Cancer Vaccination at Older Age

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Abstract

Cancer vaccination is less effective at old than at young age, due to T cell unresponsiveness. This is caused by various age-related changes of the immune system, such as lack of naïve T cells, defects in activation pathways of T cells and antigen-presenting cells, and age-related changes in the tumor microenvironment. Natural killer, natural killer T cells, and γδT cells of the innate immune system also change with age but these responses may be more susceptible for improvement than adaptive immune responses at older age. This chapter compares various studies involving adaptive and innate immune responses in elderly and cancer patients, as well as cancer vaccination at young and old age. Finally, potential new directions in cancer vaccination at older age are discussed.

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With the current rise of the elderly population, cancer is becoming an increasingly frequent disease and cause of death. From 2010 to 2030, the total projected cancer incidence in the United States for older adults will increase by approximately 67% [1]. Indeed, metastatic cancer has surpassed heart disease as the primary cause of death in people younger than age 85 [2]. Therefore, in spite of some improvements in prevention and treatment, metastatic cancer is now the most frequent cause of death in the elderly, with co-morbid conditions complicating further treatment. When metastatic, cancer often needs aggressive, second-line treatment, for which there are few options. This is particularly challenging for frail, elderly cancer patients in which co-morbidity plays an important role. Immunotherapy may be our best and most benign option for preventing or curing metastatic cancer in such patients. Unfortunately, cancer immunotherapy is less effective at old than at young age, due to T cell unresponsiveness, especially in the tumor microenvironment (TME) [3, 4]. Various age-related changes of the immune system, such as lack of naïve T cells, defects in activation pathways of T cells and antigen-presenting cells (APC), and immune suppression in the TME contribute to T cell unresponsiveness at older age [4].
Analysis of various vaccine studies in preclinical cancer models at young and old age showed that vigorous anti-tumor responses could be obtained by tailoring vaccination to older age, but in most cases T cell responses were hardly detectable. Therefore, we questioned the feasibility of T cell activation in the TME by vaccination at older age, and whether activation of innate immune responses against cancer could be a more feasible approach since innate immune responses seems less affected by aging than adaptive immune responses. To answer these questions, we reviewed adaptive and innate immune responses in elderly and cancer patients, and compared vaccine studies in preclinical models at young and old age. These studies strongly suggest that adaptive and innate immune responses should be activated against cancer through vaccination or immunotherapy, respectively, at older age. Finally, we propose new vaccine and immunotherapeutic strategies focusing on improvement of adaptive and innate immune responses at older age, respectively.

Decreased Immune Responses in Elderly

Adaptive Immune Responses
One of the most important changes in the immune system at older age is the decline in responsiveness of T cells to new antigens. This is mainly caused by a strong decrease in the number of naïve T cells (capable of reacting to new antigens) and an increase in the number of memory T cells (capable of reacting to previously exposed antigens) at old compared to young age [5]. However, other possible causes for decreased T cell responses in aged humans and mice have also been described, such as defects in T cell receptor (TCR)/CD3-mediated phosphorylation events or aberrant regulation of tyrosine kinases associated with the TCR [6], and an age-related decrease in the αβ repertoire of the human TCR [7]. The TCR is expressed by T cells, and is required for recognition of foreign antigens in association with self-major histocompatibility complex (MHC) molecules, presented by APC to the immune system, and for subsequent activation of T cells. In addition, an age-related decrease in the expression of CD28 on the cell membrane of T cells, which provides a secondary signal for T cell activation when ligated to the B7 molecule on APC, has been reported [8]. Decreased production of interleukin (IL)-2 or interferon (IFN)γ at old compared to young age in individuals vaccinated with influenza virus or in vitro upon stimulation with influenza virus has been shown as well [9].

Innate Immune Responses
Cumulative evidence indicates that aging exerts significant effects on all cells of the innate immune system [10]. This includes natural killer (NK) cells, natural killer T (NKT) cells, γδT cells, dendritic cells (DC), macrophages, and neutrophils. NK cells are the most well-known cells of the innate immune system. NK cell function has been extensively studied in relation to aging in mice and humans. Although in
25-month-old mice NK cell number and function, such as the production of IFNγ, IL-2 of perforin, is decreased at old compared to 8-week-old mice, it has been reported that in human healthy centenarians NK cytotoxicity by activation with IL-12, IFNα, and IFNγ is well preserved, but somewhat decreased in less healthy elderly [11]. In our studies we found that the production of IFNγ by NK cells induced by vaccination with an attenuated *Listeria monocytogenes*-based vaccine was almost as good in old as in young mice [unpubl. data].

