assay [13]. Results are given as percent $CD45.2^+$ donor cell chimerism in the peripheral blood or BM at the times noted.

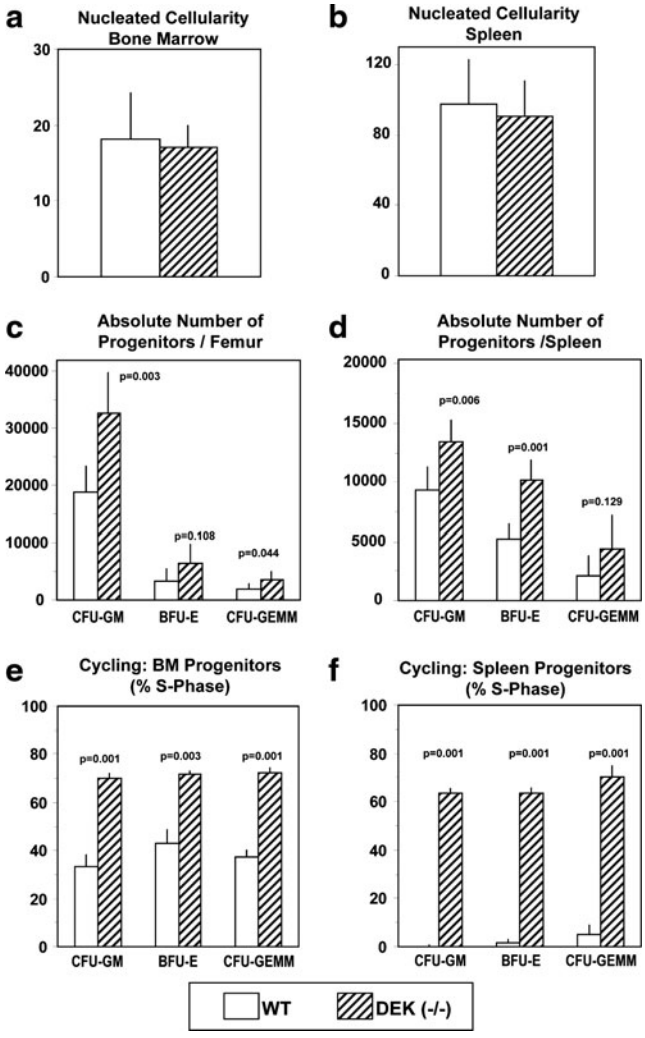

FIG. 1. Hematopoietic progenitor cell numbers and cycling characteristics (% in S-Phase of the cell cycle) in bone marrow (femur) and spleens of DEK −/− and WT control mice. Results (mean ± 1 SEM) are based on 6 individually assessed mice per group for each progenitor cell population in a total of 2 separate experiments. *P* values compare DEK −/− with WT mice. WT, wild type; SEM, standard error of the mean; CFU-GM, colony forming unit-granulocyte macrophage; BFU-E, burst forming unit-erythroid; CFU-GEMM, colony forming unit-granulocyte erythroid macrophage megakarocyte.

Statistical analysis

In vitro and in vivo differences between WT and DEK −/− mice were assessed by double-tailed student's *t*-test. Significance for single cell assays was determined by Fischer's exact test. *P* values of at least $P < 0.05$ were considered significant.

Results and Discussion

HPC proliferation in DEK −/− mice

To determine whether DEK had an effect on steady state hematopoiesis, BM and spleen cells from DEK −/− mice were compared with that of WT control mice for absolute numbers and cycling status of CFU-GM, BFU-E, and CFU-GEMM. Although there were no differences in BM (Fig. 1a) or spleen (Fig. 1b) nucleated cellularity between DEK −/− and WT mice, the absolute numbers of CFU-GM, BFU-E, and CFU-GEMM per femur (Fig. 1c) and per spleen (Fig. 1d) were increased in DEK −/− mice with significant increases in BM CFU-GM and CFU-GEMM, and in splenic CFU-GM and BFU-E. Increases in DEK −/− progenitors in BM and spleen were consistent with significantly increased percentages of CFU-GM, BFU-E, and CFU-GEMM in S-Phase of the cell cycle in DEK −/− BM (Fig. 1e) and spleen (Fig. 1f).

