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The Hayflick Limit and Maladaptive T cell Aging

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At the turn of the 19th century, the field of biology that studies why and how we age -- called 'Biogerontology' -- was considered a "Black Art"(1). From the development of cell culturing techniques in the early 1900's, until the 1960's, it was believed that cells can be immortalized *in vitro*, provided they are surrounded by optimal conditions (2). Due to this central dogma, biogerontology research was focused on extracellular determinants, rather than intracellular events. However; as time passed, the original experiments on which this model was based could not be reproduced.

In 1961, Leonard Hayflick and Paul Moorhead overthrew this dogma by discovering that cultured human cells have a finite number of replications, beyond which cells enter a state of cell-cycle arrest known as 'senescence'(3). The experiment involved mixing male fibroblasts in their fortieth cell-cycle with female fibroblasts in their tenth cell-cycle, with unmixed cell populations as controls (3). At the time when the male control group of cells ceased to divide, the mixed group was studied and only female fibroblasts were observed in the once-mixed culture. This revealed that the older male cells stored a memory of their number of cell divisions, despite the extracellular factors of the surrounding young female cells, and favourable environmental conditions. This work suggested that cells have a cell-division 'counting' mechanism, which is not directly related to time but rather to rounds of replication, hence the name 'replicometer' (1).

The molecular basis of the replicometer is linked to the age-related attrition of protective caps on the ends of chromosomes known as 'telomeres'. Telomeres are non-coding repetitive DNA sequences that prevent the unravelling of protein-coding genes and end-to-end fusion of chromosomes during cell division. Due to an imperfect system of DNA replication, the base pairs at the ends of telomeres cannot fully replicate. Consequently, telomeres shorten with every round of replication, a phenomenon known as 'the end replication problem' (4). When the telomere length (TL) drops below a critical point, the cell ceases to divide, and thus has reached its 'Hayflick limit' (5).

One would assume that rebuilding telomeres would be the key to cellular longevity, by preventing cells from reaching their Hayflick limit. In 1978, Elizabeth Blackburn discovered an enzyme that is capable of just that, which she named telomerase (6). Telomerase is expressed in some classes of cells that have a high turnover rate, including foetal tissue, bone marrow stem cells, testes, peripheral blood lymphocytes, skin epidermis, and intestinal cells (7). However promising this discovery was, telomerase was later found to be associated with uncontrolled cell growth, with about 90% of all human cancers showing telomerase activity (8).

Adaptive immune system most affected by aging

There is still much to be learned about the cellular basis of aging. There is an already booming field observing the correlation of TL shortening with disease states, but this field focuses on a broad group of immune cells not specific to the adaptive immune system (AIS), known as leukocytes. Short mean leukocyte TL has been associated with a broad set of age-related diseases such as diabetes (9,10), heart disease (11,12), cancer (13) and other diseases of old-age (14). The AIS is the part of the immune system that is responsible for storing a memory of pathogens, in order to mount a more robust response upon re-exposure, and is the part most affected by aging. Although there is a robust link between shorter mean leukocyte TL and age-related diseases, this metric is a highly simplified representation of aging in the AIS, which is a highly complex and dynamic system.

T lymphocytes (T cells) form part of the AIS, and are exposed to abundant encounters with pathogens and other substances, the combination of which is unique to each individual. The human T cell repertoire can be broadly divided into antigen-inexperienced or 'naïve' cells and antigen-experienced 'memory' cells.

The naïve T cell pool size and composition requires maintenance to sustain a diverse repertoire. This is achieved through continuous, low-rate cellular proliferation. As we age, the ratio of the numbers of naïve versus memory T cells decreases, due to the diminishing output of naïve T cells by the thymus (the site of naïve T cell maturation) and the differentiation of naïve T cells into memory cells (15).

In healthy aging, the mechanism of cell generation is utilized successfully, yet subsets of T cells pay the price of imposing replicative stress, pushing these cells toward their Hayflick limit. As the AIS immune system ages, it commits considerable resources to negative regulatory pathways to control clonal expansion in acute T cell responses and to prevent memory inflation resulting from chronic stimulation. If an individual is exposed to a chronic, latent, or repeated infection, this will require adaptation from the immune system. Maladaptive T cell aging in response to proliferative pressure and chronic stimulation leads to inflammatory effector functions and sustains chronic, tissue-injurious inflammation. This inflammation involves the secretion of harmful proteins known as cytokines.

Measuring immunological cost

The existence of a Hayflick limit implies that infections have a long-term immunological cost (IC) to the individual as they drive immune cells towards their Hayflick limit. Eventually, the majority of these cells are unable to respond to other infections, while the remaining cells secrete cytokines that can be injurious to the individual. One could measure the TLs of the various T cell subsets and make inferences about their replicative capacity. In combination with other markers, such measurements have the potential to yield an estimate of the residual capacity of the AIS to respond to pathogens.

In high-functioning older persons, a measure of inflammation can identify those at a much higher risk of mortality and a possibly higher risk of functional decline (16). However, current measurements yield only a mean estimate for TL for a T cell population, and do not indicate the proportion of cells from the various T cell subsets that have a short TL. More refined measurements are needed to enable the development of robust metrics of IC, that will have much better correlations with susceptibility to disease. In particular, accurate measurements of IC need to combine high-resolution TL measurements with measurements of inflammation such as levels of circulating cytokines IL-6, IL-1b, and TNF-α production (16).

The last century has been a prolific time for the advancement of the field of biogerontology. Age-related cellular senescence influences mortality by causing dysfunction of the immune system. The AIS is made up of a complex repertoire of cells that needs to be maintained in size and composition with age. Infections can have a long lasting effect on the T cell repertoire as chronic stimulation of T cells will lead them faster towards their Hayflick limit, and this effect can be summarized into IC. IC is challenging to quantify, but should involve a high-resolution measurement of TL of different T cell compartments, combined with a measurement of the circulating cytokine milieu. Nevertheless, the development of an accurate IC measurement will lead to improved prediction of the age-related decline of the immune system, and would constitute a very useful adjunct to emerging, personalised approaches to healthcare.

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