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The importance of the age factor in cancer vaccination at older age

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Abstract

Cancer is an age-related disease, and with the graying of the society there is an increasing need to optimize cancer management and therapy to elderly patients. Vaccine therapy for cancer is less toxic than chemotherapy or radiation and could be, therefore, especially effective in older, more frail cancer patients. However, it has been shown that older individuals do not respond to vaccine therapy as well as younger adults. This has been attributed to T cell unresponsiveness, a phenomenon also observed in cancer patients per se. Therefore, research is needed to establish whether age-specific tumor-immunological variables permit optimal use of cancer vaccines and therapy in the elderly. This review summarizes the current knowledge of T cell unresponsiveness in cancer patients and elderly, and the results of cancer vaccination in preclinical models at young and old age. Finally, new directions that may lead to effective cancer vaccination at older age will be proposed.

Keywords

Cancer vaccines; T cell unresponsiveness; Old age; Metastatic breast cancer; Mage-b DNA vaccine

T cell unresponsiveness in cancer patients

Cytotoxic T lymphocytes (CTL) are considered to be the most important players in anti-tumor reactions. The T cell receptor (TCR) of CTL recognizes tumor-associated antigens (TAA) in association with major histo-compatibility complex (MHC) molecules on the tumor cells. As has become evident from in vitro studies, these CTL are activated when exposed simultaneously to both TAA/self-MHC complexes and co-stimulatory molecules, resulting in CTL-mediated tumor cell cytolysis. In cancer patients, CTL are often found at the site of the tumor, but have evidently been unable to destroy the tumor cells [1].

Multiple possible causes have been described for this unresponsiveness of the CTL in cancer patients [2, 3]. For example, low expression of the co-stimulatory molecule B7.1 on human metastatic carcinoma cells in gastrointestinal tumors [4] has been suggested as a possible

cause of T cell unresponsiveness because the interaction between co-stimulatory molecules and its ligand is an absolute need for T cell activation. Other described co-stimulatory or adhesion molecules that may play a role in T cell unresponsiveness are intercellular adhesion molecules (ICAM)-1, vascular cellular adhesion molecules (VCAM)-1 or epidermal lymphocyte adhesion molecules (ELAM)-1 [5]. In mice, the importance of co-stimulatory molecules such as 4-1BBL or B7.1, for CTL activation, has been reported as well [6, 7].

T cell unresponsiveness could also be due to low expression of self-MHC on tumor cells, which is required for recognition of tumor-specific antigens by the TCR. Low expression of MHC molecules has been commonly found in human metastatic tumor cells, such as metastatic melanoma, breast cancer or colon cancer [8]. Similarly, low expression of MHC molecules has been observed in mouse tumors, such as primary brain tumors [9], fibrosarcoma [10], melanoma [11], or lung carcinoma [12]. As discussed later in this article, vaccination based on TAA could circumvent both problems. Even then, however, loss of TAA expression will allow tumor cells to escape vaccine-induced T cell responses [13, 14]. This underlines the need of multi-antigen vaccines.

