THE ROLE OF IMMUNOSENESCENCE IN THE DEVELOPMENT OF AGE-RELATED DISEASES

TAMAS FÜLÖP1*, GILLES DUPUIS2, JACEK M. WITKOWSKI3 AND ANIS LARBI4

1Research Center on Aging and 2Department of Biochemistry, Graduate Program in Immunology, Faculty of Medicine and Health Sciences, University of Sherbrooke, Sherbrooke, Quebec, Canada; 3Department of Pathophysiology, Medical University of Gdansk, Gdansk, Poland; 4Singapore Immunology Network (SIgN), Biopolis, Agency for Science Technology and Research (A*STAR), Singapore

ABSTRACT

Aging is a complex phenomenon leading to numerous changes in the physiological systems of the body. One of the most important changes, called immunosenescence, occurs in the immune system. Immunosenescence covers changes in the innate and the adaptive immune systems and is associated with a low-grade inflammation called inflammaging. Aging, likely via inflammaging, is also associated with the emergence of chronic diseases including cardiovascular and neurodegenerative diseases, cancer, and diabetes mellitus type 2. The origin of this inflammaging is not known with certainty, but several concurrent contributing factors have been suggested, such as aging-associated changes in the innate and adaptive immune response, chronic antigenic stimulation, the appearance of endogenous macromolecular changes, and the presence of senescent cells exhibiting a senescence-associated secretory phenotype. A better understanding of the multiple biological phenomena leading to these diseases via the immunosenescence associated with inflammaging provides a powerful target for interventions to increase the healthspan of elderly subjects. (REV INVES CLIN. 2016;68:84-91)

Key words: Inflammaging. Immunosenescence. Chronic diseases.

INTRODUCTION

Aging is a complex biological and physiological process1,2. The exact cause of aging is not known. However, we know that with aging, the incidence and prevalence of chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative diseases are increasing3,4. Indeed, the most important risk factor for the occurrence of these diseases is age. The relationship between aging and these age-related diseases is still actively being searched for. Recently, a new concept called “geroscience” was proposed to understand the putative role of aging in the appearance of age-related diseases and seeks to develop novel multi-disease preventative and therapeutic approaches5-7. The corollary of this concept is “healthspan” or a substantial extension in healthy life expectancy. This states that by decreasing/decelerating the rate...
of aging we can increase the time spent in health before the appearance of these age-associated diseases. In this review we will describe the age-related changes, especially in the immune system, which can lead to the development of these age-related chronic diseases.

AGE-RELATED CHANGES IN THE IMMUNE SYSTEM

Aging is associated with several changes in the physiology of many organs and systems underlined by molecular, cellular, tissue, and organismal changes. In a recent work, nine hallmarks of aging were described. Among these hallmarks, what emerged very recently as one of the most determinant is changes in the immune system. These changes in the immune response are presently called “immunosenesence”. This concept is not clearly defined and recent research questioned many paradigms linked to this concept. In spite of this debate, numerous experimental data support the changes in the immune system with aging, even in humans.

Immunosenescence

This concept suggests that the immune system as a whole is aging, not in one block, but with certain parts aging more than others. The immune response is composed by two distinct, but closely interrelated parts: the innate and the adaptive parts. It is overwhelmingly recognized that the immune changes occurring with aging affect the adaptive part. However, recently it was also recognized that there are substantial changes in the innate immune system.

The innate immune system is composed of several cells, including neutrophils, monocytes/macrophages, and dendritic cells. In spite of the fact that natural killer (NK) cells are part of the innate-like lymphocyte group, we will discuss their changes in this paragraph.

Neutrophils are the first to arrive at the site of aggression. Their lifespan is quite short as, if unstimulated, they die by apoptosis, but their lifespan can be increased by pro-inflammatory stimuli such as lipopolysaccharide (LPS) and granulocyte-macrophage colony stimulating factor (GM-CSF) including chemotaxis, intracellular killing, and respiratory burst resulting in free radical production. Interestingly, in most studies the phagocytosis and adherence did not show age-related alterations. It is of note that the changes in these effector functions can be, on the one hand, explained by the activation of these neutrophils already in the quiescent state manifested by the increased free-radical production, cytokine production, and metalloproteinase production, concomitantly to the sustained nuclear factor kappa B (NF-kB) activation, and on the other hand by the alteration of the signaling pathways. The most important signaling pathway is PI3K, which has been found to be altered in neutrophils with aging.

