

Terminal Deoxynucleotidyl Transferase (TdT) Inhibition of Cord Blood Derived B and T Cells Expansion

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Abstract

Purpose: Terminal deoxynucleotidyl transferase (TdT) is a DNA polymerase that is present in immature pre-B and pre-T cells. TdT inserts N-nucleotides to the V (D) J gene segment during rearrangements of genes, therefore, it plays a vital role in the development and variation of the immune system in vertebrates. Here we evaluated the relationship between cytokines like interleukin-2 (IL-2), interleukin-7 (IL-7), and interleukin-15 (IL-15) and TdT expression in cord blood mononuclear cells and also effect of inhibition in the expansion of B and T cells derived from cord blood.

Methodes: The cord blood mononuclear cells were cultured with different combination of cytokines for 21 days, which they were harvested in definite days (7, 14 and 21) and evaluated by flow cytometry.

Results: Our data indicated that TdT expression increased in cord blood mononuclear cells using immune cell key cytokines without being dependent on the type of cytokines. TdT inhibition reduced both the expansion of B and T cells derived from cord blood and also declined the apoptosis and proliferation. Considered together, TdT played an important role in the control of the expansion of B and T cells derived from cord blood.

Conclusion: considered together, it was observed that TdT expression was increased by cytokines and TdT inhibition not only reduced B and T cells derived from cord blood, but it also affected the rate of apoptosis and proliferation.

Introduction

Terminal deoxynucleotidyl transferase (TdT) is a nuclear enzyme in one unique parcel of the pol X family of DNA polymerase.^{1,2} In human, TdT activity is present in the immature fraction of thymocytes and bone marrow cells.^{3,4} TdT plays a vital role in the development and variation of the immune system in vertebrates.⁵⁻⁷ TdT contributes to the variation of antigen receptors by random addition of nucleotides to single-stranded DNA at the junctions recombination of immunoglobulin heavy-chain genes in B and T cells development.^{8,11}

V(D)J recombination creates diversity of antigen receptors in immune system.¹² TdT is responsible for inserting nucleotides to the junctions of gene segment during V (D) J recombination.¹³⁻¹⁵ TdT gene is present in pre-B and pre-T cells and increases in some human leukemia's.^{9,16} For this reason, TdT is an effective biochemical marker for classifying leukemia's.¹⁷

TdT has been identified in childhood and adult acute lymphoblastic leukemia (ALL) and often appear in chronic myelogenous leukemia (CML), acute nonlymphocytic leukemia (ANLL) and acute myeloid

leukemia (AML). Also TdT expression level is elevated in Non-Hodgkin's lymphoma (NHL) patients.¹⁶⁻¹⁹

Interleukin-2 (IL-2), interleukin-7 (IL-7), and interleukin-15 (IL-15) are certain cytokines which play an important role in generation, hematopoiesis and differentiation of hematopoietic stem cells (HSCs).^{20,21} IL-2 acts in hematopoiesis and proliferation of T cells.²²⁻²⁴ IL-7 affects the B cells development and T cells differentiation.²⁵ Additionally, IL-15 influences the homeostasis, development and proliferation of T cells.²⁶ It seems there are clear interactions between TdT and B and T cell rearrangements and immune cell development and hemostasis. It is important to study these contexts in cord blood since umbilical cord blood contains a plentiful source of CD34 positive cells that can be used in HSC-transplantation in immunotherapy for different cancers. Therefore, it could probably be applicable in combination of TdT inhibition for leukemia treatment. However the role of TdT in the generation of cord blood derived B and T cells is unclear.

The aim of this study is to evaluate TdT expression in cord blood mononuclear cells using different key

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immune cell cytokines like IL-2, IL7, and IL15. Moreover, it is to indicate the influence of TdT inhibition by genistin on the generation of B and T cells.