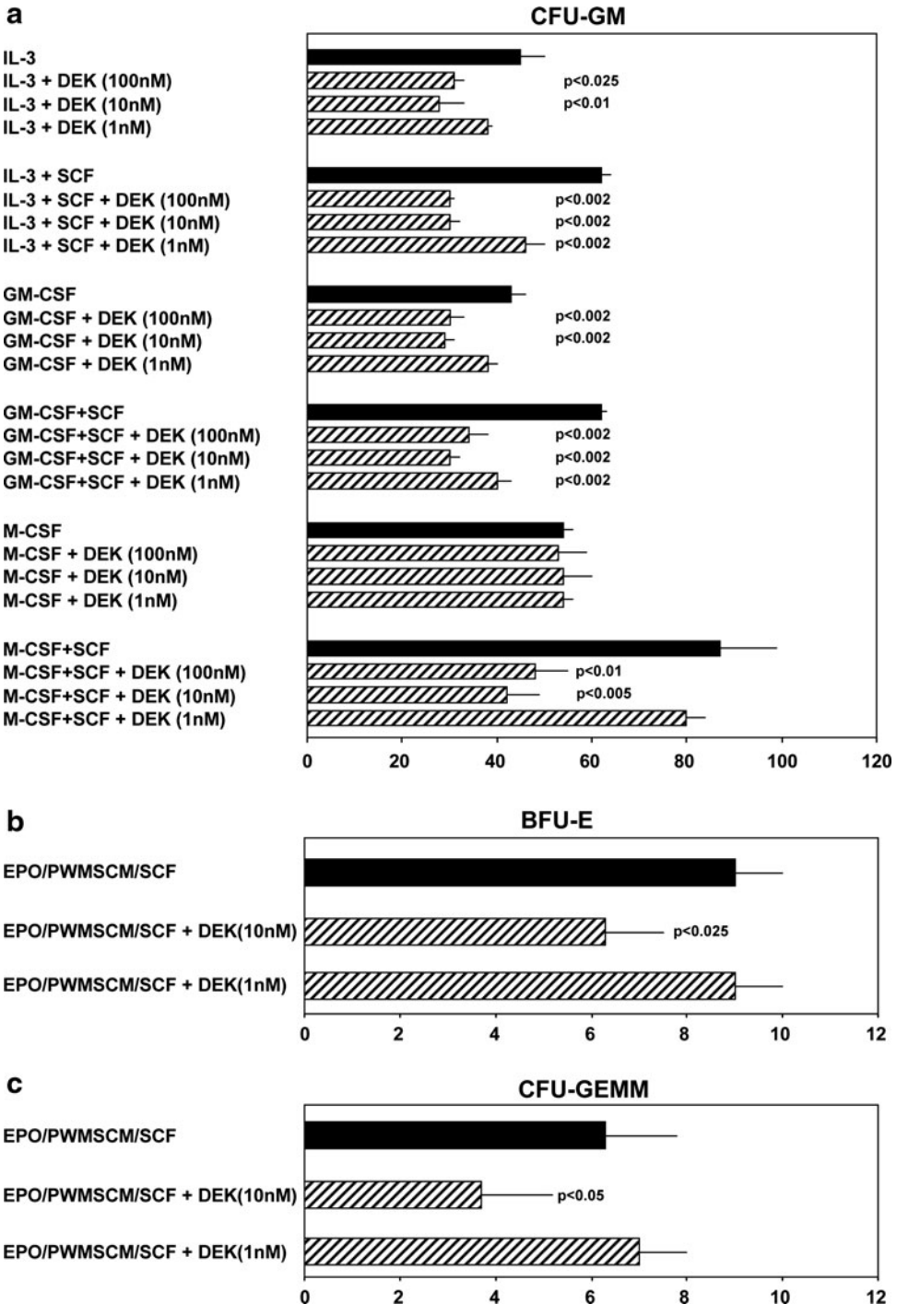

FIG. 2. Influence of rhu DEK on colony formation CFU-GM **(a)**, BFU-E **(b)**, and CFU-GEMM **(c)** in 5×10^4 unseparated WT control mouse bone marrow cells per milliliter. Results (mean ± 1 SEM) shown are 1 of 3 reproducible experiments. *P* values are compared with control values without added DEK. Rhu, recombinant human.

Increased numbers and cycling of HPCs in DEK −/− BM and spleen suggest that DEK acts as a negative regulator of HPC proliferation in vivo.

