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Chapter 4:

No synergistic effect of co-transplantation of MSC and ex vivo TPO expanded CD34+ cord blood cells on platelet recovery and bone marrow engraftment in NOD SCID mice

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Abstract

After cord blood transplantation, early platelet recovery in immune deficient mice is obtained by expansion of cord blood (CB) CD34+ cells with TPO as single growth factor. Moreover, improvement of hematopoietic engraftment has been shown by co-transplantation of Mesenchymal Stem Cells (MSC). We investigated whether a combination of both approaches would further enhance the outcome of CB transplantation in NOD SCID mice. NOD SCID mice were transplanted with either CB CD34+ cells, CD34+ cells with MSC, TPO expanded CD34+ cells or TPO expanded CD34+ cells with MSC. We analyzed human platelet recovery in the peripheral blood (PB) from day 4 after transplantation onwards and human bone marrow (BM) engraftment at week 6. The different transplants were assessed in vitro for their migration capacity and expression of CXCR4. TPO expansion improved the early platelet recovery in the PB of the mice. Co-transplantation of MSC with CD34+ cells improved BM engraftment and platelet levels in the PB 6 weeks after transplantation. Combining TPO expansion and MSC co-transplantation however, neither resulted in a more efficient early platelet recovery nor in better BM engraftment and even very low or absent BM engraftment occurred. In vitro, MSC boosted the migration of CD34+ cells, suggesting a possible mechanism for the increase in engraftment. Our results show that co-transplantation of MSC with TPO expanded CD34+ cells at most combines, but does not increase the separate advantages of these different strategies. A combination of both strategies even adds a risk of non engraftment.
Introduction

Cord blood (CB) is an alternative hematopoietic graft source for almost 20% of the patients for whom no HLA matched donor can be found. However, CB contains relatively low numbers of hematopoietic stem cells (HSCs), which translates into delayed neutrophil recovery and slow and impaired platelet engraftment when compared to transplantation with bone marrow (BM) or G-CSF mobilized peripheral blood stem cell (PBSC) grafts. Several strategies to overcome this are under investigation such as the selection of CB units containing large cell numbers, double CB transplantation, the ex vivo manipulation of CB cells and co-transplantation of accessory cells, such as MSC. Ex vivo culture of CB cells, depending on culture conditions and growth factors, often alters the functionality of the cord blood cells and/or the composition of the cord blood graft. In this respect, ex vivo culture with TPO as single growth factor accelerates platelet recovery in the PB of mice, without impairment of engraftment in the BM. These platelets are derived from TPO induced lineage negative (CD34-CD61-Lin-) cells preceding megakaryocyte formation.

Mesenchymal stem cells (MSC) are also investigated to boost engraftment. These multipotent stromal cells are characterized by three characteristics, 1) plastic adhesion in culture, 2) the expression of a set of distinct markers and 3) the ability to differentiate into three mesodermal lineages. MSC can be isolated from both adult and fetal tissues. MSC have anti-proliferative, immunosuppressive and anti-inflammatory effects and are currently evaluated in clinical studies for the treatment of immune mediated disorders such as Crohn’s disease, systemic lupus and systemic sclerosis. In hematopoietic stem cell transplantation (HST), post-transplant infusions of MSC as well as co-transplantation of MSC are explored for the treatment and prophylaxis of graft versus host disease and/or graft rejection. In animal models, co-transplantation of MSC, improves engraftment after HST. Clinical studies have so far shown variable results when MSC co-transplantation was compared to neutrophil and/or platelet recovery of historical controls. While improved neutrophil recovery is reported more consistently, the median time to platelet engraftment remained delayed, compared to unrelated BM or PBSC transplants.

Combining TPO expansion of CB CD34+ cells and MSC co-transplantation could enhance the CD34+ cell fraction preceding platelet recovery as well as BM engraftment. In this study, we therefore compared the engraftment potential of both approaches in an NOD SCID mouse model.

