Published in final edited form as:

Exp Hematol. 2007 February ; 35(2): 326–334.

HEMATOPOIETIC PROGENITOR CELLS (HPC) FROM MOBILIZED PERIPHERAL BLOOD DISPLAY ENHANCED MIGRATION AND MARROW HOMING COMPARED TO STEADY-STATE BONE MARROW HPC

Halvard Bonig, Gregory V Priestley, Vivian Oehler, and Thalia Papayannopoulou

Department of Medicine/Hematology, University of Washington, Seattle, WA

Abstract

Objective—Faster engraftment of G-CSF mobilized peripheral blood (MPB) transplants compared to steady-state bone marrow (ssBM) is well documented and clinically relevant. A number of different factors likely contribute to this outcome. In the present study we explored whether independent of cell number there are intrinsic differences in the efficiency of progenitor cell homing to marrow between MPB and ssBM.

Methods—Mobilization was achieved by continuous infusion of G-CSF alone or in combination with other mobilizing agents. *In vivo* homing assays, *in vitro* migration assays, gene expression analysis and flow cytometry were utilized to compare homing-related *in vivo* and *in vitro* properties of MPB and ssBM HPC.

Results—Marrow homing of murine MPB HPC, generated by different mobilizing schemes, was reproducibly significantly superior to that of ssBM, in lethally irradiated as well as in non-irradiated hosts. This phenotype was independent of MMP9, selectins, $\beta 2$ - and $\alpha 4$ -integrins. Superior homing was also observed for human MPB HPC transplanted into NOD/SCID $\beta 2$ microglobulin-/-recipients. Inhibition of HPC migration abrogated the homing advantage of MPB but did not affect homing of ssBM HPC, whereas enhancement of motility by CD26 inhibition improved marrow homing only of ssBM HPC. Enhanced SDF-1 dependent chemotaxis and low CD26 expression on MPB HPC were identified as potential contributing factors. Significant contributions of the putative alternative SDF-1 receptor, RDC1, were unlikely based on gene expression data.

Conclusion—The data suggest increased motility as a converging end point of complex changes seen in MPB HPC which is likely responsible for their favorable homing.

Introduction

The favorable kinetics of neutrophil reconstitution of Granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood (MPB) grafts compared to steady-state bone marrow (ssBM) have boosted MPB as a favored source of hematopoietic “stem cell” transplantation. [1-3] The benefits of the earlier engraftment are significant, since the incidence of severe bacterial infections is reduced, and hospitalization is abbreviated.[1,4] The number of clonogenic cells infused with MPB grafts often exceeds that of ssBM grafts, and the earlier

Corresponding author: Halvard Bonig, M.A., M.D., University of Washington Department of Medicine/Hematology, 1959 NE Pacific Street, Seattle, WA 98195-7720, Phone (206)543-2368, Fax (206)543-3050, hbonig@u.washington.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

engraftment has sometimes been attributed to this fact.[5,6] However, earlier engraftment was also observed when similar numbers of MPB and ssBM CD34+ cells were transplanted, which suggests some additional inherent functional differences between the donor cells.[7] Despite numerous studies describing differences between ssBM and MPB immature hematopoietic cells at gene expression, protein and *in-vitro* functional level, the specific feature(s) responsible for this engraftment benefit was previously not experimentally tested. In this report we describe a differential homing pattern between MPB and ssBM colony-forming unit-culture (CFU-C), and validate an intrinsic migratory phenotype of MPB cells as likely responsible for this effect.

Materials and Methods

Mice

B6×129 mice were used as donors and recipients unless otherwise stated; they were obtained from Jackson Laboratories (Bar Harbor, ME). MMP-9^{-/-}, CD18^{-/-}, CD62ELP^{-/-} or Rosa26βgeo mice (on a B6×129 genetic background), bred and housed at our facility, were used in some experiments; these mice were previously described.[8,9] For human HPC transplantation, NOD/SCIDβ2microglobulin^{-/-}-mice (NOD/SCIDβ2m^{-/-}, Jackson Laboratories) were used as recipients. Mice were housed in the Specific Pathogen Free mouse vivarium of the Department of Comparative Medicine at the University of Washington, Seattle, WA, with autoclaved chow and water *ad libitum*. NOD/SCIDβ2m^{-/-}-mice were housed in a Helicobacter-free room within the same facility. Studies and procedures were approved by the University of Washington IACUC.

Cells

Mouse: To generate MPB HPC, anaesthetized mice received subcutaneous implants of model 2001 osmotic minipumps (Alzet, Palo Alto, CA) continuously releasing 100 µg rhuG-CSF/kg*day (Neupogen, Amgen, Thousand Oaks, CA). Some animals received a single intraperitoneal injection of 4 mg (ca. 200 mg/kg) Cyclophosphamide one day prior to pump implantation, as described.[10] Mice were exsanguinated on day 5 of mobilization. ssBM cells were isolated from naïve (i.e., not G-CSF treated) age- and sex-matched donors by flushing long bones. Erythrocytes in ssBM and MPB samples were lysed by brief incubation in hypotonic buffer at 37 °C prior to transplantation. Where indicated, c-kit⁺ ssBM or MPB cells were then enriched by immunomagnetic purification (Miltenyi Biotech, Auburn, CA) as described.[11] **Human:** Samples of immunomagnetically enriched CD34⁺ cells from cadaveric ssBM or MPB, collected from volunteers with permission of the University of Washington Review Board in accordance with the Helsinki declaration, were received from the NIH repository at the Fred Hutchinson Cancer Research Center.

