CD26+ Cord Blood Mononuclear Cells Significantly Produce B, T, and NK Cells

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ABSTRACT

Background: Umbilical cord blood (UCB) is an alternative source of hematopoietic stem cell transplantation (HSCT), used in Leukemia treatment. CD26+ cells, a fraction of CD34 positive cells, are a major population of UCB cells which negatively regulate the *in vivo* homing and engraftment of HSCs. CD26 is highly expressed in various cells such as HSCs, immune cells, fibroblasts, and epithelial cells. It has been shown that the inhibition of the CD26 on CD34+ cells improves the efficiency of Hematopoietic Stem and Progenitor Cell (HPC) transplantation. **Objective:** To evaluate the relationship between the production of B, T, and NK cells from the CD26 positive fraction of cord blood mononuclear cells. **Methods:** Cord blood mononuclear cells were cultured for 21 days using different combinations of stem cell factors (SCF), Flt3 ligand (FL), IL-2, IL-7, and IL-15. The harvested cells were then analyzed by flowcytometry every week for 21 days. **Results:** T cell differentiation from CD26 subset of cord blood mononuclear cells increased by using IL-2 and IL-7. Our data showed that IL-2 and IL-7 significantly affected the generation of B cells from CD26 positive cord blood mononuclear cells. On the other hand, NK (NKp46+) derived CD26+ cells increased by IL-15 and IL-2. **Conclusion:** Taking all into account, we conclude that B, T, and NK cells can differentiate from the CD26+ subset of mononuclear cord blood cells by using key regulatory cytokines.

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Keywords: CD26 Cells, Cytokine, Immune Cells, Umbilical Cord Blood

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INTRODUCTION

Bone marrow (BM) is not the only the source of hematopoietic cells used in transplantation. Umbilical cord blood (UCB) transplantation is an alternative source of hematopoietic stem and progenitor cells. UBC stem cells have a greater proliferative and self-renewal capacity than those from BM. UCB is more easily obtainable and has a reduced risk of graft-versus-host disease, compared to BM (GVHD) (1). It has been shown that UCB myeloid progenitor cells are relatively chemo-resistant (2).
However, this source of HSC has some disadvantages; low number of cells per delivery, low neutrophil recovery, and a high rate of mortality due to infection are just a number of the challenging issues in cord blood transplantation(3).
In order to make an effective Cord blood transplantation, many laboratories perform *ex vivo* stem cell expansion before transplantation. Therefore, they use some extrinsic regulators, like cytokines for the cord blood mononuclear cell expansion. For example: Stem cell factors (SCFs) have been used to improve the homing and proliferation of UCB cells in preclinical models (4-5), fetal liver tyrosine kinase-3-ligand (Flt3) increase the proliferation and differentiation of HSCs in a short-term expansion (6). Previous studies have illustrated a mutual role of IL-7 in B cell development and NK cells differentiation (7,8). IL-15 is crucial factor for NK cell differentiation and activation (8-10). IL-2 is a T cell growth factor, also mediating in activated B cell proliferation and NK cells differentiation (11-13).
CD26+ cells, as a fraction of CD34+ cells, are a big population of mononuclear cells which negatively regulate *in vivo* homing and migration(14). They are highly expressed on hematopoietic stem cells, immune cells including T, B, and NK cells, the liver, and lungs (15-20). CD26 is a cell surface dipeptidylpeptidase which can cleave the N-terminal dipeptide of several cytokines, including chemokine SDF-1 (CXCL12) (21,22). SDF has been shown to act as chemoattractant via cell surface CXCR4, also it regulates the trafficking of CD34+ HSCs, pre-B lymphocytes, and T lymphocytes in the bone marrow (1). These findings suggest that inhibition of CD26 may be a promising strategy for improving clinical outcomes. Enhancement of HSCs homing may reduce the number of required cells for UCB transplantation (23). Therefore, it is propitious to evaluate the potential of the CD26 fraction of cord blood mononuclear cells into B, T, and NK cells.
In this study, we have evaluated the generation of B, T, and NK cells from CD26+/- fraction of cord blood mononuclear cells by using related cytokines.

MATERIALS AND METHODS

Cell Isolation. Five cord blood samples were collected from full-term normal deliveries. All samples were diluted a 2:1 solvent with phosphate-buffered saline (PBS) (SIGM). Subsequently, the mononuclear cells were isolated by centrifugation on Ficoll-paque (GE healthcare -1.078 g/ml) at 850 *gm* for 25 minutes. The mononuclear cells were then collected, washed twice in RPMI1640 (Gibco), supplemented with 10% FBS (Gibco), and resuspended in RPMI1640 supplemented with 20% FBS and 1% penicillin/streptomycin either for culturing or freezing.
Cell Culture and Culture Condition. The 10^5 cord blood mononuclear cells were seeded in 96-well plates in 250 μL of RPMI1640 (Gibco) containing 20% fetal bovine serum (FBS; Gibco), 1% penicillin/streptomycin (Gibco), supplemented with cytokines

with final concentrations of: SCF (40 ng/ml), Flt3 ligand (FL, 40 ng/mL), interleukin-7 (IL-7, 40 ng/mL), IL-15 (40 ng/mL), and IL-2 (40 ng/mL) (all cytokines were purchased from PeproTech, USA).

