REVIEW

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Dendritic cell-based cancer immunotherapies

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Abstract

Because of their unique role in linking the innate and adaptive immune systems, dendritic cells (DCs) have been a logical focus for novel immunotherapies. However, strategies employing active immunization with *ex vivo* generated and antigen--pulsed DCs have shown limited efficacy in clinical trials. These past approaches did not take into account the complex interactions between cells of the innate immune system and DCs during DC maturation, antigen processing, and presentation to naïve T cells. By better understanding the natural sequence of events occurring *in vivo* during an effective immune response, we can tailor antitumor immunotherapeutic strategies to augment aspects of this response from the activation of innate immune cells to antigen uptake and DC maturation to priming of naïve T cells and, ultimately, to the establishment of antitumor immunity. Current DC vaccination strategies utilize a number of methods to recapitulate the cascade of events that culminate in a protective antitumor immune response.

Key words: dendritic cells (DCs), adjuvant, *ex vivo* DC therapy, *in vivo* DC therapy, immunotherapy.

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INTRODUCTION

Dendritic cells (DCs) interact with many cells of the immune system. Their interactions with naïve T cells during antigen presentation and T-cell priming have been well studied. Because of DCs' potent ability to present antigen, vaccines utilizing these cells should stimulate a protective immune response in cancer patients that is superior to other vaccination strategies (Finn 2003). However, clinical trials utilizing these vaccines have demonstrated rates of objective clinical benefit in less than 10% of vaccine recipients (Rosenberg et al. 2004). Therefore, improvements in DC vaccines are necessary before they can be considered a useful adjunct to conventional cancer treatment modalities. Current DC vaccine strategies use one of two approaches to augment antitumor immunity: *ex vivo* DC therapy, using *ex vivo* generated DCs loaded with tumor antigen to stimulate cytotoxic T cells, and *in vivo* DC therapy, using tumor cells or antigens linked to DC maturation stimuli to promote antigen uptake in a pro-inflammatory environment.

To evaluate the efficacy of *ex vivo* DC therapy, a number of factors need to be considered: the number of injected DCs (Inaba et al. 1992), the method of loading antigen onto DCs, the route of administration, the methods of immune monitoring, and study design. In particular, there is a question of whether *ex vivo* immune-monitoring methods such as tetramer staining, ELISPOT, immunostaining, and cytokine analysis accurately reflect *in vivo* immunological function. Several studies utilizing *ex vivo* generated DCs demonstrated a correlation between the persistence of antigen in the DCs and the magnitude of the immune response. In addition to the *ex vivo* approach, a number of studies have recently focused on targeting antigen to *in vivo*

DCs for processing and presentation. This strategy is comprised of two steps. The first targets antigen to DCs via specific DC receptors and the second involves the elaboration of danger signals to induce *in situ* DC maturation, thus linking innate and adaptive immunity. In this review we examine the recent advances in *ex vivo* and *in vivo* DC immunotherapy and their efficacy in the induction of protective antitumor immunity.

EX VIVO DC THERAPY

Most DC immunotherapies use *ex vivo* DCs generated from monocytes or $CD34^+$ hematopoietic stem cells obtained from cancer patients during leukocyte apheresis. To obtain large numbers of cells suitable for therapeutic applications under Good Manufacturing Practice (GMP)-grade conditions, it is recommended to isolate and enrich $CD14^+$ cells from apheresis products using immunomagnetic isolation devices (CliniMACS) or cell separators (Elutra) (Finn 2003; Rosenberg et al. 2004). In many studies, isolated monocytes are cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4, followed by incubation with a cocktail of maturation cytokines, including IL-1β, IL-6, tumor necrosis factor (TNF)-α, and prostaglandin E_2, in order to mature DCs for use in clinical trials (Jonuleit et al. 1997).

Cytokines can be used to skew the *ex vivo* differentiation of DCs into various phenotypes with vastly different functions. After activation, monocytes that encounter IL-4 become DCs known as IL-4-DCs (immature DCs) (Romani et al. 1994; Sallusto and Lanzavecchia 1994). By contrast, incubation with interferon (IFN)-α, TNF-α, or IL-15 differentiates activated monocytes into IFN-α-DCs (Luft et al. 1998; Paquette et al. 1998; Santini et al. 2000), TNF-α-DCs ($CD1a^+$ Langerhans cells and $CD14^+$ interstitial DCs) (Chomarat et al. 2003), or IL-15-DCs (Mohamadzadeh et al. 2001), respectively. The importance of these phenotypic variations has been demonstrated in studies that showed IFN-α- or IL-15-DCs pulsed with melanoma-derived peptide were more efficient than IL-4-DCs in the induction of antigen-specific cytotoxic T lymphocytes (CTLs) (Mohamadzadeh 2001; Santini et al. 2000).

Methods of loading antigen onto DCs are another consideration in studies using *ex vivo* generated DCs. In particular, antigen loading onto MHC class I and class II molecules differentially induces $CD8^+$ and $CD4^+$ T cells. Access to either the class I or class II pathway depends on the form of antigen loaded onto DCs. Different forms of antigen are currently being studied, including peptides, whole protein, tumor lysates, apoptotic tumor bodies, DCs fused with tumor cells, and DCs transfected with cDNA or mRNA encoding tumor antigens. Studies have shown that persistence of antigen in *ex vivo* loaded DCs is important in the induction of an effective antitumor immune response (Antonia et al. 2004; Davis et al. 2003; Finn 2003; Gilboa 2007). This is most likely due to the fact that it takes time for peripherally injected DCs to reach the lymph nodes where lymphocyte priming occurs.

