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Enhancing Cancer Immunotherapy by Intracellular Delivery of Cell-Penetrating Peptides and Stimulation of Pattern-Recognition Receptor Signaling

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Abstract

The importance of T-cell-mediated antitumor immunity has been demonstrated in both animal models and human cancer immunotherapy. In the past 30 years, T-cell-based immunotherapy has been improved with an objective clinical response rate of up to 72%. Identification of MHC class I- and II-restricted tumor antigens recognized by tumor-reactive T cells has generated a resurgence of interest in cancer vaccines. Although clinical trials with cancer peptide/protein vaccines have only met a limited success, several phase II/III clinical trials are either completed or ongoing with encouraging results. Recent advances in immunotherapy have led to the approval of two anticancer drugs (sipuleucel-T vaccine and anti-CTLA-4 antibody) by the US FDA for the treatment of meta-static castration-resistant prostate cancer and melanoma, respectively. Intracellular delivery of antigenic peptides into dendritic cells (DCs) prolongs antigen presentation of antigen-presenting cells to T cells, thus further improving clinical efficacy of peptide/protein cancer vaccines. Because innate immune responses are critically important to provide sensing and initiating of adaptive immunity, combined use of cell-penetrating peptide vaccines with stimulation of innate immune signaling may produce potent anti-tumor immune responses. We will discuss the recent progress and novel strategies in cancer immunotherapy.

1. INTRODUCTION

The host immune system consisting of innate and adaptive immunity plays an essential role in immunosurveillance, recognition, and destruction of cancer cells (Schreiber *et al.*, 2011; Vesely *et al.*, 2011). T cell-mediated antitumor immunity has been demonstrated in murine tumor models 30 years ago by adoptive transfer experiments (Greenberg, 1991; Rosenberg, 1990; Rosenberg *et al.*, 2008). Recently, adoptive T-cell therapy has been successfully used to treat many human cancers such as melanoma, renal cell carcinoma, and lymphoma with varying degrees of tumor regression (Lesterhuis *et al.*, 2011; Rosenberg, 2011; Rosenberg *et al.*, 2008; Tey *et al.*, 2006). Although $CD4^+$ and $CD8^+$ T cells are the major components of T-cell-mediated antitumor immunity, natural killer (NK), NK1.1 T (NKT), and γδ T cells may also play a role in immunosurveillance against cancer (Diefenbach and Raulet, 2001; Vesely *et al.*, 2011; Wang, 2001). Using tumor-reactive T cells, many immunogenic tumor antigens have been identified (Boon *et al.*, 1994; Wang and Rosenberg, 1999). Clinical studies using molecularly defined MHC class I-restricted tumor antigens show that peptide vaccines can induce antigen-specific immune responses in the patients who received peptide vaccines, but overall immune responses elicited by the use of $CD8^+$ T-cell peptides are weak in several early clinical studies. However, several tumor antigens, including gp100 and

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MAGE-3, have been used in phase II and III clinical trials with encouraging results. Recently, antigen-presenting cells (APCs) loaded with tumor antigens have been approved by US Federal Food Administration (FDA) for the treatment of prostate cancer. This is an important milestone in the field of cancer immunology. However, blood APC-based vaccines require autologous cells from the patients and are expensive ($93,000 for a course of three treatments) with an average extension of survival of 4.1 months. To further improve broad application and clinical efficacy of cancer vaccines, we need to develop peptide/protein-based vaccines as a more broadly applicable and less expensive anticancer drugs. Further, recent studies have identified several checkpoints or roadblocks for T-cell activation and function (Callahan *et al.*, 2010; Chambers *et al.*, 2001; Zhu *et al.*, 2011). These include coinhibitory molecules and other negative regulators in T cells and dendritic cells (DCs), as well as immune suppression mediated by regulatory T cells (Zhu *et al.*, 2011). To enhance antitumor immunity, it is necessary to remove these roadblocks so that T cells can be fully activated and to mediate the eradication of cancer cells through modulating innate immune signaling. Importantly, anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) antibody therapy has a proven therapeutic effect on metastatic melanoma and has recently been approved by US FDA for the treatment of metastatic melanoma (Hodi *et al.*, 2010; Royal *et al.*, 2010). With encouraging results of clinical trials and FDA approval of two anticancer immunotherapy drugs, development of synthetic peptide/protein-based cancer vaccines will be the next frontline in the field of cancer immunotherapy and vaccines. In this chapter, we discuss a novel strategy for enhancing antitumor immune responses by intracellular delivery of peptides into DCs and stimulation of innate immune signaling to overcome immune suppression.

2. ADOPTIVE CELL THERAPY: HISTORY AND RECENT PROGRESSES

2.1. LAK and cytokine-induced killer cell therapy

In the early 1980s, lymphocyte-activated killer (LAK) cells were first used in mouse models and human cancer patients (Grimm *et al.*, 1982; Muul *et al.*, 1987). LAK cells are generated from the peripheral blood mononuclear cells (PBMCs) following *ex vivo* expansion in the presence of interleukin (IL)-2 and are capable of killing fresh tumor cells without requirement of MHC restriction. Clinical studies show modest efficacy against melanoma and renal carcinoma cancer (Rosenberg *et al.*, 1987; Takayama *et al.*, 2000). In the early 1990s, cytokine-induced killer (CIK) cells are generated from PBMCs in the presence of interferon (IFN)-γ on day 0, followed by adding anti-CD3 and IL-2 on day 1, with continued addition of IL-2 during 21–28 days of culture (Schmidt-Wolf *et al.*, 1991). These $CD3^+$ $CD56^+$ CIK cells are converted from $CD3^+$ $CD56^-$ cells and expanded up to 1000-fold after 21–28 days with mixed cell populations (2% NK cells, and >90% CD3 and $CD56^+$ T cells) and show strong cytotoxicity against many tumor cell targets without MHC restriction (Linn *et al.*, 2002). Thus, these cells may function similar to NK-like T cells. It is not clear how they recognize tumor targets and what antigens they recognize on tumor cells. Although CIK cells have been tested in a clinical setting for a variety of cancers, data on the efficacy of CIK therapy are limited. The first report of the international registry on CIK treatment of 426 patients shows that a large-scale expansion of CIK cells *ex vivo* is possible and cell infusion is safe (Hontscha *et al.*, 2011). The clinical response rate of CIK is 51/384 (13%) with complete and partial responses, 40/384 (10%) with minor response, and 161/384 (42%) with a stable disease (Hontscha *et al.*, 2011). Several groups report that combination of CIK or tumor-infiltrating lymphocyte (TIL) with chemotherapy produces better clinical response rates and reduces the percentage of relapse compared with chemotherapy alone (Dudley *et al.*, 2002; Jiang *et al.*, 2005). With encouraging clinical evidence of CIK therapy, it is important to conduct randomized clinical trials to determine the clinical benefit of cancer patients who receive CIK alone, chemotherapy alone, and CIK plus chemotherapy in the near future.

2.2. Adoptive T-cell therapy with TILs

Adoptive cell therapy (ACT) with TILs has emerged as the most effective treatment for patients with metastatic melanoma. TIL-based therapy was first reported in 1988, and clinical studies with TIL plus IL-2 showed 34% objective response rate compared with 16% clinical response with IL-2 alone (Rosenberg, 2000; Rosenberg *et al.*, 1988, 1994). Importantly, ACT with TILs has been dramatically improved for clinical efficacy by introducing treatment with a nonmyeloablative preparative immunodepleting regimen consisting of cyclophosphamide and fludarabine before T-cell infusion (Dudley *et al.*, 2002). Objective clinical response rates have been observed in 49–72% of patients with metastatic melanoma refractory to all other treatments (Rosenberg, 2011; Rosenberg *et al.*, 2008). This regimen depletes circulating lymphocytes as well as those at tumor sites for a week before host hematopoietic cell recovery, thus likely removing suppressive Treg cells and allowing antitumor T cells to survive and expand in patients after adoptive transfer. The unique features of ACT are that clinical responses can be durable (>5 years) and low percentages of relapse (Rosenberg *et al.*, 2011). More recently, whole body irradiation was added to the cyclophosphamide and fludarabine lymphodepleting preparative regimen before adoptive T-cell transfer. These further modifications result in the highest objective clinical responses in all patients receiving treatment (Wrzesinski *et al.*, 2010). However, because the difficulties associated with generation and expansion of TILs derived from tumor tissues and variable availability of such tumor tissues in cancer patients, ACT with autologous TILs is not suitable for many cancer patients, in particular, those with cancer other than melanoma. The success rate for generating TILs from other types of cancer is much lower than that seen in melanoma. Thus, use of T cells transduced with retro-viruses or lentiviruses encoding T-cell receptors (TCRs) that recognized tumor antigens or with genes encoding cytokines or costimulatory molecules has made ACT available for patients with different types of cancer. Alternatively, these peripheral T cells will be stimulated with antigenic peptides to generate antigen-specific T cells. This approach has been successfully used to treat lymphoma and melanoma with antigen-specific T cells (Hunder *et al.*, 2008; Leen *et al.*, 2006; Wolf *et al.*, 2003; Yee *et al.*, 2000, 2002). Despite impressive clinical response rates of ACT in cancer therapy in phase I/II clinical studies, so far no ACT treatment has been approved as an anticancer drug. This may be due to difficulties in patentability of many immunotherapeutic strategies or technologies, individualized treatment associated with high costs, and lack of funding and interests by the pharmaceutical companies. However, with recent successes and promising clinical results, ACT or other immune cell transfer therapies are likely to move into phase III trials and obtain FDA approval as anticancer drugs.

