doi 10.18699/bgrs2024-13-33

Transcriptional signatures and age-related changes in CD8+HLA-DR+ regulatory T cells

Matveeva K.[1]*, Kolmykov S.[2], Shevyrev D.[3]

[1] *Department of Immunobiology and Biomedicine, Sirius University of Science and Technology, Sirius, Russia*

[2] *Department of Computational Biology, Sirius University of Science and Technology, Sirius, Russia*

[3] *Resource Center of Cell Technology and Immunology, Sirius University of Science and Technology, Sirius, Russia*

** ksum.miha@gmail.com*

Key words: immunology; regulatory T cells; aging; single cell transcriptomics

Motivation and Aim: The subset of $CD8^+HLA\text{-}DR^+$ regulatory T lymphocytes ($CD8^+$Treg) was first described in 2014 [1]. These cells are known to suppress effector T cells with the involvement of checkpoint inhibitor molecules and have some properties of conventional $CD4^+$Treg [2]. Nevertheless, the nature and function of this subset remain poorly understood. Moreover, the study of the properties of this population becomes important in the context of general age-associated changes in the human immune system and higher vulnerability of $CD8^+$ T lymphocytes to these changes. In preliminary experiments, the $CD8^+HLA\text{-}DR^+$ population was found to segregate into two subpopulations based on surface expression of CD127 (IL7R). Based on this, the $CD8^+$Treg phenotype was defined as $CD3^+CD8^+HLA\text{-}DR^+CD127^{low}$. In this work, we aimed to determine transcriptional signatures and age-related changes in gene expression within the $CD8^+$Treg population using publicly available single-cell RNA-seq (scRNA-seq) data. Our objectives were as follows: (1) Identify the $CD8^+$Treg cluster; (2) Determine transcriptional signatures of $CD8^+$Treg; (3) Evaluate age-related gene expression changes in the $CD8^+$Treg cell population.

Methods and Algorithms: In this study, we analyzed peripheral blood mononuclear cells (PBMCs) scRNA-seq data from 982 donors, aged 19 to 97 y.o., from the OneK1K cohort [3] and 99 healthy donors, aged 22 to 75 y.o., from the SLE study [4]. The Scanpy package (version 1.9.8) [5] was used to analyze scRNA-seq data. Initially, data preprocessing steps such as normalization, logarithmization, scaling, dimensionality reduction (by PCA), and batch correction were performed. Subsequently, cells were clustered using the Leiden algorithm. The Celltypist package [6] was utilized to annotate the cell clusters and identify cluster of $CD8^+$ T effector cells ($CD8^+$Teff). Then, to more accurately represent CD8+Teff cells in reduced dimensionality embedding, the cells in a given cluster were returned logarithmized gene counts and the preprocessing steps from scaling to batch correction were performed repeatedly. Next, re-clustering and further manual annotation were conducted to identify the $CD8^+HLA\text{-}DR^+CD127^{low}$ cell subpopulation. The cell populations were pooled to conduct a pseudobulk differential expression analysis using edgeR (version 4.0.16) [7]. Functional analysis of the differentially expressed genes was performed using the g:Profiler web tool [8].

The described analyses are available as Jupyter-notebooks on a GitHub page: https://github.com/estakathersun/cd8treg_sc.

Results: To identify the signatures of the CD8$^+$Treg, data from donors under 60 years of age were utilized, as T cells at this age typically don't exhibit significant age-related changes. It's worth mentioning, these cells weren't segregated into a distinct cluster but distributed among the CD8$^+$Teff population. Therefore, CD8$^+$Teff cells displaying increased HLA-DR (HLA-DRA, HLA-DRB1 and HLA-DRB5 genes) and decreased IL7R expression levels were considered as the CD8$^+$Treg subpopulation. Differential expression analysis of the single-cell data revealed that the cell population, compared to the overall CD8$^+$Teff cell population, exhibited increased expression levels of genes associated with MHCII-mediated antigen presentation, cytotoxicity, and cytoskeleton organization (Fig. 1). However, at the pseudo-bulk level, no statistically significant differences were observed between the CD8$^+$Treg and CD8$^+$Teff populations.

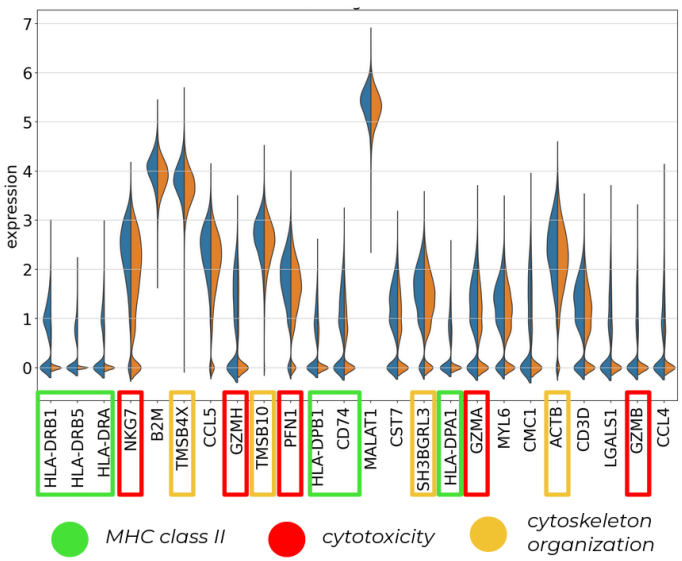

Fig. 1. Differential gene expression of CD8$^+$Treg (blue) in relation to the common CD8$^+$Teff population (orange). Colored boxes signify genes related to the corresponding function

