Chapter 4
Immune Responses in the Elderly

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4.1 Immune Senescence and Aging

Aging is mostly associated with immune changes showing a decline and/or enhancement of many parameters when compared to young, healthy individuals, defined as immune senescence. Immune dysfunction in the elderly is characterized by increased susceptibility to infections and the decline in immune responses to vaccines [1, 2]. These changes occur mainly due to the failure of aged T cells to translate recognition of non-self-antigens (bacteria and viruses) with HLA and to induce T cell activation, clonal expansion, and differentiation into effector cells. In parallel with this scenario, aging is associated with increased subclinical pro-inflammatory responses, and inflame-aging suggested to play a role in many diseases in the elderly such as cancer and autoimmune diseases [3]. Many gerontologists view immune senescence as an adaptive response needed for survival and longevity rather than leading to various diseases. In addition to increased infections, age-related decline in immune functions is associated with an increased incidence of autoimmunity due to its impact on immune regulatory and tolerogenic mechanisms of the immune system [4, 5]. In this chapter, we will cover some of the main aspects of these seemingly paradox issues of immune senescence.

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4.2 Innate Immune System and Aging

Aging affects innate immune responses by a wide diversity of changes, mostly affecting macrophage functions, including toll-like receptor signaling, phagocytosis, natural killer (NK) cell functions, and others.

4.2.1 Macrophages and Aging

Macrophages play a leading role in the battle against pathogenic microorganisms via phagocytosis and the production of reactive oxygen species. Besides, macrophages release a vast range of mediators such as pro-inflammatory cytokines and chemokines that are crucial to the adaptive immune responses, antigen presentation, and activation of both B and T cells. In tissues, macrophages express a range of germline-encoded pathogen recognition receptors (PRRs), the binding of which enable macrophages to recognize conserved microbial products defined as pathogen-associated molecular patterns (PAMPs). These include toll-like receptors (TLRs), Nod-Like receptors (NLRs), and RIG-1 like receptors (RLRs) [6, 7]. The activation of TLRs in macrophages induces the production of inflammatory cytokines such as TNF-α, IL-6, and IL-1. It has been shown that many aspects of macrophage functions are down-regulated in the process of aging. In many studies, macrophages from old mice secreted lower amounts of TNF-α and IL-6 compared to macrophages from young mice in response to LPS or other TLR ligands. On the other hand, macrophages from old mice secreted increased amounts of IL-10 and prostaglandin E2 following their stimulation with LPS. Of the possible mechanisms explaining the above dis-regulations is their impaired intracellular signaling, namely the reduction of LPS induced phosphorylation of p38 and JNK mitogen-activated protein [8]. In another study, the finding of decreased TLR4 expression was suggested to be responsible, in part, for the observed alteration in TLR signaling in macrophages of older mice. Age-related reduction in IL-6 and TNF-α by human monocytes following TLR1/2 ligands was also reported. In addition to this finding, decreased surface expression of TLR1,4 on aged human monocytes has been shown as well. Macrophages from old mice expressed lower MHC class II molecules on cell surface compared to macrophages from younger mice when stimulated with IFN-γ, thus contributing to their impaired antigen presentation. Reduced STAT-1 phosphorylation in macrophages from old mice in response to IFN-γ suggests that aging is associated with defects in intracellular signaling in macrophages. Finally, aged macrophages failed to maintain sufficient clearance of pathogenic microorganisms when compared to young mice. Impaired phosphorylation by CD14+ monocytes was also reported in older human individuals [9].

4.2.2 NK Cells and Aging

Natural killer (NK) cells are essential effector cells of the innate immune system in the defense line against viruses. In older individuals, increased incidence of viral infections was found to be associated with defects in NK cell activity, cytotoxicity, and their ability to secrete immune-regulatory cytokines and chemokines. Immune senescence of NK cells is different between the various subsets [10]. CD56 (bright) cells are decreased in elderly individuals, whereas CD56 (dim) cells and CD57+ (highly differentiated NK cells) are increased. This NK redistribution in the elderly explains their altered proliferation and the failure to maintain CD16-dependent cytotoxicity. Functionally, CD56 (negative) NK cells are of reduced cytotoxic capacity and IFN-γ production and are characterized by a low KIR expression. They were found to be in association with the accumulation of end-stage-differentiated T cells and reduced CD4/CD8 ratio [11, 12]. In respect to this, CD56 (negative) NK cells are expanded in individuals >60 years of age and CMV+EBV+ elderly individuals, suggesting that they may play a role in the susceptibility of older people to these infections [13].

