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# Identification and characterization of senescent cells in human and nonhuman primate peripheral blood CD3+ T-lymphocyte populations. †

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**Abstract:** The development of age-related diseases and immune system dysfunction are associated with accumulation of senescent cells characterized by a senescent-associated secretory phenotype (SASP). It is widely recognized as a key mechanism of pathologies in the elderly that increase the risk of cardiovascular, neurodegenerative, autoimmune, and cancer diseases, reducing the effectiveness of vaccinations, which increases the burden on the health care system. Investigations into the immunosenescence mechanisms suggest a new approaches for the prevention and therapy of age-associated diseases. In this study, senescent immune cells were measured using a senescence-associated beta-galactosidase (SA-β-Gal) activity assay based on FACS. The number of CD3+ T-lymphocytes with SA-β-Gal$^{high}$ activity increases significantly in over 60 y.o. donors peripheral blood in comparison with 20-30 y.o. The most dramatic differences in the number of senescent cells were observed in the CD8+ lymphocytes population, while for CD4+ T-lymphocytes differences were less pronounced. In agreement with previously published data SA-β-Gal$^{high}$ lymphocytes express p16 and p21 (cell cycle arrest proteins). Additional verification by secretion of inflammatory (SASP) cytokines, extranuclear localization of HMGB1, analysis of histone H2AX phosphorylation (γH2AX) and functional tests will confirm senescent status of indicated above SA-β-Gal$^{high}$ cells populations. Thus, this will allow to create a convenient model for testing laboratory substances approved by Food and Drug Administration (FDA) as potential anti-aging drugs and will serve as a basis for the development of new effective methods to support an active and healthy longevity.

**Keywords:** senescence; immunosenescence; SA-b-Gal; ageing; drug testing models

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## Introduction

The development of age-related diseases and immune system dysfunction are associated with accumulation of senescent cells characterized by a senescent-associated secretory phenotype (SASP). It is widely recognized as a key mechanism of pathologies in the elderly that increase the risk of cardiovascular, neurodegenerative, autoimmune, and cancer diseases [1-4], reducing the effectiveness of vaccinations, which increases the burden on the health care system. Thus, the study of the specific features of immune cell aging is becoming one of the most relevant areas of modern investigations aimed at the development of new approaches to the prevention and therapy of age-associated diseases. Validation of the methodology for the detection of potentially senescent immune cells demonstrating SA-b-Gal activity for the non-human primate *Macaca fascicularis* is the first step in the development of a comprehensive algorithm for identifying specifically

senescent immune cells using additional recognised biomarkers of ageing, which will further provide a convenient model for studying the physiology of the immune system ageing, developing laboratory diagnostic assays and testing anti-ageing drugs such as senolytics and senomorphics.

**Materials and methods.**

Healthy donors 20-30 and 60-70 years old (y.o.) and *M. fascicularis* of the age group corresponding to young donors (5-6 y.o.) took part in the study. Peripheral blood mononuclear cells (PBMCs) were isolated in a ficoll-paque gradient according to a standard protocol. Then, we assessed the number of potentially senescent immune cells in CD3+ T-lymphocyte populations using a senescence-associated beta-galactosidase (SA-β-Gal) activity assay (CellEvent™ Senescence Green Detection Kit (ThermoScientific, USA) based on FACS (CytoFLEX B5-R3-V4, Beckman Coulter, USA). The following panel of fluorochromes and antibodies was used to identify different immune cell populations for both human and *M. fascicularis* samples: FVS780 (BD, USA), CD3 PerCP-Cy5.5 (clone SP34-2, BD, USA), CD4 AF700 (clone OKT4, Thermo Fisher, USA), CD8 BV711 (clone RPA-T8, BD, USA).