Homing assay

Where indicated, B6×129 mice were lethally irradiated (1200 cGy single dose, using a cesium source at the dose rate of >100 cGy/min).[11] NOD/SCIDβ2m^{-/-}-mice used for 18-hour homing (not engraftment) studies were likewise subjected to lethal irradiation (1050 cGy), as previously reported,[12,13] to avoid contamination with donor CFU-C, as occurs in sublethally irradiated animals. Donor cells were injected i.v. into the lateral tail vein. A fraction of the transplanted inoculum (generally 30,000 - 100,000 total nucleated cells/mL) was plated in commercially available growth factor-enriched methylcellulose media (MC, StemCell Technologies, Vancouver, BC or Miltenyi Biotech, for murine or human CFU-C, respectively), to quantify the number of injected CFU-C after 1 or 2 weeks in culture for murine or human CFU-C, respectively. BFU-E, CFU-mixed and CFU-GM were distinguished using a 2.5× magnification dissecting microscope. Marrow homing was quantified at the time points indicated as previously described.[13] At least 5 animals per group were tested. Homing results

are displayed as the fraction of the total injected CFU-C homed to the entire bone marrow, based on earlier estimates of femur contribution to total bone marrow mass.[14]

Migration assay

Transwell migration through 5 μ m pore-size transwells (Corning Costar) was assessed after 4 h in DMEM+10% FCS $\pm$ SDF-1 (Peprotech, Rocky Hill, NJ), using flow cytometry, [13] or colony assays.

Inhibition of CD26, CXCR4 or Gi-protein signaling on transplanted cells

To inhibit CD26 activity, cells were incubated in PBS/BSA in the presence of DiprotinA (Sigma, St. Louis, MO) at a concentration of 5 mM at 37 °C for 30 minutes, as described. [15] To render the cells unresponsive to SDF-1 they were incubated in PBS/BSA with the CXCR4 antagonist AMD3100 (Sigma) for 15 minutes at 37 °C before injection, as described. [13,15] Cell suspensions were then used directly for transplantation without washing. To block Gi protein signaling cells were incubated in serum-free medium with Pertussis toxin (PTX, List Biochemicals, Campbell, CA) for 4 hours prior to transplantation, then washed and resuspended in PBS/BSA for transplantation, as described.[13] α 4-integrin was blocked with endotoxin-free PS/2 antibody (kindly provided by Dr. Roy Lobb (Biogen, Cambridge, MA)), as described.[16]

Flow cytometry

Cells were labeled with directly fluorescence-conjugated CXCR4 or CD26 or isotype control antibodies (all from Becton-Dickinson, San Jose, CA) according to standard protocols and analyzed by multi-parameter flow cytometry (FACSCalibur, Becton-Dickinson) and CellQuest software (Becton-Dickinson).

Gene expression analysis

Gene expression analysis using HG-U133A microarrays (Affymetrix, Santa Clara, CA) was performed on 8 and 10 samples of ssBM and MPB CD34+ cells, respectively. Five μ g of non-amplified high quality RNA was biotin-labeled using the Enzo BioArray™ High Yield™ RNA Transcript Labeling Kit (Affymetrix). Standard Affymetrix protocols and guidelines were followed as published in the GeneChip™ Expression Analysis Technical Manual. Image (DAT), cell intensity (CEL), and chip (CHP) files were generated using MAS 5.0 software (Affymetrix). Individual arrays were screened for quality. Any array with a 3'/5' GAPDH or β -actin ratio of > 1.5 or background >100 was eliminated from the analysis. Expression values for individual probe sets were extracted from CEL files using the Bioconductor™[17,18] and gcRMA [19,20] programs to background adjust, normalize and log-transform the data. GenePlus™software, which uses estimating equation techniques, was used to determine differential expression between the two groups using gene-by-gene ANOVA analysis to calculate p-values and the number of false discoveries.[21] Analysis relating to adhesion and cell motility was performed using Gene Ontology categories.

Reverse transcriptase-polymerase chain reaction

Total RNA was isolated using Qiagen RNeasy kits (Quiagen, Valencia, CA) according to manufacturer's protocol and suspended in RNase-free water, followed by reverse transcription using Superscript Reverse Transcriptase (Invitrogen, Carlsbad, CA) and oligo-dT primers. cDNA generated from the equivalent of 50 ng total RNA was analyzed by PCR for expression of β -actin and RCD1 message. Oligonucleotide primers (20-mers) were synthesized by IDT (Integrated DNA Technologies, Coralville, IA). Gene and cDNA sequences were drawn from NCBI Entrez Nucleotide (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide&itool=toolbar>),

oligonucleotide sequences were generated in Primer3 input (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Sequence specificity was assured by insertion of oligonucleotide sequences into BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). In addition, bands for RDC1 of the expected length were excised and sequenced at the UW Dept. of Biochemistry sequencing core, to confirm their identity. Oligonucleotide sequences for β -actin were 5' agccatgtacgtagccatc and 3' ttgatgtcacgcagcatt, to yield a 250 bp fragment, and those for RDC1 were 5' atagagcacgggttgacac and 3' ccaggagaagcacagtagcc, for a fragment with an expected length of 214 bp. PCR was performed on an Eppendorf Mastercycler (Westbury, NY) with 3 min initial denaturation, followed by 30 (β -actin, RDC1) or 40 (RDC1) cycles of 30 sec denaturation, 30 sec annealing (60 °C) and 90 sec primer extension, plus a final 10 min primer extension. $MgCl_2$ concentration was 1.5 mM or 3 mM for β -actin or RDC1, respectively. Taq was from Promega (Madison, WI). PCR products were visualized on ethidium bromide stained TAE-Agarose gels.

Statistical analysis of homing and migration assays

Descriptive statistics and t-tests were calculated using Excel (Microsoft, Redmond, WA). A $p < 0.05$ was considered statistically significant.