The monocyte/macrophage lineage also shows age-related changes, although interestingly this is much less studied in humans. However, the results indicate that most of the effector functions of these cells are decreased, including cytotoxicity, intracellular killing, and antigen presentation. The recently discovered toll-like receptor (TLR) functions are also altered in these cells. Some monocyte subpopulations, mainly with an inflammatory phenotype such as CD14⁺CD16⁺, are increased. This is manifested by the increase of pro-inflammatory cytokine production at the quiescent state, while they are decreased during stimulation. Similar changes were observed in plasmacytoid and myeloid dendritic cells, resulting in impaired antigen presentation and CD4⁺ T-cell activation.

The NK cells have important killing functions toward virus-infected and cancerous cells. Two distinct populations exist considering the CD56 cell surface markers. With aging, the CD56 bright subpopulation decreases while the CD56 dim subpopulation increases. On a single cell basis, the cytotoxic activity of NK cells decreases, but their greater number compensates for this decrease. Together these changes observed in the innate system indicate that with aging there is a basal activation state, which manifests itself by increased pro-inflammatory mediators concomitantly with decreased receptor signaling and effector function decrease. The question arises, why is that so? The origin of these changes is not well understood; however, a chronic low-level stimulation
by infectious agents, some related to gut microbiota, can be postulated. The barriers (especially in the gastrointestinal system) are more permeable, especially in the presence of low-grade inflammation, and many substances can be found in the circulation or in tissues even if at the origin these substances may be beneficial or neutral. Many other antigens are also chronically generated, such as cancer-related antigens, cellular debris, modified DNA, and oxidatively modified proteins, which interact with danger-associated molecular patterns (DAMP) to generate and sustain the basal activation of innate immune cells. This is the basis of the inflamming concept proposed by Franceschi, et al. The most marked changes with aging occur in the adaptive immune system; this is affected phenotypically as well as functionally. The phenotypic changes that were observed pertain to a decrease in the naive T-cell populations and an increase in the memory populations, especially in the CD8+ T-cell compartment. This decrease in the naive T-cells with age is mainly caused by the thymic function diminution, but is partly compensated by homeostatic proliferation at the periphery. The ultimate consequence of this decrease is loss of diversity of the TCR repertoire, especially in CD4+ naive cells and in CD8+ late-differentiated memory cells. This may result in both increased susceptibility to new infections and decreased response to vaccines as well as poorer memory for previously encountered pathogens.

In the meantime, the number of exhausted/terminally differentiated (the so-called senescent) CD28– T-cells increases, mainly in the CD8+ T-cell subpopulation. The cause of this shift is likely the chronic antigen stimulation originating either from micro-organismal sources or from internal altered tissue and molecular debris. The most accepted antigenic stimulation in this context is the cytomegalovirus (CMV), which has a tendency to reactivate when the immune surveillance decreases. These observations lead to the determination of the immune risk phenotype (IRP) following the Swedish Octo and Nona studies and were linked to higher mortality during the follow-up period.

It is of note that there are not only phenotypical changes, but also functional changes either at the level of the T-cell subpopulations or in individual T-cells with aging. There are profound changes in the CD4+ T-cell subpopulations in that the number of T-helper type 2 cells (Th2) and T regulatory cells (Treg) increases with aging. This leads to a further decrease in the adequate adaptive immune response towards new antigens, also altering the memory response. Besides these changes, there are intrinsic alterations in T-cells resulting in altered activation. The membranes of these cells become more viscous because of the increase in cholesterol content with aging. These alterations lead to changes in the signaling abilities of different surface receptors including TCR/CD3 complex, cytokine, and co-stimulatory receptors. Age-associated differences in signaling can be found at almost all stages of the intracellular pathways, but the most important are at the early stages, involving the Src tyrosine kinases (e.g. Lck) and the protein tyrosine phosphatases (e.g. Src homology region 2 domain-containing phosphatase-1, SHP-1). Our recent data suggest that modulating protein tyrosine phosphatases may increase T-cell responsiveness in the elderly, as was also described by other groups.

Together, these changes in the innate and adaptive immunity favor the development of chronic, low-grade (subclinical) inflammatory process (inflammaging) and decrease efficient responses to new infections, cancer, and endogenous tissue injuries, as well as compromising immunity to some previously encountered pathogens.