This study has been performed in 6 distinct groups containing:

1. No cytokine,
2. SCF+Flt3
3. SCF+FL+IL-2,
4. SCF+FL+IL-7,
5. SCF+FL+IL-15,
6. SCF+FL+IL-2+IL-7+IL-15

Cells were cultured at 37°C for 21 days, and half of the culture medium was renewed every week. At the indicated days, cells were harvested and analyzed by FACS for B (CD20), T (CD3), NK (NKP46), CD26, and CD34 positive cells.

Monoclonal Antibodies and Flowcytometry. All antibodies were from BD Biosciences unless stated otherwise. Monoclonal antibodies (conjugated with different fluorochromes) used to stain the cell-surface antigens were: CD34 (581; Abcam), CD26 (M-A261), CD20 (2H7), CD3 (UCHT1; R&D), and NKP26 (9E2/Nkp46). The cells were incubated with Antibody cocktails for 20 minutes in 4 degree.

We evaluated the cultured cells using flowcytometry in days 0, 7, 14, and 21. Propidium iodide (1.0 mg/mL; Invitrogen) was used to exclude dead cells from the analysis. Between 10,000 to 30,000 events were collected and analyzed by BD caliber (BD-ebioscience) using flowing software (Perttu Terho, version: 2.5.1.). Gating on FACS plots has been performed as shown in Figures 1 and 3.

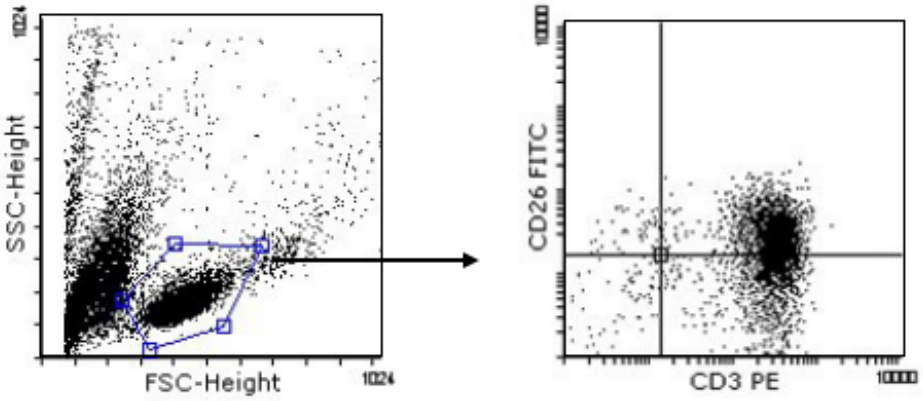

Figure 1. Phenotype of CD3+ cells derived in vitro from CD26+ and CD26- cells. 10^5 cord blood mononuclear cells were cultured with different combination of cytokines. Ater 21 days in culture, labeling was performed using PE-conjugated anti-CD3 and FITC-conjugated anti-CD26. The cells were analyzed by flowcytometry in distinct time points. The experiments were repeated three times.

Statistical Analysis. All results are expressed as mean (SD). The statistical significance between groups was determined using the student *t-test* and one-way ANOVA. $p<0.05$ was considered as statistically significant. The analysis was performed by Graph Pad Prism software (version: 5.04). The ethical issues of this experiment have all been approved by the Ethical Committee of Tabriz University of Medical Sciences.

RESULTS

Effect of Cytokines on the Generation of T Cells from Cord Blood CD26+/- Cells. We cultured 1x 10^5 cord blood mononuclear cells for 21 days in the presence of different combination of SCF, FL, IL-2, IL-7, and IL-15. Harvested cells were evaluated by FACS in distinct time points. On FACS plots, the mononuclear cells were gated on forward and side scatter (R1). CD3+CD26+ and CD3+CD26- were evaluated as shown in (Figure 1).

