Dendritic-Cell-Based Therapeutic Cancer Vaccines

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The past decade has seen tremendous developments in novel cancer therapies through the targeting of tumor-cell-intrinsic pathways whose activity is linked to genetic alterations and the targeting of tumor-cell-extrinsic factors, such as growth factors. Furthermore, immunotherapies are entering the clinic at an unprecedented speed after the demonstration that T cells can efficiently reject tumors and that their antitumor activity can be enhanced with antibodies against immune-regulatory molecules (checkpoint blockade). Current immunotherapy strategies include monoclonal antibodies against tumor cells or immune-regulatory molecules, cell-based therapies such as adoptive transfer of ex-vivo-activated T cells and natural killer cells, and cancer vaccines. Herein, we discuss the immunological basis for therapeutic cancer vaccines and how the current understanding of dendritic cell and T cell biology might enable the development of next-generation curative therapies for individuals with cancer.

Introduction

Vaccination represents one of the most effective methods of preventing disease (Finn and Edwards, 2009; Nabel, 2013; Subbarao et al., 2006). Preventive vaccines are designed to block the spread of infection, and their activity correlates with the induction of specific antibodies and long-lived memory B cells (Pulendran and Ahmed, 2011). Cellular immunity can also be induced, especially with vaccines composed of attenuated microbes (Pulendran and Ahmed, 2011). On the other hand, therapeutic vaccines are designed to eliminate the cause of a given disease, e.g., to eliminate cancer cells or virally infected cells and to treat the disease. Their activity is mostly dependent on antigen-specific $CD8^+$ T cells that generate cytotoxic T lymphocytes (CTLs) to reject cancer or infected cells. Ideally, therapeutic vaccines should both prime naive T cells and modulate existing memory T cells, i.e., induce a transition from nonprotective $CD8^+$ T cells to healthy $CD8^+$ T cells able to yield effective CTLs (Figure 1). Indeed, cancer is a chronic disease and, as such, it is associated with skewed T cell memory, e.g., chronically activated $CD8^+$ T cells that express programmed cell death 1 (PD-1) and are anergic (Freeman et al., 2006). In addition, vaccination should lead to the generation of long-lived memory $CD8^+$ T cells that will act to prevent relapse (Figure 1).

The numerous clinical studies assessing therapeutic vaccination in cancer during the past two decades have helped us define the desired properties of vaccine-elicited $CD8^+$ T cells associated with the rejection of cancer (Appay et al., 2008). These include (1) high T cell receptor (TCR) affinity and high T cell avidity for peptide major histocompatibility complexes (MHCs) expressed on tumor cells (Appay et al., 2008), (2) high amounts of granzymes and perforin (Appay et al., 2008), (3) expression of surface molecules that allow T cell trafficking into the tumor (e.g., CXCR3 [Mullins et al., 2004]) and persistence in the tumor site (e.g., integrins CD103 [Le Floc'h et al., 2007] and CD49a [Sandoval et al., 2013]), and (4) high expression of costimulatory molecules (e.g., CD137 [Wilcox et al., 2002]) or low expression of inhibitory molecules (e.g., cytotoxic T lymphocyte antigen 4 [CTLA-4] [Peggs et al., 2009] or PD-1 [Freeman et al., 2006]). The immune system components necessary for the induction of such $CD8^+$ T cells include (1) the presentation of antigen by appropriate antigen-presenting cells (APCs) (Joffre et al., 2012; Lizée et al., 2013) and (2) the generation of $CD4^+$ T cells producing cytokines helping $CD8^+$ T cell proliferation and differentiation, e.g., IL-21 (Spolski and Leonard, 2008) (Figure 2).

Numerous avenues of therapeutic vaccination against cancer are currently being pursued (Finn, 2008). Searching for the term "cancer vaccines" at http://www.clinicaltrials.gov yields 1,307 clinical studies (as of July 2013), 152 of which are in phase III clinical trials and 591 of which are in phase II clinical trials, highlighting the clinical activity in the field. A common feature among these studies, and a critical step in vaccination, is the efficient presentation of cancer antigens to T cells (Figure 2). Because dendritic cells (DCs) are the most efficient APCs (Banchereau and Steinman, 1998), exploiting their diversity (in terms of both subsets and plasticity) is likely to yield improved therapeutic vaccines.

DCs are an essential component of vaccination through their capacity to capture, process, and present antigens to T cells (Banchereau and Steinman, 1998). Although immature DCs in peripheral tissues efficiently capture antigens (Mellman and Steinman, 2001), antigen presentation usually results in immune tolerance because of the lack of costimulatory molecules (Steinman et al., 2003; Tarbell et al., 2007). Induction of immune tolerance occurs through various mechanisms, including T cell deletion and expansion of regulatory T (Treg) cells (Steinman et al., 2003; Tarbell et al., 2007). Activated (mature), antigen-loaded DCs initiate the differentiation of antigen-specific T cells into effector T cells that display unique functions and cytokine profiles. DC maturation is associated with a wide variety of cellular changes, including (1) decreased antigen-capture activity, (2) increased expression of surface MHC class II molecules and costimulatory molecules, (3) acquisition of chemokine receptors (e.g., CCR7), which guide their migration (Trombetta and Mellman, 2005), and (4) the ability to secrete different cytokines (e.g., interleukin-12 [IL-12]) that control T cell differentiation. It is now accepted that vaccine adjuvants act by inducing