3. APC-BASED VACCINES: THE FIRST FDA APPROVED DRUG FOR PROSTATE CANCER

Professional APCs such as DCs can potently induce antigen-specific T cells. DC loaded with cancer antigens or tumor lysates has been used to vaccinate cancer patients with limited success. However, the US FDA recently approved the first therapeutic cancer vaccine drug (Provenge, Dendreon) for the treatment of metastatic castration-resistant prostate cancer. The sipuleucel-T vaccines consist of blood APCs, including monocytes and DCs, loaded with a fusion protein (PA2024) of the prostate antigen prostatic acid phosphatase linked to an immunostimulatory granulocyte–macrophage colony-stimulating factor. Although tumor regressions, radiographic, and prostate-specific antigen responses occurred very rarely, an overall survival benefit of 4.1 months was observed for the sipuleucel-T group versus the placebo group. This constitutes an important milestone in the field of cancer immunotherapy. However, because of the individual patient-specific nature and the high cost of this treatment ($93,000 for three infusions), it is very challenging to implement this treatment in the large eligible patient population. Direct *in vivo* targeting and activation of

DC with broadly injectable immunogens for cancer treatment are desirable. Moreover, the clinical efficacy of cancer vaccines remains to be improved. To achieve this purpose, the most appropriate tumor antigens and/or immune targets for immunotherapy of distinct cancer types need to be identified.

4. TUMOR ANTIGENS RECOGNIZED BY TUMOR-REACTIVE T CELLS

4.1. Tumor antigens recognized by CD8$^+$ T cells

In 1991, the first human tumor antigen was identified by screening cDNA expression library with tumor-reactive CD8$^+$ T cells (Van der Bruggen *et al.*, 1991). Since then, many tumor antigens have been identified using T cells with antitumor activity (Table 6.1). These tumor antigens can be classified into several types: (1) tissue-specific tumor antigens with higher expression in cancer cells compared with normal cells; (2) tumor-specific and shared antigens that are expressed in cancer and testis, but not in other normal tissues; (3) tumor-specific and unique antigens. Some of them, including gp100, MAGE-3, and NY-ESO-1, have been testing in phase II/III clinical trials with encouraging results. It should be noted that chronic infection or chronic inflammation has caused about 20% of human cancers (Coussens and Werb, 2002; De Marzo *et al.*, 2007). Epstein–Barr virus (EBV), a human gamma herpesvirus with tropism for B cells, has been implicated in the pathogenesis of a variety of human tumors, including immunoblastic lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, and Hodgkin disease (Leen *et al.*, 2007; Young and Rickinson, 2004). Infection with oncogenic human papilloma virus (HPV) is causally linked to the development of cervical cancer (Melief and van der Burg, 2008). Hepatitis B virus (HBV) and HCV infection of liver cells is a primary factor in the development of liver cancer (Coussens and Werb, 2002; Karin *et al.*, 2006; Peto, 2001). Viral antigens derived from EBV, HPV, and HBV are not the subject of this review but have been extensively used to develop prophylactic and therapeutic vaccines against viral infection and cancer (Huang *et al.*, 2011; Kwak *et al.*, 2011; Long *et al.*, 2011; van der Burg and Melief, 2011).

4.2. Tumor antigens recognized by CD4$^+$ Th and Treg cells

Given the importance of CD4$^+$ T cells in antitumor immunity, it is critical to identify MHC class II-restricted tumor antigens capable of stimulating CD4$^+$ Th cells. There are several strategies to identify MHC class II antigens recognized by CD4$^+$ T cells: (1) genetic targeting expression (GTE) system using tumor-reactive human T cells isolated from patients with cancer or other immune-related diseases, (2) use of HLA-DR transgenic (Tg) mice for identification of MHC class II-restricted antigens *in vivo*, (3) peptide stimulation *in vitro* using candidate antigens over-expressed in cancer cells. Many tumor antigens capable of stimulating CD4$^+$ T cells have been identified (Table 6.2).

4.2.1. GTE system—We have developed GTE system for identification of antigens recognized by CD4$^+$ T cells (Wang and Rosenberg, 1999; Wang *et al.*, 1999a, b). The GTE system comprises two essential components: (A) generation of a highly transfectable HEK293IMDR cell line and (B) the creation of an Ii fusion library from tumor cells such that the Ii fusion proteins are targeted to the endosomal/lysosomal compartment for efficient antigen processing and presentation for T-cell recognition (Wang and Rosenberg, 1999; Wang *et al.*, 1999b). Using this system, many tumor antigens have successfully been identified. For example, we identified a mutated fibronectin as a tumor antigen recognized by HLA-DR2-restricted CD4$^+$ T cells. A mutation in this gene results in the substitution of lysine for glutamic acid and gives rise to a new T-cell epitope recognized by CD4$^+$ T cells (Wang *et al.*, 2002b). Analysis of cytokine profiles and suppressive activity of these T cells reveal that they are CD4$^+$ Th1 cells, secreting IFN-γ and IL-2, but no suppressive function. Because elevated percentage of CD4$^+$ Treg cells are present in tumor tissues, we have

recently generated many such tumor/antigen-specific $CD4^+$ Treg cell clones from TILs in surgically removed tumor samples. Using the same strategy for Th1 cells, we identified LAGE1 and ARTC1 as antigenic ligands for $CD4^+$ Treg cells, providing direct evidence that antigen-specific $CD4^+$ Treg cells are present at tumor sites and mediate antigen-specific and local immune suppression of antitumor immunity (Wang *et al.*, 2004, 2005).

4.2.2. Use of HLA-DR Tg mice and *in vitro* peptide stimulation—MHC class II-restricted epitopes can be identified by using HLA-DR Tg mice in combination peptide stimulation *in vitro*. HLA-DR4 Tg mice were used to identify $CD4^+$ T-cell epitopes from candidate antigens (Touloukian *et al.*, 2000; Zeng *et al.*, 2000). HLA-DR Tg mice might have advantages for identifying putative peptides, as they should have a high precursor frequency of antigen-specific T cells after immunization. Once candidate peptides are known, one can generate antigen-specific $CD4^+$ T cells from human PBMCs stimulated with synthetic candidate peptides. Therefore, the combined use of immunization of DR Tg mice with the intact protein antigens and stimulated with the peptides predicted by a computer-assisted algorithm may avoid the need to stimulate human PBMCs with a large number of peptides. NY-ESO-1 is a potent immunogen recognized by both antibody and T cells (Chen *et al.*, 1997; Jager *et al.*, 1998; Wang *et al.*, 1998b). Of particular interest is that 10–13% of patients with advanced cancer developed a high titer of antibody (Stockert *et al.*, 1998; Zeng *et al.*, 2000). We identified a T-cell epitope presented by HLA-DP4, a predominant allele expressed in 40–70% of the population (Zeng *et al.*, 2001). Identification of DP4-restricted T-cell peptides from MAGE-3 and NY-ESO-1 could be of great benefit for more than 50% of patients with cancer. These studies suggest that, unlike LAGE-1, NY-ESO-1 may preferentially activate $CD4^+$ Th cells. However, a recent study shows that NY-ESO-1 can also induce Treg cells (Vence *et al.*, 2007). Interestingly, several MHC class II peptides recognized by $CD4^+$ T cells have been identified from EBNA1 as well as other viral antigens (Bickham *et al.*, 2001; Leen *et al.*, 2001; Munz *et al.*, 2000; Paludan and Munz, 2003; Voo *et al.*, 2002). T-cell peptides derived from EBV viral antigens are capable of stimulating $CD4^+$ Th1 and Treg cells (Marshall *et al.*, 2003; Voo *et al.*, 2005). It appears that the same T-cell epitope from EBNA1 can stimulate both Th1 and Treg cells (Voo *et al.*, 2005). Thus, it is likely that both tumor and viral antigens can activate effector and Treg cells, depending on particular epitope affinity and cytokine milieu. Although IL-17-producing T (Th17) cells are present in many human cancer tissues, very little is known about their antigen specificity. In particular, the role of Th17 cells in cancer immunity and tumor progression is not clear and requires further study.

5. CURRENT PROGRESSES OF SYNTHETIC PEPTIDE VACCINES

Identification of these MHC class I-restricted antigens has set the stage for developing peptide-based cancer vaccines, although some evidence for a therapeutic effect on tumor growth inhibition and regression was observed in patients who received peptide vaccines (Marchand *et al.*, 1999; Rosenberg *et al.*, 1998). However, objective complete clinical responses were sporadic, even though CTL reactivity was clearly evident after one round of stimulation *in vitro* of PBMC from the majority of vaccinated patients (Rosenberg *et al.*, 2004). Analysis of the infiltrating lymphocytes in skin and tumor biopsies using T-cell-specific peptide–major histocompatibility complex tetramers showed generation of antigen-specific $CD8^+$ T cells (Yee *et al.*, 2000). Recently, a multicenter phase II clinical trial of melanoma patients with gp100 peptide with or without IL-2 shows that the gp100 peptide vaccine plus IL-2-treated group had a significant improvement in overall clinical response compared with the IL-2-only group (16% vs. 6%) as well as longer progression-free survival (Schwartzentruber *et al.*, 2011). The median overall survival was also improved in the gp100 peptide vaccine plus IL-2 group compared to the IL-2-only group (17.8 vs. 11.1 months). Similarly, MAGE-3 peptide/protein vaccines are ongoing in phase III clinical trials (Bilusic

and Madan, 2011; Cecco *et al.*, 2011). The clinical efficacy of peptide vaccines may be further improved by the use of DCs loaded with cancer peptides or by the use of synthetic long peptides harboring both CD4 and CD8 T-cell epitopes (Melief and van der Burg, 2008; Chapters 3 and 4). A major challenge in cancer vaccine development is how to generate strong and long-lasting antitumor immunity through optimal delivery of well-chosen tumor-associated antigens.

