Published in final edited form as:

Transfusion. 2013 April ; 53(4): 878–887. doi:10.1111/j.1537-2995.2012.03826.x.

Loss of CD26 Protease Activity in Recipient Mice during Hematopoietic Stem Cell Transplantation Results in Improved Transplant Efficiency

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Abstract

BACKGROUND—A firm understanding of the biology of hematopoietic stem and progenitor cell (HSC/HPC) trafficking is critical to improve transplant efficiency and immune reconstitution during hematopoietic stem cell transplantation (HSCT). Our earlier findings suggested that suppression of CD26/DPPIV (dipeptidylpeptidase IV) proteolytic activity in the donor cell population can be utilized as a method for increasing transplant efficiency. However, factors in the recipient should not be overlooked, given the potential for the bone marrow (BM) microenvironment to regulate HSCT.

STUDY DESIGN AND METHODS—We first evaluated CD26 expression and then investigated the effects of the CD26 inhibitor, Diprotin A, and the absence of CD26 (CD26^{−/−}) in recipient mice on HSC/HPC homing and engraftment using an *in vivo* congenic mouse model of HSCT.

RESULTS—A significant increase in donor cell engraftment into the peripheral blood (PB), and to a lesser extent homing into the BM, was observed in CD26^{−/−} mice or CD26 inhibitor-treated mice. Increased PB engraftment of CD26^{−/−} mice was significant at 3 and 6, but not 1 month, post-transplant. It was noted that the increased homing was statistically greater with donor cell manipulation [CD26^{−/−} donor cells] than with recipient manipulation [CD26^{−/−} recipient mice].

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Conversely, donor and recipient manipulation both worked well the increase PB engraftment at 6 months.

CONCLUSION—These results provide pre-clinical evidence of CD26, in the HSCT recipient, as a major regulator of HSC/HPC engraftment with minor effects on HSC/HPC homing and suggest the potential use of CD26 inhibitors in HSCT patients to improve transplant efficiency.

Keywords

Stem Cell Transplant; Engraftment; Trafficking; Protease; Chemokines; CXCL12

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a curative treatment strategy for many patients with severe hematologic diseases. However, large numbers of transplantable cells are needed and patient survival is compromised when donor cell numbers are limited. Donor cell numbers may be limited when umbilical cord blood (CB) is utilized as a donor source for transplantation of stem cells into adult patients¹⁻³ or when peripheral blood stem cell (PBSC) donors fail to mobilize adequate numbers of stem cells.⁴⁻⁶

Accordingly, our long-term goal is to improve transplant efficiency, the measurable extent to which hematopoietic reconstitution occurs post-HSCT as quantitatively determined by the amount of output (homing and engraftment into the recipient) relative to the amount of input (a fixed number of donor cells). One approach is to modulate the trafficking of hematopoietic stem and progenitor cells (HSC/HPC) from the circulation into the bone marrow during HSCT. Such trafficking is believed to involve interactions with molecules important for proper recruitment and retention; namely selectins, chemokines, and integrins. Each of these interactions provides an opportunity to intervene in order to achieve improved HSCT outcomes. An important question in the field is whether to target the donor cell population, processes in the transplant recipient, or perhaps both. In this study, we investigated regulatory processes in the transplant recipient, especially the role of chemokines and their modulation by recipient factors such as CD26.

Chemokines are a large family of cytokines that not only have a traditional role as chemo-attractants and activators of leukocytes, but also have been implicated in the regulation of hematopoietic development, angiogenesis, tumor growth, and metastasis.^{7,8} Chemokines act through chemokine receptors. The chemokine CXCL12, also known as stromal-cell derived factor-1 (SDF-1), has been shown to attract hematopoietic stem and progenitor cells¹⁰, and both murine and human HSCs express the corresponding chemokine receptor, CXCR4.¹¹ CXCL12/CXCR4 signaling has been implicated in HSC/HPC retention by the HSCT recipient; including migration into and out of the bone marrow and recruitment of other hematopoietic cells to various parts of the body.^{12,13}