T cell tolerance may be the most important obstacle for successful immunotherapy against cancer [15]. Since most TAA are weakly expressed on normal cells, the immune system will recognize them as self. Earlier in life T cells are taught in the primary lymphoid organs not to respond to self-antigens. Therefore, it is difficult to induce a strong immune response against most TAA. Evidence exists in humans and mice that natural regulatory T cells (T_{regs}), expressing $CD4^+CD25^+$, are crucial for maintaining T cell tolerance to self-antigens. More recent studies have shown that transcription factor forkhead box P3 (FOXP3) plays a critical role in the development of functional T_{regs} ($CD4^+CD25^+FOXP3^+$). Natural T_{regs} are different from inducible T_{regs} . Inducible T_{regs} arise during inflammatory processes such as infections and cancers [16]. Although inducible T_{regs} express CD4, CD25, and FoxP3 like natural T_{regs} , they are functionally different. In contrast to natural T_{regs} , inducible T_{regs} suppress immunity through the production of soluble factors such as IL-10 and transforming growth factor (TGF) β or through direct cell–cell contact [17]. It has been shown in mice [18, 19] and man [20, 21] that inducible T_{regs} inhibit vaccine-induced T cell responses as well as NK cell responses. Many strategies have been discussed and tested in animal models that target the inactivation or elimination of inducible T_{regs} , in order to enhance vaccine efficacy. For instance, treatment with a tumor cell-based vaccine secreting granulocyte-macrophage colony stimulating factor (GM-CSF), combined with anti-CTLA-4 antibodies (CTLA-4 is constitutively expressed in T_{regs}) in the B16/B6 melanoma mouse tumor model resulted in eradication of primary tumors and prevention of lung metastases. This was accompanied with improved anti-tumor responses [22]. However, in humans with stage IV melanoma or renal cancer administration of anti-CTLA-4-specific antibodies did not inhibit suppressive activity of T_{regs} in vitro or in vivo [23]. Moreover, anti-CTLA-4 antibodies, often used in combination with cancer vaccination, may induce autoimmune responses [79]. Inducible T_{regs} may become an important target for cancer immunotherapy, but more research is needed to identify which $CD4^+CD25^+$ T cells are suppressive, and how inducible T_{regs} can be targeted without depletion of natural T_{regs} . Depletion of natural T_{regs} leads to autoimmune diseases [24–26].

Yet, other factors may play a role in inhibition of vaccine-induced T cell responses in cancer patients. In humans and mice, many tumors secrete lymphokines or factors that inhibit vaccine-induced immune responses. Examples are TGF β , IL-6, IL-10, cyclooxygenase-2 (COX-2) and its products prostaglandine E2 (PGE₂), PD1-ligand, or indolamine 2,3-dioxygenase (IDO) [27]. TGF β inhibits antigen presentation by dendritic cells (DC), resulting in inhibition of T cell function [28], or facilitates the induction of T_{regs} resulting in suppression of tumor-specific CD8 T cell cytotoxicity in vivo [29]. IL-6 is a potent regulator of DC differentiation in vivo [30], and is able to initiate the expression of a signal transducer and activator of transcription (STAT)3 in DC. However, high levels of STAT3 can prevent the maturation from DC, and subsequent presentation of antigens [30], resulting in T cell inhibition. IL-10 inhibits maturation of DC and T cell function through T_{regs} [31]. COX-2 and its product PGE₂ induce the expression of T_{reg} cell-specific transcription factor Foxp3 and increase T_{reg} activity [32]. PGE₂ also downregulates IL-12 production by macrophages and differentiation of Th1 responses [33]. Engagement of PD-1 by PD-1 ligand leads to inhibition of T cell receptor-mediated lymphocyte proliferation and cytokine secretion [34]. IDO inhibits T cell proliferation through tryptophan degradation [35]. Reduction of these lymphokines or factors may enhance vaccine efficacy.

Over the last few years, more attention has been given to macrophages. Macrophages in the microenvironment of tumors, may also produce IL-6, TGF β , PGE₂, and IL-10, and subsequently inhibit T cell activation [36]. However, macrophages may also stimulate T cells through the production of IFN γ or IL-12 [37], or produce TNF α facilitating Fas-mediated tumor cell apoptosis by CD8 T cells [38], or express membrane bound TNF α , facilitating apoptosis by the interaction with the TNFR1 [39], or become so-called “killer macrophages” when producing TNF α and IFN γ [40, 41]. Macrophages play a crucial role in suppression and activation of the immune system in cancer patients, and may become an important target for anti-cancer therapies.

An overview of tumor-immunological parameters potentially involved in T cell unresponsiveness in cancer patients is shown in Table 1. To develop effective cancer vaccines, many of the above-described obstacles have to be overcome. However, an additional problem almost totally ignored in the development of cancer vaccines, is ageing of the immune system.

