SA-β-galactosidase activity in effector and regulatory T cells

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INTRODUCTION

According to WHO, the size of population aged over 60 is expected to double by 2050. The ageing of the population puts a heavy social and economical strain, since ageing involves significantly increased risk of developing and severe course of various autoimmune, oncological and infectious diseases.

All age-related chronic diseases are considered to be associated with key ageing attributes convergence, those attributes underlie age-related tissue dysfunction caused by senescent cells accumulation. The age-conditioned accumulation of ageing and senescent cells causes negative changes in body functioning and its ageing in general. In 1995 it was proposed that ageing cells are characterized by increased expression of β-galactosidase (β-Gal), which stays active at a biased optimum of pH = 6.0. Such senescence-associated β-Gal has been called SA-β-Gal, and nowadays is considered most versatile and widely used marker of senescent cells.

Regulatory T cells (Treg) play a key role in maintaining peripheral self-tolerance and are involved in limiting an excessive immune response. The imbalance between effector and regulatory chains of immunity may be caused by Treg ageing and transition to senescent state. Despite an intensive research in the field of immunity system ageing, the properties of senescent Treg cells have not been studied sufficiently. Therefore, it appears relevant to investigate ageing features and mechanisms of Treg cells transition into a senescent state.

THE AIM OF THE STUDY

To examine various populations of human regulatory and effector T lymphocytes for assessing their senescent status depending on age by β-Gal activity assay.

MATERIALS AND METHODS

The objects of the study were populations of $CD3^+CD4^+CD25^{high}CD127^{dim/neg}$ ($CD4^+$Treg) and $CD3^+CD8^+CD127^-HLA\text{-}DR^+$ ($CD8^+$Treg) T lymphocytes isolated from the fraction of peripheral blood mononuclear cells (PBMCs) of conditionally healthy donors in two age groups (23-32 years old and 50-61 years old). The subject of the study was examination of β-Gal activity in various populations of effector and Treg cells in human peripheral blood.

β-Gal activity was assessed in populations of $CD3^+CD4^+$-, $CD3^+CD8^+$-, $CD4^+$Treg- and $CD8^+$Treg-lymphocytes. Additionally, β-Gal activity was assessed in activated ($CD25^+$ and $HLA\text{-}DR^+$) and non-activated T lymphocyte subsets.

The β-Gal activity in PBMCs was assessed using CellEvent™ Senescence Green Detection Kit (Thermo Fisher Scientific, USA) in accordance with the manufacturer's protocol via flow cytometry. Cells were preliminarily stained with antibodies against surface markers. Statistical processing of the data was performed by means of GraphPad Prism 7.03 software.

RESULTS

$CD4^+$Treg lymphocytes demonstrate reduced β-Gal activity in contrast to $CD4^+$ lymphocytes. Among the $CD8^+$ lymphocyte subsets, the $CD3^+CD8^+CD127^+HLA\text{-}DR^+$ population has higher β-Gal activity compared to $CD8^+$ lymphocytes, what characterizes them as activated cells. However, there were no significant differences found in β-Gal activity for the $CD8^+$Treg population compared to $CD8^+$ lymphocytes. Nor were found significant differences in β-Gal activity between the $CD4^+$ and $CD8^+$ T cell populations (Fig. 1).

$CD25^+$ and $HLA\text{-}DR^+$ T lymphocyte subsets demonstrate higher β-Gal activity compared to non-activated T lymphocytes (Fig. 2).

There were no statistically significant differences found when comparing the studied subpopulations among donors in two age groups. However, the percentage of $CD8^+HLA\text{-}DR^+$ (Fig. 3, a) and $CD4^+HLA\text{-}DR^+$ (Fig. 3, b) lymphocytes positively correlated with the age of the donors.

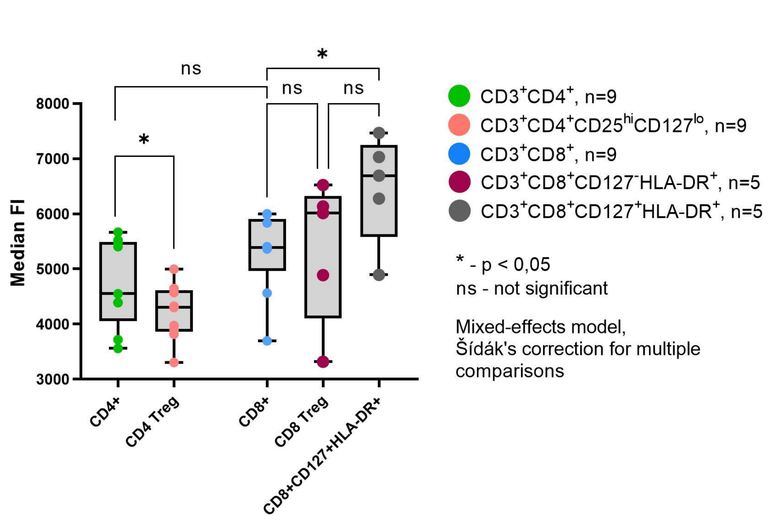

Fig. 1. β-Gal activity in the main populations of CD3+ lymphocytes, including Treg lymphocytes.