Immunotherapy with tumor-associated antigen peptide

Many studies have evaluated the efficacy of loading DCs with short peptides corresponding to MHC class I or class II tumor epitopes. Although peptides can be chemically synthesized and are readily available in GMP grade, the logistical advantage of using peptides is limited by the availability of characterized tumor epitopes, the decreased stability of exogenous peptide-MHC complexes, and patient-specific MHC haplotype restrictions. Moreover, another level of complexity is added with the use of modified heteroclitic peptides, which are usually derived from immunodominant antigens on tumor cells and may be modified to improve their immunogenicity by increasing their affinity for MHC class I molecules (Parkhurst et al. 1996; Salgaller et al. 1996; Solinger et al. 1979). However, T cells elicited by vaccination with such molecules demonstrated poor recognition and cytolytic function compared with endogenous antigen--specific T cells (Stuge et al. 2004). Therefore loading DCs with total antigen, such as recombinant proteins, dying tumor cells, and exosomes, or transducing DCs with viral vectors and mRNA improves the efficacy of antigen presentation by allowing natural processing and epitope selection.

DCs have been shown to process peptides from phagocytosed dead tumor cells efficiently and present them to $CD8^+$ T cells. Our group demonstrated that CD34-derived DCs pulsed with irradiated tumor cells can induce an antigen-specific T cell response (Fujii et al. 1999). In other studies comparing the efficacy of cross-presentation using necrotic cells versus apoptotic cells it was shown that exposure to necrotic tumor cells, more than to apoptotic cells, induced the maturation of immunostimulatory DCs (Larmonier et al. 2006; Melcher et al. 1999; Sauter et al. 2000; Schnurr et al. 2002). This may be due to the release of heat-shock proteins (HSPs) gp96, calreticulin, Hsp90, and Hsp70, which occurs when cells undergo necrosis, but not apoptosis (Basu et al. 2000). Palucka et al. recently showed that vaccination with DCs pulsed with killed allogeneic melanoma cell lines from Colo829 cells led to the induction of MART-1-specific $CD8^+$ T cells (Palucka et al. 2006).

mRNA-transfected DC therapy

DCs can be transfected with antigen-encoding mRNA for processing and epitope presentation on MHC class I molecules. This approach has the advantage of extended periods of antigen persistence in transfected DCs. Vaccination with DCs transfected with mRNA encoding tumor antigen stimulated robust CTL responses and antitumor immunity in mice and humans

(Gilboa 2007). The use of whole antigen has the advantage of allowing specific epitope selection for different MHC molecules, thus generating CTL responses across MHC classes. In particular, specific CTLs have been reported for such whole-tumor antigens as carcinoembryonic antigen (CEA) (Nair et al. 1998), prostate-specific antigen (PSA) (Heiser et al. 2000), and telomerase reverse transcriptase (TERT) (Nair et al. 2000; Su et al. 2005).

In phase I/II prostate cancer and renal cancer clinical trials, vaccination with mRNA-transfected DCs induced tumor antigen-specific $CD8^+$ T-cell responses in the majority of patients (Gilboa and Vieweg 2004). Gilboa et al. reviewed a number of vaccination studies in which DCs were transfected with mRNA encoding tumor antigens such as PSA or TERT as well as unfractionated tumor-derived mRNA and found virtually all vaccinated patients developed tumor-specific $CD8^+$ T-cell responses (Gilboa and Vieweg 2004; Heiser et al. 2001; Su et al. 2005). Furthermore, clinically related responses, such as reduction in PSA level, were also seen in vaccinated patients (Heiser et al. 2002). Improvements to this approach could be made by enhancing MHC class I antigen presentation through the addition of adjuvant and selecting better DCs for antigen mRNA transfection.

IN VIVO DC THERAPY

Currently there are several strategies for modulating the function of DCs *in vivo*. One method delivers antigen to DCs through antibodies targeting specific DC lectin molecules, such as CD205 (DEC205), the mannose receptor, DC-SIGN, and Langerin (Tacken et al. 2007). Another uses adjuvants, such as GM-CSF, that attract DCs to the site of antigen deposition. Finally, dying cells can also be used as a means of antigen delivery in a pro-inflammatory environment. Methods of *in vivo* DC maturation include targeting Toll-like receptor (TLR) ligands as well as activation of innate lymphocytes (Fig. 1).

GM-CSF gene-transduced immunotherapy

GVAX therapy uses autologous cancer cells transduced with retroviral or adenoviral vectors carrying the GM-CSF gene (Dranoff et al. 1993). In this strategy, GM-CSF secreted by the tumor cells recruit DCs to the vaccination site and enhances DC antigen uptake and presentation to both T cells and B cells. Clinically, this technique has been shown to be safe and effective in boosting immune responses to a number of different cancers, including multiple myeloma, acute myeloid leukemia (AML), non-small-cell lung carcinoma (NSCLC), and pancreatic cancer (Simons et al. 1997; Soiffer et al. 1998).

Phase I/II clinical trials have been conducted using autologous tumor cells mixed with an allogeneic GM--CSF-secreting cell line (erythroleukemia cell line K562, i.e. K562/GM). This method avoids the necessity of genetically transducing autologous tumor cells and demonstrated a 25-fold increase in vaccine GM-CSF secretion. However, an objective clinical benefit over autologous GVAX vaccines was not demonstrated. Next, GVAX trials using K562/GM and autologous tumor cells have been reported in patients with multiple myeloma, AML, NSCLC, and pancreatic cancer and these turned out to be safe, although the clinical response in the NSCLC study was less favorable (Hege et al. 2006; Nemunaitis et al. 2006).