Results

Homing assays

The size of the transplanted inoculum varied between experiments and ranged between 9,000 and 43,000 CFU-C for MPB, and between 12,000 and 45,000 CFU-C for ssBM. The total transplanted cell number per recipient was between 5 and 33×10^6 for ssBM and between 11 and 100×10^6 for MPB. Over this range, the % CFU-C homing was constant for either of the sources of donor cells, in keeping with previous systematic studies to this effect [22], suggesting that within this range of donor cell numbers, cell number does not affect the efficiency of homing. Colony assays from the injected and the recovered cell populations were scored for CFU-GM, CFU-mixed and BFU-E. For mouse the relative distribution between transplanted or recovered CFU-C lineages did not differ between cell sources (ca. 5-10% BFU-E), and the efficiency of marrow homing was the same for CFU-GM and BFU-E. The frequency of BFU-E was greater in human MPB than in ssBM, as reported,[12] but again the efficiency of homing did not differ between CFU-GM and BFU-E. Therefore, all results are reported here as total CFU-C.

Superior marrow homing of murine and human MPB-CFU-C

Measured 3 and 18 hours after transplantation, the mean marrow homing/retention of MPB CFU-C was 41% and 45% better than the mean homing of ssBM CFU-C (Fig. 1A, D). HPC mobilized with the combination of Cyclophosphamide + G-CSF or Flt3L + G-CSF were similarly tested and showed the same superior homing as MPB HPC (Fig. 1B and data not shown). In order to determine contributions of irradiation-induced changes in the marrow microenvironment, cells from Rosa26 β geo mice were used as donors for lethally irradiated or non-irradiated recipients. Marrow homing was assessed 48 hours later, using G418-enriched colony assay medium, to kill host-derived CFU-C, as described.[11] The later time point was selected in these studies not only to overcome technical limitations (low frequency of G418 resistant CFU-C within the host marrow in non-irradiated recipients), but also to inquire whether the better CFU-C homing persisted. Contributions of irradiation-induced changes in the marrow microenvironment to the differential homing were not observed, since whether irradiated or non-irradiated hosts were used the recovery of CFU-C from the marrow of recipients of MPB donor cells was significantly greater than the one using ssBM cells (Fig. 1C). As the proportions of mature cells in ssBM vs. MPB differs markedly, it was important

to rule out chaperoning effects of cotransplanted mature “companion” leukocytes. Both enriched c-kit⁺ cells from MPB and total nucleated cells from MPB were used as donor populations. The marrow homing of CFU-C from these sources was equally efficient, and both were superior to homing of ssBM total nucleated cells ($p < 0.05$, data not shown). Similarly, homing of human CD34⁺ cells from MPB displayed significantly better CFU-C homing to marrow than CD34⁺ cells from ssBM (Fig. 1E).

Enhanced chemotaxis of MPB-CFU-C

Chemokinetic and chemotactic migratory activity of ssBM and MPB were compared using *in vitro* transwell assays. c-kit⁺ cells from MPB displayed much higher degree of chemokinesis and chemotaxis to SDF-1 than ssBM c-kit⁺ cells (Fig. 2A). While chemokinesis of CFU-C was undetectable, chemotaxis of MPB CFU-C was likewise significantly greater than that of ssBM CFU-C (Fig. 2B). HPC mobilized with Cyclophosphamide+G-CSF had similarly increased migration (data not shown). This result was observed despite reproducibly down-regulated CXCR4 on murine c-kit⁺ MPB-cells compared to c-kit⁺ ssBM cells ($p < 0.005$, Fig. 2C) or on human CD34⁺ cells (data not shown and [23,24]). Gene expression studies of human CD34⁺ cells from ssBM or MPB additionally confirmed significant downregulation of CXCR4 mRNA in MPB ($p < 10^{-5}$, data not shown). An alternative novel receptor for SDF-1, RDC1, was recently described.[25] mRNA for RDC1 was expressed at very low levels in murine MPB mononuclear cells (MNC), since a band was only seen after 40 PCR cycles (Fig. 2E), and also very low levels were detected by gene expression arrays in human CD34⁺ cells (data not shown).

Relationship between migration and marrow homing

If the exaggerated motility of MPB cells played a role in the superior marrow homing of MPB, we reasoned that manipulations of this motile behavior would result in differential effects on the marrow homing for these two donor cell types (Fig. 3A). The Gi-protein inhibitor Pertussis toxin (PTX) and the small-molecule CXCR4 antagonist AMD3100 block chemotactic migration to SDF-1 *in vitro* ([11,26] and Fig. 2D). ssBM or MPB cells were therefore incubated with AMD3100 or PTX and transplanted as described.[13] As previously shown,[13] homing of ssBM-CFU-C was not affected by this treatment. In contrast, marrow homing of MPB was decreased by AMD3100 or PTX by >50% ($p < 0.005$, Fig. 3B,C). The CD26 inhibitor DiprotinA increases *in vitro* migration and marrow homing of ssBM CFU-C, as was previously reported and confirmed by us (Fig. 3D).[15] However, marrow homing of MPB was not affected (Fig. 3D), which was not unexpected, considering our novel finding that CD26 is virtually absent from c-kit⁺ MPB-cells (Fig. 3E).