6. ENHANCING ANTITUMOR IMMUNITY BY INTRACELLULAR DELIVERY OF PEPTIDES INTO DCs

Although mature DCs are more potent than immature DCs in priming and eliciting T-cell responses (Apetoh *et al.*, 2011; Delamarre and Mellman, 2011; Tacken and Figdor, 2011), they lose the ability to efficiently take up exogenous antigens, particularly for MHC class II-restricted antigens (Banchereau and Steinman, 1998). As a result, peptide-pulsed DCs as vaccines have several limitations. For example, peptide degradation, rapid MHC class I turnover, and the disassociation of peptide from MHC class I molecules during the preparation and injection of DC/peptides may result in short half-lives of MHC class I/peptide complexes on the DC surface, leading to weak T-cell responses. We developed a novel strategy to overcome these problems by the use of cell-penetrating peptides (CPPs) for intracellular delivery of cancer peptides into DCs (Wang and Wang, 2002; Wang *et al.*, 2002a). Intracellular delivery of peptides into DCs could allow DCs to continuously process and present the internalized peptides to T cells for an extended period of time. Several potent CPPs have been identified from proteins, including the Tat protein of human immunodeficiency virus, the VP22 protein of herpes simplex virus, and fibroblast growth factor (Berry, 2008; Deshayes *et al.*, 2005; Edenhofer, 2008; Gupta *et al.*, 2005; Torchilin, 2006), although some long synthetic peptides can also penetrate selectively into DCs (Melief and van der Burg, 2008). Among them, the 11-mer TAT peptide (YGRKKRRQRRR) and other CPPs have been well studied for the transduction of biologically active proteins into cells both *in vitro* and *in vivo* (Gupta *et al.*, 2005). We found that both CPP1-TRP2 or TAT-TRP2 peptides can translocate intracellularly into mature DCs and prolong DCs to process the internalized peptides and to present MHC–peptide complexes to antigen-specific T cells (Wang and Wang, 2002; Wang *et al.*, 2002a). Immunization of mice with DCs transduced with CPP1-TRP-2 or TAT-TRP2 resulted in complete protection against B16 tumor as well as significant inhibition of the preestablished B16 tumor (Wang and Wang, 2002; Wang *et al.*, 2002a). Although both DC/TRP2 and DC/TAT-TRP2 immunization increased the number of TRP2-specific $CD8^+$ T cells detected by K^b/TRP2 tetramers, T-cell activity elicited by DC/TAT-TRP2 was 3- to 10-fold higher than that induced by DC/TRP2 (Wang *et al.*, 2002a). Consistent with previous studies showing that $CD4^+$ T cells are required for an antitumor effect, our studies show a similar requirement for DC/TAT-TRP-2-induced antitumor immunity, suggesting that both CPP1- and TAT-mediated antigen delivery of a self-peptide may have general applications for enhancing T-cell-based cancer therapy, and $CD4^+$ T-cell response is required for generating optimal antitumor immunity. More recently, we initiated a phase I clinical trial for prostate cancer using TAT-NY-ESO-1 peptides. Clinical studies show that TAT-NY-ESO-1 peptide vaccines are safe and induced antigen-specific T-cell responses (R.-F.W. Guru Sonpavde and Teresa G. Hayes, unpublished data).

7. ENHANCING IMMUNE RESPONSES AND BLOCKING IMMUNE SUPPRESSION BY STIMULATION OF INNATE IMMUNE RECEPTORS

7.1. Innate immune receptors and signaling

Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and AIM-2-like receptors have emerged as innate pattern recognition receptors (PRRs) that can

detect a variety of invading pathogens and intracellular ligands, thus serving as a first line of defense against infectious pathogens and cancer. These germline-encoded PRRs are expressed in DCs and other immune cells, and can recognize structure-conserved molecules, such as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Recognition of PAMPs or DAMPs by PRRs triggers the activation of several key signaling pathways, including NF-κB, type I IFN, and inflammasome, leading to the production of inflammatory cytokines, which, in turn, promote DC maturation programs for the induction of adaptive immune responses (Iwasaki and Medzhitov, 2004; Takeda and Akira, 2005). TLRs are expressed on the cell surface (TLR1, TLR2, TLR4, and TLR5) or in the endosome (TLR3, TLR7, TLR8, and TLR9). By contrast, several intracellular PRRs such as RIG-I and MDA5 are in the cytoplasm and responsible for the recognition of invading viruses (Akira *et al.*, 2006; Kato *et al.*, 2005; Yoneyama *et al.*, 2004). NLRs represent a large family of protein receptors/regulators harboring an initiating signal domain, such as the caspase recruitment domain (CARD), pyrin domain (PYRIN) or baculovirus inhibitor-of-apoptosis repeat (BIR) domain, a nucleotide-binding oligomerization domain (NOD), and an LRR domain. Activation of such cytoplasmic receptors by invading pathogens including bacteria and viruses activates inflammasome consisting of caspase-1 and ASC and leads to the production of proinflammatory cytokines such as IL-1b and IL-18. Thus, TLRs, NLRs, and RLRs are critical in bridging innate and adaptive immune responses by activating several key signaling pathways and in producing many important cytokines as mediators. Thus, they represent a potent means of modulating immune responses in cancer immunotherapy. Both natural and synthetic ligands for TLRs and RLRs have been identified and characterized for their recognition, but only a limited number of ligands have been identified for NLRs. These ligands of PRRs, in particular TLRs, have been used as potent vaccine adjuvants to enhance immune responses. The most significant development of cancer vaccine studies is to include various TLR agonists to vaccine formulations, including TLR-3 (poly I:C), TLR-4 (monophosphoryl lipid A; MPL), TLR-5 (flagellin), TLR-7 (imiqui-mod), and TLR-9 (CpG) (Duthie *et al.*, 2011). The types of signaling and cytokines by immune cells after TLR stimulation control $CD4^+$ T-cell differentiation into Th1, Th2, Th17, and Treg cells. However, stimulation of immune cells such as DCs and T cells by most TLR-based adjuvants produces proinflammatory cytokines and promotes Th1 and $CD8^+$ T responses (Manicassamy and Pulendran, 2009).

7.2. Blocking negative regulators of innate immune signaling

Although innate immune responses are critically important as sensors to induce adaptive immunity, tight regulation of innate signaling pathways is essential for both innate and adaptive immunities; otherwise, aberrant immune responses may occur, leading to severe or even fatal consequences. Similarly, DC maturation and activation are controlled by both positive and negative regulators, thus leading to immunity or tolerance induction. To generate potent antitumor immunity, we need to block negative regulators in immune cells such as DCs and T cells. For example, A20-silenced DCs produce potent antitumor immunity (Song *et al.*, 2008). We recently identified several negative regulators (NLRC5 and NLRX1) that inhibit NF-κB and type I IFN signaling (Cui *et al.*, 2010; Xia *et al.*, 2011). Similarly, several key negative regulators such as CTLA-4 and programmed cell death 1 (PD-1) play a critical role in the inhibition of T-cell activation. Blockade of CTLA-4 and/or PD-1 results in T-cell activation and enhances nonspecific immune responses (Chambers *et al.*, 2001; Zhu *et al.*, 2011). These antibody-based immunotherapies are currently under active clinical trials (Callahan *et al.*, 2010), and anti-CTLA-4 antibody therapy has been approved by US FDA for the treatment of metastatic melanoma (Hodi *et al.*, 2010).

7.3. Overcoming Treg cell-mediated immune suppression by TLR signaling

As Treg cells have accumulated in the tumor microenvironment, it is necessary to develop novel strategies to overcome this type of immune suppression; otherwise, the immune response induced by cancer vaccines will be weak and transient. To block immune suppression mediated by Treg cells, we found that TLR8 ligands (ssRNA40 and Poly-G10 oligonucleotides) can directly reverse the suppressive function of human (but not murine) Treg cells in the absence of DCs. Using RNA interference technology, we identified the TLR8–MyD88 signaling pathway that is required for the reversal of Treg cell function by Poly-G oligonucleotides (Peng *et al.*, 2005). More importantly, we demonstrated that the suppressive function of $CD8^+$ Treg cells and γδ-TCR Treg cells can also be reversed after Poly-G oligonucleotide treatment (Kiniwa *et al.*, 2007; Peng *et al.*, 2007), suggesting that these cells share a common TLR8 signaling-mediated mechanism. Recent studies show that TLR2 ligands can reverse the suppressive function of human Treg cells (Nyirenda *et al.*, 2011; Oberg *et al.*, 2010). However, murine TLR2-deficient mice reduce the number of $CD4^+$ $CD25^+$ Treg cells (Netea *et al.*, 2004). Activation of TLR2 with its ligand (Pam3Cys) directly increases the proliferation of murine Treg cells and transiently reverses their suppressive function (Liu *et al.*, 2006; Sutmuller *et al.*, 2006). However, engagement of TLR2 with polysaccharide A of *Bacteroides fragilis* enhances the suppressive function of Treg cells (Round *et al.*, 2011). These studies demonstrated that TLR signaling is critically important in modulating immune responses.

8. CONCLUSIONS

In the past 30 years, significant progress has been made in the field of cancer immunotherapy. While clinical validation and development has been slow, recent approval of two immunotherapy drugs (sipuleucel-T and anti-CTLA-4 antibody) has boosted the development of immunotherapy as important and promising treatment of patients with cancer. As ACT is the most powerful treatment of cancer with up to 72% objective clinical response rate, it is important to move forward with phase III clinical trials with tumor-specific T cells and CIK effector cells. Like APC-based therapy, the limitation of ACT is individualized medicine, and it is very challenging to broaden its application with retroviruses or lentiviruses expressing antigen-specific TCR. For these reasons, development of peptide/protein cancer vaccines has great potential. With encouraging results from recent multicenter clinical trials with gp100 and MAGE-A3, many therapeutic companies will step in the field and develop therapeutic peptide cancer vaccines for many types of cancer. The key issues will be how to further improve immune responses and clinical efficacy of peptide cancer vaccines. To achieve this goal, (1) we need to develop *in vivo* targeted delivery of peptides into APCs such as DCs with CPPs-linked cancer peptides (long peptide)/proteins, or nanoparticles; (2) we need to overcome immune suppression mediated by Treg cells and other immune cells; (3) importantly, peptide vaccines should be combined with strategies that block negative regulators or immune suppression to achieve maximal antitumor immunity and clinical responses. However, because immune responses measured in the blood do not necessarily correlate with clinical efficacy or survival, it is important to probe the reasons for these clinical observations. Because pretreatment of patients with chemodepleting regimens improves the clinical benefit of ACT, it is reasonable to believe that combined use of immunotherapy with chemotherapy can enhance immune responses and clinical outcomes. Recent advances and rapid progress in the field of cancer immunotherapy represent an unprecedented opportunity for the development of therapeutic cancer vaccines in the next few years.

