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Dendritic cells in cancer immunotherapy: vaccines or autologous transplants?

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Abstract

Dendritic cells (DCs) are the most powerful immunostimulatory cells specialized in the induction and regulation of immune responses. Their properties and the feasibility of their large-scale ex vivo generation led to the application of ex vivo-educated DCs to bypass the dysfunction of endogenous DCs in cancer patients and to induce therapeutic anti-cancer immunity. While multiple paradigms of therapeutic application of DCs reflect their consideration as cancer “vaccines”, numerous features of DC-based vaccination resemble those of autologous transplants, resulting in challenges and opportunities that distinguish them from classical vaccines. In addition to the functional heterogeneity of DC subsets and plasticity of the individual DC types, the unique features of DCs are the kinetic character of their function, limited functional stability, and the possibility to imprint in maturing DCs distinct functions relevant for the induction of effective cancer immunity, such as the induction of different effector functions or different homing properties of tumor-specific T cells (delivery of “signal 3” and “signal 4”). These considerations highlight the importance of the application of optimized, potentially patient-specific conditions of ex vivo culture of DCs and their delivery, with the logistic and regulatory implications shared with transplantation and other surgical procedures.

Keywords

Dendritic cells; IL-12; Immunotherapy; Cancer; Vaccines; Cytokines; Chemokines; Chemokine receptors; Th1; CTL; NK; Treg

DCs and cancer immunotherapy

Despite advances in cancer prevention and therapy, resulting in the recent decrease in cancer-related death rates, cancer remains a leading cause of mortality in the developed world [1]. Many groups of cancer patients and especially patients with advanced disease lack not only curative therapies, but also treatment options that offer significant impact upon cancer progression and symptom palliation. Standard therapies are effective in eliminating tumor bulk, but often lack the capacity to eliminate residual cancer cells and to prevent cancer recurrence. This particular deficit provides the rationale to include the means to mobilize the antitumor functions of immune system that specializes in eliminating rare “non-self” cells, in comprehensive cancer care. Following the original demonstration by William B. Coley that the immune system can be mobilized to fight established cancer [2, 3], massive research efforts of the past century helped to develop an understanding of the basic paradigms of immune recognition and elimination of tumor cells, facilitating the clinical introduction of multiple forms of cancer immunotherapy. Within the last 12 months alone, three novel therapies, Sipuleucel-T (Provenge; Dendreon), a cellular product involving antigen-loaded DCs and T cells [4–10], and Ipilimumab (Bristol-Myers Squibb),

an antibody blocking the CTLA-4-mediated suppressive events [11], as well as pegylated IFN α (peginterferon alfa-2b, Sylatron; Schering) have been approved by the FDA for the treatment of advanced prostate cancer and melanoma, reflecting the increasing contribution of immunotherapy to cancer management [11, 12].

In contrast to non-specific immunotherapies (adjuvants, cytokines, and checkpoint blockade) that activate multiple cell types (tumor, stroma, and different subsets of immune cells), therapeutic cancer vaccines attempt to instruct selected tumor-specific immune system to kill cancer cells. This narrow focus offers unique advantages of low toxicity and prolonged effects (immunosurveillance), due to the selective activation of relatively few, but long-lived tumor-specific memory cells. While most types of vaccines, involving antigenic peptides, proteins or genetically modified tumor cells or viruses, all depend on antigen cross-presentation by patients' endogenous DCs, the dysfunction of DCs in cancer patients [13–18] suggested the use of ex vivo-generated DCs as superior inducers of immune response [19]. DCs, discovered by Ralph M. Steinman in 1970s [20–23], are antigen-presenting cells uniquely specialized in inducing primary immune responses, supporting survival and effector functions of previously primed T cells, as well as mediating effective communication between other components of the immune system [24, 25]. Importantly for their clinical application, human DCs can be generated in large numbers from blood [26, 27] or bone marrow [28] progenitors. Since, in contrast to endogenous DCs in cancer patients that develop in the presence of tumor-associated suppressive factors [13–18], mature DCs acquire significant resistance to suppression [29–31], ex vivo-generated functional DCs have been introduced as immunization tools.

Despite the early promise of DCs in inducing antitumor responses and delaying cancer progression [32–35], the overall effectiveness of the currently available DC vaccines (and other therapeutic vaccines against cancer) remains dramatically lower than the effectiveness of protective vaccines against infectious agents and tumors caused by such agents [12, 36–43]. In particular, despite the ability of therapeutic vaccines to stabilize cancer progression and/or prolong patients' survival [5, 41, 44–46], their effectiveness in inducing regression of bulky tumors, as measured by RECIST or WHO criteria, remains low [5, 33, 40, 44, 45, 47].

Limits of the vaccine paradigm

Protection versus therapy

Comparison of the goals and challenges facing the therapeutic use of DCs and the traditional use of protective vaccines reveals significant differences. While some goals of “therapeutic vaccines” are shared with protective vaccines (for example the need to induce high numbers of T cells specific against unique antigens: delivery of “signal 1” and “signal 2” [12, 36, 47–49]), several aspects of vaccination in therapeutic settings pose additional challenges in patients with advanced cancer (Figs. 1, 2): In contrast to recall responses to tissue-invading microorganisms, the vaccination-induced T cells of cancer patients are not exposed to pro-inflammatory alarm signals from infected tissues and innate immune cells, known to facilitate the development of effector functions and attracting effector cells to the sites of pathogen entry. This introduces more stringent requirements for therapeutic vaccines, which in addition to driving the expansion of cancer-specific T cells, also need to substitute for the missing pathogenic challenge and acute inflammatory response which are critical to promote T-cell cytolytic effector functions and their acquisition of peripheral homing function [50–56]. In addition, in contrast to viral and bacterial infections that act as sources of the effector cell-attracting chemokines at the sites of pathogen entry [48, 54, 57], therapeutic vaccines either need to induce T cells that respond to the chemokines spontaneously expressed by tumors (that themselves use chemokines for growth, metastatic spread, and survival [58–

63]), or be combined with additional factors able of modulating tumor-associated chemokine environments.

Furthermore, therapeutic cancer vaccines need to function in the presence of an established tumor mass and tumor-induced immune dysfunction, associated with the expansion and hyperactivation of regulatory T cells (T_{reg}) and myeloid-derived suppressor cells (MDSC) [64–67]. Patients with melanoma, ovarian-, breast-, renal, prostate-, lung- and head-and-neck-cancer also show profound dysfunction of endogenous DCs and other antigen-presenting cells (APCs), and overproduction of the factors that suppress the functions of endogenous or adoptively transferred DCs: IL-10, TGF- β , VEGF, IL-6, and COX2 products (such as PGE₂) [13–18, 49, 68–77]. In contrast to the developing DCs, fully mature DCs acquire significant resistance to such inhibitory factors [29–31], providing the rationale for the ex vivo generation of DCs for the therapy of cancer patients. Unfortunately, while pre-existing T_{regs} are known to limit the effectiveness of cancer vaccines [66, 78], T_{reg} numbers can be even further expanded by the some of the previously tested DC vaccines [67, 79].

The above data raise concerns whether the traditional paradigms developed based on experience with protective vaccines are indeed relevant or sufficient to the development of therapeutic vaccines against cancer.

DCs versus other immunogens

In addition to the above challenges related to their application in therapeutic settings, dendritic cells also differ from traditional vaccines with regard to their physical character. The use of autologous live cells as a source of immunogenic signals offers several distinct advantages compared with such stabilized forms of antigens as proteins, peptides or even genetically modified viruses or tumor cells. The main advantage of DC therapy is that while all the latter immunogens rely on their cross-presentation by endogenous APCs, ex vivo generation of the DCs allows avoiding endogenous DC dysfunction. Direct presentation of antigen by the ex vivo-generated DCs also facilitates the delivery to T cells of the most desirable signals that can be imprinted in DCs by the conditions of their development and maturation (*see below*). In addition, the ex vivo loading of tumor-associated antigens facilitates their selective delivery to DCs and limits their presentation by non-professional APCs or tumor-affected endogenous DCs, both of which can be tolerogenic.

At the same time, immunization with live cells needs to take into account the limited stability of their functions over time [29, 80–82], their limited life span in vivo and the possibility of their elimination by pre-existing effector T cells (induced by the growing tumors or active immunization) [83–88]. Since DC immunization aims to deliver not only antigen, but also additional information required by T cells (*see below*), this form of therapy makes sense mostly if the original DCs (rather than antigens released from DCs) are able to contact the lymph node-based T cells.