Materials and methods

CD34+ cell purification. Umbilical cord blood (CB) was collected with written consent from the mother according to Netcord-FACT standards and with ethical permission from the medical ethical board of the Leiden University Medical Center (LUMC). Mononuclear cells were isolated from CB using a ficoll density gradient. The CD34+ cell fraction was isolated using magnetic CD34+ isolation beads (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of the isolated CD34+ cell fraction was verified by flow cytometry (Beckman Coulter, Woerden, The Netherlands) with CD45-FITC and CD34-PE (Beckman Coulter). The percentage of CD34+/CD45+ cells in the isolated fraction was 91±3%.

Expansion of the CD34+ cells. CD34+ cells were cultured at 37°C and 5% CO2 in a humidified atmosphere in IMDM medium (Gibco, Breda, The Netherlands) supplemented with 20% (v/v) AB heparin plasma (Sanquin Blood Supply Foundation, Rotterdam, The
Culture of mesenchymal stem cells. Mesenchymal stem cells were obtained from fetal lung tissue as previously described. The cells were cultured in M199 supplemented with 10% FCS, 1% pen/strep, 20 µg/ml EGF and 8 U/ml heparin, in gelatin coated tissue culture flasks at 37°C and 5% CO₂ in a humidified atmosphere.

Transplantation in NOD/scid mice. Female, 5-6 weeks old, NOD SCID mice (Charles River, France) were kept the animal facilities of the LUMC. The animal ethical committee of the LUMC approved all animal experiments. The mice were irradiated sub lethally (3.5 Gy) 24 hours before i.v. transplantation with the different transplants: 1) 2*10⁵ CD34+ cells (hereafter referred to as CD34), 2) 2*10⁵ CD34+ cells 10 days expanded with TPO (hereafter referred to as CD34-E), 3) 2*10⁵ CD34+ cells + 1x10⁶ FL MSC (hereafter referred to as CD34/MSC) and 4) 2*10⁵ CD34+ cells 10 days expanded with TPO + 1x10⁶ FL MSC (hereafter referred to as CD34-E/MSC). A schematic representation of the experiment is shown in figure 1.

Blood collection via tail vein incision was performed twice weekly during the first 3 weeks after transplantation and once weekly thereafter. Blood collection and human platelets measurements were performed as described previously. Briefly, human platelets were stained with a non-cross reactive mouse-anti-human CD41-PE (Beckman Coulter) and erythrocytes were lysed with IQTest3 Lysing solution (Beckman Coulter). Flow-Count™ fluorospheres (Beckman Coulter) were added to enable the measurement of the absolute number of circulating human platelets. The detection limit was 1x10⁵ platelets/ml of PB. Analysis was performed with flow cytometry (EPICS® XL-MCL, Beckman Coulter) running system II software.

Six weeks after transplantation, mice were sacrificed and the bone marrow was obtained from the femur. Cells were resuspended in IMDM and cells were labeled with goat-anti-mouse-CD45-PE (LCA, Ly-5, 30-F11, Pharmingen), mouse-anti-human CD45-FITC (Beckman Coulter), and the appropriate isotype controls. Subsequently erythrocytes were lysed with IQ Test 3 Lysing solution according to the manufactures procedures (Beckman Coulter). Analysis was performed with flow-cytometry (EPICS® XL-MCL, Beckman Coulter) running system II software.

Migration experiments. Four different cell suspensions were prepared identical to the in vivo experiment. After 30 minutes of incubation cells were analyzed for the expression of CD45, CD34, CD61, CXCR4, CD49d and CD49e with flow cytometry and placed in the upper compartment of a trans-well plate [Costar, Amsterdam, the Netherlands], with a 100 ng/ml SDF gradient in the lower compartment, both containing IMDM (Gibco). Plates were incubated for 5 hours at 37°C and 5% CO₂ in a humidified incubator. After incubation, cells were harvested from both compartments. After incubation all cells were...
analyzed for the expression of CD45, CD34, and CD61 (all Beckman Coulter) to calculate the number of cells that have migrated.