**Inflamming**

Inflamming as described above is a state associated with increased proinflammatory mediators, which develops gradually through continuous antigenic stimulation in aged subjects. This antigenic stimulation can be provided either by pathogens such as CMV and herpes simplex virus-1, or by cellular and molecular debris arising from transformations caused by reactive oxygen species (ROS), by the Maillard reaction (e.g. advanced glycation end products), by nitrosylation and cancer cells. These constantly generated antigens already stimulate at the resting state both innate and adaptive immunity, resulting in low-grade inflammation.

The most common chronic diseases associated with aging and representing an important threat for survival are related to a low-grade inflammatory process. Thus, aging is the most important risk factor for these chronic diseases because of immunosenescence associated with the inflamming.
important of these diseases are atherosclerosis, obesity, diabetes, and neurodegenerative diseases. In each of these diseases, the trigger DAMP interacting with pattern recognition receptors is different as, for atherosclerosis, the oxidized lipoproteins may be the initiator triggers, while in diabetes type 2 these can be the glycated end products, and in neurodegenerative processes these can be the viral products or misfolded proteins. Together, these facts underline the importance of immunosenescence-related inflammaging as a major factor to influence, via these deadly chronic diseases, the longevity of humans. These data further suggest that immune system alterations with aging will be important for determining longevity. Thus, the question arises whether the effects of the altered immune response with aging occur only via disease susceptibility, or if there is another independent mechanism by which altered immunity influences human aging.

In this context, we should also mention that more and more there is a complementary concept stating that the origin of the pro-inflammatory cytokines may be the senescent cells acquiring a senescent-associated secretory phenotype (SASP)\(^{57,58}\). These cells seem to accumulate during the aging process and secrete pro-inflammatory cytokines and concomitantly they cannot be eliminated by the immune system when they arise\(^{59}\). This cell senescence also in essence is a beneficial process to avoid the development of cancer, but can become dysregulated and contribute to the phenomenon of inflammaging. We have further drawn attention to the fact that pro-inflammatory cytokines alone cannot explain the inflammaging, as this is a very complex process with various interactions between the pro- and anti-inflammatory molecules and the innate immune system\(^{60}\). Increased interleukin (IL)-4 production and increased numbers of suppressor cells, such as \(T_{\text{reg}}\) or myeloid-derived suppressor cells, also exemplify this anti-inflammatory reactive phenomenon, which is most probably not efficient enough in the face of the sustained inflammatory processes with aging\(^{61-64}\). Alternatively, it can be too efficient to suppress specific responses, but not efficient enough against sustained, non-specific and multiple-origin, low-level, pro-inflammatory "storm".

Recently, the trained innate memory concept was described\(^{65}\). This signifies that innate immune cells may remember the insult far beyond the presence of the insult in the environment. Concomitantly, it became also apparent that an epigenetic memory exists, which can perhaps also maintain the inflammatory state without the original stimulus\(^{66,67}\). Finally, the long-lasting pro-inflammatory milieu could also contribute to maintenance of the supranormal immune reaction by self-maintaining it, creating a vicious cycle. It seems that this pro-inflammatory phenotype can at least be detected up to three months after the original stimulation, but presently there are no data for longer follow-up\(^{68}\).

Further studies on the molecular basis of this long-term memory demonstrated that the trained monocytes/macrophages were characterized by a global increase in epigenetic regulation by H3K4me3 on the promoter region of genes involved in immune signaling and metabolism\(^{69,70}\). More recently, the metabolic basis of the trained immunity was uncovered\(^{71}\). Thus, the various stimuli for trained immunity include microbes, nutrients, and other stimulating agents that are able to induce a metabolic shift from oxidative phosphorylation to aerobic glycolysis (the Warburg effect). This happens via the activation of mammalian target of rapamycin (mTOR) and its effector, hypoxia-inducible factor (HIF)-1\(\alpha\). So, the mTOR- and HIF-1\(\alpha\)-induced metabolic shift play a major role in epigenetic reprogramming of monocytes, leading to the trained innate immunity phenotype of macrophages\(^{72}\). Summarizing, we can hypothesize that the trained status of innate immune cells via epigenetic memory presents a persisting pro-inflammatory phenotype maintained by the age-related constant challenges, resulting in maintenance of the functioning of the immune system and also contributing to the appearance of various age-related, chronic inflammatory diseases.

