Immune Cells in Oxi-Inflamm-Aging

Subjects: Immunology

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Since immune cells need to produce oxidant and inflammatory compounds to carry out their defensive function, when uncontrolled, they may be responsible for the generation of oxidative-inflammatory stress that would not only cause their functional deterioration (immunosenescence) but could also increase these stresses in the body, accelerating the aging process. Given that phagocytes (neutrophils in humans and macrophages in mice) are the main immune cell type that generates oxidants throughout the “respiratory burst” in which NADPH oxidase and xanthine oxidase enzymes participate, they were proposed to play a central role in oxi-inflamm-aging.

aging immune cells oxidative stress inflammatory stress biological age

1. Introduction

The aging process can have multiple definitions depending on the perspective from which it is considered. From a biological point of view, the aging process may be defined as the progressive and general deterioration of the functions of the organism that leads to a lower ability to react to changes and preserve homeostasis adaptively [1]. Homeostasis includes all processes that organisms use to actively maintain or adjust to appropriate conditions necessary for survival. Thus, although aging should not be considered a disease (it would be absurd to think of an illness that affects 100% of people), it is the main risk factor for the occurrence of chronic age-related diseases [2]. There are three physiological systems, the nervous, endocrine, and immune systems, in charge of maintaining body homeostasis. Moreover, these systems are in continuous communication, constituting a neuroimmunoendocrine system, which allows the preservation of homeostasis and, therefore, of health [3]. With aging, there is a functional decline of these homeostatic systems and an impairment in the communication between them [2][4], which translates into a worse capacity to mount an adequate response to a variety of stressors. The decay of this capacity, which has also been referred to as decreased homeodynamic space [5][6] or decreased homeostatic resilience [1], is what results in higher morbidity and mortality. Nevertheless, the age-related changes in these homeostatic systems are established at different rates in each subject, which translates into a different rate of aging or biological age of individuals with identical chronological age [2][4].

2. Following the Free Radical and Mitochondrial Theory of Aging

Many theories were proposed to explain how the process of aging occurs. Among them, the free radical theory of aging proposed by Harman [7] and further developed by several authors [8][9][10][11] is probably the most widely accepted one. This epigenetic theory proposes that aging is the consequence of damage accumulation by

deleterious oxidation of biomolecules caused by the high reactivity of the free radicals and reactive oxygen species (ROS) produced in our cells because of the necessary use of oxygen. Oxygen (O 2) is essential for the synthesis of adenosine triphosphate (ATP) in the mitochondrial respiratory chain, which is believed to be the primary site of ROS production, acting as the final acceptor of four electrons, generating one molecule of water. However, when the reduction in oxygen is not full, reactive oxygen species are generated. Thus, when oxygen captures one electron, the superoxide anion (O 2−) is formed, which can lead to hydrogen peroxide (H 2O 2) and hydroxyl radical (OH −). O 2− and OH − are free radicals given that they have an unpaired electron, which makes them highly reactive towards all biomolecules, whereas H 2O 2 is not. Even though H 2O 2 is not a free radical, it can result in OH − being considered as an important oxidant. Hence, the term reactive oxygen species (ROS) is generally used to include them all. These ROS act as second messengers and coordinate several molecular pathways within the cells [12]. Nevertheless, they have to be quickly neutralized by antioxidant defenses to avoid the generation of oxidative damage to the different cellular components. Thus, all aerobic organisms have developed antioxidant defenses, both enzymatic and non-enzymatic, to keep these ROS between appropriate ranges. However, with aging, there is an imbalance between oxidant and antioxidant compounds in favor of the former due to uncontrolled production of oxidants and/or due to a decrease in antioxidant defenses, which generates what is known as an oxidative stress situation. The establishment of oxidative stress exposes cells to a pro-oxidant environment that entails the accumulation of damage of the different biomolecules (proteins, lipids and, nucleic acids), loss of function, and cell death.