**Statistical Analysis.** All statistics were done with SPSS, version 20. All results are presented as mean±SEM. To compare groups, a student’s T test (normally distributed) or Mann Whitney test (not normally distributed) was used. Differences were considered significant when p<0.05. If multiple groups were compared, an ANOVA or a Holm’s sequential Bonferroni adjustment was applied.

*Figure 1:* flow chart of the transplantation experiment. Four different types of transplants were prepared (cell numbers/mouse): **CD34**: 2*10^5 un-manipulated CD34+ cells (control group) **CD34/MSC**: 2*10^5 un-manipulated CD34+ cells with 1*10^6 fetal lung MSC **CD34-E/MSC**: the total expansion product of 2*10^5 CD34+ cells expanded with TPO with 1*10^6 fetal lung MSC **CD34-E**: the total expansion product of 2*10^5 CD34+ cells expanded with TPO
Results

Platelet recovery in the peripheral blood of NOD-SCID mice
To study the different strategies to overcome delayed and reduced engraftment of CB cells, mice were transplanted with either unmanipulated CD34+ cells (CD34 group), TPO expanded CD34+ cells (CD34-E group), unmanipulated CD34+ cells with MSC (CD34/MSC group) or TPO expanded CD34+ cells with MSC (CD34-E/MSC group) while platelet recovery as well as bone marrow engraftment were studied.

Similar to earlier studies\(^{11-13,42}\), in the first week after transplantation, mice that received TPO expanded cells (CD34-E and CD34-E/MSC groups) showed accelerated early platelet (plt) recovery in the PB when compared to mice transplanted with only CD34+ cells (CD34 group) (figure 2A and 2C), as shown by significantly higher platelet concentrations in the PB of the mice 8 days after transplantation (CD34-E group: 7.8±2.5x10\(^3\) plt/ml PB, CD34-E/MSC group: 8.3±2.7 x10\(^3\) plt/ml, compared to the control group, CD34: 1.4±0.6x10\(^3\) plt/ml, p<0.005 for both groups). The TPO induced increased PB platelet concentration was present for two weeks after transplantation while after week 2, the mean PB platelet levels in all groups slowly increased. Co-transplantation of MSC in contrast, did not significantly increase early platelet repopulation as compared to CD34+ cells or TPO expanded CD34+ cells respectively (CD34/MSC: 1.9±0.8x10\(^3\) plt/ml compared to CD34: 1.4±0.6x10\(^3\) plt/ml and CD34-E/MSC: 8.3±2.7x10\(^3\) plt/ml compared to CD34-E: 7.8±2.5x10\(^3\) plt/ml). Compared to unmanipulated CD34+ grafts, all mice that received a manipulated graft and/ or additional MSCs showed higher platelet concentrations six weeks after transplantation (mean platelet concentration ±SEM in PB: CD34/MSC: 207.4±68.2x10\(^3\), CD34-E: 216.2±112.2x10\(^3\) and CD34-E/MSC: 127.0±60x10\(^3\) plt/ml) as compared to the mice that received unmanipulated CD34+ cells (CD34: 49.8±21.0x10\(^3\) plt/ml PB). However, only for the CD34/MSC group this difference was significant (p<0.01). The mean increase of long term platelet engraftment in the CD34-E group was due to a wide SD not significant because a number of mice engrafted exceptionally well, thereby increasing the mean value of the group. The median values of these groups, 18.0x10\(^3\) plt/ml PB for CD34-E versus 17.4x10\(^3\) plt/ml PB for CD34, were similar. Vice versa, the (non-significant) lower mean platelet concentration of the group that was transplanted with CD34-E/MSC was the result of a number of mice with very low or non-engraftment.