Effectiveness of delivery to the lymph nodes and DC dosage

The limited functional life span of migrating DCs and the eventual “exhaustion” of their cytokine-producing function [29, 80–82] suggest the importance of the route of DC delivery, which should allow their entry into lymph nodes within short period of time after injection. Unfortunately, the efficacy of the most frequently used routes of DC delivery (i.d. or s.c) allows only 0.1–2% of injected DCs to ever reach the vaccine-draining LNs, with the majority of the DCs reaching the lymph nodes only after the 24–48 h [89–92]. While the traditional solution to this problem has been the administration of high numbers of DCs ($3 \times [106]$ to $30 \times [106]$, and sometimes exceeding $100 \times [106]$ cells per injection), such an approach cannot compensate for the delayed arrival of DCs to the nodes, at time points

known to be associated with significant reduction of DCs ability to produce Th1 cell- and CTL-activating cytokines (DC “exhaustion”) [29, 80–82]. Moreover, administration of large numbers of DCs increases the chances of the uptake of cancer-associated antigen released from DCs by endogenous, cancer-suppressed DCs and other non-professional APCs.

Another consideration is an identification of an optimal dose of DCs for effective immunization. The method of long-term cannulation of human afferent lymphatic vessels [93–95] allowed demonstrating that the development of even such powerful responses as contact allergy [96–98] involves no more than several hundred thousand DCs per day [97]. While for most of cancer therapeutic agents, the higher dose (close to the maximal tolerated dose; MTD) is presumed to be better, resulting in the typical pattern of phase 1 studies that aim to identify the MTD, the same assumption may not be valid for DCs, due to potential phenomena of high-dose tolerance [99, 100] and undesirable cross-presentation of DC-carried antigens by endogenous cells.

Duration of DC delivery and their persistence in lymph nodes

The induction of allergic reaction to DNCB is associated with the continued lymph flow of antigen-containing endogenous DCs for at least 22 h after antigen application (although no longer at 51 h) [97]. Since activated T cells require prolonged TCR occupancy to assure effective T-cell activation and development of memory and effector functions [101–106], and effective preventive vaccines are known to require prolonged antigen release (depot effect) [107, 108], the effectiveness of DC vaccines is also likely to depend on the prolonged presence of DCs in the lymph nodes. In contrast to classical vaccines (peptides, proteins), which release the antigens in a prolonged fashion, the stability of DC-expressed peptide/MHC complexes, as well as the functional stability of DCs and their overall survival, are limited. These considerations suggest that in contrast to typical vaccines, the immunogenic activity of antigen-loaded DC may benefit from their repetitive administration over each course of immunization and from their protection from exhaustion and apoptosis. In line with this possibility, mouse studies have documented that transfection with antiapoptotic genes [109], inhibitors of pro-apoptotic genes [110, 111], or survival factors such as GM-CSF, all increase the ability of adoptively transferred DCs to induce therapeutic anti-tumor immunity [112], while additional advantage can be offered by extending the duration of antigen persistence within DCs [113].

Autologous cells versus off-the-shelf vaccines: Logistic and regulatory issues

Typical vaccines, even vaccines containing live organisms (modified viruses or bacteria) can be mass-produced and effectively preserved, allowing their prolonged shelf life and widespread distribution. The use of standardized resources and uniform operating procedures (SOPs) allow high batch-to-batch uniformity.

In contrast, several aspects of production and administration of DCs resemble the steps involved in bone marrow- or pancreatic islets transplantation, or blood transfusion. Since the process starts with a patient-specific population of precursor cells, even strict adherence to SOPs during DC generation cannot guarantee the uniform quality of different batches of DCs, nor their uniform numbers, which may be intrinsically different between patients.

These considerations raise the possibility that the optimal quality of the cellular product for each individual patient may benefit from the use of flexible or alternative SOPs, which would allow adjustment of the process of DC generation (for example the duration of DC maturation or its specific conditions), based on the quality of the precursor cells, interim analysis of the product, or a small-scale test run to identify the most-effective option. Furthermore, the resulting cellular product shows relatively limited stability, and while it

can be cryopreserved, its final processing and administration needs to take into account the changing functions of DCs prior to and after administration to patients, and the need to achieve the DC-T-cell contact within short period of time following injection, resulting in the importance of the mode of DC application. Therefore, taking autologous DC therapies to the level of “personalized” medicine, based on patient-specific cellular makers, may allow them to fully realize their potential.

From the regulatory standpoint, these considerations raise the question whether DC therapies are a vaccine product or a medical procedure. While the complexity of cellular manipulation of DCs is higher compared with bone marrow transplant, cellular therapies with DCs do not involve the risks associated with myeloablative conditioning regimens, and as autologous cells, have significantly lower risks and minimal or absent toxicity.

Making the most of DCs: exploiting signal 1, signal 2, signal 3, and signal 4

Generation of DCs ex vivo allows their loading with the desirable antigens, in order to assure effective delivery of “signal one” and “signal two” (antigen and costimulation) [114–116] to tumor-specific T cells (Fig. 2). It also allows imprinting additional DC features important for effective cancer immunity, such as their ability to preferentially interact with selected subsets of immune cells (effector rather than regulatory), induce desirable effector mechanisms (delivery of “signal three”) [25] to selectively enhance the Th1-, CTL- and NK cell-mediated type-1 immunity [25, 114–123], desirable in cancer [124], and to enhance the tumor-relevant homing properties of activated T cells (delivery of “signal four”) [125].

Delivery of antigen (signal 1)

DCs are specialized in cross-presentation of different forms of antigen, including whole tumor cells [49, 126–128]. These properties allow the use of not only peptides or recombinant proteins, but also allogeneic or autologous tumor material as the source of DC-cross-presented antigen [129], allowing for application of DCs against tumors with undefined tumor-rejection antigens and to immunize against unique patient-specific antigens. However, the ability to cross-present antigens differs between different DC subsets, stages of DC development and maturation, and is affected by the conditions of DC activation/maturation [130–133]. While the early stages of DC maturation are in general believed to be optimal for antigen uptake and its cross-presentation [27, 134–136], the effectiveness of tumor cell cross-presentation can be significantly affected by the selection of factors that are used in the maturation of DCs [137].

Costimulatory molecules (signal 2) and lymph node-homing: Role of DC maturation

Effective induction of anti-tumor CTL responses requires mature DCs that express high levels of costimulatory molecules and which can migrate in response to CCL19 or CCL21, the lymph node-produced CCR7 ligands [89, 90, 138, 139]. In order to overcome the limitations of immature or partially mature DCs (constituting the “first generation” of DC vaccines), numerous protocols were developed to induce fully mature DCs for clinical use. Initially, two modalities involving PGE₂: monocyte-conditioned medium [140, 141] and a cytokine cocktail involving IL-1 β , TNF α , IL-6, and PGE₂ [142] were used to induce mature DCs with high expression of co-stimulatory molecules and CCR7 (and the high migratory responsiveness to lymph-node-produced chemokines CCL19 and CCL21) [143, 144]. The IL-1 β /TNF α /IL-6/PGE₂-matured DCs [142] showed enhanced immunogenicity in vitro and in vivo in healthy volunteers, [90, 138] as well as improved migratory responses to LN-associated chemokines, when compared with immature DCs [89, 143, 144], and have been tested in numerous clinical trials. Unexpectedly, their randomized comparison with dacarbazine in a III trial in advanced melanoma showed very limited ability to induce

clinical responses (less than 5%) and the lack of detectable impact on patients' survival [145]. While additional factors, such as the preparation of DC in different laboratories, might have negatively affected the results of this multi-center trial, the negative impact of PGE₂ on the production of IL-12p70 [30, 31, 146, 147] (central to the induction and survival of type-1 immune cells) [120] is a possible culprit. These clinical data highlight the functional importance of the ability of DCs to produce IL-12p70, which has been demonstrated to be the best indicator of anti-tumor potency of DCs in vitro [148, 149] and in vivo [46] identified to date.

Induction of anti-tumor effector functions (signal 3)

The character of immune response (its Th1 dominance and avoidance of Treg activation), rather than its magnitude, has been shown to predict therapeutic activity of vaccination in mouse [124]. In accordance with these data, high secretion of IL-12p70 has been shown to strongly enhance DCs ability to induce tumor-specific Th1 cells and CTLs, and to promote tumor rejection in therapeutic models [150–160]. These murine model data have been more recently supported by human preclinical and clinical studies, demonstrating that the ability of antigen-loaded DCs to produce IL-12p70 is the predictive indicator of their ability to induce tumor-specific CTLs in vitro [148, 149] and clinical benefits in vivo [46].

