Immunologic Changes in Frail Older Adults

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Abstract - Several studies have shown a heightened inflammatory state in frail older adults, marked by high serum levels of interleukin-6 and C-reactive protein and an increased number of circulating leukocytes. Activation of monocytes and macrophages, marked by increased levels of neopterin, may contribute to chronic inflammation in the frail older adult. However, the reduced mononuclear cell response to lipopolysaccharide *in vitro* suggests the existence of defective activation pathways within the innate immune system possibly due to desensitization. Conversely, the expansion of $CD8^+$ T cells, and specifically those expressing the CCR5 chemokine receptor, above and beyond the levels observed in senescence, points to the involvement of adaptive immune pathways. In line with these observations, frail older adults exhibit a reduced antibody response to pneumococcal and influenza vaccines. Collectively, these observations support the existence of a dysregulated immune system in frail older adults and highlight the need for strategies to improve its function.

Abbreviations - AIDS, acquired immunodeficiency syndrome; CCL, CC-chemokine receptor ligand; CCR, CC-chemokine receptor; CHS, Cardiovascular Health Study; CMV, cytomegalovirus; GTP, guanosine trisphosphate; HAART, highly active anti-retroviral therapy; HIV, human immunodeficiency virus; IDO, indoleamine-pyrrole 2,3-dioxygenase; IL, interleukin; IFN, interferon; MACS, Multicenter AIDS Cohort Study; NH2PPP, dihydro-neopterin trisphosphate; Tc, T cytotoxic; TCR, T-cell receptor; TEMRA, T effector memory cells re-expressing CD45RA; Th, T helper; TNF, tumor necrosis factor; WHAS, Women's Health and Aging Study

Aging is currently viewed as an active process, in which a number of factors concur to the progressive disruption of homeostasis in multiple physiologic systems, ultimately resulting in the development of age-related diseases. The aging of the immune system is associated with a decline in immunity against infections and a departure from the finely-tuned balance between physiologically beneficial, protective inflammation and inappropriate, undesirable inflammation. Among the factors involved in this process are a number of cytokines and mediators produced by cells of the immune system, most prominently interleukin (IL)-6 and tumor necrosis factor (TNF)-α. These are produced by cells of both the innate and adaptive axes of the immune system and repeatedly can be accounted for the chronic inflammatory changes underlying a diverse host of clinical manifestations in the aged individual, such as cardiovascular disease, autoimmune disease, osteoarthritis, cancer, dementia, and a few others [9]. While these changes may suggest a hyperactive, uncontrolled state of the aging immune system, they ultimately originate from a progressive immune defect [24].

T-cell senescence and the aging immune system

The immune system can be targeted by several environmental insults, including radiations, chemicals, pathogens, and pharmacologic or biologic agents. Lymphocyte numbers can be rapidly and greatly reduced in response to exposure to immunosuppressive agents. Under physiologic conditions, these can be restored via the homeostatic expansion of surviving clones and the new generation of naïve clones from lymphopoietic tissues, namely the thymus and the bone marrow [29]. However, while homeostatic proliferation constitutes a positive selective pressure for clones responsive to persisting antigens, only active lymphopoiesis can ensure the reconstitution of a comprehensive repertoire of antigen specificities [18]. With advancing age, the contribution of lymphopoiesis becomes less prominent. It is well known that the thymus, the gland in which T cells develop and mature, undergoes involution throughout most of adult life. Thymic atrophy and, consequently, the dramatically reduced numbers of thymic emigrants in chronologic aging, are thought to be the primary reason for the progressive contraction of the naïve T-cell fraction and the relative expansion of the memory subset [24].