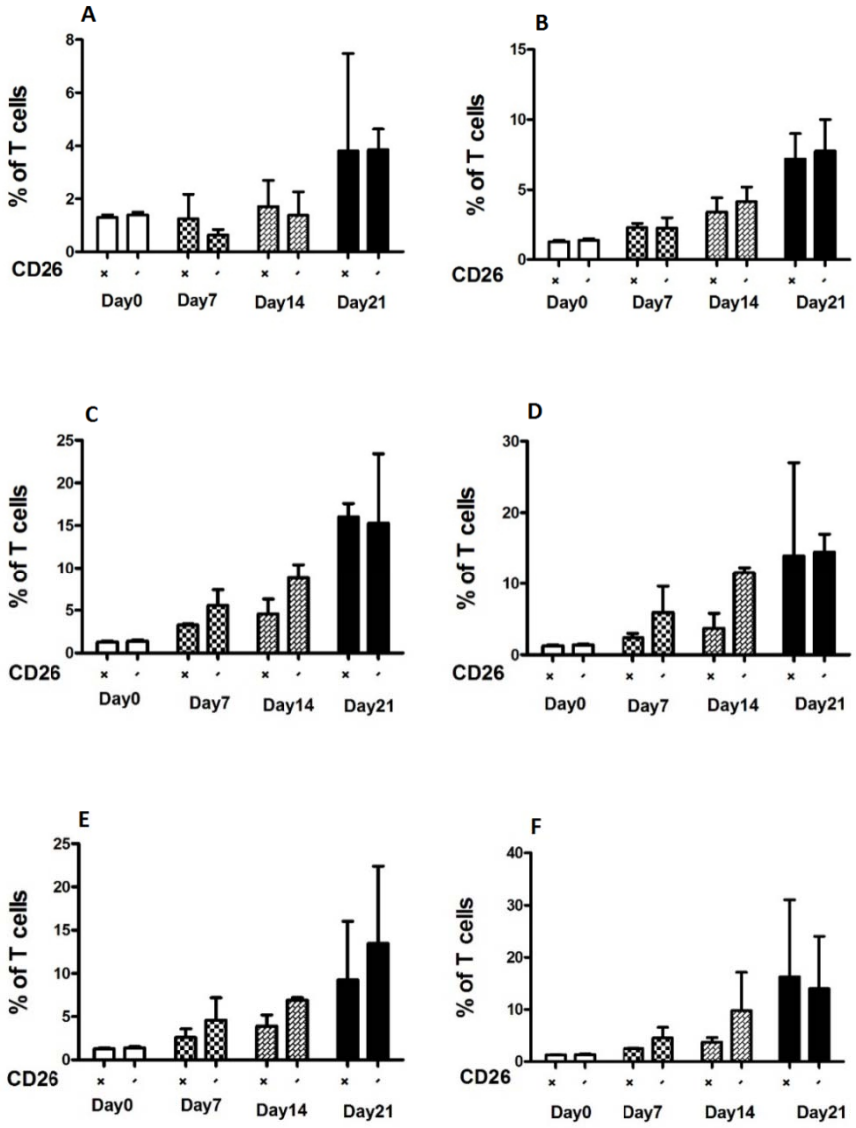

Figure 2. Percentage of CD3+CD26+ and CD3+CD26- cells derived from cord blood mononuclear cells. Flowcytometry and mean (SD) were used to evaluate the expression of CD3+ cells in different time points in presence of different combination of cytokines: no cytokines (A), SCF+FL (B), SCF+FL+IL-2 (C), SCF+FL+IL-7 (D) SCF+FL+IL-15(E), and SCF+FL+IL-2+IL-7+IL-15 (F). The percentage of CD3+CD26+ and CD3+CD26- cells increased significantly after 21 days of culture in the presence of cytokines. Data represent the mean (SD) proportion of positive wells from 3 independent experiments, with 22 to 34 wells analyzed in each experiment. The values considered significant were from p <001 to p < 0.05.

In the presence of a combination of the mentioned cytokines, the percentage of T cells increased from day 7 to day 21, significantly (Figure 2). IL-2 produced a high percentage of CD26+ T cells (16%) (Figure 2C) in comparison to either additional IL-7 or IL-15, since their percentage of CD26+T cells were 13.9% and 9%, respectfully (Figure 2D and E). Moreover, in a combination of all cytokines the percentage of T cells was (16.3%), just like IL-2 alone (Figure 2F). No significant difference was detected between the expansion of CD3+CD26+ and CD3+CD26-, in different time points (Figure 2).

Cytokines in the Generation of B Cells from Cord Blood CD26+/- Cells. We evaluated the effect of SCF, FL, IL-2, IL-7, and IL-15 on B cell derived CD26+/- umbilical cord blood cells for 21 days in culture conditions. The harvested cells analyzed for B cells by FACS in days 0,7,14, and 21. The CD20+CD26+ and CD20+CD26- cells were determined by sub-gating on the lymphoid mononuclear cells gated on FSC versus SSC (R1) (Figure 3).