Figure 2. A: platelet concentration in the peripheral blood (PB) of each of the mice 8 days after transplantation. Bars represent the mean platelet concentration of each group. On average, both groups that were transplanted with TPO expanded cells had significantly higher concentrations of platelets in the PB (\(p<0.02\)). B: platelet concentration of each of the mice 40 days after transplantation. Bars represent the mean platelet concentration of each group. On average, all groups had higher concentrations of platelets in the PB than the control group, but this difference was only significant for the group that was transplanted with CD34+ cells and MSC (\(p<0.05\)). C: kinetics of platelet recovery in the mice throughout the experiment. Shown are the mean±SEM values of the four different transplants.
Human engraftment in the bone marrow

The percentage of human CD45 cells in the bone marrow 6 weeks after transplantation of the mice is shown in figure 3A. Co-transplantation of MSC increased the engraftment when compared to transplantation of CD34+ cells alone with higher percentages of human cells in the BM (CD34/MSC: 24.5±6.3% vs. CD34: 8.9±3.7%; p<0.05). In line with the 6 weeks PB platelet counts, TPO expanded cells also induced engraftment, but again this was not significant (CD34-E: 20.2±7.4%; p=0.402 compared to the CD34 group).

Interestingly, whereas co-transplantation of MSC with CD34+ cells significantly improved the BM engraftment of the mice at week 6, co-transplantation of MSC with TPO expanded CD34+ cells did not enhance BM engraftment and seemed even less favorable when compared to transplantation of TPO expanded cells alone (%human CD45 cells in the BM for CD34-E/MSC: 14.7±6.2% vs. 20.2±7.4% for CD34-E). Again, as seen with the low platelet counts at week 6, low or non-engraftment in a subset of mice did account for this difference. To study if this finding was related to the quality of a particular CB unit, we compared the mean BM engraftment of mice that received cells from the same cord blood unit (figure 3B). Again, co-transplantation of MSC with CD34+ cells increased the engraftment (on average 7.1±2.1 fold) for all of the five different cord blood units that were used in this study. Co-transplantation of MSC with TPO expanded cells, however, again showed very low engraftment for one of the cord blood units and no engraftment for a second unit (0-0.1% of human CD45+ cells in the bone marrow). Whereas co-transplantation of MSC with TPO expanded cells of these two specific units impaired engraftment, co-transplantation of MSC with the unmanipulated CD34+ cells of these two units increased the engraftment 5 and 17 fold respectively. The latter was in line with the average increase in engraftment (7.1±2.1 fold) when MSC are co-transplanted with unmanipulated CD34+ cells, whilst they decreased the engraftment (1.1±0.4 fold) when co-transplanted with TPO expanded cells.
Co-transplantation of MSC with TPO expanded cells on different time points

TPO expanded grafts contain a higher cell number as compared to unmanipulated CD34+ cells. Thus, mice transplanted with CD34-E grafts receive more cells. Co-transplantation of MSC with these high cell numbers might result in e.g. obstruction of the lung circulation and impairment of the expected positive influence of MSC on marrow engraftment contributing to the non-engraftments we observed in some cases. To prevent this possible blocking of circulatory beds by MSC, we performed a pilot experiment in which we infused MSC 4 hours after TPO expanded cells. As shown in figure 4, this later timing of MSC co-transplantation did not significantly alter the level of platelets in the PB 6 weeks after transplantation (Fig 4A, 125.8±58.2 plt/ml PB for MSC transplantation concomitantly with the hematopoietic cell transplantation vs. 134.4±62.6 x10³ plt/ml PB for MSC transplantation 4 hours after hematopoietic cell transplantation, p=0.873). Moreover, the percentage of human CD45+ cells in the bone marrow was not significantly changed when MSC were directly infused with CD34+ cells or after 4 hours (Fig 4B, 22.2±6.4% vs 16.1±8.3%, p=0.522).

Figure 3. A: Percentage of human CD45 cells as a percentage of the total CD45 cells in the BM of the mice 6 weeks after transplantation. Bars represent mean values of each group. All groups had higher mean percentages of human CD45 in their BM than the control group, but this was only significant for the group that received CD34+ cells and MSC (p<0.05). B: The mean percentage of human CD45+ cells of the mice that were transplanted with cells from the same cord blood unit were calculated and compared for each cell type (CD34+ cells or TPO expanded cells) when transplanted with or without MSC, showing an increase or a decrease in engraftment when MSC were added to the CD34+ cells or the TPO expanded cells of this cord blood. Co-transplantation of MSC with CD34+ cells increased the engraftment of the cells of all of the cord blood units. Co-transplantation of MSC with TPO expanded cells only increased the engraftment of the cells of three cord blood units. The average fold increase in engraftment by the co-transplantation of MSC with CD34+ cells was 7.1±2.1. The average fold increase in engraftment by the co-transplantation of MSC with TPO expanded cells was 1.1±0.4.
Migration properties and expression of migration related molecules of the transplanted cells