Unfortunately, the “second-generation” DCs, matured in the presence of an IL-1 β /TNF α /IL-6/PGE₂-containing cytokine cocktail [142], showed a reduced ability to produce bioactive IL-12p70, and the phenomenon is referred to as DC “exhaustion” [29, 80, 161]. In an attempt to boost the clinical efficacy of cancer vaccines, we and other groups have demonstrated the feasibility of inducing “non-exhausted” mature DCs, by exposing immature DCs to type-1 and type 2 interferons and TLR ligands, or alternatively, to IL-18-activated NK cells or memory-type CD8⁺ T cells [30, 161–168]. The resulting “type-1 polarized” DCs (DC1) show a strongly enhanced capacity to induce long-lived tumor-specific T cells with pronounced anti-tumor effector functions in human in vitro and mouse in vivo models, as well as enhancement of tumoricidal functions in resting NK cells. Our initial studies [30, 161] and the data from other laboratories [166, 169] showed that the combination of IFN- γ with LPS (or its clinically compatible form, MPLA), or with TNF α and IL-1 β can overcome the maturation-associated DC “exhaustion”, resulting in polarized DC1s that produce elevated levels of IL-12p70 upon interaction with CD40L-expressing CD4⁺ Th cells and induce stronger Th1 and CTL responses [30, 166]. The additional presence of IFN α and polyinosinic:polycytidylic acid (poly-I:C; TLR3 ligand) in the maturation-inducing cocktail, further enhances the ability of maturing DC1s to express CCR7 [161], and instructs them to preferentially interact with naïve, memory, and effector T cells, rather than with the undesirable T_{regs} [147] (*see below*). In accordance with the ability of polarized DCs to induce qualitatively improved immune responses, “alpha-type-1-polarized DCs” (α DC1s) induce on average 20-fold higher numbers of long-lived functional melanoma-specific CTLs in a single round of in vitro sensitization [161] when compared with the DCs matured by IL-1 β /TNF α /IL-6/PGE₂ [142]. So far, our data from melanoma [161], CLL [170], prostate [171], glioblastoma [46] and several other cancers uniformly demonstrate the feasibility of generating type-1-polarized DCs from patients with advanced cancer, their loading with peptide antigens [46, 161] or apoptotic tumor cells [137, 170, 171] and their effectiveness in inducing tumor-specific CTLs. In addition, it was recently demonstrated that α DC1s also show superior activity in activating NK cells [172]. While our own work focused on IFN α -supported DC1s (α DC1s) [161, 167] and DC1s induced by autologous NK cells or memory CD8⁺ T cells [164, 165, 168], several other groups [166, 167, 169] showed the feasibility of generating clinical-grade DC1s using the combination of IFN- γ with MPLA, a “detoxified” form of LPS [30, 166, 167, 169] and on alternative ways of enhancing the desirable properties of DCs (that could be combined with DC1

polarization), such as the use of IL-15 (instead of IL-4) to promote early DC development [173], B7-DC-cross-linking [174], inhibition of p38MAPK [175, 176] or genetic manipulation of DCs to over-express *t-bet*.

While polarized and non-polarized DCs both effectively induce the expansion of naïve CD8⁺ T cells and their CD45RA to CD45RO conversion, polarized DC1s show advantage in inducing T-cell expression of granzyme B and perforin, and their cytolytic activity against tumor targets. The advantage of DC1s in inducing qualitatively superior CTLs was observed both in the case of polyclonally activated naïve cells, and recall responses to tumor-specific antigens (such as MART-1), but DC1 involvement was particularly important for naïve cells, suggesting their key role in the de novo CTL induction, rather than selection of the previously induced CTLs.

Cumulatively, these data suggest that the effectiveness of DCs as inducers of antitumor responses can be modulated by the factors regulating their ability to produce IL-12p70 (and possibly other Th1-, CTL- and NK cell-activating cytokines). We are currently evaluating this hypothesis in phase I/II trials in patients with cutaneous T-cell lymphoma, glioma, colon and prostate cancers, as well as melanoma (respectively, NCT00099593, NCT00766753, NCT0055 8051, NCT00970203, and NCT00390338). The recently completed phase I/II trial in patients with the recurrent high-grade malignant glioma demonstrated the ability to prolong the progression free survival (PFS) to at least 12 months (compared with the expected PFS of 3–4 months for this patient group) in 9 of 22 patients [46, 82, 177]. Radiological tumor shrinkage was observed in two of these patients. Importantly, the ability of the individual αDC1 vaccines to produce IL-12p70 was the best predictive marker of the prolonged PSF in the individual patients [46].

Induction of tumor-homing properties in tumor-specific T cells (signal 4)

While the activation of naïve T cells is generally considered to be associated with the acquisition of their ability to home peripheral tissues, T-cell activation by different types of DCs has been shown to be associated with the induction of their different homing patterns in mouse models [178–184]. Importantly for the application of human differentially matured DCs in cancer immunotherapy, the MART-1_{27–35}-specific CD8⁺ T cells from HLA-A2⁺ melanoma patients sensitized by polarized DC1 showed elevated levels of CCR5 (receptors for CCR1, CCR2, and CCR5) and CXCR3 (receptor for CXCL9, CXCL10 and CXCL11), the peripheral tissue-type chemokine receptors involved in the T-cell entry into melanomas and other tumors [59, 82, 185–188], compared with the cells sensitized by and nonpolarized DCs.

Programming the DCs to interact with desirable types of immune cells

Several recent trials demonstrated that standard DC vaccines may promote the undesirable expansion of T_{reg} cells in cancer patients [67, 79, 82, 189–191], raising the question whether the pattern of interaction of DCs with T_{regs} (and resulting T_{reg} activation) can be modulated independently from the DC interaction with naïve, central memory, and effector cells.

Consistent with such possibility, the elimination of PGE₂ and inclusion of IFNα in the DC-maturation cocktails is the enhanced production of CXCL9, CXCL10, CXCL11, and CCL5 by resulting mature DCs and their decreased production of CCL22, promoting their interaction with the desirable, CXCR3- and CCR5-expressing CTLs, Th1, and NK cells, and to avoid the attraction of Tregs and other suppressor cells known to express CCR4 (receptor for CCL22) [92, 147, 172]. The observed stability of such maturation-induced differences in chemokine profiles of DCs [92, 147] suggests that the differentially matured DCs can be used to selectively expand these subsets and/or support their functions.

Promoting rapid delivery of DCs to the lymph nodes: Immunotherapy in surgical context

Accelerated delivery of DCs to T-cell-containing lymph nodes may enhance their immunologic and clinical activity, by limiting the time-associated exhaustion of their IL-12p70-producing function. Ultrasound-guided intranodal injection of DCs represents the most direct way of promoting rapid contact of DCs with the lymph node-based T cells and has been used in multiple clinical trials. Unfortunately, as showed by Jolanda de Vries and colleagues, even highly trained radiologists can miss the lymph node or deliver the DCs superficially, resulting in their expulsion from the lymph node and peri-nodal localization in up to 40% of injections, limiting the effectiveness of this procedure [192]. The direct intranodal route also makes it difficult to (re)administer multiple doses of DCs, due to the logistical limitations (need for a trained radiologist for each injection) and progressive structural damage to the targeted lymph nodes.

Driven by past utilization of intralymphatic injections as a way to deliver DCs [166, 193–197] and other immunomodulators [198–200], and the feasibility of long-term lymphatic annulations to harvest lymph-born DCs [93–98], we tested the feasibility of prolonged semi-continuous intralymphatic delivery of antigen-loaded DCs in two clinical trials in colorectal cancer and melanoma. This method has been successfully applied in over 20 cancer patients, allowing reliable delivery of multiple doses of low numbers of antigen-loaded DCs (12 injections per course; 25,000 or 250,000 DCs per injection) over a 4-day-long course of vaccination. The possibility of repetitively administering DCs directly into the lymphatics over prolonged periods of time obviates many of the logistic and biologic problems associated with the direct intranodal injections. It also allows for the delivery of DCs in combination with factors that regulate their *in vivo* production of different classes of T-cell-activating, T-cell-attracting and T-cell-regulating factors.

Vaccination or autologous transplantation?

The biology of DCs, their adaptability to environmental conditions, and the “kinetic” character of their functions create unique opportunities as well as logistic and regulatory challenges, differentiating DC therapies from other forms of immunization. In contrast to standardized off-the-shelf vaccines, the therapeutic application of DCs needs to accommodate the intrinsic differences of the cells generated from different patients, the changing functions of DCs prior to and after their administration, and the need to assure the ability of DCs to interact with T cells within a short period of time following injection. On the other hand, the use of *ex vivo*-matured DCs uniquely allows to bypass the dysfunction of the endogenous APCs in cancer-bearing patients and deliver to the lymph nodes additional pro-inflammatory signals, (“signal 3” and “signal 4”) typically provided by infectious agents themselves but missing in the settings of cancer. These considerations highlight the analogy to several aspects of transplantation, similarly aiming to restore lost functionality of an organ or system, and implicate that a personalized approach to DC therapies may help to fully realize their therapeutic potential.

