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Review

# Immunity, Cancer and Aging: Lessons from Mouse Models

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**ABSTRACT: The deterioration of immune function with advancing age is associated with an increased incidence of cancer. Most of the studies to evaluate the effect of immunotherapy on cancer have been conducted in the young without considering the effect of age-associated changes in immune function. Studies from my laboratory and others groups indicate that immunotherapeutic interventions could be effective in young animals, but that the same therapies are not as effective in old animals. The present review summarizes some defects found in the old immune system affecting the activation of antitumor immune responses, the strategies used to activate a more robust antitumor immune response in the old and the description of a preclinical tumor model indicating possible strategies for optimization of immunotherapeutic interventions in the old.**

***Key words:*** Aging; Cancer; Immunity; Intervention; Immunotherapy

Statistically it has been established that the incidence of cancer is increased with age [1, 2]. Although the underlying mechanism is not completely clear of why there is an increase in the number of cancers after the age of 65, it is believed that it is due to the cumulative number of events such as; exposure to carcinogens, accumulation of mutations and a diminishing of immune function. Based on animal and human data there is strong evidence suggesting that the immune system is critical in defending and preventing the formation of tumors in which this process is defined as immunosurveillance and immuno-editing of cancer [3]. Using knockout mouse models such as INF-γ, RAG2, perforin and others, it has been demonstrated that these animals are more susceptible to tumor formation following carcinogen exposure[4-7]. These studies indicate that immunosurveillance is an important mechanism that provides immune protection resulting in the inhibition of carcinogenesis and maintaining normal cellular homeostasis.

With the advancement of age there are characteristics and functions of the immune system that show a dysregulated response. These changes or alterations are observed in the innate and adaptive immune cells [8-10]. As such, it has been hypothesized that due to these alterations the elderly are less protected and consequently more susceptible to infectious diseases and cancer. There is not one particular factor or cause that can be pointed to as the mechanism for the age related changes in the immune function, rather it is an accumulation of events that deteriorate the immune responses. A major characteristic in the T cell system is that in the aged there is a decrease in the naïve T cell population and an increase of memory T cells creating an imbalance in memory/naïve T cell populations which may, in part, account for the hyporesponsive state in the aged [11, 12]. In addition there are a lower number of available naïve T cells in the old with a reduced capacity to react to new antigens [13]. The majority of immune cells in the old are associated with defects or alterations making the elderly more susceptible to cancer.

## Inflamm-aging and immune system

It is well established that aging is characterized by a pro-inflammatory status with an increase in the level of cytokines, chemokines and other factors. This state of sub-clinical, chronic inflammation has been called

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"inflamm-aging" [14]. It is believed that inflamm-aging results from exposure to continuous antigenic stimulation of inflammatory cells such as macrophages (MØ) or dendritic cells (DCs) [15]. Inflamm-aging is associated with higher levels of cytokines such as IL-1β, IL-6, IL-18, TNF-α and chemokines such as RANTES, MIP-α, IL-8 and MCP-1 [15, 16]. Inflamm-aging can trigger a series of diseases with an inflammatory pathogenesis such as diabetes, neurodegeneration, cardiovascular pathologies, and cancer. It is thought that chronic CMV infection or other infections could trigger inflamm-aging, however old animals kept under sterile conditions still suffer from inflamm-aging. Therefore, there are other mechanisms that can trigger and/or influence inflamm-aging. The inflammatory status in the old does not only originate from cells of the immune system but it is also influenced by other non-immune cells which have undergone genotoxic stress-induced senescence and can secrete many inflammatory factors, called senescence-associated secretory phenotype (SASP) [17]. Additionally, this chronic inflammatory status can also modulate the function of several immune cell types by altering or dysregulating the properties of the immune system in the old [18, 19]. This is reflected in the poor immune responses to infection or vaccination strategies by the elderly, who also suffer from recurrent bacterial and viral infections [20-22].

## T cells and dysregulation in the old

To identify which biological pathways truly affect the function of aged T cells and define differences between young and old naïve and memory CD4+ and CD8+ T cells, microarray analysis was performed pre- and post-TCR stimulation. For these experiments young and old CD4+ and CD8+ naïve (CD44low/CD62Lhigh) and memory (CD44high/CD62Llow) T cells were isolated and either unstimulated or stimulated with anti-CD3 plus anti-CD28 mAb for 4, 12, 24 and 72 hours: At these time points cells were collected, RNA was isolated, labeled and hybridized to a whole mouse genome chip for microarray analysis. Data analysis was approached from two perspectives: 1) to reveal the innate differences between young and old naïve CD4+ and CD8+ T cells; and 2) to discover the changes in T cell function in old T cells as defined by altered responses after TCR stimulation. The combination of both analyses resulted in insights into the fundamental differences that exist in the transcriptome of matched old and young T cell populations in contrast to the acquired functional differences in aged cells. Although the scope of this review does not focus on a detailed discussion of transcriptional data, a brief example of the type of analysis performed is as follows. Genes that were innately differentially regulated and those which were differentially expressed upon TCR activation in old CD4+ or CD8+ T cells compared to young T cells were tabulated. These differentially regulated gene candidates were grouped into gene ontologies to examine which cellular processes were particularly differentially regulated over time. Overall, the data indicates that alterations in immune-functions in old CD4+ and CD8+ T cells is due to modifications in various cellular functions such as inflammation, migration, adhesion and numerous signal transduction pathways [23]. In addition, this type of analyses also allows us to have a general picture about the behavior of the transcriptome. Based on kinetic analysis it is possible to determine whether or not differential expression was observed prior to stimulation or if it was maintained or acquired post-stimulation. This information is critical to optimize the CD4+ and CD8+ T cell responses in the old. We certainly do not understand the implications of all the genes that are differentially expressed between young and old T cells. However, this type of analysis reveals the transcriptional modulation of specific genes which might reflect functional changes in the aged immune system. Such information allows us to postulate new hypotheses addressing why old T cells are dysregulated compared to young T cells.

