ELSEVIER

Aging, immunity and cancer

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Immunosenescence, the progressive decline in immune function that develops with age, results from cumulative alterations in critical B- and T-cell subpopulations. Decreases in circulating memory B cells and in germinal center formation are evident in the elderly, possibly due to diminished follicular dendritic-cell function. T-cell dysfunction is associated with reduced thymic generation of naïve T cells, virus-induced expansion of terminal effectors and increased levels of memory cells producing type I and II cytokines. The diversity of the T-cell receptor repertoire is diminished by the first two changes, and elevated type I cytokines might contribute to the pro-inflammatory cytokine milieu present in the elderly.

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Abbreviations

FDC	follicular dendritic cell
IFN	interferon
IL	interleukin
LIF	leukemia inhibitory factor
M-CSF	macrophage colony-stimulating factor
NK	natural killer
OSM	oncostatin M
SCF	stem-cell factor
TCR	T-cell receptor
TEC	thymic epithelial cell
TNF	tumor necrosis factor
TREC	TCR rearrangement excision circle

Introduction

Current analysis of the increase in cancer incidence that accompanies aging primarily focuses on the multistep process of tumorigenesis. Yet, progressive T- and B-cell functional deficits develop with aging, a decline termed immunosenescence [1,2,3•], which may also play a role. With aging, the thymus involutes and the supply of naïve T cells gradually falls. The cumulative expansion of memory cells increases the production of type I and type II cytokines, with inflammatory mediators often predominating. The T-cell receptor (TCR) repertoire becomes skewed and oligoclonal as terminally differentiated effector subpopulations accumulate. These changes alter both humoral and cellular immune competence. This review will summarize recent work on the changes of the immune system with aging, with a particular focus on changes in T-cell subpopulations and their relevance to cancer susceptibility and therapy.

A key problem in studies of aging has been that of distinguishing changes prognostic of susceptibility to infection, autoimmunity and cancer from those changes resulting from these very conditions. Most of the studies are cross-sectional, comparing aged and younger groups. Furthermore, the health status of many of the elderly in these studies is poorly defined. This concern has been addressed by longitudinal studies of the aged and by the establishment of performance status criteria under the SENIEUR protocol, defining the healthy aged [4]. Longitudinal studies of the healthy aged have identified immune risk phenotypes (IRPs) by correlating immune changes, such as low $CD4^+$ cell numbers and inverted CD4:CD8 ratios, with poor prognosis [5,6]. Although variations have been found [3•], these studies provide the strongest characterization of immune system changes during aging.

Alterations in hematopoiesis, and in innate and humoral immunity

Cells of the immune system are constantly renewed from hematopoietic stem cells. With age, a reduction in the overall capacity for renewal of these stem cells as a whole has been observed [7]. The proliferative activity of bone marrow (measured by the proportion of $Ki67^+$ cells) peaks in middle age and then gradually decreases, although reductions in marrow cellularity are found only with extreme age [8], perhaps associated with increased apoptosis [9]. Consistent with this, $CD34^+$ stem cells mobilize less effectively in the elderly when compared to younger donors [10]. Moreover, both a reduction in commitment to lymphopoiesis [11] and a reduction in the ability of marrow stroma to support lymphopoiesis have been reported with aging [12]. Thus, some of the deficits of immunosenescence begin with stem cells.

Within the innate immune system, natural killer (NK) cells play an important role in inhibiting tumor growth and metastases. In a prospective study, following 3500 middle-aged and elderly Japanese over 11 years, the incidence of cancer was increased in those with lower initial NK cytotoxic activity [13]. In gastric cancer patients, lower NK cytolytic activity at diagnosis correlates with higher tumor

volume, metastases and worse prognosis [14]. Unlike T and B cells, the absolute number of NK cells is increased in aged individuals in comparison to young or middle aged groups, and IFN-γ production and phagocytosis are also increased [15,16]. Total NK-cell cytotoxicity is stable, however, so the NK-cell cytotoxicity on a 'per-cell' basis is impaired, as is the response of NK cells to IL-2 [15]. Bonafe [17] suggested that the age-associated increases in NK cells and in T cells expressing NK receptors play a beneficial role in immunosurveillance. Individuals with elevated levels of these effectors might blunt the growth of neoplastic cells.