T cell unresponsiveness in the elderly

Ageing of the immune system leads to T cell unresponsiveness in elderly [42]. Alteration in several immunological parameters that may contribute to the age-related decline in T cell responses is discussed below. For instance, a decrease in the number of naive 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) in elderly humans as compared to young adults have been reported [43]. It has been suggested that continual activation of the immune system by new antigens during the life span would lead to a depletion of naive T cells from the thymus, and a clonal expansion of memory T cells. With the involution of the thymus almost complete at the age of 60 years, new naive T cells at old age can no longer be generated [44]. The host is then dependent on the pool of naïve T cells generated earlier in

life. Analogous to the situation in humans, a decrease of naïve T cells and an increase of memory T cells have also been described for aging mice [45]. Other possible causes for decreased T cell responses in aged humans and mice have been described, such as defects in TCR/CD3-mediated phosphorylation events or aberrant regulation of tyrosine kinases associated with the TCR [46]. An age-related decrease in the $\alpha\beta$ repertoire of the human TCR has been described that may lead to diminished T cell responses [47]. Another molecule important for T cell activation is CD28. CD28 is expressed at the cell membrane of T cells, which is the receptor for B7 expressed on antigen-presenting cells (APC). Clinical studies have documented that high proportions of CD8 T cells that lack CD28 are correlated with a reduced antibody response to influenza vaccination [48]. Also in mice, CD8 T cells lacking CD28 expression have been reported [49]. Moreover, it has been shown that CD28-lacking CD8 T cells can suppress antigen-specific CTL responses [50].

Studies have been performed in the mouse to evaluate the involvement of natural T_{regs} in age-related decline in T cell-mediated immune responses. Suggestive evidence exists that age-related decline in immune responses is ascribed to changes in the $CD4^+CD25^-Fox3^+$ T cell population and not to a functional augmentation of suppressive $CD4^+CD25^+Fox3^+$ T_{regs} [51]. Others have shown that the number of human peripheral blood $CD4^+CD25^{\text{high}}$ T_{regs} increased with age but their function appeared to be unaltered in comparison to young age [52]. The relevance of $CD4^+CD25^{\text{high}}$ T_{regs} in relation to immune senescence as yet remains unclear.

In addition to the problems at the level of T cells and/or tumor cells, defects in cytokine production have been observed in aged humans. An example is a human vaccine study in which significantly lower levels of IL-2 were produced by T cells of older individuals stimulated with an influenza vaccine in vitro compared to those of young individuals [53]. Similarly, significantly lower levels of $IFN\gamma$ were produced by peripheral blood mononuclear cells (PBMC) from elderly individuals immunized with an influenza vaccine compared to young individuals [54]. IL-2 promotes T cell activation and proliferation, as well as release of $IFN\gamma$ by T cells. The lower IL-2 production following in vitro stimulation with the influenza vaccine may explain the lower $IFN\gamma$ production. $IFN\gamma$ is involved in activation of APC such as macrophages. These macrophages are important for CTL priming.

Defects in other cell types than CTL and/or tumor cells may also explain T cell unresponsiveness in aging. APC such as DC in blood or Langerhans cells in skin, play a central role in T cell activation. These cells are often called “professional” APC because they enable efficient processing of foreign proteins into peptides, and because they express MHC and co-stimulatory molecules at the high levels required for optimal presentation of the peptides to the immune system and subsequent stimulation of T cells. Tumor cells are poor APC, because co-stimulatory molecules and self-MHC, as well as TAA required for CTL stimulation are often weakly expressed. Therefore, DC loaded with peptides or fused with autologous tumor cells are frequently used in clinical trials as an anticancer immunotherapy to stimulate T cells in cancer patients. Since APC play such an important role in T cell activation, one might question whether this cell type could be involved in the age-related decline of T cell responsiveness. Different results have been published about the function of DC at older age. For instance, it has been demonstrated that blood DC from old