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References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011; 61(2):69–90. [PubMed: 21296855]

2. Coley WB. The treatment of inoperable sarcoma by bacterial toxins (the mixed toxins of the *Streptococcus erysipelas* and the *Bacillus prodigiosus*). *Proc R Soc Med*. 1910; 3:1–48. *Surg Sectc*. [PubMed: 19974799]
3. Nauts HC, Swift WE, Coley BL. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., reviewed in the light of modern research. *Cancer Res*. 1946; 6:205–16. [PubMed: 21018724]
4. Wu Y, Rosenberg JE, Taplin ME. Novel agents and new therapeutics in castration-resistant prostate cancer. *Curr Opin Oncol*. 2011; 23(3):290–6. [PubMed: 21311328]
5. Small EJ, Schellhammer PF, Higano CS, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J Clin Oncol*. 2006; 24(19):3089–94. [PubMed: 16809734]
6. Harzstark AL, Small EJ. Immunotherapy for prostate cancer using antigen-loaded antigen-presenting cells: APC8015 (Provenge). *Expert Opin Biol Ther*. 2007; 7(8):1275–80. [PubMed: 17696825]
7. Rethinking therapeutic cancer vaccines. *Nat Rev Drug Discov*. 2009; 8(9):685–6. [PubMed: 19721436]
8. Drake CG. Immunotherapy for prostate cancer: walk, don't run. *J Clin Oncol*. 2009; 27(25):4035–7. [PubMed: 19635998]
9. Higano CS, Schellhammer PF, Small EJ, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer*. 2009; 115(16):3670–9. [PubMed: 19536890]
10. Lassi K, Dawson NA. Emerging therapies in castrate-resistant prostate cancer. *Curr Opin Oncol*. 2009; 21(3):260–5. [PubMed: 19363343]
11. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. *Nat Rev Drug Discov*. 2010; 9(10):767–74. [PubMed: 20811384]
12. Schlom J, Arlen PM, Gulley JL. Cancer vaccines: moving beyond current paradigms. *Clin Cancer Res*. 2007; 13(13):3776–82. [PubMed: 17606707]
13. Bell D, Chomarat P, Broyles D, et al. In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. *J Exp Med*. 1999; 190(10):1417–26. [PubMed: 10562317]
14. Toriyama K, Wen DR, Paul E, Cochran AJ. Variations in the distribution, frequency, and phenotype of Langerhans cells during the evolution of malignant melanoma of the skin. *J Invest Dermatol*. 1993; 100(3):269S–73S. [PubMed: 7680054]
15. Troy AJ, Summers KL, Davidson PJ, Atkinson CH, Hart DN. Minimal recruitment and activation of dendritic cells within renal cell carcinoma. *Clin Cancer Res*. 1998; 4(3):585–93. [PubMed: 9533525]
16. Zou W, Machelon V, Coulomb-L'Hermin A, et al. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med*. 2001; 7(12):1339–46. [PubMed: 11726975]
17. Almand B, Resser JR, Lindman B, et al. Clinical significance of defective dendritic cell differentiation in cancer. *Clin Cancer Res*. 2000; 6(5):1755–66. [PubMed: 10815894]
18. Della Bella S, Gennaro M, Vaccari M, et al. Altered maturation of peripheral blood dendritic cells in patients with breast cancer. *Br J Cancer*. 2003; 89(8):1463–72. [PubMed: 14562018]
19. Shimizu K, Kurosawa Y, Taniguchi M, Steinman RM, Fujii S. Cross-presentation of glycolipid from tumor cells loaded with alpha-galactosylceramide leads to potent and long-lived T cell mediated immunity via dendritic cells. *J Exp Med*. 2007; 204(11):2641–53. [PubMed: 17923500]
20. Steinman RM, Adams JC, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice IV. Identification and distribution in mouse spleen. *J Exp Med*. 1975; 141(4):804–20. [PubMed: 1127378]
21. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med*. 1973; 137(5):1142–62. [PubMed: 4573839]
22. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J Exp Med*. 1974; 139(2):380–97. [PubMed: 4589990]

23. Steinman RM, Lustig DS, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties in vivo. *J Exp Med.* 1974; 139(6):1431–45. [PubMed: 4598015]
24. Fujii S, Shimizu K, Hemmi H, et al. Glycolipid alpha-C-galactosylceramide is a distinct inducer of dendritic cell function during innate and adaptive immune responses of mice. *Proc Natl Acad Sci USA.* 2006; 103(30):11252–7. [PubMed: 16844772]
25. Kalinski P, Hilkens CM, Wierenga EA, Kapsenberg ML. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol Today.* 1999; 20(12):561–7. [PubMed: 10562707]
26. Markowicz S, Engleman EG. Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. *J Clin Invest.* 1990; 85(3):955–61. [PubMed: 2179270]
27. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med.* 1994; 179(4):1109–18. [PubMed: 8145033]
28. Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. *Nature.* 1992; 360(6401):258–61. [PubMed: 1279441]
29. Kalinski P, Schuitemaker JH, Hilkens CM, Wierenga EA, Kapsenberg ML. Final maturation of dendritic cells is associated with impaired responsiveness to IFN-gamma and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. *J Immunol.* 1999; 162(6):3231–6. [PubMed: 10092774]
30. Vieira PL, de Jong EC, Wierenga EA, Kapsenberg ML, Kalinski P. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J Immunol.* 2000; 164(9):4507–12. [PubMed: 10779751]
31. Kalinski P, Schuitemaker JH, Hilkens CM, Kapsenberg ML. Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a + CD83 + dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J Immunol.* 1998; 161(6):2804–9. [PubMed: 9743339]
32. Nestle FO, Alijagic S, Gilliet M, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med.* 1998; 4(3):328–32. [PubMed: 9500607]
33. Nestle FO, Farkas A, Conrad C. Dendritic-cell-based therapeutic vaccination against cancer. *Curr Opin Immunol.* 2005; 17(2):163–9. [PubMed: 15766676]
34. Engleman EG. Dendritic cells in the treatment of cancer. *Biol Blood Marrow Transplant.* 1996; 2(3):115–7. [PubMed: 9199753]
35. Hsu FJ, Benike C, Fagnoni F, et al. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat Med.* 1996; 2(1):52–8. [PubMed: 8564842]
36. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol.* 2005; 5(4):296–306. [PubMed: 15803149]
37. Blattman JN, Greenberg PD. Cancer immunotherapy: a treatment for the masses. *Science.* 2004; 305(5681):200–5. [PubMed: 15247469]
38. Figdor CG, de Vries IJ, Lesterhuis WJ, Melief CJ. Dendritic cell immunotherapy: mapping the way. *Nat Med.* 2004; 10(5):475–80. [PubMed: 15122249]
39. Gilboa E. The promise of cancer vaccines. *Nat Rev Cancer.* 2004; 4(5):401–11. [PubMed: 15122211]
40. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med.* 2004; 10(9):909–15. [PubMed: 15340416]
41. Srivastava PK. Therapeutic cancer vaccines. *Curr Opin Immunol.* 2006; 18(2):201–5. [PubMed: 16464565]
42. Schlom J, Gulley JL, Arlen PM. Paradigm shifts in cancer vaccine therapy. *Exp Biol Med (Maywood).* 2008; 233(5):522–34. [PubMed: 18375829]
43. Patel PH, Kockler DR. Sipuleucel-T: a vaccine for metastatic, asymptomatic, androgen-independent prostate cancer. *Ann Pharmacother.* 2008; 42(1):91–8. [PubMed: 18094343]

44. Pilla L, Patuzzo R, Rivoltini L, et al. A phase II trial of vaccination with autologous, tumor-derived heat-shock protein peptide complexes Gp96, in combination with GM-CSF and interferon-alpha in metastatic melanoma patients. *Cancer Immunol Immunother.* 2006; 55(8):958–68. [PubMed: 16215718]
45. Peoples GE, Holmes JP, Hueman MT, et al. Combined clinical trial results of a HER2/neu (E75) vaccine for the prevention of recurrence in high-risk breast cancer patients: U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02. *Clin Cancer Res.* 2008; 14(3):797–803. [PubMed: 18245541]
46. Okada H, Kalinski P, Ueda R, et al. Induction of CD8 + T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. *J Clin Oncol.* 2011; 29(3):330–6. [PubMed: 21149657]
47. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature.* 2007; 449(7161):419–26. [PubMed: 17898760]
48. Mantovani A, Romero P, Palucka AK, Marincola FM. Tumour immunity: effector response to tumour and role of the microenvironment. *Lancet.* 2008; 371(9614):771–83. [PubMed: 18275997]
49. Melief CJ. Cancer immunotherapy by dendritic cells. *Immunity.* 2008; 29(3):372–83. [PubMed: 18799145]
50. Pearce EL, Shen H. Making sense of inflammation, epigenetics, and memory CD8 + T-cell differentiation in the context of infection. *Immunol Rev.* 2006; 211:197–202. [PubMed: 16824128]
51. Cooper AM, Khader SA. The role of cytokines in the initiation, expansion, and control of cellular immunity to tuberculosis. *Immunol Rev.* 2008; 226:191–204. [PubMed: 19161425]
52. Harty JT, Badovinac VP. Influence of effector molecules on the CD8(+) T cell response to infection. *Curr Opin Immunol.* 2002; 14(3):360–5. [PubMed: 11973135]
53. van Leeuwen EM, Sprent J, Surh CD. Generation and maintenance of memory CD4(+) T Cells. *Curr Opin Immunol.* 2009; 21(2):167–72. [PubMed: 19282163]
54. Haring JS, Badovinac VP, Harty JT. Inflaming the CD8 + T cell response. *Immunity.* 2006; 25(1):19–29. [PubMed: 16860754]
55. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol.* 2004; 22:745–63. [PubMed: 15032595]
56. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol.* 2000; 18:593–620. [PubMed: 10837070]
57. Hartl D, Krauss-Etschmann S, Koller B, et al. Infiltrated neutrophils acquire novel chemokine receptor expression and chemokine responsiveness in chronic inflammatory lung diseases. *J Immunol.* 2008; 181(11):8053–67. [PubMed: 19017998]
58. Mantovani A, Allavena P, Sozzani S, Vecchi A, Locati M, Sica A. Chemokines in the recruitment and shaping of the leukocyte infiltrate of tumors. *Semin Cancer Biol.* 2004; 14(3):155–60. [PubMed: 15246050]
59. Mrowietz U, Schwenk U, Maune S, et al. The chemokine RANTES is secreted by human melanoma cells and is associated with enhanced tumour formation in nude mice. *Br J Cancer.* 1999; 79(7–8):1025–31. [PubMed: 10098731]
60. Payne AS, Cornelius LA. The role of chemokines in melanoma tumor growth and metastasis. *J Invest Dermatol.* 2002; 118(6):915–22. [PubMed: 12060384]
61. Bonfil RD, Chinni S, Fridman R, Kim HR, Cher ML. Proteases, growth factors, chemokines, and the microenvironment in prostate cancer bone metastasis. *Urol Oncol.* 2007; 25(5):407–11. [PubMed: 17826661]
62. Raman D, Baugher PJ, Thu YM, Richmond A. Role of chemokines in tumor growth. *Cancer Lett.* 2007; 256(2):137–65. [PubMed: 17629396]
63. Walser TC, Fulton AM. The role of chemokines in the biology and therapy of breast cancer. *Breast Dis.* 2004; 20:137–43. [PubMed: 15687714]