Humoral immunity in the elderly often involves an increase in autoantibodies [18] but a concurrent reduction in vaccine responses [19]. Although T-cell dysfunctions play a significant role in age-related humoral immune changes [20], alterations in B cells have also been detailed. In murine studies, a progressive decline in germinal center formation is observed with age [21]. Aged follicular dendritic cells (FDCs) stimulate B cells 70% less well than those from young mice [22•]; diminished FDC function could result in reduced persistence of antigen deposits and correspondingly reduced maintenance of functional memory B cells. Indeed, the frequency of circulating $CD27^+$ memory B cells is reduced in the infirm elderly, although the deficit is minor in healthy centenarians [23,24]; reductions in $CD27^+$ memory B cell numbers in nursing home residents not only correlate with low T-cell numbers but also with reduced T-cell functions [24]. A decrease in $CD5^+$ B cells, which are associated with T-independent antibody production, was also found with aging, and there was also a decrease in expression of CD40 on B cells, a molecule needed for cognate interactions between B and T cells [23]. Thus, the induction of both T-independent and T-dependent B-cell responses may be reduced, and the persistence of memory B-cell populations may be limited in the elderly.

Alterations in cellular immunity

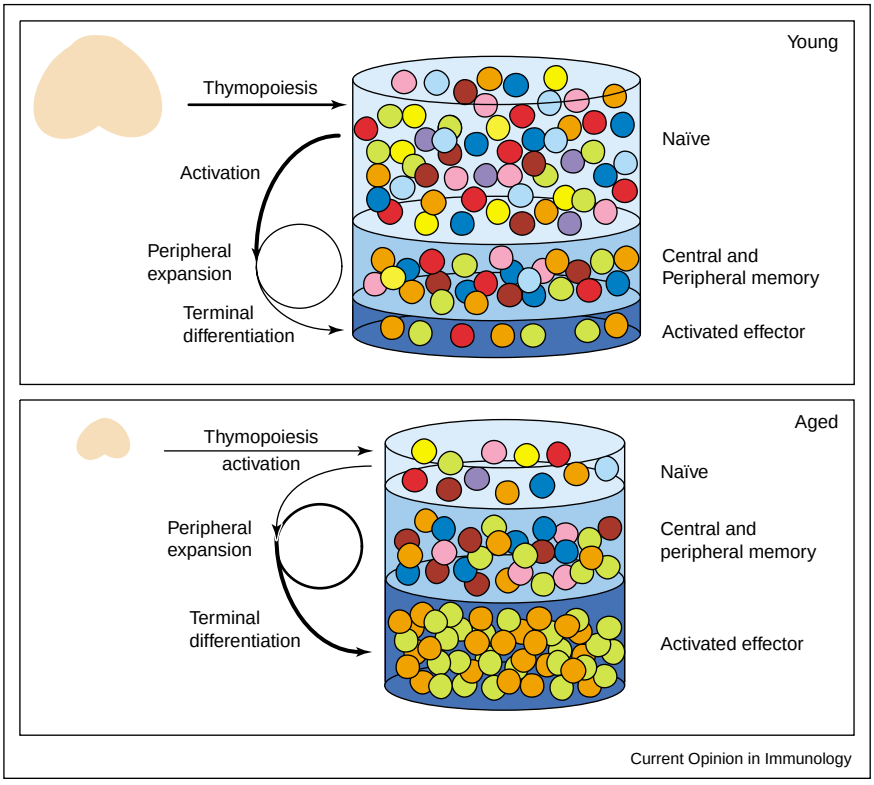
The critical changes characteristic of immunosenescence occur in the T-cell populations. Although overall numerical shifts have been observed [25], it is the changes in subpopulations that underlie the functional deficits of aging and exacerbate recovery from therapy. Three primary changes have been observed: a decline in the number of naïve cells due to diminished thymopoiesis; an increase in the number of memory cells resulting in increased cytokine production; and a dysfunctional accumulation of activated effector cells of limited T-cell repertoire occupying T-cell space (see Figure 1).

Reduction in naïve T cells and thymopoiesis

There is now evidence that thymopoiesis continues throughout life at some level, despite the gradual involution of the thymus [26]. Ongoing thymopoiesis provides a continuing supply of phenotypically naïve T cells ($CD45RA^+$, $CD62L^+$, $CD27^+$, $CD28^+$, $CD11a^{dull}$; [27]). These data are substantiated by the measurable presence in the peripheral blood of T cells containing TCR rearrangement excision circles (TRECs), non-replicating episomal DNA circles generated during thymocyte development. Because TRECs are rapidly diluted following activation-induced T-cell proliferation, their presence is consistent with recent thymic emigration. The critical consequence of thymopoiesis is the generation of a broad repertoire of diverse T-cell receptors. Spectratype analysis has demonstrated that the T-cell receptor Vβ repertoire diversity of naïve $CD4^+$ and $CD8^+$ T cells is maintained in the elderly [28]. Overall diversity of the total T-cell repertoire in the elderly is skewed and marked by oligoclonal expansions, however, due to the limited frequency of naïve cells. Although the thymus remains functionally competent, the diminished export rate associated with aging is insufficient to replace naïve T cells lost daily from the periphery. This loss results in a gradual dwindling in naïve cells and, hence, in repertoire diversity (Figure 1).