individuals can still function as powerful antigen-presenting cells when exposed to purified protein derivate (PPD) of *Mycobacterium tuberculosis* [55]. Similarly, the responsiveness of blood DC to stimulation with influenza vaccine was unimpaired at old age [56]. In both cases, expression patterns of MHC molecules and co-stimulatory molecules were found to be similar on blood DC of young and old individuals. However, it has been shown in mice and humans that the number of Langerhans cells decreases with age, resulting in impaired immune function of the skin [57, 58]. It has also been shown that DCs from aged individuals are more mature and have impaired ability to produce IL-12 [59], or that secretion of TNF α and IL-6 significantly increased upon stimulation with LPS and ssRNA in DC of aged compared to young individuals [60]. Others found reduced phosphorylation of Akt in DCs of aged individual [60], resulting in decreased activation of phosphatidylinositol 3-kinase (PI3K) pathway. PI3K-signaling regulates phagocytosis, and migration, as well as toll-like receptor (TLR) signaling. TLR are pattern recognition receptors that recognize conserved molecular patterns on microbes and activate the innate and adaptive immune system. In contrast to DC, macrophages appeared to secrete significantly lower levels of IL-6 and TNF α when stimulated with known ligands for the TLR1/2, TLR2/6, TLR3, TLR4, TLR5, and TLR9 in old than young mice [61].

Hence, in cancer patients, T cell functions are not only inhibited by the tumors but also impaired as a result of aging. An overview of age-related changes in the immune system potentially involved in T cell unresponsiveness in the elderly is shown in Table 2.

Lessons from preclinical mouse tumor models

More than 50% of all cancer patients are 65 years or older [62]. However, as discussed earlier, the immune system at older age is impaired, due to T cell unresponsiveness. In most preclinical studies, cancer vaccines have been tested at young age. Over the last decade, results of a number vaccine studies in preclinical models at old age became available, including studies with Mage-b vaccination performed in our laboratory. These are the first studies that show that cancer vaccines are less effective at old than at young age.

The first reported vaccine study in young and old mice with cancer is from Provinciali et al. [63]. A syngeneic mammary adenocarcinoma cell line TS/A was genetically engineered to release IL-2 (TS/A–IL-2). Young and old mice were immunized with TS/A–IL-2 cells and subsequently challenged with the parental TS/A cell line. While TS/A–IL-2 protected 90% of the young mice, only 10% of the old mice were protected. CD8 and CD4 T cells were detected in tumors of young but hardly in tumors of old mice, while macrophages and neutrophils were abundantly present in tumors of mice from both ages. Yet, it has not been proven whether these CD8 and CD4 T cells were functionally active.

Many vaccine studies have been performed with the Her2/neu DNA vaccine in young mice. Provinciali et al. [64] developed a pCMVneuNT DNA vaccine and tested its efficacy in young and old mice that were subsequently challenged with syngeneic TUBO cells, overexpressing HER2/neu. Young mice were completely protected while less than 60% of the old mice were protected against TUBO challenge. Anti-neu antibodies, induced by the vaccine, and proliferation after restimulation in vitro, was higher at young than at old age.

Lustgarten et al. [65] immunized young and old mice with a syngeneic pre-B cell lymphoma cell line (BM-185), expressing enhanced green fluorescent protein (EGFP) and a co-stimulatory molecule CD80 (B7.1). While the young mice developed a long-lasting memory response capable of rejecting BM-185 wild type tumors, the old mice did not develop long-lasting memory responses. However, when the BM-185-EGFP-CD80 plus agonist anti-OX40 mAb were injected in old mice, long-lasting memory responses were capable of rejecting BM-185 wild type tumor cells with the same vigor as in young mice. In vivo depletion of CD8 T cells resulted in decreased survival of mice immunized with BM-185-EGFP, and challenged with wild type BM-185. However, these studies were performed at young age only. They also analyzed the number of CD8 T cells specific for EGFP and found cytotoxic activity against BM-185 wild type tumor cells in spleen cultures stimulated with BM-185-EGFP-CD80 in old and young mice. However, the spleen cultures were from mice without tumors. In another study by the group of Lustgarten [66], DC vaccination plus rIL-2 protected 60% of the young mice from challenge with syngeneic TRAMP-C2 tumor cells (adenocarcinoma of the prostate), while only a minimal effect was observed in the old mice. However, when co-administered with anti-OX-40 or anti-4-1BB mAbs (leukocyte differentiation antigen on T and NK cells, and DC) a vigorous anti-tumor response in both young (85–90%) and in old (70–75%) mice was observed. Cytolytic activity in spleen cultures of young and old mice immunized with DC pulsed with apoptotic TRAMP-C2 cells, and anti-OX40 or anti-4-1BB antibodies were observed. However, the spleen cultures were from mice without tumors.