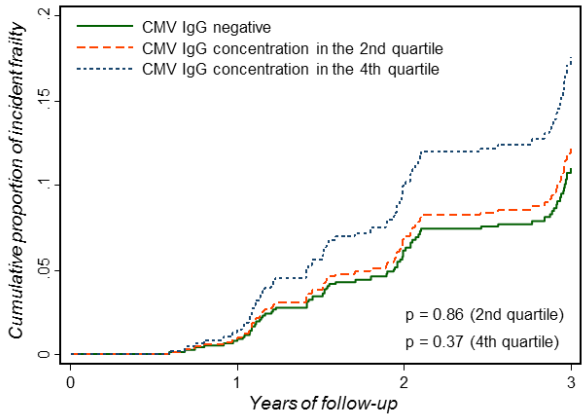
As a consequence of a failing reservoir of newly generated T cells, the aging immune system is characterized by a reduced level of diversity [31]. In this context, pre-existing clones reactive to less prevalent, occasional antigens will gradually be competed out and supplanted by clones expanding in response to such persisting antigens as cytomegalovirus (CMV) [1]. Remarkably, while at least 10^5-10^6 clones account for the functional diversity of the T-cell receptor (TCR) repertoire in the healthy adult memory T-cell population, these can be reduced by up to 2 orders of magnitude in the senescent immune system [17,22]. This reduction in TCR repertoire diversity likely contributes to the reduced response to vaccines and the overall increased susceptibility to infections and tumors in older age. On the other hand, the relative prevalence of clones responsive to self antigens and chronic pathogens increases dramatically. For instance, clones specific for certain CMV antigens, e.g. pp65, may account for as much as 25% of the total $CD8^+$ T-cell population in seropositive older adults [19]. However, sustained clonal expansion of these cells leads to their progressive senescence and exhaustion, which is accompanied by unique phenotypic and functional traits that have been extensively characterized in the $CD8^+$ subset over the past few years. Indeed, it has been shown that persistent CMV infection is associated with a higher fraction of these terminally differentiated, senescent $CD8^+$ T cells, typically referred to as T_{EMRA}, or T effector memory cells re-expressing the naïve marker CD45RA [11]. Besides the CD45RA reversion, these cells also exhibit markedly reduced levels of the lymph node homing receptors CD62L and CCR7, and of the costimulatory receptors CD27 and CD28 [2,10]. The cells exhibit other signs of senescence, including shortened telomeres and a low proliferative response to mitogens, yet they are remarkably resistant to apoptosis, express higher levels of cytotoxicity effectors, e.g. granzyme B and perforin, and exhibit a raised level of activation at baseline possibly responsible for the increased serum levels of interferon (IFN)-γ commonly documented in older age [2,9,10,24].

A chronic immune burden in the development of frailty

The immune changes that occur in the aging host have been likened to those occurring in human immunodeficiency virus (HIV)-infected patients prior to the introduction of highly active anti-retroviral therapy (HAART) [10]. Acquired immunodeficiency syndrome (AIDS) is characterized by a low naïve to memory ratio and a reduced T-cell repertoire as the result of reduced thymic function. While not directly affected by the primary infectious agent, $CD8^+$ T cells undergo, in response to the sustained antigenic burden from HIV as well as other pathogens, e.g. CMV, a premature senescence process with expansion of $CD28^-$ effector memory clones, heightened IFN-γ and TNF-α production and chronic inflammatory changes mediated by a host of innate immunity cytokines such as IL-6 [5]. Interestingly, in middle-aged patients enrolled in the Multicenter AIDS Cohort Study (MACS) prior to the HAART era an association was found between the degree of $CD4^+$ T-cell depletion and a clinical picture virtually indistinguishable from that of frailty of older age [12]. Frailty was in that study defined by the coexistence of at least three of the five criteria defined by Fried and collaborators in the Cardiovascular Health Study (CHS) and subsequently validated in the Women's Health and Aging Studies (WHAS): namely, weight loss, weakened handgrip, exhaustion, reduced gait speed, and reduced activity [3,14]. By adoption of these criteria the prevalence of this condition in older people not affected by other conditions has been estimated to range from 4% to 12% in different populations and age groups [13].