## Innate immune system, induction of antitumor response and aging

The innate immune response relies on the recognition of foreign antigens by receptors that recognize specific structures found exclusively in microbial pathogens termed pathogen-associated molecular patterns (PAMPs) [24, 25]. The recognition of PAMPs by antigen presenting cells (APCs) is mediated by the Toll-like receptor (TLRs) family [26, 27]. There are more than 10 known TLR family members capable of sensing bacterial components such as Poly-I:C (TLR-3), LPS (TLR-4), flagellin (TLR-5), imiquimod (TLR-7), CpG-ODN (TLR-9) and other microbial products [28]. The TLRs have distinct patterns and locations of cellular expression. A wide variety of TLRs are expressed in immature or mature DCs, macrophages (MΦ) and monocytes; and these receptors control the activation of those APCs. Now that specific ligands have been identified for most of the TLRs, it is finally possible for immunotherapy to move away from the nonspecific effects of whole bacterial extracts and determine whether the same or even better therapeutic responses may be induced using synthetic TLR ligands. There is accumulating evidence indicating that targeting APC with different types of TLR-ligands results in the

induction of a strong antitumor immune response resulting in the rejection of tumors [29-32]. With respect to aging and TLRs, Renshaw et al. [33] demonstrated that the expression of TLRs in splenic and thioglycolate-elicited macrophages was reduced in aged animals which is associated with lower secretion of cytokines following stimulation with various TLR-ligands. Boehmer et al. [34] also showed that age-related deterioration of TLR-mediated signaling was due to the decreased expression of mitogen-activated protein kinases. We evaluated whether in vivo targeting of APCs with TLR-ligands results in the restoration of the immune responses and activation of antitumor responses in the old. We compared the antitumor potential of TLR ligands such as Poly-I:C, LPS, flagellin, Imiquimod and CpG-ODN in young and old tumor bearing mice. Our results indicated that only intratumoral (i.t.) injections of CpG-ODN induced a complete rejection of tumors in young and old mice. Intratumoral injections of Poly-I:C also induced the rejection of tumors in the young but not the old. We observed significant differences in the activation of immune responses following CpG-ODN and Poly-I:C injections in the aged. The induction of an antitumor response by CpG-ODN correlates with the activation of a Th1 type pro-inflammatory response resulting in the generation of CD4+ T cell, CD8+ T cell and NK cell responses, activation of APCs and a significant reduction in the number of Tregs in the old [35]. These studies indicate that not all TLR ligands have the same effector function in the young and the old. Additionally, the selection of an adjuvant (e.g. CpG-ODN) is critical in optimizing a vaccination strategy for the young and the old. Taken together, these results indicate that there is a TLR age defect altering the function of the old innate immune system [36].

**Immune Suppression and Aging**

The most common regulatory cells are the Tregulatory cells (Tregs) [37, 38]. Tregs can be CD4+CD25+ or CD8+CD25+ T cells which are characterized by the expression of the forkhead lineage specific transcription marker, Foxp3 [39]. Tregs maintain and induce immune cell tolerance [38] by directly inhibiting T cells, NK cells and DCs through direct cell-cell contact mechanisms [37, 38]. Depletion of Tregs leads to organ specific autoimmune disorder [40, 41]. We have recently demonstrated that old mice have twice the number of CD4+CD25+Foxp3+ and CD8+CD25+Foxp3+ cells in spleen and lymph nodes when compared to spleens and lymph nodes from young mice [42]. Consequently, depletion of CD25+ cells with anti-CD25 mAb in old mice resulted in the rejection of the immunogenic BM-185-EGFP tumor cells and restored antitumor T cell cytotoxic activity against the surrogate EGFP-tumor antigen. These results indicate that the accumulation of Tregs in the old inhibit or prevent the activation of immune responses. These results are in agreement with the findings of Gregg et al. [43] that show increased numbers of CD4+CD25hi T cells (Tregs) in humans. Recently Lages et al. [44] showed a higher total number of Tregs in humans and mice that alters immune responses against *Leishmania major* infections. The imbalance of Treg homeostasis could then predispose the aged to immune dysfunction resulting in a higher risk of immune-mediated diseases, cancer or infections. Additionally, the accumulation or increased numbers of Tregs will affect or disturb the activation of antitumor immune responses in the old [35-42]and the depletion or reduction of Tregs might be critical to optimally activate an immune response in the aged.

Myeloid derived suppressor cells (MDSC) are a heterogeneous population comprised of macrophages, neutrophils, granulocytes and dendritic cells. These cells are characterized by the expression of CD11b and Gr-1 cellular markers. MDSC can suppress the activation of CD4+ and CD8+ T cells inhibiting the generation of an antitumor response [45-47] MDSC are thought to be induced by a variety of cytokines and growth factors (e.g. TGF-β and VEGF) which are produced within the tumor microenvironment [48, 49]. Though the biology and pathological functions of MDSC under non inflammatory conditions are not fully understood, we have evaluated whether there were differences in the level of CD11b+Gr-1+ cells between young and old mice. Our results indicate that old mice have a higher accumulation of CD11b+Gr-1+ cells in spleen and bone marrow of old mice when compared to young mice. Furthermore, tumor samples of old mice have a higher incidence of CD11b+Gr-1+ cells than young tumors (N.M. and J.L. unpublished results). These results are in agreement with the recent report by Grizzle et al. [50] where 12 month old BXD12 mice show a higher accumulation of CD11b+Gr-1+ cells than in two month old BXD12 mice. Furthermore, they demonstrated that old CD11b+Gr-1+ cells are more suppressive than young CD11b+Gr-1+ cells. This raises the question as to why there is an accumulation of CD11b+Gr-1+ cells in the old and why they are more suppressive. A simple explanation for these findings could be as follows: Typically, freshly isolated CD11b+Gr-1+ cells do not have the capacity to inhibit T cells [51]. Only CD11b+Gr-1+ cells isolated from an inflammatory environment such as a tumor have the capacity to inhibit T cells [52, 53]. There is cumulative evidence indicating that the production of a number of inflammatory

cytokines such as IL-4, IL-10, TGF-β and others are elevated in the old [54, 55]. Perhaps this inflammatory condition of the old promotes the accumulation and activation of CD11b+Gr-1+ cells that subsequently could inhibit the activation of immune responses.

Indoleamine 2,3-Dioxygenase (IDO) is an immunosuppressive molecule capable of inhibiting T cells and other immune cells [56]. IDO is primarily present in antigen presenting cells. The expression of IDO on tumor cells is implicated in suppression of immune responses and tumors become resistant to immunologic rejection [57, 58]. Expression of IDO is correlated with poor clinical prognosis in several types of cancer [59, 60]. 1-methyl tryptophan (1MT) is an important competitive inhibitor of IDO [61, 62]. On the basis of these findings, 1MT is now tested in clinical trials. We tested whether old animals have higher levels of IDO than young animals and indeed our results indicate that this is the case. Using a breast tumor model, when old tumor bearing mice were treated with 1MT plus immunotherapy, we observed an improvement in the antitumor response compared to animals treated with immunotherapy alone (S.Z.M and J.L. unpublished results). Recently, Pertovaara et al. [63] showed that IDO was significantly higher in nonagenarian compared to young. It is not clear yet to us as to why IDO is up-regulated in the old, but taken together these results suggest that elevation of IDO is another possible mechanism by which T cell responses are inhibited in the old.

