Brief report

Sustained alterations in biodistribution of stem/progenitor cells in Tie2Cre$^{+}\alpha4^{f/f}$ mice are hematopoietic cell autonomous

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We have generated Tie2Cre$^{+}\alpha4^{f/f}$ mice with documented α4-integrin ablation in hematopoietic and endothelial cells. A prominent feature in this model is a sustained, significant increase in circulating progenitors at levels higher than the levels seen with Tie2Cre^{+}VCAM-1$^{f/f}$ mice. To test whether phenotypic differences are due to contributions by ligands other than VCAM-1 in bone marrow, or to α4-deficient endothelial cells or pericytes, we carried out transplantation experiments using these mice as donors or as recipients. Changes in progenitor biodistribution after transplantation were seen only with α4-deficient donor cells, suggesting that these cells were necessary and sufficient to reproduce the phenotype with no discernible contribution by α4-deficient nonhematopoietic cells. Because several similarities are seen after transplantation between our results and those with CXCR4$^{-/-}$ donor cells, the data suggest that VLA4/VCAM-1 and CXCR4/CXCL12 pathways contribute to a nonredundant, ongoing signaling required for bone marrow retention of progenitor cells during homeostasis. (Blood. 2007;109:109-111)

Introduction

The use of Cre-lox strategy to mediate conditional deletion of embryonic lethal genes in a tissue-restricted manner has enabled the investigation of their functional roles in adult life. A commonly used approach to delete chosen floxed genes in hematopoietic cells is the interbreeding with MxCre transgenic mice harboring an interferon-inducible transgene.[1-5] Although efficient deletion in hematopoietic cells has been documented using this strategy, the extent of deletion in nonhematopoietic cells has not been rigorously assessed previously. The α4 integrin is expressed widely in hematopoietic and nonhematopoietic cells, such as endothelial cells[6] or pericytes[7] within the bone marrow (BM). In fact, its presence in endothelial cells is critical in tumor progression, in neoangiogenesis, and in developing blood vessels.[6,7] Conditional deletion of α4 integrin in adults has led to sustained changes in biodistribution of stem/progenitor cells at steady-state hematopoiesis.[5] A similar, albeit transient, picture has been described with the use of antifunctional α4-integrin antibodies in vivo.[8] Using either strategy, it has not been clear to what extent the phenotype is solely attributed to deletion of α4 in hematopoietic cells or contribution by similarly affected cells in BM microenvironment, as has been recently advocated.[9-11] To address this issue the following approaches were used. First, we generated mice in which the α4 integrin is ablated both in hematopoietic and in endothelial cells, by breeding α4 floxed mice (f/f) with Tie2Cre^{+} transgenic mice with the expected α4 ablation in hematopoietic and endothelial cells.[12] Second, we carried out transplantation experiments using either donor cells from these mice or using them as recipients of normal cells. The results from these experiments provide definitive conclusions about the contribution of α4-integrin ablation only in hematopoietic cells to the phenotype seen at homeostasis.

Materials and methods

Mice

(C57Bl/6 × 129) mice homozygous for the floxed α4 allele (α4$^{f/f}$) were crossed to C57Bl/6 mice expressing Cre under the control of the Tie2 promoter to generate Tie2Cre$^{+}\alpha4^{f/f}$ mice. Genotyping was performed by polymerase chain reaction (PCR) using the following primers: F-1: 5′-CGGGATCAGAAAGAATCCA-3′; F-2: 5′-CCACCTGGTGTATGAAAGC-3′; Rev: 5′-GATCACATACAGTTGCAAGC-3′ for discrimination of wild-type (WT), f, and Δ alleles, respectively. Tie2Cre^{+}/VCAM-1$^{f/f}$ mice were previously described.[13]

Antibodies

Directly conjugated antibodies from BD Biosciences (San Diego, CA) were B220, CD3, CD4, CD8, CD45, TER119, and GR1. Directly conjugated CD49d antibody was from Southern Biotechnology (Birmingham, AL). BM or blood cells stained with these antibodies were analyzed on a FACSCalibur (BD Biosciences) using CellQuest software (BD Biosciences).

Clonogenic progenitor assays

Colony-forming unit in culture (CFU-C) assays were performed from BM, spleen, or peripheral blood (PB) samples using a methylcellulose mixture (Methocult GF; StemCell Technologies, Vancouver, BC, Canada), as described previously.[5]

