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Research Paper

Role of macrophages in age-related oxidative stress and lipofuscin accumulation in mice

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ABSTRACT

The age-related changes in the immune functions (immunosenescence) may be mediated by an increase of oxidative stress and damage affecting leukocytes. Although the "oxidation-inflammation" theory of aging proposes that phagocytes are the main immune cells contributing to "oxi-inflamm-aging", this idea has not been corroborated. The aim of this work was to characterize the age-related changes in several parameters of oxidative stress and immune function, as well as in lipofuscin accumulation ("a hallmark of aging"), in both total peritoneal leukocyte population and isolated peritoneal macrophages. Adult, mature, old and long-lived mice (7, 13, 18 and 30 months of age, respectively) were used. The xanthine oxidase (XO) activity-expression, basal levels of superoxide anion and ROS, catalase activity, oxidized (GSSG) and reduced (GSH) glutathione content and lipofuscin levels, as well as both phagocytosis and digestion capacity were evaluated. The results showed an age-related increase of oxidative stress and lipofuscin accumulation in murine peritoneal leukocytes, but especially in macrophages. Macrophages from old mice showed lower antioxidant defenses (catalase activity and GSH levels), higher oxidizing compounds (XO activity/expression and superoxide, ROS and GSSG levels) and lipofuscin levels, together with an impaired macrophage functions, in comparison to adults. In contrast, long-lived mice showed in their peritoneal leukocytes, and especially in macrophages, a well-preserved redox state and maintenance of their immune functions, all which could account for their high longevity. Interestingly, macrophages showed higher XO activity and lipofuscin accumulation than lymphocytes in all the ages analyzed. Our results support that macrophages play a central role in the chronic oxidative stress associated with aging, and the fact that phagocytes are key cells contributing to immunosenescence and "oxi-inflamm-aging". Moreover, the determination of oxidative stress and immune function parameters, together with the lipofuscin quantification, in macrophages, can be used as useful markers of the rate of aging and longevity.

1. Introduction

Aging is associated with a progressive decline of physiological functions, which leads to aged-related pathologies and, ultimately, to death. The immune system is especially affected by this process, causing an increased susceptibility to infections and mortality, as well as a major incidence of immune diseases and cancer in the elderly [1–3]. The age-related impairment of the immune system, which is referred to as immunosenescence, involves remodeling changes in its organization and functionality that negatively impact the health of older adults [1,2,4]. Although the immune cells change their functional competence with aging, not all immune cell types or all functions of an immune cell show a significant impairment. In fact, a decrease in several lymphocyte functions (e.g. chemotaxis and proliferation) has been described, but an increase in other functions, especially those carried out by phagocytic cells (e.g. digestion capacity, ROS production), has also been observed [1–4]. In addition, it has been proposed that the basis of immunosenescence, is the same as that responsible for the senescence of other cells in the organism, namely the chronic oxidative stress, linked to the unavoidable use of oxygen to support cellular functions, and the consequent damage [2–4].

The immune cells continuously generate oxidants and inflammatory compounds, in order to carry out their defensive functions. The free radicals and the reactive oxygen or nitrogen species (ROS and RNS, respectively), which are produced by mitochondria, but especially in different metabolic processes, are necessary for the destruction of

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pathogens and tumor cells [1,2,4]. This fact occurs principally in phagocytes (e.g. macrophages, neutrophils, etc.), in which high amounts of ROS (mainly superoxide anion and hydrogen peroxide) are produced in order to carry out the microorganism destruction. This process involved the "respiratory burst" with participation of enzymes such as NADPH oxidase and xanthine oxidase (XO) [5,6]. However, although the ROS and RNS are beneficial to maintain both cellular signal transductions and functions, and therefore are necessary to maintain homeostasis, when they are up-regulated, their higher concentrations may contribute to increase the oxidative stress and the consequent damage in cells and tissues [2–4]. To avoid these harmful effects, in the immune cells and in the surrounding cells, leukocytes generally have higher concentrations of antioxidant enzymes (e.g. catalase, etc.) and non-enzymatic compounds (e.g. glutathione, etc.) than other cells [7,8]. Thus, in the immune cells more than in other cells, a perfect oxidant-antioxidant balance is necessary [4,9]. Furthermore, it is important to note that immune cells also produce inflammatory compounds for their defensive functions, however, if it is production is not well-controlled by anti-inflammatory compounds, this leads to an inflammatory stress situation. Because of both oxidation and inflammation are interlinked process and have many feedback loops [10], an excessive production of ROS and RNS by leukocytes could also induce an inflammatory response in these immune cells [11]. Thus, ROS can also activate the transcription factor NF-KB, which stimulates the expression of genes programming the production of inflammatory mediators in leukocytes (e.g. proinflammatory cytokines, chemokines, etc.) and these could also induce an oxidative stress situation [10,12]. If these pathways are not well regulated, a vicious circle of oxidation-inflammation could be established, which would increase the oxidative stress associated with aging, and consequently accelerate this process [3,4,10]. In this context, the "oxidative-inflammatory" theory of aging was proposed [2]. This theory suggested that the age-related oxidative-inflammatory stress affects all cells, but especially those of the regulatory systems, such as the nervous, endocrine and immune systems, resulting in an impairment of their functions, as well as in the communication between them. Moreover, this theory proposes a key involvement of the immune system in "oxi-inflamm-aging", and therefore in the rate of aging in the organism [2,3,12]. Thus, the age-related impairment of immune functions may be mediated by an increase of both oxidative stress and cellular accumulation damage affecting leukocytes. In this regard, previous studies of our group revealed that the higher oxidative stress state (e.g. increased intracellular superoxide anion, oxidized glutathione, XO activity, etc.) and oxidative damage (e.g. increased levels of malondialdehyde, 8-oxodeoxyguanosine, etc.) observed in leukocytes from old mice, as well as in peripheral blood immune cells from older humans, were coincident with the impaired immune responses (e.g. phagocytosis, chemotaxis, lymphoproliferation, etc.), whereas healthy human centenarians and long-lived mice, who show preserved immune functions, show a lower expression of proinflammatory genes and a well-controlled oxidative stress in their immune cells, which could explain their successful aging [2,12–14]. In this context, it is important to note that phagocytes, such as macrophages and neutrophils, have been proposed to be the main cells responsible for the chronic oxidative-inflammatory stress associated with immunosenescence [1,2]. Thus, as a result of the age-related oxidative injury, these cells could lose their capacity to regulate their own redox and inflammatory balance, producing more and more oxidant and inflammatory compounds, and therefore, contributing to the increased oxidative-inflammatory stress observed in other physiological systems with aging. However, although some studies conducted on peritoneal macrophages and peripheral blood neutrophils, from mice and humans, respectively, have showed that these cells generate higher levels of oxidant compounds than those produced by lymphocytes, and these levels significantly increase with age [1,2], this idea has been scarcely studied.

