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Ageing and frailty immune landscape

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Frailty is an important age-related prognostic for mortality, with little known about the immune landscape. Using single cell profiling, *Luo et al.* gather a comprehensive understanding of the immune changes that happen from birth to old age[1]. Therefore, providing new insights into the often overlooked state of frailty.

Time marches inexorably on bringing with it a plethora of changes to our bodies. No tissues or bodily functions are exempt from ageing and the immune system is no exception. Immune ageing, sometimes referred to as immunosenescence, has been the subject of study for decades[2–4]. However, not all chronological ageing brings with it significant malaise or co-morbidity, but some people do go on to develop frailty. Frailty is characterised by multiple symptoms including a reduction in physical strength, increased vulnerability to disease, and overall reduced resilience[5,6]. [7] To better understand the immune phenotype associated with healthy aging or frailty, *Luo et al.* collected blood samples from the umbilical cord of new-borns, young adults, and healthy and frail elderly and perform single-cell RNA-Seq analysis as well as T-cell receptor (TCR) V(D)J-Seq and TotalSeq-C, thus combining single-cell transcriptomic analysis with TCR repertoire and cell-surface protein expression data.

These data will prove invaluable for other ageing researchers looking to mine deposited datasets to address some of their own questions. In addition, the authors use their novel data to highlight how ageing at the systemic level correlates with cellular ageing in blood. The researchers showcase phenotypic divergence of immune cell populations during healthy and frailty. They confirm previous findings of a decrease in naïve CD4 and CD8 T cell numbers and TCR repertoires[8], but pseudotime analysis of naïve CD4 cells showed a clear divergence between old and frail individuals. These frail naïve CD4 T cells exhibited a number of differentially expressed genes compared to those from the old cohort, but more work will be

required to understand these changes more clearly. Not only focusing on T cells, the authors also discovered differential cell trajectories for natural killer (NK) cell subsets. Of the three separate subsets of NK cells identified, the authors determined that the two most prominent of these subsets showed distinct immune ageing trajectories.

The authors go on to show that, as has been known, older and frail people exhibit expanded memory T cell clonotypes and thus an overall contraction of the TCR repertoire. As might be expected, the effector memory (T_{EM}) repertoire is severely lacking in cord blood, but relatively stable from young age onwards. Interestingly, they observed that while the naïve CD8 T cell compartment showed age-dependent TCR restriction, naïve CD4 T cells were relatively stable in healthy older people. However, the frail cohort showed a decrease of TCR landscape. Thus, one might be able to use CD4 T cell TCR repertoire as a biomarker for frailty, though more work will be needed to verify this. Indeed, the search for valid biomarkers and diagnostic factors for frailty are still somewhat elusive, so this approach might be relevant clinically. Similarly, the expansion of CMV-specific CD8 T cell clones in persons with frailty could prove another useful marker in the diagnosis and prognosis of frailty. Though still disputed, and caveated by the fact that CMV-specific TCRs have appreciable cross-reactivity to other epitopes[11], CMV^+ serology has previously been linked to frailty and overall decline in function with age. This study by *Luo et al.* provides further indication of a link between CMV and frailty, but more work needs to be done to firm up this link[9,10].Expanding on the idea of CMV-specific TCR cross-reactivity, the authors also show substantial overlap in TCR clonotypes between T_{EM}, CD4 $T_{CM,}$ Natural Killer T (NKT) and CD8 mucosal-associated invariant T (MAIT) cells, suggesting a drive towards broad-spectrum T cell function in the ageing immune system. This is in keeping with ideas that have been mooted by others whereby the immune system adapts to cope with the changes occurring in the body as a whole with age, such as increased tissue senescence and cancer[12,13].

In addition to using the CD4 TCR repertoire as a potential biomarker for frailty, the authors suggest a use for some of the more specific gene signatures they uncovered in better understanding the mechanisms that underlie the development of frailty. For example, increased expression of transcription factors such as DUSP5, NFKB1, and NR4A3 were universal in multiple cell types in persons with frailty. More specifically, chemokine ligand genes such as CCL3 and CCL4 were specifically elevated in frail NK and myeloid cells, suggesting a functional role of these chemokines in the progression or development of frailty.

Lastly, the authors observed specific upregulation of NEAT1 and NEAT2 (also known as MALAT1), two long non-coding RNAs implicated in multiple cancers and positively correlated with poorer survival[14]. The authors discovered increases in NEAT2 in naïve CD4 T cells in

persons with frailty, but perhaps more strikingly saw that the increase of NEAT1 and NEAT2 went hand in hand with a peculiar novel monocyte subset unique to the cohort of persons with frailty. This subset exhibited an overall increase in non-specific inflammatory responses, but decreased exocytosis, myeloid-specific activation, antigen presentation, and apoptosis. The suggestion that these monocytes are inherently inflammatory cells is of interest with regards to frailty, given previous suggestions that inflammation is a prognostic for frailty[15]. However, as of yet the ontogeny of these monocytes is unknown.

While certainly pointing out novel cellular subsets and specific markers of interest, more work will be required to understand the role of NEAT1 and NEAT2 in the context of immunity. Indeed, the appearance of a new subset of monocytes in older people with frailty is intriguing and begs the question how it arises. Is there a particular inflammatory environment that drives this effect? Is there a link to HIV or CMV status, thus linking these monocytes to particular T cell clonotypes? Would targeting these cells alleviate the symptoms of frailty? Indeed, many interesting questions arise as a result of the work of *Luo et al*.

In summary, in this issue of Nature Aging, *Luo et al.* uncover global immune changes that characterise frailty thereby providing a framework that will help classify human biological ageing at a cellular level and identify healthy compared to unhealthy ageing. The combination of multiple omics tools such as RNA-Seq, TotalSeq, and TCR V(D)J-Seq used by the researchers is an immensely powerful one and enables us to explore not just immunity at different stages of life, but also of health by intersecting their data with clinical information on a population of older people with frailty.

Conflict of interest

The authors declare no competing interests.

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