Materials and Methods

Sample collection

Cord blood samples were collected in 50 mL falcon tubes from full-term normal deliveries by using sterile techniques in Tabriz Alzahra hospital (Tabriz city, East Azerbaijan). Ficoll-Hypaque (GE Healthcare, Piscataway, NJ, USA) was used for mononuclear cells (MNCs) isolation. Cord blood was diluted 1:2 with phosphate buffered saline (PBS, pH 7.4) and 10% fetal bovine serum (FBS), centrifuged at 850 g for 25 min, buffy coat layer was collected with syringes, washed twice and re-suspended in RPMI1640 (Gibco) with 10% FBS (Gibco) and collected MNCs were either used for culture or kept for freezing.

Monoclonal antibodies and flow cytometry

Monoclonal antibodies, conjugated with different fluorochromes, were used to stain cell surface markers including: anti-CD3 (UCHT1; R&D, Minneapolis, MN, USA), anti-CD20 (clone 2H7; BD Biosciences), anti-KI67(clone: 20Raj1, eBioscience, CA 92121, USA), anti-caspase3 (Cat:51-68655X; BD Biosciences) and TdT (cat:51-35404X.4; BD Biosciences). Matched isotype control with appropriate fluoro-chrome was used. Propidium Iodide (1.0mg PI/ml; Invitrogen) was used to exclude dead cells from analysis. Cells were incubated with related antibodies for 20 minutes. Afterwards samples being analyzed on FACSCalibur (BD Bioscience), between 30000 and 80000 events were collected and data analysis was performed using FlowJo software.

Cell culture and cytokines

The 5×10^5 cord blood MNCs were plated in 96-well plates in 250 μ L of RPMI1640 (Gibco) plus 20% Fetal Bovine Serum (FBS; Gibco) and containing 1% penicillin/streptomycin (Gibco) supplemented with cytokines with final concentrations(50 ng/mL for each cytokine): SCF, FLT3 ligand(FL), IL-7, IL-15, and IL-2 (all purchased from PeproTech, NJ, United States). Cells were kept at 37 °C for 21 days, and half of the co-culture medium was replaced weekly. Cells were harvested in definite days (7, 14 and 21) and evaluated for different properties.

For inhibition of TdT expression, MNC cells harvested on the 21st day of the culture, incubated with 50 μ M of genistin (Santa Cruz biotechnology) for 48 hours in incubator (37°C, 5% Co₂). Afterwards, the samples were evaluated for different aims by flow cytometry.

Results

Effects of selected cytokines on TdT expression

We cultured 5×10^5 cord blood mononuclear cells with different combinations of cytokines including SCF, FLT3,

IL-2, IL7 and IL-15 for 21days. Harvested cells were evaluated for TdT expression (Figure 1A).

The percentage of TdT expression increased from day 7 to 21. TdT expression was around 3 and 6 in days 7 and 14 respectively, and reached to 8 on day 21 ($p = 0.0379$), ($p = 0.0158$) (Figure 1B).

However, there was no significant difference between the types of cytokines with the TdT expression. The percentage of TdT expression in the different conditions using IL2, IL7 and IL15 was around 6% on day 14 (Figure 1C).