Grolleau-Julius et al. [67] showed that Ag-pulsed DC vaccines of old mice have impaired in vivo antitumor activity. Using the B16-OVA C57BL/6 melanoma model, tumors appeared to be significantly smaller in mice receiving “young (3 months)” OVA-pulsed DC compared to mice that received “old (21–24 months)” OVA-pulsed DC. They also reported that young-derived DC vaccines in old tumor-bearing mice were less effective than in young tumor-bearing mice, indicating the altered tumor microenvironment at older age and its effect on vaccination.

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 model, 4TO7cg and 4T1, both over expressing Mage-b in metastases and primary tumors [68, 69]. The 4TO7cg model is mildly metastatic (range 3–20 metastases per mouse), while 4T1 is a highly aggressive with poorly immunogenic tumors and metastases (range 5–300 metastases per mouse). Vaccination of the 4TO7cg mice with Mage-b was highly effective against metastases and young age (90% of the mice were free of metastases) but not at old age (40% of the mice were free of metastases), and this correlated with strong Mage-b-specific immune responses (production of IL-2 and IFN γ in vitro after re-stimulation with Mage-b) at young but not at old age [70]. Vaccination of the highly aggressive 4T1 model with Mage-b alone was not effective. Therefore, we optimized the immunization protocol. To recruit APC more effectively, thioglycollate was injected into the peritoneal cavity (pc), prior to each vaccination. To improve the activation and maturation of APC, the pcDNA3.1-Mage-b DNA vaccine was mixed with plasmid DNA secreting GM-CSF. With this improved immunization protocol, a significant effect was observed on the metastases at young age and old age [71]. As expected, Mage-b-specific immune responses were induced in spleen and

draining LN (after re-stimulation with Mage-b) of young tumor-bearing mice. However, Mage-specific immune responses were completely absent in spleen and draining LN of old tumor-bearing mice and could not explain the significant effect of the vaccine on the metastases at old age. In order to evaluate what type(s) of cells were activated in vivo after vaccination, draining LN of young and old tumor-bearing mice, vaccinated with Mage-b, TGB and GM-CSF, were directly analyzed without any prior re-stimulation. It appeared that CD4 and CD8 T cells produced intracellular IFN γ and expressed IL-2 receptors at young but not at old age [71], while macrophages and NK cells produced intracellular IFN γ and expressed IL-2 receptors at old but not at young age. Moreover, immunohistochemical analysis indicated that the number CD8 T cells significantly increased in the metastases of young mice vaccinated with Mage-b, TGB and GM-CSF compared to the control groups, but were completely absent in the metastases of old mice (manuscript in preparation). Based on these results we concluded that adaptive CD8 T cell responses to Mage-b vaccination could not be induced in older mice with 4T1 tumors and metastases, but that macrophages and NK still seems active at old age. So far, we have not identified the discrepancy between the presence of intracellular levels of IFN γ and the absence of extracellular IFN γ in macrophages and NK cells at old age. This discrepancy could be related to the difference in vitro restimulations and in vivo situation, or an aging-related phenomenon.

Concluding remarks

The main conclusion from studies in preclinical models at young and old age is that cancer vaccines are less effective at older than at younger age. These results may imply that vaccines may not be very effective in cancer patients, which are usually elderly. However, these studies also demonstrate that optimization of vaccines for older age is feasible and crucial. A number of approaches that may lead to improvement of cancer vaccination at older age are proposed below.