Influence of rhu DEK on colony formation in vitro by HPCs

To assess this negative role for DEK further, rhu DEK was tested for effects on HPC proliferation using unseparated mouse BM (Fig. 2) and low density hu CB (Fig. 3) cells. DEK, dose-dependently suppressed colony formation by mouse BM CFU-GM stimulated by either IL-3 or GM-CSF, each alone; it did not influence colony formation stimulated by M-CSF alone (Fig. 2a). However, it dose-dependently inhibited CFU-GM colony formation by either IL-3, GM-CSF, or M-CSF when these cytokines were combined with the potent co-stimulating cytokine SCF. In fact, inhibition by DEK was greater on CFU-GM stimulated by the combination of IL-3, GM-CSF, or M-CSF, each in the presence of SCF, compared with CFU-GM stimulated by IL-3, GM-CSF, or M-CSF each alone in terms of percent inhibition, and the amount of DEK required to inhibit colony formation. Although the lowest amount of DEK that could inhibit colony formation of CFU-GM stimulated by IL-3 or GM-CSF alone was 10 nM, concentrations as low as 1 nM DEK could inhibit colony formation stimulated by IL-3 plus SCF, or GM-CSF plus SCF. Although DEK did not inhibit colony formation of CFU-GM stimulated by M-CSF at up to 100 nM, it was active at concentrations as low as 10 nM in suppressing M-CSF plus SCF stimulated colony formation. DEK at concentrations of 10 nM also inhibited colony formation by BFU-E (Fig. 2b) and by CFU-GEMM (Fig. 2c) each stimulated by a combination of growth factors (EPO, PWMSCM, and SCF). It is known that HPCs stimulated by a single CSF (eg, IL-3, GM-CSF, or M-CSF) represent a more mature subset of HPCs than those stimulated by a combination of cytokines (such as a CSF plus SCF) [1], suggesting that immature subsets of HPCs are more sensitive in vitro to the suppressive effects of DEK than are the more mature HPCs.

Similar results were noted for HPCs present in hu CB (Fig. 3). DEK was an inhibitor of CFU-GM (Fig. 3a) and this inhibition was greater and apparent at lower concentrations of DEK when colonies formed from CFU-GM were stimulated by combinations of cytokines, compared with those stimulated by a single cytokine. Colony formation by BFU-E (Fig. 3b) and CFU-GEMM (Fig. 3c) in hu CB were also suppressed by DEK.

The activity of DEK in vitro in inhibiting colony formation by CFU-GM, BFU-E, and CFU-GEMM (Figs. 2 and 3) is consistent with the increased numbers and cycling of these progenitors in DEK −/− compared with WT BM and spleen (Fig. 1c–f), confirming the negative regulatory effects of DEK on proliferation of HPCs. Since the HPCs in Figs. 2 and 3 were present in a relatively unseparated population of