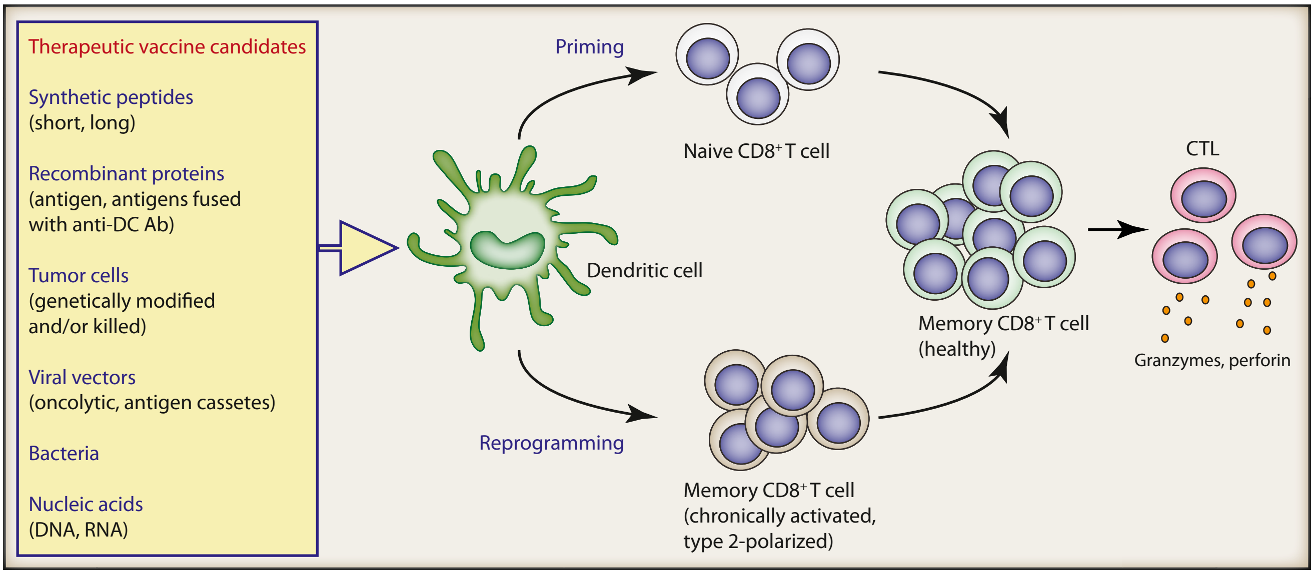

Figure 1. Therapeutic Vaccines Act via Dendritic Cells to Generate Protective CD8$^+$ T Cell Immunity
Therapeutic vaccines are designed to elicit cellular immunity. In this goal, they are expected to prime new T cells and induce a transition from chronically activated nonprotective CD8$^+$ T cells to healthy CD8$^+$ T cells able to (1) generate CTLs that reject cancer and (2) provide long-lived memory CD8$^+$ T cells able to rapidly generate new effector T cells that secrete cytotoxic molecules, thereby preventing relapse. Numerous approaches to therapeutic vaccines currently being pursued are illustrated. Their common denominator is the action via DCs for either random or specific targeting.

DC maturation (Steinman and Banchereau, 2007). Vaccines can also reach lymph-node-resident DCs directly through the lymphatics (Itano et al., 2003). Recent years brought about an increased understanding of DC biology, the existence of distinct DC subsets with specific functions, and a distinct molecular mechanism that DCs use to regulate the immune response. Hereunder, we will discuss how this progress can be harnessed for improved vaccination against cancer.

Human DC Subsets

Human DCs in the steady state were first studied in whole blood and skin. Three cell-surface markers characterize blood DCs: CD303, expressed on plasmacytoid DCs (pDCs), and CD1c and CD141, both expressed on circulating DCs (Dzionek et al., 2000; Dzionek et al., 2001; MacDonald et al., 2002). Both CD1c$^+$ and CD141$^+$ DCs can produce IL-12, thereby enabling the generation of interferon-γ (IFN-γ)-secreting type 1 CD4$^+$ T (Th1) cells and the priming of naive CD8$^+$ T cells (Meixlsperger et al., 2013; Schlitzer et al., 2013). Both CD1c$^+$ and CD141$^+$ DCs, isolated from blood or tissues, are able to cross-present long peptides of melanoma-tissue-derived antigen (MART-1) to T cell lines (Segura et al., 2012) and acquire viral antigens and drive antiviral effector CD8$^+$ T cell responses (Yu et al., 2013). However, they also display unique features. CD141$^+$CD1c$^-$ DCs, the human counterpart of mouse CD8α^+ DCs, produce very large amounts of IFN-α upon recognition of synthetic double-stranded RNA (Meixlsperger et al., 2013) and, when activated with poly I:C, efficiently cross-prime CD8$^+$ T cells (Bachem et al., 2010; Crozat et al., 2010; Haniffa et al., 2012; Jongbloed et al., 2010; Lauterbach et al., 2010; Mittag et al., 2011; Poulin et al., 2010). CD1c$^+$ DCs from both blood and lungs are uniquely able to drive the differentiation of CD103$^+$CD8$^+$ mucosal T cells with high retention capacity in the lungs (Yu et al., 2013).