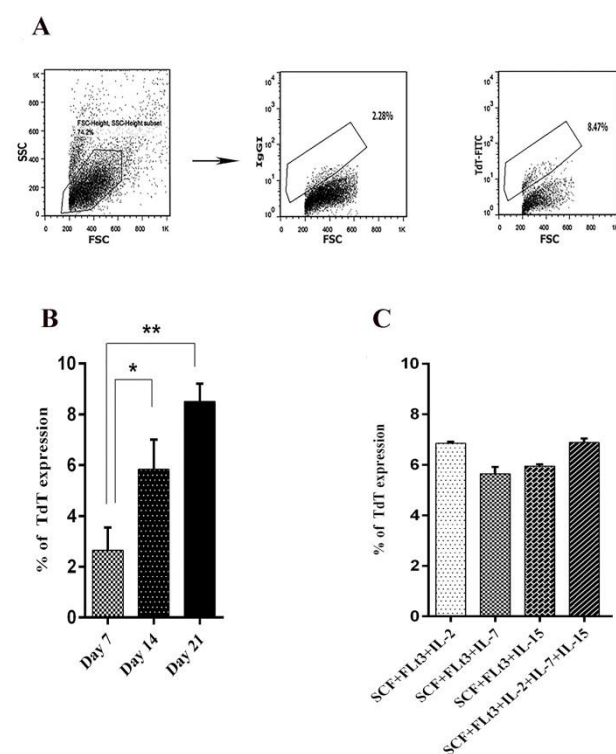


Figure 1. TdT expression in cord blood mononuclear cells. Representative FACS plots were used to evaluate percentage of TdT expression in indicated time points (A). TdT expression in indicated time points (culture supplemented with SCF+FLT3 (B) TdT expression using different combination of indicated cytokines in day 14(C). Values shown are mean \pm SD from 3 independent experiments with 25 to 36 wells analyzed. Differences between groups are significant at ($*p = 0.0379$), ($**p = 0.0158$).

The impact of TdT inhibition on B and T cells

As a previous study stated, genistin inhibits TdT in B and T cells development.²⁷ In this study, we cultured mononuclear cells for 21 days and continued incubation for 48 hours with genistin. We showed that TdT expression reduced about 17% (Figure 2A).

Harvested cells were evaluated for percentage of T and B cells. T and B cells expression was reduced by TdT inhibition, although the reduction in B cells expression(from 10 to 1%) was significantly more than the decrease in T cells expression ($p = 0.0194$), ($p = 0.0074$) (Figure 2B).

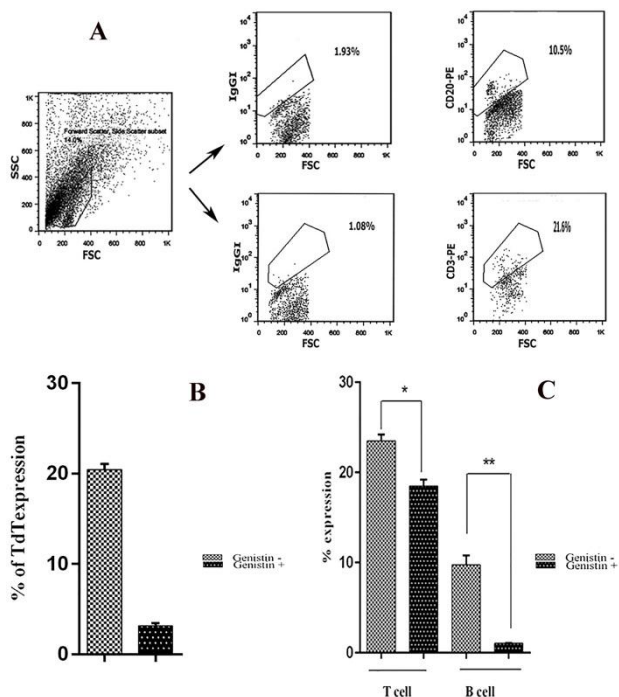


Figure 2. Inhibition of TdT on cord blood derived B and T cells. TdT expression following TdT inhibition using genistin (B) Values shown are mean \pm SD from 5 independent treatment. Expansion of B and T cells following using genistin in day 21 supplemented with SCF+FLT3+IL-2+IL-7+IL-15 cytokines(C). Culture incubated with genistin (genistin+), culture incubate without genistin(genistin-). Values shown are mean \pm SD from 8 independent experiment (* p = 0.0194), (** p = 0.0074).

The impact of TdT inhibition on apoptosis and proliferation

Harvested cells were evaluated with flow cytometry at day 21 (Figure 3A). As genistin is a candidate inhibitor for leukemia, it could alter proliferation and differentiation. We evaluated caspase3 and KI67 expression for apoptosis and differentiation respectively in cord blood mononuclear cells using genistin for TdT inhibition. Percentage of caspase3 expression reduced from 44 to 26% (Figure 3B). Also KI67 expression reduced from 35 to 19% (p = 0.228), (p = 0.0069) (Figure 3B).