Favorable homing of MPB persists in the absence of MMP9, $\beta 2$ -integrins, VLA-4 or selectins

Potential roles of L-selectin, endothelial selectin ligands, $\beta 2$ integrins or MMP-9 for marrow homing were previously proposed, but not stringently tested.[14,27-38] Such contributions, as well as differential contributions to the homing of grafted MPB or ssBM CFU-C could be definitively tested by studying genetic mouse models. Marrow homing of genetically deficient cells (MMP9^{-/-}, $\beta 2$ ^{-/-}, L-selectin) or in genetically deficient hosts (E-selectin^{-/-}), whether from MPB or from ssBM, was equally efficient as that of WT cells (Fig. 4A-D), and the favorable homing of MPB was maintained in all instances. Of note, mobilization of MMP-9 mice was as efficient as that of WT mice, as also noted earlier.[16,39] In keeping with earlier findings, reduced surface expression of $\alpha 4$ integrin was observed on MPB c-kit⁺ cells ([33,35] and data not shown). Reduced $\alpha 4$ expression and $\alpha 4$ -mediated adhesion were also reported for CD34⁺ cells,[40,41] a phenotype which was suggested to favor engraftment, possibly by promoting reversible adhesion and increased migration.[42,43] We additionally tested homing of ssBM and MPB cells treated with or without $\alpha 4$ -blocking antibodies. Anti- $\alpha 4$ incubated samples,

both ssBM and MPB, showed a similar reduction in marrow homing by 30-40%, so that marrow homing of MPB remained superior to that of ssBM even in the absence of functional $\alpha 4$ integrin (data not shown). Seeking to identify further differences between MPB and ssBM, gene expression arrays were performed on 8 or 10 samples of cadaveric ssBM CD34+ cells or volunteer donor MPB CD34+ cells, respectively, averaged and compared to each other. With the exception of CXCR4, expression of which was down-regulated ca. 10-fold in MPB CD34+ cells (vide supra), these analyses were not contributory; specifically, differential regulation of RDC1, CD26, integrins, metalloproteinases/ADAMs, selectins or fucosyl transferases at gene expression level was not observed (data not shown).

Discussion

We have documented here that MPB CFU-C home with significantly greater efficiency than ssBM CFU-C, and this applied to all mobilizing regimens used, and to both murine and human CFU-C. The order of magnitude by which marrow homing differs compares favorably with earlier described independent homing-enhancing approaches.[15,44] As we are showing, superior marrow homing of MPB is observed irrespectively of whether total nucleated cells or c-kit+ or CD34+ cells were injected. Since in these experiments HPC were not enriched to homogeneity we can not prove that the greater homing efficiency is strictly a consequence of an HPC-intrinsic property. These studies nevertheless show that the property governing the superior homing of MPB HPC to marrow is independent of accompanying mature leukocytes or of certain host conditions and lies within a cell population which is enriched for HPC. Although several studies have shown [45] or implied [46-48] the potential for superior marrow homing of MPB HPC, opposing views, showing similar [49] or even inferior [10] homing of MPB, have also been reported. Reasons for the discrepancies are not clear but may include insufficient sample size [49] and immunophenotypic instead of functional definition of HPC. [10] Well-established functional *in vivo* assays were used to compare marrow homing of CFU-C present in ssBM or MPB.[50] These assays give reproducible results, score for live clonogenic cells seeded in marrow, and correlate with CFU-S seeded in marrow, [50] *i.e.* the cells responsible for early, short-term engraftment. Clearly, marrow homing is required for engraftment, and improving marrow homing, particularly with limited donor cell numbers, enhances engraftment.[15]

Our data confirm and extend previous data [15,44,51] by demonstrating that superior migration is not simply associated with, but likely causally related to the superior marrow homing of MPB CFU-C. Increase of migration by CD26 blockade was recently proposed as a strategy to increase marrow homing/engraftment of transplanted HPC.[15] Since CD26 is almost completely down-regulated on MPB HPC and CD26 blockade had no effect on marrow homing of MPB HPC, this strategy may not be equally applicable to MPB cells. The seemingly counter-intuitive increase of SDF-1-induced chemotaxis of MPB CFU-C despite down-regulated CXCR4 was previously reported, but not explained.[24,27,47,52,53] Since CXCR4 is re-expressed on HPC after brief cytokine exposure [54,55] or early after transplantation [47], the physiologic relevance of down-regulated CXCR4 on MPB HPC found *in vitro* is not clear, as it may not represent the status of the cells exposed to the *in vivo* environment in the conditioned host. Furthermore, the correlation between CXCR4 expression and *in vitro* migration may not be straight forward. Thus Broxmeyer et al. demonstrated several-fold different migration towards SDF-1 of AMD3100+G-CSF MPB and G-CSF MPB, despite similarly low CXCR4 expression.[56] Our data suggest that loss of CD26 expression may be instrumental in the exquisite sensitivity of MPB HPC to SDF-1 despite low CXCR4, since CD26 constitutes a negative feed-back mechanism of SDF-1 induced chemoattraction by cleaving SDF-1.[15] The mechanism of CD26 downregulation is unclear but does not seem to occur at gene expression level.(data not shown and [57,58]) If G-CSF directly induces increased motility of ssBM, then mobilized BM may be expected to also have enhanced homing compared to ssBM. However,

BM cells from G-CSF treated mice neither display the same enhanced motility shown by MPB, nor the enhanced marrow homing, at least when tested on day 5, at the peak of mobilization (data not shown). This in addition to CD26 down-regulation may be interpreted to suggest an instrumental role of the mobilized blood milieu with its soluble factors in eliciting the phenotype required for superior marrow homing of G-CSF mobilized HPC. Consistent with this concept is the suggestion that “supernatants of leukapheresis products” from G-CSF MPB may sensitize HPC to SDF-1-dependent migration and consequently may contribute to the improved marrow homing of MPB cells.[48,59] The molecular mechanisms enabling this behavior remain to be elucidated. Alternatively, it is conceivable that after G-CSF stimulation the cells with inherently greater migratory propensity preferentially leave the marrow, to accumulate in MPB. Accumulated evidence suggests that the enhanced migratory property of MPB HPC is the nexus of a number of different intermediate mechanisms. As G-CSF mobilized cells display a complex phenotype, it will be difficult to isolate one in the absence of all others. Recently a novel SDF-1 receptor, RDC1 or Cmkor1, was described, [25] although previously a monogamous ligand-receptor relationship had been assumed for SDF-1/CXCR4. If RDC1 was expressed and active on immature hematopoietic cells, then this might contribute to the prominent chemotactic response of MPBHPC despite down-regulated CXCR4. This question was not previously addressed. However, RDC1 mRNA expression was very low in murine MPB MNC by PCR (Fig. 2E), so that our data do not support significant contributions of RDC1 to HPC migration and homing. Low expression of RDC1 mRNA in MPB MNC is in keeping with array data on murine bone marrow side population and main population cells published in the NIH’s GEO project database (dataset GDS1010), where RDC1 message was below the detection limit for gene expression array chips, and is also in agreement with our own gene expression array data on human CD34+ cells from ssBM and MPB.