Studies of human cutaneous DCs demonstrated their phenotypic and functional heterogeneity (Klechevsky et al., 2008; Nestle et al., 2009; Joffre et al., 2012). In particular, Langerhans cells (LCs) specialize in priming CD8$^+$ T cell immunity, whereas interstitial (dermal) CD14$^+$ DCs promote humoral immunity (Klechevsky et al., 2008). The efficiency of LCs in priming naive CD8$^+$ T can be partially explained by their ability to produce IL-15 (Banchereau et al., 2012a; Romano et al., 2012) and/or upregulate CD70 (van der Aar et al., 2011). Interstitial DCs can either act directly on B cells (Dubois et al., 1997) or prime CD4$^+$ T cells to differentiate into T follicular helper (Tfh) cells that help B cell differentiation in germinal centers (GCs) (Crotty, 2011). They induce the differentiation of Tfh cells through the production of IL-12 (Schmitt et al., 2013). Interstitial DCs can generate type 2 CD8$^+$ T cells that produce low amounts of granzyme A and display poor CTL functions, a property that can be inhibited by the blocking of ILT4 (Banchereau et al., 2012b). Thus, vaccines that target interstitial DCs might raise good antibody responses but poor CD8$^+$ T cell immunity.

DCs express numerous nonclonal pattern-recognition receptors (PRRs), which permit sensing and transmission of danger signals to adaptive immunity. PRRs include membrane C-type lectins and Toll-like receptors (TLRs) and cytoplasmic NOD-like receptors, as well as DNA and RNA sensors (Barber, 2011; Desmet and Ishii, 2012). These receptors allow DCs to sense pathogens, apoptotic and necrotic cells, and stressed cell products, e.g., extruded DNA (Caielli et al., 2012). Herein, we will only discuss a few examples of these recognition mechanisms to illustrate how these DC properties can be harnessed for the generation of more efficient cancer vaccines. Interested readers can find more in-depth discussion in recent reviews (Coffman et al., 2010; Desmet and Ishii, 2012; Latz et al., 2013).

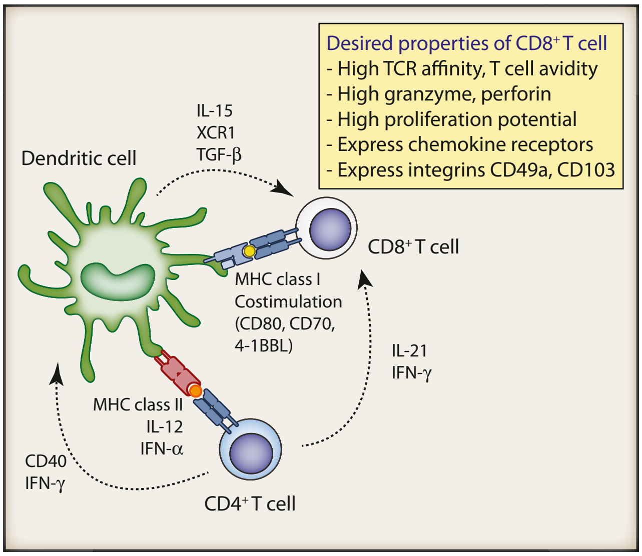

Figure 2. Dendritic Cells Play a Central Role in Vaccination

The desired properties of vaccine-elicited CD8$^+$ T cells include (1) high TCR affinity and high T cell avidity, (2) high levels of granzymes and perforin, (3) trafficking into the tumor and persistence in the tumor site, and (4) high proliferation potential. Naive CD8$^+$ T cells initiate a CTL differentiation program upon encounter with DCs presenting tumor-derived peptides via MHC class I. This is supported by costimulation mediated by CD80, CD70, and 4-1BB and by DC-derived cytokines such as IL-15. XCR1 chemokine secreted by DCs facilitates the interaction with naive CD8$^+$ T cells. TGFβ expressed by DCs is critical for CD8$^+$ T cells to express CD103 and acquire a mucosal phenotype. CD8$^+$ T cell differentiation, especially generation of memory, is dependent on the quality of CD4$^+$ T cell help. The latter one is partially dependent on the IL-12 secreted by DCs. CD4$^+$ T cells producing IFN-γ and/or IL-21 can help CD8$^+$ T cell expansion and differentiation. Treg cells might play a critical role during the selection of high-avidity CD8$^+$ T cells. This might be ascribed to the crosstalk between DCs and CD4$^+$ T cells where CD4$^+$ T cells control DC functions. There, Treg cells can suppress DCs via IL-10 production and also regulate the production of chemokines, thereby limiting the interactions between DCs and low-avidity T cells. CD4$^+$ T cells can also provide DC maturation signals via CD40.