Inhibition of TdT by genistin significantly reduced both apoptosis and proliferation.

Discussion

Terminal deoxynucleotidyl transferase (TdT) or terminal transferase as a DNA polymerase is expressed in pre B, pre T cells and in acute lymphoblastic leukemia (ALL) cells. recombination-activating genes (RAGs) and TdT are composite elements of V(D)J rearrangement.²⁸ RAG-1 and RAG-2 proteins are present at breaks of double-strand at the border of recombination signal sequence (RSS, #250) and a coding segment during V(D)J rearrangements.²⁹ T cell receptors (TCR) as an analogous to immunoglobulins as well as B cell receptor (BCR) are complex. The diversity of T and B cells receptors are ascribable to the junctional diversity generated during gene

recombination.^{30,31} In BCR and TCR genes, TdT adds N-nucleotides to the V, D, and J exons during the gene rearrangements due to diversity and their important role in the evolution of immune cells.

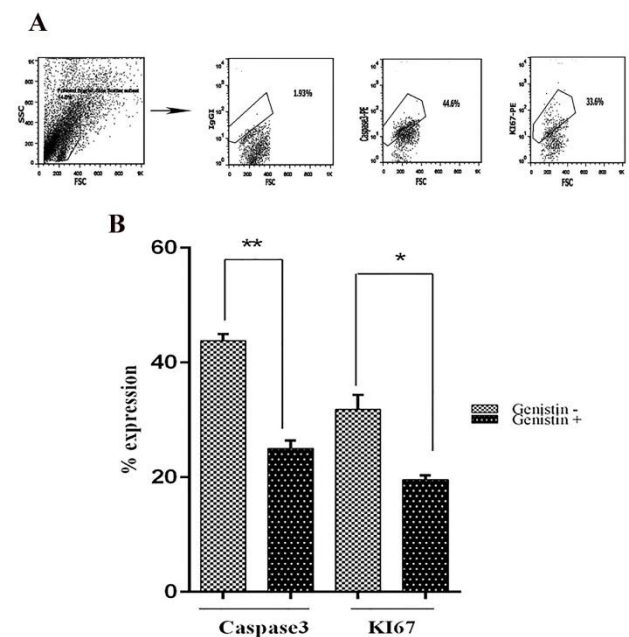


Figure 3. Genistin reduced apoptosis and proliferation in cord blood mononuclear cells.

Representative FACS profiles for evaluation of KI67 and caspase 3 expression in cord blood mononuclear cells following TdT inhibition using genistin (B) Culture incubated with genistin (genistin+), culture incubate without genistin(genistin-). Values shown are mean \pm SD from 8 independent experiments (* p = 0.228), (** p = 0.0069).

In the present study, TdT expression was evaluated in cord blood mononuclear cells in presence of SCF and Flt3 as well as additional IL-2, IL-7 and IL-15. We found that TdT expression increased using different cytokines; however, it was not dependent on the type of cytokines. Inhibition of TdT by genistin reduced B and T cell expansion. As well as reducing apoptosis and proliferation.

Apoptosis regulation is important for the both development and maintenance of the immune system.^{32,33} Interleukins, including IL-2, IL-4, IL-7 and IL-15, extensively effect on lymphocytes survival during V(D)J rearrangement and later in cellular homeostasis,³⁴⁻³⁶ and play an important role in lymphoid cell development.³⁷ It is well documented that IL-7 has important role in T cell development and B cell differentiation.³⁸⁻⁴⁰ As well as in activation of RAG gene.⁴¹ IL-2 can stimulate T cell proliferation.⁴² and IL15 can be useful for generation of T cells.^{43,44} Therefore probably these cytokines have key role in TCR β V-D-J rearrangements and also could influence on TdT expression.³⁷

Based on above information, there are some interactions between cytokine, TdT activity and B and T cell development. Furthermore proliferation and apoptosis

are the key cell activity to balance the B and T cell development and hemostasis, therefore B and T cells could be affected by any TdT alteration. To sum up, TdT is a factor that interacts and is influenced by cytokines, and also could affect the rate of apoptosis and proliferation to balance B and T cell development. As shown TdT expression increases in lymphoma,⁴⁵ maybe these findings are beneficial in the treatment of lymphoma. The inhibition of TdT could play an important role in cancer therapy, particularly in parallel with cord blood stem cell transplantation.