4.2.3 Innate Lymphoid Cells—Group 2 (ILC2) and Aging

Groups 2 innate lymphoid cells (ILC2) are essential members of the innate immune system, playing a role in tissue repair during many infections, mainly respiratory ones. They are the dominant lymphoid population in the lung, being front protective responders by producing type 2 cytokines [14]. Interleukin-33 (IL-33) was shown to mediate the activation of both ILC2 and T regulatory cells during parasite infections, promoting metabolic resources necessary to protect the host. During aging and with high-fat diet-induced obesity, ILC2 induced protective responses are diminished, and their role in pulmonary infections is compromised [15]. The effect of aging on ILC2 development and function was the subject of recent studies. One of these reported that aging induces compartmentalized changes in ILC2 development. Aging enhances bone marrow early ILC2 development through Notch signaling, but the newly generated circulating ILC2 were unable to settle in the lungs and to replace the declining mature lung ILC2 compartment in aged mice. Aged lung ILC2 are functionally compromised and fail to produce protective cytokines during influenza infection. Transfer of lung ILC2 from young mice increased resistance to influenza infection in old mice. In another recent study, group 2 innate lymphoid cells were shown to be implicated in defense responses, tissue repair, and immunopathology of several diseases of the human respiratory system [16, 17]. The exact role played by ILCs in human health and disease, namely, in young versus aged people, remains poorly understood.

4.3 Adaptive Immune Responses and Aging

4.3.1 T Cells and Aging

T cells are the leading players on the ground of adaptive immune responses, responsible for the recognition and response of both self and non-self-antigens. Chronic immune-mediated inflammation is mediated by different phenotypes of effector T-helper (Th) cells. On the other hand, the maintenance of self-tolerance and suppression of autoimmune disorders is achieved when T regulatory cells are numerally and functionally available [18]. The proper definition and understanding of all subtypes of effector T cells are crucial for the classification and diagnosis of all rheumatic diseases. This will enable clinicians to target these cells better and inhibit their enhanced pro-inflammatory function. Not less important is the ability to improve the function of T regulatory cells by targeting their relevant molecules. CD4+ T cell compartment shrinks with aging while the T cell receptor diversity is well maintained until the eighth decade of life, but then it collapses. It is also well shown that the reactivation of the thymus and the repopulation of the peripheral T cell compartment do not take place in most individuals older than 50 years. Stimulation of the TCR initiates a cascade of tyrosine phosphorylation signals regulated by a network of tyrosine kinases and phosphatases of both activating and inhibiting functions. The mutation or deletion of some of these phosphatases was shown to induce autoimmunity. In bone marrow transplant studies, reactivation of the thymus and repopulation of the peripheral T cell compartment was no longer achievable in individuals older than 50 years. Aged individuals fail to develop a compensatory increase in peripheral T cell turnover, consistent with thymic production being irrelevant at this stage [18]. Besides, age-associated repertoire skewing is accelerated by increased T cell loss due to defective DNA repair mechanisms and compensatory increased peripheral replication leading to telomere shortening and TCR repertoire contraction. Telomeres are essential in maintaining chromosome integrity and in controlling cellular replication. Telomerase activity decline with age in activated T-cells attributed in part, to the change in physiological conditions such as increased blood glucose, and pro-inflammatory cytokines such as interleukin-6 [19]. Compared to younger adults, CD4 memory T cells from healthy older individuals exhibit a higher up-regulation of oxidative phosphorylation with increased production of reactive oxygen species and intra-cellular secreted ATP and increased catabolic state in lipid metabolism [20]. The so-called dangerous γδT cells are of limited clonal diversity and found to be strongly expanded in lymph nodes of aging mice. They are characterized by the swift production of IL-17 upon ex-vivo stimulation and of impaired anti-tumor responses in old mice, proposing a link between γδT cells and increased risk of cancer development in aged mice [21]. In a recent study, IL-17-producing auto-reactive CD4-intermediate T cells were increasingly observed in aged mice. However, they were found to be different from typical Th17 cells by expressing higher levels of immune-suppressive receptor PD-1 [22].