SDF-1α is the main chemo-attractant for hematopoietic cells to home to the bone marrow and migration capacity of cells towards SDF-1α is therefore a crucial step in engraftment. To investigate if TPO expansion and/or MSC affect the migratory capacity of the cells, we analyzed the migration capacity of the grafts towards a 100 ng/ml SDF-1α gradient in Transwell plates (figure 5A). Addition of MSC improved the migration of the CD34+ cells by 3 fold (21.6±4.8% with MSC vs. 6.7±2.4% for CD34+ cells alone, p<0.05). Also TPO expansion improved the migration of the remaining CD34+ cells in the transplant 8-9 fold (48.7±8.9% p<0.005). Addition of MSC did not further enhance the already increased migration of residual CD34+ cells in the TPO expanded CB (57.2±10.5%).

Because TPO expansion generated CD34-CD61-Lin- cells that establish PB platelet recovery, we also investigated the effect of MSC on migration of this sub-population. Figure 5B in this respect shows that, similarly as with residual CD34+ cells, co-transplantation of MSC does not alter the migration of CD34-CD61- cells (37.0±4.1% for TPO expanded transplants, 33.2±2.1% for TPO expanded cells with MSC, p=0.818).

The expression of CXCR4, the receptor for SDF1α, on (residual) CD34+ (figure 5C) or CD34-CD61-Lin- cells (figure 5D) was analyzed with flow cytometry (figure 5C and 5D) but was not significantly altered by their incubation with MSC nor by TPO expansion alone or in combination with MSC. However, the expression of CD49d and CD49e was upregulated in TPO expanded CD34+ cells as opposed to non expanded CD34+ cells and CD34+ cells that were incubated with MSC.
**Figure 5.**

**A:** Percentage of CD34+ cells that have migrated through a trans-well system towards the lower compartment of the plate containing medium with 100ng/ml SDF-1α gradient (black bars) or medium alone (gray bars, spontaneous migration). CD34+ cells are either un-manipulated cells (control group and group that was transplanted with CD34+ cells and MSC) or the CD34+ subpopulation of the cells after expansion with TPO. Both addition of MSC and TPO expansion improved the migration of the CD34+ cells significantly. Addition of MSC to TPO expanded cells did not improve the migration of the subpopulation (*p<0.05).*

**B:** Percentage of cells of the CD34-CD61-Lin- subpopulation found after TPO expansion of CD34+ cells that have migrated through a trans-well system towards the lower compartment of the plate containing medium with 100ng/ml SDF-1α gradient (black bars) or medium alone (gray bars, spontaneous migration). Addition of MSC to TPO expanded cells did not improve the migration of the subpopulation.

**C:** Percentage of CD34+ cells expressing CXCR4, CD49d or CD49e. There was no difference in the expression of CXCR4 between the different transplants suggesting that the differences found in the transwell migration are not due a difference in the expression of the receptor for SDF-1α. TPO expanded cells did express higher percentages of CD49d and to a lesser extend CD49e (*p<0.0001).*

**D:** Percentage of cells of the CD34-CD61- subpopulation found after TPO expansion of CD34+ cells expressing CXCR4. No differences in the expression of the receptor of SDF-1α were found.
Discussion

