Published in final edited form as:

Blood Cells Mol Dis. 2012 June 15; 49(1): 29–31. doi:10.1016/j.bcmd.2012.03.005.

IS THE POST-TRANSPLANTATION TREATMENT WITH AMD BENEFICIAL?

Yi Jiang¹, Tatiana Ulyanova¹, and Thalia Papayannopoulou¹

¹Dept. of Medicine, Div. of Hematology, University of Washington, Seattle, WA

Abstract

Recent data have suggested novel ways to enhance donor cell engraftment by treating transplanted recipients with CXCR4/CXCL12 inhibitors, thereby expanding the biologic potential of these molecules primarily used for mobilization purposes. We tested whether repeated pulse inhibitions of CXCR4/CXCL12 signaling using AMD, an inhibitor of CXCR4/CXCL12 signaling, would enhance engraftment in non-myeloablative murine recipients, similar to data published in a myeloablative setting. We documented an increased proportion of circulating neutrophils (both donor- and host-derived) in the AMD-treated group, but this increase was not kinetically influenced by AMD treatment and multilineage engraftment was not enhanced. Although our results with neutrophils are similar to recent clinical data in neutropenic patients chronically treated with AMD, the absence of multilineage engraftment diverges from data in myeloablative recipients. We conclude that pulses of mobilization by AMD post transplantation do not enhance multilineage engraftment.

Keywords

Bone marrow transplantation; AMD; engraftment

INTRODUCTION

The chemokine SDF-1, has been established as a major chemoattractant for hematopoietic stem/progenitor cells responsible for their physiologic retention within BM and for their quiescence and self renewal properties [1,2,3,4]. Apart from guiding the stem cell's migratory properties, SDF-1 also influences the physiologic migration of mature cells, like granulocytes and lymphocytes [5,6].

Several of these properties have been exploited for clinical purposes, such as the mobilization and collection of donor cells for transplantation following treatment with FDA approved CXCR4/SDF-1 inhibitor molecules, like AMD3100 [7,8,9]. Of further interest were propositions-not yet validated-to harness the stem/progenitor cell mobilizing ability of CXCR4/SDF-1 inhibitors for enhancing engraftment of donor cells with no recipient conditioning [10], or more intriguingly for boosting donor cell engraftment in fully

Corresponding Author: Thalia Papayannopoulou, UW Campus Box 357710, Seattle, WA 98195, thalp@uw.edu, Phone: 206-543-5756, Fax: 206-543-3560.

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AUTHOR CONTRIBUTION

Yi Jiang and Tatiana Ulyanova performed the experiments and Thalia Papayannopoulou wrote this manuscript.

conditioned recipients with repeated applications of transient blockade of CXCR4/SDF-1 signaling for several weeks [11]. These strategies were mechanistically interpreted as enhancing the availability of stem cell BM “niches”, either at the time of transplantation [10] or through pulsed releases of endogenous stem cells repeatedly applied post transplantation [11].

We reasoned that, if correct, the above strategy should be applicable or would work even better using non-myeloablated recipients. Such a scheme is increasingly applied in transplants for non-malignant hematopoiesis and enhancement of donor cell engraftment would be beneficial in cases of limited donor cell availability, or after in vitro incubation of cells, such as for gene therapy. We tested this possibility by transplanting BM/GFP+ cells and evaluating engraftment for several weeks in AMD-treated vs. non-treated murine recipients. Although we found that an increased proportion of circulating GR-1+ cells (both donor- and host- derived) in the treated group became evident at 6–8 weeks after transplant, this was not accompanied by multilineage engraftment, in contrast to previous data. Furthermore, the kinetics of an increase in circulating white cells, both in our experiments and those previously published, was not influenced by the treatment with AMD and may not be effective to counteract the early period of cytoreduction after transplantation in conditioned recipients. Thus our data suggest that repeated post transplantation treatments with the antiCXCR4 inhibitor, AMD, at least in the non-myeloablative setting, is not an efficient strategy to boost engraftment.