Nucleic acid detection can lead to the production of protective type I IFN via endosomal or cytoplasmic sensors (Barber, 2011; Desmet and Ishii, 2012; Zhang et al., 2011a; Zhang et al., 2011b). This offers a venue for the development of potent vaccine adjuvants generating high levels of type I IFN, such as poly I:C binding TLR3 and cytoplasmic sensors, Imiquimod binding TLR7, and CpG oligonucleotides binding TLR9 (Coffman et al., 2010). Some lectins harbor in their cytoplasmic regions signaling motifs that deliver activation signals when engaged by ligands expressed on necrotic cells (Sancho and Reis e Sousa, 2013). For example, macrophage-inducible C-type lectin detects nuclear ribonucleoproteins released from damaged cells (Sancho and Reis e Sousa, 2013), whereas CLEC9A, expressed uniquely on CD141$^+$ DCs, detects actin exposed on necrotic cells (Ahrens et al., 2012; Zhang et al., 2012) and thereby facilitates cross-presentation of necrotic cell antigens (Sancho et al., 2009). DCs also express inflammasome components that regulate the release of caspase-activation-dependent cytokines, including IL-1β, IL-18, and high-mobility group box 1 (HMGB1) (Latz et al., 2013). Inflammasome activation in DCs can occur through the recognition of microbial ligands, such as flagellin, or through indirect mechanisms resulting from the phagocytosis of particles, including alum, uric acid, and biodegradable particles that are currently being tested as vaccine adjuvants (Coffman et al., 2010; Latz et al., 2013). Activation of the inflammasome also plays a very important role in the response to cancer therapy via so-called "immunogenic cancer cell death" (Kroemer et al., 2013). There, certain types of anti-cancer chemotherapy drugs such as anthracyclines or oxaliplatin can induce immunogenic cancer cell death, which is characterized by the secretion of HMGB1 from dying cells (this secretion engages TLR4 on DCs) (Kroemer et al., 2013). This signal facilitates cancer antigen processing and presentation by DCs to T cells (Kroemer et al., 2013). This in turn plays an important role in boosting anti-cancer immunity via endogenous vaccination. Indeed, the absence of HMGB1 expression by dying tumor cells compromises DC-dependent T cell priming by tumor-associated antigens (Yamazaki et al., 2013). Exploiting these unique molecular pathways for antigen delivery and DC activation represents another way of harnessing DCs for vaccination.

DC-Based Vaccines

DCs can be exploited for vaccination against cancer through various means, including (1) nontargeted peptide- or protein- and nucleic-acid-based vaccines captured by DCs in vivo, (2) vaccines composed of antigens directly coupled to DC antibodies, or (3) vaccines composed of ex-vivo-generated DCs that are loaded with antigens. We will discuss selected examples of current therapeutic vaccination approaches to illustrate these key concepts. All these approaches are being assessed in ongoing clinical trials.

Nontargeted Vaccines

Vaccines composed of short 9–10 aa peptides, with or without adjuvants, demonstrated that MHC-class-I-restricted antigen-specific CD8$^+$ T cell immunity can be mounted in individuals with metastatic disease (Boon et al., 2006; Rosenberg et al., 1998; Speiser et al., 2008). However, the clinical success was limited (Rosenberg et al., 2005), possibly because of the lack of CD4$^+$ T cell help, which we now know is necessary for the generation of potent CTLs and long-lived memory CD8$^+$ T cells (Janssen et al., 2005; Filipazzi et al., 2012). Long synthetic peptides of ~25–50 aa have the advantage of potentially inducing broad immunity with both CD8$^+$ T cell and CD4$^+$ T cell responses against multiple epitopes (Quakkelaar and Melief, 2012). Vaccination of 20 individuals with high-grade vulvar intraepithelial neoplasia with a long peptide covering the two oncogenic proteins E6 and E7 of high-risk human papilloma virus type 16 (HPV16) led to complete regression of all lesions and eradication of virus in nine individuals (Kenter et al., 2009). A high ratio of vaccine-antigen-specific effector T cells to CD4$^+$CD25$^+$Foxp3$^+$ Treg cells was predictive of clinical benefit (Welters et al., 2010). Vaccination of subjects suffering from recurrent ovarian cancer with long peptides covering p53 led to the expansion of p53-specific CD4$^+$ T cells in blood and tumors (Leffers et al., 2009). However, no impact on the clinical course of the disease was observed (Leffers et al., 2009). The lack of clinical responses might be explained by the domination of the immune response to vaccine antigens by CD4$^+$ T cells that secrete type 2 cytokines (IL-4 and IL-5) rather than IFN-γ. Indeed, type 2 CD4$^+$ T cells might not be protective against cancer. Durable expansion of p53-specific type 2 CD4$^+$ T cells was also observed in subjects with colorectal cancer (Speetjens et al., 2009). Combining the long p53 peptide vaccine with IFN-α resulted in increased expansion of antigen-specific IFN-γ-secreting CD4$^+$ T cells, although the impact on clinical efficacy remains to be established (Zeestraten et al., 2013). These results further illustrate the challenges that the reprogramming of pre-existing T cell memory represents and the need to identify vaccines that will enable priming of a new T cell repertoire.

With the advances of proteomics, vaccines can now be prepared with peptides representing antigens identified from affected individuals' tumors. The peptides are combined with granulocyte-macrophage colony-stimulating factor (GM-CSF), which can attract and activate DCs and a low dose of cyclophosphamide in an effort to control Treg cells. This regimen led to immune responses that were associated with clinical responses (Walter et al., 2012). Although it is difficult to assess which component of this therapy accounted for good immune and clinical efficacy, shifting from shared tumor antigens common to many subjects to individual-specific neo-antigens might enable the efficient activation of an available T cell repertoire against which Treg cells might not have developed. The concept of subject-specific vaccines was initiated more than two decades ago with idiotype vaccines in lymphoma (Kwak et al., 1992), where tumor idiotypicF determinants were conjugated to the immune carrier keyhole limpet hemocyanin (KLH) (Kwak et al., 1992). A phase III trial in subjects with lymphoma showed that such a vaccine combined with GM-CSF can lead to significant prolongation of disease-free survival (Schuster et al., 2011).