**Neurodegenerative diseases**

Dementia is highly prevalent among the elderly and increases with age. The question naturally arising in this context is whether low-grade inflammation related to immunosenescence contributes to the development of Alzheimer’s disease (AD) by altering the adaptive immune response and favoring an inflammatory status, or whether these changes are only epiphenomena. Recent studies concluded that brain inflammation mediated by microglia is ongoing during the whole neurodegenerative process\(^{73-74}\). This has
different intensity in the prodromal mild cognitive impairment stage compared to clinically full-blown AD. Microglia are continuously secreting pro-inflammatory cytokines, while their capacity to phagocytose amyloid beta peptide (Aβ) is seriously compromised as the disease progresses76. The production of inflammatory cytokines originating from the innate immune response is stimulated by the increased production of aggregating Aβ73,77. These cytokines could have a dual role, since they can be protective by increasing the elimination of Aβ, or they can cooperate as co-stimuli during chronic Aβ stimulation, thereby enhancing a pro-apoptotic effect. Moreover, Aβ primes T-cells, eliciting the appearance of autoreactive T-cells, which can participate either directly or by the production of interferon-γ (IFN-γ) in the destruction of neurons and formation of plaques. It was recently shown that dramatic alterations in naive and memory subsets of CD4+ T-cells occur in patients with mild AD, with greatly decreased percentages of naive cells, elevated memory cells, and increased proportions of CD4+ as well as CD8+ cells lacking the important co-stimulatory receptor CD28. These data provide evidence for more highly differentiated CD4+ as well as CD8+ T-cells in AD patients, consistent with an adaptive immune system undergoing persistent antigenic challenge and possibly manifesting dysregulation as a result, and as such contributing to the pathogenesis of AD78. Together, the role of neuroinflammation in AD pathogenesis gains more and more support from experimental data supporting the systemic disease nature of AD and the vicious cycle existing between the periphery and the brain by the re-circulation of the inflammatory cells such as macrophages73. Thus, alterations of the immune system contributing to low-grade inflammation with aging might contribute to the development of AD, but the exact role played is still under intense scrutiny.

**Diabetes type 2**

Diabetes is one of the most common diseases of the elderly, with almost 10% suffering overtly and probably another 10% unrecognized. Diabetes type 2 (T2DM) alone or associated with metabolic syndrome is related to chronic inflammation52,54,79,80, which in turn is related to the pro-inflammatory activity of the adipose tissue, leading to some degree of insulin resistance and decreased insulin production by pancreatic Langerhans islet cells. It has also been shown that some inflammatory markers, such as F2 isoprostane in urine, IL-6, tumor necrosis factor (TNF), and C-reactive protein (CRP), are increased in T2DM53. It has been suggested that the increase of these parameters is associated with increased oxidative stress81. T2DM is often associated with complications at the levels of arteries, eyes, and kidneys82. These complications are further associated with an inflammatory process exaggerated by the presence of advanced glycation end products. Thus, diabetes is itself an inflammatory disease and, combined with other alterations and with immunosenescence, further alters the immune response.

**Atherosclerosis**

Atherosclerosis is a typical inflammatory disease and is the pathological basis of cardiovascular diseases, which are extremely frequent in the elderly83. This inflammatory disease may be initiated by certain auto-antigens, such as possibly Hsp65, modified low-density lipoproteins, or by infectious agents such as Chlamydia pneumoniae84. These agents stimulate cellular immune infiltration of the intima of the arterial wall. The most abundant infiltrating cells are CD4+ T-cells, which bear activation markers85. This leads to the secretion of IFN-γ, which in turn stimulates innate immune responses and creates a vicious cycle, leading to clinical events such as acute coronary syndrome with rupture of the plaque83. Moreover, a link between the IRP-related CD8+CD28− T-cell population and coronary artery disease was demonstrated independently of any CMV infection86. This latter finding further suggests that T-cell subset changes during immunosenescence may contribute to the development and progress of atherosclerosis. These cells are now considered as senescent T-cells with SASP58,59. Recent work concerning the mechanisms of atherosclerosis also found a persistent pro-inflammatory behavior in monocytes/macrophages with disease progression. In this disease it was found that the inflammatory nature of the peripheral monocytes might predict future cardiovascular events66. It is of note that atherosclerosis develops as a life-long process, which starts at a young age and only manifests itself clinically at more advanced age. Together, it seems clear that alterations of the immune system with aging contribute to the development of clinically manifested atherosclerosis such as coronary heart disease.
Chronic heart failure

