Editorial

Elevated metallothionein expression in long-lived species

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Frailty syndrome is a stronger indicator for biological ageing and survival than chronological age. Symptoms include loss of reserves (energy, physical ability, cognition, health), and increased vulnerability to endogenous or exogenous stressors, culminating in a higher risk of negative health outcomes. Less efficient repair mechanisms, insufficient removal of abnormal and degenerated cells, reduced detoxification and deficient immune responses trigger the accumulation of insults. This finally drives "inflammaging", a state of chronic low-grade immune activation manifested in ageing adults. Inflammaging is a major driving force of frailty, as it contributes to the development of age-related, chronic and degenerative diseases, increased susceptibility to infections and diminished response to vaccines. Therefore, it is of utmost relevance to define biomarkers that may indicate the onset and progression of such processes at early stages. A major challenge is to distinguish accelerated or frailty-related immune activation from immunosenescence processes that intrinsically accompany healthy aging.

Defining abundance, activity and/or differentiation of lymphocyte populations may uncover underlying immunological conditions. Ageing-associated lymphocyte changes include decreased production of T and B cells, diminished export of naive cells to the periphery and relative increases of various memory T cells [1]. Reduced total blood lymphocyte levels or percentages of several subsets were associated with frailty and its severity in different populations of older adults. A cross-sectional study of Spanish older adults reported a significant increase in the CD4$^+$/CD8$^+$ ratio and a decrease in the percentage of CD19$^+$ cells in frail individuals compared to non-frail controls, however only inflammatory plasma markers such as IL-6 and sTNF-RII but not lymphocyte populations progressively increased with frailty severity [2]. Moreover, reduced cytokine production is associated with aging, often correlating with poor prognosis in case of bacterial infection and sepsis.

Metabolites are important systemic mediators of immune homeostasis. They shape the interaction of immune cells with the microenvironment as well as a variety of other processes in parallel. One key example is the accelerated immune-mediated catabolism of the essential amino acid tryptophan along the kynurenine axis by indoleamine 2,3-dioxygenase 1 (IDO-1). The kynurenine to tryptophan ratio in plasma acts as a proxy for IDO-1 activity, if concentrations of inflammatory markers such as neopterin or pro-inflammatory cytokines rise simultaneously. Depletion of tryptophan reduces the proliferation of invading pathogens and under chronic conditions of immune cells, as tryptophan is also the least abundant amino acid in proteins. Persistent reduction of tryptophan is a tolerogenic immunosuppressive mechanism. Kynurenine downstream products may impinge on immune processes, e.g. by selectively inducing apoptosis in lymphocytes. Some are ligands of the environment-sensing aryl hydrocarbon receptor, which is also involved in coordinating activation, differentiation and proliferation processes of immune cells, and hematopoiesis. Moreover, several kynurenine catabolites are neuroactive. Tryptophan is a precursor of the neurotransmitter serotonin, and thus feeds the melatonin pool as well. There is also a regulatory role of tryptophan on protein synthesis in neurodegenerative protein-aggregation. Disturbed tryptophan metabolism is associated with a variety of consequences depending on other underlying conditions, ranging from neuropsychiatric and cognitive disturbances to tumor-associated cachexia and anemia. In addition, the impact of microbial derived-tryptophan and derivatives on the gut-brain-immune axis is of relevance. Furthermore, kynurenine affects skeletal muscle functioning which links tryptophan metabolism to age-related physical impairments.

Certainly, immune-mediated accelerated tryptophan metabolism is part of a larger crosstalk and consequences that are not isolated from other metabolic and redox changes that occur during inflammation. The cytokine interferon (IFN)-γ plays a major role, and some associated pathways are highly susceptible to modulation by lifestyle factors [3, 4].

Human genetic data and various animal models suggest that balanced metabolism of tryptophan may support longer and/or disease-free survival. Several factors along the IDO-1 activation cascade may participate, including polymorphisms of the IFN-γ gene. Increased kynurenine to tryptophan ratios and levels of neopterin, an associated IFN-γ induced marker of cellular immune activation, were predictors of coronary events in the Hordaland prospective health study. Low serum tryptophan in coronary artery disease patients was

associated with reduced life expectancy in the Ludwigshafen Risk and Cardiovascular Health study.

Increased tryptophan breakdown and kynurenine levels were observed in frail older adults [5, 6]. These data, and our recent study reporting the association with oxidative DNA damage and a trend to decreased concentrations of vitamin E, highlight the importance of amino acid metabolism and underline that frailty development is linked to a degree of immune activation and oxidative stress beyond what could be expected from biological age alone. Hence, tryptophan and/or its degradation products seem to be suitable candidates for frailty biomarkers [7].

A decrease of serum/plasma total tryptophan and increases of both the kynurenine to tryptophan ratio (and neopterin) were observed also during healthy aging [3, 8]; however sex-related differences and the nutritional status of study participant also play a role. The ratio of free to total tryptophan may be relevant for transport and further metabolic routes, too [3]. A larger systematic analysis of the parameters across lifespan would be needed to gain further information of age-grouped normal concentration ranges in the healthy population.