The B7 family consists of activating and inhibitory co-stimulatory molecules that positively and negatively regulate immune responses [50, 64]. The B7 family and other co-stimulatory molecules used by APCs directly influence and/or fine-tune T cell responses [65]. Currently the B7 family is comprised of seven members, which are B7.1 (CD80), B7.2 (CD86), B7-DC (PD-L2 or CD273), B7-H1 (PD-L1 or CD274), B7-H2 (ICOSL), B7-H3 (CD276) and B7-H4 (B7S1 or B7x) [66]. B7.1 and B7.2 provide T cell signaling through CD28 and cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptors. The interaction of B7.1/B7.2 and CD28 provides a positive signal stimulating the immune response [67], whereas the interaction of B7.1/B7.2 with CTLA-4 is inhibitory and will subdue the immune response [68]. B7-H1 binds to the programmed cell death 1 (PD-1; also known as CD279) receptor [69]. Engagement of PD-1 with B7-H1 suppresses T cell responses [70]. Furthermore, PD-1 null mice develop strain specific autoimmune syndromes later in life [71], and B7-H1 knockout mice showed an increase susceptibility to experimental autoimmune diseases such as hepatitis and encephalomyelitis [72]. B7-H1 is highly up-regulated in tumors, impairing T cell anti-tumor responses [73]. These results indicate that the interactions of B7-H1:PD-1 have a suppressive function inhibiting T cell effector responses [74]. Expression of B7-H1 on T cells transmits signals regulating the function of these cells [75], which indicates that B7-H1 can act both as a ligand and receptor to execute immunoregulatory functions. The interaction of B7-H2:ICOS is critical for the generation of Th2 type responses and regulating humoral immunity [76]. B7-H3 costimulation increases proliferation of CD4 and CD8 T cells, enhances IFN-γ production and cytotoxic activity [77]. B7-H4 remains an orphan ligand and currently the known functions of B7-H4 are exclusively inhibitory [78]. There is no existing information about the expression of B7-family and CD28-family receptors in the old. Considering that the interactions of B7-family receptors with their respective ligands could fine tune immune responses, we sought to evaluate whether differences in B7-family and CD28-family receptor expression between young and old macrophages, dendritic cells, CD4+ and CD8+ T cells could contribute to the immune dysfunction in the aged. Although we observed that higher percentages of young macrophages, DCs, CD4+ and CD8+ T cells express B7.1, B7.2 and ICOS, the most surprising result was that higher percentages of old CD8+ T cells express B7-H1 compared to young CD8+ T cells. Based on the regulatory-function of these molecules, it can be hypothesized that the differential expression of the B7-receptors could modulate or interfere with the effector-function of T cells and modify regulation of immune responses. Since B7.1, B7.2 and ICOS positively regulate immune responses and B7-H1 negatively regulates immune responses, we were more interested in examining the expression of B7-H1 in old CD8+ T cells for three fundamental reasons: 1) CD8+ T cells are critical cells for tumor rejection, viral clearance and other immune responses; 2) numerous old CD8+ T cells expressing B7-H1 could inhibit the activation of immune responses in the aged; and 3) for therapeutical purposes it is more accessible to block or down regulate the expression of a receptor than to increase its expression. We hypothesized that the expression of B7-H1 could impair the proper activation of old CD8+ T cells and that blockade of B7-H1/PD1 interaction could enhance or restore the old CD8+ T cell responses. Indeed, blockade of B7-H1 in old CD8+ T cells restored the proliferation of these cells, and *in vivo* treatment with anti-B7-H1 mAb restored effective immune responses in aged animals. Furthermore, treatment with anti-B7-H1 also restored the expression of TCR complexes, which were otherwise constitutively down-regulated in old CD8+ T cells. The results imply that the expression of B7-H1 in

old CD8+ T cells and blockade of B7-H1:PD-1 interactions could be used to manipulate the aged immune responses.

## Immune-costimulation, antitumor responses and aging

Often the addition of co-stimulatory signals are used to enhance and augment the immunogenicity of tumors [79, 80]. For example, expression of B7.1 or B7.2 by tumor cells enhance the antitumor response resulting in the rejection of the tumor [81, 82]. In addition, molecules of the TNF receptor family such as CD27, CD30, CD40, 4-1BB and OX40 have gained importance as co-stimulatory molecules delivering signals that prolong and propagate T cell responses [83]. For example, the administration of monoclonal antibodies against OX40 or 4-1BB induce a vast amplification of T cell mediated immune responses [84], inhibit apoptotic cell death [85], stimulate long-lived T cell responses [86] and significantly enhance antitumor immune responses [87]. We tested whether the expression of CD80 could enhance or restore the antitumor responses against the BM-185-EGFP cells in old mice. When CD80 molecules were expressed in BM-185-EGFP tumors (BM-185-EGFP-CD80) old mice were able to reject the primary tumors, however, old animals could not develop memory responses [88]. The results indicate that the addition of CD80 partially restored the immune responses in old. Next, we evaluated the effect of adding anti-OX40 or anti-4-1BB mAb. The addition of anti-OX40 or anti-4-1BB mAb injections, markedly improved the ability of aged animals to respond, as 80% of animals injected with BM-185-EGFP and anti-OX40 or anti-4-1BB mAb cleared the tumor [88]. However, only ~30-40% of the old animals developed a protective memory response. Only when BM-185-EGFP-CD80 tumors were given in combination with anti-OX40 or anti-4-1BB mAb, 100% of the old mice rejected the primary tumor and developed long term protective memory responses capable of rejecting a challenge against wild type tumors [88]. In another study, we compared the efficacy of DC-vaccination in young and old mice. Young and old mice were immunized with young and old DCs pulsed with apoptotic TRAMP-C2 tumor cells. Our results showed that DC-vaccination in young animals induced an antitumor response resulting in ~60% tumor growth inhibition, while minimal protection was observed in old animals [89]. DC vaccination plus rIL-2 further enhanced the antitumor response in young animals (~70-75% tumor growth inhibition), while it was ineffective in old animals. Only when DC-vaccination and anti-OX40 or anti-4-1BB mAb were combined enhanced antitumor immune responses were generated inhibiting tumor growth in both young and old mice [89]. These results suggest that deficiencies in T cell function associated with aging may be due to insufficient or inappropriate costimulatory molecule displayed by the APC or that the aged T cell requires a different level of help than do young to achieve an effective primary response giving rise to memory cells. These results have important implications for the development of vaccination strategies in the elderly; indicating that the aged immune repertoire can be exploited for the induction of tumor immunity and that it is possible to convert aged animals from non-responder to responder status with the inclusion of additional costimulation [88-90].