MATERIAL AND METHODS

Mice used in this study were: C57Bl/6J and C57BL/6-Tg(UBC-GFP)30Scha/J as recipient and donor mice respectively for transplantation. The GFP+ mice have GFP in all their hematopoietic cells including RBCs and Platelets. Recipient mice (n=20) were irradiated with 300cGy (using a Cesium source) and transplanted with 5x10E6 BM/GFP+ cells. Half of the mice, starting two days after transplant, were treated with AMD3465 (5mg/Kg body weight) three times weekly until nine weeks after transplant. AMD3465 is a monomacrocyclic anti-CXCR4 antagonist of high potency [12] and generously provided to us by Genzyme Corp, Cambridge, MA. At 4–9 weeks post-transplant, complete blood counts and proportions of donor-derived GFP+/GR-1+, GFP+/RBCs and GFP+/Platelets were assessed. All mice were sacrificed after 9 weeks and BM, (two femurs from each), spleen and Peripheral blood were evaluated for cellularity, % LSK (Lineage /Sca1+/kit+) and total CFU (Colony Forming Units) content of donor or host origin. The latter was evaluated by genotyping single colonies using GFP- specific primers (primer sequences were taken from The Jackson Laboratory website, Bar Harbor, MN).

RESULTS AND DISCUSSION

Our findings at 4, 6, 8 and 9 weeks after transplantation in the PBS-treated controls and AMD treated animals are presented in Figs. 1,2 and Tables 1,2 and can be summarized as follows:

We found that **a)** the proportion of Neutrophils, as determined by Hemavet (Drew Scientific, Dallas, TX) was significantly higher in the AMD treated group at weeks 6 and 8 after transplant. (Fig. 1A). Both host- (GFP-) and donor-derived (GFP+) GR1+ cells determined independently by FACS (Fig. 1B) were higher at these times. **b)** In contrast to these data, Hct, Hgb, and platelets as determined by Hemavet and the proportion of donor-derived GFP+/RBCs and GFP+/Platelets were not significantly different at any point after transplant. (Table 1 and Fig. 2). **c)** To test whether findings in PB also reflected those present in BM and spleen of these mice, the animals were sacrificed at week 9 and total cellularities,

progenitor content (CFU), proportion of LSK cells were assessed, as described in Methods. Both host and donor-derived (GFP+) cells in BM, as well as proportions of LSK cells were not significantly different between the two groups. Likewise, total cellularity and donor-derived proportions were similar in spleens of these mice. Total colonies, assessed both in BM and spleen, were present in similar numbers. To assess donor-derived colonies, at least 100 colonies were picked individually from methylcellulose plates and genotyped by PCR. As seen in Table 2, their proportions were similar between the two groups.

Collectively our data suggest, that although multilineage engraftment was not enhanced in the AMD-treated group, there were more circulating Neutrophils in the latter group, probably contributing to the radioprotection survival advantage seen previously in lethally irradiated mice transplanted with limited numbers of cells by Kang et al. [11]. Our interpretation of enhanced release of neutrophils by chronic AMD treatment is also supported by recently published data of patients with WHIM (warts, hypogammaglobulinemia, immunodeficiency and myelokathexis) syndrome treated chronically with AMD3100 maintaining higher numbers of circulating neutrophils [13,14]. All the above clinical data support a dominant role of CXCR4 in neutrophil trafficking, as well as other mature cells, like B-cells (CD19+) and cannot be used to support multilineage engraftment [13,14,15]. However this advantage, both in our case and in Kang et al. is evident after the first month post transplantation, suggesting that the kinetics of donor post transplantation recovery were not influenced by AMD treatment. Furthermore, since we transplanted more cells, the cell dose did not appear to influence the kinetics of recovery. Although our data and those of Kang et al. [11] showed increases in circulating cells with the same kinetics, differences in BM engraftment were not confirmed in our mice. Whether conditioning differences account for the divergence in our data is unclear.

To interpret the enhancement in multilineage engraftment with frequent pulses of AMD treatment, Kang et al. [11] proposed that such a treatment increased the “niche” availability in BM though mobilization of residual recipient stem cells. Mobilization of these cells was not assessed directly, but rather deduced from a reduction of recipient KLS cells in BM. PB was not evaluated and spleen content of recipient progenitors was not increased, as usually occurs with increased PB mobilization. Instead, AMD treatment at 72hrs post-transplant increased mobilization of donor cells and their splenic homing. Furthermore, if availability of BM “niches” was indeed enhanced, such a treatment should be effective shortly after transplantation, without the need of AMD treatment for several weeks thereafter.