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References

Aarnoudse CA, van den Doel PB, Heemskerk B, Schrier PI. Interleukin-2-induced, melanoma-specific T cells recognize CAMEL, an unexpected translation product of LAGE-1. Int J Cancer. 1999; 82:442–448. [PubMed: 10399963]

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006; 124:783–801. [PubMed: 16497588]

Apetoh L, Locher C, Ghiringhelli F, Kroemer G, Zitvogel L. Harnessing dendritic cells in cancer. Semin Immunol. 2011; 23:42–49. [PubMed: 21295491]

Ayyoub M, Stevanovic S, Sahin U, Guillaume P, Servis C, Rimoldi D, Valmori D, Romero P, Cerottini JC, Rammensee HG, et al. Proteasome-assisted identification of a SSX-2-derived epitope recognized by tumor-reactive CTL infiltrating metastatic melanoma. J Immunol. 2002; 168:1717–1722. [PubMed: 11823502]

Bakker ABH, Schreurs MWJ, de Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG. Melanocyte lineage-specific antigen gp100 is recognized by melanocyte-derived tumor-infiltrating lymphocytes. J Exp Med. 1994; 179:1005–1009. [PubMed: 8113668]

Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998; 392:245–252. [PubMed: 9521319]

Benlalam H, Linard B, Guilloux Y, Moreau-Aubry A, Derre L, Diez E, Dreno B, Jotereau F, Labarriere N. Identification of five new HLA-B*3501-restricted epitopes derived from common melanoma-associated antigens, spontaneously recognized by tumor-infiltrating lymphocytes. J Immunol. 2003; 171:6283–6289. [PubMed: 14634146]

Berry CC. Intracellular delivery of nanoparticles via the HIV-1 tat peptide. Nanomedicine. 2008; 3:357–365. [PubMed: 18510430]

Bickham K, Munz C, Tsang ML, Larsson M, Fonteneau JF, Bhardwaj N, Steinman R. EBNA1-specific CD4+ T cells in healthy carriers of Epstein-Barr virus are primarily Th1 in function. J Clin Invest. 2001; 107:121–130. [PubMed: 11134187]

Bilusic M, Madan RA. Therapeutic cancer vaccines: The latest advancement in targeted therapy. Am J Ther. 2011 In Press (Epublication Feb. 2011).

Boel P, Wildmann C, Sensi ML, Brasseur R, Renauld JC, Coulie P, Boon T, Van der Bruggen P. BAGE: A new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity. 1995; 2:167–175. [PubMed: 7895173]

Boon T, Cerottini JC, Van Den Eynde B, Van der Bruggen P, Van Pel A. Tumor antigens recognized by T lymphocytes. Annu Rev Immunol. 1994; 12:337–365. [PubMed: 8011285]

Brichard VG, Herman J, Van Pel A, Wildmann C, Gaugler B, Wolfel T, Boon T, Lethe B. A tyrosinase nonpeptide presented by HLA-B44 is recognized on a human melanoma by autologous cytolytic T lymphocytes. Eur J Immunol. 1996; 26:224–230. [PubMed: 8566071]

Callahan MK, Wolchok JD, Allison JP. Anti-CTLA-4 antibody therapy: Immune monitoring during clinical development of a novel immunotherapy. Semin Oncol. 2010; 37:473–484. [PubMed: 21074063]

Castelli C, Storkus WJ, Maeurer MJ, Martin DM, Huang EC, Pramanik BN, Nagabhushan TL, Parmiani G, Lotze MT. Mass spectrometric identification of a naturally processed melanoma peptide recognized by CD8+ cytotoxic T lymphocytes. J Exp Med. 1995; 181:363–368. [PubMed: 7807017]

Castelli C, Tarsini P, Mazzocchi A, Rini F, Rivoltini L, Ravagnani F, Gallino F, Belli F, Parmiani G. Novel HLA-Cw8-restricted T cell epitopes derived from tyrosinase-related protein-2 and gp100 melanoma antigens. J Immunol. 1999; 162:1739–1748. [PubMed: 9973437]

Cecco S, Muraro E, Giacomin E, Martorelli D, Lazzarini R, Baldo P, Dolcetti R. Cancer vaccines in phase II/III clinical trials: State of the art and future perspectives. Curr Cancer Drug Targets. 2011; 11:85–102. [PubMed: 21062241]

Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol. 2001; 19:565–594. [PubMed: 11244047]

Chaux P, Luiten R, Demotte N, Vantomme V, Stroobant V, Traversari C, Russo V, Schultz E, Cornelis GR, Boon T, et al. Identification of five MAGE-A1 epitopes recognized by cytolytic T lymphocytes obtained by in vitro stimulation with dendritic cells transduced with MAGE-A1. J Immunol. 1999a; 163:2928–2936. [PubMed: 10453041]

Chaux P, Vantomme V, Stroobant V, Thielemans K, Corthals J, Luiten R, Eggermont AM, Boon T, van der Bruggen P. Identification of MAGE-3 epitopes presented by HLA-DR molecules to CD4(+) T lymphocytes. J Exp Med. 1999b; 189:767–778. [PubMed: 10049940]

Chen YT, Scanlan MJ, Sahin U, Tureci O, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci USA. 1997; 94:1914–1918. [PubMed: 9050879]

Chiari R, Hames G, Stroobant V, Texier C, Maillere B, Boon T, Coulie PG. Identification of a tumor-specific shared antigen derived from an Eph receptor and presented to CD4 T cells on HLA class II molecules. Cancer Res. 2000; 60:4855–4863. [PubMed: 10987298]

Consogno G, Manici S, Facchinetti V, Bachi A, Hammer J, Conti-Fine BM, Rugarli C, Traversari C, Protti MP. Identification of immunodominant regions among promiscuous HLA-DR-restricted CD4+ T-cell epitopes on the tumor antigen MAGE-3. Blood. 2003; 101:1038–1044. [PubMed: 12393675]

Coulie PG, Brichard V, Van Pel A, Wolfel T, Schneider J, Traversari C, Mattei S, De Plaen ED, Lurquin C, Szikora JP, et al. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med. 1994; 180:35–42. [PubMed: 8006593]

Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; 420:860–867. [PubMed: 12490959]

Cox AL, Skipper J, Cehn Y, Henderson RA, Darrow TL, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CL. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science. 1994; 264:716–719. [PubMed: 7513441]

Cui J, Zhu L, Xia X, Wang HY, Legras X, Hong J, Ji J, Shen P, Zheng S, Chen ZJ, et al. NLRC5 negatively regulates the NF-kappaB and type I interferon signaling pathways. Cell. 2010; 141:483–496. [PubMed: 20434986]

De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG, Nakai Y, Isaacs WB, Nelson WG. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007; 7:256–269. [PubMed: 17384581]

Delamarre L, Mellman I. Harnessing dendritic cells for immunotherapy. Semin Immunol. 2011; 23:2–11. [PubMed: 21377379]

Deshayes S, Morris MC, Divita G, Heitz F. Cell-penetrating peptides: Tools for intracellular delivery of therapeutics. Cell Mol Life Sci. 2005; 62:1839–1849. [PubMed: 15968462]

Diefenbach A, Raulet DH. Strategies for target cell recognition by natural killer cells. Immunol Rev. 2001; 181:170–184. [PubMed: 11513138]

Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002; 19:19.

Duffour MT, Chaux P, Lurquin C, Cornelis G, Boon T, van der Bruggen P. A MAGE-A4 peptide presented by HLA-A2 is recognized by cytolytic T lymphocytes. Eur J Immunol. 1999; 29:3329–3337. [PubMed: 10540345]

Duthie MS, Windish HP, Fox CB, Reed SG. Use of defined TLR ligands as adjuvants within human vaccines. Immunol Rev. 2011; 239:178–196. [PubMed: 21198672]

Edenhofer F. Protein transduction revisited: Novel insights into the mechanism underlying intracellular delivery of proteins. Curr Pharm Des. 2008; 14:3628–3636. [PubMed: 19075739]

Fleischhauer K, Avila D, Vilbois F, Traversari C, Bordignon C, Wallny HJ. Characterization of natural peptide ligands for HLA-B*4402 and -B*4403: Implications for peptide involvement in allorecognition of a single amino acid change in the HLA-B44 heavy chain. Tissue Antigens. 1995; 44:311–317. [PubMed: 7878657]

Fujie T, Tahara K, Tanaka F, Mori M, Takesako K, Akiyoshi T. A MAGE-1-encoded HLA-A24-binding synthetic peptide induces specific anti-tumor cytotoxic T lymphocytes. Int J Cancer. 1999; 80:169–172. [PubMed: 9935194]

Gaugler B, Van Den Eynde B, Van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, Lethe B, Brasseur F, Boon T. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med. 1994; 179:921–930. [PubMed: 8113684]

Greenberg PD. Adoptive T cell therapy of tumors: Mechanisms operative in the recognition and elimination of tumor cells. Adv Immunol. 1991; 49:281–355. [PubMed: 1853786]

Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982; 155:1823–1841. [PubMed: 6176669]

Gupta B, Levchenko TS, Torchilin VP. Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. Adv Drug Deliv Rev. 2005; 57:637–651. [PubMed: 15722168]

Heidecker L, Brasseur F, Probst-Kepper M, Gueguen M, Boon T, Van den Eynde BJ. Cytolytic T lymphocytes raised against a human bladder carcinoma recognize an antigen encoded by gene MAGE-A12. J Immunol. 2000; 164:6041–6045. [PubMed: 10820289]

Herman J, van der Bruggen P, Luescher IF, Mandruzzato S, Romero P, Thonnard J, Fleischhauer K, Boon T, Coulie PG. A peptide encoded by the human MAGE3 gene and presented by HLA-B44 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE3. Immunogenetics. 1996; 43:377–383. [PubMed: 8606058]

Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363:711–723. [PubMed: 20525992]

Hontscha C, Borck Y, Zhou H, Messmer D, Schmidt-Wolf IG. Clinical trials on CIK cells: First report of the international registry on CIK cells (IRCC). J Cancer Res Clin Oncol. 2011; 137:305–310. [PubMed: 20407789]

Huang LQ, Brasseur F, Serrano A, De Plaen E, van der Bruggen P, Boon T, Van Pel A. Cytolytic T lymphocytes recognize an antigen encoded by MAGE-A10 on a human melanoma. J Immunol. 1999; 162:6849–6854. [PubMed: 10352307]

Huang LM, Lu CY, Chen DS. Hepatitis B virus infection, its sequelae, and prevention by vaccination. Curr Opin Immunol. 2011; 23:237–243. [PubMed: 21257300]

Hunder NN, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, Jungbluth A, Gnjatic S, Thompson JA, Yee C. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med. 2008; 358:2698–2703. [PubMed: 18565862]

Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004; 5:987–995. [PubMed: 15454922]

Jager E, Chen YT, Drijfhout JW, Karbach J, Ringhoffer M, Jager D, Arand M, Wada H, Noguchi Y, Stockert E, et al. Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: Definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. J Exp Med. 1998; 187:265–270. [PubMed: 9432985]

Jiang H, Liu KY, Tong CR, Jiang B, Lu DP. The efficacy of chemotherapy in combination with auto-cytokine-induced killer cells in acute leukemia. Zhonghua Nei Ke Za Zhi. 2005; 44:198–201. [PubMed: 15840260]

Kang XQ, Kawakami Y, Sakaguchi K, El-Gamil M, Wang RF, Yannelli JR, Appella E, Rosenberg SA, Robbins PF. Identification of a tyrosinase epitope reocognized by HLA-A24 restricted tumor-infiltrating lymphocytes. J Immunol. 1995; 155:1343–1348. [PubMed: 7543520]

Karin M, Lawrence T, Nizet V. Innate immunity gone awry: Linking microbial infections to chronic inflammation and cancer. Cell. 2006; 124:823–835. [PubMed: 16497591]

Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, Tsujimura T, Takeda K, Fujita T, Takeuchi O, et al. Cell type-specific involvement of RIG-I in antiviral response. Immunity. 2005; 23:19–28. [PubMed: 16039576]

Kawakami Y, Eliyahu S, Delgaldo CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA. 1994a; 91:3515–3519. [PubMed: 8170938]

Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JB, Appella E, Rosenberg SA. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2 restricted tumor infiltrating lymphocytes. J Exp Med. 1994b; 180:347–352. [PubMed: 7516411]

Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang X, Southwood S, Robbins PF, Sette A, Appella E, Rosenberg SA. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J Immunol. 1995; 154:3961–3968. [PubMed: 7706734]

Kawakami Y, Robbins PF, Wang X, Tupesis JP, Parkhurst MR, Kang X, Sakaguchi K, Appella E, Rosenberg SA. Identification of new melanoma epitopes on melanosomal proteins recognized by tumor infiltrating T lymphocytes restricted by HLA-A1, - A2, and -A3 alleles. J Immunol. 1998; 161:6985–6992. [PubMed: 9862734]

Kawashima I, Tsai V, Southwood S, Takesako K, Celis E, Sette A. Identification of gp100-derived, melanoma-specific cytotoxic T- lymphocyte epitopes restricted by HLA-A3 supertype molecules by primary in vitro immunization with peptide-pulsed dendritic cells. Int J Cancer. 1998; 78:518–524. [PubMed: 9797143]

Kiniwa Y, Miyahara Y, Wang HY, Peng W, Peng G, Wheeler TM, Thompson T, Old LJ, Wang RF. CD8+ Foxp3+ regulatory T cells mediate immunosuppression in prostate cancer. Clin Cancer Res. 2007; 13:6947–6958. [PubMed: 18056169]

Kittlesen DJ, Thompson LW, Gulden PH, Skipper JC, Colella TA, Shabanowitz J, Hunt DF, Engelhard VH, Slingluff CL Jr, Shabanowitz JA. Human melanoma patients recognize an HLA-A1-restricted CTL epitope from tyrosinase containing two cysteine residues: Implications for tumor vaccine development. J Immunol. 1998; 160:2099–2106. [PubMed: 9498746]

Kobayashi H, Kokubo T, Takahashi M, Sato K, Miyokawa N, Kimura S, Kinouchi R, Katagiri M. Tyrosinase epitope recognized by an HLA-DR-restricted T-cell line from a Vogt-Koyanagi-Harada disease patient. Immunogenetics. 1998; 47:398–403. [PubMed: 9510558]

Kobayashi H, Lu J, Celis E. Identification of helper T-cell epitopes that encompass or lie proximal to cytotoxic T-cell epitopes in the gp100 melanoma tumor antigen. Cancer Res. 2001a; 61:7577–7584. [PubMed: 11606397]

Kobayashi H, Song Y, Hoon DS, Appella E, Celis E. Tumor-reactive T helper lymphocytes recognize a promiscuous MAGE-A3 epitope presented by various major histocompatibility complex class II alleles. Cancer Res. 2001b; 61:4773–4778. [PubMed: 11406551]

Kwak K, Yemelyanova A, Roden RB. Prevention of cancer by prophylactic human papillomavirus vaccines. Curr Opin Immunol. 2011; 23:244–251. [PubMed: 21185706]

Lapointe R, Royal RE, Reeves ME, Altomare I, Robbins PF, Hwu P. Retrovirally transduced human dendritic cells can generate T cells recognizing multiple MHC class I and class II epitopes from the melanoma antigen glycoprotein 100. J Immunol. 2001; 167:4758–4764. [PubMed: 11591807]

Leen A, Meij P, Redchenko I, Middeldorp J, Bloemena E, Rickinson A, Blake N. Differential immunogenicity of Epstein-Barr virus latent-cycle proteins for human CD4(+) T-helper 1 responses. J Virol. 2001; 75:8649–8659. [PubMed: 11507210]

Leen AM, Myers GD, Sili U, Huls MH, Weiss H, Leung KS, Carrum G, Krance RA, Chang CC, Molldrem JJ, et al. Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. Nat Med. 2006; 12:1160–1166. [PubMed: 16998485]

Leen AM, Rooney CM, Foster AE. Improving T cell therapy for cancer. Annu Rev Immunol. 2007; 25:243–265. [PubMed: 17129181]

Lesterhuis WJ, Haanen JB, Punt CJ. Cancer immunotherapy—Revisited. Nat Rev Drug Discov. 2011; 10:591–600. [PubMed: 21804596]

Linn YC, Lau LC, Hui KM. Generation of cytokine-induced killer cells from leukaemic samples with in vitro cytotoxicity against autologous and allogeneic leukaemic blasts. Br J Haematol. 2002; 116:78–86. [PubMed: 11841399]

Liu H, Komai-Koma M, Xu D, Liew FY. Toll-like receptor 2 signaling modulates the functions of CD4+CD25+ regulatory T cells. Proc Natl Acad Sci USA. 2006; 103:7048–7053. [PubMed: 16632602]

Long HM, Taylor GS, Rickinson AB. Immune defence against EBV and EBV-associated disease. Curr Opin Immunol. 2011; 23:258–264. [PubMed: 21269819]

Lupetti R, Pisarra P, Verrecchia A, Farina C, Nicolini G, Anichini A, Bordignon C, Sensi M, Parmiani G, Traversari C. Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared human melanoma antigen not expressed in normal cells of the melanocytic lineage. J Exp Med. 1998; 188:1005–1016. [PubMed: 9743519]

Mandruzzato S, Brasseur F, Andry G, Boon T, van der Bruggen P. A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. J Exp Med. 1997; 186:785–793. [PubMed: 9271594]

Manicassamy S, Pulendran B. Modulation of adaptive immunity with Toll-like receptors. Semin Immunol. 2009; 21:185–193. [PubMed: 19502082]

Manici S, Sturniolo T, Imro MA, Hammer J, Sinigaglia F, Noppen C, Spagnoli G, Mazzi B, Bellone M, Dellabona P, et al. Melanoma cells present a MAGE-3 epitope to CD4(+) cytotoxic T cells in association with histocompatibility leukocyte antigen DR11. J Exp Med. 1999; 189:871–876. [PubMed: 10049951]

Marchand M, van Baren N, Weynants P, Brichard V, Dreno B, Tessier MH, Rankin E, Parmiani G, Arienti F, Humblet Y, et al. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA- A1. Int J Cancer. 1999; 80:219–230. [PubMed: 9935203]

Marshall NA, Vickers MA, Barker RN. Regulatory T cells secreting IL-10 dominate the immune response to EBV latent membrane protein 1. J Immunol. 2003; 170:6183–6189. [PubMed: 12794149]

Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer. 2008; 8:351–360. [PubMed: 18418403]

Munz C, Bickham KL, Subklewe M, Tsang ML, Chahroudi A, Kurilla MG, Zhang D, O'Donnell M, Steinman RM. Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. J Exp Med. 2000; 191:1649–1660. [PubMed: 10811859]

Muul LM, Nason-Burchenal K, Carter CS, Cullis H, Slavin D, Hyatt C, Director EP, Leitman SF, Klein HG, Rosenberg SA. Development of an automated closed system for generation of human lymphokine-activated killer (LAK) cells for use in adoptive immunotherapy. J Immunol Methods. 1987; 101:171–181. [PubMed: 3611795]

Netea MG, Sutmuller R, Hermann C, Van der Graaf CA, Van der Meer JW, van Krieken JH, Hartung T, Adema G, Kullberg BJ. Toll-like receptor 2 suppresses immunity against Candida albicans through induction of IL-10 and regulatory T cells. J Immunol. 2004; 172:3712–3718. [PubMed: 15004175]