Several studies have shown that co-transplantation of MSC improves the bone marrow engraftment in animal models\textsuperscript{18,31-35}, but the effect on the speed of platelet recovery has not been investigated. In this study, we observed that MSC co-transplantation with either unmanipulated CD34+ cells or with TPO expanded CD34+ cells had no effect on the recovery of platelets in the peripheral blood within 2 weeks after transplantation. The early platelet recovery seen after transplantation of CB cells expanded with TPO originates from CD34-CD61-Lin- cells. This population that is (partly) committed to the megakaryocyte lineage is unique for CB and not observed among TPO expanded adult stem cell sources and this maturation pattern may contribute to the delayed and slow platelet recovery observed after CB transplantation.\textsuperscript{12,47} The absence of this Lin-neg population in unmanipulated CD34+ CB explains the lack of improvement of early platelet recovery when MSC are co-transplanted with uncultured CB CD34+ cells. Also, in line with earlier studies in NOD SCID mice showing that MSC co-transplantation improved BM engraftment and repopulation for CD34+ CB cells\textsuperscript{12,13,31-34}, we observed that MSC boosted both PB platelet levels after 6 weeks and BM engraftment.

The mechanism behind the improved engraftment by MSC is still unknown. We found enhanced SDF-1\(\alpha\) migration capacity of CD34+ cells by MSC in vitro, which was not related to a change in the expression of CXCR4. This suggests that MSC induced improvement of engraftment might be partly attributed to this increased migration capacity. However, the interaction between MSC and stem cells on homing is complex\textsuperscript{48,49} and conclusive proof for this or other mechanisms is still lacking. Homing of MSC to the marrow was studied as determinant for the observed engraftment potentiating effect and these studies have shown conflicting results. Both Noort et al. and Kim et al. did not find MSC in the BM of the mice after transplantation\textsuperscript{18,34}. Noort at al analyzed the presence of MSC in multiple organs with RT PCR and did not find any MSC in the BM, spleen, liver or thymus, but only sequestration of the MSC in the lung. This 'lung barrier' was corroborated by Schrepfer et al\textsuperscript{50}, who IV injected labeled MSC and found high bioluminescence in the lungs with in vivo imaging and ex vivo analysis in contrast to only trace signals from other organs such as the spleen, the tibia and the liver. Moreover, efforts from Noort et al to bypass the lung barrier by intra-cardiac injection did not result in the detection of MSC in the BM either. Only Hiwase et al. suggested that MSC and HSC can migrate in conjunction to the marrow\textsuperscript{32}. Other mechanisms, induced by MSC secreted cytokines and growth factors might also play a role. MSC are known to support and maintain blood vessels\textsuperscript{51} and might contribute to marrow regeneration by inducing vascularization. Even MSC mediated immunomodulation enhancing allogeneic tolerance has to be considered\textsuperscript{36}.

The aim of our study was to investigate whether co-transplantation with MSC had a synergistic effect on accelerated platelet recovery and/or improved BM engraftment of TPO expanded CB. We observed that MSC did not potentiate short term platelet engraftment of TPO expanded CD34+ cells. This could be associated with an unchanged migration pattern of CD34-CD61-Lin- cells towards an SDF-1\(\alpha\) gradient in vitro since MSC do not influence the homing capacity of the TPO generated CD34-CD61-Lin- cells responsible for early platelet repopulation. However, a causal role on the lack of synergy in platelet recovery between the two populations is elusive. Furthermore the increase in BM engraftment, seen with the co-transplantation of MSC with unmanipulated CD34+ cells is not seen when MSC are co-transplanted with TPO expanded cells. Strikingly, in some situations the combination seemed to decrease the 6 week PB platelet numbers as well as human CD45+ BM engraftment. This could largely be attributed to the fact that some mice treated with the combination of TPO expansion and MSC virtually showed non-
engraftment (only 0-0.1% of human CD45 cells in the bone marrow). If these ‘non’ or ‘very low’ engrafters were not taken into account when calculating the mean engraftment percentage of this group, the level of human CD45+ cells in the BM rose from 14.7% to 22.0%, which is similar to mice transplanted with only TPO expanded cells (20.2%). A synergy of the two approaches which each separately have shown to improve platelet recovery or BM engraftment in NOD/SCID mice was clearly absent. Most importantly, adding MSC to TPO expanded cells may result in engraftment failure.

