Published in final edited form as:
Crit Rev Oncog. 2013 ; 18(6): 573–583.

Optimization of Immunotherapy in Elderly Cancer Patients

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Abstract

Increasing evidence has revealed the incidence of cancer augments with aging, which could be attributed to a multitude of age-associated changes including the dysregulation of the immune system. Although many reports demonstrate the efficacy of cancer immunotherapies in numerous preclinical studies, most experiments have been performed in young animals. Studies from our group and others show that cancer immunotherapy could be ineffective in old mice, even though the same therapeutic treatment works efficiently in young mice. Given that cancer occurs mostly in the elderly, we should take age-associated immune dysregulation into consideration to achieve the effectiveness of immunotherapeutic interventions in the old. Understanding both age-related and tumor-related immune alterations might be equally important in improving the effectiveness of immunotherapy. This article reviews a number of age-associated immune alterations with specific attention given to the impact on antitumor responses, and also discusses possible strategies for optimization of immunotherapeutic interventions in the elderly.

Keywords

Immunosenescence; elderly; immunotherapy; optimization

I. INTRODUCTION

Evidence has suggested that host immunity plays an important role in preventing and fighting cancer.[1–6] Therefore, cancer immunotherapies are considered to be a promising approach to treat these diseases, and numerous studies have demonstrated the successful outcomes of antitumor immune therapy in animal models. However, the clinical efficacy of immune therapies in cancer patients, many of whom are elderly, remains modest. In general, most preclinical studies of antitumor immune therapy have been conducted in young animals. However, studies, which particularly addressed the age-associated alteration in an immune response, mainly by comparing the difference in the reaction against immune

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The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

therapies between young and old animals, identified a clear discrepancy in the responses to immune therapies. Thus, age-associated immune dysfunction has been established.[7–10]

The increased incidence and development of many diseases including autoimmune diseases, infections, and cancers in the elderly may be caused, at least in part, by age-associated immune dysfunction. [11–13] Both animal studies and human data have revealed that age-associated alterations of the cellular components of innate and adaptive immunity, such as the reduced proliferative capacity of T cells,[14,15] reduced effector function of T cells,[16,17] reduced cytotoxic activity of T cells,[18,19] reduced capacity of response to exogenous antigens of B cells,[20–22] decreased number or impaired function of antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages,[23–25] qualitative alteration of natural killer (NK) and natural killer T (NKT) cells,[26–29] and accumulation of regulatory cells such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs),[30–33] may contribute to the poor efficacy of cancer immunotherapies. Therefore, it is important to consider these age-associated changes in immune function while designing effective cancer immunotherapeutic interventions in the aged individuals.

II. T CELL RESPONSE AND ANTITUMOR IMMUNITY IN AGING

Both experimental animal studies and human data have revealed that age-associated immune declines are mainly due to diminished T cell reactivity, including a reduced capacity of T cells to proliferate and survive after TCR stimulation.[14,15] Moreover, aged T cells exhibit decreased rescue from activation-induced cell death by co-stimulation.[16] Therefore, it is important to understand how advancing age affects immune responses within the T cell compartment with respect to tumor immunity. Early reports regarding age-associated decline in the function of the T cell compartment described profound impairment of endogenous polyclonal T cell responses mediated by both CD4 and CD8 T cells.[34–36] However, recent evidence suggests that there may be a functional difference on a cellular level between CD4 and CD8 T cells with age, particularly age-associated declines in T cell function occurring within CD4 T cells.[37,38] CD4 T cells play an important role by mediating both humoral and cellular immune responses in antitumor immunity.[39] Recent evidence has strongly suggested that the age-associated declines in CD4 T cells may contribute to the development of cancer in the elderly. The proliferative ability of naïve CD4 T cells from aged mice is significantly reduced, which may be associated with the reduced ability of CD4 T cells to secrete IL-2 and express the IL-2 receptor.[40,41] In addition, surface-activation markers, including CD154, CD25, and CD62L located on the effector T cells derived from aged CD4 T cells, are significantly decreased compared with those derived from young CD4 T cells. These defects could be overcome by additional IL-2 treatment *in vitro*, suggesting that the set of defects may be ascribed to lower production of IL-2 by aged naïve T cells.[42,43] One of the most notable age-associated changes in the peripheral T cell compartment is the decrease in the number of naïve T cells, which favors the increase of memory T cells. Advancing age is associated with an increased number of memory T cells; however, these cells are markedly defective in proliferation and cytokine production during recall responses. Haynes et al. found that memory T cells derived from old naïve CD4 T cells exhibit markedly reduced proliferation, cytokine production, and cognate helper function compared with those derived from young CD4 T cells.[44] Furthermore, these aged memory T cells were largely nonfunctional, whereas young memory T cells retain their function in aged mice. The upregulation of surface-expressed inhibitory receptors (e.g., PD-1) may cause the age-dependent functional decline in effector-memory T cells.[45–47]