A key protease that affects CXCL12/CXCR4 is CD26, also known as dipeptidyl peptidase IV (DPP-IV). CD26 is a membrane-bound extracellular peptidase that cleaves dipeptides from the amino-terminus of polypeptide chains after a x-proline or x-alanine.¹⁴ Originally described as a T-cell activation molecule, it is now regarded as a non-lineage-specific antigen whose expression is regulated by differentiation and activation.¹⁵⁻¹⁷ CD26 is also present in a catalytically-active soluble form in plasma.¹⁸ The specificity of CD26 is very narrow and is known to include chemokines, the pancreatic polypeptide family (including neuropeptide Y and peptide YY), and the glucagon family (glucagon, glucagon-like peptide-1, and glucagon-like peptide-2).¹⁹

Importantly, CD26 can regulate post-translational modification of chemokines through a process we refer to as chemokine amino-terminal proteolytic processing (CATPP). CD26 does this through selective cleavage of chemokines that contain an amino-terminal X-Pro or X-Ala motif. However, even with this motif, some chemokines resist cleavage by CD26, suggesting that not all chemokines serve as *in vivo* substrates.^{20–29} Two chemokines in particular, CXCL12 and CCL22, are *in vitro*, preferentially processed proteolytically by CD26, making them likely *in vivo* targets.³⁰ When full length CXCL12 is processed by CD26, it generates a (–2) amino-terminus truncated chemokine analog of CXCL12. This is important because chemokines bind chemokine receptors via interactions at their amino-terminus, and removal of amino acids from this region reduces receptor activation and/or binding affinity.³¹

In line with our long-term goal of improving HSCT efficiency, we developed a novel method for increasing the number of mouse donor HSC/HPC that traffic properly to recipient's bone marrow (BM). We did this by targeting CD26 in the donor cell population.³² Our first reported data described the ability of CD26 to negatively regulate the *in vitro* migratory response of human CD34⁺ CB cells and normal mouse Sca-1⁺c-kit⁺lin[–] BM cells to CXCL12.^{33,34} In addition, we showed that the (–2) truncated CXCL12 analog cannot induce migration of hematopoietic stem and progenitor cells and inhibits the ability of full-length CXCL12 to stimulate migration during a chemotaxis assay.³³ We have also shown in mice that CD26 is important for G-CSF-induced mobilization of HPC into peripheral blood (PB).^{34,35} Lastly, we found that inhibition of CD26 activity on CD34⁺ or lin[–] human umbilical cord blood (CB) donor cells improves their engraftment into immunodeficient recipient mice.^{36,37} Although treatment of CD34⁺ cells from human G-CSF-mobilized PB donor cells with a CD26 inhibitor, Diprotin A, did not enhance engraftment, treatment of immunodeficient recipient mice with a CD26 inhibitor at the same time as cell infusion increased levels of engraftment.³⁸ CD26 deficient mice (CD26^{–/–}) in general have a normal phenotype under homeostatic conditions but do exhibit increased glucose tolerance and subtle changes in T- and NK-cell subsets.^{39,40} Given the possibility that the specific CD26 inhibitor used may have had off target effects on other proteases, and our need to identify the step during HSC/HPC trafficking which endogenous CD26 in the HSCT recipient is important, we set out to determine the *in vivo* effect of CD26 proteolytic deficiency in recipient mice during HSCT.

MATERIALS & METHODS

Mice

CD26 deficient (CD26^{–/–}) mice³⁹ generated on a C57BL/6 background have been previously utilized by us to demonstrate the importance of CD26 to donor cell populations in hematopoietic stem and progenitor cell trafficking.^{32,34,35} B6.SJL-*Ptprca*^a *Pepcb*^b/BoyJ and C57BL/6 age- and sex-matched mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained under pathogen-free conditions and all animal experimentation was performed under an IACUC approved animal welfare protocol.

Histological Analysis

In order to evaluate mouse CD26 tissue expression, histological analysis was done on formalin-fixed paraffin-embedded tissues. Freshly isolated tissues were fixed in a 4% solution of paraformaldehyde (10% formalin) for 18 h. Femurs were then isolated and decalcified for seven days in 5% formic acid, refreshing the solution every 3 days. Femurs and soft tissues were dehydrated through a series of increasing ethanol concentrations, embedded in paraffin, and cut into 3 micron thick sections. 10 mm citrate buffer antigen retrieval was done. Slides, of tissue sections were incubated for 18 h at 4° C in 15 µg/ml

polyclonal Biotinylated Anti-mouse CD26 (R&D Systems, Minneapolis, MN) in 0.1% goat serum. Slides were rinsed and incubated in VECTASTAIN Elite ABC reagent (Vector Labs, Burlingame, CA), and then stained, using a DAB peroxidase substrate solution (Vector Labs) and counterstained with hematoxylin. Images were obtained using a Nikon Elipse E200 microscope equipped with a 100x/1.25 numeric aperture oil objective and a Nikon DS-5MC Cooled Camera (Nikon, Melville, NY).