Chronic heart failure (CHF) affects not only the cardiovascular system, but also neuroendocrine, renal, and immune systems; CHF is associated with a state of chronic inflammation. TNF, IL-6, and IL-1β in the myocardium and peripheral tissues have been shown to play an important role in the pathogenesis and progression of myocardial dysfunction. Indeed, plasma levels of these pro-inflammatory cytokines predict short- and long-term survival in patients with CHF. Furthermore, in hearts of patients with CHF, the accumulation of these TLR-4-regulated pro-inflammatory cytokines and expression of TLR-4 receptor itself have been reported to be increased. Recently, the expression of TLR-4 and TLR-2 was measured on monocytes from CHF patients. Monocyte TLR-4 expression was found to be increased in patients with CHF, and fluvastatin was able to inhibit the excessive innate immune response via inhibition of monocyte TLR signaling. Such increased TLR-4 expression could be a link to the increased proinflammatory cytokines found in CHF. Similar alterations were observed in the TLR system of leukocytes with aging.

Geroscience

As mentioned earlier, the most important risk factor for all these diseases is aging. Thus, diseases seriously limit not only life expectancy, but also the newly used concept, healthspan. This makes reference to the part of the lifespan that is spent in health and adequate functioning. The aim of geroscience is to understand how aging enables chronic diseases, and seeks to develop novel multi-disease preventive and therapeutic approaches. This approach is much larger than what was discussed earlier, as inflammation is only one of the seven pillars of aging, which includes macromolecular damage, epigenetics, adaptation to stress, proteostasis, stem cell and regeneration and metabolism. These putative causes emerged from animal studies and served as a base for intervention studies, which increased longevity and healthspan; however, the basic understanding of the aging process should be the target of intensive basic research. The challenge is to transfer all this knowledge experimentally and therapeutically to humans, aiming to slow the aging process to prolong human healthspan and consequently decrease the appearance of age-related chronic diseases. However, we can envisage that this will be the future for humans with the collaboration of multidisciplinary teams to prevent and avoid the disastrous effects of chronic diseases individually. Thus, targeting aging may allow early interventions and damage avoidance, maintaining a fully functional life and healthspan.

CONCLUSIONS

Aging is a very complex biological and physiological process. Aging results from genetic, epigenetic, and environmental events interacting throughout life. One corollary of aging is that it is the most important risk factor for age-related chronic diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, and diabetes. It was shown that the most common underlying physiopathological process of all these diseases is inflammation. It was also stated that aging is accompanied by changes in the immune system called immunosenescence, characterized by multiple alterations in the phenotypes and functions of the innate and adaptive immune cells. One important characteristic of this immunosenescence is a low-grade inflammation called inflammaging. There is overwhelming evidence that this inflammaging contributes, if not being the origin of, most age-related chronic diseases. The etiology of this inflammaging is still largely unknown, but certainly the dis-equilibrium between the relatively functioning innate and the more altered adaptive immune system is a contributing factor. The presence of the senescence-associated secretory phenotype is another contributor, and finally, the presence of endogenous damaged macromolecules and cells as well as the leakage of the gut microbiota also participate. Certainly inflammaging is not the only cause of these age-related chronic diseases, but it may represent a sort of common pathway. These chronic diseases shorten the lifespan and also the healthspan. Recently, new efforts of geroscience aimed to more deeply uncover the biological aging process as observed in animal models to apply to humans, and to find pathway targets for slowing down the aging process, leading to a more healthy and functional lifespan by delaying the emergence of these chronic age-related diseases. Among the interventions, those targeting the immunosenescence by thymic replacement, effective vaccine against CMV, targeted anti-inflammatory interventions, or those...
targeting senescent cells or the inflammasome have shown promise, but presently mainly in animal models. The way will be very long to get these interventions real in humans.

ACKNOWLEDGMENTS
This work was partly supported by grants from the Canadian Institutes of Health Research (CIHR) (No. 106634 and No. 106701), Canada Research Chairs (SRC) the Université de Sherbrooke, and the Research Center on Aging. Anis Larbi is funded by the Agency for Science Technology and Research. Jacek M. Witkowski is funded by a statutory grant from Polish Ministry of Science and Higher Education 02-0058/07/262.

REFERENCES
5. Seals DR, Justice JN, LaRocca TJ. Physiological gerosclerosis: targeting function to increase healthspan and achieve optimal longevity. J Physiol. 2015 [Epub ahead of print].