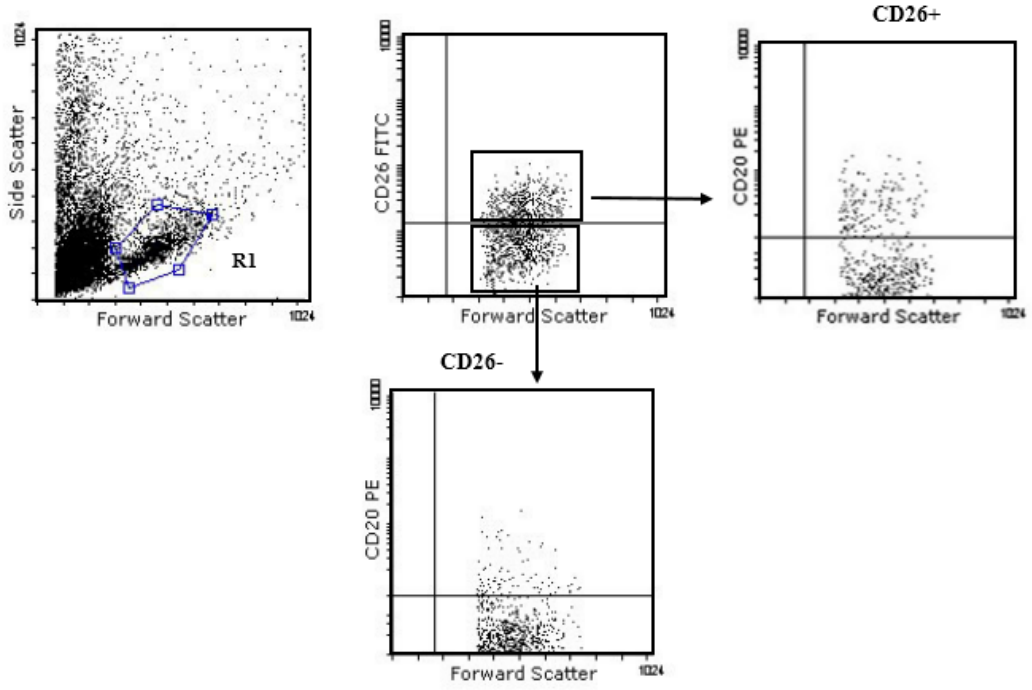

Figure 3. Representative FACS profile of 10^5 cultured cord blood mononuclear cells in identical time points**.** $CD20^+CD26^+$ (shown CD26+) and $CD20^+CD26^-$(shown CD26-) evaluated by gating on the lymphoid population in FSC versus SSC.Phenotypic analysis of CD20 positive cells derived from CD26 positive and negative fractions. Representative FACS profiles of 10^5 cord blood mononuclear cells were cultured with indicated combinations of cytokines and analyzed in identical time points. CD20+CD26+ (shown CD26+) and CD20+CD26-(shown CD26-) analyzed using CD20-PE and CD26-FITC.

Our data illustrated the percentage of CD20+CD26+ cells which increased in groups A (1.5%), C (6.4%), D (5.2%), and F (6.4%) in day 14, but did not changed in group B, despite its slight decrease in group E (Figure 4). The percentage of CD20+CD26+ cells remained constant in day 21 except in groups D and F. The percentage of cells

significantly increased during 21 days in the presence of SCF+FL+IL-2 and in a combination of all cytokines (group F). There was no significant difference in the percentage of CD20+CD26+ and CD20+CD26- derived cells (Figure 4).