Peptide-protein vaccines are poorly immunogenic by themselves unless adjuvants are added for the generation of robust antitumor immune responses. Many adjuvants are currently under evaluation as constituents of cancer vaccines (Dubensky and Reed, 2010). These include agonists of various TLRs, such as TLR3 (poly I:C), TLR4 (monophosphoryl lipid A [MPL]), TLR5 (flagellin), TLR7 (Aldara [Imiquimod]), TLR7-TLR8 (Resiquimod), and TLR9 (CpG) (Dubensky and Reed, 2010). Combinations of adjuvants targeting different pathways might synergize to generate more potent immune responses because their combination can activate DCs in a synergistic fashion (Coffman et al., 2010). A promising candidate is GlaxoSmithKline's AS15 adjuvant system, which incorporates MPL that acts via TLR4, the saponin QS-21, and CpG oligonucleotides that act via TLR9 (Cluff, 2010). Vaccines composed of recombinant MAGE-A3 and AS15 elicited specific immune responses and clinical activity in both a phase II study in subjects with metastatic melanoma (NCT00086866) and a phase II study in subjects with resected non-small-cell lung cancer (NSCLC) (NCT00290355) (Brichard and Lejeune, 2007). Phase III trials are currently ongoing in two settings: (1) in subjects with resectable regionally advanced melanoma (DERMA phase III trial, NCT00796445) (Kirkwood, 2011, J. Clin. Oncol., abstract) and (2) in subjects with MAGE-A3-expressing NSCLC with minimal residual disease after surgery (NCT00480025). Clearly, a better understanding of DC biology will provide a fertile ground for discovery of novel adjuvants.

DCs are also engaged in response to complex vaccine preparations, such as GVAX tumor-cell-based vaccines, for which cancer cells are genetically modified to express GM-CSF, which attracts and activates DCs (Le et al., 2010). Such GVAX vaccines have shown some immune and clinical activity in pancreatic cancer (Thomas et al., 2004; Lutz et al., 2011) and other types of solid tumors (Dranoff, 2002). Another vaccine platform is based on recombinant *Listeria monocytogenes* (*Lm*), an intracellular bacterium that targets DCs in vivo and utilizes both class I and II antigen-processing pathways (Brockstedt et al., 2004; Le et al., 2012). The live mutant *Lm*-based vaccine that expresses mesothelin elicits mesothelin-specific T cells in mice and humans (Le et al., 2012). Engineered viruses can ferry selected antigens and costimulation cassettes (Larocca and Schlom, 2011). In a randomized phase II trial with a poxvirus-based vaccine expressing prostate-specific antigen (PSA) (PROSTVAC) and TRICOM (CD54, CD58, and CD80), men with metastatic prostate cancer showed an improved overall survival (8.5 months) (Kantoff et al., 2010b). Another strategy is based on intratumoral delivery of oncolytic viruses, i.e., viruses that preferentially infect and kill cancer cells. These can be modified to express GM-CSF to attract DCs and lymphocytes at the lysed tumor site (Russell et al., 2012). A phase II study of GM-CSF-oncolytic herpes virus in individuals with stage IIIc and stage IV melanoma indicated durable regression in both injected and noninjected lesions, suggesting a systemic effect (Senzer et al., 2009). The recent data from a randomized prospective phase III clinical trial showed tumor regression lasting at least 6 months in 16% of individuals treated with the recombinant virus. Only 2% of individuals treated with GM-CSF in the control arm showed such a response (OPTiM, Oncovex Pivotal Trial in Melanoma, Amgen website). A formal analysis of the trial is expected later this year. Viral vectors to deliver antigens to DCs, either directly by encoded genes or indirectly via tumor lysis, is an attractive strategy because it mimics the natural way of infection and generation of protective immunity. However, the immunogenicity of these vectors might prevent their efficacy upon boosting, therefore calling for prime-boost strategies in which a second vector is used for boosting the specific immune response. This strategy is currently being developed in the context of HIV vaccines (both preventive and therapeutic) and could be applied to cancer in case of success.

Vaccination with Ex-Vivo-Generated DCs

DCs can be generated ex vivo, loaded with different forms of antigens, activated, and injected in affected individuals (Palucka and Banchereau, 2012). Clinical studies from the past 15 years have analyzed (1) different DC vaccine preparations, (2) different DC activators, (3) different forms of antigen preparations from short peptides to complex whole-tumor-cell hybrids, and (4) different routes of DC injection. These studies were initially performed as single treatments, but combination studies are now being assessed with agents such as systemic adjuvants, e.g., poly I:C (Aarntzen et al., 2008; Kalinski et al., 2013; Palucka and Banchereau, 2012; Schuler, 2010). These studies concluded that DC-based vaccines are safe and can induce the expansion of circulating CD4$^+$ T cells and CD8$^+$ T cells specific to tumor antigens. Although objective clinical responses have been observed in certain affected individuals, there is a discrepancy between the blood immune response and the rate of clinical responses, as we will later discuss. The clinical response takes time to build up, but remissions can be long lasting. The United States Food and Drug Administration has approved the treatment of metastatic prostate cancer with Sipuleucel-T, a cellular product composed of enriched blood APCs cultured with a fusion protein of prostatic acid phosphatase (PAP) and GM-CSF. Treatment with Sipuleucel-T resulted in a ~4-month-prolonged median survival in subjects with prostate cancer (Kantoff et al., 2010a). Another subset of blood DCs, plasmacytoid DCs, which represent the main source of type I IFN upon viral infection, have also been assessed as the basis for cancer vaccines (Liu, 2005; Tel et al., 2013). Some metastatic-melanoma-affected individuals, who were vaccinated with activated pDCs loaded with tumor-antigen peptides, showed antigen-specific CD4$^+$ and CD8$^+$ T cell responses (Tel et al., 2013).