Conclusion

All taken into consideration, it was found out that not only TdT expression increased by cytokines and TdT inhibition decreased B and T cells derived from cord blood, but also it altered the rate of proliferation and apoptosis.

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Ethical Issues

Not applicable.

Conflict of Interest

All authors declare complete responsibility of the content of the study with no conflict of interests.

References

- Nick McElhinny SA, Ramsden DA. Sibling rivalry: competition between Pol X family members in V(D)J recombination and general double strand break repair. *Immunol Rev* 2004;200(1):156-64. doi: 10.1111/j.0105-2896.2004.00160.x
- Nick McElhinny SA, Havener JM, Garcia-Diaz M, Juarez R, Bebenek K, Kee BL, et al. A gradient of template dependence defines distinct biological roles for family X polymerases in nonhomologous end joining. *Mol Cell* 2005;19(3):357-66. doi: 10.1016/j.molcel.2005.06.012
- Pahwa RN, Modak MJ, McMorrow T, Pahwa S, Fernandes G, Good RA. Terminal deoxynucleotidyl transferase (TdT) enzyme in thymus and bone marrow. I. Age-associated decline of TdT in humans and mice. *Cell Immunol* 1981;58(1):39-48.
- Chang LM. Development of terminal deoxynucleotidyl transferase activity in embryonic calf thymus gland. *Biochem Biophys Res Commun* 1971;44(1):124-31. doi: 10.1016/s0006-291x(71)80167-5
- Motea EA, Berdis AJ. Terminal deoxynucleotidyl transferase: the story of a misguided DNA polymerase. *Biochim Biophys Acta* 2010;1804(5):1151-66. doi: 10.1016/j.bbapap.2009.06.030
- Komori T, Okada A, Stewart V, Alt FW. Lack of N regions in antigen receptor variable region genes of TdT-deficient lymphocytes. *Science* 1993;261(5125):1171-5. doi: 10.1126/science.8356451
- Bertocci B, De Smet A, Weill JC, Reynaud CA. Nonoverlapping functions of DNA polymerases mu, lambda, and terminal deoxynucleotidyltransferase during immunoglobulin V(D)J recombination in vivo. *Immunity* 2006;25(1):31-41. doi: 10.1016/j.immuni.2006.04.013
- Landau NR, Schatz DG, Rosa M, Baltimore D. Increased frequency of N-region insertion in a murine pre-B-cell line infected with a terminal deoxynucleotidyl transferase retroviral expression vector. *Mol Cell Biol* 1987;7(9):3237-43. doi: 10.1128/mcb.7.9.3237
- Isobe M, Huebner K, Erikson J, Peterson RC, Bollum FJ, Chang LM, et al. Chromosome localization of the gene for human terminal deoxynucleotidyltransferase to region 10q23-q25. *Proc Natl Acad Sci U S A* 1985;82(17):5836-40. doi: 10.1073/pnas.82.17.5836
- Desiderio SV, Yancopoulos GD, Paskind M, Thomas E, Boss MA, Landau N, et al. Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells. *Nature* 1984;311(5988):752-5. doi: 10.1038/311752a0
- Benedict CL, Gilfillan S, Kearney JF. The long isoform of terminal deoxynucleotidyl transferase enters the nucleus and, rather than catalyzing nontemplated nucleotide addition, modulates the catalytic activity of the short isoform. *J Exp Med* 2001;193(1):89-99. doi: 10.1084/jem.193.1.89
- Schatz DG. V(D)J recombination. *Immunol Rev* 2004;200:5-11. doi: 10.1111/j.0105-2896.2004.00173.x
- Boubakour-Azzouz I, Bertrand P, Claes A, Lopez BS, Rougeon F. Terminal deoxynucleotidyl transferase requires KU80 and XRCC4 to promote N-addition at non-V(D)J chromosomal breaks in non-lymphoid cells. *Nucleic Acids Res* 2012;40(17):8381-91. doi: 10.1093/nar/gks585
- Bertocci B, De Smet A, Berek C, Weill JC, Reynaud CA. Immunoglobulin kappa light chain gene rearrangement is impaired in mice deficient for DNA polymerase mu. *Immunity* 2003;19(2):203-11.
- Boule JB, Rougeon F, Papanicolaou C. Terminal deoxynucleotidyl transferase indiscriminately incorporates ribonucleotides and deoxyribonucleotides. *J Biol Chem* 2001;276(33):31388-93. doi: 10.1074/jbc.M105272200
- McCaffrey R, Bell R, Lillquist A, Wright G, Baril E, Minowada J. Selective killing of leukemia cells by inhibition of TdT. *Haematol Blood Transfus* 1983;28:24-7. doi: 10.1007/978-3-642-68761-7_4