Noppen C, Levy F, Burri L, Zajac P, Remmel E, Schaefer C, Luscher U, Heberer M, Spagnoli GC. Naturally processed and concealed HLA-A2.1-restricted epitopes from tumor-associated antigen tyrosinase-related protein-2. Int J Cancer. 2000; 87:241–246. [PubMed: 10861482]

Nyirenda MH, Sanvito L, Darlington PJ, O'Brien K, Zhang GX, Constantinescu CS, Bar-Or A, Gran B. TLR2 Stimulation drives human naive and effector regulatory T cells into a Th17-like phenotype with reduced suppressive function. J Immunol. 2011; 187:2278–2290. [PubMed: 21775683]

Oberg HH, Ly TT, Ussat S, Meyer T, Kabelitz D, Wesch D. Differential but direct abolishment of human regulatory T cell suppressive capacity by various TLR2 ligands. J Immunol. 2010; 184:4733–4740. [PubMed: 20363971]

Oiso M, Eura M, Katsura F, Takiguchi M, Sobao Y, Masuyama K, Nakashima M, Itoh K, Ishikawa T. A newly identified MAGE-3-derived epitope recognized by HLA-A24-restricted cytotoxic T lymphocytes. Int J Cancer. 1999; 81:387–394. [PubMed: 10209953]

Paludan C, Munz C. CD4+ T cell responses in the immune control against latent infection by Epstein-Barr virus. Curr Mol Med. 2003; 3:341–347. [PubMed: 12776989]

Panelli MC, Bettinotti MP, Lally K, Ohnmacht GA, Li Y, Robbins P, Riker A, Rosenberg SA, Marincola FM. A tumor-infiltrating lymphocyte from a melanoma metastasis with decreased expression of melanoma differentiation antigens recognizes MAGE-12. J Immunol. 2000; 164:4382–4392. [PubMed: 10754339]

Parkhurst MR, Fitzgerald EB, Southwood S, Sette A, Rosenberg SA, Kawakami Y. Identification of a shared HLA-A*0201-restricted T-cell epitope from the melanoma antigen tyrosinase-related protein 2 (TRP2). Cancer Res. 1998; 58:4895–4901. [PubMed: 9809996]

Peng G, Guo Z, Kiniwa Y, Voo KS, Peng W, Fu T, Wang DY, Li Y, Wang HY, Wang RF. Toll-like receptor 8 mediated-reversal of CD4+ regulatory T cell function. Science. 2005; 309:1380–1384. [PubMed: 16123302]

Peng G, Wang HY, Peng W, Kiniwa Y, Seo K, Wang RF. Tumor-infiltrating gamma-delta T cells suppress T and dendritic cell function via mechanisms controlled by a unique Toll-like receptor signaling pathway. Immunity. 2007; 27:334–348. [PubMed: 17656116]

Peto J. Cancer epidemiology in the last century and the next decade. Nature. 2001; 411:390–395. [PubMed: 11357148]

Pieper R, Christian RE, Gonzales MI, Nishimura MI, Gupta G, Settlage RE, Shabanowitz J, Rosenberg SA, Hunt DF, Topalian SL. Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T cells. J Exp Med. 1999; 189:757–766. [PubMed: 10049939]

Robbins PF, El-Gamil M, Li YF, Kawakami Y, Loftus D, Appella E, Rosenberg SA. A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J Exp Med. 1996; 183:1185–1192. [PubMed: 8642260]

Robbins P, El-Gamil M, Li YF, Fitzgerald E, Kawakami Y, Rosenberg SA. The intronic region of an incompletely spliced gp100 gene transcript encodes an epitope recognized by melanoma-reactive tumor-infiltrating lymphocytes. J Immunol. 1997; 159:303–308. [PubMed: 9200467]

Rosenberg SA. Adoptive immunotherapy for cancer. Sci Am. 1990; 262:62–69. [PubMed: 2333496]

Rosenberg SA. Interleukin-2 and the development of immunotherapy for the treatment of patients with cancer. Cancer J Sci Am. 2000; 6 (Suppl 1):S2–S7. [PubMed: 10685650]

Rosenberg SA. Cell transfer immunotherapy for metastatic solid cancer—what clinicians need to know. Nat Rev Clin Oncol. 2011; 8:577–585. [PubMed: 21808266]

Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med. 1987; 316:889–897. [PubMed: 3493432]

Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, et al. Use of tumor infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A Preliminary report. N Engl J Med. 1988; 319:1676–1680. [PubMed: 3264384]

Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, Seipp CA, Einhorn JH, White DE. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst. 1994; 86:1159–1166. [PubMed: 8028037]

Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Dudley ME, Schwarz SL, Spiess PJ, et al. Immunologic and therapeutic evaluation of a synthetic tumor-associated peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med. 1998; 4:321–327. [PubMed: 9500606]

Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: Moving beyond current vaccines. Nat Med. 2004; 10:909–915. [PubMed: 15340416]

Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: A clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008; 8:299–308. [PubMed: 18354418]

Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, Citrin DE, Restifo NP, Robbins PF, Wunderlich JR, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011; 17:4550–4557. [PubMed: 21498393]

Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science. 2011; 332:974–977. [PubMed: 21512004]

Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, Sherry RM, Topalian SL, Yang JC, Lowy I, et al. Phase 2 trial of single agent ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother. 2010; 33:828–833. [PubMed: 20842054]

Russo V, Tanzarella S, Dalerba P, Rigatti D, Rovere P, Villa A, Bordignon C, Traversari C. Dendritic cells acquire the MAGE-3 human tumor antigen from apoptotic cells and induce a class I-restricted T cell response. Proc Natl Acad Sci USA. 2000; 97:2185–2190. [PubMed: 10681453]

Schmidt-Wolf IG, Negrin RS, Kiem HP, Blume KG, Weissman IL. Use of a SCID mouse/human lymphoma model to evaluate cytokine-induced killer cells with potent antitumor cell activity. J Exp Med. 1991; 174:139–149. [PubMed: 1711560]

Schneider J, Brichard V, Boon T, Meyer zum Buschenfelde KH, Wolfel T. Overlapping peptides of melanocyte differentiation antigen Melan-A/MART- 1 recognized by autologous cytolytic T lymphocytes in association with HLA-B45.1 and HLA-A2.1. Int J Cancer. 1998; 75:451–458. [PubMed: 9455808]

Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331:1565–1570. [PubMed: 21436444]

Schroers R, Huang XF, Hammer J, Zhang J, Chen SY. Identification of HLA DR7-restricted epitopes from human telomerase reverse transcriptase recognized by CD4+ T-helper cells. Cancer Res. 2002; 62:2600–2605. [PubMed: 11980655]

Schultz ES, Lethe B, Cambiaso CL, Van Snick J, Chaux P, Corthals J, Heirman C, Thielemans K, Boon T, van der Bruggen P. A MAGE-A3 peptide presented by HLA-DP4 is recognized on tumor cells by CD4+ cytolytic T lymphocytes. Cancer Res. 2000; 60:6272–6275. [PubMed: 11103782]

Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011; 364:2119–2127. [PubMed: 21631324]

Sensi M, Pellegatta S, Vegetti C, Nicolini G, Parmiani G, Anichini A. Identification of a novel gp100/pMel17 peptide presented by HLA-A*6801 and recognized on human melanoma by cytolytic T cell clones. Tissue Antigens. 2002; 59:273–279. [PubMed: 12135425]

Skipper JC, Kittlesen DJ, Hendrickson RC, Deacon DD, Harthun NL, Wagner SN, Hunt DF, Engelhard VH, Slingluff CL Jr. Shared epitopes for HLA-A3-restricted melanoma-reactive human CTL include a naturally processed epitope from Pmel-17/gp100. J Immunol. 1996; 157:5027–5033. [PubMed: 8943411]

Slager EH, Borghi M, van der Minne CE, Aarnoudse CA, Havenga MJ, Schrier PI, Osanto S, Griffioen M. CD4+ Th2 cell recognition of HLA-DR-restricted epitopes derived from CAMEL: A tumor antigen translated in an alternative open reading frame. J Immunol. 2003; 170:1490–1497. [PubMed: 12538712]

Song XT, Evel-Kabler K, Shen L, Rollins L, Huang XF, Chen SY. A20 is an antigen presentation attenuator, and its inhibition overcomes regulatory T cell-mediated suppression. Nat Med. 2008; 14:258–265. [PubMed: 18311150]

Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, Karbach J, Arand M, Knuth A, Old LJ. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med. 1998; 187:1349–1354. [PubMed: 9547346]

Sutmuller RP, den Brok MH, Kramer M, Bennink EJ, Toonen LW, Kullberg BJ, Joosten LA, Akira S, Netea MG, Adema GJ. Toll-like receptor 2 controls expansion and function of regulatory T cells. J Clin Invest. 2006; 116:485–494. [PubMed: 16424940]

Tacken PJ, Figdor CG. Targeted antigen delivery and activation of dendritic cells in vivo: Steps towards cost effective vaccines. Semin Immunol. 2011; 23:12–20. [PubMed: 21269839]

Tahara K, Takesako K, Sette A, Celis E, Kitano S, Akiyoshi T. Identification of a MAGE-2-encoded human leukocyte antigen-A24-binding synthetic peptide that induces specific antitumor cytotoxic T lymphocytes. Clin Cancer Res. 1999; 5:2236–2241. [PubMed: 10473111]

Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, Shimada K, Sakamoto M, Hirohashi S, Ohashi Y, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: A randomised trial. Lancet. 2000; 356:802–807. [PubMed: 11022927]

Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol. 2005; 17:1–14. [PubMed: 15585605]

Tanaka F, Fujie T, Tahara K, Mori M, Takesako K, Sette A, Celis E, Akiyoshi T. Induction of antitumor cytotoxic T lymphocytes with a MAGE-3-encoded synthetic peptide presented by human leukocytes antigen-A24. Cancer Res. 1997; 57:4465–4468. [PubMed: 9377553]

Tanzarella S, Russo V, Lionello I, Dalerba P, Rigatti D, Bordignon C, Traversari C. Identification of a promiscuous T-cell epitope encoded by multiple members of the MAGE family. Cancer Res. 1999; 59:2668–2674. [PubMed: 10363990]