**Results.**

We observed an age-associated increase in the percentage of potentially senescent cells showing high SA-b-Gal activity in both CD8+ and CD4+ T-lymphocyte populations (Figures 1, 2) in donors 60-70 y. o. in comparison with donors 20-30 y. o. The age-related increase in the percentage of SA-b-Gal$^{high}$ cells was more pronounced in the CD8+ T-lymphocyte population, reaching 68.63±12.04% in donors 60-70 y.o. (in some donors >85%), which is in good agreement with previously published data (Figure 2) [5]. The explanation of this phenomenon requires further investigations and might be an important step towards a better understanding of the mechanisms of immune system aging at the cellular level, which will help in the development of new effective therapeutic approaches for the treatment of age-associated.

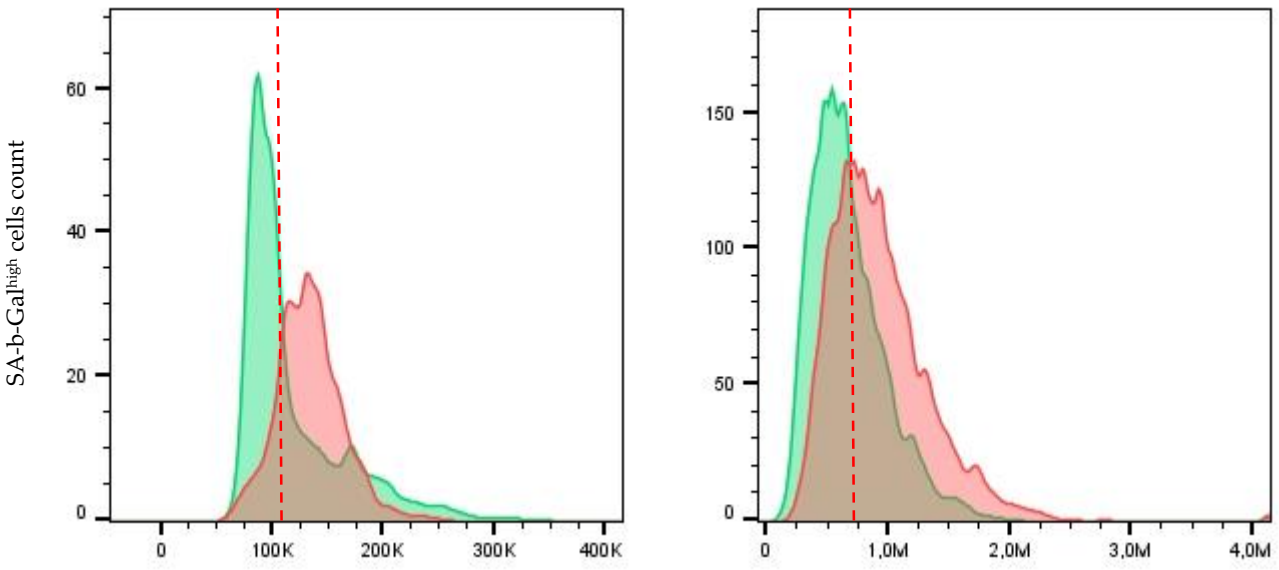


**Figure 1.** Representative SA-b-Gal intensity profiles and the gating strategy used to quantify potentially senescent SA-b-Gal$^{high}$ cells in CD8+ (left) and CD4+ (right) lymphocyte populations from donors 20-30 (green) and 60-70 y. o. (red).

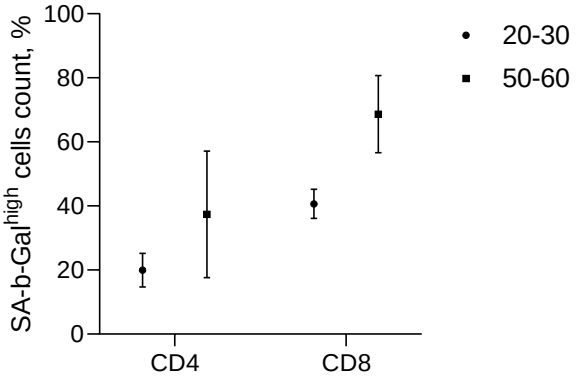


**Figure 2.** Percentage content of potentially senescent SA-b-Gal$^{high}$ cells in CD4+ and CD8+ T-lymphocyte populations in donors 20-30 y. o. in comparison with donors 60-70 y. o. (n = 3, Mean ± SEM).