Congenic Murine Model of Homing & Engraftment

In vivo mouse models of HSCT were used to test the ability of mouse HSC/HPC to home and engraft into recipient mice efficiently. Short-term homing (24 hours), short-term engraftment (1 and 3 months), and long-term engraftment (6 months) experiments were used to ascertain the transplant efficiency of ten-week old B6.SJL-*Ptprca*^a *Pepc*^b/BoyJ (CD45.1⁺) donor mouse bone marrow (BM) cells into lethally irradiated (950 cGy) ten-week old C57BL/6 or CD26^{-/-} (CD45.2⁺) congenic recipient mice. A group of C57BL/6 recipient mice were treated with a CD26 inhibitor (5μmol Diprotin A, 2x/day) based on our previous studies.^{34,35} The first Diprotin A treatment (IP) was provided at the time of transplantation; the second was given 6 hours later. Pooled donor mononuclear cells (MNC) used for transplantation were obtained using lympholyte-M (Cederlane Laboratories, Ontario, Canada). The short-term homing of donor cells was done in the same manner as we have previously described.³² Donor cells were monitored by flow cytometric analysis of cells in the bone marrow of recipient mice by evaluating CD45.1⁺ and CD45.2⁺ 24 hours after transplantation of 20x10⁶ bone marrow MNC. The engraftment of donor cells was evaluated by establishing the contribution of CD45.1⁺ donor cells to hematopoiesis in the context of CD45.2⁺ recipient cells in the peripheral blood 1, 3, and 6 months after transplantation of 5x10⁵ donor MNC.³² For the studies described here, donor MNC were transplanted into C57BL/6, Diprotin A treated (5μmol Diprotin A, 2x/day), or CD26^{-/-} congenic recipient mice.

Following euthanization of recipient mice by CO₂ inhalation, peripheral blood was collected via cardiac puncture using a 25 gauge needle and a syringe containing the anticoagulant EDTA (Sarstedt, Germany). Bone marrow cells were flushed from the femurs through a 25G needle with PBS. Cells were then pelleted by centrifugation, and red blood cells were lysed as necessary with an ACK lysis solution (155 mM NH₄Cl, 0.1 mM Na₂EDTA, 10 mM KHCO₃, pH 7.2). Cells were assayed immediately by antibody staining for subsequent flow cytometry. Homing efficiency was calculated as the % Sca-1⁺lin⁻ donor cells which home into the recipient bone marrow. Donor cell engraftment was calculated as % donor cell contribution to hematopoiesis in recipient peripheral blood or bone marrow. Data for both homing and engraftment is presented as mean ± SEM and significance was determined by a Mann-Whitney U rank sum test as determined by data variance using SigmaPlot software (version 10.0, Systat Software, Inc.).

Flow Cytometry

Multivariate flow cytometric analysis was performed on cell populations isolated from bone marrow and peripheral blood taken from C57BL/6, C57BL/6 CD26 inhibitor treated, and CD26^{-/-} mice. 1 × 10⁶ cells were stained with appropriate combinations of 1 μg of each primary antibody, using a previously described staining method.^{32,34,41,42} To analyze donor contributions to short-term homing and long-term engraftment, fluorescently conjugated anti-CD45.1, and anti-CD45.2 antibodies were used. To analyze hematopoietic stem/progenitor cell populations during short-term homing studies, the following antibodies were used: anti-lineage (lineage cocktail comprised of anti-CD3e, anti-B220, anti-Gr-1, anti-Mac-1, and TER-199) and anti-Sca-1. Data collection was done using a BD LSRII flow

cytometer and analysis was performed using BD FACSDiva software. Data are expressed as mean $\pm$ SEM for the % of cells positive compared to the Isotype control.