4.3.2 T Regulatory Cells and Aging

The aging process is characterized by the imbalance between pro- and anti-inflammatory mechanisms leading to the loss of compensatory reserve and accumulation of unrepaired damage. The finding of a chronic low-grade inflammatory state which exists in many older individuals, even when they are apparently healthy, was reported to be associated with T regulatory cell and NF-kB dysregulation [23]. Single-cell RNA sequencing and multi-dimensional protein analyzes were assessed in thousands of CD4+ T cells obtained from young and old mice, aiming to define these dysregulated functions. Cytotoxic and activated regulatory T cells were found to be rare in young mice but gradually accumulate with age, providing some explanation for the existing chronic inflammation and immunity decline in aged mice [24]. Conventional T cells, mainly T regulatory, are elevated in adipose tissues during aging and have been implicated in the pathogenesis of metabolic diseases. These changes contribute to the associated metabolic dysfunctions, including insulin resistance and inflammation in adipose tissue, namely the so-called "inflamm-aging" [25]. Aging is associated with an increased incidence of cancer being the result of decreased anti-tumor immune responses. This was shown to be in part due to changes in T-cell function in the elderly. In lymph nodes of aged mice, T regulatory cells are characterized by increased expression of many regulatory markers such as CTLA-4, PD-1, ICOS, LAG-3, and IL-10, compared to T regulatory cells from young mice. In respect to these findings, elderly tumor-bearing mice demonstrated decreased IFN-γ by CD8+ and CD4+ T cells within tumors, compared to young mice [26]. The relation between aging and induced T regulatory cells (iTreg) was assessed in a mouse model of hepatic ischemia-reperfusion injury (IRI). In this model, aged mice suffered more serious injury than young mice, with higher serum levels of liver enzymes and higher histological scores from liver biopsies. Induced Treg cells from young mice demonstrated stronger immune-suppressive ability in vitro. Adoptive transfer of iTregs ameliorated liver IRI and was followed by liver recovery in association with decreased levels of IFN-γ, and IL-17. This suggests that liver injury in aged-mice is a result of decreased iTreg function [27]. In a very recent study, an increased number of circulating T follicular regulatory cells (Tfr) defined to be FoxP3+ was found to correlate significantly with aging in healthy volunteers. The suppressive effect of Tfr cells on B cell function in elderly subjects was diminished when compared to that in younger individuals. This was attributed to their failure to produce the regulatory cytokine IL-10 [28].

4.4 B Cells and Aging

Immunosenescence is characterized by a decrease in total B cells (CD19+), contributing to the insufficient ability of the elderly to control infectious diseases and to their inadequate response to new antigens and vaccination. CD19+ B cell