Recently Ruby and Weinberg [91, 92] reported that the activity of anti-OX40 in middle-aged (12-month old) and elderly (20-month old) tumor-bearing mice did not have the desired antitumor responses and correlated with diminished numbers of differentiated effector T cells. The age-related dysfunction appeared not to be associated with the responding T cells, but more likely the cells of the host environment that promote and support T cell responses. However, co-administration of anti-OX40 and IL-12 restored the critical T cell responses and tumor regression in the old. Perhaps the optimal strategy for use of anti-OX40 or anti-4-1BB costimulatory molecules is to combine it with molecules that stimulate the innate immune system.

## Preclinical tumor models, aging and antitumor responses

The group of Yung [93] recently analyzed the ability of bone-marrow derived CD11c + CD4- CD8- DCs, obtained from young (3–6 months) and old (21–24 months) C57BL/6 mice, to induce regression of the pre-established B16-OVA melanoma model. After establishing the tumor in young C57BL/6 mice, they were injected with young and old OVA-peptide pulsed DCs. Their results indicate that Ag-pulsed old DCs have impaired in vivo antitumor activity and tumors grew faster. In other studies the Yung group also observed a lower expression level of DC-SIGN in aged DCs, altering their function. Similarly our laboratory compared the effectiveness of young and old DCs pulsed with apoptotic tumors and the results show that old DCs do not have the ability to be stimulated and promote an antitumor response when compared to young pulsed DCs. These results indicate that it might be necessary to optimize the preparation and use of old DCs for immunotherapy cancer in the old.

Provinciali et al. [94] demonstrated that young and old mice immunized with TS/A tumors secreting IL-2

and challenged with wild type cells resulted in 90% protection in young animals while only 10% of old animals were protected. The group of Provinciali also demonstrated that old BALB/c mice transplanted with a tumor cell line derived from a spontaneous tumor from Her-2/neu mice and vaccinated with DNA-Her-2/neu plasmids had lower protective immunity when compared to young mice [95]. Similarly, Gravekamp et al. [96] demonstrated that DNA-vaccination against Mage-b is more effective in reducing tumor metastases in young mice when compared to old mice. Our group utilized a simple model to evaluate antitumor immune responses in the old. The BM-185 is a pre-B lymphoma cell line that causes 100% mortality in Balb/c mice [88]. It has been established that Enhanced Green Fluorescent Protein (EGFP) is an antigenic molecule capable of eliciting an immune response and tumor cells expressing this protein are rejected. This cell line was transduced with the EGFP gene (BM-185-EGFP) and we utilized the EGFP as a surrogate tumor antigen. We evaluated whether there were differences in the ability of young and old mice to mount an immune response against the BM-185-EGFP tumor. Our data indicate that young Balb/c animals were successful in eradicating the tumor and were additionally protected against subsequent challenges with either the EGFP-modified or wild-type tumor [97]. In contrast, aged Balb/c mice succumbed to the BM-185-EGFP tumor. Even though BM-185-EGFP cells are highly immunogenic, old mice cannot mount an immune response against an immunogenic tumor.

Although the use of the BM-185-EGFP or TUBO tumor models provides valuable information on the behavior and how it might be possible to manipulate the old immune system, these tumor models and many other tumor models used by other investigators rely on immunogenic tumors. As such, it will be more difficult to translate the results from these immunogenic tumor models into a clinical setting for the treatment of tumors in the old. It is well established that the T cell component of the aged immune system is dramatically compromised, i.e., the immune response is impaired and the repertoire is constricted. To date very little data exists on the immune responses against self tumor antigens in the aging population. So far there are no reports evaluating antitumor immune responses in aged tumor models where tolerance and spontaneous tumor progression are present simultaneously. The effect of aging on T cell tolerance remains to be elucidated. Therefore, it is clear that there is a need for relevant animal tumor models which include aspects of self-tolerance and development of spontaneous primary and metastatic tumors in the elderly. Models like this are critical for the development and optimization of more accurate cancer-related immunotherapeutic strategies for the elderly.

The Her-2/neu transgenic mice over express the rat Her-2/neu oncogene under the control of the MMTV promoter [98, 99]. These animals develop spontaneous tumors and the clinical progression and pathogenesis of the disease closely resembles what is seen in human patients with breast cancer. Therefore, the neu mouse is a clinically relevant animal tumor model that can be used to: 1) define the nature of the responsiveness to self-tumor antigens; 2) analyze the requirements for initiating and sustaining antitumor responses in tolerant hosts; and 3) evaluate strategies for overcoming or circumventing tolerance to self tumor antigens that can be effectively used as targets for immunotherapy. Over the past few years, my laboratory has studied the immune responses of Her-2/neu transgenic mice (neu mice). In order to be able to evaluate peptide specific immune responses in neu mice, we crossed the neu mice with the A2.1/Kb transgenic mouse (A2xneu) [100, 101] so that we could evaluate A2.1-Her-2/neu responses against the p369-377 and p773-782 peptides that we have identified [102]. Our results indicate that T cells obtained from A2xneu mice were less efficient in recognizing target cells loaded with peptides when compared to T cells derived from A2xFVB mice (control animals, A2.1/Kb transgenic mice crossed with FVB mice) [100, 101]. A2xneu mice contained a low avidity repertoire to neu antigens indicating that these animals are tolerant to neu antigens [100, 101]. Analysis of the antitumor responses indicate that multiple immunizations with dendritic cells (DCs) pulsed with the neu-antigens is not effective to control the tumor growth in these animals [103, 104]. As described above the use of intratumoral injections of CpG-ODN is an effective method for the induction of antitumor responses. Recent work from my laboratory indicates that intratumoral injection of CpG-ODN plus depletion of Tregs completely rejected the primary tumor in A2xneu mice and animals developed protective memory responses [35]. These results suggest that some immunotherapeutic strategies are able to overcome tolerance and are suitable for tumor elimination.