Although considerable progresses have been made over the years, additional studies are required to fully reveal the potential immunotherapeutic impact of ex-vivo-generated DCs. Most studies have been performed in late-stage subjects who display strong immunosuppression mechanisms, e.g., Treg cells that counteract the induction of effective immunity to vaccine antigens. Nevertheless, there are two ongoing phase III trials assessing in comparative studies the clinical efficacy of monocyte-derived ex-vivo-generated DC vaccines. One trial is testing a DC vaccine in individuals with a newly diagnosed brain tumor (glioblastoma) after surgery as an add-on to the standard of care, which combines radiation and chemotherapy (NCT00045968; Northwest Therapeutics). The DCs are loaded with autologous tumor lysate. The second trial is testing a DC vaccine in subjects with advanced kidney cancer (renal carcinoma) as an add-on to targeted therapy with Sunitinib, a receptor tyrosine kinase inhibitor (NCT01582672; ADAPT trial, Argos Therapeutics). The DCs are loaded with autologous tumor RNA. The three common features of these two trials are (1) the vaccination of subjects with resected tumors and thus lower tumor burden, (2) vaccination in combination with other therapy, and (3) loading DCs with autologous tumor preparations. Time will show whether the promising phase II data observed with these vaccines will be confirmed in phase III.

In Vivo DC Targeting

Pioneering studies from Ralph Steinman and Michel Nussenzweig demonstrated the principle of targeting antigens to DCs in vivo through the coupling of antigens to antibodies specific to DC surface receptors such as DEC205 or DCIR (Bonifaz et al., 2002; Hawiger et al., 2001; Soares et al., 2007). Importantly, in the absence of adjuvants, targeting antigens to DEC205$^+$ DCs in vivo induces antigen-specific tolerance (Hawiger et al., 2001), which can be used as treatment against autoimmune diseases such as type 1 diabetes (Steinman, 2012). Administration of these complex vaccines with DC activators such as TLR3, TLR7-8, or CD40 agonists enables the maturation of DCs and thus the establishment of immunity rather than tolerance (Steinman, 2012). The induced immunity was shown to be protective in a number of diseases, including various infections (e.g., malaria and HIV) and cancer (Steinman, 2012; Tacken and Figdor, 2011). DC-targeting-based vaccination studies in nonhuman primates demonstrated robust T cell immunity in a prime-boost design with HIV gag-DEC205-targeting vaccine (Flynn et al., 2011).

Currently, numerous in vitro and in vivo studies in humans and mice are focused on developing DC-targeting vaccines. For example, targeting antigens through the DC surface lectins DCIR (Klechevsky et al., 2010; Meyer-Wentrup et al., 2009), DC-SIGN (Dakappagari et al., 2006), dectin 1 (Ni et al., 2010), CLEC9A (Sancho et al., 2008), and Langerin (Flacher et al., 2009) results in humoral and cellular responses, including those of both CD4$^+$ and CD8$^+$ T cells. As observed in the original studies with DEC205, the presence or absence of adjuvants has a profound impact on immune responses. Thus, in the absence of adjuvants, injection of antigens coupled to antibodies against CLEC9A results in strong antibody responses, which are linked to the generation of Tfh cells (Caminschi et al., 2012). It also results in priming of Treg cell immunity(Joffre et al., 2010), but not CD8$^+$ T cell immunity, despite the capture and the cross-presentation of targeted antigens by CD8α^+ DCs (Sancho et al., 2008). This can be skewed by the addition of adjuvants, e.g., poly I:C, at which point targeting of antigen to DCs via CLEC9A results in potent and robust antitumor CD4$^+$ and CD8$^+$ T cell immunity (Sancho et al., 2008; Joffre et al., 2010). In mice, in vivo studies comparing immunogenicity of HIV antigens linked with antibodies to Langerin (CD207), DEC205 (CD205), and CLEC9A receptors, along with CD40 antibody, to induce DC activation resulted in comparable levels of gag-specific Th1 and CD8$^+$ T cells (Idoyaga et al., 2011). These target molecules are expressed by CD8α^+ DCs, and the responses were more robust than those obtained by gag targeting to CD8α^- DCs via DCIR (Idoyaga et al., 2011). Thus, when the appropriate DC subset is targeted with a vaccine antigen with appropriate adjuvants, several different receptors expressed by that subset are able to initiate T cell immunity.