Complete allogeneic GVAX therapy using allogeneic cell lines such as PANC have been studied in patients with pancreatic cancer (Jaffee et al. 2001; Jaffee et al. 1998). In phase I/II trials in patients with prostate cancer, two GM-CSF-modified cell lines, LNCaP and PCs, were tested and found to be safe and effective in eliciting high-titer antibody responses to cell-line antigens (Higano et al. 2008; Small et al. 2007; Urba et al. 2008). This immunotherapy proved to be immunologically active and safe enough for clinical use in 21 hormone-naive prostate cancer patients (Simons et al. 2006). The most recent phase II trial evaluated the efficacy of allogeneic GVAX-modified immunotherapy in 55 chemotherapy-naive patients with hormone-refractory prostate cancer (Small et al. 2007). Patients with both radiologic metastases and rising PSA levels were treated with high-dose immunotherapy (3×10^8 cells) and demonstrated a longer median survival time than those treated with low-dose immunotherapy (1×10^8 cells) and those not treated with GVAX cells. Another phase I/II dose-escalation study found that GVAX immunotherapy provided a longer median survival for patients with hormone refractory prostate cancer, regardless of changes in serum PSA levels (Higano et al. 2008). However, Cell Genesys announced in 2008 that a phase III study in patients with symptomatic hormone-refractory advanced prostate cancer was halted by the study's independent data monitoring committee due to a disproportionate number of deaths in patients treated with GVAX plus Taxotere versus those in the control arm treated with Taxotere plus prednisone.

Immunotherapy using TLR ligands

Innate immunity is linked to adaptive immunity through DC maturation, which has a number of triggers, including TLRs (Fig. 1). DC maturation via TLRs leads to a Th1 response. Because of this, a number of TLR agonists have been investigated for use as adjuvants to immunotherapy. Animal studies demonstrate that TLR agonists can elicit robust and specific immune responses. Because they are located on the cell surface, TLR1, 2, 4, 5, 6, and 11 detect external pathogen products, such as lipopeptides (TLR1, 2, and 6), LPS (TLR2 and 4), flagellin (TLR5), and profilin (TLR11) (van Duin et al. 2006; Yarovinsky et al. 2006). TLR3, 7, 8, and 9 are located in the endoplasmic reticulum and the endosomal/lysosomal compartment, where they detect intracel-

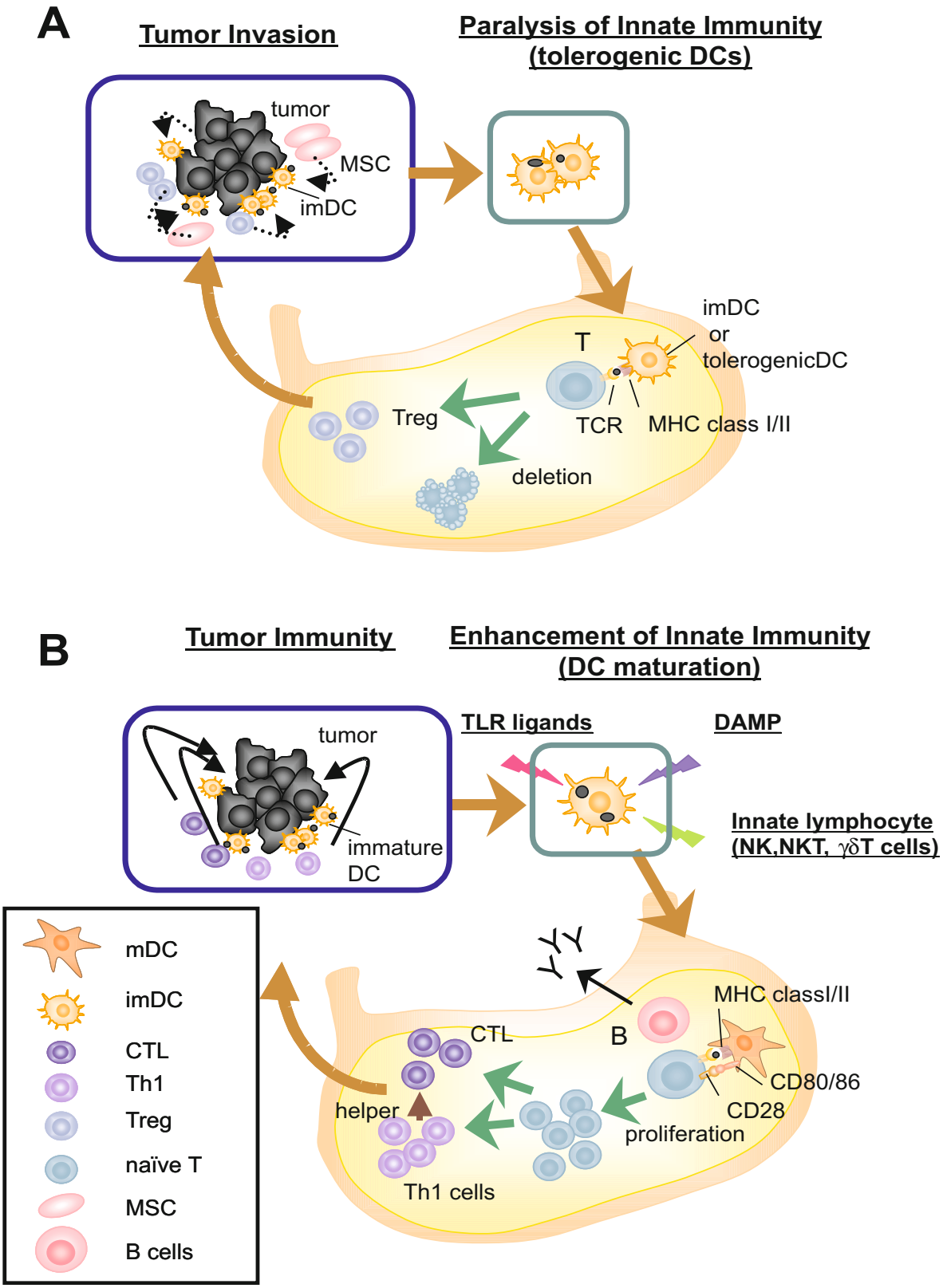

Fig. 1. DC maturation leads to adaptive immunity. **(A)** In the tumor microenvironment, immature DCs (imDCs) or tolerogenic DCs capture tumor antigen in the absence of inflammatory mediators. Naïve T cells that respond to antigen presented by imDCs or tolerogenic DCs are either deleted at secondary lymphoid organs or become specific T regulatory cells (Treg). Tumor invasion is further enhanced by Treg and myeloid suppressor cells (MSC) present at the tumor site. **(B)** When imDCs capture tumor antigens and undergo maturation, tumor antigen-specific $CD4^+$ T (Th1) cells and $CD8^+$ T (CTLs) cells are generated in secondary lymphoid tissues. Damage-associated molecular pattern (DAMP) molecules, Toll-like receptor (TLR) ligands (e.g. LPS, CpG-ODN, and PolyIC) and activated NKT cells at the tumor site all serve as maturation stimuli for immature DCs. T cells primed against tumor antigen by mature DCs kill local tumor and migrate to metastases at other sites. Abbreviations: mDC – mature dendritic cells, CTL – cytotoxic T lymphocytes, Th1 – Th1 helper T cells, Treg – regulatory $CD4^+CD25^+$ T cells.