Preventive vaccination at young age, offers great promise for elderly cancer patients. A first immunization at young age, when sufficient naïve T cells are still present, followed by recall at old age may improve T cell responses at older age. This approach has shown to be effective for improving Ab responses at older age in mice [72]. TAA expressed by the tumor but not by normal cells could be used for preventive vaccination, but improvement of T cell activation against weak immunogenic TAA will be necessary (for a review see [3]). Another approach could be addition of a DNA sequence encoding IL-7 [73] to the vaccine antigen in order to recruit only those naïve T cells that react with the vaccine antigen.

However, lack of naïve T cells is not the only hurdle to overcome, and it is clear that the immune system needs help to activate T cells against cancer cells. DNA vaccines are of great value since any DNA sequence can be added to the vaccine vector that may improve T cell activation with minimal toxicity. Our studies showed a complete lack of IL-2 and IFN γ secretion in spleen and LN at older age. Inclusion of IL-2 and IFN γ sequences into the vaccine vector may lead to T cell activation at older age. Other sequences such as IL-12 or IL-21 could be included as well. Also, activation of co-stimulatory molecules by anti-OX-40 and anti-4-1BB Abs seems to be a promising approach as shown by Sharma et al. [66]. Our own results suggest that the innate immune system seems still intact. Therefore, activation of

the innate immune system such as killer macrophages may be a useful alternative or addition to activation of the adaptive immune system. One way of activating killer macrophages is by a lipophilic glycopeptide liposyl muramyl phosphatidylethanolamine (L-TMP-PE) [74]. Based on previous studies [50], elimination of CD4⁺CD25⁺FOXP3⁺ T cells [51] or CD8⁺CD28⁺T cells may enhance vaccine efficacy at older age.

However, ageing is not the only problem that leads to T cell unresponsiveness. Other obstacles, such as immune suppression induced by large tumors or the low immunogenicity or genetic instability of tumors, as discussed in the section of T cell unresponsiveness in cancer patients, may occur at young and old age and needs to be overcome as well. Poor vaccine efficacy in “less old” cancer patients (51 years and younger) has been discussed by Rosenberg et al. [80]. Therefore, additional strategies that may improve vaccine efficacy in general are equally important. For instance, multi-antigen vaccines may prevent escape of genetically unstable tumor cells that have lost antigen expression. Many tumors produce lymphokines or factors that may inhibit vaccine-induced T cells responses such as TGFβ, IL-6, COX-2 and its products PGE₂, PD1-ligand, or IDO [27, 30]. Reduction of these factors may enhance vaccine efficacy as well. An example is the combination of cancer vaccination and curcumin, an Indian spice that inhibits IL-6. Also macrophages may produce these factors such as IL-6, IL-10, PGE₂, or TGFβ. Therefore, elimination of macrophages that produce IL-6, TGFβ, PGE₂ or IL-10 may improve vaccine efficacy. Improved delivery systems resulting in improved expression of the vaccine antigen in vivo, not discussed in this review, will certainly improve vaccine efficacy in general. A schematic view of the various approaches that may lead to improvement of cancer vaccination at older age is shown in Fig. 1.

Finally, it is obvious that in most of the vaccine studies discussed here, immunological responses were analyzed in old (and young) mice without tumors, while the primary tumors are crucial in the development of vaccine-induced immune responses. As discussed earlier, primary tumors may inhibit vaccine-induced immune responses, may lose antigen expression in vivo and therefore escape vaccine-induced immune responses. Tumors may also stimulate macrophages resulting in the production of factors that may inhibit vaccine-induced immune responses. It is also obvious that none of the vaccine studies in which vaccination was tailored to old age, and despite good anti-tumor responses, activation of CD8 T cells was shown in mice with tumors. Our own results with the 4T1 model did not show any CD8 response, while a significantly lower number of metastases were observed. Preliminary results suggest that innate rather than the adaptive immune responses were activated by vaccination. Currently, we are analyzing whether activation of innate immune responses could be used as a therapy against cancer at older age.