The pathogenesis of frailty of older age is postulated to involve multiple physiologic systems, including the immune system [15,28]. Data from the CHS showed that frail older adults, compared with non-frail ones, have elevated levels of C-reactive protein, even after excluding individuals with cardiovascular disease and diabetes and adjusting for age, sex, and race [26]. Clinical evidence from the MACS as well as studies of patients with systemic autoimmune conditions, together with the appreciation of consistent immune and inflammatory changes, lend support to the idea that sustained exposure to a persistent antigen(s), either infectious or autologous, might play a critical role as either a causative or precipitating factor [12,16]. Along this line, an association between frailty status, plasma IL-6 concentrations, and CMV seropositivity was found in a recent collaborative study by our group [27]. The study, involving 635 participants from the WHAS cohort in 1992 and 2002 (WHAS I and II), identified distinct levels of risk relationship between serum anti-CMV antibody (IgG) concentration and prevalent frailty, across groups of subjects categorized according to tertiles of plasma IL-6 concentrations. That is, the higher the plasma IL-6 concentration, the steeper the curve of the relationship between anti-CMV IgG level and the likelihood of prevalent frailty. Within each tertile, the odds ratio for frailty was generally higher with higher CMV IgG levels, reaching about 5.18% (95% confidence interval, 0.99-27.05), for IgG concentrations exceeding 18 IU/mL at the third tertile of IL-6 concentration [27]. In addition, the data suggested that individuals who were not frail at the beginning of the study were more likely to develop incident frailty over three years of follow up, although the relationship was not statistically significant. Higher plasma IL-6 levels were associated with steeper curves in this CMV IgG-incident frailty relationship (Figure 1). Although it is possible that confounding variables, especially socioeconomic status, may have played a non-trivial role in our study [30], these findings are suggestive of a connection between a sustained adaptive response to CMV, the activation of an innate immune response, characterized by increased production of IL-6, and the development of frailty.

A. Incident frailty at lower plasma IL-6 concentration



B. Incident frailty at higher plasma IL-6 concentration

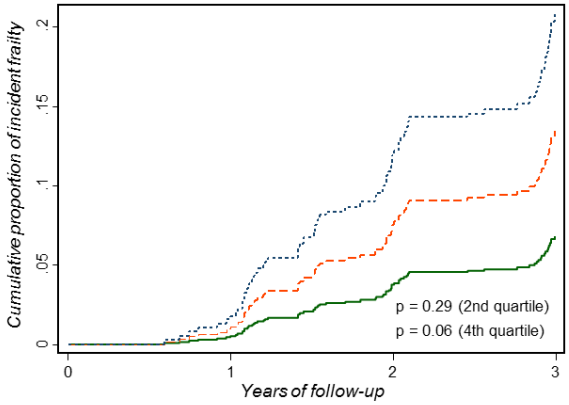


Figure 1. Adjusted cumulative proportion curves of incident frailty according to CMV IgG antibody and IL-6 concentration. Cumulative proportions of incident frailty for older women (N = 299) in 3 contrasting categories of CMV IgG antibody concentration (seronegative, second quartile [10.17-14.55 IU/mL], and fourth quartile [18.17-150 IU/mL]), are shown separately at 2 contrasting plasma IL-6 concentrations of 1.6 pg/mL (panel A) and 4.8 pg/mL (panel B). Curves were adjusted for age, race, high school education, coverage by private medical insurance, pack-years of smoking, cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), diabetes mellitus, and plasma IL-6 concentration, with the use of Cox proportional hazards model. Interaction between CMV antibody and IL-6 concentration was modeled. Age was set to 70. Variables other than age, CMV, and IL-6 were set to 0. The p values indicated in each panel denote comparisons between women in the second quartile of CMV antibody concentration and CMV seronegative women, and between women in the fourth quartile of CMV antibody concentration and CMV seronegative women, respectively.