17. Greenwood MF, Coleman MS, Hutton JJ, Lampkin B, Krill C, Bolium FJ, et al. Terminal deoxynucleotidyltransferase distribution in neoplastic and hematopoietic cells. *J Clin Invest* 1977;59(5):889-99. doi: 10.1172/jci108711
18. Motea EA, Lee I, Berdis AJ. A non-natural nucleoside with combined therapeutic and diagnostic activities against leukemia. *ACS Chem Biol* 2012;7(6):988-98. doi: 10.1021/cb300038f
19. Drexler HG, Gaedicke G, Minowada J. Biochemical enzyme analysis in acute leukaemia. *J Clin Pathol* 1985;38(2):117-27. doi: 10.1136/jcp.38.2.117
20. Copley MR, Beer PA, Eaves CJ. Hematopoietic stem cell heterogeneity takes center stage. *Cell stem cell* 2012;10(6):690-7. doi: 10.1016/j.stem.2012.05.006
21. Zhang CC, Lodish HF. Cytokines regulating hematopoietic stem cell function. *Curr Opin Hematol* 2008;15(4):307-11. doi: 10.1097/MOH.0b013e3283007db5
22. Gaffen SL, Liu KD. Overview of interleukin-2 function, production and clinical applications. *Cytokine* 2004;28(3):109-23. doi: 10.1016/j.cyto.2004.06.010
23. Frauwirth KA, Thompson CB. Regulation of T lymphocyte metabolism. *J Immunol* 2004;172(8):4661-5. doi: 10.4049/jimmunol.172.8.4661
24. Kandasamy K, Mohan SS, Raju R, Keerthikumar S, Kumar GS, Venugopal AK, et al. NetPath: a public resource of curated signal transduction pathways. *Genome Biol* 2010;11(1):R3. doi: 10.1186/gb-2010-11-1-r3
25. Janot-Sardet C, Assouline B, Cheynier R, Morre M, Beq S. A validated assay to measure soluble IL-7 receptor shows minimal impact of IL-7 treatment. *J Immunol Methods* 2010;353(1-2):115-23. doi: 10.1016/j.jim.2009.12.003
26. Schluns KS, Williams K, Ma A, Zheng XX, Lefrancois L. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. *J Immunol* 2002;168(10):4827-31. doi: 10.4049/jimmunol.168.10.4827
27. Uchiyama Y, Tagami J, Kamisuki S, Kasai N, Oshige M, Chiku H, et al. Selective inhibitors of terminal deoxyribonucleotidyltransferase (TdT): baicalin and genistin. *Biochim Biophys Acta* 2005;1725(3):298-304. doi: 10.1016/j.bbagen.2005.06.017
28. Mansilla-Soto J, Cortes P. VDJ recombination: Artemis and its in vivo role in hairpin opening. *J Exp Med* 2003;197(5):543-7.
29. Akamatsu Y, Oettinger MA. Distinct roles of RAG1 and RAG2 in binding the V(D)J recombination signal sequences. *Mol Cell Biol* 1998;18(8):4670-8. doi: 10.1128/mcb.18.8.4670
30. Zhu L, Chang CH, Dunnick W. Excessive amounts of mu heavy chain block B-cell development. *Int Immunol* 2011;23(9):545-51. doi: 10.1093/intimm/dxr049
31. Hodges E, Krishna MT, Pickard C, Smith JL. Diagnostic role of tests for T cell receptor (TCR) genes. *J Clin Pathol* 2003;56(1):1-11. doi: 10.1136/jcp.56.1.1