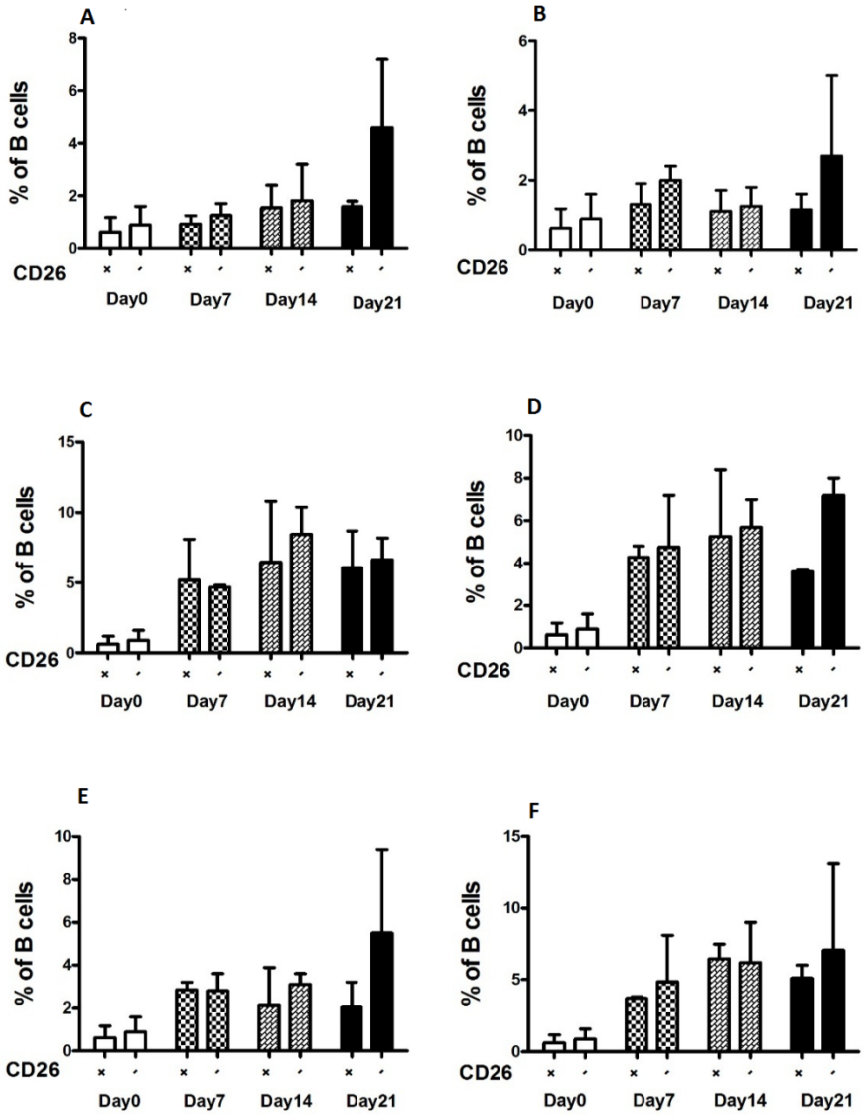

Figure 4. Evaluation of $CD20^{+}CD26^{+}$ and $CD20^{+}CD26^{-}$ cells derived from cord blood mononuclear cells in different time points. The data of the harvested cells, evaluated by FACS and mean (SD), are shown in groups using no cytokines (A), SCF+FL(B) SCF+FL+IL-2 (C), SCF+FL+IL-7 (D), SCF+FL+IL-15 (E), and SCF+FL+IL-2+IL-7+IL-15 (F). In groups C and F, the CD20+ cells increased significantly in day 21. Data represent the mean (SD) proportion of positive wells from 3 independent experiments, with 18 to 29 wells analyzed in each experiment. The values considered significant were between $p<0.003$ and $p<0.05$.

Role of Cytokines in the Generation of NK Cells from Cord Blood CD26+/- Cells. We evaluated NKP46+ cells derived from cultured cord blood mononuclear cells in different combinations of IL-2, IL-7, and IL-15, for 21 days. The harvested cells were

analyzed by FACS in days 0,7,14, and 21 and the percentages of NKp46+CD26+ and NKp46+CD26- cells were determined (Figure 5).

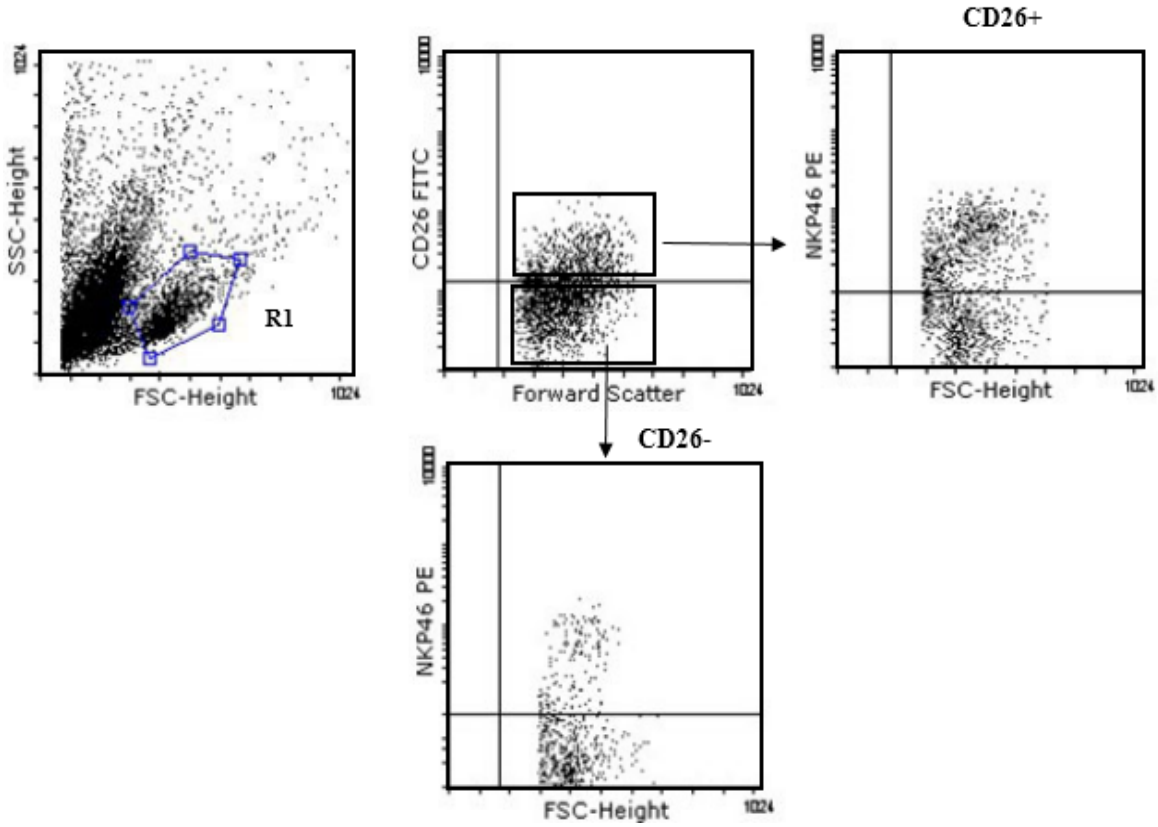

Figure 5. Representative FACS profile of 10^5 cultured cord blood mononuclear cells in identical time points. $NKp46^+CD26^+$ (shown CD26+) and $NKp46^+CD26^-$ (shown CD26-) evaluated by gating on the lymphoid population in FSC versus SSC.