NKT cells are considered to be a member from the innate immune system because of their early response against infection and perhaps against cancer. They represent a heterogeneous T cell population that shares some functional and phenotypical characteristics with NK cells. It has been reported that the number of NKT cells increases with age [12], while their Th1 cytokines decreases with age [13]. However, liver NKT cells bearing TCRγδ are not only strongly increased in number but their functions are also well preserved in very old mice and humans [14].

γδT cells also belong to the innate immune system because of their early response against infection and perhaps against cancer. They are characterized by their ability to respond to non-processed and non-peptidic phosphoantigens in a MHC-unrestricted manner [15]. In human peripheral blood, two main populations of γδT cells have been identified based on their TCR composition. The predominant subset expresses the Vδ2 chain associated with Vγ9 and represents 70% of the circulating γδT cells in adults [15]. It has been reported that the percentage of TNFα-producing γδT cells increased with age, while the percentage of IFNγ-producing γδT cells did not alter with age [16].

DC in blood or Langerhans’ cells in skin play a central role in T cell activation, but the results reported so far are variable. For instance, it has been demonstrated that blood DC from old individuals can still function as powerful APC when exposed to purified protein derivate of *Mycobacterium tuberculosis* or influenza vaccine [17, 18], while others have shown that DCs from aged individuals are more mature and have impaired ability to produce IL-12 [19], or that secretion of tumor necrosis factor (TNF)α and IL-6 significantly increased upon stimulation with lipopolysaccharide and ssRNA in DC of aged compared to young individuals [20].

**Decreased Immune Responses in Cancer Patients**

*Adaptive Immune Responses*

In cancer patients, cytotoxic T lymphocytes (CTL), recognizing tumor-associated antigens (TAA) in association with MHC molecules on the tumor cells through their TCR, and expected to destroy tumor cells when exposed simultaneously to both TAA/self-MHC complexes and co-stimulatory molecules, are often found at the site of the tumor, but have evidently been unable to destroy the tumor cells [21]. Multiple possible causes have been described for this unresponsiveness of the CTL in cancer...
patients [for a review, see 3]. This includes decreased expression of MHC, TAA, or co-stimulatory molecules by tumor cells, and immune suppression induced by the primary tumors. In humans and mice, many tumors secrete lymphokines or factors that inhibit vaccine-induced T cell and NK cell responses. Examples are transforming growth factor (TGF)β, IL-6, IL-10, cyclooxygenase-2, and its products prostaglandin E2, PD-1 ligand, or indolamine 2,3-dioxygenase. Immune cells in the TME attracted and activated by the primary tumor such as myeloid-derived suppressor cells (MDSC) also suppress T cell and NK cell responses by the production of IL-6, IL-10, TGFβ, reactive oxygen species, inducible nitric oxide synthase or arginase [22], while tumor-associated macrophages and M2 macrophages strongly suppress T cell responses through the production of IL-6, IL-10, TGFβ in the TME [23]. Interestingly, it has been reported that the TME changes with age, i.e. it appeared that the number of MDSC increases with age, and that this contributed to the T cell unresponsiveness at older age [24]. So far, little research has been performed on MDSC and T cell unresponsiveness in relation to aging. Inducible T_{regs} also play an important role in suppression of the immune system in cancer patients, through the production of soluble factors such as IL-10 and TGFβ or through direct cell-cell contact, resulting in the inhibition of T cell and NK cell responses [25]. Moreover, evidence exists that the number of T_{regs} increases with age [26].

**Innate Immune Responses**

In vivo depletion of NK cells leads to a poor control of tumor growth in various cancer models, indicating the importance of NK cells in anti-tumor responses and tumor surveillance [27]. Evidence exists from mice and humans that NK cells alter with age, but that they still function at older age. However, the effect of aging on NK cells against cancer has been far less extensively studied than T cells. A few reports describe that NK cells of elderly had a lower ability to respond to IL-2, lower spontaneous cytolytic activity towards tumors than young adults [28]. However, NK cells can also be used to kill tumor cells through other pathways than perforin-mediated tumor cell destruction. For instance, a clinical trial is ongoing with bortezomib which sensitzes tumor cells for TRAIL- and FasL-mediated destruction by NK cells in cancer patients between 20 and 70 years (NCT00720785) [29]. We found NK cell responses (producing IFNγ) in vivo in old mice with metastatic breast cancer after vaccination with pcDNA3.1-Mage-b [3], or with Listeria-Mage-b [unpubl. results].