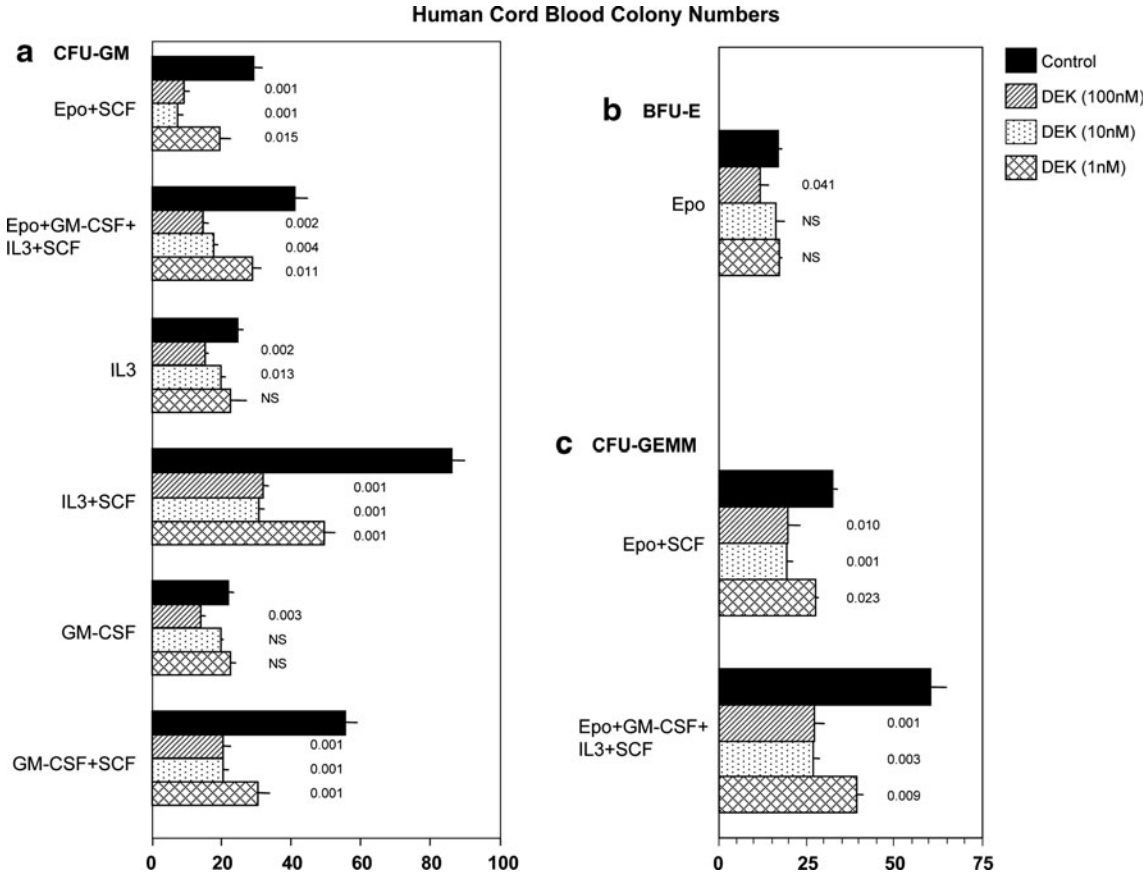

FIG. 3. Influence of rhu DEK on colony formation by CFU-GM **(a)**, BFU-E **(b)**, and CFU-GEMM **(c)** per milliliter in low density human cord blood. Results (mean ± 1 SEM) shown are 1 of 2 reproducible experiments. *P* values are compared with control values without added DEK.

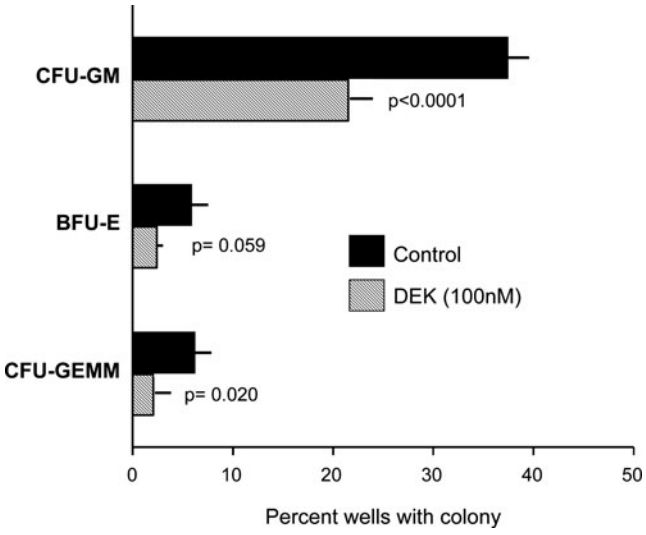

FIG. 4. Influence of rhu DEK directly on hematopoietic progenitor cells. DEK was assessed on colony formation by single isolated $CD34^+$ human cord blood cells each in a single well. Results (mean ± 1 standard deviation) of each experimental point are based on analysis of 288 wells.

mouse BM and low density cord blood cells, the suppressive effects of DEK on colony formation could have been due to direct effects on the HPCs, or indirectly mediated by effects on accessory cells. To determine if the DEK effects were directly or indirectly manifesting on the HPCs, DEK was assessed for effects on single isolated $CD34^+$ cord blood cells [10], each in a single well stimulated by EPO, GM-CSF, IL-3, and SCF. As seen in Fig. 4, DEK significantly decreased the number of wells containing a CFU-GM-, BFU-E-, or CFU-GEM- colony. The BFU-E effects were borderline significant, but scoring the numbers of wells with either a BFU-E- or CFU-GEMM-colony together resulted in inhibition that attained a P value of $P < 0.0013$. This demonstrates that DEK directly initiates its suppressive effect on HPC. This however, does not rule out the possibility that DEK may also act on other, possibly accessory, cells to influence HPC proliferation.

Engrafting capability of DEK −/− mouse BM cells

The competitive repopulating assay in vivo allows assessment of the short- and longer-term repopulating HSC, and transplantation of BM cells from primary to secondary lethally-irradiated recipients in a noncompetitive assay describes the longer-term repopulating HSC [1,14], and can offer information on the self-renewal capacity of this population of HSCs. As shown in Fig. 5a, although there was no difference in the HSC repopulating capacity of DEK −/− and WT shorter-term repopulating cells (months 1 and 2 for blood chimerism), there was a significant decrease in DEK −/− compared with WT BM cell repopulation at month 4 in the blood, and month 6 in the BM. This decreased repopulation of DEK −/− compared with WT HSC was even more apparent in secondary mouse recipients (Fig. 5b) suggesting that DEK played a positive role in engraftment of longer-term repopulating HSCs, and perhaps in the self-renewal capacity of mouse BM HSCs. Exactly how DEK acts to influence long term repopulating self-renewing HSCs remains to be determined. The effects may be direct acting on the HSCs, or indirectly mediated by accessory cells in the mouse.