RESULTS

Histological Analysis of CD26 Expression

CD26 expression, as indicated by positive DAB staining, was clearly visible in the spleen, bone marrow, and liver (Figure 1). CD26 staining was absent in all tissues examined from CD26^{-/-} mice, thereby validating the antibody. The expression pattern in the spleen appeared to be consistent with staining of T-lymphocytes in the white pulp/follicle. There was background staining of the stroma and trabecular structure, especially at the parafollicular zone, where endothelial staining may contribute to the penicillar vessels and sheathed arterioles that feed into the sinusoidal structure of the red pulp. Positive staining for CD26 was also prevalent in the bone marrow of decalcified sections in control C57BL/6 mice, but not in CD26^{-/-} mice. It is less clear which cells are responsible for the bone marrow expression pattern. Staining of the liver was consistent with staining of the hepatic sinusoidal endothelium and the bile canaliculus, as previously reported by others.⁴³

Short-Term Homing (24 Hours) Comparing Donor Cell vs Recipient Manipulation

Short-term homing of BoyJ (CD45.1⁺) donor cells into lethally irradiated untreated C57BL/6 mice, CD26 inhibitor (Diprotin A)-treated C57BL/6 mice, or CD26^{-/-} mice (CD45.2⁺) was monitored by flow cytometric analysis of the bone marrow at 24 h post-transplant. We observed a 14.74% $\pm$ 0.96%, 17.81 $\pm$ 1.68%, and 20.74 $\pm$ 2.22% homing efficiency of Sca-1⁺lin⁻ cells into the bone marrow of untreated C57BL/6 control mice, Diprotin A treated C57BL/6 mice, and CD26^{-/-} recipient mice, respectively (Figure 2A). Compared to control mice, this represents a 20.8% increase in of Sca-1⁺lin⁻ cell homing efficiency resulting from CD26 inhibitor treatment of the recipient mice on the day of transplant, and a 40.7% increase in homing efficiency resulting from the use of CD26^{-/-} recipient mice ($p < 0.05$, $n = 5$ mice per group collected from two experiments). We previously reported short-term homing of untreated, Diprotin A treated, and CD26^{-/-} MNC as 13.94 $\pm$ 0.87%, 20.12 $\pm$ 0.49%, and 36.33 $\pm$ 3.11% homing efficiency of Sca-1⁺lin⁻ cells into the bone marrow, respectively (Figure 2A; reprinted with permission from AAAS) following the transplant of MNC.³² This represents a 44% and 160% increase, respectively, in homing efficiency compared to untreated control cells. Analysis of the raw short-term homing data of untreated donor cells into untreated control recipient mice verified that there was no statistical difference in control animals between current studies and our prior studies ($p = 0.55$). Not shown here are our previous observations that Sca-1⁺lin⁻ cells have a higher homing efficiency when transplanted as a component of the whole MNC fraction rather than as a cell-sorted population.³² When the effect of donor cell manipulation [use of CD26^{-/-} cells] is compared to recipient manipulation [use of CD26^{-/-} recipient mice] there is a statistically greater level of homing efficiency resulting from the use of CD26^{-/-} cells than from the use of CD26^{-/-} recipient mice ($p < 0.01$) (Figure 2A).

Long-Term Engraftment (6 Months) Comparing Donor Cell vs Recipient Manipulation

Long-term engraftment of BoyJ (CD45.1⁺) donor cells into lethally irradiated untreated C57BL/6 mice, CD26 inhibitor (Diprotin A) treated C57BL/6 mice, or CD26^{-/-} mice (CD45.2⁺) was monitored by flow cytometric analysis of peripheral blood at 6 months post-transplantation. We observed 42.89 $\pm$ 3.46%, 71.14 $\pm$ 3.72%, and 90.02 $\pm$ 2.11% donor contribution to hematopoiesis in the peripheral blood of, respectively, C57BL/6 mice, Diprotin A treated C57BL/6 mice, and CD26^{-/-} recipient mice (Figure 3). This represents a 66% increase in engraftment with CD26 inhibitor treatment and a 110% increase resulting from the use of the CD26^{-/-} recipient compared to control C57BL/6 recipient mice ($p < 0.01$,