We have observed that one of the consequences of crossing FVB-Her-2/neu mice with HLA-A2 mice (A2xneu) is that spontaneous tumors appear in these animals when they are 22-27 months old. Therefore, the A2xneu mouse model represents a unique model where aging, tolerance and spontaneous tumor progression are present simultaneously. There are several reasons as to why there is a lack of more studies evaluating antitumor responses in old mice and one critical factor is the extended time period which is necessary to age these mice and the costs incurred towards this aging. To test

the antitumor responses in the A2xneu mice, we have developed a tumor model utilizing a tumor cell line derived from spontaneous tumors (N202.A2 cell line) that facilitates the rapid evaluation of the antitumor responses. We have previously demonstrated that N202.A2 cells grow in young A2xneu as a consequence of immune-tolerance, but are rejected by young A2xFVB mice [100]. When old A2xFVB and A2xneu mice were implanted s.c. with N202.A2 cells, surprisingly we observed that tumors formed in old A2xFVB and as expected in A2xneu mice [105]. Analysis of immune responses against the p773 indicate that the CTL activity from young A2xFVB or A2xneu mice was stronger when compared with the CTL activity of A2xFVB or A2xneu old mice. Interestingly, a very weak CTL activity was detected in old A2xneu mice [105]. Taken together, these results clearly demonstrate that aging severely compromises the immune system and that old A2xneu mice do not have the same capacity to prime a T cell response as young A2xneu mice. In agreement with our previous reports [42], old A2xFVB and A2xneu mice have higher numbers of Tregs when compared to young A2xFVB and A2xneu mice. Our previous studies indicate that intratumoral injections of CpG-ODN could rescue the immune responses in the old and promote antitumor responses in young Her-2/neu mice. We tested the effect of intratumoral injections of CpG-ODN plus Treg depletion in young and old A2xneu mice. As we have observed previously, young A2xneu mice rejected the tumor and developed protective memory responses. In contrast, this combination treatment only prolonged the survival of old A2xneu mice, however, none of the animals could reject or control tumor growth [105]. These results indicate that although it was possible to restore the antitumor responses in young tolerant hosts after targeting CpG-ODN to the tumor site plus Treg depletion, the same therapy was not as effective in old tolerant hosts. These results raise an important question: why do old A2xneu mice, after CpG-ODN and Treg depletion, not mount an immune response capable of rejecting the tumor? Considering that old mice have an excess of networks of immune regulation (more Tregs, MDSC, IDO, etc.), we thought that if intratumoral injections of CpG-ODN were combined with Treg depletion and inhibition of IDO, it could result in a stronger antitumor response. However, our results indicate that animals treated with this triple combination became very sick and seventy percent of the animals died. These results suggest that we should keep a balance between activating a safe and effective antitumor immune response and inducing an autoimmune reaction. Although we might be able to develop immunotherapeutic strategies capable of overcoming tolerance and activating effective antitumor responses in young tolerant hosts, the same therapies may be not equally effective in the old.

## Concluding Remarks

There is undisputable evidence that the occurrence of cancer augments with aging. This could be attributed to a multitude of factors including the dysregulation of the immune system. The immune system of the elderly is very different from the young and it is difficult to extrapolate results obtained in the young, for use in the old. Most of the studies to evaluate the effect of immunotherapy on cancer have been conducted in the young without considering the effect of age-associated changes in immune function. Studies from my laboratory and groups of others indicate that immunotherapeutic interventions could be effective in young animals, but that the same therapies are not as effective in old animals. Considering that the majority of cancers occur in the elderly and that the incidence of cancers is expected to increase due to the expansion of the aging population, it is imperative to pay particular attention to the effect that age imposes on the immune system to assure the effectiveness of immunotherapeutic interventions in the old. Although it is important to consider factors such as T cell tolerance, expression of relevant TAA and inhibition of immunosuppressor cells to activate effective antitumor immune responses in the young and the old. While not the focus of this review, it is worth mentioning that the functional decline of the immune response in the context of aging can also be related to intrinsic susceptibilities, apart from the accumulation of genetic mutations, at the hematopoietic stem cell or progenitor cell stage in the development of cancer. Additionally, aging immune cells can also exhaust their proliferative potential due to increased levels of Hayflick factors resulting in the amassing of senescent cells. Understanding intrinsic changes in the old immune response might be equally important in improving the effectiveness of immunotherapy in the old. Animal models like the A2xneu mice where tolerance and aging are present at the same time will enable us to uncover some of the cellular and molecular basis for the decline in immune function in the elderly and determine conditions and strategies to improve the antitumor activity against self-tumor antigens in the aged. Importantly the information generated from these animals will be more comparable to the aging environment and the results obtained from this animal tumor model would have a better chance to be translated in the clinical setting for the treatment of cancer in the old. Only with this knowledge will we be able to

successfully customize tumor vaccines to be effective for the treatment of tumors in the old.

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**References**

[1] Miller RA (1991). Gerontology as oncology. Research on aging as the key to the understanding of cancer. Cancer, 68: 2496-2501

[2] Yancik R (1997). Cancer burden in the aged: an epidemiologic and demographic overview. Cancer, 80: 1273-1283

[3] Dunn GP, Bruce AT, Ikeda H, Old LJ and Schreiber RD (2002). Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol, 3: 991-998

[4] Russell JH and Ley TJ (2002). Lymphocyte-mediated cytotoxicity. Annu. Rev. Immunol., 20: 323-370

[5] Smyth MJ, Thia KY, Street SE, Cretney E, Trapani JA, Taniguchi M, Kawano T, Pelikan SB, Crowe NY and Godfrey DI (2000). Differential tumor surveillance by natural killer (NK) and NKT cells. J. Exp. Med., 191: 661-668

[6] Street SE, Cretney E and Smyth MJ (2001). Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis. Blood, 97: 192-197

[7] van den Broek ME, Kagi D, Ossendorp F, Toes R, Vamvakas S, Lutz WK, Melief CJ, Zinkernagel RM and Hengartner H (1996). Decreased tumor surveillance in perforin-deficient mice. J. Exp. Med., 184: 1781-1790

[8] Saurwein-Teissl M, Romani N and Grubeck-Loebenstein B (2000). Dendritic cells in old age--neglected by gerontology? Mech. Ageing Dev., 121: 123-130