32. Marsden VS, Strasser A. Control of apoptosis in the immune system: Bcl-2, BH3-only proteins and more. *Annu Rev Immunol* 2003;21:71-105. doi: 10.1146/annurev.immunol.21.120601.141029
33. Rathmell JC, Thompson CB. The central effectors of cell death in the immune system. *Annu Rev Immunol* 1999;17:781-828. doi: 10.1146/annurev.immunol.17.1.781
34. Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. *Immunity* 1995;2(3):223-38. doi: 10.1016/1074-7613(95)90047-0
35. Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J Exp Med* 1994;180(5):1955-60. doi: 10.1084/jem.180.5.1955
36. Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature* 2003;426(6967):671-6. doi: 10.1038/nature02067
37. Blom B, Spits H. Development of human lymphoid cells. *Annu Rev Immunol* 2006;24:287-320. doi: 10.1146/annurev.immunol.24.021605.090612
38. Tan JT, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg KI, et al. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc Natl Acad Sci U S A* 2001;98(15):8732-7. doi: 10.1073/pnas.161126098
39. Aliyari Z, Alami F, Mostafavi T, Taiefi Nasrabadi H, Soleimanirad J, Nozad Charoudeh H. The Roles of IL-2, IL-7, and IL15 Ligands in B Cells Development from Cord Blood Mononuclear Cells. *Iran J Ped Hematol Oncol* 2015;5(3):155-60.
40. Tani-ichi S, Shimba A, Wagatsuma K, Miyachi H, Kitano S, Imai K, et al. Interleukin-7 receptor controls development and maturation of late stages of thymocyte subpopulations. *Proc Natl Acad Sci U S A* 2013;110(2):612-7. doi: 10.1073/pnas.1219242110
41. Hikida M, Nakayama Y, Yamashita Y, Kumazawa Y, Nishikawa SI, Ohmori H. Expression of Recombination Activating Genes in Germinal Center B Cells: Involvement of Interleukin 7 (IL-7) and the IL-7 Receptor. *J Exp Med* 1998;188(2):365-72. doi: 10.1084/jem.188.2.365
42. Nelson BH. IL-2, regulatory T cells, and tolerance. *J Immunol* 2004;172(7):3983-8. doi: 10.4049/jimmunol.172.7.3983
43. Weng NP, Liu K, Catalfamo M, Li Y, Henkart PA. IL-15 is a growth factor and an activator of CD8 memory T cells. *Ann N Y Acad Sci* 2002;975:46-56. doi: 10.1111/j.1749-6632.2002.tb05940.x

44. Mueller YM, Petrovas C, Bojczuk PM, Dimitriou ID, Beer B, Silvera P, et al. Interleukin-15 Increases Effector Memory CD8+ T Cells and NK Cells in Simian Immunodeficiency Virus-Infected Macaques. *J Virol* 2005;79(8):4877-85. doi: 10.1128/jvi.79.8.4877-4885.2005
45. Habeshaw JA, Catley PF, Stansfield AG, Ganeshaguru K, Hoffbrand AV. Terminal deoxynucleotidyl transferase activity in lymphoma. *Br J Cancer* 1979;39(5):566-9. doi: 10.1038/bjc.1979.102