NKT cells also have anti-tumor activity in mice, including lung and hepatic cancer metastases when activated by α-galactosylceramide (αGalCer), by secreting large amounts of IFNγ and IL-4, resulting in activation of other cells of the immune system including NK cells [30, 31]. In a phase I clinical trial with αGalCer in patients with solid tumors, the effect was dependent on the high number of NKT cells present pre-treatment [32]. Since the number of NKT cells increases with age, αGalCer could be a potential candidate to activate NKT cells against cancer at older age.
It has been reported that the percentage of γδT cells producing TNFα decreased in melanoma patients, but that the percentage of γδT cells producing IFNγ stayed unaltered independent of age [15]. Moreover, patients with lymphoid malignancies treated with IL-2 showed improved γδT cell responses in correlation with improved objective responses to therapy [33]. The anti-tumor effect of γδT cells was confirmed by in vitro assays showing that γδT cells recognize and kill a broad spectrum of B-cell lymphomas in vitro. The absence of effect of aging on the production of IFNγ by γδT cells and their anti-tumor effect makes γδT cells a highly attractive target for immunotherapy against cancer at older age.

**Improvement of Cancer Vaccination at Old Age in Preclinical Models**

More than 50% of all cancer patients are 65 years or older. The vaccine studies discussed below show that cancer vaccination is less effective at old than at young age, but that tailoring cancer vaccination to older age is feasible. Moreover, innate immune responses may also be a potential target for immunotherapy against cancer.

The research group of Provinciali [34] reported that immunization with a highly engineered mammary adenocarcinoma TS/A-IL-2, protected both young and old mice from TS/A challenge which was not possible without IL-2. CD4 and CD8 T cells were present in tumors of young but hardly detectable in tumors of old mice, while macrophages and neutrophils were detected at both ages. However, protective memory responses that could reject tumor cells upon re-challenge of tumor-free mice was only obtained in young mice. Another study by Provinciali’s group [35] showed that vaccination with pCMV-neuNT against Her2/neu-expressing breast tumor cells (TUBO) completely protected young mice but only 60% of the old mice from TUBO challenge, and correlated with proliferation of spleen cells of young compared to old mice, in vitro upon re-stimulation with the Her2/neu antigen. In a later study, Provinciali et al. [36] showed that cytotoxicity of CD8 T cells was improved at old age by improved DNA uptake using the combination of intramuscular immunization and electroporation, compared to intramuscular immunization only and that this correlated with complete rejection of TUBO cells in old mice. These results suggest a poor uptake of DNA by APC at old age, and that this could be avoided by delivering the plasmid DNA by electroporation.

The group of Lustgarten [37] also found that cancer vaccination was less effective at old than at young age. They showed that young but not old mice developed long-lasting memory responses to a pre-B-cell lymphoma (BM-185). However, inclusion of CD80 to the BM-185 cell line (BM-185-CD80) plus agonist anti-OX-40 or anti-4-1BB (receptor for co-stimulation on T cells) mAb induced equally strong long-lasting memory responses at young and old age, suggesting the involvement of T cell responses. In another study they also found that adding anti-OX40 or anti-4-1BB mAb to a DC vaccine resulted in vigorous anti-tumor responses in a syngeneic
TRAMP-C2 model at young and old age, while without anti-OX40 or anti-4-1BB, protection was significantly better in young than in old mice [38]. Moreover, immunization of young and old mice with DC-TRAMP-C2 vaccine plus anti-OX40 or anti-4-1BB mAb resulted in improved CTL responses to apoptotic TRAMP-C2 cells in vitro upon re-stimulation, compared to the same vaccination without OX40 or anti-4-1BB mAb at old age, but the CTL responses were less vigorous compared to the same immunizations at young age.

Grolleau-Julius et al. [39] showed that vaccination with a DC-OVA vaccine derived from young mice was less effective against B16-OVA melanoma tumors in old than in young mice, indicating the altered TME at older age and its effect on vaccination. The group of Zhang [24] also found that the TME was altered at old compared to young age. They demonstrated that the number of MDSC increased in the tumor environment of old compared to young mice, and that this contributed to the age-related T cell unresponsiveness.

In our laboratory, we developed a DNA vaccine of Mage-b (pcDNA3.1-Mage-b) and tested this vaccine at young and old age in two syngeneic metastatic mouse breast tumor models, 4TO7cg and 4T1, both overexpressing Mage-b in metastases and primary tumors [3]. Vaccination of both models with Mage-b was highly effective against metastases at young but not at old age, and this correlated with strong Mage-b-specific T cell responses in vitro and in vivo at young but not at old age [3]. Interestingly, we found that Mage-b vaccination activated macrophages and NK cells (producing IFNγ) in old mice [3]. In another more recent vaccine study with Mage-b delivered through a highly attenuated *L. monocytogenes*, we found a dramatic effect on the metastases in the 4T1 model at young age [40]. However, we discovered that this was not solely due to Mage-b, but rather to the direct infection and kill of tumor cells by *Listeria* [40]. Since *Listeria*-infected tumor cells highly express *Listeria* proteins, the tumor cells become a highly sensitive target for NK cells and *Listeria*-specific CTL [40]. We found that the *Listeria*-based vaccine was equally effective against metastatic breast cancer at young and old age [unpubl. results]. NK cell responses were also strongly activated by *Listeria* at young and old age, and may have contributed to the reduced growth of metastases at both ages as well.