Involution of the thymus with age involves complex changes in gene expression, as the thymus is a chimeric organ that contains cells of several lineages [29]. In thymocytes, no changes in mRNA levels of pre-Tα and its transcriptional regulator HEB have been found with aging. The expression of E2A, a transcriptional regulator critical for TCRβ rearrangement, however, declines with age, whereas expression of LMO2 (a negative regulator of E2A activity) increases [30••]. Recombinase-activating gene 1 (Rag1) and Rag 2 expression also decline in aged mice, but cocultures have demonstrated that this is controlled by thymic epithelial cells (TECs; [31]). A comparison of thymocyte-depleted stromal cell preparations from young and aged mouse thymuses has found decreased mRNA for IL-7, M-CSF and SCF, as well as for the transcriptional factor Foxn1 (the *nude* gene, critical for TECs during organogenesis [30••,32]). Both connexin 42 and keratin 8 (also associated with the cortical stroma) were stable at seven months of age, suggesting that the reduction in IL-7 was not merely a decline in the relative proportion of cortical TECs, but a specific change in the regulation of IL-7 expression [30••]. Consistent with these findings, injection of IL-7, but not SCF, resulted in an increase in thymocyte numbers in aged mice [33]. By contrast, in human studies using RNA from total thymus tissue, IL-7 does not decrease, and in addition, SCF and M-CSF increase, together with leukemia inhibitory factor (LIF), oncostatin M (OSM), and IL-6 [34]. Interestingly, it was found that adipose tissue expresses LIF, OSM, SCF, IL-7, IL-15, IL-6 and M-CSF mRNA at levels equal to those seen in aged thymic tissue. As adipose tissue replaces thymic volume during aging in man, this finding suggests that adipose tissue might exert

Figure 1



Young and aged thymocyte populations. The upper cylinder represents the peripheral T-cell compartment in the young, and the lower cylinder represents the smaller T-cell population found in the elderly. Naïve (pale blue), memory (mid-blue) and activated effector cells (dark blue) are found at both ages, but the proportions change. The naïve cells comprise the largest proportion of T cells in the young, but are relatively few in the elderly. These naïve cells are the product of the thymus, which is large in the young but progressively diminishes with age. Naïve cells have the greatest TCR repertoire diversity, as indicated by the range of colors on the cells. Upon activation, naïve cells move into the memory pool, where they may undergo peripheral expansion (circular arrow). The memory population is the source of most type 1 and type 2 cytokines. The enlarged memory component in the elderly may give rise to the increase in cytokines observed. Overall, the repertoire of memory cells in the elderly is less diverse than in the young due to a reduced input from the small naïve cell population. Upon repeated stimulation, memory cells give rise to terminally differentiated effectors. These activated effectors have the most severely limited repertoire within the three T-cell pools. Dysfunctional cells of this oligoclonal population accumulate in the elderly.

an additional level of control on thymic function. *In vivo* administration of OSM, LIF or IL-6 produces thymic involution in mice, apparently by increasing corticosteroid production that affects cortical thymocytes [34,35]. In man, plasma IL-6 levels significantly increase with aging [36•], perhaps with comparable effects.

Increase in memory T cells

The second major change in T-cell populations with age is the increase in memory T cells with defined cytokine phenotype and consequent changes in the overall production of cytokines. With increasing age, memory and activated effector T cells predominate, both due to the accumulation of memory responses following antigen activation and from homeostatic proliferation to maintain T-cell levels. IL-2 production *in vitro* in stimulated T cells is reduced in most studies using SENIEUR donors [3•]. This change may result from the loss of naïve populations, which are high producers of IL-2 [37], and the expansion of memory T cells. In a recent study of healthy (but not SENIEUR assessed) elderly, the frequency of $CD8^+$ cells producing type 1 cytokines (IFN-γ and TNF) increased with aging, particularly in the 'cytotoxic' $CD28^-CD8^+$ subset [38•]. Similarly, the frequency of type 2 (IL-4 and IL-10) cytokine-producing memory $CD8^+$ cells, although much lower than type 1 producers, also increased with aging [38•]. Such studies are limited by being primarily cross-sectional rather than longitudinal, and are complicated by a lack of consistency in T-cell stimulation and criteria for inclusion as elderly. Nonetheless, repeated observations have suggested that elevated levels of inflammatory cytokines (IFN-γ, TNF-α) exist, and this could contribute to the overall pro-inflammatory state in many of the elderly, whereas an increased number of IL-4-producing cells could be driving autoimmune B-cell hyperactivity.