Tey SK, Bollard CM, Heslop HE. Adoptive T-cell transfer in cancer immunotherapy. Immunol Cell Biol. 2006; 84:281–289. [PubMed: 16681825]

Topalian SL, Gonzales MI, Parkhurst M, Li YF, Southwood S, Sette A, Rosenberg SA, Robbins PF. Melanoma-specific CD4+ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. J Exp Med. 1996; 183:1965–1971. [PubMed: 8642306]

Topalian SL, Gonzales MI, Ward Y, Wang X, Wang RF. Revelation of a cryptic major histocompatibility complex class II- restricted tumor epitope in a novel RNA-processing enzyme. Cancer Res. 2002; 62:5505–5509. [PubMed: 12359760]

Torchilin VP. Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. Annu Rev Biomed Eng. 2006; 8:343–375. [PubMed: 16834560]

Touloukian CE, Leitner WW, Topalian SL, Li YF, Robbins PF, Rosenberg SA, Restifo NP. Identification of a MHC class II-restricted human gp100 epitope using DR4-IE transgenic mice. J Immunol. 2000; 164:3535–3542. [PubMed: 10725708]

Traversari C, Van der Bruggen P, Luescher IF, Lurquin C, Chomez P, Van Pel A, De Plaen E, Amar-Costesec A, Boon T. A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. J Exp Med. 1992; 176:1453–1457. [PubMed: 1402688]

Tsai V, Southwood S, Sidney J, Sakaguchi K, Kawakami Y, Appella E, Sette A, Celis E. Identification of subdominant CTL epitopes of the GP100 melanoma-associated tumor antigen by primary in vitro immunization with peptide-pulsed dendritic cells. J Immunol. 1997; 158:1796–1802. [PubMed: 9029118]

Van der Bruggen P, Traversari C, Chomez P, Lurquin C, DePlaen E, Van Den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991; 254:1643–1647. [PubMed: 1840703]

Van der Bruggen P, Bastin J, Gajewski T, Coulie PG, Boel P, de Smet C, Traversari C, Townsend A, Boon T. A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. Eur J Immunol. 1994a; 24:3038–3043. [PubMed: 7805731]

Van der Bruggen P, Szikora JP, Boel P, Wildmann C, Somville M, Sensi M, Boon T. Autologous cytolytic T lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing Cw1601. Eur J Immunol. 1994b; 24:2134–2140. [PubMed: 7522162]

van der Burg SH, Melief CJ. Therapeutic vaccination against human papilloma virus induced malignancies. Curr Opin Immunol. 2011; 23:252–257. [PubMed: 21237632]

Vence L, Palucka AK, Fay JW, Ito T, Liu YJ, Banchereau J, Ueno H. Circulating tumor antigen-specific regulatory T cells in patients with metastatic melanoma. Proc Natl Acad Sci USA. 2007; 104:20884–20889. [PubMed: 18093940]

Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011; 29:235–271. [PubMed: 21219185]

Visseren MJ, van der Burg SH, van der Voort EI, Brandt RM, Schrier PI, van der Bruggen P, Boon T, Melief CJ, Kast WM. Identification of HLA-A*0201-restricted CTL epitopes encoded by the tumor-specific MAGE-2 gene product. Int J Cancer. 1997; 73:125–130. [PubMed: 9334819]

Voo KS, Fu T, Heslop HE, Brenner MK, Rooney CM, Wang RF. Identification of HLA-DP3-restricted peptides from EBNA1 recognized by CD4(+) T cells. Cancer Res. 2002; 62:7195–7199. [PubMed: 12499257]

Voo KS, Peng G, Guo Z, Fu T, Li Y, Frappier L, Wang RF. Functional characterization of EBV-encoded nuclear antigen 1-specific CD4+ helper and regulatory T cells elicited by in vitro peptide stimulation. Cancer Res. 2005; 65:1577–1586. [PubMed: 15735048]

Wang RF. The role of MHC class II-restricted tumor antigens and CD4+ T cells in antitumor immunity. Trends Immunol. 2001; 22:269–276. [PubMed: 11323286]

Wang RF, Rosenberg SA. Human tumor antigens for cancer vaccine development. Immunol Rev. 1999; 170:85–100. [PubMed: 10566144]

Wang RF, Wang HY. Enhancement of antitumor immunity by prolonging antigen presentation on dendritic cells. Nat Biotechnol. 2002; 20:149–156. [PubMed: 11821860]

Wang RF, Appella E, Kawakami Y, Kang X, Rosenberg SA. Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. J Exp Med. 1996a; 184:2207–2216. [PubMed: 8976176]

Wang RF, Parkhurst MR, Kawakami Y, Robbins PF, Rosenberg SA. Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. J Exp Med. 1996b; 183:1131–1140. [PubMed: 8642255]

Wang RF, Johnston S, Southwood S, Sette A, Rosenberg SA. Recognition of an antigenic peptide derived from TRP-2 by cytotoxic T lymphocytes in the context of HLA-A31 and -A33. J Immunol. 1998a; 160:890–897. [PubMed: 9551926]

Wang RF, Johnston SL, Zeng G, Schwartzentruber DJ, Rosenberg SA. A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. J Immunol. 1998b; 161:3596–3606.

Wang RF, Wang X, Atwood AC, Topalian SL, Rosenberg SA. Cloning genes encoding MHC class II-restricted antigens: Mutated CDC27 as a tumor antigen. Science. 1999a; 284:1351–1354. [PubMed: 10334988]

Wang RF, Wang X, Rosenberg SA. Identification of a novel MHC class II-restricted tumor antigen resulting from a chromosomal rearrangement recognized by CD4+ T cells. J Exp Med. 1999b; 189:1659–1667. [PubMed: 10330445]

Wang HY, Fu T, Wang G, Gang Z, Donna MPL, Yang JC, Restifo NP, Hwu P, Wang RF. Induction of CD4+ T cell-dependent antitumor immunity by TAT-mediated tumor antigen delivery into dendritic cells. J Clin Invest. 2002a; 109:1463–1470. [PubMed: 12045260]

Wang HY, Zhou J, Zhu K, Riker AI, Marincola FM, Wang RF. Identification of a mutated fibronectin as a tumor antigen recognized by CD4+ T cells: Its role in extracellular matrix formation and tumor metastasis. J Exp Med. 2002b; 195:1397–1406. [PubMed: 12045238]

Wang HY, Lee DA, Peng G, Guo Z, Li Y, Kiniwa Y, Shevach EM, Wang RF. Tumor-specific human CD4+ regulatory T cells and their ligands: Implication for immunotherapy. Immunity. 2004; 20:107–118. [PubMed: 14738769]

Wang HY, Peng G, Guo Z, Shevach EM, Wang RF. Recognition of a new ARTC1 peptide ligand uniquely expressed in tumor cells by antigen-specific CD4+ regulatory T cells. J Immunol. 2005; 174:2661–2670. [PubMed: 15728473]

Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B. Increase of regulatory T cells in the peripheral blood of cancer patients. Clin Cancer Res. 2003; 9:606–612. [PubMed: 12576425]

Wolfel T, Van Pel A, Brichard V, Schneider J, Seliger B, Meyer Zum Buschenfelde KH, Boon T. Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. Eur J Immunol. 1994; 24:759–764. [PubMed: 8125142]

Wolfel T, Hauer M, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer Zum Buschenfelde KH, Beach D. A p16INK4a-insensitive CDK4 mutant targeted by

cytolytic T lymphocytes in a human melanoma. Science. 1995; 269:1281–1284. [PubMed: 7652577]

Wrzesinski C, Paulos CM, Kaiser A, Muranski P, Palmer DC, Gattinoni L, Yu Z, Rosenberg SA, Restifo NP. Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells. J Immunother. 2010; 33:1–7. [PubMed: 19952961]

Xia X, Cui J, Wang HY, Zhu L, Matsueda S, Wang Q, Yang X, Hong J, Songyang Z, Chen Z, et al. NLRX1 negatively regulates TLR-induced NF-κB signaling by targeting TRAF6 and IKK. Immunity. 2011; 34:843–853. [PubMed: 21703539]

Yee C, Thompson JA, Roche P, Byrd DR, Lee PP, Piepkorn M, Kenyon K, Davis MM, Riddell SR, Greenberg PD. Melanocyte destruction after antigen-specific immunotherapy of melanoma. Direct evidence of T cell-mediated vitiligo. J Exp Med. 2000; 192:1637–1644. [PubMed: 11104805]

Yee C, Thompson JA, Byrd D, Riddell SR, Roche P, Celis E, Greenberg PD. Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: In vivo persistence, migration, and antitumor effect of transferred T cells. Proc Natl Acad Sci USA. 2002; 99:16168–16173. [PubMed: 12427970]

Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol. 2004; 5:730–737. [PubMed: 15208624]

Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. Nat Rev Cancer. 2004; 4:757–768. [PubMed: 15510157]

Zarour HM, Kirkwood JM, Kierstead LS, Herr W, Brusic V, Slingluff CL Jr, Sidney J, Sette A, Storkus WJ. Melan-A/MART-1(51–73) represents an immunogenic HLA-DR4-restricted epitope recognized by melanoma-reactive CD4(+) T cells. Proc Natl Acad Sci USA. 2000; 97:400–405. [PubMed: 10618430]

Zeng G, Touloukian CE, Wang X, Restifo NP, Rosenberg SA, Wang RF. Identification of CD4+ T cell epitopes from NY-ESO-1 presented by HLA-DR molecules. J Immunol. 2000; 165:1153–1159. [PubMed: 10878395]

Zeng G, Wang X, Robbins PF, Rosenberg SA, Wang RF. CD4+ T cell recognition of MHC class II-restricted epitopes from NY-ESO-1 presented by a prevalent HLA-DP4 allele: Association with NY-ESO-1 antibody production. Proc Natl Acad Sci USA. 2001; 98:3964–3969. [PubMed: 11259659]