The studies shown in Figs. 1–5 demonstrate a here-to-fore unknown role for DEK in the regulation of HPCs, HSCs, and hematopoiesis. DEK could have separate effects on HPC and HSC, as suggested by the direct acting effects of DEK on single HPC. Alternatively, DEK may alone or in addition allow HSC to favor a self-renewal, versus a differentiation pathway to HPC. Exactly how DEK mediates this regulation

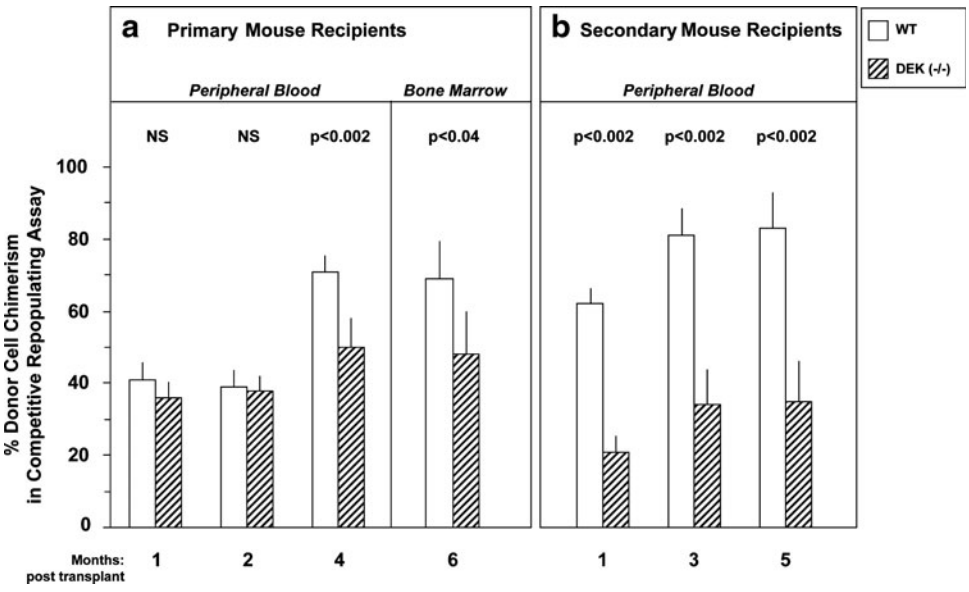

FIG. 5. Primary and secondary engrafting capability of DEK −/− and WT control donor hematopoietic stem cells. Experiments were designed as noted in the Materials and Methods section. Results are given as donor cell ($CD45.2^+$) chimerism in blood and bone marrow of lethally-irradiated (F1; dual $CD45.2^+$/$CD45.1^+$) recipients. Primary engraftment was with competitor cells (BoyJ; $CD45.1^+$); secondary transplants did not use additional competitor cells. Results are expressed as mean ± 1 SEM. For primary transplants, bone marrow cells from 2 WT and 2 DEK −/− mice were used. Cells from each mouse were combined with competitor cells and intravenously infused into 5 lethally irradiated recipients each for WT cells, and into 4 and 5 recipients each for DEK −/− cells cells, for a total of 10 primary recipients for WT and 9 primary recipients for DEK −/−. Bone marrow from each group of primary mice, 6 months after transplant were separately pooled (10 for WT; 9 for DEK −/−) and these pools injected without additional competitor cells into 10 (WT) and 9 (DEK −/−) recipients.

in the cell is not yet known. This may entail transcriptional effects. DEK is heavily posttranslationally modified, and is acetylated in vivo at lysine residues in the first 70 *N*-terminal amino acids [15]. Both acelylation and phosphorylation of DEK decrease its affinity for DNA elements in transcriptional promoter regions, consistent with its involvement in transcriptional modulation [3,6,10]. Our recent observation that DEK is vital to global heterochromatin integrity in vivo suggests that this activity may also play a role in the way DEK affects hematopoiesis [3].

It is possible that DEK may be of potential clinical value for enhancing HSC activity/proliferation in vivo, or in an ex-vivo situation. Either of these possibilities would have clinical applicability and relevance, and further assessment of these activities are warranted.

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Author Disclosure Statement

The authors have no conflicts of interest to disclose.

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