Expansion of cytotoxic CD4⁺ T cells in supercentenarians revealed by single cell transcriptomics

What underlies an exceptionally long life span? Researchers at Keio University School of Medicine (Tokyo, Japan) studied immune systems in supercentenarians—individuals at least 110 years of age—to find clues to their incredibly long health spans. Using single cell analysis, they found that expansion of cytotoxic T cells, particularly the usually rare CD4⁺ cytotoxic T cells, may confer protection against cancer and viral infection. K Hashimoto et al., *PNAS*. (2019).

Snapshot	10x Genomics product
Research area: Cellular & Molecular Immunology **Organism:** Human **Sample type:** PBMCs isolated from blood **Research questions:** How do the immune systems of supercentenarians uniquely protect against aging? Can single cell analysis help unlock the secrets of immunological success?	**Chromium Single Cell Gene Expression Solution** • Chromium Single Cell 3′ Library and Gel Bead Kit v2 • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines **Chromium Single Cell Immune Profiling Solution** • Chromium Single Cell 5′ Library and Gel Bead Kit • Chromium Single Cell V(D)J Enrichment Kit, Human T Cell • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines

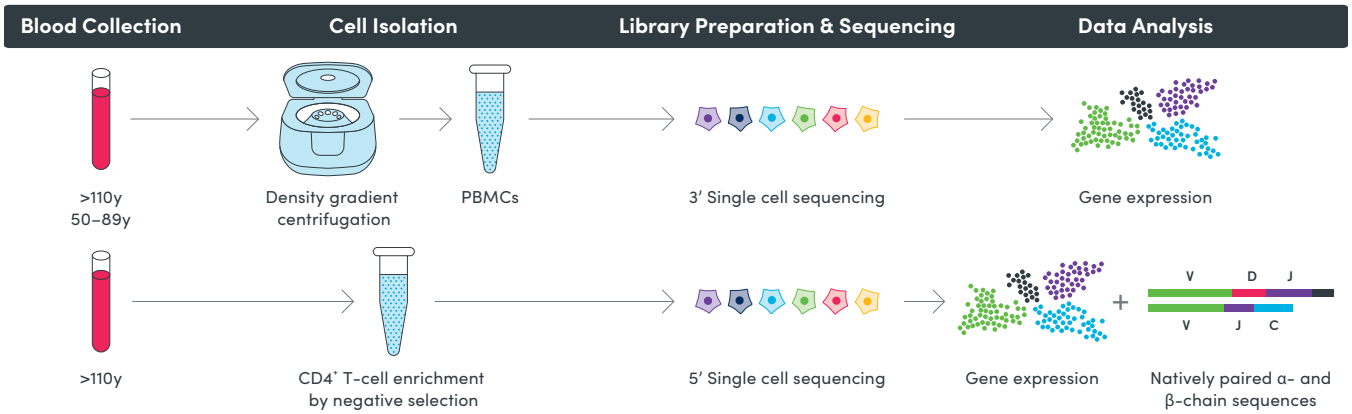
Experiment overview

Comparison of supercentenarians and adult controls

- Collection of blood samples from 7 supercentenarians (≥110 years) and 5 control adults (50–89 years)
- Isolation of PBMCs from whole blood by density gradient centrifugation
- Single cell gene expression analysis of 61,202 total cells using the Chromium Single Cell Gene Expression Solution

Characterization of CD4⁺ T cells

- Collection of second blood draw from 2 supercentenarians
- Enrichment of CD4⁺ T cells using negative selection
- Single cell sequencing of whole transcriptome and full-length, paired T-cell receptors using the Chromium Single Cell Immune Profiling Solution



Why single cell?

Single cell analysis provides insight not only into the proportion of CD4⁺/CD8⁺ T cells, but also into different cell states defined by gene expression signatures. Here, single cell transcriptome analysis revealed how CD4⁺ cytotoxic T cells differentiate in supercentenarians. Full-length, paired T-cell receptor sequencing coupled with gene expression analysis showed massive clonal expansion of CD4⁺ cytotoxic T cells, suggesting conversion of CD4⁺ T cells from a helper to cytotoxic phenotype may promote longevity.

Computational analysis

Creation of a pseudotemporal path of T-cell differentiation helped explain how differentiation of T cells in supercentenarians enables the increase in CD4⁺ cytotoxic T cells. First, Cell Ranger was used to cluster and annotate cell types from PBMCs, and only the two T-cell clusters from initial gene expression analysis were selected for trajectory analysis. Monocle 2 estimated the differentiation trajectory, with every T cell given a pseudotime value between 0 and 12. Ordering the cells in pseudotime enabled tracking of established marker gene expression changes over time, highlighting the changing proportion of cell types. Supercentenarians were shown to have more T cells from later pseudotime points compared to controls.

Results

Expansion of cytotoxic CD4⁺ T cells

Comparison of PBMCs from supercentenarians and controls showed a reduction in B cells, mirroring previous aging studies. T-cell proportions were similar, but supercentenarians had an expansion of cytotoxic T cells at the expense of non-cytotoxic T cells. Cytotoxic T cells were further divided into γδ, CD8⁺, and CD4⁺ cytotoxic T cells, with the CD4⁺ population dramatically overrepresented in supercentenarians (Figure 1).

Limited immune repertoire of cytotoxic CD4⁺ T cells

Paired analysis of gene expression and full-length, paired T-cell receptor repertoire in isolated CD4⁺ T cells indicated massive clonal expansion of CD4⁺ cytotoxic T cells in supercentarians (Figure 1). Within cytotoxic CD4⁺ T cells, 70% of the total repertoire was made up of only 10 clones, with a single clonotype dominating.

The limited clonal diversity in CD4⁺ cytotoxic T cells of supercentenarians suggests their increased numbers may be due to repeated activation and expansion. Cytotoxic CD4⁺ T cells have previously been implicated in immunosurveillance, tumor immunity, and viral response, suggesting they may play important roles in protecting supercentenarians from disease and enabling extreme long life.

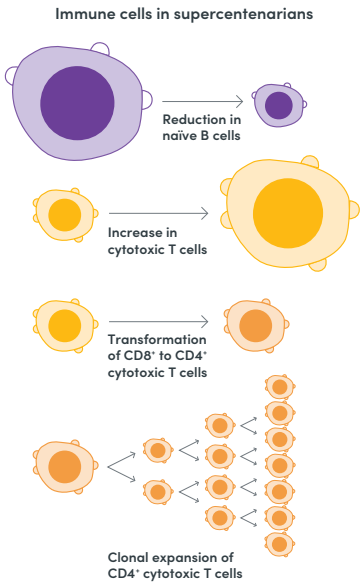


Figure 1. Unique immune cell changes in supercentenarians. Through single cell transcriptome and immune profiling, unique aspects of supercentenarian immune systems were revealed. A reduction in the number of B cells was confirmed. Cytotoxic T cells were found to increase, and the unusual transcriptional profile of cytotoxic T cells in supercentenarians identified them as CD4⁺. Pseudotime analysis suggested a common differentiation pathway for CD4⁺ cytotoxic T cells as CD8⁺ cytotoxic T cells. Single Cell Immune Profiling showed massive clonal expansion of CD4⁺ cytotoxic T cells, with a single clonotype sometimes accounting for 35% of the cells.

References

1. K Hashimoto, T Kouno, T Ikawa et al., Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians. *PNAS*. 116, 24242–24251 (2019).

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