These non or very low engraftment cases in the combined approach could hypothetically be caused by a phenomenon called the lung barrier. Because of their larger size, MSC might become trapped in the lung. In addition, MSC can adhere to hematopoietic stem cell, further impairing their homing to the marrow. The high cell numbers in the TPO expanded transplants in combination with the MSC may be critical to develop this complication. Although we did observe acute deaths in both groups that were co-transplanted with MSC, possibly caused by pulmonary embolism, non engrafters were only found in the group with TPO expanded cells combined with MSC. To see whether the simultaneous presence of high numbers of cells transplanted after TPO expansion together with MSC influenced the engraftment, we performed additional experiments in which we transplanted the MSC 4 hours after the transplantation of the TPO expanded cells. Although no differences in the engraftment of both platelets in the PB and human CD45 cells in the BM after 6 weeks were discerned, one mouse again showed hardly any BM engraftment after 6 weeks. Interestingly, this mouse was given MSC 4 hours after infusion of TPO expanded cells. Thus, entrapment of MSC adhered to TPO expanded progenitor cells in the lung does not seem to be an explanation for non-engraftment.

TPO expanded CD34+ cells migrate better in vitro than fresh CD34+ cells or fresh CD34+ cells with MSC. Whether this improved in vitro migration also translates into better in vivo homing is not definite. A previously conducted homing study with 99mTc-tropolone-labeled cells which showed a similar proportion (approximately 0.5%) of the fresh CD34+ or TPO expanded CD34+ cells that were transplanted homed towards the femur. In these experiments fresh CD34+ cells or their expanded equivalent were transplanted, i.e. higher numbers of cells were transplanted in the TPO expanded group. The absolute number of homing cells is therefore higher in the group that was transplanted with TPO expanded cells. However, the experiment did not discern the proportion of each of the three major subpopulations found after TPO expansion that homed to the BM. Although more cells homed to the BM in the mice that received TPO expanded cells and the largest population of these cells (CD61+) migrated less in vitro than the two other populations (CD34+ and CD34-CD61- cells (48.7±8.9% and 37.0±4.1% respectively vs. 15.4±2.1% for CD61+ cells), we cannot with certainty conclude that TPO expanded CD34+ cells home better to the BM than fresh CD34+ cells. Despite the higher migration rate of TPO expanded cells in vitro, the lack of improvement in engraftment suggests that other characteristics such as TPO induced changes to their immature characteristics, their stemness and thus long term engraftment potential, are more important in this respect.

In this paper we additionally focused on the added engraftment effect by MSC co-transplantation with TPO expanded cells. It is likely that MSC do not improve the homing capacity of TPO expanded CD34+ cells since they do not affect migration in vitro and transplantation of MSC four hours after the transplantation of CD34+ cells does not alter the engraftment of the cells.

Superior migration of TPO expanded cells over the fibronectin coated transwell plates despite a lack of difference in CXCR4 expression might be explained by their higher expression of adhesion molecules for which fibronectin is a ligand, such as CD49d and CD49e. Blocking of CD49d and CD49e with antibodies reduces the migration of CD34+...
cells in fibronectin coated plates. In the migration experiments, short term incubation with MSC did not lead to a change in the expression of adhesion markers on CD34+ cells. The increased migration and enhanced engraftment of non expanded CD34+ cells in the presence of MSC can therefore not be explained by a change in adhesion molecule expression. Strikingly, expansion with CD34+ cells, either with MSC or TPO might change the longer term the engraftment capacity of the cells. This was suggested by studies showing that after transplantation of one CB unit expanded by culture ex vivo with MSC or TPO and one unmanipulated unit, the unmanipulated CB graft establishes long-term engraftment.

In conclusion, co-transplantation of MSC can improve engraftment after 6 weeks while TPO expansion improves early platelet recovery. MSC co-transplantation combined with TPO expansion at best combines but gives no synergy on either of these effects. However, the combination of high cell numbers introduces the risk of non-engraftment. More precise characterization of these non-engrafting events will be essential to combine these approaches.
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