Published in final edited form as:

Immunol Res. 2011 December ; 51(2-3): 153–160. doi:10.1007/s12026-011-8255-5.

Generation of immunogenic and tolerogenic Clinical-grade dendritic cells

Tahereh Kalantari¹, Eskandar Kamali-Sarvestani², Bogoljub Ciric¹, Mohamad H. Karimi³, Mohsen Kalantari⁴, Alireza Faridar¹, Hui Xu¹, and Abdolmohamad Rostami¹

¹Department of Neurology, Thomas Jefferson University, Philadelphia, PA, USA

²Immunology Department and Autoimmune Diseases Research Center, University of Medical Sciences, Shiraz, Iran

³Transplant Research Center, University of Medical Sciences, Shiraz, Iran

⁴School of Veterinary Medicine, Shiraz, Iran

Abstract

Immunotherapy with dendritic cells (DCs) that have been manipulated ex vivo to become immunogenic or tolerogenic, has been tested in clinical trials for disease therapy. DCs are sentinels of the immune system, which after exposure to antigenic or inflammatory signals and crosstalk with effector CD4⁺ T cells express high levels of costimulatory molecules and cytokines. Suppression of either costimulatory molecules or cytokines generates tolerogenic DCs (TDCs), which induce T regulatory cells (Tregs) and a state of tolerance.

Nevertheless, DC vaccination is still at an early stage, and improvements are mainly needed in generating clinical-grade DC products and in quality control of monitoring assays to assess the effect of DC vaccination in future clinical trials. Here, we review the recent work in DC generation and monitoring approaches for DC-based trials with immunogenic or tolerogenic DCs.

Keywords

dendritic cell; vaccines; autoimmune diseases; cancer; quality control; immunotherapy

Introduction

In 1868, Paul Langerhans observed a population of dendritically shaped cells in the epidermis of the skin. He at first mistook these cells for nerve cells both because of their dendrite-like morphology and also their interaction with gold salt [1]. These cells remained an enigma for over a century until 1973, when Ralph Steinman introduced them as a new type of cell, dendritic cells (DCs), of which Langerhans cells (LCs) were a subset [2].

DCs as antigen-presenting cells (APCs) are critical to the initiation of CD4⁺ T-cell responses. Resting or immature DCs (iDCs) migrate from peripheral tissues to T-dependent areas of lymphoid organs when they are exposed to pathogens or inflammatory mediators. During this migration DCs mature, a process characterized by an increase in expression of

Correspondence to: Abdolmohamad Rostami, Department of Neurology, Jefferson Hospital for Neuroscience, Thomas Jefferson University, 900 Walnut Street, JHN 300, Philadelphia PA 19107, USA; Tel.: + 215-503 8100, A.M.Rostami@jefferson.edu.

Authorship

Conflict-of-interest disclosure: The authors declare no competing financial interests.

major histocompatibility complex (MHC) and costimulatory molecules such as CD80 and CD86[3,4]. DCs are heterogeneous in their origin, maturation, morphology, phenotype, and function [5]. Two major types of DCs were originally defined as conventional (cDCs) and plasmacytoid (pDCs) dendritic cells in both human and mice. At first it was thought that cDCs and pDCs were of myeloid and lymphoid origin, respectively, but later it was shown that both subsets of DCs could be generated from either myeloid (CMP: Common Myeloid Precursors) or lymphoid (CLP: Common Lymphoid Precursors) progenitors [6, 7]. However, it has also been shown that DCs develop more from CMP than CLP [7].

Immunogenic DCs generally promote the development of effector T cells, while TDCs (TDCs) promote the development of suppressive Treg cells [6]. Three signals (signal 1: T cell receptor /MHC, signal 2: costimulatory molecules, and signal 3: cytokines) participate in DC-T cell crosstalk and downregulation in any of these signals leads to generation of TDC [8]. Thus, DCs may have a tolerogenic function rather than an immunogenic effect both in the thymus and the periphery [9]. Recent studies have shown that iDCs [10] are not alone in having tolerogenic properties but that semi-mature DCs [11] and mature DCs (mDCs) [12] can also be tolerogenic. The major characteristics of tolerogenic DCs are reduced expression of costimulatory molecules such as CD40, CD80, and CD86, and high expression of inhibitory receptors such as immunoglobulin-like transcripts (ILTs) [13]. In addition, tolerogenic DCs (TDCs) usually show reduced IL-12 and increased IL-10 production at an early stage of maturation [14]. It has also been demonstrated that, in spite of producing a large amount of type I interferon (IFN) and having a potent anti-viral activity, pDCs are often tolerogenic when compared with cDCs [15].

Up to now DCs have been tested for therapy of infectious diseases such as HIV-1 [16], various cancers[17], post-transplant graft versus host disease (GVHD) [18] and various autoimmune diseases. [19]. Many clinical trials of DC-based immunotherapy show that this method is reliable, safe and therapeutically efficient [20]. However, the major problem that prevents routine clinical use of DCs in therapy of various diseases is the lack of a universally accepted quality control and standardization for generated therapeutic DCs [21]. The aim of this review is to give an overview of the methods used for in vitro generation of immunogenic and tolerogenic DCs and also a brief review of quality control of generated DCs.