Our data showed that the NKp46+CD26+ cells increased slightly until day 21. However, in the condition with no cytokines, the percentage of NKp46+ cells was constant from day 7 to day 21. Nevertheless, the percentage of NKp46+ increased dramatically by using IL-2 (8.4%), IL-15(9.3%), and in combinations (9.7%)(Figure 6C and D). The NKp46+CD26- cells increased in day 21 in all groups (Figure 6). In the presence of IL-15, the NK cells significantly increased (Figure 6E). Importantly, the percentages of NKp46+CD26+ and NKp46+CD26- did not change, significantly (Figure 6).

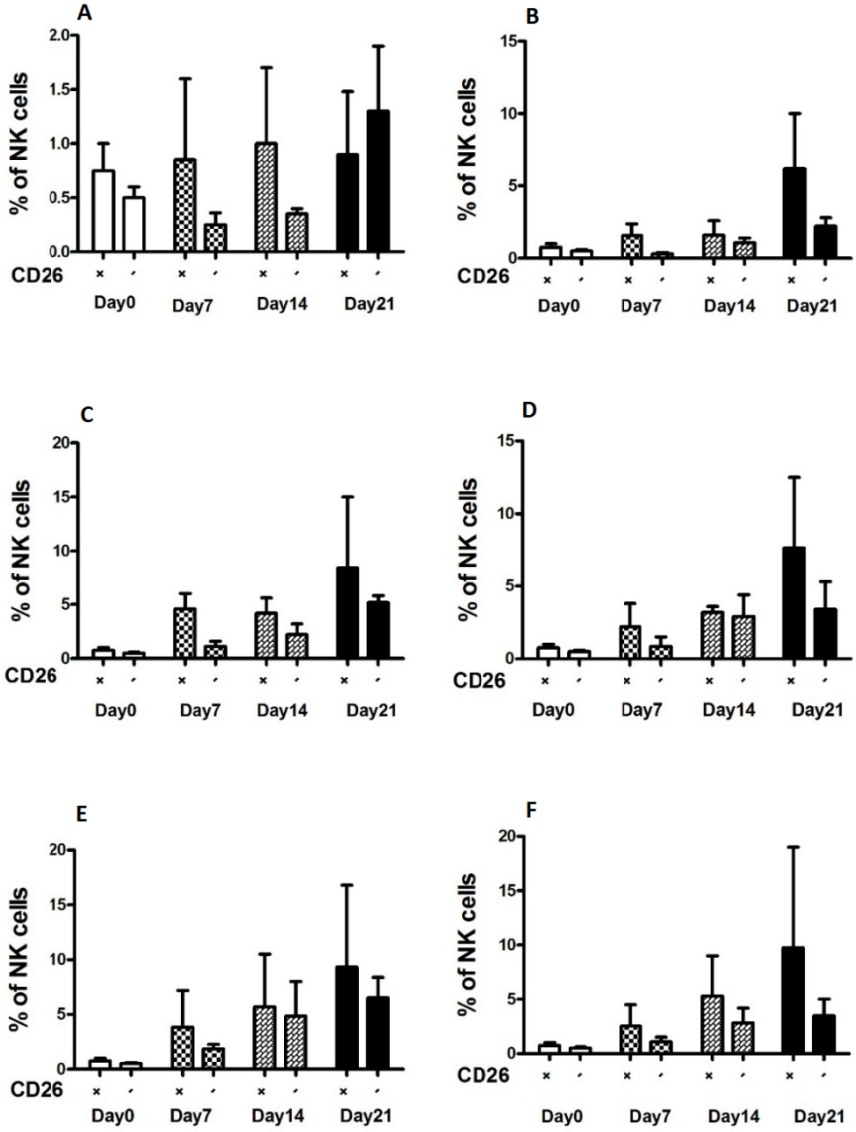

Figure 6. Effects of different combination of cytokines on NKp46+CD26+ and NKp46+CD26- cell production. Cord blood mononuclear cells were cultured for 21 days and analyzed by FACS at specified times. No cytokines (A), SCF+FL(B) SCF+FL+IL-2 (C), SCF+FL+IL-7 (D), SCF+FL+IL-15 (E), and SCF+FL+IL-2+IL-7+IL-15 (F). Data represents means ± SD. The NKp46+ cells increased significantly in group E in day 21. Data represents the mean (SD) proportion of positive wells from 3 independent experiments, with 20 to 29 wells analyzed in each experiment. Significant value is considered $p < 0.01$.

DISCUSSION

Stem cell transplantation is a standard treatment for hematological disorders. Umbilical cord blood, as an alternative source of hematopoietic stem cells, is being used for transplantation in leukemia patients (26). However, the limiting number of cells in the cord blood is a challenging factor in CB transplantation, especially in adult recipients, who need a high number of cells in transplantation (3). CD26+ cells are a big

population of UCB cells which negatively regulate the *in vivo* homing and engraftment of HSCs, so inhibition of this population is a promising strategy for enhancing the efficiency of transplantation(23). In this study, we evaluated the potential of UCB CD26+ cells for differentiation into immune cells and also, the relationship between CD26 expansion and cytokine conditions.

We compared the percentage of B, T, and NK cells derived from CD26+ and CD26- in the presence of different combinations of cytokines. The harvested cells were later evaluated to understand the effects of cytokines on the expansion of CD26 subsets to immune cells.