Oxidative stress leads to an elevated oxidation of macromolecules, such as DNA, lipids, and proteins. It was postulated that age-related accumulation of damaged, oxidized, and aggregated compounds might contribute to the aging process. In this regard, lipofuscin is considered one of the best "hallmark of aging", due to the fact that the amount of lipofuscin increases with age, but also, and more importantly because the rate of lipofuscin accumulation correlates negatively with longevity [15–17]. Lipofuscin and lipofuscin-like compounds, are formed by polymeric substances, primarily composed of cross-linked lipid and protein residues due to iron-catalyzed oxidative processes, and should be regarded as aggregates of undigested cell materials [17]. The ineluctable formation and accumulation of lipofuscin in, especially, postmitotic cells, seems to lead to a variety of defects in cellular function and homeostasis. The indigestible nature of this material is associated with progressive diminution of lysosomal function and alterations in both phagocytosis and autophagy processes, which has secondary effects on many different cellular activities [17]. Moreover, it has been described that higher accumulation of lipofuscin may promote generation of ROS, sensitizing cells to oxidative injury through lysosomal destabilization, making cells considerably more vulnerable to oxidative stress [17]. Although it has been observed that lymphocytes from aged mice showed abundant granules of lipofuscin [1] and it is known that lipofuscin may act as danger signals, stimulating the release of proinflammatory chemokines and cytokines, contributing to the activation of macrophages, and therefore, leading to chronic oxidative-inflammatory process [18], to our knowledge, there are no studies about the age-related variations of the lipofuscin accumulation in phagocytes, and even less in comparison to other immune cell types.

In view of all of the above, it seems evident that immune cells, and especially macrophages, can play an important role in the increase oxidative-inflammatory stress and damage associated with aging, which could also contribute to the impairment of the immune functions, and the increase of the rate of aging. Therefore, the aim of this study was to investigate the age-related changes in several parameters of oxidative stress and immune functions, as well as in the lipofuscin accumulation, in isolated peritoneal macrophages of mice, as well as in the total population of peritoneal leukocytes, containing mainly B and T lymphocytes, macrophages, and NK cells. For this purpose, the total population of peritoneal leukocytes, as well as both isolated macrophages and lymphocytes from ICR/CD1 female mice of different ages, that is, adult, mature, old and natural extreme long lived were used, in order to analyze a variety of oxidant compounds (XO activity and expression, intracellular superoxide anion and ROS levels and oxidized glutathione content) and antioxidant protectors (namely catalase activity and reduced glutathione levels), as well as their redox balance (XO/CAT and GSSG/GSH ratios). Furthermore, the degree of lipofuscin accumulation in both isolated macrophages and lymphocytes was measured.

2. Materials and methods

2.1. Animals and experimental conditions

For this study, ex-reproductive females of outbred ICR/CD-1 mice (*Mus musculus*) were used, purchased from Harlan Interfauna Ibérica (Barcelona, Spain) at the young adult age (28 ± 4 weeks of age). The mice were specifically pathogen free as tested by Harlan according to the Federation of European Laboratory Science Association recommendations. They were housed 6 ± 1 per cage, at a constant temperature (22 ± 2 °C), in sterile conditions inside an aseptic air negative-pressure environmental cabinet (Flufrance, Cachan, France) and on a 12/12 h reversed light/dark cycle. All animals had access to tap water and standard Sander Mus pellets (A04 diet from Panlab L.S. Barcelona, Spain) *ad libitum*. This diet was in accordance with the recommendations of the American Institute of Nutrition for laboratory animals. This cross-sectional study was performed simultaneously on mice of differ-