[9] Song L, Kim YH, Chopra RK, Proust JJ, Nagel JE, Nordin AA and Adler WH (1993). Age-related effects in T cell activation and proliferation. Exp. Gerontol., 28: 313-321

[10] Taub DD and Longo DL (2005). Insights into thymic aging and regeneration. Immunol. Rev., 205: 72-93

[11] Fagnoni FF, Vescovini R, Passeri G, Bologna G, Pedrazzoni M, Lavagetto G, Casti A, Franceschi C, Passeri M and Sansoni P (2000). Shortage of circulating naive CD8(+) T cells provides new insights on immunodeficiency in aging. Blood, 95: 2860-2868

[12] Gupta S, Bi R, Su K, Yel L, Chiplunkar S and Gollapudi S (2004). Characterization of naive, memory and effector CD8+ T cells: effect of age. Exp. Gerontol., 39: 545-550

[13] Thoman ML (1997). Effects of the aged microenvironment on CD4+ T cell maturation. Mech. Ageing Dev., 96: 75-88

[14] Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E and De Benedictis G (2000). Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci., 908: 244-254

[15] Franceschi C (2007). Inflammaging as a major characteristic of old people: can it be prevented or cured? Nutr. Rev., 65: S173-176

[16] Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC and Salvioli S (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech. Ageing Dev., 128: 92-105

[17] Davalos AR, Coppe JP, Campisi J and Desprez PY (2010). Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev., 29: 273-283

[18] Linton PJ, Li SP, Zhang Y, Bautista B, Huynh Q and Trinh T (2005). Intrinsic versus environmental influences on T-cell responses in aging. Immunol. Rev., 205: 207-219

[19] Sansoni P, Vescovini R, Fagnoni F, Biasini C, Zanni F, Zanlari L, Telera A, Lucchini G, Passeri G, Monti D, Franceschi C and Passeri M (2008). The immune system in extreme longevity. Exp. Gerontol., 43: 61-65

[20] Loo DS (2007). Onychomycosis in the elderly : drug treatment options. Drugs Aging, 24: 293-302

[21] McElhaney JE (2009). Prevention of infectious diseases in older adults through immunization: the challenge of the senescent immune response. Expert Rev Vaccines, 8: 593-606

[22] Meyer KC (2004). Lung infections and aging. Ageing Res Rev, 3: 55-67

[23] Mirza N, Pollock K, Hoelzinger DB, Dominguez AL and Lustgarten J (2011). Comparative kinetic analyses of gene profiles of naive CD4(+) and CD8(+) T cells from young and old animals reveal novel age-related alterations. Aging Cell, 10: 853-867

[24] Janeway CA, Jr., Dianzani U, Portoles P, Rath S, Reich EP, Rojo J, Yagi J and Murphy DB (1989). Cross-linking and conformational change in T-cell receptors: role in activation and in repertoire selection. Cold Spring Harb. Symp. Quant. Biol., 54 Pt 2: 657-666

[25] Trinchieri G and Sher A (2007). Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol, 7: 179-190

[26] Kaisho T and Akira S (2003). Regulation of dendritic cell function through Toll-like receptors. Curr Mol Med, 3: 373-385

[27] Takeda K, Kaisho T and Akira S (2003). Toll-like receptors. Annu. Rev. Immunol., 21: 335-376

[28] Hawlisch H and Kohl J (2006). Complement and Toll-like receptors: key regulators of adaptive immune responses. Mol. Immunol., 43: 13-21

[29] Kawarada Y, Ganss R, Garbi N, Sacher T, Arnold B and Hammerling GJ (2001). NK- and CD8(+) T cell-mediated eradication of established tumors by peritumoral injection of CpG-containing oligodeoxynucleotides. J. Immunol., 167: 5247-5253

[30] Yang Y, Huang CT, Huang X and Pardoll DM (2004). Persistent Toll-like receptor signals are required for reversal of regulatory T cell-mediated CD8 tolerance. Nat Immunol, 5: 508-515

[31] Salem ML, Kadima AN, Cole DJ and Gillanders WE (2005). Defining the antigen-specific T-cell response to vaccination and poly(I:C)/TLR3 signaling: evidence of enhanced primary and memory CD8 T-cell responses and antitumor immunity. J. Immunother., 28: 220-228

[32] Bullock TN and Yagita H (2005). Induction of CD70 on dendritic cells through CD40 or TLR stimulation contributes to the development of CD8+ T cell responses in the absence of CD4+ T cells. J. Immunol., 174: 710-717

[33] Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J and Sambhara S (2002). Cutting edge: impaired Toll-like receptor expression and function in aging. J. Immunol., 169: 4697-4701

[34] Boehmer ED, Goral J, Faunce DE and Kovacs EJ (2004). Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. J. Leukoc. Biol., 75: 342-349

[35] Sharma S, Dominguez AL, Hoelzinger DB and Lustgarten J (2008). CpG-ODN but not other TLR-ligands restore the antitumor responses in old mice: the implications for vaccinations in the aged. Cancer Immunol. Immunother., 57: 549-561

[36] van Duin D and Shaw AC (2007). Toll-like receptors in older adults. J. Am. Geriatr. Soc., 55: 1438-1444

[37] Miyara M and Sakaguchi S (2007). Natural regulatory T cells: mechanisms of suppression. Trends Mol Med, 13: 108-116

[38] Sakaguchi S, Yamaguchi T, Nomura T and Ono M (2008). Regulatory T cells and immune tolerance. Cell, 133: 775-787

[39] Hori S, Nomura T and Sakaguchi S (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science, 299: 1057-1061

[40] Green EA, Gorelik L, McGregor CM, Tran EH and Flavell RA (2003). CD4+CD25+ T regulatory cells control anti-islet CD8+ T cells through TGF-beta-TGF-beta receptor interactions in type 1 diabetes. Proc. Natl. Acad. Sci. U. S. A., 100: 10878-10883

[41] Samy ET, Parker LA, Sharp CP and Tung KS (2005). Continuous control of autoimmune disease by antigen-dependent polyclonal CD4+CD25+ regulatory T cells in the regional lymph node. J. Exp. Med., 202: 771-781

[42] Sharma S, Dominguez AL and Lustgarten J (2006). High accumulation of T regulatory cells prevents the activation of immune responses in aged animals. J. Immunol., 177: 8348-8355