CD8 T cells also play a crucial role in the control of antitumor immune responses, and age-associated changes in CD8 T cell-mediated immune responses have been demonstrated. In addition to the increased frequency of memory-phenotype CD4 T cells with age, advancing

age is also associated with the magnitude of the memory response in the CD8 T cell compartment. Previous studies have reported the accumulation of memory-phenotype CD8 T cells during aging both in animals and humans.[48] However, several recent cellular-based studies in mice have revealed that the age-associated defects in CD8 T cell responses are not likely the consequence of changes that are intrinsic to CD8 T cells, and that the effector function of CD8 T cells remains intact during aging. In the study reported by Li et al., Ag-specific CD8 T cell function was evaluated using both young and old TCR transgenic mice.[49] Those authors showed that aged naïve CD8 T cells differentiated into cytotoxic T cells as efficiently as their young counterparts. Similarly, in the study performed by Lyse et al., CD8 T cell activation and effector function were examined using TCR transgenic mice.[50] The above-mentioned authors also demonstrated an absence of intrinsic defects in the ability of aged naïve CD8 T cells to become primary effector T cells and to reject tumor challenges. These results indicate that the CD8 T cell function remains largely intact as a consequence of aging. Overall, these results suggest that, at least in mice, age-related alterations in the T cell compartment may be the consequence of the increased frequency of memory-phenotype T cells, rather than of the inherent decline in the function of T cells.

Gamma-delta T cells are also considered important effector cells of the immune system. These T cells constitute 5%–10% of all T lymphocytes and play an important role in immune defense against microbial phathogens.[51,52] Recent studies have also suggested that gamma-delta T lymphocytes may contribute to antitumor immune surveillance in the periphery.[53–55] In contrast to the large majority of T cells, so-called alpha-beta T cells, this class of T cells recognizes small non-peptidic antigens via gamma-delta antigen receptors (TCR) in an MHC-unrestricted manner, suggesting that they could exert antitumor effects despite decreased expression of MHC and tumor antigens on cancer cells. Therefore, their potential application in cancer immunotherapy has generated considerable interest. Several studies have also demonstrated the numerical and functional impairment of gamma-delta T cells in the elderly. Caruso's group has reported age-related numerical and functional alterations, as evidenced by the reduced number of circulating gamma-delta T cells, an impaired ability of *in vitro* expansion through different stimuli, enhanced expression of the early activation marker CD69, and increased production of tumor necrosis factor (TNF)-alpha by these cells in old people in comparison with young subjects.[56] Similarly, in the study performed by Provinciali's group, decreased numbers of circulating gamma-delta T cells was observed in old subjects, although the actual cytotoxic activity of sorted populations of the cells was preserved.[57] Moreover, in their analysis of melanoma patient samples, they revealed that the absolute number of circulating gamma-delta T cells and the ability of *in vitro* expansion of these cells were significantly reduced in melanoma patients, whereas these numerical and functional impairments were not correlated with age.[58] Further studies will be required to elucidate whether the age-related alterations of gamma-delta T cells contribute to the impairment of antitumor immune responses in the elderly.

III. DENDRITIC CELL FUNCTION AND ANTITUMOR IMMUNITY IN AGING

Dendritic cells (DCs) play a pivotal role in antitumor immunity by presenting tumor antigens to T cells, which results in the initiation of antigen-specific immune responses or immune tolerance.[59] Both experimental and clinical studies have demonstrated that the age-associated alterations in DC number and function may contribute to the impairment of immune function during aging. Decreased numbers or impaired function of DCs was demonstrated in aged mice. In several mouse strains, the numerical density of epithelial DC populations in aged mice was reduced compared with that observed in young mice.[60–63] Age-associated phenotypic alteration of DCs has also been demonstrated. The surface expression of co-stimulatory molecules such as MHC class II molecules and the intercellular adhesion molecule-1 (ICAM-1) on DCs in aged mice was significantly lower compared with

that observed in young mice.[60] In addition, the stimulatory capacity of DCs in aged mice was reduced compared with that detected in young mice. Moreover, splenic DC number and DC generation from bone marrow precursors in mice with spontaneous murine prostate cancer decreased with age.[64]