**Concluding Remarks**

The main conclusion from the studies analyzed here is that the innate immune system should also be considered for testing as a potential candidate for immunotherapy at older age. This is based on the following findings. While the effect of cancer vaccination on growth of tumors and metastases could be strongly improved by tailoring the vaccine to older age, as shown in the preclinical studies analyzed here, in most cases improvement was not the result of T cell activation but rather the result of other immune cells stimulated by the vaccine. Although various functions of NK, NKT, and...
γδT cells are decreased at old age, it is far less dramatic than the age-related decline in T cell function, and these cells play an important role in anti-tumor responses. However, improvement of T cell activation against cancer through vaccination at older age should also be further optimized. Below, new strategies to improve adaptive and innate immune responses against cancer at older age through vaccination or immunotherapy, respectively, are proposed below and summarized in figure 1.

As mentioned above, innate immune responses should be considered as a potential target for improvement of immunotherapy against cancer at older age. For instance,
NK cells and NKT cells could be activated by attenuated *Listeria* or αGalCer, both cell types are present in sufficient numbers at older age, and both cell types exhibit anti-tumor activity. γδT cells could also be a new target for cancer immunotherapy at older age. The production of IFNγ by γδT cells seems to be unaffected by age. Moreover, patients infected with *L. monocytogenes* showed higher percentage of γδT cells than uninfected controls [41]. It has also been shown that γδT cells exhibit anticancer activity [33].

MDSC increases at older age and is responsible for the age-related T cell unresponsiveness in the TME. Elimination of MDSC may result in reduced immune suppression in the TME. It has been reported that CpG ODN, vitamin A, curcumin and several chemotherapeutica eliminate MDSC [42, 43]. It appears that CpG seems especially good at enhancing cellular and humoral immunity and promoting Th1-type responses in old mice [44]. We found that *Listeria* reduced the number of MDSC at young and old age [unpubl. results]. Elimination of immune suppressing tumor-associated and M2 macrophages may also lead to improved T cell activation in the TME at young and old age.

T cells could also be activated through other strategies. For instance, the problem of lack of naïve T cells, one of the most important changes at older age, could be avoided by immunizing at young age when sufficient naïve T cells are present, followed by recall at old age to reactivate memory T cells. Such an approach has been successfully used for improving antibody production at older age [45]. Also, naïve T cells could be recruited by IL-7 [46]. However, lack of naïve T cells is not the only hurdle to overcome. TAA are weakly immunogenic and T cells need help to become activated against TAA expressed by cancer cells. As shown in the studies discussed here, just adding IL-2 to TS/A tumor cells will improve anti-tumor responses but not memory responses to the tumor at old age. The best results so far have been shown by the group of Lustgarten [38] by activating T cells against cancer through vaccination plus co-stimulation using anti-OX40 or 1-4BB mAb at young and old age. Also, elimination of Tregs could improve T cell activation at older age [25].

Finally, we have shown that an attenuated *L. monocytogenes* can be used to deliver genes directly and selectively in tumor cells in vivo [40]. Also other non-pathogenic bacteria are currently under investigation for the delivery of genes selectively into tumor cells such as *Lactococcus lactis* and *Escherichia coli* [47]. Our results suggest that such an approach could be effective at young and old age. Also magnetic beads can be used to improve the selective delivery of agents at the tumor site that improves anti-tumor responses or kill tumor cells directly, with minor side effects on normal tissues [48].

In summary, despite all the obstacles that need to be overcome, vaccination against cancer is potentially the most promising approach. While cancer vaccination has limited success against late stage tumor development, it can be particularly effective where almost all other therapies struggle, i.e. against metastases and recurrence of cancer. The vaccine studies analyzed here show that improvement of vaccine efficacy
at older age is possible, but that in addition to activation of T cells, the innate immune system should also be considered as a possible target for immunotherapy against cancer at older age. The advantage of activating adaptive immune responses by vaccination is its prophylactic and therapeutic application, while activating innate immune responses by immunotherapy can only be applied therapeutically. Finally, the results of these studies demonstrate the need of testing and tailoring cancer vaccines to older age in preclinical models before entering the clinic.

References

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