In aggregate, we are thus showing that marrow homing of MPB HPC is more efficient than marrow homing of ssBM HPC, likely by virtue of the greater chemotactic migration of MPB HPC towards SDF-1. This finding may explain, at least in part, the favorable engraftment kinetics of MPB grafts. More avid chemotactic migration of MPB HPC was observed despite down-regulated CXCR4 and without apparent contribution of the novel alternative SDF-1 receptor, RDC1. Down-regulated CD26 is a novel contributor to this functional phenotype, along with other previously identified and unidentified parameters. Although the functional behavior of G-CSF mobilized cells may not be indicative of the reason for their egress, it certainly facilitates their return to bone marrow.

Acknowledgements

This study was supported by an ASH Fellow Scholar Award (HB), and NIH Grant HL58734 (TP). Some of the data represented in this manuscript were previously presented in abstract form (Papayannopoulou T *et al.* Blood. 1999;94:689a, Bonig H *et al.* Blood. 2005;106:142a).

References

- [1]. Chao NJ, Schriber JR, Grimes K, et al. Granulocyte colony-stimulating factor “mobilized” peripheral blood progenitor cells accelerate granulocyte and platelet recovery after high-dose chemotherapy. Blood 1993;81:2031–2035. [PubMed: 7682454]
- [2]. Ringden O, Remberger M, Runde V, et al. Peripheral blood stem cell transplantation from unrelated donors: a comparison with marrow transplantation. Blood 1999;94:455–464. [PubMed: 10397713]
- [3]. Schmitz N, Linch DC, Dreger P, et al. Randomised trial of filgrastimmobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. Lancet 1996;347:353–357. [PubMed: 8598700]
- [4]. Mertelsmann R, Herrmann F, Hecht T, Schulz G. Hematopoietic growth factors in bone marrow transplantation. Bone Marrow Transplant 1990;6:73–77. [PubMed: 1698494]

- [5]. Hassan HT, Zeller W, Stockschrader M, Kruger W, Hoffknecht MM, Zander AR. Comparison between bone marrow and G-CSF-mobilized peripheral blood allografts undergoing clinical scale CD34+ cell selection. *Stem Cells* 1996;14:419–429. [PubMed: 8843543]
- [6]. Tricot G, Jagannath S, Vesole D, et al. Peripheral blood stem cell transplants for multiple myeloma: identification of favorable variables for rapid engraftment in 225 patients. *Blood* 1995;85:588–596. [PubMed: 7529066]
- [7]. Verfaillie CM, Almeida-Porada G, Wissink S, Zanjani ED. Kinetics of engraftment of CD34(-) and CD34(+) cells from mobilized blood differs from that of CD34(-) and CD34(+) cells from bone marrow. *Exp Hematol* 2000;28:1071–1079. [PubMed: 11008020]
- [8]. Papayannopoulou T, Priestley GV, Nakamoto B, Zafiroopoulos V, Scott LM. Molecular pathways in bone marrow homing: dominant role of alpha(4)beta(1) over beta(2)-integrins and selectins. *Blood* 2001;98:2403–2411. [PubMed: 11588037]
- [9]. Kennedy DW, Abkowitz JL. Mature monocytic cells enter tissues and engraft. *Proc Natl Acad Sci U S A* 1998;95:14944–14949. [PubMed: 9843995]
- [10]. Wagers AJ, Allsopp RC, Weissman IL. Changes in integrin expression are associated with altered homing properties of Lin(-/lo)Thy1.1(lo)Sca-1(+)-c-kit(+) hematopoietic stem cells following mobilization by cyclophosphamide/granulocyte colony-stimulating factor. *Exp Hematol* 2002;30:176–185. [PubMed: 11823053]
- [11]. Bonig H, Priestley GV, Nilsson LM, Jiang Y, Papayannopoulou T. PTXsensitive signals in bone marrow homing of fetal and adult hematopoietic progenitor cells. *Blood* 2004;104:2299–2306. [PubMed: 15217839]
- [12]. Bonig H, Chang KH, Nakamoto B, Papayannopoulou T. The p67 laminin receptor identifies human erythroid progenitor and precursor cells and is functionally important for their bone marrow lodgment. *Blood* 2006;108:1230–1233. [PubMed: 16609068]
- [13]. Bonig H, Priestley GV, Papayannopoulou T. Hierarchy of molecular-pathway usage in bone marrow homing and its shift by cytokines. *Blood* 2006;107:79–86. [PubMed: 16141352]
- [14]. Katayama Y, Hidalgo A, Furie BC, Vestweber D, Furie B, Frenette PS. PSGL-1 participates in E-selectin-mediated progenitor homing to bone marrow: evidence for cooperation between E-selectin ligands and alpha4 integrin. *Blood* 2003;102:2060–2067. [PubMed: 12763924]
- [15]. Christopherson KW 2nd, Hangoc G, Mantel CR, Broxmeyer HE. Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* 2004;305:1000–1003. [PubMed: 15310902]
- [16]. Papayannopoulou T, Priestley GV, Bonig H, Nakamoto B. The role of Gprotein signaling in hematopoietic stem/progenitor cell mobilization. *Blood* 2003;101:4739–4747. [PubMed: 12595315]
- [17]. Gentleman, R. Dudoit, and Hornick. The Bioconductor FAQ; 2003. <http://www.bioconductor.org>.
- [18]. Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 2004;5:R80. [PubMed: 15461798]
- [19]. Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003;4:249–264. [PubMed: 12925520]
- [20]. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 2004;20:307–315. [PubMed: 14960456]
- [21]. Zhao LP, Prentice R, Breeden L. Statistical modeling of large microarray data sets to identify stimulus-response profiles. *Proc Natl Acad Sci U S A* 2001;98:5631–5636. [PubMed: 11344303]
- [22]. Szilvassy SJ, Bass MJ, Van Zant G, Grimes B. Organ-selective homing defines engraftment kinetics of murine hematopoietic stem cells and is compromised by Ex vivo expansion. *Blood* 1999;93:1557–1566. [PubMed: 10029584]
- [23]. Larochelle A, Krouse A, Metzger M, et al. AMD3100 mobilizes hematopoietic stem cells with long-term repopulating capacity in nonhuman primates. *Blood* 2006;107:3772–3778. [PubMed: 16439684]
- [24]. Voermans C, Gerritsen WR, von dem Borne AE, van der Schoot CE. Increased migration of cord blood-derived CD34+ cells, as compared to bone marrow and mobilized peripheral blood CD34+ cells across uncoated or fibronectin-coated filters. *Exp Hematol* 1999;27:1806–1814. [PubMed: 10641598]

