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Journal of Leukemia

Editorial

Brief Note on Human Hematopoietic Subpopulations

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ABSTRACT

The Aiolos record factor assumes a significant part in the control of lymphocyte separation and multiplication. The statement of Aiolos isoform has been concentrated in lymphoid pathologies yet nothing is thought about its appearance in unaffected human hematopoietic subpopulations. In this original copy we show interestingly the differential Aiolos articulation at the RNA and protein level in hematopoietic cell subpopulations. B cells express more elevated levels of Aiolos than NK and T cells while monocytes express practically imperceptible degrees of Aiolos. Also, human CD34 (+) begetters don't communicate Aiolos. We didn't notice huge contrast when contrasting gullible with memory T and B cells, however we noticed a significant distinction among Bright and Dim NK cells. Moreover, we show that, notwithstanding hematopoietic cells, non-hematopoietic cell lines, for example, MCF-7, SW480, HEK, PC3 and HeLa additionally express Aiolos.

INTRODUCTION

Mononuclear cells isolated from peripheral blood were prepared by Ficoll gradient centrifugation and stained with anti-hCD4-PE,anti-hCD8-FITC, anti-hCD19-FITC,antihCD27 PE mAbs (BD Biosciences),anti-hCD45RA-PC7,anti-hCD16-FITC,antihCD56-PC5 mAbs (Beckman Coulter). Isolation of lymphocyte subpopulations was performed using FacsVantage (Becton Dickinson). Each fraction, containing 106 cells was sorted and the purity of isolated cells was >95%.

Total RNA was extracted from isolated cell populations from healthy donors and umbilical cord blood using Quiagen RNeasy kit according to manufacturer's intructions. RNA was reversed transcribed and amplified by Superscript VILO cDNA synthesis kit (Invitrogen). Quantitative PCR was carried out on an ABI PRISM 7300 detection system. We used the TaqMan gene expression assay (Applied Biosystems) for quantitative detection of human Aiolos (Hs00232635 m1) and the absolute qPCR Rox mix (Abgene) according to the manufacturer's instructions. The absolute quantification in each sample was determined by the standard curve method. Data from triplicates are expressed as normalized expression by using the Abelson gene (Hs00245445 m1) expression as a reference. Cells were lysed in Laemmli sample buffer and extracts separated by SDS-PAGE, transferred to nitrocellulose, blocked (5% non-fat dry milk in TBS (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.05% Tween-20) and incubated with primary antibody in TBS. Membrane was washed with and incubated with secondary antibody. Proteins were detected using the ECL system.

It is known that murine T and B cells express Aiolos. On the contrary, nothing is known concerning Aiolos expression in different human healthy subpopulations. In order to answer this question, we investigated the expression pattern of Aiolos in peripheral blood mononuclear cells (PBMC) from healthy donors as well as in blood cord and hematopoietic progenitors. The FACS profiles of CD4 (+) T, CD8 (+) T, B and NK cells and the selected subpopulations. The level of Aiolos expressed in different primary cells: B cells express the highest level of total Aiolos compared to T, NK, monocytes and CD34 (+) hematopoietic progenitors isolated from bone marrow. T and NK cells express comparable levels of Aiolos and monocytes express almost undetectable level of Aiolos while CD34 (+) progenitors are negative for Aiolos expression. The absence of Aiolos expression in human CD34 (+) progenitors is in agreement with the lack of expression detected in murine hematopoietic stem cells. The correlation between Aiolos RNA and protein expression, showing that B cells express the highest level of Aiolos protein, T and NK cells express comparable levels, monocytes express very weak level of Aiolos and CD34 (+) progenitors do not express Aiolos. This result strongly suggests a

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Received date: Aug 24, 2021; **Accepted date:** November 10, 2021; **Published date:** November 22, 2021

Citation: Agarwal S (2021) Brief note on human hematopoietic subpopulations.J Leuk, 9:P51.

direct correlation between the RNA and the protein expression. Among T cells, CD8 (+) T cells express slightly higher level of total Aiolos than CD4 (+) T cells.

CONCLUSION

When Aiolos expression was analyzed in T and B cells and monocytes isolated from human cord blood, we observed that B cells express high level of Aiolos compared to T cells while monocytes, express almost undetectable levels of Aiolos. When compared Aiolos expression among B cells from adults PBMC and cord blood, we observe that T cells and monocytes express similar levels. On the contrary, B cells from cord blood express higher level of total Aiolos than B cells from PBMC. Regarding subpopulations in cord blood, naive B cells express higher level of Aiolos than naive CD4 (+) or CD8 (+) of human cord blood. This result allowed us to conclude that total B cells or naive B cells from human cord blood express higher level of Aiolos that total or naive B cells from PBMC. However, we do not know whether this increase in Aiolos expression detected in cord blood corresponds to an increase in the Aiolos wild type isoform, to an increase in the expression of dominant negative isoforms or both.