lular nucleic acids such as bacterial or viral nucleic acid, PolyIC (TLR3 and MDA5), R848 (TLR7 and 8), and CpG-ODN (TLR9) (van Duin et al. 2006).

TLR ligand binding to protein antigens has been reported to enhance antigen presentation to T cells (Heit et al. 2005; Wille-Reece et al. 2005). In addition, the immunostimulatory sequence of CpG-ODN in combination with antigen conjugates has proven to be an efficient Th1-inducing adjuvant (Datta et al. 2004). This suggests that both the antigen and TLR ligand need to co-localize within the same phagosome for efficient MHC class II antigen presentation to link innate and adaptive immunity.

When a ligand binds to a TLR, it activates an intracellular signaling pathway through NF-κB, mitogen-activated protein kinases, and IFN regulatory factor 3, which leads to the production of pro-inflammatory cytokines and type I IFN and, finally, to the expression of cell surface co-stimulatory molecules (Creagh and O'Neill 2006).

Clinical studies using TLR agonists have begun for patients with several types of cancer. Imiquimod, an FDA-approved topically applied TLR7 agonist, has shown clinical efficacy against basal cell carcinoma (Beutner et al. 1999) and has been used in combination with NY-ESO-1 protein in patients with malignant melanoma (Adams et al. 2008). A phase II study in stage IIIB and IV NSCLC patients comparing taxane and platinum chemotherapy plus PF-3512676, a synthetic TLR9-activating oligodeoxynucleotide, demonstrated improved objective responses and median survival time (Manegold et al. 2008). Another phase II study evaluated the efficacy of polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) in the treatment of patients with recurrent anaplastic gliomas (Butowski et al. 2009). This study demonstrated that poly-ICLC was well tolerated, but did not improve six-month progression-free survival. Common adverse events included pancytopenia and a flu-like syndrome. Additional studies will need to be done to evaluate the safety and clinical efficacy of this therapy further.

Immunotherapy using anti-CD40 agonist antibody

CD40, a member of the TNF receptor superfamily, is expressed on the cell surface of DCs, B cells, and monocytes and broadly regulates the immune response (Grewal and Flavell 1998). Its ligand, CD154, is expressed on the surface of activated T lymphocytes and serves as a co-stimulatory molecule providing activation to antigen-presenting cells (APCs) through CD40. Agonistic CD40 antibodies can serve as a substitute for activated $CD4^+$ T lymphocytes in murine models of T cell-mediated immunity (Ridge et al. 1998; Schoenberger et al. 1998). In addition, combined triggering of CD40 and TLR ligands has been shown to lead to an expansion of $CD8^+$ T cells and increased CD70 expression on DCs (Ahonen et al. 2004; Sanchez et al. 2007).

CD40 is also expressed on 30–70% of primary solid tumor cells. Engagement of CD40 on tumor cells results in tumor-cell apoptosis *in vitro* and impaired tumor growth *in vivo*. In a phase I trial, a human CD40 agonist monoclonal antibody, CP-870893, was well tolerated and demonstrated an objective partial response in 27% (n=4) of melanoma patients enrolled in the study (Vonderheide et al. 2007). The most common adverse event was cytokine release syndrome. The danger of this systemic inflammatory response is highlighted in a phase I study in which six healthy volunteers received a dose of the superagonistic anti-CD28 antibody TGN1412 and experienced a massive systemic release of pro-inflammatory cytokines, leading to multi-system organ failure within 16 h (Suntharalingam et al. 2006).

Immunotherapy with HSPs

Prokaryotic and eukaryotic cells respond to stress with the production of a number of highly conserved proteins known as HSPs (Binder et al. 2004; Todryk et al. 2000). Depending on their location inside or outside the cell, HSPs, such as Hsp27, Hsp70, and Hsp90, can serve as molecular chaperons or mediators of the immune system (Basu et al. 2001; Schmitt et al. 2007; Srivastava 2002a; Srivastava 2002b).

As a cytoprotective function, HSPs serve as inhibitors of apoptosis, interacting with various components of the tightly regulated programmed cell death machinery. Because of this, HSPs can have tumorigenic properties and are over-expressed in some tumor cells. In addition, the ability of HSPs to stabilize mutant proteins, such as bcr-abl and p53, also enhances tumor survival. Hsp27 has been found in breast cancer, osteosarcoma, endometrial and gastric cancer associated with metastases, and poor-prognosis leukemia (Gehrmann et al. 2003; Schmitt et al. 2007). Hsp90 is also over-expressed in breast tumors, lung cancer, leukemias, Hodgkin's disease, and B-cell non-Hodgkin's lymphomas (Schmitt et al. 2007). Phase I and II studies were conducted in melanoma patients using Hsp90 inhibitors, such as 17-AAG, and only short-lived effects were found with safety and no objective antitumor responses (Schmitt et al. 2007; Solit et al. 2008).