Several cytokines are known to up regulate and control the generation of T cells. For instance, IL-2 is a T cell growth factor, and IL-7 is involved in the proliferation and survival of T cells (11-13). Based on previous studies, which have indicated a relationship between IL-2 and IL-7 with T cell differentiation (11-13), we tested T cell differentiation from cord blood mononuclear cells in the presence of IL-2 and IL-7. We found that IL-2, in comparison to IL-7, has more influence on T cell differentiation from CD26+ HSCs (Figure 2C). We obtained the same results in agreement with previous studies that declared the percentage of T cells increased in the presence of IL-7, Marcel R. M *et al* 2001 (27). However, IL-15 did not increase T cell differentiation. IL-7 has been identified as a critical regulator of B cells, and both IL-2 and IL-15 as stimulants of the proliferation, survival, and functional activities of NK cells and activated T and B cells (7-10,24). Our data showed that the percentage of B cells derived from cord blood mononuclear cells increased, on the same level, in the presence of either IL-7 or IL-2.

IL-15 is a key cytokine for the development, proliferation, and differentiation of different subsets of NK cells (10,25). IL-7 also plays a role in NK cells` differentiation. It has been shown that IL-2 and IL-15 stimulate the proliferation, survival, and functional activities of NK cells as well as activated T and B cells (7,8). Also, Anne Caignard, *et al.* demonstrated that IL-15 is an essential requirement for the generation of CD56+ NK cells from CD34+ cells (13). Thus, in the absence of stromal cells, the combination of SCF and IL-2 leads to the production of a fewer number of CD56+ cells. Caignard, *et al.* showed the addition of 20 ng/ml IL-15 increased the numbers of CD56+ NK cells, significantly (13). Here, we showed that NK (NKp46+) derived from CD26+ cells increased in the presence of IL-15 (9.3%) and IL-2 (8.4%).

As we have shown, immune cells like B, T, and NK cells can differentiate from the CD26+ subset of CD34+ mononuclear cord blood cells. Perhaps the CD26+ cells should be differentiated in vitro before being used in transplantation. These findings are very important in cord blood transplantation as they provide an answer to whether or not cytokine therapy should be conducted parallel to it. As researchers have shown, the negative role of CD26+ cells in transplantation is a very critical issue for using cytokines such as IL-2, IL-7, and IL-15 during the treatment of leukemia. Our data has shown the effects of cytokines on B cells derived from CD26+ cells, which is very essential for preventing infection in the transplanted donors. However, the functionality of derived B cells still remains unclear. Even so, these findings hold great merit since they reduce the CD26+ cells, by differentiation of CD26 cells to B cells, in transplanted donors.

As we showed, IL-2 and IL-15 increase the NK cells derived CD26+ cells. NK cells have a positive role in engraftment, without changing in GVHD in donors. NK cells kill leukemia cells without any pre activation. Therefore, our findings suggest that using IL-2 and IL-15 during transplantation may increase the differentiation of CD26 positive

cells into NK cells. When Cd26+ cells differentiate to NK cells, we expect reduction in the number of CD26+ cells and a decline in their negative role in homing during transplantation. This is very prominent finding which can increase the efficiency of cord blood transplantation by increasing the NK cells differentiation and reduction of CD26+ cells, in the same time.

However, in this regard, the cytokines also increase T cells. Possibly, depletion or repletion of T cells in culture conditions or during transplantation can reduce this negative factor. For further studies, it is vital to evaluate the function of immune cells derived from the Cd26`s positive fraction.

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REFERENCES

1. Delaney C, Ratajczak MZ, Laughlin MJ. Strategies to enhance umbilical cord blood stem cell engraftment in adult patients. Expert review of hematology. 2010;3(3):273-83.
2. Toren A, Einat M, Fabian I, Nagler A. Human umbilical cord blood myeloid progenitor cells are relatively chemoresistant: A potential model for autologous transplantations in HIV-infected newborns. American journal of hematology. 1997;56(3):161-7.
3. Gluckman E, Rocha V. Cord blood transplantation: state of the art. Haematologica. 2009;94(4):451-4.
4. Ueda T, Tsuji K, Yoshino H, Ebihara Y, Yagasaki H, Hisakawa H, et al. Expansion of human NOD/SCID-repopulating cells by stem cell factor, Flk2/Flt3 ligand, thrombopoietin, IL-6, and soluble IL-6 receptor. Journal of Clinical Investigation. 2000;105(7):1013-21.
5. Ren Z, Jiang Y. Umbilical Cord Blood Hematopoietic Stem Cell Expansion Ex Vivo. J Blood Disorders Transf S. 2013;3:2.
6. Hofmeister C, Zhang J, Knight K, Le P, Stiff P. Ex vivo expansion of umbilical cord blood stem cells for transplantation: growing knowledge from the hematopoietic niche. Bone marrow transplantation. 2007;39(1):11-23.
7. Parrish YK, Baez I, Milford TA, Benitez A, Galloway N, Rogerio JW, et al. IL-7 Dependence in human B lymphopoiesis increases during progression of ontogeny from cord blood to bone marrow. J Immunol. 2009 Apr 1;182(7):4255-66.
8. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, De Coene C, Selz F, Le Deist F, et al. Role of interleukin-2 (IL-2), IL-7, and IL-15 in natural killer cell differentiation from cord blood hematopoietic progenitor cells and from gamma c transduced severe combined immunodeficiency X1 bone marrow cells. Blood. 1996;88(10):3901-9.
9. Vitale M, Sivori S, Pende D, Augugliaro R, Di Donato C, Amoroso A, et al. Physical and functional independency of p70 and p58 natural killer (NK) cell receptors for HLA class I: their role in the definition of different groups of alloreactive NK cell clones. Proceedings of the National Academy of Sciences. 1996;93(4):1453-7.
10. Cheng M, Charoudeh HN, Brodin P, Tang Y, Lakshmikanth T, Höglund P, et al. Distinct and overlapping patterns of cytokine regulation of thymic and bone marrow-derived NK cell development. The Journal of Immunology. 2009;182(3):1460-8.
11. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. Nature Reviews Immunology. 2006;6(8):595-601.
12. Meazza R, Azzarone B, Orengo AM, Ferrini S. Role of common-gamma chain cytokines in NK cell development and function: perspectives for immunotherapy. BioMed Research International. 2011;2011.