The free radical theory of aging has been criticized by several authors, doubting its usefulness to explain how the aging process occurs, indicating that oxidative damage does not represent the cause of aging [13][14]. For example, Gladyshev [13] concludes that the role of ROS in aging is not universal, with the idea that aging still occurs under anaerobic conditions in yeast cells. However, it is not correct to apply the idea of aging to unicellular organisms since aging should be understood in the physiological context of pluricellular animals with sexual reproduction. Some other concerns that were put forward against the role of ROS in aging were based on some works in which increased oxidative stress has led to increased longevity [15][16]. These results, however, far from dismantling the free radical theory of aging, can be explained due to a hormetic response. Thus, a short-term increase in ROS production can cause an adaptive response by increasing antioxidant expression [17][18][19], whereas chronic ROS levels beyond a certain threshold are still damaging for cellular components. Other claims against the free radical theory of aging are some studies in which the use of antioxidants did not increase longevity in mammals, as some authors stated [14]. Nevertheless, confusion between maximum and mean longevity is one of the reasons for this criticism. Actually, species with higher longevity have fewer antioxidants because they do not need them since they produce a lower amount of ROS [20]. In addition, there are some other examples in which ingestion of a diet enriched in antioxidants increases longevity, as will be discussed in the last section of this review. Another argument against the theory is the fact that overexpression of antioxidant enzymes does not extend the lifespan of invertebrates (Drosophila melanogaster) and mammals (Mus musculus) [21][22]. However, caution should be taken when interpreting the causes of aging by the use of genetically manipulated animals, as they can develop other adaptive mechanisms to counteract a specific mutation. Interestingly, a 20% increase in lifespan was observed when upregulation of catalase expression was targeted to the mitochondria specifically [23].

3. Oxidation and Inflammation, Always Together. Oxi-Inflamm-Aging

Even though inflamm-aging is not included as one of the nine hallmarks of aging, possible because these hallmarks were focused on markers within the cells, there is a universal agreement that aging is accompanied by the establishment of a low-grade chronic inflammation at the systemic level [24]. This chronic inflammatory stress is established when there is an imbalance between pro-inflammatory compounds and anti-inflammatory compounds in favor of the former. It is known that immune cells need to produce pro-inflammatory mediators to carry out their defensive functions. Thus, inflammation is not a negative phenomenon per se since it is needed to maintain life through a constant struggle to preserve the integrity of the individuals [24][25]. However, this response has to be tightly regulated and finished shortly after the resolution of the noxious agent, which is mainly achieved by the triggering of an anti-inflammatory response by immune cells. Nevertheless, as we age, this transient inflammatory process becomes chronic [24][25]. It was suggested that this could happen due to the persistence of the antigenic challenge or weakening of the regulatory systems of the immune response [26]. We propose that this chronic inflammatory stress can be the result of the establishment of chronic oxidative stress by the immune system's activity. Currently, it is clear that oxidation and inflammation are linked processes since excessive or uncontrolled free radical production can induce an inflammatory response, and free radicals are inflammatory effectors [25]. Indeed, both oxidation and inflammation occur when the immune system responds to the invasion of pathogens. This chronic inflammation is characterized by mononuclear immune cell infiltration (monocytes, macrophages, and lymphocytes) to different tissues where these cells produce excessive ROS and pro-inflammatory mediators to conclude this situation but, at the same time, generate tissue damage and fibrosis. Therefore, a continued and active oxidant response by immune cells can lead to cellular damage due to ROS overproduction, which can also recruit other inflammatory cells leading to additional pro-inflammatory and oxidant production amplifying cellular damage [27][28]. Different pathways were proposed to mediate the connection between both inflammation and oxidation (reviewed in [4][25]). Continuing with the idea that mitROS production is the first event in the aging process, it was demonstrated that mtDNA fragments generated due to continued ROS leakage in the mitochondria over time act as danger or damage-associated molecular patterns (DAMPs) that can bind to pattern recognition receptors (PRR) and through activation of the nuclear transcription factor kappa b (NF-κB) could activate the expression of pro-inflammatory cytokines, boosting inflamm-aging [29][30]. Moreover, it is also known that mitROS can activate NACHT, LRR, and PYD domains containing protein 3 (NLRP3) inflammasome, which leads to the processing and secretion of the pro-inflammatory cytokines interleukin-1 and 18 [31][32][33][34].