However, different DC receptors can deliver different signals to the same DC, leading to distinct types of immune responses. For example, targeting antigens to DC-ASGPR in the absence of adjuvants favors the generation of antigen-specific IL-10-secreting CD4$^+$ T cells with regulatory properties both in vitro in humans and in vivo in nonhuman primates. Targeting the same DC population with antibodies to LOX-1 results in the generation of antigen-specific IFN-γ-secreting CD4$^+$ T cells (Li et al., 2012). Furthermore, targeting different human DC receptors revealed the importance of antigen internalization into either early or late endosomes (Chatterjee et al., 2012). Thus, in human BDCA1$^+$ and monocyte-derived DCs, antibodies to CD40 and mannose receptor targeted antigens to early endosomes, whereas antibodies to DEC205 targeted antigens primarily to late compartments. CD40, the receptor that was least efficient at internalization, turns out to be the most efficient at cross-presentation because it promotes limited intraendosomal degradation (Chatterjee et al., 2012). Similarly, the targeting of different DC receptors generates quantitatively and qualitatively different T cell responses in vivo in mice (Dudziak et al., 2007; Soares et al., 2007). There, unlike CD8α^+ DCs, which express DEC205, CD8α^- DCs, which express 33D1 antigen, are specialized for presentation of targeted antigen on MHC class II. This difference in antigen processing was shown to be intrinsic to the DC subsets and associated with increased expression of proteins involved in MHC processing (Dudziak et al., 2007). Thus, it will be essential to refine the understanding of DC biology to guide the processing of targeted antigen and subsequent presentation resulting in CD8$^+$ T cell immunity.

CD8$^+$ T Cell Immunity

Therapeutic vaccination aims at expanding high-avidity CD8$^+$ T cells that can differentiate into CTLs able to kill cancer cells and can generate long-lived memory CD8+T cells. This could be accomplished through either the priming of naive T cells or the reprogramming of memory T cells that differentiate earlier in an environment not conducive to the generation of potent cytotoxic T cells (Figure 1). Naive CD8$^+$ T cells differentiate into CTLs in lymphoid organs upon encounter with DCs presenting tumor-derived peptides (Bousso and Robey, 2003) (Figure 2) in the context of costimulation through CD80, CD70, and 4-1BB (Shuford et al., 1997), as well as DC-derived cytokines such as IL-12 and IL-15 (Araki et al., 2010; Waldmann, 2006; Zhang and Bevan, 2011). The priming of the new repertoire of T cells might be critical for clinical success. Studies with adoptive T cell transfer showed that effector cells derived from naive CD8$^+$ T cells expressed higher CD27 and retained longer telomeres, suggesting that these cells have a greater proliferative potential (Hinrichs et al., 2011; Klebanoff et al., 2012).

Circulating memory CD8$^+$ T cells include both central memory and effector cells that circulate between secondary lymphoid organs and peripheral tissues. A third category, i.e., tissue-resident memory T cells, has been recently identified (Jiang et al., 2012; Mueller et al., 2013) and shown to be superior to circulating memory T cells at providing rapid long-term protection against reinfection (Gebhardt et al., 2009; Jiang et al., 2012). CD103 ($\alpha E\beta 7$) integrin allows peripheral CD8$^+$ T cell retention in epithelial compartments (Sheridan and Lefrançois, 2011). In the context of cancer, the expression of CD103 by CTLs facilitates their adherence to cancer cells expressing E-cadherin, eventually leading to tumor cell lysis and rejection (Le Floc'h et al., 2007). Indeed, for mucosal cancer vaccines, the homing to and retention of CD8$^+$ T cells in the mucosa are critical for efficacy (Sandoval et al., 2013). In this context, the growth of orthotopic head and neck or lung cancers can be inhibited by a cancer vaccine provided that it is administered by the intranasal mucosal route, but not the intramuscular route (Sandoval et al., 2013). This is explained by the induction through intranasal vaccination of mucosal CD8$^+$ T cells expressing the mucosal integrin CD49a, the expression of which is essential for the efficacy of cancer vaccines (Sandoval et al., 2013). The critical role of tissue DCs in imprinting the trafficking patterns of elicited T cells explains the critical role of the route of immunization (Mullins et al., 2003; Sheasley-O'Neill et al., 2007) (Mora et al., 2003). The current challenge is to find out how to control T cell differentiation and trafficking in affected individuals.

Designing Tomorrow's Therapeutic Cancer Vaccines

The challenge for next-generation vaccines is to resolve the discrepancy between the immune and clinical efficacy measured by the rate of cancer rejection. We will summarize herein the three key aspects that when combined can bring the resolution to this challenge: (1) the quality of vaccine-elicited CD8$^+$ T cell immunity, (2) the quality of vaccine-elicited CD4$^+$ T cells, and (3) the barriers that vaccine-elicited CD8$^+$ T cells must confront to access and reject cancer.

As discussed at the beginning of this review, studies in adoptive T cell transfer and cancer vaccines yielded a better understanding of what constitutes a potent antitumor CD8$^+$ T cell immunity. Thus, next-generation DC vaccines need to be based on those DC subsets that are best equipped to elicit CD8$^+$ T cells that fulfill these criteria. For example, targeting cancer antigens to CD141$^+$ DCs would allow the generation of highly potent CTLs. On the other hand, targeting the antigen to CD1c$^+$ DCs would allow the expansion of CD103$^+$CD8$^+$ T memory T cells able to reside in the tissue.