In addition to the above mechanism, the authors [11] proposed that AMD treatment attenuated the effects of a cytokine storm that is usually seen post irradiation or post chemotherapy. Indeed less inflammatory cytokines or chemokines were measured in the AMD treated group in BM. Whether these differences in cytokine levels or differences in SDF-1 levels-not assessed by Kang et al.-are responsible for our different outcome is unclear, since low levels of irradiation were used for our recipients. It was further argued [11] that, if high SDF-1 levels post irradiation contribute to HSC quiescence, then its inhibition by AMD shortly after transplant should reverse the proliferation-inhibitory effect of SDF-1 for both host and donor cells. Such an argument, advocated but not documented by Kang et al., did not appear to exert any major influence post transplantation in our setting and it is not supported by in vitro data using AMD [15].

Future carefully designed experiments with AMD-treated recipients are needed to advance our understanding of mechanistic issues involved before any clinical recommendations are proposed.

Acknowledgments

These experiments were funded by NIH grant P01 HL53750 Project 3.

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Figure 1.

A. Proportions of GR-1+ cells in PB are shown and include calculations of host and donor GFP+ cells among GR-1+ cells and AMD-treated and control mice at 4, 6, 8 and 9 weeks post transplantation (AMD was given up to 8 weeks), *p<0.05.

B. Representative Histograms from one recipient mouse at 8 weeks after transplantation. Note that due to survival differences proportions of donor (GFP+), red blood cells/platelets are higher than donor GR-1+/GFP+ cells.

C. Representative dot plots of GFP+/CD45+ BM cells in recipient non-transplanted mice (left panel), in donor BM used for transplantation (middle panel), and in one recipient mouse at 9 weeks post transplantation (right panel).

Table showing quantitative data for RBCs and Platelets (total and donor-derived=green) from control and AMD-treated groups of mice.

Table 1

	4 wks			6 wks			8 wks		
	RBCs M/ μ L	Plts K/ μ L	RBCs M/ μ L	Plts K/ μ L	RBCs M/ μ L	Plts K/ μ L	RBCs M/ μ L	Plts K/ μ L	RBCs M/ μ L
AMD Treated (n=10)	9.54 $\pm$ 0.11 14.0 $\pm$ 1/1%	628 $\pm$ 33.37 13.8 $\pm$ 1.5%	9.46 $\pm$ 0.13 15.9 $\pm$ 1.1%	680 $\pm$ 40.02 23.8 $\pm$ 2.3%	9.57 $\pm$ 0.17 22.6 $\pm$ 2.4%	708.9 $\pm$ 24.73 29.7 $\pm$ 2.7%			
Control (n=10)	9.62 $\pm$ 0.12 13.6 $\pm$ 1.1%	635 $\pm$ 15.80 13.3 $\pm$ 0.9%	9.17 $\pm$ 0.08 16.9 $\pm$ 1.2%	780.7 $\pm$ 15.05 24.01 $\pm$ 2.6%	9.79 $\pm$ 0.14 21.2 $\pm$ 2.2%	715.3 $\pm$ 19.88 27.9 $\pm$ 3.0%			

Table 2

Table showing quantitative data on total and donor-derived (GFP+) cells and CFU-C in BM and spleen of recipients sacrificed at 9 weeks after transplant.

	BONE MARROW				SPLEEN	
	Cells/femur X 10exp6	CFU-C/femur	LSK/femur	Cells/spleen X 10exp6	CFU-C/spleen	
AMD	26.02±0.91	62,575±2,605	7.76±0.57	234.5±14.21	20,162±1,868	
Treated (n=10)	21.84±3.17%	24.36% *	24.42±3.0%	22.43±2.39%		
Control	26.09±0.82	64,310±3,324	8.21±0.52	220.13±11.9		
(n=10)	19.74±1.43%	24.02% **	21.55±3.55%	20.09±11.9%	21,747±1,617	

* 57 donor CFU-C out of 234 colonies (from 4 mice) genotyped

** 80 donor CFU-C out of 333 colonies (from 7 mice) genotyped