Based on this link between oxidation and inflammation, the oxidative-inflammatory theory of aging emerged [2] to provide a more complete and integrative vision of the most relevant processes involved in the aging process. Thus, aging would be the consequence of chronic oxidative stress, associated with inflammatory stress, which would cause the deterioration of the function of all cells of the individual, but would have a greater impact on those of the homeostatic systems, that is, the nervous, endocrine, and immune systems, which would explain the lower ability to maintain homeostasis that occurs with aging and leads to increased morbidity and mortality. Furthermore, this theory introduced the involvement of the immune system in the greater or lesser oxidation and inflammation that appears as we age. Since immune cells need to produce oxidant and inflammatory compounds to carry out their

defensive function, when uncontrolled, they may be responsible for the generation of oxidative-inflammatory stress that would not only cause their functional deterioration (immunosenescence) but could also increase these stresses in the body, accelerating the aging process. Given that phagocytes (neutrophils in humans and macrophages in mice) are the main immune cell type that generates oxidants throughout the “respiratory burst” in which NADPH oxidase and xanthine oxidase enzymes participate, they were proposed to play a central role in oxi-inflamm-aging [2][35].

4. Can Immunosenescence Be a Marker of the Rate of Aging in Each Individual?

As was previously mentioned, the age-related decline that the homeostatic systems undergo does not take place at the same rate in a group of individuals of the same species and the exact chronological age. This fact led to the concept of biological age, which means the real rate of aging of an individual. However, several different parameters were proposed as biomarkers of biological age (telomere length [36], DNA methylation [37], plasma proteome profile [38], among others; we focus on those involving the function and redox state of the immune cells.

In order to be a marker of the rate of aging, the values of a given parameter have to be related to the lifespan of an individual. Thus, our research group focused on finding which of the age-related changes in the functions exerted by immune cells can be related to the longevity of mice. In this context, we first demonstrated that prematurely aging mice that have an inadequate response to stress show at the adult age immune function parameters and oxidative stress parameters closer to old animals, and they have a shorter lifespan [39][40]. Afterward, a battery of immune function and oxidative stress parameters were analyzed in adult mice and then left to naturally age until death, and the individual lifespan of each mouse was written down. Throughout multiple linear regression, we were able to develop mathematical models for lifespan prediction based on the values of immune function and oxidative stress parameters that mice showed at the adult age [41], demonstrating that both the function and oxidative stress of immune cells from mice at the adult age relate to their lifespan. With these results, and with the previous investigations showing that the age-related changes in the function and oxidative stress parameters follow similar patterns in mice and humans [39][40], we focused on developing a mathematical model throughout multiple linear regression for estimation of the biological age based on the immune cells’ function, which we called the ImmunolAge, in humans, with the Immunity Clock [42]. The Immunity Clock encompasses five immune function variables: neutrophil chemotaxis and phagocytosis abilities, lymphocyte chemotaxis and proliferation abilities, as well as cytotoxic natural killer activity. Based on the status of these immune functions in an individual, we can estimate their aging rate. Accordingly, we demonstrated that women with anxiety, as is further discussed in the next section, have a higher ImmunolAge than their chronological age, which means that they are aging at a faster rate. On the opposite side, we found out that centenarians exhibited a lower ImmunolAge than their chronological age, which confirms the idea of extremely long-lived people aging at a slower rate.

With respect to oxidative stress parameters, we established a Redox signature of Aging and Longevity throughout principal components analysis, by which in a 2D-graph we were able to differentiate age groups based on their antioxidant and oxidant markers [40]. While adult, mature and elderly were different groups, nonagenarians showed

overlapping areas with adult signatures, suggesting that a controlled redox balance underlies healthy aging. Centenarians, in this case, were characterized by the highest antioxidant capacities, which could indicate that at this age, they need this high antioxidant component to maintain appropriate redox balance or that only those that can express high antioxidant defenses are those that reach high longevity.

In this context, some other research groups also proposed other markers of immunosenescence to estimate the rate of aging. For example, Alpert and collaborators proposed an IMM-AGE score [43] based on different immune subsets frequency dynamics through aging, and it was found to predict mortality better than other aging clocks based on epigenetic parameters such as DNA methylation, i.e., Epigenetic Clock [37]. More recently, Sayed and colleagues proposed an inflammatory clock of aging (iAge) developed by machine learning based on soluble markers of chronic inflammation [44]. Altogether, our results and others demonstrate that a small battery of immune function and redox parameters of immune cells, as well as inflammatory markers, can be useful for the determination of the aging rate of an individual, that is, the quantification of their biological age.

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