64. Zou W. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol.* 2006; 6(4): 295–307. [PubMed: 16557261]
65. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med.* 2004; 10(9):942–9. [PubMed: 15322536]
66. Vieweg J, Su Z, Dahm P, Kusmartsev S. Reversal of tumormediated immunosuppression. *Clin Cancer Res.* 2007; 13(2 Pt 2):727s–32s. [PubMed: 17255301]
67. Banerjee DK, Dhodapkar MV, Matayeva E, Steinman RM, Dhodapkar KM. Expansion of FOXP3^{high} regulatory T cells by human dendritic cells (DCs) in vitro and after injection of cytokine-matured DCs in myeloma patients. *Blood.* 2006; 108(8):2655–61. [PubMed: 16763205]
68. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol.* 2007; 25:267–96. [PubMed: 17134371]
69. Gabrilovich D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. *Nat Rev Immunol.* 2004; 4(12):941–52. [PubMed: 15573129]
70. Gabrilovich D, Pisarev V. Tumor escape from immune response: mechanisms and targets of activity. *Curr Drug Targets.* 2003; 4(7):525–36. [PubMed: 14535653]
71. Almand B, Clark JI, Nikitina E, et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J Immunol.* 2001; 166(1):678–89. [PubMed: 11123353]
72. Gabrilovich DI, Velders MP, Sotomayor EM, Kast WM. Mechanism of immune dysfunction in cancer mediated by immature Gr-1 + myeloid cells. *J Immunol.* 2001; 166(9):5398–406. [PubMed: 11313376]
73. Young MR, Wright MA, Lozano Y, et al. Increased recurrence and metastasis in patients whose primary head and neck squamous cell carcinomas secreted granulocyte-macrophage colonystimulating factor and contained CD34 + natural suppressor cells. *Int J Cancer.* 1997; 74(1): 69–74. [PubMed: 9036872]
74. Uchida K, Schneider S, Yochim JM, et al. Intratumoral COX-2 gene expression is a predictive factor for colorectal cancer response to fluoropyrimidine-based chemotherapy. *Clin Cancer Res.* 2005; 11(9):3363–8. [PubMed: 15867236]
75. Williams C, Shattuck-Brandt RL, DuBois RN. The role of COX-2 in intestinal cancer. *Ann NY Acad Sci.* 1999; 889:72–83. [PubMed: 10668484]
76. Inaba T, Sano H, Kawahito Y, et al. Induction of cyclooxygenase-2 in monocyte/macrophage by mucins secreted from colon cancer cells. *Proc Natl Acad Sci USA.* 2003; 100(5):2736–41. [PubMed: 12598658]
77. Soumaoro LT, Uetake H, Higuchi T, Takagi Y, Enomoto M, Sugihara K. Cyclooxygenase-2 expression: a significant prognostic indicator for patients with colorectal cancer. *Clin Cancer Res.* 2004; 10(24):8465–71. [PubMed: 15623626]
78. Dannull J, Su Z, Rizzieri D, et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. *J Clin Invest.* 2005; 115(12):3623–33. [PubMed: 16308572]
79. Yamazaki S, Inaba K, Tarbell KV, Steinman RM. Dendritic cells expand antigen-specific Foxp3 + CD25 + CD4 + regulatory T cells including suppressors of alloreactivity. *Immunol Rev.* 2006; 212:314–29. [PubMed: 16903923]
80. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat Immunol.* 2000; 1(4):311–6. [PubMed: 11017102]
81. Camporeale A, Boni A, Iezzi G, et al. Critical impact of the kinetics of dendritic cells activation on the in vivo induction of tumor-specific T lymphocytes. *Cancer Res.* 2003; 63(13):3688–94. [PubMed: 12839960]
82. Watchmaker P, Berk E, Muthuswamy R, et al. Independent regulation of chemokine responsiveness and cytolytic function versus CD8 + T cell expansion by dendritic cells. *J Immunol.* 2010; 184:591–7. [PubMed: 20018619]

83. Hermans IF, Ritchie DS, Yang J, Roberts JM, Ronchese F. CD8 + T cell-dependent elimination of dendritic cells in vivo limits the induction of antitumor immunity. *J Immunol.* 2000; 164(6):3095–101. [PubMed: 10706699]
84. Ronchese F, Hermans IF. Killing of dendritic cells: a life cut short or a purposeful death? *J Exp Med.* 2001; 194(5):F23–6. [PubMed: 11535638]
85. Yang J, Huck SP, McHugh RS, Hermans IF, Ronchese F. Perforin-dependent elimination of dendritic cells regulates the expansion of antigen-specific CD8 + T cells in vivo. *Proc Natl Acad Sci USA.* 2006; 103(1):147–52. [PubMed: 16373503]
86. Nakamura Y, Watchmaker P, Urban J, et al. Helper function of memory CD8 + T cells: heterologous CD8 + T cells support the induction of therapeutic cancer immunity. *Cancer Res.* 2007; 67(20):10012–8. [PubMed: 17942935]
87. Watchmaker P, Urban J, Berk E, et al. Memory CD8 + T cells protect dendritic cells from CTL killing. *J Immunol.* 2008; 180:3857–65. [PubMed: 18322193]
88. Guarda G, Hons M, Soriano SF, et al. L-selectin-negative CCR7-effector and memory CD8 + T cells enter reactive lymph nodes and kill dendritic cells. *Nat Immunol.* 2007; 8(7):743–52. [PubMed: 17529983]
89. De Vries IJ, Krooshoop DJ, Scharenborg NM, et al. Effective migration of antigen-pulsed dendritic cells to lymph nodes in melanoma patients is determined by their maturation state. *Cancer Res.* 2003; 63(1):12–7. [PubMed: 12517769]
90. de Vries IJ, Lesterhuis WJ, Scharenborg NM, et al. Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. *Clin Cancer Res.* 2003; 9(14):5091–100. [PubMed: 14613986]
91. Barratt-Boyes SM, Figdor CG. Current issues in delivering DCs for immunotherapy. *Cytotherapy.* 2004; 6(2):105–10. [PubMed: 15203986]
92. Muthuswamy R, Mueller-Berghaus J, Haberkorn U, Reinhart TA, Schadendorf D, Kalinski P. PGE(2) transiently enhances DC expression of CCR7 but inhibits the ability of DCs to produce CCL19 and attract naive T cells. *Blood.* 2010; 116(9):1454–9. [PubMed: 20498301]
93. Olszewski WL, Grzelak I, Ziolkowska A, Engeset A. Immune cell traffic from blood through the normal human skin to lymphatics. *Clin Dermatol.* 1995; 13(5):473–83. [PubMed: 8665458]
94. Galkowska H, Olszewski WL. Immune events in skin. I. Spontaneous cluster formation of dendritic (veiled) cells and lymphocytes from skin lymph. *Scand J Immunol.* 1992; 35(6):727–34. [PubMed: 1604244]
95. Yawalkar N, Brand CU, Braathen LR. IL-12 gene expression in human skin-derived CD1a + dendritic lymph cells. *Arch Dermatol Res.* 1996; 288(2):79–84. [PubMed: 8932585]
96. Brand CU, Yawalkar N, Hunziker T, Braathen LR. Human skin lymph derived from irritant and allergic contact dermatitis: interleukin 10 is increased selectively in elicitation reactions. *Dermatology.* 1997; 194(3):221–8. [PubMed: 9187837]
97. Hunger RE, Yawalkar N, Braathen LR, Brand CU. CD1a-positive dendritic cells transport the antigen DNCB intracellularly from the skin to the regional lymph nodes in the induction phase of allergic contact dermatitis. *Arch Dermatol Res.* 2001; 293(8):420–6. [PubMed: 11686518]
98. Yawalkar N, Brand CU, Braathen LR. Interleukin-12 expression in human afferent lymph derived from the induction phase of allergic contact dermatitis. *Br J Dermatol.* 1998; 138(2):297–300. [PubMed: 9602878]
99. LaSalle JM, Hafler DA. T cell anergy. *FASEB J.* 1994; 8(9):601–8. [PubMed: 8005388]
100. Miller JF, Kurts C, Allison J, Kosaka H, Carbone F, Heath WR. Induction of peripheral CD8 + T-cell tolerance by cross-presentation of self antigens. *Immunol Rev.* 1998; 165:267–77. [PubMed: 9850866]
101. Gett AV, Sallusto F, Lanzavecchia A, Geginat J. T cell fitness determined by signal strength. *Nat Immunol.* 2003; 4(4):355–60. [PubMed: 12640450]
102. Iezzi G, Karjalainen K, Lanzavecchia A. The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity.* 1998; 8(1):89–95. [PubMed: 9462514]
103. Iezzi G, Scotet E, Scheidegger D, Lanzavecchia A. The interplay between the duration of TCR and cytokine signaling determines T cell polarization. *Eur J Immunol.* 1999; 29(12):4092–101. [PubMed: 10602020]