CD4$^+$ T cells regulate CD8$^+$ T cell immunity in both the priming and the effector phases. For example, Treg cells can inhibit the effector functions of CD8$^+$ T cells, thereby preventing tumor rejection (Tanchot et al., 2012). However, Treg cells also play a critical role during the priming by promoting the selection of high-avidity CD8$^+$ T cells (Pace et al., 2012). Although they mostly help tumor rejection, Th1 cells might contribute to tumor escape via secretion of IFN-γ that triggers expression of PDL-1 in tissues, thus providing an off signal to effector CD8$^+$ T cells (Sharpe et al., 2007). Th17 cells (Dong, 2008) exert either protumor or antitumor activity depending on the tissue environment in which they reside (reviewed in Wei et al., 2012). Indeed, IL-17 can synergize with IFN-γ to induce tumor cells to secrete CXCL9 and CXCL10, which attract cytotoxic CD8$^+$ T cells (Wei et al., 2012). Thus, it will now be critically important to unravel molecular factors governing CD4$^+$ T cell programming and differentiation and DC molecules that can control such factors. Again, the functional specialization among human DC subsets can be harnessed here. Indeed, as we discussed above, CD14$^+$ DCs are able to prime Tfh. Meanwhile, LCs prime Th2 cells (Klechevsky et al., 2008), and CD1c$^+$ DCs, but not CD141$^+$ DCs, are molecularly equipped to generate Th17 responses in humans (Schlitzer et al., 2013). This knowledge can be applied to the design of next-generation vaccines for directing the differentiation of antigen-specific CD4$^+$ T cells to a desired phenotype and function.

Last but not least, once elicited, CD8$^+$ T cells must confront numerous barriers, including (1) intrinsic regulators, such as CD28-CTLA-4, PD1-PDL1, and ILTs (Pardoll, 2012), and extrinsic regulators, such as Treg cells (Fehérvari and Sakaguchi, 2004) or myeloid-derived suppressor cells (MDSCs) (Gabrilovich and Nagaraj, 2009); (2) a corrupted tumor microenvironment with protumor inflammation (Coussens et al., 2013; Klebanoff et al., 2011); (3) antigen loss and immune evasion of tumor targets (Klebanoff et al., 2011); and (4) tissue-specific alterations, such as fatty cells in breast cancer or desmofibrosis in pancreatic cancer stroma (Figure 3). Defining strategies for bypassing these obstacles is the object of intense studies to improve the clinical efficacy of vaccination via DCs. A logical approach to addressing these issues is the combination of DC vaccine candidates and agents that target different pathways. For example, checkpoint inhibitors such as antagonists to CTLA-4 or PD-1 might offset inhibitor signals (Figure 3) (see review by Chen and Mellman, 2013, in this issue of *Immunity*). The combination of GVAX and CTLA4 antibody (Ipilimumab) has proven to be safe (van den Eertwegh et al., 2012), and preclinical models show increased effector CD8$^+$ T cells and enhanced tumor-antigen-directed CTL function (Wada et al., 2013).

We foresee tomorrow's vaccines as based on DC antibodies, which, thanks to progresses in antibody engineering, can be

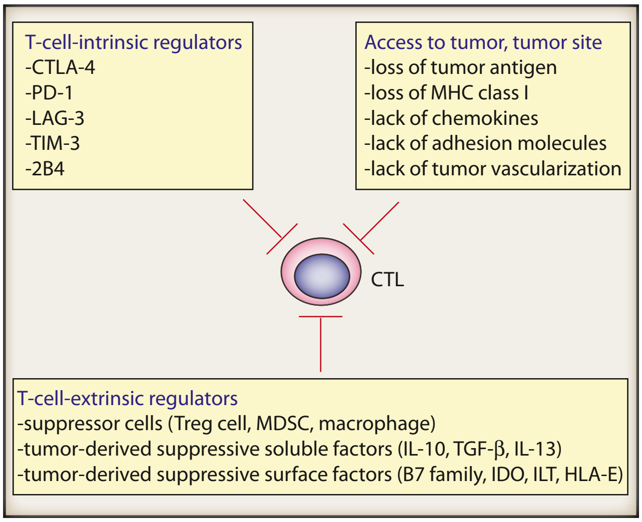

Figure 3. The Barriers for CD8$^+$ T-Cell-Mediated Tumor Rejection
The next-generation vaccines must confront and address numerous barriers that CD8$^+$ T cells face, including (1) T cell access to the tumor site, (2) T-cell-intrinsic regulators, e.g., CD28-CTLA-4 and PD1-PDL1, and (3) T-cell-extrinsic regulators such as suppressor cells (Treg cells, MDSCs, or protumor macrophages), tumor-secreted suppressive factors (including IL-10), and suppressive surface molecules (including coinhibitory molecules from the B7 family).

made into polyvalent vaccines targeting distinct yet specific DC subsets to trigger an ideal composite anti-cancer immune response. Such vaccines will also carry DC activators and immunomodulatory molecules to neutralize inhibitory signals, e.g., anti-PDL-1. This will keep us busy for a while.

Conclusions

We have come a long way since the first clinical trial with ex vivo DCs was launched in 1996 (Hsu et al., 1996) with regard to our understanding of the main problem: what is needed to elicit therapeutic immunity when cancer escapes the natural barrier of protective immunity. The considerable progress made in the understanding of the biology of DCs and effector and Treg cells has opened avenues for the development of new vaccine strategies. Progresses in ‘‘omics’’ will enable linking genetic alterations with the type of immune response. Novel protocols will be tailored to the individual-specific mutations (Schreiber et al., 2011) and immune alterations the affected individuals display. Thus, there has never been a more exciting time for working on cancer vaccines.

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