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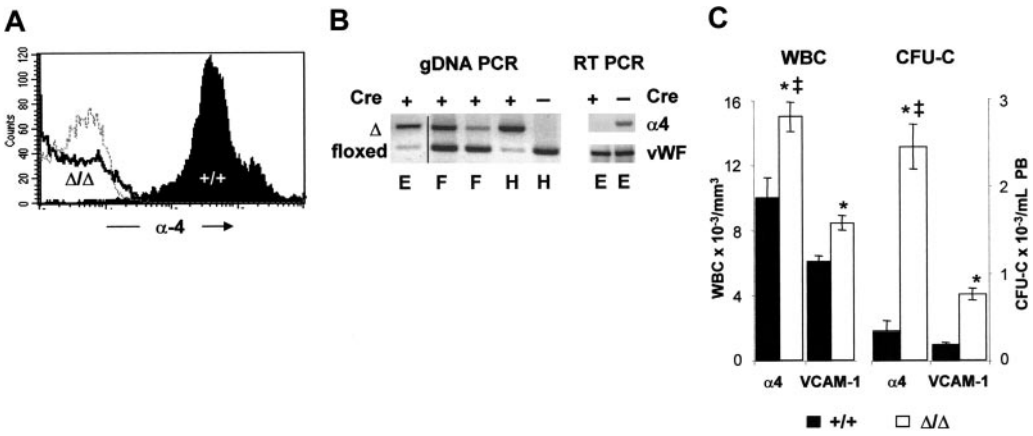

Figure 1. Deletion of α4 gene in hematopoietic and nonhematopoietic cells in Tie2Cre^{+}α4$^{f/f}$ mice. (A) Surface expression of α4 integrin on circulating WBCs in Tie2Cre^{+}α4$^{f/f}$ (open histogram, thick line) and control (filled histogram) mice. FACS analysis with PS/2, anti-α4 antibody showed that in Tie2Cre^{+}α4$^{f/f}$ mice, 3.1% ± 0.2% (n = 20) of circulating WBCs and 2.6% ± 0.6% (n = 4) of BM cells (not shown) are α4^{+}. Isotype-matched immunoglobulin (open histogram, dashed line) served as a negative control. (B) Deletion of α4 integrin in endothelial cells (E), fibroblasts (F), and hematopoietic BM cells (H) at the genomic level (left panel) and at the mRNA level (right panel). Note only partial deletion of α4 gene in fibroblasts. (C) Deletion of α4 integrin results in increase in WBCs and in circulating hematopoietic progenitors. Circulating CFU-C levels in Tie2Cre^{+}a4$^{f/f}$ mice (2446 ± 256, n = 17) are significantly increased compared with control levels (338 ± 113, n = 9, $P < .01$). Circulating WBC and CFU-C levels in Tie2Cre^{+}VCAM-1$^{f/f}$ mice are shown for comparison. The asterisk indicates significant difference over controls, $P < .01$; ‡, significant difference over Tie2Cre^{+}VCAM-1$^{f/f}$ mice, $P < .05$. Error bars indicate standard error of the mean (SEM).

Transplantation experiments

BM cells (0.5, 2.5, or 5.0 × 10^{6}/recipient) from α4-deficient or from normal mice were infused into lethally irradiated normal or α4-deficient recipients (1150 cGy whole body irradiation with a ^{137}Cs source). Recipients were analyzed at multiple points after transplantation. Donor reconstitution was verified by fluorescence-activated cell sorting (FACS) or PCR or both.

BM stromal endothelial cell cultures

Endothelial cells were procured from cleansed, flushed, and minced long and pelvic bones and cultured as described.[13] For generating BM-derived fibroblasts the cells were cultured in DMEM with 20% FCS for 4 to 7 weeks. Contaminating macrophages were removed by depletion of CD45^{+} cells, whereas endothelial cells were identified by several endothelial-specific markers.[13] Reverse transcription-PCR (RT-PCR) was performed as described.[13] Primers for α4: forward, 5′-TGTGGAAGGCTGGATTCTTT-3′, reverse, 5′-CGGGTCTTCTGAACAGGATT-3′; VWF: forward, 5′-AGAGGAGAGTACATCTGGGAG-3′, reverse, 5′-AGAACACCTGCACTGCATGGC-3′.

Statistical analysis

All statistical analyses were performed with a 2-tailed Student *t* test.

Results and discussion

Tie2Cre^{+}α4$^{f/f}$ mice are viable and healthy with no apparent ill effects when observed for more than a year. Phenotypically all circulating PB or BM cells were virtually α4^{-} (Figure 1A-B), as were BM endothelial cells, whereas α4 deletion in bone fibroblasts/mesenchymal cells was partial at best (Figure 1B). Detailed studies on hematopoiesis disclosed that white blood cell (WBC) counts were above control levels (Figure 1C) with no deviation in lineage-specific contributions (data not shown). Circulating progenitors were several-fold higher than controls (Figure 1C) and this difference was maintained through 16 months of postnatal life (Figure S1A, available on the *Blood* website; see the Supplemental Figure link at the top of the online article). Overall the hematopoietic phenotype was similar to that previously described for Tie2Cre^{+}VCAM-1$^{f/f}$ mice.[13] However, circulating progenitors in Tie2Cre^{+}α4$^{f/f}$ were higher (Figure 1C). The fact that α4 integrin has additional ligands other than VCAM-1, (ie, fibronectin, osteopontin, VWF, ADAMS28, α4-chain itself) could explain the more efficient release of α4$^{Δ/Δ}$ progenitors. Alternatively, one may suggest that efficient excision of α4 integrin from endothelial cells or other microenvironmental cells in this model may have a contributing role.[11]