Extracellularly located or membrane-bound HSPs act as key regulators of the host's immune system. HSP-chaperoned tumor-derived peptides are internalized by APCs via endocytosis and loaded onto MHC class I molecules for activation of $CD8^+$ T cells. In DCs, these antigens can additionally be presented on MHC class II molecules for stimulation of $CD4^+$ T cells. For example, Hsp70 initiates antitumor immunity by activating DCs via the TLR2 or TLR4 pathway, resulting in TNF-α, IL-6, and IL-1β production (Asea et al. 2000; Schmitt et al. 2007; Wang et al. 2002). Because of this, HSP peptides are being evaluated in many phase I–III clinical trials (Lewis 2004; Mazzaferro et al. 2003; Testori et al. 2008). A study of 29 colorectal patients demonstrated immunological responses in more than half of those

given a Gp96-peptide vaccine (Mazzaferro et al. 2003). Clinical responders showed a two-year overall survival rate of 100%, compared with 50% for non-responders. However, in a similar study, renal cell carcinoma patients given the HSP Gp96 (vitespen) showed no difference in disease-free survival compared with those receiving no treatment after nephrectomy for renal cell carcinoma (Wood et al. 2008). Furthermore, Hsp70 triggers NK-cell activation, particularly in NK cells expressing CD94 (Schmitt et al. 2007). Acute and chronic myeloid leukemia cells frequently express Hsp70 (Gehrmann et al. 2003). Stress-inducible increases in Hsp70 density are correlated with an increased tumor--cell sensitivity to NK cell-mediated killing. Because of this, some types of chemotherapy for some hematological malignancies might be augmented with NK cell--based immunotherapies.

Immunotherapy using iNKT-cell activation

DC maturation leads to an upregulation of co--stimulatory molecules, enhancement of antigen-presenting capacity, and expression of chemokine receptors that promote migration to nodal T-cell areas (Fig. 1). Targeting antigen to DC receptors without providing proper maturation stimuli results in tolerance in mice. Our group has studied ways of directing specific tumor antigens linked with maturation stimuli to *in vivo* DCs. We found that activation of the innate immune system, particularly invariant natural killer T (iNKT) cells, led to the maturation of *in vivo* DCs and a polarized Th1 immunogenic response to a number of tumor antigens.

α-Galactosylceramide (α-GalCer) is a glycosphingolipid compound extracted from the marine sponge *Agelas mauritianus*. It is capable of activating iNKT cells *in vitro* and *in vivo*, thus enabling them to mature DCs via cytokine secretion and cell-cell interactions. Even transient activation of iNKT cells is sufficient for the development of adaptive T-cell immunity; however, administration of unbound α-GalCer can drive iNKT cells into an anergic state for a long-term period (Bezbradica et al. 2005; Fujii et al. 2002; Parekh et al. 2005). Recently, Silk et al. demonstrated that iNKT cells stimulated by nonglycosidic CD1d lipid ligands, such as threitolceramide, were initially refractory to a subsequent *in vitro* α-GalCer challenge, but recovered their ability to produce IFN-γ (Silk et al. 2008). Our group has shown that adoptive transfer of tumor cells loaded with α-GalCer led to both innate and adaptive immunity after tumor antigen cross-presentation by host DCs (Shimizu et al. 2007b). Specifically, administration of B16 melanoma cells loaded with α-GalCer (B16/Gal) or B16 melanoma cells transduced to express high levels of CD1d and loaded with α-GalCer ($CD1d^{hi}$B16/Gal) protected mice from the development of B16 lung metastases after tumor challenge. This resistance to tumor challenge continued for more than six months after vaccination and has been replicated using a number of different tumor cell lines. In all cases, this resistance was dependent on NK and iNKT cells.

Mice given i.v. tumor cells loaded with α-GalCer (tumor/Gal) developed innate iNKT cell-dependent immunity against subsequent i.v. tumor challenge, as expected. Surprisingly, these mice also became resistant to subcutaneous challenge with tumor cells, unlike mice given DCs loaded with α-GalCer (DC/Gal) i.v. (Fujii et al. 2007; Shimizu et al. 2007a). The $CD4^+$ and $CD8^+$ T cell-mediated resistance to tumor challenge was due to antigen uptake by host DCs and their subsequent maturation by activated iNKT cells. After tumor regression occurred, T cells responding to a variety of specific tumor antigens, such as Trp2, Tyrp, Dct, and gp100, persisted as memory T cells. When glycolipid-loaded tumors are captured by DCs *in situ*, DCs perform two kinds of cross-presentation: the presentation of antigen peptide to $CD8^+$ T cells and the presentation of glycolipids to NKT cells.

iNKT cell-based immunotherapy using α-GalCer--loaded DCs has been well tolerated in clinical trials (Chang et al. 2005; Ishikawa et al. 2005; Nieda et al. 2004) and is effective in mediating iNKT-cell activation and antitumor cytotoxic effects, leading in some patients to prolonged stable disease or extending the time to tumor progression.

CONCLUSION

Both *ex vivo* and *in vivo* DC immunotherapies have been well tolerated in clinical trials and offer new strategies for the treatment of cancer. We now have a better understanding of the importance of linking DC maturation with antigen acquisition in the development of protective antitumor immunity. Using several strategies, including TLR ligands, CD40 agonists, *ex vivo* DC maturation, GM-CSF expression by tumor cells, and *in vivo* activation of iNKT cells, DC-based immunotherapies better recapitulate the natural cascade of events necessary for the induction of a cytotoxic immune response. Taken together, patient responses to *ex vivo* DC vaccinations were higher (9.5%) than that of antitumor immunotherapies using peptides (2.7%), viral vectors (1.9%), or irradiated tumor cells (4.6%) (Banchereau and Palucka 2005).