104. Valitutti S, Dessing M, Aktories K, Gallati H, Lanzavecchia A. Sustained signaling leading to T cell activation results from prolonged T cell receptor occupancy. Role of T cell actin cytoskeleton. *J Exp Med*. 1995; 181(2):577–84. [PubMed: 7836913]
105. Obst R, van Santen HM, Mathis D, Benoist C. Antigen persistence is required throughout the expansion phase of a CD4(+) T cell response. *J Exp Med*. 2005; 201(10):1555–65. [PubMed: 15897273]
106. Jusforgues-Saklani H, Uhl M, Blachere N, et al. Antigen persistence is required for dendritic cell licensing and CD8 + T cell cross-priming. *J Immunol*. 2008; 181(5):3067–76. [PubMed: 18713977]
107. Schijns VE. Induction and direction of immune responses by vaccine adjuvants. *Crit Rev Immunol*. 2001; 21(1–3):75–85. [PubMed: 11642615]
108. Zhao Z, Leong KW. Controlled delivery of antigens and adjuvants in vaccine development. *J Pharm Sci*. 1996; 85(12):1261–70. [PubMed: 8961136]
109. Kim TW, Hung CF, Ling M, et al. Enhancing DNA vaccine potency by coadministration of DNA encoding antiapoptotic proteins. *J Clin Invest*. 2003; 112(1):109–17. [PubMed: 12840065]
110. Kang TH, Lee JH, Noh KH, et al. Enhancing dendritic cell vaccine potency by combining a BAK/BAX siRNA-mediated antiapoptotic strategy to prolong dendritic cell life with an intracellular strategy to target antigen to lysosomal compartments. *Int J Cancer*. 2007; 120(8):1696–703. [PubMed: 17230516]
111. Peng S, Kim TW, Lee JH, et al. Vaccination with dendritic cells transfected with BAK and BAX siRNA enhances antigen-specific immune responses by prolonging dendritic cell life. *Hum Gene Ther*. 2005; 16(5):584–93. [PubMed: 15916483]
112. Ozawa H, Ding W, Torii H, et al. Granulocyte-macrophage colony-stimulating factor gene transfer to dendritic cells or epidermal cells augments their antigen-presenting function including induction of anti-tumor immunity. *J Invest Dermatol*. 1999; 113(6):999–1005. [PubMed: 10594743]
113. Arthur JF, Butterfield LH, Roth MD, et al. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther*. 1997; 4(1):17–25. [PubMed: 9012447]
114. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature*. 1998; 392(6673):245–52. [PubMed: 9521319]
115. Schuler G, Schuler-Thurner B, Steinman RM. The use of dendritic cells in cancer immunotherapy. *Curr Opin Immunol*. 2003; 15(2):138–47. [PubMed: 12633662]
116. Schuler G, Steinman RM. Dendritic cells as adjuvants for immune-mediated resistance to tumors. *J Exp Med*. 1997; 186(8):1183–7. [PubMed: 9379142]
117. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol*. 2003; 3(12):984–93. [PubMed: 14647480]
118. Ikeda H, Chamoto K, Tsuji T, et al. The critical role of type-1 innate and acquired immunity in tumor immunotherapy. *Cancer Sci*. 2004; 95(9):697–703. [PubMed: 15471553]
119. Pulendran B. Modulating TH1/TH2 responses with microbes, dendritic cells, and pathogen recognition receptors. *Immunol Res*. 2004; 29(1–3):187–96. [PubMed: 15181281]
120. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol*. 2003; 3(2):133–46. [PubMed: 12563297]
121. Palucka K, Banchereau J. How dendritic cells and microbes interact to elicit or subvert protective immune responses. *Curr Opin Immunol*. 2002; 14(4):420–31. [PubMed: 12088675]
122. Czerniecki BJ, Cohen PA, Faries M, Xu S, Roros JG, Bedrosian I. Diverse functional activity of CD83 + monocyte-derived dendritic cells and the implications for cancer vaccines. *Crit Rev Immunol*. 2001; 21(1–3):157–78. [PubMed: 11642602]
123. Kalinski P, Moser M. Consensual immunity: success-driven development of T-helper-1 and T-helper-2 responses. *Nat Rev Immunol*. 2005; 5(3):251–60. [PubMed: 15738955]
124. Driessens G, Gordower L, Nuttin L, et al. Therapeutic efficacy of antitumor dendritic cell vaccinations correlates with persistent Th1 responses, high intratumor CD8 + T cell recruitment and low relative regulatory T cell infiltration. *Cancer Immunol Immunother*. 2008; 57(12):1745–56. [PubMed: 18369621]

125. Kalinski P, Okada H. Polarized dendritic cells as cancer vaccines: directing effector-type T cells to tumors. *Semin Immunol.* 2010; 22(3):173–82. [PubMed: 20409732]
126. Amigorena S, Savina A. Intracellular mechanisms of antigen cross presentation in dendritic cells. *Curr Opin Immunol.* 2010; 22(1):109–17. [PubMed: 20171863]
127. Ma Y, Aymeric L, Locher C, Kroemer G, Zitvogel L. The dendritic cell-tumor cross-talk in cancer. *Curr Opin Immunol.* 2010; 23(1):146–52. [PubMed: 20970973]
128. Vyas JM, Van der Veen AG, Ploegh HL. The known unknowns of antigen processing and presentation. *Nat Rev Immunol.* 2008; 8(8):607–18. [PubMed: 18641646]
129. van der Bruggen P, Van den Eynde BJ. Processing and presentation of tumor antigens and vaccination strategies. *Curr Opin Immunol.* 2006; 18(1):98–104. [PubMed: 16343880]
130. Amigorena S, Savina A. Intracellular mechanisms of antigen cross presentation in dendritic cells. *Curr Opin Immunol.* 22(1):109–17. [PubMed: 20171863]
131. Segura E, Villadangos JA. Antigen presentation by dendritic cells in vivo. *Curr Opin Immunol.* 2009; 21(1):105–10. [PubMed: 19342210]
132. Shortman K, Heath WR. The CD8 + dendritic cell subset. *Immunol Rev.* 2010; 234(1):18–31. [PubMed: 20193009]
133. Manfredi AA, Capobianco A, Bianchi ME, Rovere-Querini P. Regulation of dendritic- and T-cell fate by injury-associated endogenous signals. *Crit Rev Immunol.* 2009; 29(1):69–86. [PubMed: 19348611]
134. Macagno A, Gillet M, Sallusto F, Lanzavecchia A, Nestle FO, Groettrup M. Dendritic cells up-regulate immunoproteasomes and the proteasome regulator PA28 during maturation. *Eur J Immunol.* 1999; 29(12):4037–42. [PubMed: 10602014]
135. Lanzavecchia A. Mechanisms of antigen uptake for presentation. *Curr Opin Immunol.* 1996; 8(3):348–54. [PubMed: 8794000]
136. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med.* 1995; 182(2):389–400. [PubMed: 7629501]
137. Lopez-Albaitero A, Mailliard R, Hackman T, et al. Maturation pathways of dendritic cells determine TAP1 and TAP2 levels and cross-presenting function. *J Immunother.* 2009; 32(5):465–73. [PubMed: 19609238]
138. Dhodapkar MV, Steinman RM, Sapp M, et al. Rapid generation of broad T-cell immunity in humans after a single injection of mature dendritic cells. *J Clin Invest.* 1999; 104(2):173–80. [PubMed: 10411546]
139. Adema GJ, de Vries IJ, Punt CJ, Figdor CG. Migration of dendritic cell based cancer vaccines: in vivo veritas? *Curr Opin Immunol.* 2005; 17(2):170–4. [PubMed: 15766677]
140. Bender A, Sapp M, Schuler G, Steinman RM, Bhardwaj N. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J Immunol Methods.* 1996; 196(2):121–35. [PubMed: 8841451]
141. Reddy A, Sapp M, Feldman M, Subklewe M, Bhardwaj N. A monocyte conditioned medium is more effective than defined cytokines in mediating the terminal maturation of human dendritic cells. *Blood.* 1997; 90(9):3640–6. [PubMed: 9345048]
142. Jonuleit H, Kuhn U, Muller G, et al. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *Eur J Immunol.* 1997; 27(12):3135–42. [PubMed: 9464798]
143. Luft T, Jefford M, Luetjens P, et al. Functionally distinct dendritic cell (DC) populations induced by physiologic stimuli: prostaglandin E(2) regulates the migratory capacity of specific DC subsets. *Blood.* 2002; 100(4):1362–72. [PubMed: 12149219]
144. Scandella E, Men Y, Gillesen S, Forster R, Groettrup M. Prostaglandin E2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells. *Blood.* 2002; 100(4):1354–61. [PubMed: 12149218]
145. Schadendorf D, Ugurel S, Schuler-Thurner B, et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic

- melanoma: a randomized phase III trial of the DC study group of the DeCOG. *Ann Oncol.* 2006; 17(4):563–70. [PubMed: 16418308]
146. Kalinski P, Vieira PL, Schuitemaker JH, de Jong EC, Kapsenberg ML. Prostaglandin E(2) is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer. *Blood.* 2001; 97(11):3466–9. [PubMed: 11369638]
 147. Muthuswamy R, Urban J, Lee JJ, Reinhart TA, Bartlett D, Kalinski P. Ability of mature dendritic cells to interact with regulatory T cells is imprinted during maturation. *Cancer Res.* 2008; 68(14): 5972–8. [PubMed: 18632653]
 148. Butterfield LH, Gooding W, Whiteside TL. Development of a potency assay for human dendritic cells: IL-12p70 production. *J Immunother.* 2008; 31(1):89–100. [PubMed: 18157016]
 149. DeBenedette MA, Calderhead DM, Tcherepanova IY, Nicolette CA, Healey DG. Potency of mature CD40L RNA electroporated dendritic cells correlates with IL-12 secretion by tracking multifunctional CD8(+)/CD28(+) cytotoxic T-cell responses in vitro. *J Immunother.* 2011; 34(1): 45–57. [PubMed: 21150712]
 150. Zitvogel L, Mayordomo JJ, Tjandrawan T, et al. Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell 1-associated cytokines. *J Exp Med.* 1996; 183(1):87–97. [PubMed: 8551248]
 151. Zitvogel L, Robbins PD, Storkus WJ, et al. Interleukin-12 and B7.1 co-stimulation cooperate in the induction of effective antitumor immunity and therapy of established tumors. *Eur J Immunol.* 1996; 26(6):1335–41. [PubMed: 8647214]
 152. Furumoto K, Arai S, Yamasaki S, et al. Spleen-derived dendritic cells engineered to enhance interleukin-12 production elicit therapeutic antitumor immune responses. *Int J Cancer.* 2000; 87(5):665–72. [PubMed: 10925360]
 153. Furumoto K, Mori A, Yamasaki S, et al. Interleukin-12-gene transduction makes DCs from tumor-bearing mice an effective inducer of tumor-specific immunity in a peritoneal dissemination model. *Immunol Lett.* 2002; 83(1):13–20. [PubMed: 12057850]
 154. Nishioka Y, Hirao M, Robbins PD, Lotze MT, Tahara H. Induction of systemic and therapeutic antitumor immunity using intratumoral injection of dendritic cells genetically modified to express interleukin 12. *Cancer Res.* 1999; 59(16):4035–41. [PubMed: 10463604]
 155. Okada N, Iiyama S, Okada Y, et al. Immunological properties and vaccine efficacy of murine dendritic cells simultaneously expressing melanoma-associated antigen and interleukin-12. *Cancer Gene Ther.* 2005; 12(1):72–83. [PubMed: 15389286]
 156. Redlinger RE Jr, Mailliard RB, Barksdale EM Jr. Advanced neuroblastoma impairs dendritic cell function in adoptive immunotherapy. *J Pediatr Surg.* 2003; 38(6):857–62. [PubMed: 12778381]
 157. Satoh Y, Esche C, Gambotto A, et al. Local administration of IL-12-transfected dendritic cells induces antitumor immune responses to colon adenocarcinoma in the liver in mice. *J Exp Ther Oncol.* 2002; 2(6):337–49. [PubMed: 12440225]
 158. Shimizu T, Berhanu A, Redlinger RE Jr, Watkins S, Lotze MT, Barksdale EM Jr. Interleukin-12 transduced dendritic cells induce regression of established murine neuroblastoma. *J Pediatr Surg.* 2001; 36(8):1285–92. [PubMed: 11479878]
 159. Yamanaka R, Zullo SA, Ramsey J, et al. Marked enhancement of antitumor immune responses in mouse brain tumor models by genetically modified dendritic cells producing Semliki Forest virus-mediated interleukin-12. *J Neurosurg.* 2002; 97(3):611–8. [PubMed: 12296646]
 160. Zhang S, Zeng G, Wilkes DS, et al. Dendritic cells transfected with interleukin-12 and pulsed with tumor extract inhibit growth of murine prostatic carcinoma in vivo. *Prostate.* 2003; 55(4): 292–8. [PubMed: 12712408]
 161. Mailliard RB, Wankowicz-Kalinska A, Cai Q, et al. alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. *Cancer Res.* 2004; 64(17):5934–7. [PubMed: 15342370]
 162. Kalinski P, Giermasz A, Nakamura Y, et al. Helper role of NK cells during the induction of anticancer responses by dendritic cells. *Mol Immunol.* 2005; 42(4):535–9. [PubMed: 15607810]
 163. Kalinski P, Mailliard RB, Giermasz A, et al. Natural killer-dendritic cell cross-talk in cancer immunotherapy. *Expert Opin Biol Ther.* 2005; 5(10):1303–15. [PubMed: 16197336]

164. Mailliard RB, Egawa S, Cai Q, et al. Complementary dendritic cell-activating function of CD8 + and CD4 + T cells: helper role of CD8 + T cells in the development of T helper type 1 responses. *J Exp Med*. 2002; 195(4):473–83. [PubMed: 11854360]
165. Mailliard RB, Son YI, Redlinger R, et al. Dendritic cells mediate NK cell help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function. *J Immunol*. 2003; 171(5):2366–73. [PubMed: 12928383]
166. Xu S, Koski GK, Faries M, et al. Rapid high efficiency sensitization of CD8 + T cells to tumor antigens by dendritic cells leads to enhanced functional avidity and direct tumor recognition through an IL-12-dependent mechanism. *J Immunol*. 2003; 171(5):2251–61. [PubMed: 12928369]
167. Wesa A, Kalinski P, Kirkwood JM, Tatsumi T, Storkus WJ. Polarized type-1 dendritic cells (DC1) producing high levels of IL-12 family members rescue patient TH1-type ant melanoma CD4 + T cell responses in vitro. *J Immunother*. 2007; 30(1):75–82. [PubMed: 17198085]
168. Kalinski P, Nakamura Y, Watchmaker P, Giermasz A, Muthuswamy R, Mailliard RB. Helper roles of NK and CD8 + T cells in the induction of tumor immunity. Polarized dendritic cells as cancer vaccines. *Immunol Res*. 2006; 36(1–3):137–46. [PubMed: 17337774]
169. Ten Brinke A, Karsten ML, Dieker MC, Zwaginga JJ, van Ham SM. The clinical grade maturation cocktail monophosphoryl lipid A plus IFN γ generates monocyte-derived dendritic cells with the capacity to migrate and induce Th1 polarization. *Vaccine*. 2007; 25(41):7145–52. [PubMed: 17719152]
170. Lee JJ, Foon KA, Mailliard RB, Muthuswamy R, Kalinski P. Type 1-polarized dendritic cells loaded with autologous tumor are a potent immunogen against chronic lymphocytic leukemia. *J Leukoc Biol*. 2008
171. Wieckowski E, Chatta GS, Mailliard RM, et al. Type-1 polarized dendritic cells loaded with apoptotic prostate cancer cells are potent inducers of CD8(+) T cells against prostate cancer cells and defined prostate cancer-specific epitopes. *Prostate*. 2010; 71(2):125–33. [PubMed: 20717900]
172. Gustafsson K, Ingelsten M, Bergqvist L, Nystrom J, Andersson B, Karlsson-Parra A. Recruitment and activation of natural killer cells in vitro by a human dendritic cell vaccine. *Cancer Res*. 2008; 68(14):5965–71. [PubMed: 18632652]
173. Dubsky P, Saito H, Leogier M, et al. IL-15-induced human DC efficiently prime melanoma-specific naive CD8 + T cells to differentiate into CTL. *Eur J Immunol*. 2007; 37(6):1678–90. [PubMed: 17492620]
174. Nguyen LT, Radhakrishnan S, Ciric B, et al. Cross-linking the B7 family molecule B7-DC directly activates immune functions of dendritic cells. *J Exp Med*. 2002; 196(10):1393–8. [PubMed: 12438431]
175. Wang S, Yang J, Qian J, Wezeman M, Kwak LW, Yi Q. Tumor evasion of the immune system: inhibiting p38 MAPK signaling restores the function of dendritic cells in multiple myeloma. *Blood*. 2006; 107(6):2432–9. [PubMed: 16291589]
176. Jarnicki AG, Conroy H, Brereton C, et al. Attenuating regulatory T cell induction by TLR agonists through inhibition of p38 MAPK signaling in dendritic cells enhances their efficacy as vaccine adjuvants and cancer immunotherapeutics. *J Immunol*. 2008; 180(6):3797–806. [PubMed: 18322186]
177. Lipscomb MW, Chen L, Taylor JL, et al. Ectopic T-bet expression licenses dendritic cells for IL-12-independent priming of type 1 T cells in vitro. *J Immunol*. 2009; 183:7250–8. [PubMed: 19915058]
178. Mora JR, Bono MR, Manjunath N, et al. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature*. 2003; 424(6944):88–93. [PubMed: 12840763]
179. Mora JR, Cheng G, Picarella D, Briskin M, Buchanan N, von Andrian UH. Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. *J Exp Med*. 2005; 201(2):303–16. [PubMed: 15642741]
180. Mora JR, von Andrian UH. Retinoic acid: an educational “vitamin elixir” for gut-seeking T cells. *Immunity*. 2004; 21(4):458–60. [PubMed: 15485623]