Studies in humans have documented decreased numbers and dysfunction of DCs in the elderly. It has also been demonstrated that the density of human gingival epithelium DCs in the elderly is significantly lower compared with that detected in a younger group.[65] Furthermore, it has been shown that decreased numbers of epidermal DCs in the elderly may be correlated with the development of skin cancer.[66] Recent studies have revealed that although the number of myeloid DCs was not altered with age, the number of plasmacytoid DCs was significantly altered during aging. This suggests that the age-related alteration of plasmacytoid DCs plays a crucial role in the development of immune suppression in the elderly.[67–69]

Several reports have demonstrated differences in surface phenotype and function of monocyte-derived DCs between elderly and young individuals. Steger et al. reported an absence of differences in the expression of several surface antigens on peripheral blood monocyte-derived DCs in old compared with those in young subjects.[70] Those authors also reported that monocyte-derived DCs from both the elderly and young have a similar capacity of antigen presentation and induced the antigen-specific T cell response equally.[71] Lung et al. also reported that the generation of peripheral blood monocyte-derived DCs was similar in the elderly and young.[72] These results suggest that DCs generated *ex vivo* from old subjects have the capacity to stimulate the immune response that is similar to that of young subjects. In contrast, there is increasing evidence that *in vivo* DCs in the elderly have impaired function and phenotypic alteration. Pietschmann et al. demonstrated the decreased expression of HLA-DR in the peripheral blood DCs of the elderly compared with the young, although the expression level of various other surface markers was not different between the two groups.[73] Della Bella et al. reported that DCs in the elderly exhibit a more mature phenotype and reduced ability of cytokine production.[69] Varas et al. showed a decrease in the level of several surface molecules, including MHC class II, CD86, CD40, and CD54 on thymic DCs from the elderly. In addition, these thymic DCs from old individuals had an impaired ability to induce allogeneic T cell responses.[73,74]

More recently, Grolleau-Julius et al. reported that DC-specific intracellular adhesion molecule type 3-grabbing, non-integrin (DC-SIGN), which has been identified as being expressed on APCs and having a function in T cell co-stimulation, decreased in aged DCs.[75] Moreover, Castle et al. revealed that increased production of IL-10 in DCs in the elderly is associated with age-dependent impaired proliferation of peripheral mononuclear cells.[76] Taking all these findings into consideration, *ex vivo*-generated DCs from precursor cells of old individuals are functionally and phenotypically similar to those from young individuals, whereas *in vivo* DCs in elderly subjects are phenotypically altered and functionally decreased compared with those of young subjects.

Several other groups have shown that DCs from the frail elderly are functionally decreased compared with those from the healthy elderly.[77] These results strongly indicate that age-associated declines in DC function impair the efficacy of DC-mediated antitumor immunity in the elderly. Grolleau-Julius et al. reported the impaired induction of the antitumor immune response by aged DCs in a murine melanoma model.[78] In their study, the growth of established melanoma tumors in mice injected with peptide-pulsed aged DCs was significantly faster than that of mice injected with peptide-pulsed young DCs. Similarly, Sharma et al. compared the vaccination efficacy of old and young DCs pulsed with apoptotic

tumor cells in young recipients, and demonstrated that old DCs presented a significantly reduced antitumor response compared with young DCs.[79]

In addition to the intrinsic deficiencies observed in aged DC, several recent studies have demonstrated that DC function may be affected by the aging microenvironment indirectly. Shi et al. have reported that the antitumor efficacy of young DC-based vaccination in aged mice was significantly impaired compared with that observed in young mice.[80] The decreased antitumor effect of DC vaccination in aged mice was potentially associated with increased number of NK1.1+CD3+NKT cells.[80] In agreement with this report, Grolleau-Julius et al. suggested in their review that the efficacy of vaccination with young DCs was significantly decreased in aged melanoma tumor-bearing mice compared with that recorded in young melanoma tumor-bearing mice.[78] Thus, alterations in DC-mediated antitumor T cell responses in aging are likely attributable to both intrinsic and environmental influences.[81]