- [25]. Balabanian K, Lagane B, Infantino S, et al. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. *J Biol Chem* 2005;280:35760–35766. [PubMed: 16107333]
- [26]. Juarez J, Bradstock KF, Gottlieb DJ, Bendall LJ. Effects of inhibitors of the chemokine receptor CXCR4 on acute lymphoblastic leukemia cells in vitro. *Leukemia* 2003;17:1294–1300. [PubMed: 12835717]
- [27]. Janowska-Wieczorek A, Marquez LA, Nabholz JM, et al. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. *Blood* 1999;93:3379–3390. [PubMed: 10233890]
- [28]. Watanabe T, Dave B, Heimann DG, Lethaby E, Kessinger A, Talmadge JE. GM-CSF-mobilized peripheral blood CD34+ cells differ from steady-state bone marrow CD34+ cells in adhesion molecule expression. *Bone Marrow Transplant* 1997;19:1175–1181. [PubMed: 9208110]
- [29]. Watanabe T, Dave B, Heimann DG, Jackson JD, Kessinger A, Talmadge JE. Cell adhesion molecule expression on CD34+ cells in grafts and time to myeloid and platelet recovery after autologous stem cell transplantation. *Exp Hematol* 1998;26:10–18. [PubMed: 9430509]
- [30]. Sackstein R. The bone marrow is akin to skin: HCELL and the biology of hematopoietic stem cell homing. *J Invest Dermatol Symp Proc* 2004;9:215–223.
- [31]. Dimitroff CJ, Lee JY, Raffi S, Fuhlbrigge RC, Sackstein R. CD44 is a major E-selectin ligand on human hematopoietic progenitor cells. *J Cell Biol* 2001;153:1277–1286. [PubMed: 11402070]
- [32]. Schreiber TH, Cain DW, Dimitroff CJ, Sackstein R. G-CSF mobilization radically upregulates alpha-1,3-fucosyltransferases-4 and -7 generating high avidity E-selectin ligands on circulating nucleated cells. *Blood* 2003;102:115a.
- [33]. Mohle R, Murea S, Kirsch M, Haas R. Differential expression of L-selectin, VLA-4, and LFA-1 on CD34+ progenitor cells from bone marrow and peripheral blood during G-CSF-enhanced recovery. *Exp Hematol* 1995;23:1535–1542. [PubMed: 8542944]
- [34]. Gigant C, Latger-Cannard V, Bensoussan D, et al. CD34+ cells homing: quantitative expression of adhesion molecules and adhesion of CD34+ cells to endothelial cells exposed to shear stress. *Biorheology* 2003;40:189–195. [PubMed: 12454404]
- [35]. Gigant C, Latger-Cannard V, Bensoussan D, Feugier P, Bordigoni P, Stoltz JF. Quantitative expression of adhesion molecules on granulocyte colony-stimulating factor-mobilized peripheral blood, bone marrow, and cord blood CD34+ cells. *J Hematother Stem Cell Res* 2001;10:807–814. [PubMed: 11798507]
- [36]. Sovalat H, Racadot E, Ojeda M, Lewandowski H, Chaboute V, Henon P. CD34+ cells and CD34+CD38-subset from mobilized blood show different patterns of adhesion molecules compared to those from steady-state blood, bone marrow, and cord blood. *J Hematother Stem Cell Res* 2003;12:473–489. [PubMed: 14594504]
- [37]. Asosingh K, Renmans W, Van der Gucht K, et al. Circulating CD34+ cells in cord blood and mobilized blood have a different profile of adhesion molecules than bone marrow CD34+ cells. *Eur J Haematol* 1998;60:153–160. [PubMed: 9548413]
- [38]. Ford CD, Greenwood J, Anderson J, Handrahan D, Petersen FB. Good and poor mobilizing patients differ in mobilized CD34+ cell adhesion molecule profiles. *Transfusion* 2004;44:1769–1773. [PubMed: 15584993]
- [39]. Levesque JP, Liu F, Simmons PJ, et al. Characterization of hematopoietic progenitor mobilization in protease-deficient mice. *Blood* 2004;104:65–72. [PubMed: 15010367]
- [40]. Lichterfeld M, Martin S, Burkly L, Haas R, Kronenwett R. Mobilization of CD34+ haematopoietic stem cells is associated with a functional inactivation of the integrin very late antigen 4. *Br J Haematol* 2000;110:71–81. [PubMed: 10930981]
- [41]. Prosper F, Stronck D, McCarthy JB, Verfaillie CM. Mobilization and homing of peripheral blood progenitors is related to reversible downregulation of alpha4 beta1 integrin expression and function. *J Clin Invest* 1998;101:2456–2467. [PubMed: 9616217]
- [42]. Orschell-Traycoff CM, Hiatt K, Dagher RN, Rice S, Yoder MC, Srour EF. Homing and engraftment potential of Sca-1(+)lin(-) cells fractionated on the basis of adhesion molecule expression and position in cell cycle. *Blood* 2000;96:1380–1387. [PubMed: 10942381]