n=16 mice per group collected from three experiments). We previously reported long-term engraftment of untreated mice, Diprotin A treated mice, and CD26^{-/-} mice as, respectively, 46.72 ± 4.73%, 76.64 ± 4.20%, and 95.74 ± 0.50% donor contribution to peripheral blood cells (Figure 2B, reprinted with permission of AAAS) following the transplant of MNC.³² This represents a 64% increase in engraftment with CD26 inhibitor treatment of donor cells and a 104% increase resulting from the use of the CD26^{-/-} donor cells compared to untreated control donor cells. Analysis of the raw long-term engraftment data of untreated donor cells into untreated control recipient mice verified that there was no statistical difference in control animals between current studies (42.89 ± 3.46%) and our prior studies (46.72 ± 4.73%) (p=0.68). When the effect of donor cell manipulation [use of CD26^{-/-} cells] is compared to recipient manipulation [use of CD26^{-/-} mice] there is no statistical difference in engraftment (p=0.38) (Figure 2B).

Temporal Changes in Engraftment (1, 3, & 6 Months)

Short- and long-term engraftment of BoyJ (CD45.1⁺) donor cells into lethally irradiated untreated C57BL/6 or CD26^{-/-} mice (CD45.2⁺) was monitored by flow cytometric analysis of PB and BM at 1, 3, and 6 months post-transplantation. In the PB of C57BL/6 control mice donor cell engraftment was 38.5 ± 10.4% at 1 month post-transplant (Figure 3A). PB engraftment at 3 months (57.4 ± 4.3%) and 6 months (42.9.5 ± 3.5%) post-transplant was not statistically different than 1 month in control C57BL/6 recipient mice. Donor cell PB engraftment into CD26^{-/-} mice at 1 month post-transplant (54.1 ± 12.69%) was not statistically different from C57BL/6 control mice at 1 month post-transplant. However, donor cell engraftment in the PB at 3 months (80.2 ± 4.7%) and 6 months (90.2 ± 2.1%) post-transplant was significantly higher than in C57BL/6 control mice at the same timepoint (Figure 3A, p 0.01). In the BM of C57BL/6 control mice a high percentage (83.1 ± 12.7%) of donor cell engraftment was observed that remained unchanged at 3 and 6 months post-transplant. This was also observed in the BM of CD26^{-/-} mice (83.3 ± 5.7%). No statistical differences in BM engraftment were observed when comparing C57BL/6 control mice with CD26^{-/-} mice at 1, 3, or 6 months post-transplant (Figure 3B).

DISCUSSION

The questions we wished to answer were: 1) Does CD26 in the transplant recipient act as a negative regulator of hematopoietic stem cell homing and/or engraftment? 2) Is it possible to improve transplant efficiency by inhibition or loss of CD26 in the recipient? The data presented here indicate that it is possible to increase significantly the homing efficiency of Sca-1⁺lin⁻ cells within the donor MNC population into the bone marrow by transplanting donor cells into CD26^{-/-} recipient mice (p=0.04) but not by treating the recipient mouse with the CD26 inhibitor, Diprotin A (p=0.15), as compared to untreated normal control C57BL/6 recipients. The resulting increase in homing efficiency was modest (40% for CD26^{-/-} recipient mice). Also shown, is that the use of a CD26 inhibitor in recipients or the use of CD26^{-/-} recipient mice significantly increases the engraftment as represented by donor contribution to hematopoiesis in the peripheral blood (p 0.01). It is possible that treatment of the recipient with the CD26 inhibitor may have had an additional direct effect on the donor cells. This is unlikely since use of CD26^{-/-} mice validates that the involvement of CD26 expressed in the recipient is real. It is also possible that endogenous recovery of hematopoiesis following exposure to irradiation in residual recipient CD26^{-/-} cells may have been muted. This possibility is unlikely since CD26^{-/-} cells engraft well when used as a donor cell source. In addition, the absolute numbers of residual recipient cells in the PB post-transplant was not different when comparing CD26^{-/-} to C57BL/6 control. Lastly, it is important to note that the increase in engraftment (66% for Diprotin A treatment; 110% for

CD26^{-/-} recipient mice) was more substantial than the increase in homing efficiency (21% for Diprotin A treatment; 40% for CD26^{-/-} recipient mice).