In the past, obstacles to the success of DC-based cancer vaccines included the induction of tolerance through the generation of antigen-specific suppressor cells, such as $CD4^+$ (Curiel 2008) and $CD8^+$ regulatory T cells (Endharti et al. 2005; Rifa'I et al. 2004), type II NKT cells (Berzofsky and Terabe 2008; Terabe and Berzofsky 2007), myeloid suppressor cells (MSCs) (Delano et al. 2007; Nagaraj and Gabrilovich 2008; Nagaraj et al. 2007), and tumor-induced $CD4^+$ T cells capable of producing IL-13 (Aspord et al. 2007) (Fig. 1). In particular, the suppressive effects of regulatory T cells on DCs may impair vaccine response by inhibiting DC antigen presentation (Larmonier et al. 2007;

Misra et al. 2004). The efficacy of conventional therapies, such as chemotherapy and radiation therapy, and novel therapies, such as blocking antibodies for CTLA-4, TGF-β, or IL-10, may be partly due to the eradication of regulatory T cells present in tumor tissue. Numerous studies have shown inactivation of regulatory T cells may enhance the effects of DC-based therapy. Prasad et al. showed that treatment with anti-CD25 antibody to deplete $CD4^+CD25^+$ regulatory T cells before DC vaccination considerably improved the effect of vaccines (Prasad et al. 2005). The effect of MSCs and immature and tolerogenic DCs on the induction of T-cell tolerance, Treg expansion, and tumor-induced immunosuppression needs to be further delineated (Huang et al. 2006; Marigo et al. 2008; Serafini et al. 2008). The experience of the anti-CD28 Ab TGN1242 clinical trial highlights the importance of the immune balance between cytotoxic and regulatory T cells.

Therapies that combine the inhibition of regulatory factors and the augmentation of antigen presentation by DCs may prove more effective in treating cancers that are resistant to conventional therapeutic methods. Because DCs uniquely bridge the gap between the innate and adaptive immune system, immunotherapies targeting these cells are a vital area of research for the development of future cancer therapies.

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REFERENCES

Adams S, O'Neill DW, Nonaka D et al (2008) Immunization of malignant melanoma patients with full-length NY-ESO-1 protein using TLR7 agonist imiquimod as vaccine adjuvant. J Immunol 181:776–784

Ahonen CL, Doxsee CL, McGurran SM et al (2004) Combined TLR and CD40 triggering induces potent CD8+ T cell expansion with variable dependence on type I IFN. J Exp Med 199:775–784

Antonia S, Mule JJ, Weber JS (2004) Current developments of immunotherapy in the clinic. Curr Opin Immunol 16:130–136

Asea A, Kraeft SK, Kurt-Jones EA et al (2000) HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6:435–442

Aspord C, Pedroza-Gonzalez A, Gallegos M et al (2007) Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. J Exp Med 204:1037–1047

Banchereau J and Palucka AK (2005) Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol 5:296–306

Basu S, Binder RJ, Ramalingam T et al (2001) CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity 14:303–313

Basu S, Binder R, Suto R et al (2000) Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-κB pathway. Int Immunol 12:1539–1546

Berzofsky JA and Terabe M (2008) NKT cells in tumor immunity: opposing subsets define a new immunoregulatory axis. J Immunol 180:3627–3635

Beutner KR, Geisse JK, Helman D et al (1999) Therapeutic response of basal cell carcinoma to the immune response modifier imiquimod 5% cream. J Am Acad Dermatol 41:1002–1007

Bezbradica JS, Stanic AK, Matsuki N et al (2005) Distinct roles of dendritic cells and B cells in Vα14Jα18 natural T cell activation in vivo. J Immunol 174:4696–705

Binder RJ, Vatner R, Srivastava P (2004) The heat-shock protein receptors: some answers and more questions. Tissue Antigens 64:442–441

Butowski N, Lamborn KR, Lee BL et al (2009) A North American brain tumor consortium phase II study of poly-ICLC for adult patients with recurrent anaplastic gliomas. J Neurooncol 91:183–189

Chang DH, Osman K, Connolly J et al (2005) Sustained expansion of NKT cells and antigen-specific T cells after injection of α-galactosyl-ceramide loaded mature dendritic cells in cancer patients. J Exp Med 201:1503–1517

Chomarat P, Dantin C, Bennett L et al (2003) TNF skews monocyte differentiation from macrophages to dendritic cells. J Immunol 171:2262–2269

Creagh EM and O'Neill LA (2006) TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. Trends Immunol 27:352–357

Curiel TJ (2008) Regulatory T cells and treatment of cancer. Curr Opin Immunol 20:241–246

Datta SK, Cho HJ, Takabayashi K et al (2004) Antigen-immunostimulatory oligonucleotide conjugates: mechanisms and applications. Immunol Rev 199:217–226

Davis ID, Jefford M, Parente P et al (2003) Rational approaches to human cancer immunotherapy. J Leukoc Biol 73:3–29

Delano MJ, Scumpia PO, Weinstein JS et al (2007) MyD88-dependent expansion of an immature GR-1(+)CD11b(+) population induces T cell suppression and Th2 polarization in sepsis. J Exp Med 204:1463–1474

Dranoff G, Jaffee E, Lazenby A et al (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophate colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 90:3539–3543

Endharti AT, Rifa'I M, Shi Z et al (2005) Cutting edge: CD8+CD122+ regulatory T cells produce IL-10 to suppress IFN-gamma production and proliferation of CD8+ T cells. J Immunol 175:7093–7097

Finn OJ (2003) Cancer vaccines: between the idea and the reality. Nat Rev Immunol 3:630–641

Fujii S, Fujimoto K, Shimizu K et al (1999) Presentation of tumor antigens by phagocytic dendritic cell clusters generated

from human CD34+ hematopoietic progenitor cells: induction of autologous cytotoxic T lymphocytes against leukemic cells in acute myelogenous leukemia patients. Cancer Res 59:2150–2158

Fujii S, Shimizu K, Hemmi H et al (2007) Innate Vα14$^+$ natural killer T cells mature dendritic cells, leading to strong adaptive immunity. Immunol Rev 220:183–198

Fujii S, Shimizu K, Kronenberg M et al (2002) Prolonged interferon-γ producing NKT response induced with α-galactosylceramide-loaded dendritic cells. Nat Immunol 3:867–874