Immunologic markers of frailty

In further support of a substantial role of the innate immune system in the development of frailty, a study comparing non-frail to frail or prefrail elder populations—this latter defined by satisfying one or two of the Fried's criteria for frailty—detected significantly elevated serum levels of IL-6 and of the monocyte-derived molecule, neopterin, which correlated with the severity of the frailty status [20]. Interestingly, neopterin plasma levels have also been associated with cognitive decline in the elderly, being significantly more elevated in subjects with amnestic mild cognitive impairment and much more so in patients with Alzheimer's disease [23]. Plasma levels of IL-6 and IFN-γ are also elevated in these conditions relative to controls matched by age and other variables, both of which are significantly correlated with neopterin levels [23]. This is in agreement with the notion that IFN-γ, along with other inflammatory factors, is a strong inducer of neopterin production in human macrophages and dendritic cells [6]. Neopterin is a pteridine produced from guanosine trisphosphate (GTP) via dihydro-neopterin trisphosphate (NH_2PPP), a reaction catalyzed by the IFN-γ-induced enzyme, GTP-cyclohydrolase 1. Accumulation of neopterin is favored by the production of reactive oxygen species in IFN-γ-activated macrophages, which interferes with the conversion of NH_2PPP to tetrahydropterin, a necessary coenzyme for the synthesis of tyrosine and 5-hydroxytryptophan from phenylalanine and tryptophan, respectively. This results in defective production of such key neurotransmitters as catecholamines and serotonin, responsible in turn for mood, cognitive and motor dysregulation. Reduced tryptophan availability also results from IFN-γ-mediated activation of the enzyme, indoleamine-pyrrole 2,3-dioxygenase (IDO), and the ensuing production of kynurenines further contributes to neuromotor dysfunction. Notably, IDO activation also results in immune suppression and reduced surveillance for pathogens and tumors [25].

Taken together, these observations point to increased production of IFN-γ from cells of the adaptive immune system as a key step in the age-related chronic inflammatory process, eventually linked to multiorgan dysfunction in the elderly and substantially more so in the frail older adult. As an initial approach to functionally defining the cellular sources of IFN-γ in this condition, we compared the T-cell surface and effector phenotype in frail *vs.* nonfrail octogenarians [8]. Among other markers, we looked at the surface expression of CCR5, a receptor for the chemokines, CCL3, CCL4 and CCL5, which was previously found to be expressed at higher levels in the aging host and in T cells is associated with IFN-γ production and with a polarized Th1/Tc1 phenotype [7,21]. By flow-cytometric determination we preliminarily found that circulating T cells that express this receptor are an almost exclusive source of IFN-γ upon mitogenic stimulation, while production of IL-4 and other Th2/Tc2 cytokines is only observed in $CCR5^-$ cells that express the prostaglandin D_2 chemotactic receptor,

CD294 [4,7]. In frail donors we detected significantly increased numbers of circulating T cells expressing CCR5, along with significantly increased numbers of $CD8^+$ and significantly reduced $CD4^+$ T cells [8]. These changes were graded across frailty scores, supporting a direct correlation with the severity of the clinical picture [8]. $CCR5^+$ T cells from frail donors predominantly expressed a $CD8^+CD45RA^+CD28^-$ phenotype, reminiscent of the T_{EMRA} clones described in earlier studies [8,11]. Indeed, almost twice as many $CD8^+CD45RA^+$ T cells capable of producing IFN-γ—a fraction approximating 100% in some cases—were found in frail *vs.* nonfrail donors (G.C.W. and V.C., unpublished observations), strongly supporting the idea that the prevalence of T_{EMRA} may be considered in itself a reliable marker in discriminating frailty from relatively healthy senescence. Importantly enough, regardless of other conditions, the frequencies of these T-cell clones are significantly and markedly more elevated in CMV-seropositive individuals, further stressing the possible contributive role of this pathogen, and the ensuing adaptive immune response, in the pathogenesis of frailty of older age.

Concluding remarks

Recent work is shedding new light on the mechanisms that account for the immune derailment in frail older people and immune senescence in general. Thymic atrophy, coupled with chronic antigenic burdens, may explain the reduction in T-cell repertoire and the enrichment for terminally differentiated, senescent clones, which in turn promote chronic inflammation and multiorgan deterioration by sustained induction of IL-6 and other cytokines. Prevalent frailty and the risk for frailty are increased in older individuals with high titers of CMV IgG and IL-6 plasma concentrations. A graded increase in plasma IL-6 can be seen in prefrail and frail relative to nonfrail older individuals, and is correlated with plasma concentrations of neopterin, a marker of IFN-γ-mediated macrophage activation. Our evidence for an increased frequency of IFN-γ-producing, $CCR5^+CD8^+$ cells is the first to document significant changes in T-cell phenotypes in frail older adults. We show that these cells mostly express a T_{EMRA} surface phenotype, characterized by re-expression of the naïve marker CD45RA and loss of the costimulatory marker CD28. This T-cell terminally differentiated population expands in response to persisting CMV antigen load and may therefore represent a link between chronic CMV infection and inflammation in frailty.

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