Altogether, monitoring of tryptophan metabolism and related immunobiochemical pathways provides information on IFN-γ dependent immune activation and can support the early detection of dysregulated immu-nologic, neuropsychiatric and physiological processes. Robust and cost-efficient analytical methods for the determination of the most important metabolites are available. This may promote personalized strategies to predict and counteract inflammaging, in an early stage and to accompany and to evaluate treatment strategies. Metallothioneins (MTs) are a family of small (6-7 kDa) cysteine-rich metal-binding proteins that have been extensively studied for their function in homeostatic regulation of zinc (Zn) and copper (Cu) as well as for their role in heavy metal detoxification.

Further studies have contributed to move the attention around the function of MTs from toxicology to biogerontology [1]. Indeed, MTs have been shown to protect cells against oxidative, UV and even viral [2] forms of damage. The gene expression of MTs increases in multiple tissues in response to aging and to anti-aging interventions including caloric restriction (CR) and inhibition of the insulin/insulin-like signaling (IIS) pathway [1].

MTs display all the features of strong candidate longevity genes. Overexpression of MTs was found to lengthen mouse lifespan [3] while MT-knockout shortens lifespan and promotes cancer [4]. The lifespan extension mediated by overexpression of MTs has been further observed even in a mouse model of amyotrophic lateral sclerosis (Tokuda E, et al. Mol Genet 2014) and after cardiac-specific overexpression of the human MT2 isoform in mice [5].

The potential mechanisms underlying these beneficial effects of MTs are still not completely understood. Protection from multiple stressors by MTs is in line with modern stress resistance and damage-based theories of aging. For example, we know that fibroblasts derived from long-lived species resist cell death induced by Cd, paraquat or other stressors and that long-lived mice show higher expression of cytoprotective genes regulated by the Nrf2 transcription factor. MTs are among the genes induced by Nrf2, placing them as effectors of this central geroprotective transcriptional network. Taking into account that loss of metallostasis and accumulation of cadmium (Cd) in multiple tissues are common features of aged organisms, it could be also argued that MTs protect from age-related Cd toxicity and metal dyshomeostasis.

In a recent paper we further strengthened the role of MTs in aging addressing the interaction among MTs, Cd and longevity [6]. We found that long-lived species accumulated Cd faster than expected by chronologic age, implying an additional factor was at play. An extensive meta-analysis and assembly of novel datasets provided evidence that long-lived mammals express more MTs at the protein and mRNA level than short-lived ones, which may explain, at least in part, the "paradoxical" faster accumulation of Cd. To further support this hypothesis, we measured Cd in several long-lived mouse models with elevated MT expression. Most of them were found to have elevated hepatic and renal Cd content with the strongest results in MT-transgenic mice. These results suggest that long-lived species have evolved a more efficient induction of MTs in response to damage, which in turn contributes to faster accumulation of tissue Cd levels. Since Cd is tightly bound to MTs, the toxicity of the metal is suppressed and its longevity costs are likely offset by the beneficial effects of MTs. It could be additionally argued that the protective effect of MTs involves also Cu, which is an essential trace element known to accumulate with aging and able to induce premature senescence in human fibroblasts (Matos L, et al. Age (Dordr) 2012).

In spite of this considerable knowledge on the role played by MTs in longevity, modulation of their expression and testing in aged non-transgenic animals is still elusive, and this is a critical step toward utilizing these mechanisms for the treatment of age-related conditions in humans.

In worm models of neurodegenerative disorders indirect induction of MTs by Zn, progesterone, quercetin, dexamethasone and apomorphine reduced the burden

associated with Amyloid β and α-synuclein while knockdown of MTs resulted in a partial loss of bioactivity of these compounds [7]. However, these and most other known MT-inducing compounds are also non-specific inducers of Nrf2 expression or are known to interfere with many other pathways thus complicating their therapeutic translation based on the specific target. By contrast, direct induction of MTs by gene therapy has been limited only to chemically-induced liver fibrosis mouse models with interesting positive results (Jiang Y and Kang YJ, Mol Ther 2004). Indeed, the therapy reversed the fibrosis along with increased hepatocyte regeneration in few days.

This technology, or alternative safe gene-delivery systems, to overexpress MTs "in vivo" clearly poses some challenges. Given the complexity of the aging process, their effectiveness may produce unsatisfactory results or unexpected side-effects. However, methods and protocols are now finally available to implement studies in geriatric mice or other preclinical models of aging. It has been recently shown that adeno-associated virus (AAV)-based therapies employing longevity associated genes are safe and can been successfully applied in the simultaneous treatment of several age-related diseases [8].

Previous studies on MTs have seen a shift from toxicology to biogerontology, we expect that the next step will be a shift from biogerontology to geroscience, which aims to directly extend organismal healthspan and ameliorate the burden of age-related diseases.

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