IV. CO-SIGNALING MOLECULES AND ANTITUMOR IMMUNITY IN AGING

Co-signaling signals are essential for the augmentation of APC-mediated T cell responses in antitumor immunity. For example, expression of CD80 (B7.1) and CD86 (B7.2) on APCs or tumor cells is crucial for promoting antitumor T cell responses.[82] Several studies have demonstrated decreased expression of these co-signaling molecules on aged DCs, which may contribute to the reduced capacity for T cell stimulation by DCs during aging.[73,74] Lustgarten et al. investigated whether CD80 expression on tumor cells was important for the enhancement of antitumor responses in aged mice. Immunization with the foreign protein enhanced GFP (EGFP)-expressing BM-185 pre-B-cell lymphoma cells (BM-185-EGFP) yielded an antitumor immune response in young, but not in aged, mice. However, immunization with CD80-expressing BM-185-EGFP tumor cells (BM-185-EGFP-CD80) yielded an antitumor immune response in both young and aged mice, although the tumor rejection was lower in aged mice compared with young mice.[83]

There is increasing evidence that several other members of the TNFR family, including OX40 (CD134), 4-1BB (CD137), CD27, and CD30, may be important as secondary co-stimulatory molecules when co-signaling molecules are reduced and insufficient for T cell stimulation during aging. In studies evaluating the effect of additional OX40 signaling in antitumor immune responses in aged mice, immunization with BM-185-EGFP-CD80 in combination with an anti-OX40 mAb markedly improved antitumor response in aged mice.[83] Similarly, an apoptotic tumor cell-pulsed DC vaccine in combination with an anti-OX40 mAb significantly enhanced the antitumor immune response in aged mice.[84] Interestingly, Ruby and Weinberg investigated the efficacy of an anti-OX40 mAb alone in middle-aged and elderly sarcoma-tumor-bearing mice, and confirmed that the administration of the mAb to these mice significantly reduced antitumor efficacy because of the decreased number of differentiated T cells, and was not due to an alteration of the surface expression of OX40 on T cells.[85,86] Furthermore, administration of the anti-OX40 mAb in combination with IL-12, a cytokine that is essential for T cell differentiation, partially restored the deficiency in OX40-mediated antitumor efficacy in older mice. 4-1BB is also a member of the TNFR family that is expressed on activated T cells and co-stimulates both CD4 and CD8 T cells. In particular, Bansal- Pakala and Croft reported that the administration of an agonist Ab to 4-1BB rescued defective T cell priming in aged mice.[87]

Similarly, Sharma et al. revealed that apoptotic tumor cell-pulsed DC vaccination in combination with an anti-4-1BB mAb significantly enhanced the antitumor immune response in aged mice.[84] These results suggest that the insufficient antitumor immune responses in aging may be restored by the efficient expression of co-stimulatory signals.

V. TLRS AND ANTITUMOR IMMUNITY IN AGING

Recent studies have revealed that innate immune responses and adaptive immune responses collaborate to induce a strong antitumor immune response. For example, the mediation of innate immune responses by members of the Toll-like receptor (TLR) family results in the subsequent induction of protective adaptive immune responses in antitumor immunity.[88,89] Several studies have demonstrated that advancing age may affect the expression and function of TLRs, and the response to TLR ligands in the innate immune system. Renshaw et al. reported that aged splenic and peritoneal macrophages express significantly lower levels of TLRs and secrete significantly lower levels of cytokines after stimulation with various TLR ligands.[90] Previous studies have shown that CpG-ODN stimulates plasmacytoid DCs to produce type I interferons (IFN α and β), which inhibit the synthesis of Th2 cytokines by CD4 T cells and induce IL-4-inhibited Th1 cells to synthesize IL-2, IL-12, and IFN-γ. In turn, these induce NK cells, NKT cells, and CTLs in the antitumor immune responses.[91] Sharma et al. reported that the intratumoral injection of CpG-ODN yielded complete rejection of *in vivo* tumors in both young and old mice; however, injection of poly I:C exhibited *in vivo* tumor rejection only in young mice.[92] The authors also revealed that the induction of the antitumor immune response by *in vivo* challenge with CpG-ODN, but not poly I:C in old mice, was correlated with the upregulation of pro-inflammatory cytokine secretion, significant accumulation of CD4 T cells, CD8 T cells, NK cells, and APCs within the tumor, and reduction of the number of Tregs within the tumor. These results indicate that the efficacy of TLR ligands in antitumor immunity may be reduced during the aging process. Moreover, there is a difference in capacity to induce immune responses among the various TLR ligands in the old mice.[92] It appears that CpG-ODN may constitute a possible therapeutic approach to overcome the age-associated immune defects in cancer immunotherapy and restore the antitumor immune response in the elderly.