Subsequently, we analyzed CD8$^+$Treg populations from two age groups: young adults, aged 20 to 35 years, and old individuals, aged 70 to 97 years. Fig. 2 shows the results of differential expression analysis at the pseudobulk level. A shift in the transcriptional profile of the studied cell population towards a terminally differentiated cell phenotype was observed. Notably, there was a decrease in the expression levels of certain cytotoxic protein genes, such as LYZ, GZMA and GZMK, while the expression of GZMH, typical of terminally differentiated CD8$^+$ T cells, increased. Additionally, we observed a positive association between age and increase in MHC-II expression level. However, there was no rise in the expression levels of genes associated with CD8$^+$ T cell exhaustion. It can be assumed that this cell population likely transitions towards a terminally differentiated phenotype with age but does not undergo cell exhaustion.

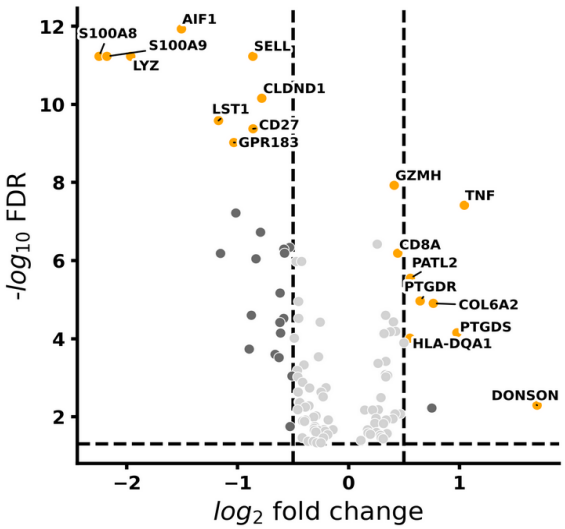

Fig. 2. Differentially expressed genes in the CD8$^+$Treg population from the old age group (70–97 y.o.)

Conclusion: CD8$^+$Treg is a heterogeneous subpopulation of effector CD8$^+$ T lymphocytes. CD8$^+$Treg tends to increase expression of genes associated with cytotoxicity, cytoskeletal rearrangement, and MHC II-mediated antigen presentation. This suggests that mechanism of CD8$^+$Treg-mediated suppression is cell-contact dependent cytolysis of target cells. In the old age group, the CD8$^+$Treg shows a change in marker gene expression to a terminal differentiated cell phenotype.

Funding: The study was financially supported by the Russian Science Foundation (project № 24-15-20003, https://rscf.ru/en/project/24-15-20003/).

References

1. Arruvito L., Payaslián F., Baz P. et al. Identification and Clinical Relevance of Naturally Occurring Human CD8+HLA-DR+ Regulatory T Cells. *J Immunol.* 2014;193(9):4469-4476
2. Machicote A., Belén S., Baz P., Billordo L.A., Fainboim L. Human CD8+HLA-DR+ Regulatory T Cells, Similarly to Classical CD4+Foxp3+ Cells, Suppress Immune Responses via PD-1/PD-L1 Axis. *Front Immunol*. 2018;9:2788
3. Yazar S., Alquicira-Hernandez J., Wing K. et al. Single-cell eQTL mapping identifies cell type – specific genetic control of autoimmune disease. *Science*. 2022;376(6589):eabf3041
4. Perez R.K., Gordon M.G., Subramaniam M. et al. Single-cell RNA-seq reveals cell type – specific molecular and genetic associations to lupus. *Science*. 2022;376(6589):eabf1970
5. Wolf F.A., Angerer P., Theis F.J. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol.* 2018;19(1):15
6. Domínguez Conde C., Xu C., Jarvis L.B. et al. Cross-tissue immune cell analysis reveals tissue-specific features in humans. *Science*. 2022;376(6594):eabl5197
7. Chen Y., Chen L., Lun A.T.L., Baldoni P.L., Smyth G.K. edgeR 4.0: powerful differential analysis of sequencing data with expanded functionality and improved support for small counts and larger datasets. *bioRxiv.* 2024
8. Kolberg L., Raudvere U., Kuzmin I., Adler P., Vilo J., Peterson H. g:Profiler – interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update). *Nucleic Acids Res.* 2023;51(W1):W207-W212