- [43]. Christ O, Kronenwett R, Haas R, Zoller M. Combining G-CSF with a blockade of adhesion strongly improves the reconstitutive capacity of mobilized hematopoietic progenitor cells. *Exp Hematol* 2001;29:380–390. [PubMed: 11274767]
- [44]. Kimura T, Boehmler AM, Seitz G, et al. The sphingosine 1-phosphate receptor agonist FTY720 supports CXCR4-dependent migration and bone marrow homing of human CD34+ progenitor cells. *Blood* 2004;103:4478–4486. [PubMed: 14988150]
- [45]. van Hennik PB, de Koning AE, Ploemacher RE. Seeding efficiency of primitive human hematopoietic cells in nonobese diabetic/severe combined immune deficiency mice: implications for stem cell frequency assessment. *Blood* 1999;94:3055–3061. [PubMed: 10556189]
- [46]. Herrera C, Sanchez J, Torres A, Pascual A, Rueda A, Alvarez MA. Pattern of expression of CXCR4 and adhesion molecules by human CD34+ cells from different sources: role in homing efficiency in NOD/SCID mice. *Haematologica* 2004;89:1037–1045. [PubMed: 15377464]
- [47]. Plett PA, Frankovitz SM, Wolber FM, Abonour R, Orschell-Traycoff CM. Treatment of circulating CD34(+) cells with SDF-1alpha or anti-CXCR4 antibody enhances migration and NOD/SCID repopulating potential. *Exp Hematol* 2002;30:1061–1069. [PubMed: 12225798]
- [48]. Wysoczynski M, Reca R, Ratajczak J, et al. Incorporation of CXCR4 into membrane lipid rafts primes homing-related responses of hematopoietic stem/progenitor cells to an SDF-1 gradient. *Blood* 2005;105:40–48. [PubMed: 15328152]
- [49]. Szilvassy SJ, Meyerrose TE, Ragland PL, Grimes B. Differential homing and engraftment properties of hematopoietic progenitor cells from murine bone marrow, mobilized peripheral blood, and fetal liver. *Blood* 2001;98:2108–2115. [PubMed: 11567997]
- [50]. Papayannopoulou T, Nakamoto B. Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. *Proc Natl Acad Sci U S A* 1993;90:9374–9378. [PubMed: 7692447]
- [51]. Voermans C, Kooi ML, Rodenhuis S, van der Lelie H, van der Schoot CE, Gerritsen WR. In vitro migratory capacity of CD34+ cells is related to hematopoietic recovery after autologous stem cell transplantation. *Blood* 2001;97:799–804. [PubMed: 11157500]
- [52]. Ratajczak J, Reca R, Kucia M, et al. Mobilization studies in mice deficient in either C3 or C3a receptor (C3aR) reveal a novel role for complement in retention of hematopoietic stem/progenitor cells in bone marrow. *Blood* 2004;103:2071–2078. [PubMed: 14604969]
- [53]. Zheng Y, Watanabe N, Nagamura-Inoue T, et al. Ex vivo manipulation of umbilical cord blood-derived hematopoietic stem/progenitor cells with recombinant human stem cell factor can up-regulate levels of homing-essential molecules to increase their trans migratory potential. *Exp Hematol* 2003;31:1237–1246. [PubMed: 14662330]
- [54]. Hirayama F, Yamaguchi M, Yano M, et al. Spontaneous and rapid reexpression of functional CXCR4 by human steady-state peripheral blood CD34+ cells. *Int J Hematol* 2003;78:48–55. [PubMed: 12894851]
- [55]. Kollet O, Petit I, Kahn J, et al. Human CD34(+)CXCR4(-) sorted cells harbor intracellular CXCR4, which can be functionally expressed and provide NOD/SCID repopulation. *Blood* 2002;100:2778–2786. [PubMed: 12351385]
- [56]. Broxmeyer HE, Orschell CM, Clapp DW, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 2005;201:1307–1318. [PubMed: 15837815]
- [57]. Graf L, Heimfeld S, Torok-Storb B. Comparison of gene expression in CD34+ cells from bone marrow and G-CSF-mobilized peripheral blood by highdensity oligonucleotide array analysis. *Biol Blood Marrow Transplant* 2001;7:486–494. [PubMed: 11669215]
- [58]. Steidl U, Kronenwett R, Haas R. Differential gene expression underlying the functional distinctions of primary human CD34+ hematopoietic stem and progenitor cells from peripheral blood and bone marrow. *Ann N Y Acad Sci* 2003;996:89–100. [PubMed: 12799287]
- [59]. Lataillade JJ, Clay D, David C, et al. Phenotypic and functional characteristics of CD34+ cells are related to their anatomical environment: is their versatility a prerequisite for their bio-availability? *J Leukoc Biol* 2005;77:634–643. [PubMed: 15673546]

Figure 1.

Superior marrow homing of MPB grafts:

A-D: *In vivo* marrow homing of murine B6/129 CFU-C from ssBM or MPB was quantified, using colony assays.