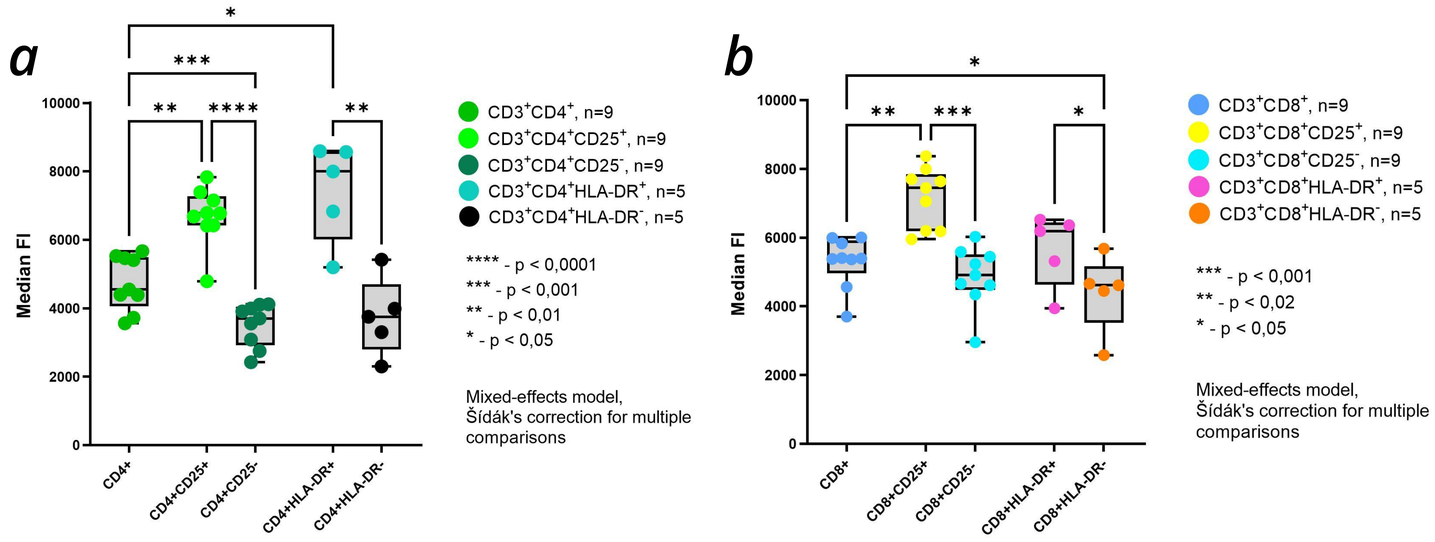

*Fig. 2. β-Gal activity in $CD3^+CD4^+$ **(a)** and $CD3^+CD8^+$ **(b)** lymphocytes subsets considered positive and negative by CD25 and HLA-DR activation markers.*

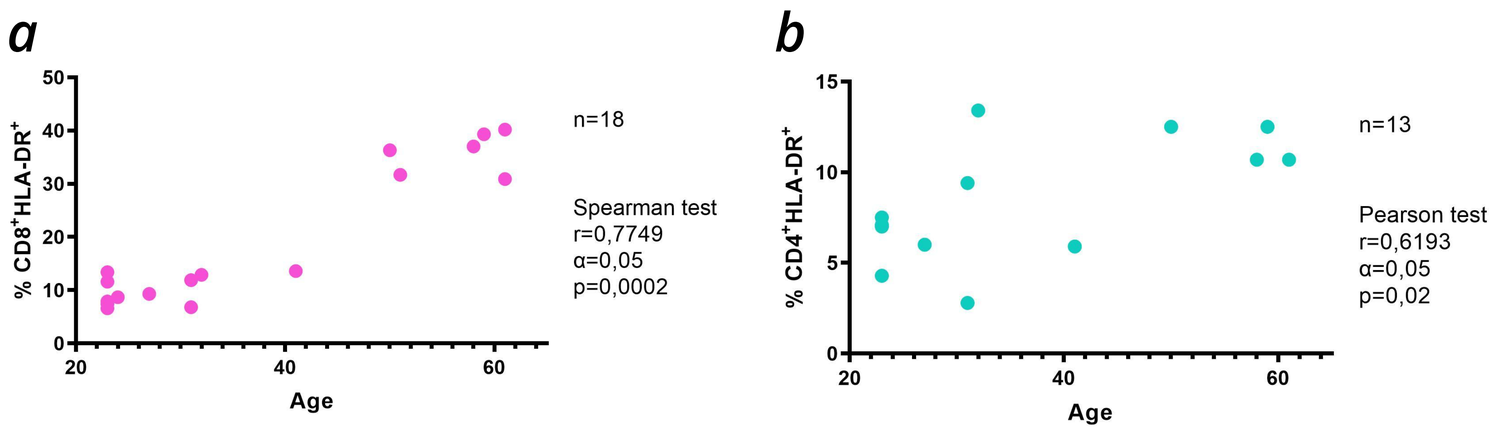

*Fig. 3. Correlation between age and percentage level of CD8+HLA-DR+- **(a)** and CD4+HLA-DR+ **(b)** lymphocytes in donors' peripheral blood*

CONCLUSION

In this way, the higher β-Gal activity in $CD25^+$ and $HLA\text{-}DR^+$ subsets of $CD4^+$ and $CD8^+$ human lymphocytes may be a reflection of a difference in metabolic activity between these populations. Reduced β-Gal activity in $CD4^+$Treg lymphocyte population in comparison to common $CD4^+$ lymphocyte population may also be an indirect reflection of energetic metabolism features of conventional $CD4^+$Treg cells. An increased percentage level of $CD4^+HLA\text{-}DR^+$ and $CD8^+HLA\text{-}DR^+$ lymphocytes in peripheral human blood correlates with an increase of donors' age and points to age-related accumulation of antigen-experienced T lymphocytes.

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