Our prior observations related to manipulation of the donor cell populations included substantial increases in homing efficiency associated with CD26 inhibitor treatment or with the use of CD26^{-/-} donor cells –44% and 160% respectively; engraftment was increased 64% and 104%, respectively.³² When recipient manipulation data is compared to donor cell manipulation data, some interesting observations can be made: 1) Manipulation of the donor cell population via inhibition/loss of CD26 substantially improves homing and engraftment; 2) Manipulation of the recipient via inhibition/loss of CD26 dramatically improves engraftment while homing is affected to a much less extent; and 3) Donor cell manipulation [use of CD26^{-/-} cells] is statistically better than recipient manipulation [use of CD26^{-/-} mice] for short-term homing efficiency but is equivalent for long-term engraftment. This suggests that regulation of HSC/HPC trafficking by donor derived CD26 may be acting through different downstream players or on different target cell populations than recipient derived CD26. Additional pre-clinical studies to examine whether manipulation of both the donor cell population and the recipient via inhibition/loss of CD26 would have a synergistic or additive effect on long-term engraftment are warranted.

In order to understand to what extent CD26 alters the kinetics of engraftment, the temporal impact of CD26 on homing and engraftment was evaluated. Comparison of short- and long-term BM and PB engraftment into CD26^{-/-} and control C57BL/6 mice at 1, 3, and 6 months post-transplant leads to insightful observations: 1) There is no significant difference in donor chimerism control C57BL/6 mice at 1, 3, or 6, months post-transplant. This suggests that transplantation of this cell dose (5×10^5) generates a stable chimera of around 80% donor in the BM and 50% donor in the PB in this model system; 2) The use of CD26^{-/-} recipient mice at this cell dose does not substantially alter the level of BM engraftment, regardless of the time point (1, 2, or 3, months post-transplant); And 3) In CD26^{-/-} recipient mice, there is a continued rise in PB engraftment over time. This continued rise in engraftment is what is responsible for the significant difference observed in CD26^{-/-} mice vs C57BL/6 mice at 3 and 6 months post-transplant but not 1 month post-transplant. The observed improvement in PB engraftment, in the absence of changes in BM engraftment, may be a result of CD26 having a greater role in the regulation of engraftment in non-BM hematopoietic compartments, and saturation of BM engraftment at a 0.5×10^6 cell dose. This phenotype observed in our study may seem to conflict with a previously published study examining CD26 inhibitor usage in recipient mice post-HSCT.⁴⁴ However, the study used a significantly higher cell dose ($10\text{--}15 \times 10^6$ unfractionated BM cells), a consequent of using a non-myeloablative conditioning regimen, which contained a low dose of total body irradiation (TBI). Our study used 0.5×10^6 MNC, in a myeloablative model (lethal dose of TBI). In addition, their data shows no improvement in engraftment in the BM at 6 months, which is consistent with our data. Our main phenotype was observed in the PB at 3 and 6 months post-transplant, with the CD26^{-/-} mice having a greater phenotype than the inhibitor treated mice. The mechanism by which engraftment into the PB occurs is not yet known.

Collectively, our data suggest that CD26 expressed in the transplant recipient is relevant as a major regulator of the engraftment of cells into the bone marrow but has minor effects on homing. In addition, CD26 expressed by the donor cell graft appears to have importance with regard to both homing and engraftment. As engraftment cannot occur without first having homing; our data suggests a paradigm shift in the manner in which we understand the biology of regulating transplant efficiency. Specifically, our data indicates that it is possible to achieve a substantial improvement in engraftment efficiency in the recipient's PB while maintaining relatively low levels of homing efficiency into the recipient's BM. This untethering of the regulation of homing and engraftment also suggests the importance of

additional regulatory mechanisms that are specific for hematopoietic stem cell engraftment. The mechanism by which CD26 alters homing or engraftment is still under investigation. Our histological evaluation of CD26 expression in recipient mice determined positive staining for CD26 in the spleen and bone marrow which had not been previously reported, as well as positive staining for CD26 in the liver which was expected. This expression data provides insight into a potential location for mechanism of action for CD26, where it may target chemokine substrates in the marrow or the spleen separately than targeting chemokine substrates in the periphery. Future studies are needed to evaluate whether CXCL12 is the *in vivo* target of CATPP by CD26 in the bone marrow and/or spleen of the HSCT recipient and whether generation of the (–2) truncated chemokine analog of CXCL12 is responsible for altering engraftment.