13. Carayol G, Robin C, Bourhis JH, Bennaceur-Griscelli A, Chouaib S, Coulombel L, et al. NK cells differentiated from bone marrow, cord blood and peripheral blood stem cells exhibit similar phenotype and functions. European journal of immunology. 1998;28(6):1991-2002.
14. Fernández MN, Regidor C, Cabrera R, García-Marco JA, Forés R, Sanjuán I, et al. Unrelated umbilical cord blood transplants in adults: Early recoveryof neutrophils by supportive co-transplantation of a low number of highlypurified peripheral blood CD34< sup>+</sup> cells from an HLA-haploidentical donor. Experimental hematology. 2003;31(6):535-44.
15. Drucker DJ. Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: preclinical biology and mechanisms of action. Diabetes Care. 2007 Jun;30(6):1335-43.
16. Vanham G, Kestens L, De Meester I, Vingerhoets J, Penne G, Vanhoof G, et al. Decreased expression of the memory marker CD26 on both CD4+ and CD8+ T lymphocytes of HIV-infected subjects. J Acquir Immune Defic Syndr. 1993 Jul;6(7):749-57.
17. Kahne T, Lendeckel U, Wrenger S, Neubert K, Ansorge S, Reinhold D. Dipeptidyl peptidase IV: a cell surface peptidase involved in regulating T cell growth (review). Int J Mol Med. 1999 Jul;4(1):3-15.
18. Beraudi A, Traversa U, Villani L, Sekino Y, Nagy J, Poli A. Distribution and expression of A< sub> 1</sub> adenosine receptors, adenosine deaminase and adenosine deaminase-binding protein (CD26) in goldfish brain. Neurochemistry international. 2003;42(6):455-64.
19. Abbott CA, McCaughan GW, Baker E, Sutherland GR. Genomic organization, exact localization, and tissue expression of the human CD26 (dipeptidyl peptidase IV) gene. Immunogenetics. 1994;40(5):331-8.
20. Inoue N, Hisamatsu T, Takayama T, Okamoto S, Ogata H, Iwao Y, et al. CD26+ Lymphocytes and serum soluble CD26/Dipeptidyl peptidase IV are involved in the Pathogenesis of Crohn's Disease: P-0131. Inflammatory Bowel Diseases. 2009;15:S47.
21. Bongers J, Lambros T, Ahmad M, Heimer EP. Kinetics of dipeptidyl peptidase IV proteolysis of growth hormone-releasing factor and analogs. Biochim Biophys Acta. 1992 Jul 31;1122(2):147-53.
22. Kobayashi S, Ohnuma K, Uchiyama M, Iino K, Iwata S, Dang NH, et al. Association of CD26 with CD45RA outside lipid rafts attenuates cord blood T-cell activation. Blood. 2004 Feb 1;103(3):1002-10.
23. Campbell TB, Hangoc G, Liu Y, Pollok K, Broxmeyer HE. Inhibition of CD26 in human cord blood CD34+ cells enhances their engraftment of nonobese diabetic/severe combined immunodeficiency mice. Stem cells and development. 2007;16(3):347-54.
24. Kang J, Der SD. Cytokine functions in the formative stages of a lymphocyte's life. Current opinion in immunology. 2004;16(2):180-90.
25. Charoudeh HN, Tang Y, Cheng M, Cilio CM, Jacobsen SEW, Sitnicka E. Identification of an NK/T cell–restricted progenitor in adult bone marrow contributing to bone marrow–and thymic-dependent NK cells. Blood. 2010;116(2):183-92.
26. Kawase T, Morishima Y, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. Blood. 2007;110(7):2235-41.
27. Alpdogan O, Schmaltz C, Muriglan SJ, Kappel BJ, Perales M-A, Rotolo JA, et al. Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-versus-host disease. Blood. 2001;98(7):2256-65.