Zhu Y, Yao S, Chen L. Cell surface signaling molecules in the control of immune responses: A tide model. Immunity. 2011; 34:466–478. [PubMed: 21511182]

Zorn E, Hercend T. A MAGE-6-encoded peptide is recognized by expanded lymphocytes infiltrating a spontaneously regressing human primary melanoma lesion. Eur J Immunol. 1999; 29:602–607. [PubMed: 10064076]

TABLE 6.1

Tumor antigens recognized by $CD8^+$ T cells

Antigens	MHC class I restrictions	Peptide epitopes	References
Tissue-specific antigens			
gp100	A2	KTWGQYWQV	Bakker *et al.* (1994)
	A2	AMLGTHTMEV	Tsai *et al.* (1997)
	A2	MLGTHTMEV	Tsai *et al.* (1997)
	A2	SLADTNSLAV	Tsai *et al.* (1997)
	A2	ITDQVPFSV	Kawakami *et al.* (1995)
	A2	LLDGTATLRL	Kawakami *et al.* (1994a)
	A2	YLEPGPVTA	Cox *et al.* (1994)
	A2	VLYRYGSFSV	Kawakami *et al.* (1995)
	A2	RLMKQDFSV	Kawakami *et al.* (1998)
	A2	RLPRIFCSC	Kawakami *et al.* (1998)
	A3	LIYRRRLMK	Kawakami *et al.* (1998)
	A3	ALNFPGSQK	Kawashima *et al.* (1998)
	A3	SLIYRRRLMK	Kawashima *et al.* (1998)
	A3	ALLAVGATK	Skipper *et al.* (1996)
	A24	VYFFLPDHL	Robbins *et al.* (1997)
	A*6801	HTMEVTVYHR	Sensi *et al.* (2002)
	B*3501	VPLDCVLYRY	Benlalam *et al.* (2003)
	Cw8	SNDGPTLI	Castelli *et al.* (1999)
MART-1/Melan-A	A2	AAGIGILTV	Coulie *et al.* (1994), Kawakami *et al.* (1994b)
	A2	ILTVILGVL	Castelli *et al.* (1995)
	A2	EAAGIGILTV	Schneider *et al.* (1998)
	B45	AEEAAGIGIL	Schneider *et al.* (1998)
gp75/TRP-1	A31	MSLQRQFLR	Wang *et al.* (1996b)
TRP-2	A2	SVYDFFVWL	Parkhurst *et al.* (1998)
	A2	TLDSQVMSL	Noppen *et al.* (2000)
	A31	LLGPGRPYR	Wang *et al.* (1996a)
	A33	LLGPGRPYR	Wang *et al.* (1998a)
	A68	EVISCKLIKR	Lupetti *et al.* (1998)
	Cw8	ANDPIFVVL	Castelli *et al.* (1999)
Tyrosinase	A1	KCDICTDEY	Kittlesen *et al.* (1998)
	A1	SSDYVIPIGTY	Kawakami *et al.* (1998)
	A2	YMDGTMSQV	Wolfel *et al.* (1994)
	A2	MLLAVLYCL	Wolfel *et al.* (1994)
	A24	AFLPWHRLF	Kang *et al.* (1995)
	B44	SEIWRDIDF	Brichard *et al.* (1996)
	B*3501	TPRLPSSADVEF	Benlalam *et al.* (2003)
Tumor-specific shared antigens			
BAGE	Cw16	AARAVFLAL	Boel *et al.* (1995)

Antigens	MHC class I restrictions	Peptide epitopes	References
CAMEL	A2	MLMAQEALAFL	Aarnoudse *et al.* (1999)
MAGE-A1	A1	EADPTGHSY	Traversari *et al.* (1992)
	A3	SLFRAVITK	Chaux *et al.* (1999a)
	A24	NYKHCFPEI	Fujie *et al.* (1999)
	A28	EVYDGREHSA	Chaux *et al.* (1999a)
	B37	REPVTKAEML	Tanzarella *et al.* (1999)
	B53	DPARYEFLW	Chaux *et al.* (1999a)
	Cw2	SAFPTTINF	Chaux *et al.* (1999a)
	Cw3	SAYGEPRKL	Chaux *et al.* (1999a)
	Cw16	SAYGEPRKL	Van der Bruggen *et al.* (1994b)
MAGE-A2	A2	KMVELVHFL	Visseren *et al.* (1997)
	A2	YLQLVFGIEV	Visseren *et al.* (1997)
	A24	EYLQLVFGI	Tahara *et al.* (1999)
	B37	REPVTKAEML	Tanzarella *et al.* (1999)
MAGE-A3	A1	EADPIGHLY	Gaugler *et al.* (1994)
	A2	FLWGPRALV	Van der Bruggen *et al.* (1994a)
	A24	TFPDLESEF	Oiso *et al.* (1999)
	A24	IMPKAGLLI	Tanaka *et al.* (1997)
	B44	MEVDPIGHLY	Fleischhauer *et al.* (1995), Herman *et al.* (1996)
	B52	WQYFFPVIF	Russo *et al.* (2000)
	B37	REPVTKAEML	Tanzarella *et al.* (1999)
	B*3501	EVDPIGHLY	Benlalam *et al.* (2003)
MAGE-A4	A2	GVYDGREHTV	Duffour *et al.* (1999)
MAGE-A6	A34	MVKISGGPR	Zorn and Hercend (1999)
	B37	REPVTKAEML	Tanzarella *et al.* (1999)
	B*3501	EVDPIGHVY	Benlalam *et al.* (2003)
MAGE-A10	A2	GLYDGMEHL	Huang *et al.* (1999)
MAGE-A12	Cw7	VRIGHLYIL	Heidecker *et al.* (2000), Panelli *et al.* (2000)
NY-ESO-1	A2	SLLMWITQCFL	Jager *et al.* (1998)
	A2	SLLMWITQC	Jager *et al.* (1998)
	A2	QLSLLMWIT	Jager *et al.* (1998)
	A31	ASGPGGGAPR	Wang *et al.* (1998b)
	B*3501	MPFATPMEA	Benlalam *et al.* (2003)
SSX-2	A2	KASEKIFYV	Ayyoub *et al.* (2002)
Tumor-specific unique antigens			
β-Catenin	A24 S	YLDSGIHF	Robbins *et al.* (1996)
Caspase-8	B35	FPSDSWCYF	Mandruzzato *et al.* (1997)
CDK-4	A2	ACDPHSGHFV	Wolfel *et al.* (1995)

TABLE 6.2

MHC class II-restricted melanoma antigens recognized by $CD4^+$ T cells

Tumor antigens	HLA restrictions	Peptides	References
Mutated/fusion antigens			
TPI	HLA-DR1	GELIG**I**LNAAKVPAD	Wang *et al.* (1999a), Pieper *et al.* (1999)
LDFP	HLA-DR1	PVIWRRAPA	Wang *et al.* (1999b)
	HLA-DR1	WRRAPAPGA	Wang *et al.* (1999b)
CDC27	HLA-DR4	FSWAMDLDPKGA	Wang *et al.* (1999a)
	Fibronectin	HLA-DR2	
		PSVGQQMIFE**K**HGFRRTTPP	Wang *et al.* (2002b)
Neo-PAP	HLA-DR7	RVIKNSIRLTL	Topalian *et al.* (2002)
ARTC1	HLA-DR1	YSVYFNLPADTIYTN	Wang *et al.* (2005)
Nonmutated antigens			
Tyrosinase	HLA-DR4	QNILLSNAPLGPQFP	Topalian *et al.* (1996)
	HLA-DR4	SYLQDSDPDSFQD	Topalian *et al.* (1996)
	HLA-DR15	FLLHHAFVDSIFEQWLQRHRP	Kobayashi *et al.* (1998)
gp100	HLA-DR4	WNRQ**LYPEWTEAQ**RLD	Touloukian *et al.* (2000)
	HLA-DR7	GPT**LIGANASFS**IALN	Kobayashi *et al.* (2001a)
	HLA-DR7/DR53	TGRA**MLGTHTMEV**TVYH	Lapointe *et al.* (2001), Kobayashi *et al.* (2001a)
	HLA-DR7	SLAV**VSTQLIMPG**QE	Kobayashi *et al.* (2001a)
MART-1	HLA-DR4	RNGYRALMDKSLHVGTQCALTRR	Zarour *et al.* (2000)
MAGE-A1	HLA-DR13	LLKYRAREPVTKAE	Chaux *et al.* (1999a)
MAGE-A2	HAL-DR1	LLKYRAREPVTKAE	Chaux *et al.* (1999a)
MAGE-3	HLA-DR11	TSYVKVLHHMVKISG	Manici *et al.* (1999)
	HLA-DR13	AELVHFLLLKYRAR	Chaux *et al.* (1999b)
	HLA-DR13	FLLLKYRAREPVTKAE	Chaux *et al.* (1999b)
	HLA-DP4	TQHFVQENYLEY	Schultz *et al.* (2000)
	HLA-DR1, 4, 7, 11	FFPVIFSKASSSLQL	Kobayashi *et al.* (2001b)
	HLA-DR1, 4, 11	RKVAELVHFLLLKYR	Consogno *et al.* (2003)
MAGE-A6	HLA-DR13	LLKYRAREPVTKAE	Chaux *et al.* (1999a)
LAGE1	HLA-DR13	RL**LQLHITMPF**SS	Wang *et al.* (2004)
CAMEL	HLA-DR11/12	PWKRSWSA	Slager *et al.* (2003)
NY-ESO-1	HLA-DR4	LPVPGV**LLKEFTVSG**NILTI	Zeng *et al.* (2000)
	HLA-DP4	**WITQCFLPV**FLAQPPSGQRR	Zeng *et al.* (2001)
hTRT	HLA-DR7	RPGLLGASVLGLDDI	Schroers *et al.* (2002)
Eph	HLA-DR11	DVTFNIACKKCG	Chiari *et al.* (2000)

Note: Amino acid sequence in bold stands for mutated or core sequence for recognition.