VI. IMMUNOSUPPRESSIVE CELLS AND ANTITUMOR IMMUNITY IN AGING

Advancing age may alter the prevalence and function of immune suppressive cells such as $CD4^+$ $CD25^+FoxP3^+$ regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). Tregs accumulate in tumor-bearing hosts and play fundamental roles in blocking antitumor immune responses.[93,94] MDSCs, a heterogeneous population comprised of immature myeloid cells, accumulate within sites of cancer, inflammation, and infection,[95,96] and have a strong capability to suppress both adaptive and innate immune responses. Accumulating evidence has revealed that the elimination of either population in a tumor-bearing host may contribute to enhanced antitumor immune responses.

Whereas the age-dependent changes in the number of Tregs and their function remain controversial, [30] several studies have suggested that Tregs accumulate with age and are involved in the age-associated immune dysfunction. Gregg et al. reported that the number of human peripheral blood Tregs increases with age, whereas the function of these cells is comparable between the young and the old.[97] Sharma et al. reported that the increased frequency of Tregs in aged mice prevented the cytotoxic T cell response in aged tumor-bearing mice.[98] Pan et al. also showed that the accumulation of Tregs in aged humans and mice was closely associated with lung tumor burden.[99] Further studies will be required to test Treg frequency and functions in the periphery versus within the tumor itself, and the impact on clinical outcome.

Similar to the Tregs, it has been demonstrated that the decline of antitumor T cell function in aged animal is also correlated with the accumulation of MDSCs with age. Grizzle et al. revealed that MDSCs are increased in the spleen of aged mice, and that adoptive transfer of these aged MDSCs delayed tumor rejection significantly in young tumor-bearing mice.[100]

More recently, the proportion of MDSCs was found to be elevated significantly in elderly patients with a history of cancer.[101]

Importantly, a number of pro-inflammatory cytokines that are required for the differentiation of MDSCs (e.g., TNF-α, IL-6, and IL-1β) are increased in the old,[101,102] suggesting that the age-related inflammatory milieu possibly promotes the accumulation and activation of MDSCs that subsequently could contribute to the increased aging-associated cancer incidence. Interestingly, it is possible to note a mutual interaction between the Treg cells and the MDSCs. In fact, MDSC contributes to Treg induction in cancer, but, in turn, Tregs may regulate MDSC expansion with a mechanism of positive feedback. In support, our group reported an age-specific inverse correlation between the prevalence of Tregs and MDSCs.[103] We examined the antitumor response to Treg depletion in the B16 melanoma model and revealed that Treg depletion alone using denileukin diftitox (DT) exerted therapeutic effects only in young mice but not aged mice. Furthermore, Treg depletion using DT in aged mice resulted in an increased number of MDSCs. MDSC depletion in combination with Treg depletion restored the impaired efficacy of Treg depletion in aged mice, suggesting that Tregs control the prevalence of MDSCs in aged mice and that Treg depletion in combination with MDSC depletion may be an effective cancer immune therapy approach for the elderly.

VII. CONCLUDING REMARKS

It is becoming evident that the deterioration of immune function with advancing age is a key factor in the increased susceptibility of elderly persons to cancer. However, this is also an area where there are few data available, and those that are tend to be controversial. Nonetheless, it has been demonstrated that age-related immune deficiency is attributable to both intrinsic alterations of the immune cellular components and extrinsic changes in the aged microenvironment. Thus, elucidating and understanding the complex among age-associated immune dysregulations in cancer development will shed new insight into developing the most optimal immunotherapeutic strategies. Importantly, data starting to accumulate using aged animals suggest that there is hope to treat the elderly more specifically and efficiently by cancer immunotherapies. However, to better uncover the cellular and molecular basis for the decline in immune function in the elderly, appropriate spontaneous animal tumor models rather than transplantable tumor system need consideration. The observations from these animals will be more comparable to the human aging environment and would have a better chance of being translated in the clinical setting in the elderly.

Acknowledgments

This work was supported partly by National Institutes of Health grants CA149669 (B.Z.) and CA170491 (T.J.C.), the Robert H. Lurie Comprehensive Cancer Center Director’s Fund (B.Z.), and P30 CA060553.

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