Despite all the obstacles that need to be overcome, vaccination against cancer is potentially the most promising approach. In most cancers, metastases and not primary tumors contribute to morbidity and mortality. In contrast to primary tumors, metastases cannot be removed by surgery or radiation, and are usually chemoresistant [81]. Our laboratory and others found that vaccination is effective against metastases [75–78]. Removal of primary tumors by surgery followed by vaccination to eliminate residual tumor cells and micrometastases may dramatically improve the clinical outcome of cancer treatment. The results discussed here

demonstrate the potential and the feasibility of cancer vaccination even for the old. However, to make vaccines effective at older age, testing and optimizing in suitable preclinical tumor models before going into clinical trials is of crucial importance.

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Fig. 1.

Improvement of cancer vaccination at older age. Various approaches are proposed that may improve vaccine-induced immune responses and subsequent in vivo efficacy at older age. This includes recruitment of naïve T cells or avoid the use of naïve T cells at older age, activation of adaptive and innate immune responses at older age, elimination of regulatory T cells or of macrophages that inhibit T cell activation, stimulation of macrophages that can kill tumor cells or that improves T cell activation, reduction of tumor-produced factors or lymphokines that inhibits T cell activation, as well as approaches that may lead to improved effect at young and old age such as improvement of DNA delivery in vivo or inclusion of DNA sequences into DNA vaccines that leads to improved T cell activation in general

Table 1

T cell unresponsiveness in cancer patients

Tumor-immunological parameters potentially involved in T cell unresponsiveness in cancer patients	
Tumors (produce lymphokines or factors that inhibits T cell activation)	
IL-6	
IL-10	
TGF β	
IDO	
COX-2	
PGE ₂	
Tumors (decreased expression of receptors and/or ligands that leads to T cell inactivation)	
MHC/TAA	
B7.1/2	
ICAM-1/2	
VCAM-1	
ELAM-1	
PD-L1-ligand	
CTL/T_{help} (decreased production of lymphokines or expression of receptors that leads to T cell inactivation)	
IL-2/IL-2 receptor	
IFN γ /IFN γ receptor	
IL-12/IL-12 receptor	
Inducible regulatory T cells (produce lymphokines or express ligands that inhibits T cell activation)	
TGF β	
IL-10	
CTLA-A4	
Macrophages/DC (produce lymphokines or factors that inhibits T cell activation)	
IL-6	
IL-10	
TGF β	
PGE ₂	

APC Antigen-presenting cells, *COX-2* cyclooxygenase-2, *CTL* cytotoxic T lymphocytes, *CTLA* cytotoxic T lymphocyte antigen, *DC* dendritic cells, *ELAM* epidermal lymphocyte adhesion molecule, *FoxP3* forkhead box P3, *ICAM* inter cellular adhesion molecule, *IDO* indolamine 2,3-dioxygenase, *IFN* interferon, *IL* interleukin, *MHC* major-histo-compatibility complex, *PGE* prostaglandine E, *TAA* tumor-associated antigens, *TGF* transforming growth factor, *T_{help}* helper T cells, *VCAM* vascular cellular adhesion molecule

Table 2

T cell unresponsiveness in the elderly

Age-related changes in the immune system potentially involved in T cell unresponsiveness in the elderly
CTL/T _{help} (decreased production of lymphokines or expression of receptors that leads to T cell inactivation)
IL-2/IL-2 receptor
IFN γ /IFN γ receptor
IL-12/IL-12 receptor
TCR
CD28
Natural regulatory T cells (different types have been identified involved in T cell inhibition)
CD4CD25Foxp3
CD4CD25 ^{high}
DC (decreased or increased production of lymphokines involved in T cell inactivation)
TNF α
IL-6
IL-12
Macrophages (decreased TLR signaling through decreased activation of PI3K pathway)
TLR1/2
TLR2/6
TLR3
TLR4
TLR5
TLR9

CD Cluster differentiation, *CTL* cytotoxic T lymphocyte, *DC* dendritic cells, *FoxP3* forkhead box P3, *IL* interleukin, *IFN* interferon, *PI* phosphatidylinositol 3-kinase, *T_{help}* helper T cells, *TCR* T cell receptor, *TLR* Toll-like receptor, *TNF* tumor necrosis factor