Then, we compared the percentage of potentially senescent SA-b-Gal$^{high}$ CD4+ and CD8+ T lymphocytes in 20-30 y. o. donors and *M. fascicularis* of the corresponding age group (Figure 3). Quantification of potentially SA-b-Gal$^{high}$ cells in both lymphocyte populations showed a similar pattern for humans and non-human primates. As in humans, a higher percentage of potentially SA-b-Gal$^{high}$ cells was observed in the CD8+ T-lymphocyte population (Table 1).

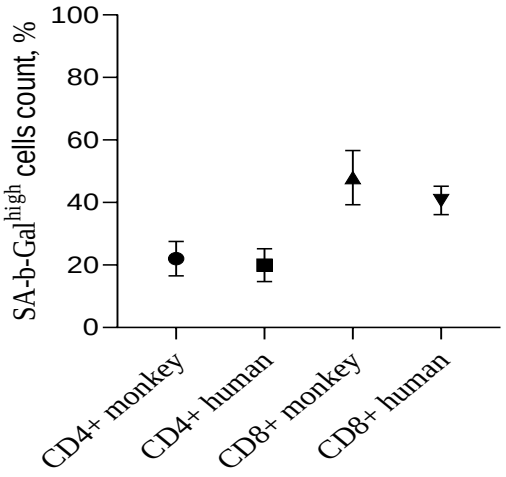


**Figure 3.** Percentage content of potentially senescent SA-b-Gal$^{high}$ cells in CD4+ and CD8+ T-lymphocyte populations in donors 20-30 y. o. (n = 3) and *M. fascicularis* (n = 7) of the corresponding age group (Mean ± SEM).

**Table 1.** Percentages of potentially senescent SA-b-Gal high cells in CD4+ and CD8+ T-lymphocyte populations from 20-30 y. o. donors (n = 3) and *M. fascicularis* (n = 7) of the corresponding age group (Mean±SEM)

| Species | CD4+ T-lymphocytes | CD8+ T-lymphocytes |
|---|---|---|
| *H. sapiens* | 19.90 ± 5.26 | 40.60 ± 4.55 |
| *M. fascicularis* | 22.10 ± 5.49 | 47.90 ± 8.67 |

In combination with other well-known biomarkers of senescence, such as secretion of inflammatory (SASP) cytokines, extranuclear localization of HMGB1, histone H2AX phosphorylation (γH2AX) and functional tests and others this data may become the basis for the development of a comprehensive algorithm for the specific identification of senescent immune cells, which is useful for further studies of the physiology of the aging immune system.

**Conclusion.**

Thus, the described method of identifying potentially senescent CD3+CD4+ and CD3+CD8+ T-lymphocytes by SA-b-Gal activity gives comparable results for humans and the nonhuman primate *M. fascicularis*, which makes it possible to further develop a comprehensive algorithm for the isolation of specifically senescent immune cells based on the combination of several different recognised biomarkers of ageing. The use of *M. fascicularis* as a model for studying the aging of the human immune system may be useful in the development of diagnostic laboratory tests, testing of anti-aging drugs and other applications.

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**Supplementary Materials:**

**Author Contributions:** Methodology, M.Yu., T.V., R.S.; investigation, M.Yu., T.V.; formal analysis, M.Yu.; writing—original draft preparation, M.Yu.; writing—review and editing, T.V.; visualization M.Yu.; supervision, project administration R.S.; funding acquisition, R.S. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** original data is available on request.

**Conflicts of Interest:** The authors declare no conflict of interest.