To test these possibilities, transplantation experiments were carried out. We transplanted α4-deficient BM cells (Figure 2A) into lethally irradiated Cre(−) littermates (C57B1/6 × 129) and assessed the levels of circulating progenitors after complete hematopoietic reconstitution. The α4$^{+/+}$ cells given to α4$^{+/+}$ or to α4$^{Δ/Δ}$ lethally irradiated recipients were also followed similarly (Figure 2B). Although α4$^{+/+}$ cells given to α4$^{+/+}$ or α4$^{Δ/Δ}$ recipients did not show increased levels of circulating progenitors after transplantation, α4-deficient cells transplanted to normal recipients had significantly increased numbers of circulating progenitors up to 29 weeks after transplantation, compared to their concurrent

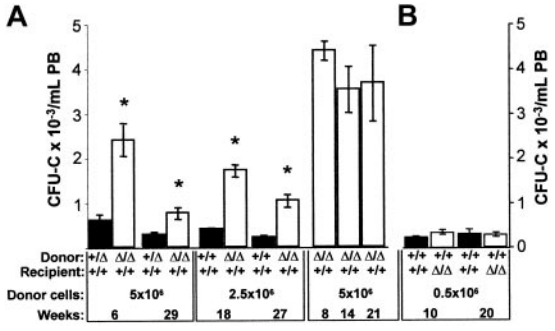

Figure 2. Posttransplantation increase in circulating progenitors is seen only with α4 integrin–deficient donor cells. (A) Transplantation experiments were performed by injecting α4-deficient (□) or α4-sufficient (+/+ or +/Δ; ■) BM cells into normal recipients (2.5-5.0 million BM cells were transplanted into 4-7 mice/group), and (B) by injecting α4$^{+/+}$ cells into normal (■) or α4-deficient (□) recipients. (One half million BM cells were transplanted to 5 mice/group.) A significant increase in circulating hematopoietic progenitor cells is seen only with α4$^{Δ/Δ}$ donor cells and is maintained up to 29 weeks after transplantation; at that time, 2.9% ± 0.3% of BM cells and 3.0% ± 0.5% of WBCs were α4^{+} in mice given transplants (Figure S1B). No increase in circulating progenitors above control levels is seen when normal cells are transplanted into α4-deficient recipients. The asterisk indicates a significant difference between transplanting α4-deficient and α4-sufficient donor cells. Error bars indicate SEM.

controls. Thus, $\alpha4^{\Delta/\Delta}$ hematopoietic cells transplanted to a normal BM environment are necessary and sufficient to provide a picture similar to one seen in animals that did not receive a transplant (Figure S1B). Alternatively, α4 deletion in endothelial cells or pericytes[7] in BM does not seem to contribute in any measurable way to the altered biodistribution of hematopoietic progenitor cells at steady state.

The data presented here and the information we recently published[14] display several common features to those described[15] with transplantation of $CXCR4^{-/-}$ fetal cells: (1) high numbers of circulating progenitor cells starting at 2 weeks after transplantation and continuing for at least 16 weeks; (2) rescue of engraftment defects when higher numbers of donor cells are used; and (3) the presence of a significant number of long-term repopulating cells in circulation, as documented by transplantation experiments using blood.[5,15] The enhanced release of progenitors from BM to blood seen in both cases is cell autonomous and would suggest that the 2 pathways, α4/VCAM-1 or CXCR4/CXCL12 (SDF-1), have a nonredundant influence on the retention of progenitors in BM at steady-state hematopoiesis.

Although alterations in progenitor biodistribution were seen previously in other genetically deficient models, that is, with Rac2 $GTPase^{-/-4}$ or $CaR^{-/-}$ cells,[16] a picture recapitulating after transplantation the phenotype seen in animals that did not receive a transplant has not been documented in these cases. A dramatic increase was seen after transplantation with $Rac2^{-/-}$ $Rac1^{\Delta/\Delta}$ doubly deficient donor cells, but this was transient, lasting 4 weeks after transplantation,[17] unlike our results. Surprisingly, transplantation of $MxCre^{+}\beta1^{f/f}$ cells and their ablation after transplantation, theoretically affecting several β1-integrin heterodimers, did not lead to any alterations in stem/progenitor biodistribution,[2] inviting the speculation that β1 integrins expressed in nonhematopoietic cells or niche cells are more important.[9,10] Although β1/β7-deficient animals display a phenotype (in BM and PB) similar to our $Tie2Cre^{+}\alpha4^{f/f}$ mice, this phenotype, unlike the one in our mice, reverts to normal with aging as a result of putative compensatory changes.[11] Whatever the case, our results clearly show that active engagement of α4 integrin in hematopoietic cells plays an important role in physiologic progenitor retention within BM.

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Authorship

Contribution: G.V.P. performed experiments and analyzed and evaluated the data; T.U. performed experiments (PCR); and T.P. directed research and wrote the manuscript.

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