A: Three hours after transplantation of lethally irradiated hosts, the mean fraction of CFU-C homed to bone marrow was 41% greater for MPB than for ssBM (* $p < 0.05$).

B: A similar marrow homing advantage as for G-CSF-MPB was observed after 18 hours for CFU-C mobilized with Cyclophosphamide+G-CSF (* $p < 0.001$) or with Flt3L+G-CSF (not shown).

C: Forty-eight hours after transplantation, recovery from recipient marrow of MPB-derived CFU-C was significantly greater compared to ssBM-derived CFU-C. This observation was made in both irradiated (left, * $p < 0.005$) and nonirradiated (right, * $p < 0.005$) hosts, ruling out a role of the irradiated marrow microenvironment in the differential marrow homing of ssBM and MPB. For both MPB and ssBM, the fraction of homed CFU-C was markedly increased 48 hours after transplantation compared to 18 hours, likely indicating early proliferation of the transplanted cells.

Depicted in Fig. 1A-C are mean+SEM for % of injected CFU-C homed to marrow (MPB , ssBM).

D: Forty-five% more efficient mean marrow homing of MPB CFU-C compared to ssBM CFU-C was observed 18 hours after transplantation of lethally irradiated isogenic recipients ($p < 0.001$). Each dot represents homing in one individual animal; bold line and error bars represent mean+SEM for % of injected CFU-C homed to marrow.

E: Marrow homing of human CD34+ cells from ssBM or MPB was tested in a xenotransplantation model. Eighteen hours after transplantation, the mean recovery of MPB

CFU-C from recipient marrow was 3-fold increased compared to ssBM (* $p < 0.001$). Each dot represents homing in one individual animal; bold line and error bars represent mean $\pm$ SEM for % of injected CFU-C homed to marrow. These data indicate that the greater marrow homing ability of MPB CFU-C is intrinsic to a population enriched for HPC, and that it applies to human as well as to mouse CFU-C.

Figure 2.

Increased migration of MPB cells:

Transwell migration of MPB c-kit⁺ cells (A), both spontaneous and SDF-1 directed, was markedly greater than that of ssBM c-kit⁺ cells (* $p < 0.005$ MPB vs. ssBM), and SDF-1 directed migration of MPB-CFU-C (B) was likewise increased (* $p < 0.005$). CFU-C did not show appreciable spontaneous migratory activity (not shown). Superior chemotactic migration was observed despite decreased CXCR4 expression ($p < 0.005$) on MPB c-kit⁺ cells. Shown is one representative histogram plot (C). Incubation with Pertussis toxin (PTX) blocked spontaneous and chemotactic migration of MPB (left) and ssBM (right) c-kit⁺ cells (D). Gene expression of the alternative SDF-1 receptor, RDC1, in MPB MNC was very low, since it was only detected after 40 PCR cycles, which makes a contributory role of RDC1 to MPB HPC migration unlikely (E). Migration is depicted as mean+SEM of % of total. Cell type is indicated in the upper right corner, cell source and stimulus below the column. The FACS histogram depicts expression of CXCR4 on c-kit⁺ cells (Isotype control lightly shaded, MPB black line, ssBM solid grey; cell counts on Y-axis, fluorescence intensity on X-axis).

Figure 3.

Increased migration is responsible for superior marrow homing of MPB CFU-C:

A: Cartoon depicting the experimental approach: The consequences of altered CFU-C migration for marrow homing were tested. This was done by blocking migration through inhibition of CXCR4 or Gi protein signaling (using AMD3100 or PTX), and by increasing migration (using DiprotinA, an inhibitor of the dipeptidylpeptidase CD26). **B-D:** AMD3100 (marrow homing assessed 3 hours after transplantation, **B**) and PTX (marrow homing assessed 18 hours after transplantation, **C**) significantly blocked marrow homing of MPB CFU-C (* $p < 0.005$ compared to untreated MPB), but had no effect on ssBM homing. In contrast, DiprotinA significantly increased marrow homing only of ssBM CFU-C (* $p < 0.05$ compared to untreated ssBM, marrow homing assessed 18 hours after transplantation, **D**). No DiprotinA effect on MPB homing was observed, likely due to loss of CD26 expression on MPB c-kit+ cells (**E**). These data suggest that the more avid migration of MPB cells, mediated through SDF-1, CXCR4 and Giprotein signals, guides their better marrow homing. Homing data are shown as mean+SEM for % of injected CFU-C homed to marrow, (MPB , ssBM , solid: - inhibitor, hatched: + inhibitor; * indicates significant difference ($p < 0.05$) compared to samples from the same source that were not treated with the inhibitor). The FACS histogram depicts expression of CD26 on c-kit+ cells (Isotype control lightly shaded, MPB black line, ssBM solid grey; cell counts on Y-axis, fluorescence intensity on X-axis).

Figure 4.

Favorable homing of MPB persists in the absence of MMP9, β 2-integrin and selectins: Previously reported differential regulation of MMP-9, β 2-integrins and selectins between MPB and ssBM HPC prompted us to test their contribution to marrow homing, using genetically deficient donors or recipients. Marrow homing of MPB or ssBM CFU-C from MMP-9^{-/-}-mice (A) CD18^{-/-}-mice (B) or CD62L^{-/-}-mice (D) in WT recipients or of WT CFU-C in CD62ELP^{-/-}-recipients (C) was entirely normal, *i.e.* no different than marrow homing in WT-to-WT transplantation, as shown in Fig. 1. The superior marrow homing of MPB-derived CFU-C was maintained (* $p < 0.005$, $p < 0.05$, $p < 0.001$, $p < 0.005$, respectively for ssBM vs. MPB). Eighteen hour homing data are shown as mean+SEM for % of injected CFU-C homed to marrow (MPB , ssBM).