[43] Gregg R, Smith CM, Clark FJ, Dunnion D, Khan N, Chakraverty R, Nayak L and Moss PA (2005). The number of human peripheral blood CD4+ CD25high regulatory T cells increases with age. Clin. Exp. Immunol., 140: 540-546

[44] Lages CS, Suffia I, Velilla PA, Huang B, Warshaw G, Hildeman DA, Belkaid Y and Chougnet C (2008). Functional regulatory T cells accumulate in aged hosts and promote chronic infectious disease reactivation. J. Immunol., 181: 1835-1848

[45] Serafini P, Borrello I and Bronte V (2006). Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. Semin. Cancer Biol., 16: 53-65

[46] Nagaraj S and Gabrilovich DI (2008). Tumor escape mechanism governed by myeloid-derived suppressor cells. Cancer Res., 68: 2561-2563

[47] Rodriguez PC and Ochoa AC (2008). Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. Immunol. Rev., 222: 180-191

[48] Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S and Carbone DP (1998). Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. Blood, 92: 4150-4166

[49] Young MR, Petruzzelli GJ, Kolesiak K, Achille N, Lathers DM and Gabrilovich DI (2001). Human squamous cell carcinomas of the head and neck chemoattract immune suppressive CD34(+) progenitor cells. Hum. Immunol., 62: 332-341

[50] Grizzle WE, Xu X, Zhang S, Stockard CR, Liu C, Yu S, Wang J, Mountz JD and Zhang HG (2007). Age-related increase of tumor susceptibility is associated with myeloid-derived suppressor cell mediated suppression of T cell cytotoxicity in recombinant inbred BXD12 mice. Mech. Ageing Dev., 128: 672-680

[51] Kusmartsev S, Nagaraj S and Gabrilovich DI (2005). Tumor-associated CD8+ T cell tolerance induced by bone marrow-derived immature myeloid cells. J. Immunol., 175: 4583-4592

[52] Bunt SK, Yang L, Sinha P, Clements VK, Leips J and Ostrand-Rosenberg S (2007). Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. Cancer Res., 67: 10019-10026

[53] Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, Divino CM and Chen SH (2006). Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res., 66: 1123-1131

[54] Huang H, Patel DD and Manton KG (2005). The immune system in aging: roles of cytokines, T cells and NK cells. Front. Biosci., 10: 192-215

[55] Fulop T, Larbi A, Douziech N, Levesque I, Varin A and Herbein G (2006). Cytokine receptor signalling and aging. Mech. Ageing Dev., 127: 526-537

[56] Munn DH and Mellor AL (2007). Indoleamine 2,3-dioxygenase and tumor-induced tolerance. J. Clin. Invest., 117: 1147-1154

[57] Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C and Mellor AL (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism. Science, 281: 1191-1193

[58] Lin YC, Chen CL, Nakano T, Goto S, Kao YH, Hsu LW, Lai CY, Jawan B, Cheng YF, Tateno C and Yoshizato K (2008). Immunological role of indoleamine 2,3-dioxygenase in rat liver allograft rejection and tolerance. J. Gastroenterol. Hepatol., 23: e243-250

[59] Brandacher G, Perathoner A, Ladurner R, Schneeberger S, Obrist P, Winkler C, Werner ER, Werner-Felmayer G, Weiss HG, Gobel G, Margreiter R, Konigsrainer A, Fuchs D and Amberger A (2006). Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. Clin. Cancer Res., 12: 1144-1151

[60] Ino K, Yoshida N, Kajiyama H, Shibata K, Yamamoto E, Kidokoro K, Takahashi N, Terauchi M, Nawa A, Nomura S, Nagasaka T, Takikawa O and Kikkawa F (2006). Indoleamine 2,3-dioxygenase is a novel prognostic indicator for endometrial cancer. Br. J. Cancer, 95: 1555-1561

[61] Friberg M, Jennings R, Alsarraj M, Dessureault S, Cantor A, Extermann M, Mellor AL, Munn DH and Antonia SJ (2002). Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. Int. J. Cancer, 101: 151-155

[62] Hou DY, Muller AJ, Sharma MD, DuHadaway J, Banerjee T, Johnson M, Mellor AL, Prendergast GC and Munn DH (2007). Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. Cancer Res., 67: 792-801

[63] Pertovaara M, Hasan T, Raitala A, Oja SS, Yli-Kerttula U, Korpela M and Hurme M (2007). Indoleamine 2,3-dioxygenase activity is increased in patients with systemic lupus erythematosus and predicts disease activation in the sunny season. Clin. Exp. Immunol., 150: 274-278

[64] Greenwald RJ, Freeman GJ and Sharpe AH (2005). The B7 family revisited. Annu. Rev. Immunol., 23: 515-548

[65] Zang X and Allison JP (2007). The B7 family and cancer therapy: costimulation and coinhibition. Clin. Cancer Res., 13: 5271-5279

[66] Zou W and Chen L (2008). Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol, 8: 467-477

[67] Korman AJ, Peggs KS and Allison JP (2006). Checkpoint blockade in cancer immunotherapy. Adv. Immunol., 90: 297-339

[68] Wolchok JD and Saenger Y (2008). The mechanism of anti-CTLA-4 activity and the negative regulation of T-cell activation. Oncologist, 13 Suppl 4: 2-9

[69] Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR and Honjo T (2000). Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J. Exp. Med., 192: 1027-1034

[70] Wang S, Bajorath J, Flies DB, Dong H, Honjo T and Chen L (2003). Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. J. Exp. Med., 197: 1083-1091

[71] Nishimura H, Nose M, Hiai H, Minato N and Honjo T (1999). Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity, 11: 141-151

[72] Dong H, Zhu G, Tamada K, Flies DB, van Deursen JM and Chen L (2004). B7-H1 determines accumulation and deletion of intrahepatic CD8(+) T lymphocytes. Immunity, 20: 327-336

[73] Strome SE, Dong H, Tamura H, Voss SG, Flies DB, Tamada K, Salomao D, Cheville J, Hirano F, Lin W, Kasperbauer JL, Ballman KV and Chen L (2003). B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer Res., 63: 6501-6505

[74] Blank C, Gajewski TF and Mackensen A (2005). Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. Cancer Immunol. Immunother., 54: 307-314

[75] Dong H, Strome SE, Matteson EL, Moder KG, Flies DB, Zhu G, Tamura H, Driscoll CL and Chen L (2003). Costimulating aberrant T cell responses by B7-H1 autoantibodies in rheumatoid arthritis. J. Clin. Invest., 111: 363-370