populations include several subsets, i.e., naïve B cells (CD27−IgD+), IgM memory (CD27+IgD+), switched memory (CD27+IgD−) and late memory double-blind (CD27−IgD−) cells [29]. Aiming to characterize B cell immunosenescence better the number of CD19+CD27+ memory cells, as well as serum levels of IgD, were analyzed in both young and older people. The percentage of memory CD19+CD27+ B cells was significantly increased in old individuals, whereas serum levels of IgD were decreased in comparison with young subjects [30]. Looking at naïve B cells (IgD+CD27−) in the elderly, they were found to be significantly diminished, whereas double negative (DN) IgD−CD27−B cells are found to be increased, explaining why IgM levels are higher in young people. These changes in B cell repertoire are suggested to be a hallmark of B cell immunosenescence [31, 32]. Increased IgD−CD27− (DN, memory B cells) in the elderly were shown to be IgG+ but of low CD80 and DR expression, indicating that they cannot serve as antigen-presenting cells. These are late memory and exhausted cells lacking the ability to interact with T cells [33]. In respect to the above, it was found that in older individuals, serum levels of B-cell activating factor (BAFF) and the proliferation-inducing ligand (APRIL), both pivotal survival factors for B cells, are decreased and in correlation with poor B cell survival [34]. The replenishment and diversity of B cell repertoire, as well as their ability to recognize and respond to new antigens, is significantly reduced in the elderly. In addition to decreased naïve B cell population in the elderly, the deterioration in B cell diversity was shown to be a consequence of reduced germinal center activity and to the progressive increase in the number of peripheral memory class-switched B cells (e.g., $IgD^{-}CD19^{+}CD27^{+}$). The above B cell aberrations lead to a selective shift toward the production of IgG/IgA, resulting in an overall contraction of the B cell repertoire, limiting the number of potential new clones available to respond to new antigens. In old individuals, activated memory B cells exhibit deterioration in their capacity to differentiate into mature plasma cells, followed by a decline in the production of specific antibodies. Increased late memory B cells ($IgD^{-}CD95^{hi}CD27^{-}$) in aged people were reported to spontaneously secrete TNF-α contributing to the well-known increased inflammatory state commonly described in elderly dysregulation of immune homeostasis and decreased B cell function [35–38]. Efficient humoral immune responses are dependent on several maturation steps, such as the generation of isotype-switched and high-affinity maturation of antibodies within germinal centers. These maturation processes are dependent in part on the activation of cytidine deaminase (AID). The stability and the production of AID are significantly reduced in aged B cells from both humans and mice. This reduction is caused in part by the reduced mRNA stability strictly related to "inflamm-ageing." In respect to this, the expression of the two pro-inflammatory micro-RNAs (miRNAs) miR-155 and miR-16, that respectively, bind the 3′-untranslated region (UTR) of AID mRNA, inducing its degradation, were found to be increased in the elderly. MiR-155 has been shown to be involved in the initiation and development of B cell malignancies, typically frequent in the elderly. It has been recently demonstrated that miR-155 is up-regulated in diffuse large B cell lymphoma and chronic lymphocytic leukemia. Moreover, it was shown that its over-expression is associated with poor prognosis in these malignancies, postulating miR-155 as a possible diagnostic and prognostic biomarker in B

cell malignancies. In addition, it was found that both miR-155 and miR-16 were demonstrated to be over-expressed in rheumatoid arthritis, demonstrating the potential pro-inflammatory role of these two miR's [39–41].

4.4.1 Reduced Ability of Older People to Respond to Newly Encountered Antigens and Vaccinations Reflects the Age-Related Impairment of Humoral Immune Response

In adults, influenza vaccine elicits both memory adaptive immune responses to epitopes that are shared with previously encountered viruses and primary (new) responses to new antigenic epitopes in the vaccine. Thus, it was speculated that memory adaptive immune responses will be predominant in the elderly due to their several previous exposures to influenza viruses or vaccines; however, it was found that there is an age-related decrease of the influenza vaccine-specific antibody response. In order to try to find the reasons for this phenomenon, several studies have been conducted. In one publication, it was reported that B cells from elderly people had a significant reduction in the expression of the co-inhibitor B and T lymphocyte attenuator (BTLA) before vaccination compared to young volunteers. It was also demonstrated that BTLA expression on mature B cells is associated with the increase in the level of influenza-specific IgG antibody titers. Moreover, it was also found that the BTLA expression is involved in the isotype switching from IgM to IgG. Thus, the decline of BTLA expression on B cells might be related to a decrease in specific antibody production in the elderly [42, 43].

4.5 Summary

Immunosenescence is responsible for the decreased ability of older people to fight infections, namely to develop a sufficient response to new antigens and vaccination. The decline in effective immunity is due to changes in T, B, and NK cell subpopulations, both in their numbers and functions. Altered immunity in the elderly is associated with increased levels of pro-inflammatory molecules and the persistence of chronic low-grade inflammation. Specific therapeutic strategies are required in order to maintain balanced and efficient immune responses, mainly by effector T and B cells.

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