Accumulation of terminally differentiated effectors

The final change in T-cell populations is the accumulation of terminally differentiated effector cells, in particular virus-reactive cells, with extremely limited TCR repertoire diversity. Longitudinal studies under the SENIEUR protocol show inversion of the CD4:CD8 ratio associated with increased mortality [6]. Individuals with an inverted ratio had not only a reduction in $CD4^+$ T-cell levels but also a doubling in $CD8^+$ T cells, dominated by cell populations that were $CD28^-$,$CD27^-$,$CD45RA^+$, $CD57^+$ [39••,40•]. This is the phenotype of activated effector $CD8^+$ T cells, which downregulate CD27 and CD28 following repeated cycles of stimulation. These cells have shortened telomeres [41] and a limited proliferative capacity [42]. The majority of these cells are clonally expanded populations reactive to cytomegalovirus (CMV) and Epstein-Barr virus (EBV) determinants, as shown by tetramer assays demonstrating the binding of viral peptides [39••,43•]. These cells are nonetheless dysfunctional, responding with low levels of IFN-γ when stimulated by viral antigens. Thus, by dominating the T-cell population these dysfunctional $CD8^+$ cells may reduce the repertoire of T cells available for responses to infection or neoplasia.

Reduced immune reconstitution after cytoreductive therapy

The problems affecting T-cell populations in the elderly are aggravated by cytoreductive cancer therapies that require immune repopulation. Chemotherapy and transplant conditioning regimens used for cancer therapy produce a severe reduction in all lymphocyte populations [44]. With age, the recovery of normal lymphocyte levels is progressively retarded [45,46]. Pediatric populations undergo thymic rebound and generate high numbers of naïve T cells within a few months [45,46]. Adult populations, by contrast, recover only after years [47]. The generation of new thymic emigrants, as measured by peripheral TREC-bearing T cells, is inversely proportional to age, with the recovery of naïve cells after the fifth decade being severely compromised [48]. Similarly, in murine studies, thymic stroma in aged hosts generates fewer T cells than that of young hosts [49]. Because of deficits in naïve cell generation, post-transplant T-cell populations characteristically include primarily memory phenotype cells, with high frequencies of dysfunctional, terminally differentiated $CD8^+$ T cells expressing CD57, but lacking CD28 [50]. Accompanying these population shifts, the TCR repertoire diversity is limited and oligoclonal post-transplant [47,51].

Cytoreductive therapies age the immune system. Certainly, the cumulative dysfunctional changes occurring over decades can be replicated in a few months following cytoreduction. This similarity underscores the critical role of thymic function in normalizing T-cell subpopulations, repertoire and function.

Immune surveillance in the elderly

Having outlined the broad spectrum of immune system changes accompanying aging, we return to the original question of the role of these changes in cancer susceptibility. Studies of knockout mice have clearly established a critical role for the immune system in controlling spontaneous tumors. As many as 50% of aged IFN-$\gamma^{-/-}$ or perforin$^{-/-}$ mice develop spontaneous lymphomas, lung adenocarcinoma or sarcomas [52••]. Crossing tumor suppressor heterozygous p53$^{+/-}$ mice onto a perforin$^{-/-}$ background markedly increases the frequency and reduces the age of onset of lymphoma [53], suggesting that the tumor suppressor deficits that occur progressively during normal aging are amplified by the absence of immune surveillance. Immune deficiency in man, however, does not result in the severe consequences of the murine knockout models. Individuals with HIV-depressed immune function do not have an increased incidence of all malignancies, but mainly lymphomas or virus-based Kaposi's sarcoma; age-related cancers by contrast are predominately carcinomas [54]. Clearly much remains unknown about the successful mechanisms of immune surveillance in the aged. The prevalence of metastatic cancer at autopsy peaks at 75–90 years and declines in 95–99 year olds and centenarians [55]. Indeed, immune changes with aging may result in more indolent tumor growth and lower levels of metastases than in younger individuals, as in breast and prostate cancer [56]. Perhaps an increased understanding of the immune system changes in these extreme elderly may provide new insights into the complex relationship between immunity and cancer.

Conclusions

Our understanding of age-related immune deficits has rapidly improved in recent years. The broadening use of the SENIEUR protocol has provided a strong foundation for identifying critical parameters. Attention has justifiably focused on the fact that thymopoiesis is critical to restoring T-cell function. Studies of the interactions between thymic epithelia and thymocytes have begun to both outline the signaling pathways critical to thymopoiesis and identify the control points to reverse age-associated involution. Treatment with IL-7, growth hormone (GH) and keratinocyte growth factor (KGF) have all resulted in increased thymopoiesis in animal models of age-induced thymic involution or immune reconstitution after marrow transplant [33,57,58]. GH therapy also enhances bone-marrow cellularity and multi-lineage hematopoiesis, although improvements in follicular dendritic cells or B cells have not been assessed [57]. Still unknown are the mechanisms that alter the normal homeostatic balance of T-cell subpopulations in the elderly, stimulating pro-inflammatory cytokine production and perpetuating large oligoclonal populations of dysfunctional cells. Restoring a youthful balance of T-cell populations and function will depend upon studies of these changes.

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