Gehrmann M, Schmetzer H, Eissner G et al (2003) Membrane-bound heat shock protein 70 (Hsp70) in acute myeloid leukemia: a tumor specific recognition structure for the cytolytic activity of autologous NK cells. Haematologica 88:474–476

Gilboa E (2007) DC-based cancer vaccines. J Clin Invest 117:1195–1203

Gilboa E, Vieweg J (2004) Cancer immunotherapy with mRNA-transfected dendritic cells. Immunol Rev 199:251–263

Grewal IS, Flavell RA (1998) CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 16:111–135

Hege KM, Jooss K, Pardoll D (2006) GM-CSF gene-modified cancer cell immunotherapies: of mice and men. Int Rev Immunol 25:321–352

Heiser A, Coleman D, Dannull J et al (2002) Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. J Clin Invest 109:409–417

Heiser A, Dahm P, Yancey D et al (2000) Human dendritic cells transfected with RNA encoding prostate specific antigen (PSA) stimulate prostate specific CTL responses in vitro. J Immunol 164:5508–5514

Heiser A, Maurice MA, Yancey DR et al (2001) Induction of polyclonal prostate cancer-specific ctl using dendritic cells transfected with amplified tumor rna. J Immunol 166:2953–2960

Heit A, Schmitz F, O'Keeffe M et al (2005) Protective CD8 T cell immunity triggered by CpG-protein conjugates competes with the efficacy of live vaccines. J Immunol 174:4373–4380

Higano CS, Corman JM, Smith DC et al (2008) Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. Cancer 113:975–984

Huang B, Pan PY, Li Q et al (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

Inaba K, Inaba M, Romani N et al (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J Exp Med 176:1693–1702

Ishikawa A, Motohashi S, Ishikawa E et al (2005) A phase I study of α-galactosylceramide (KRN7000)-pulsed dendritic cells in patients with advanced and recurrent non-small cell lung cancer. Clin Cancer Res 11:1910–1917

Jaffee EM, Abrams R, Cameron J et al (1998) A phase I clinical trial of lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene for the treatment of pancreatic adenocarcinoma. Hum Gene Ther 9:1951–1971

Jaffee EM, Hruban RH, Biedrzycki B et al (2001) Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol 19:145–156

Jonuleit H, Kuhn U, Muller G et al (1997) Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. Eur J Immunol 27:3135–3142

Larmonier N, Marron M, Zeng Y et al (2007) Tumor-derived CD4(+)CD25(+) regulatory T cell suppression of dendritic cell function involves TGF-β and IL-10. Cancer Immunol Immunother 56:48–59

Larmonier N, Merino D, Nicolas A et al (2006) Apoptotic, necrotic, or fused tumor cells: an equivalent source of antigen for dendritic cell loading. Apoptosis 11:1513–1524

Lewis JJ (2004) Therapeutic cancer vaccines: using unique antigens. Proc Natl Acad Sci USA 101(suppl 2):14653–14656

Luft T, Pang KC, Thomas E et al (1998) Type I IFNs enhance the terminal differentiation of dendritic cells. J Immunol 161:1947–1953

Manegold C, Gravenor D, Woytowitz D et al (2008) Randomized phase II trial of a Toll-like receptor 9 agonist oligodeoxynucleotide, PF-3512676, in combination with first-line taxane plus platinum chemotherapy for advanced-stage non-small-cell lung cancer. J Clin Oncol 26:3979–3986

Marigo I, Dolcetti L, Serafini P et al (2008) Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. Immunol Rev 222:162–179

Mazzaferro V, Coppa J, Carrabba M et al (2003) Vaccination with autologous tumor-derived heat-shock protein gp96 after liver resection for metastatic colorectal cancer. Clin Cancer Res 9:3235–3245

Melcher A, Gough M, Todryk S et al (1999) Apoptosis or necrosis for tumor immunotherapy: what's in a name? J Mol Med 77:824–833

Misra N, Bayry J, Lacroix-Desmazes S et al (2004) Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. J Immunol 172:4676–4680

Mohamadzadeh M, Berard F, Essert G et al (2001) Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. J Exp Med 194:1013–1020

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

Nagaraj S, Gupta K, Pisarev V et al (2007) Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. Nat Med 13:828–835

Nair SK, Boczkowski D, Morse M et al (1998) Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. Nat Biotechnol 16:364–369

Nair SK, Heiser A, Boczkowski D et al (2000) Induction of cytotoxic T cell responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. Nat Med 6:1011–1017

Nemunaitis J, Jahan T, Ross H et al (2006) Phase 1/2 trial of autologous tumor mixed with an allogeneic GVAX vaccine in advanced-stage non-small-cell lung cancer. Cancer Gene Ther 13:555–562

Nieda M, Okai M, Tazbirkova A et al (2004) Therapeutic activation of $V\alpha24^+V\beta11^+$ NKT cells in human subjects results in highly coordinated secondary activation of acquired and innate immunity. Blood 103:383–389

Palucka AK, Ueno H, Connolly J et al (2006) Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. J Immunother 29:545–557

Paquette RL, Hsu NC, Kiertscher SM et al (1998) Interferon-α and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen-presenting cells. J Leukoc Biol 64:358–367

Parekh VV, Wilson MT, Olivares-Villagomez D et al (2005) Glycolipid antigen induces long-term natural killer T cell anergy in mice. J Clin Invest 115:2572–2583

Parkhurst MR, Salgaller ML, Southwood S et al (1996) Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A*0201--binding residues. J Immunol 157:2539–2548

Prasad SJ, Farrand KJ, Matthews SA et al (2005) Dendritic cells loaded with stressed tumor cells elicit long-lasting protective tumor immunity in mice depleted of CD4+CD25+ regulatory T cells. J Immunol 174:90–98

Ridge JP, Di Rosa F, Matzinger P (1998) A conditioned dendritic cell can be a temporal bridge between a $CD4^+$ T helper and a T-killer cell. Nature 393:474–478