Several CD26 (DPP-IV) inhibitors are now being used or investigated in the management of type II diabetes, due to the endogenous biological function of the CD26 protease to down-regulate glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). GLP-1 and GIP are important for maintaining increased glucose tolerance. Sitagliptin (Januvia®, MK-0431, Merck & Co.) and Saxagliptin (Onglyza®, BMS-477118, Bristol-Myers Squibb) obtained U.S. Food and Drug Administration (FDA) approval in 2006 and 2009, respectively.^{45,46} Vildagliptin (Zomelis®, LAF237, Novartis) was approved in the European Union and southeast Asia.^{45,46} Alogliptin (SYR-322, Takeda Pharmaceutical Company), Linagliptin (BI-1356, Boehringer Ingelheim),^{45,46} and Gemigliptin (LG Life Sciences, Korea)⁴⁷ are being investigated in clinical trials. Additional DPP-IV inhibitors are reviewed by Gupta, *et al.*⁴⁸ Future pre-clinical and clinical trial studies should be done to investigate the feasibility and efficacy of using CD26 inhibitors in HSCT recipients to improve engraftment.

There are several potential clinical scenarios where one can envision a benefit from the use of CD26 inhibition as a therapeutic strategy. For instance, cord blood transplantation is associated with delayed engraftment and CD26 inhibition may be a method to accelerate engraftment, hence decreasing toxicity. Most of the CD26 inhibitors being investigated are orally bioavailable, making them convenient and less expensive than other investigative alternatives such as double cord blood transplantation or *in vitro* expansion of cord blood. In addition, advances in HLA-haploidentical hematopoietic stem cell transplantation using high dose cyclophosphamide (Cytoxan®) post-transplant to reduce GVHD are very encouraging with little to no delay in neutrophil recovery and platelet recovery but the major concern is delayed immune reconstitution.⁴⁹ Again, CD26 inhibition may decrease morbidity by an enhancement of engraftment which could result in improved immune reconstitution, leading to decreased risk of post-transplant infections and improved anti-tumor effects. Overall, during autologous or allogeneic transplant, platelet utilization is still high; any improvement in engraftment will be significant if the new strategy can result in a decrease in platelet utilization. CD26 inhibitor treatment of HSCT recipients in the setting of cord blood, double cord blood, autologous PBSC transplant, allogeneic PBSC transplant, and haploidentical transplant may be of benefit as a method to accelerate the rate of engraftment and/or improve the overall level of immune reconstitution. These clinical studies remain to be evaluated.

Acknowledgments

This work was supported primarily through a research grant from the National Blood Foundation / American Association of Blood Banks (031824) to KWC. KWC was also supported during this research period by grants from the American Association for Cancer Research (07-10-19-CHRI), the Leukemia & Lymphoma Society (6044-08), the NIH – National Institute of Diabetes and Digestive and Kidney Diseases award (DK074892), the Rush Translational Sciences Consortium (08102761), the Rubschlager Foundation, and the Coleman Foundation (5008).

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Figure 1. Histological analysis of mouse CD26 protein expression in spleen, bone marrow, and liver as shown by positive DAB (brown) staining. Images were acquired using a 100X oil immersion objective.

Figure 2.

(A) Short-term homing as quantified by % homing efficiency of donor Sca-1⁺lin⁻ cells in the recipient's bone marrow 24 hours post-transplant of 20×10^6 donor MNC. (B) Long-term engraftment as quantified by the % donor cell engraftment contributing to hematopoiesis in the recipient's peripheral blood 6 months post-transplant of 5×10^5 donor MNC. Recipient manipulation [use of Diprotin A treated mice or use of CD26^{-/-} mice as recipients] as compared to donor cell manipulation [use of Diprotin A treated donor cells or CD26^{-/-} donor cells] from previously reported studies.³²

Figure 3. Temporal evaluation of engraftment as represented by percent (%) CD45.1⁺ donor cell engraftment into □ C57BL/6 or ■ CD26^{-/-} recipient mouse (A) peripheral blood and (B) bone marrow at 1, 3, and 6 months post-transplant of 5×10^5 donor MNC.