[76] Wang S, Zhu G, Chapoval AI, Dong H, Tamada K, Ni J and Chen L (2000). Costimulation of T cells by B7-H2, a B7-like molecule that binds ICOS. Blood, 96: 2808-2813

[77] Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, Liu D, Dong H, Sica GL, Zhu G, Tamada K and Chen L (2001). B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. Nat Immunol, 2: 269-274

[78] Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H, Chapoval AI, Flies DB, Bajorath J and Chen L (2003). B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. Immunity, 18: 849-861

[79] Hurwitz AA, Kwon ED and van Elsas A (2000). Costimulatory wars: the tumor menace. Curr. Opin. Immunol., 12: 589-596

[80] Prilliman KR, Lemmens EE, Palioungas G, Wolfe TG, Allison JP, Sharpe AH and Schoenberger SP (2002). Cutting edge: a crucial role for B7-CD28 in transmitting T help from APC to CTL. J. Immunol., 169: 4094-4097

[81] Li Y, McGowan P, Hellstrom I, Hellstrom KE and Chen L (1994). Costimulation of tumor-reactive CD4+ and CD8+ T lymphocytes by B7, a natural ligand for CD28, can be used to treat established mouse melanoma. J. Immunol., 153: 421-428

[82] Raes G, Van Ginderachter J, Liu YQ, Brys L, Thielemans K, De Baetselier P and Geldhof A (1998). Active antitumor immunotherapy, with or without B7-mediated costimulation, increases tumor progression in an immunogenic murine T cell lymphoma model. Cancer Immunol. Immunother., 45: 257-265

[83] Morel Y, Truneh A, Sweet RW, Olive D and Costello RT (2001). The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. J. Immunol., 167: 2479-2486

[84] Gramaglia I, Weinberg AD, Lemon M and Croft M (1998). Ox-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. J. Immunol., 161: 6510-6517

[85] Rogers PR, Song J, Gramaglia I, Killeen N and Croft M (2001). OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T cells. Immunity, 15: 445-455

[86] Weatherill AR, Maxwell JR, Takahashi C, Weinberg AD and Vella AT (2001). OX40 ligation enhances cell cycle turnover of Ag-activated CD4 T cells in vivo. Cell. Immunol., 209: 63-75

[87] Weinberg AD, Rivera MM, Prell R, Morris A, Ramstad T, Vetto JT, Urba WJ, Alvord G, Bunce C and Shields J (2000). Engagement of the OX-40 receptor in vivo enhances antitumor immunity. J. Immunol., 164: 2160-2169

[88] Lustgarten J, Dominguez AL and Thoman M (2004). Aged mice develop protective antitumor immune responses with appropriate costimulation. J. Immunol., 173: 4510-4515

[89] Sharma S, Dominguez AL and Lustgarten J (2006). Aging affect the anti-tumor potential of dendritic cell vaccination, but it can be overcome by co-stimulation with anti-OX40 or anti-4-1BB. Exp. Gerontol., 41: 78-84

[90] Bansal-Pakala P and Croft M (2002). Defective T cell priming associated with aging can be rescued by signaling through 4-1BB (CD137). J. Immunol., 169: 5005-5009

[91] Ruby CE and Weinberg AD (2009). The effect of aging on OX40 agonist-mediated cancer immunotherapy. Cancer Immunol. Immunother., 58: 1941-1947

[92] Ruby CE and Weinberg AD (2009). OX40-enhanced tumor rejection and effector T cell differentiation decreases with age. J. Immunol., 182: 1481-1489

[93] Grolleau-Julius A, Garg MR, Mo R, Stoolman LL and Yung RL (2006). Effect of aging on bone marrow-derived murine CD11c+CD4-CD8alpha- dendritic cell function. J. Gerontol. A. Biol. Sci. Med. Sci., 61: 1039-1047

[94] Provinciali M, Argentati K and Tibaldi A (2000). Efficacy of cancer gene therapy in aging: adenocarcinoma cells engineered to release IL-2 are rejected but do not induce tumor specific immune memory in old mice. Gene Ther., 7: 624-632

[95] Provinciali M, Smorlesi A, Donnini A, Bartozzi B and Amici A (2003). Low effectiveness of DNA vaccination against HER-2/neu in ageing. Vaccine, 21: 843-848

[96] Gravekamp C, Sypniewska R, Gauntt S, Tarango M, Price P and Reddick R (2004). Behavior of metastatic and nonmetastatic breast tumors in old mice. Exp Biol Med (Maywood), 229: 665-675

[97] Marigo I, Dolcetti L, Serafini P, Zanovello P and Bronte V (2008). Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. Immunol. Rev., 222: 162-179

[98] Muller WJ, Sinn E, Pattengale PK, Wallace R and Leder P (1988). Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell, 54: 105-115

[99] Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD and Muller WJ (1992). Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc. Natl. Acad. Sci. U. S. A., 89: 10578-10582

[100] Lustgarten J, Dominguez AL and Cuadros C (2004). The CD8+ T cell repertoire against Her-2/neu antigens in neu transgenic mice is of low avidity with antitumor activity. Eur. J. Immunol., 34: 752-761

[101] Lustgarten J, Dominguez AL and Pinilla C (2006). Identification of cross-reactive peptides using combinatorial libraries circumvents tolerance against Her-2/neu-immunodominant epitope. J. Immunol., 176: 1796-1805

[102] Lustgarten J, Theobald M, Labadie C, LaFace D, Peterson P, Disis ML, Cheever MA and Sherman LA (1997). Identification of Her-2/Neu CTL epitopes using double transgenic mice expressing HLA-A2.1 and human CD.8. Hum. Immunol., 52: 109-118

[103] Cuadros C, Dominguez AL, Frost GI, Borgstrom P and Lustgarten J (2003). Cooperative effect between immunotherapy and antiangiogenic therapy leads to effective tumor rejection in tolerant Her-2/neu mice. Cancer Res., 63: 5895-5901

[104] Cuadros C, Dominguez AL, Lollini PL, Croft M, Mittler RS, Borgstrom P and Lustgarten J (2005). Vaccination with dendritic cells pulsed with apoptotic tumors in combination with anti-OX40 and anti-4-1BB monoclonal antibodies induces T cell-

mediated protective immunity in Her-2/neu transgenic mice. Int. J. Cancer, 116: 934-943

[105] Dominguez AL and Lustgarten J (2008). Implications of aging and self-tolerance on the generation of immune and antitumor immune responses. Cancer Res., 68: 5423-5431