181. Schaerli P, Loetscher P, Moser B. Cutting edge: induction of follicular homing precedes effector Th cell development. *J Immunol.* 2001; 167(11):6082–6. [PubMed: 11714765]
182. Stagg AJ, Kamm MA, Knight SC. Intestinal dendritic cells increase T cell expression of alpha4beta7 integrin. *Eur J Immunol.* 2002; 32(5):1445–54. [PubMed: 11981833]
183. Weninger W, Manjunath N, von Andrian UH. Migration and differentiation of CD8 + T cells. *Immunol Rev.* 2002; 186:221–33. [PubMed: 12234374]
184. Calzascia T, Masson F, Di Berardino-Besson W, et al. Homing phenotypes of tumor-specific CD8 T cells are predetermined at the tumor site by crosspresenting APCs. *Immunity.* 2005; 22(2): 175–84. [PubMed: 15723806]
185. Mattei S, Colombo MP, Melani C, Silvani A, Parmiani G, Herlyn M. Expression of cytokine/ growth factors and their receptors in human melanoma and melanocytes. *Int J Cancer.* 1994; 56(6):853–7. [PubMed: 7509778]
186. Kunz M, Toksoy A, Goebeler M, Engelhardt E, Bocker E, Gillitzer R. Strong expression of the lymphoattractant C-X-C chemokine Mig is associated with heavy infiltration of T cells in human malignant melanoma. *J Pathol.* 1999; 189(4):552–8. [PubMed: 10629557]
187. Wenzel J, Bekisch B, Uerlich M, Haller O, Bieber T, Tuting T. Type I interferon-associated recruitment of cytotoxic lymphocytes: a common mechanism in regressive melanocytic lesions. *Am J Clin Pathol.* 2005; 124(1):37–48. [PubMed: 15923172]
188. Mullins IM, Slingluff CL, Lee JK, et al. CXC chemokine receptor 3 expression by activated CD8 + T cells is associated with survival in melanoma patients with stage III disease. *Cancer Res.* 2004; 64(21):7697–701. [PubMed: 15520172]
189. Tarbell KV, Petit L, Zuo X, et al. Dendritic cell-expanded, islet-specific CD4 + CD25 + CD62L + regulatory T cells restore normoglycemia in diabetic NOD mice. *J Exp Med.* 2007; 204(1):191–201. [PubMed: 17210729]
190. Yamazaki S, Iyoda T, Tarbell K, et al. Direct expansion of functional CD25 + CD4 + regulatory T cells by antigen-processing dendritic cells. *J Exp Med.* 2003; 198(2):235–47. [PubMed: 12874257]
191. Dhodapkar MV, Steinman RM. Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo in humans. *Blood.* 2002; 100(1):174–7. [PubMed: 12070024]
192. de Vries IJ, Lesterhuis WJ, Barentsz JO, et al. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. *Nat Biotechnol.* 2005; 23(11):1407–13. [PubMed: 16258544]
193. Mackensen A, Krause T, Blum U, Uhrmeister P, Mertelsmann R, Lindemann A. Homing of intravenously and intralymphatically injected human dendritic cells generated in vitro from CD34 + hematopoietic progenitor cells. *Cancer Immunol Immunother.* 1999; 48(2–3):118–22. [PubMed: 10414465]
194. Fong L, Brockstedt D, Benike C, Wu L, Engleman EG. Dendritic cells injected via different routes induce immunity in cancer patients. *J Immunol.* 2001; 166(6):4254–9. [PubMed: 11238679]
195. Grover A, Kim GJ, Lizee G, et al. Intralymphatic dendritic cell vaccination induces tumor antigen-specific, skin-homing T lymphocytes. *Clin Cancer Res.* 2006; 12(19):5801–8. [PubMed: 17020987]
196. Bedrosian I, Mick R, Xu S, et al. Intranodal administration of peptide-pulsed mature dendritic cell vaccines results in superior CD8 + T-cell function in melanoma patients. *J Clin Oncol.* 2003; 21(20):3826–35. [PubMed: 14551301]
197. Lesimple T, Neidhard EM, Vignard V, et al. Immunologic and clinical effects of injecting mature peptide-loaded dendritic cells by intralymphatic and intranodal routes in metastatic melanoma patients. *Clin Cancer Res.* 2006; 12(24):7380–8. [PubMed: 17189411]
198. Freedman RS, Bowen JM, Delcos L, et al. Active intralymphatic immunotherapy of uterine cervical carcinoma with viral oncolysate: a pilot study. *Int J Gynecol Cancer.* 1994; 4(2):101–10. [PubMed: 11578392]
199. Harrer T, Schwab J, Struff WG, et al. Intralymphatic interleukin-2 in combination with zidovudine for the therapy of patients with AIDS. *Infection.* 1998; 26(6):368–74. [PubMed: 9861562]

200. Maloy KJ, Erdmann I, Basch V, et al. Intralymphatic immunization enhances DNA vaccination. *Proc Natl Acad Sci USA*. 2001; 98(6):3299–303. [PubMed: 11248073]

	Protective vaccines against bacteria or viruses	Therapeutic vaccines against cancer
Key challenges	<ul style="list-style-type: none"> • Low/moderate number of specific precursors against foreign antigens 	<ul style="list-style-type: none"> • Low number of specific precursors against tumor (modified-self) antigens • Elimination of T cells reactive with self antigens • Active immunosuppression
Key pathogen-provided signals	<ul style="list-style-type: none"> • Antigenic signals • Inflammatory signals • Induction of T cell effector functions (CTL) • Attraction of effector T cells 	<ul style="list-style-type: none"> • Antigenic signals • Suppressive /anti-inflammatory signals
Functional goals of vaccination	<ul style="list-style-type: none"> • Induce immune memory 	<ul style="list-style-type: none"> • Induce immune memory • Induce cells able of tumor killing • Induce the ability to home to tumors • Avoid suppression
Mechanistic goals of vaccination	<ul style="list-style-type: none"> • Induce pathogen-specific memory T cells • Induce pathogen-specific B cells • Induce pathogen-specific antibodies 	<ul style="list-style-type: none"> • Induce pathogen-specific memory T cells • Induce pathogen-specific effector T cells (CTL) • Induce tumor-relevant chemokine receptors • Avoid interaction with suppressor cells (and their additional activation/expansion)

Fig. 1.

Ability of DCs to regulate different aspects of T cell activity. DCs provide T cells with antigenic “signal 1” and costimulatory “signal 2” needed for the activation and expansion of tumor-specific CD4⁺ and CD8⁺ T cells. In addition, DCs provide T cells with an additional polarizing “signal 3” that selectively drives the development of different effector mechanisms. DCs can also provide T cells with an additional “signal 4”, regulating the homing pattern of tumor-specific T cells

Fig. 2.

Unique goals and challenges of DC therapies. In contrast to preventive vaccines, therapeutic application of antigen-loaded DCs needs to overcome tumor-induced dysfunction of endogenous DCs and the presence of tumor-induced suppressive immune cells, such as regulatory T cells (T_{reg}) and myeloid-derived suppressor cells (MDSC). Apart from the induction of long-lived memory T cells (a shared goal with protective vaccines against bacteria or viruses), DC therapies also need to impart the inflammatory signals (missing in the cancer settings) needed for the induction of effector functions and tumor-homing potential in tumor-specific T cells