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

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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. 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 based on FACS (CellEvent™ Senescence Green Detection Kit (ThermoScientific, 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[5] (Figure 2).

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).

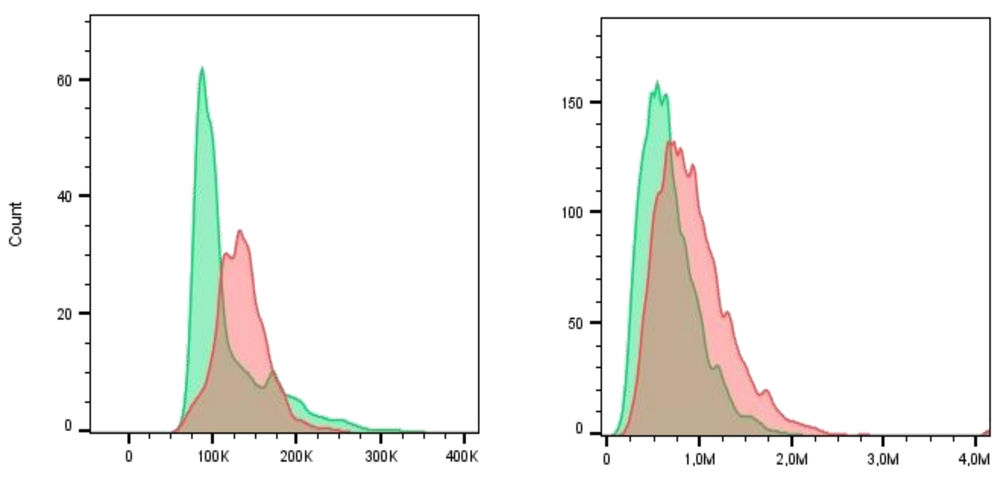

Figure 1. Representative SA-b-Gal intensity profiles and the gating strategy used to quantify potentially senescent SA-b-Gal high cells in CD8+ (left) and CD4+ (right) lymphocyte populations from donors 20-30 (green) and 60-70 y. o. (red).

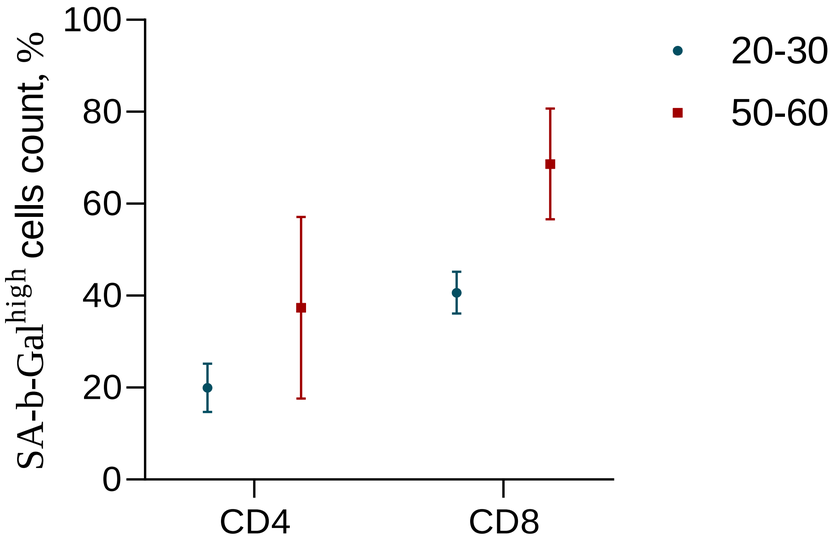

Figure 2. Percentage content of potentially senescent SA-b-Gal high cells in CD4+ and CD8+ T-lymphocyte populations in donors 20-30 y. o. in comparison with donors 60-70 y. o. (n = 3, Mean ± SEM).

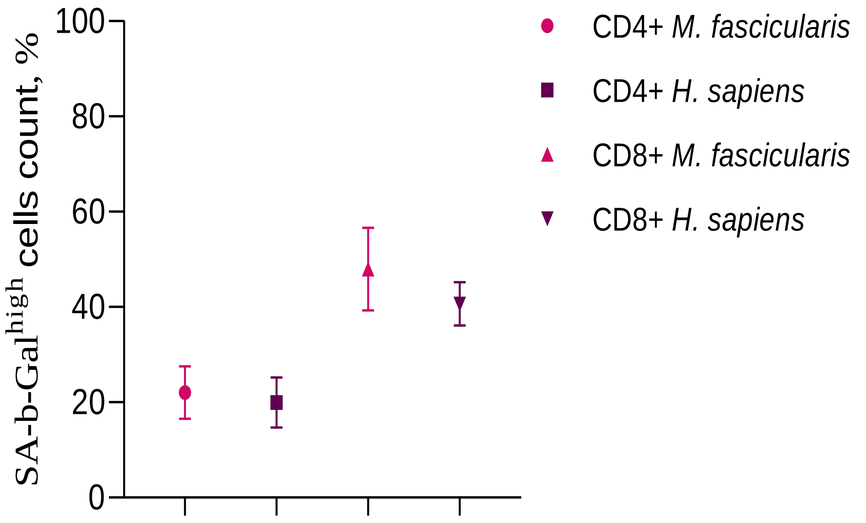

Figure 3. Percentage content of potentially senescent SA-b-Gal high cells in CD4+ and CD8+ T-lymphocyte populations in donors 20-30 y. o. (n = 3) and *M. fascicularis* (n = 7) of the corresponding age group (Mean ± SEM).

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-lymphocyes	CD8+ T-lymphocyes
H. sapiens	19.90 ± 5.26	40.60 ± 4.55
M. fascicularis	22.10 ± 5.49	47.90 ± 8.67

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, etc.

References

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