Rifa'i M, Kawamoto Y, Nakashima I et al (2004) Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. J Exp Med 200:1123–1134

Romani N, Gruner S, Brang D et al (1994) Proliferating dendritic cell progenitors in human blood. J Exp Med 180:83–93

Rosenberg SA, Yang JC, Restifo NP (2004) Cancer immunotherapy: moving beyond current vaccines. Nat Med 10:909–915

Salgaller ML, Marincola FM, Cormier JN et al (1996) Immunization against epitopes in the human melanoma antigen gp100 following patient immunization with synthetic peptides. Cancer Res 56:4749–4757

Sallusto F and Lanzavecchia A (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. J Exp Med 179:1109–1118

Sanchez PJ, McWilliams JA, Haluszczak C et al (2007) Combined TLR/CD40 stimulation mediates potent cellular immunity by regulating dendritic cell expression of CD70 in vivo. J Immunol 178:1564–1572

Santini SM, Lapenta C, Logozzi M et al (2000) Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. J Exp Med 191:1777–1788

Sauter B, Albert ML, Francisco L et al (2000) Consequences of cell death. Exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. J Exp Med 191:423–434

Schmitt E, Gehrmann M, Brunet M et al (2007) Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J Leukoc Biol 81:15–27

Schnurr M, Scholz C, Rothenfusser S et al (2002) Apoptotic pancreatic tumor cells are superior to cell lysates in promoting cross-priming of cytotoxic T cells and activate NK and γδ T cells. Cancer Res. 62:2347–2352

Schoenberger SP, Toes RE, van der Voort EI et al (1998) T-cell help for cytotoxic T lymphocytes is mediated by CD40--CD40L interactions. Nature 393:480–483

Serafini P, Mgebroff S, Noonan K et al (2008) Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer Res 68:5439–5449

Shimizu K, Goto A, Fukui M et al (2007) Tumor cells loaded with α-galactosylceramide Induce innate NKT and NK cell--dependent resistance to tumor implantation in mice. J Immunol 178:2853–2861

Shimizu K, Kurosawa Y, Taniguchi Met al (2007) Cross-presentation of glycolipid from tumor cells loaded with α-galactosylceramide leads to potent and long-lived T cell mediated immunity via dendritic cells. J Exp Med 204:2641–2653

Silk JD, Salio M, Reddy BG et al (2008) Cutting edge: nonglycosidic CD1d lipid ligands activate human and murine invariant NKT cells. J Immunol 180:6452–6456

Simons JW, Carducci MA, Mikhak B et al (2006) Phase I/II trial of an allogeneic cellular immunotherapy in hormone-naive prostate cancer. Clin Cancer Res 12:3394–3401

Simons JW, Jaffee EM, Weber CE et al (1997) Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. Cancer Res 57:1537–1546

Small EJ, Sacks N, Nemunaitis J et al (2007) Granulocyte macrophage colony-stimulating factor-secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. Clin Cancer Res 13:3883–3891

Soiffer R, Lynch T, Mihm M et al (1998) Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. Proc Natl Acad Sci USA 95:13141–13146

Solinger AM, Ultee ME, Margoliash E et al (1979) T-lymphocyte response to cytochrome c. I. Demonstration of a T-cell heteroclitic proliferative response and identification of a topographic antigenic determinant on pigeon cytochrome c whose immune recognition requires two complementing major histocompatibility complex-linked immune response genes. J Exp Med 150:830–848

Solit AB, Osman I, Polsky D et al (2008) Phase II trial of 17--allyamino-demethoxygeldanamycin in patients with metastatic melanoma. Clin Cancer Res 14:8302–8307

Srivastava P (2002a) Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immunol 2:185–194

Srivastava P (2002b) Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu Rev Immunol 20:395–425

Stuge TB, Holmes SP, Saharan S et al (2004) Diversity and recognition efficiency of T cell responses to cancer. PLoS Med 1:e28

Su Z, Dannull J, Yang BK et al (2005) Telomerase mRNA--transfected dendritic cells stimulate antigen-specific CD8+ and CD4+ T cell responses in patients with metastatic prostate cancer. J Immunol 174:3798–3807

Suntharalingam G, Perry MR, Ward S et al (2006) Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med 355:1018–1028

Tacken PJ, de Vries IJ, Torensma R et al (2007) Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. Nat Rev Immunol 7:790–802

Terabe M, Berzofsky JA (2007) NKT cells in immunoregulation of tumor immunity: a new immunoregulatory axis. Trends Immunol 28:491–496

Testori A, Richards J, Whitman E et al (2008) Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. J Clin Oncol 26:955–962

Todryk SM, Melcher AA, Dalgleish AG et al (2000) Heat shock proteins refine the danger theory. Immunology 99:334–337

Urba WJ, Nemunaitis J, Marshall F et al (2008) Treatment of biochemical recurrence of prostate cancer with granulocyte--macrophage colony-stimulating factor secreting, allogeneic, cellular immunotherapy. J Urol 180:2011–2018

van Duin D, Medzhitov R, Shaw AC (2006) Triggering TLR signaling in vaccination. Trends Immunol 27:49–55

Vonderheide RH, Flaherty KT, Khalil M et al (2007) Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. J Clin Oncol 25:876–883

Wang Y, Kelly CG, Singh M et al (2002) Stimulation of Th1--polarizing cytokines, C-C chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding fragment of heat shock protein 70. J Immunol 169:2422–2429

Wille-Reece U, Flynn BJ, Lore K et al (2005) HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and CD8+ T cell responses in nonhuman primates. Proc Natl Acad Sci USA 102:15190 –15194

Yarovinsky F, Kanzler H, Hieny S et al (2006) Toll-like receptor recognition regulates immunodominance in an antimicrobial CD4+ T cell response. Immunity 25:655–664

Wood C, Srivastava P, Bukowski R et al (2008) An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy for renal cell carcinoma: a multicenter, open--label, randomised phase III trial. Lancet 372:145–154