Cell sources used for DC generation

DCs have been selected for immunotherapeutic vaccine strategies because they have a capacity to direct immune responses either by inducing or suppressing them [6, 22]. The two starting cell populations CD34⁺ hematopoietic progenitor cells (HPCs) [23]and monocytes, have been used to generate DCs for therapy [24]. There is now a third protocol under development, in which DCs are generated from peripheral blood DCs, but because of the very low numbers of DCs in human blood (about 1% of mononuclear cells), this approach is less suitable for DC isolation than the two methods mentioned above [8].

One of the protocols for DC generation utilizes CD34⁺ HPCs from human peripheral blood. Because of the paucity of CD34⁺ progenitor cells in human blood, they are mobilized from the bone marrow to the periphery with a G-CSF or IL-3 injection. In this method iDCs, once produced by using GM-CSF / IL-4 or GM-CSF / TNF α , can be differentiated into mature DCs with a cytokine cocktail that includes TNF- α and CD40L [25–27].

CD34⁺ HPCs can also be obtained from fetal or adult bone marrow, fetal liver, and cord blood[28–30]. Other early cells that have been used for DC generation are human embryonic stem cells (hESCs) [31]; details of the procedure are given in Figure 1.

The most commonly used cell for DC generation is the peripheral blood mononuclear cell (PBMC) which can be easily obtained by leukapheresis [32]. The techniques for enrichment of monocytes and subsequent DC generation are plastic adherence [34], positive immunomagnetic selection [29], negative immunomagnetic selection [34], density gradient centrifugation [35], and elutriation [36]. One of the most common techniques for obtaining highly purified monocytes is positive immunomagnetic selection of CD14⁺ cells, but the high cost of the necessary reagents limits clinical use of this technique [34].

Role of cytokines in DC generation

Culture conditions, and especially cytokine cocktails, have the main effect on monocyte differentiation into both macrophages and DCs [35]. It has been shown that granulocyte-macrophage colony stimulating factor (GM-CSF) is involved in monocyte survival and differentiation both in humans and mice [37]. Lower concentrations of GM-CSF induce only cell survival, while higher concentrations of GM-CSF lead to cell survival and cell proliferation, differentiation or functional activation [38]. It has been recently reported that monocyte differentiation toward DC in response to GM-CSF depends on the expression level of GM-CSF receptors (GM-CSFRs) in precursor monocytes [38]. Conversely, the level of GM-CSFR expression does not have any significant effect on monocyte differentiation into DC in the presence of GM-CSF and Interleukin-4 (IL-4), demonstrating the potent effect of IL-4 [36].

iDC generation by GM-CSF/ IL-4

The oldest and most commonly used cytokine cocktail for in vitro DC generation is GM-CSF/IL-4, which was described for the first time in 1994 and is thus far the best one [39]. Treatment of human monocytes with this cocktail generates iDCs (IL-4DCs) with surface markers MHC^{lo}, CD80/CD86^{lo}, and CD83⁻ [40]. These IL-4DCs are defective in antigen presentation and migration and are not effective in DC therapy. In addition, if medium containing GM-CSF/IL-4 is replaced with culture medium without these cytokines, IL-4DCs regain macrophage features and readily re-adhere to culture plates within 3 days [41]. A two-step culture system procedure has now been developed to achieve fully mature DCs [42].

According to priority and recency, different maturation media have been used; the first maturation medium (in 1993) in which iDCs were cultured was monocyte-conditioned medium (MCM) [43]. The major characteristics of cells cultured in MCM are highly functional APCs with a high expression of CD83. In fact, MCM is the supernatant of the cultured monocytes and contains a variety of cytokines, which means that one can not have control over its cytokine content. Various cytokine cocktails have therefore been developed to replace MCM, with the goal of defining a standard procedure for clinical and experimental purposes. Cocktails that achieve the best maturation and migration capacity of IL4-DCs contain tumor necrosis factor alpha (TNF α), IL-1 β , IL-6, and prostaglandin E2 (PGE2). These matured IL4-DCs are thought to induce a Th2 response, but no Th1 response [39, 44].

However, PGE2 is supposed not only to induce Th2 immune response [45] but it may also stimulate DCs to secrete IL-10, making possible expansion of immunosuppressive Tregs [46]. It is therefore possible that many tumor vaccination trials failed because of the use of PGE2 [47]. To preclude Treg expansion, a new “megacytokine cocktail” [48] comprising TNF α , IL-1 β , Poly (I: C), Interferon-alpha (IFN α), and IFN- γ has been developed.

Cytokines involved in generation of immunogenic DCs

Adding IFN α to culture with GM-CSF instead of IL-4 leads to a rapid upregulation of MHC class II molecules, costimulatory molecules (CD80, CD86) as well as the maturation markers CD40 and CD83 [49]. A semi-mature DC phenotype has been shown after 3 days of this cell culture, which is different from the immature phenotype of IL-4-generated DCs. IFN α -DCs are more effective than immature IL-4DCs in activating Th-1 and CD8 $^{+}$ T cell responses against particular antigens in different models [50].

TNF- DC is a GM-CSF/TNF α generated DC with CD80/DC86 lo CD83 $^{-}$ CD14 $^{+}$ CD1a lo phenotype and little capacity to stimulate T cells. They can be produced from CD14 $^{+}$ monocytes cultured in the presence of TNF α with GM-CSF for 6 days. Thereafter, TNF-DCs can be converted into mature CD70 $^{+}$ DCs in the presence of lipopolysaccharide (LPS) for two days, which are able to stimulate both Th1 and Th17 immune responses [51].

Mohamadzadeh et al. were the first to demonstrate that monocytes may develop into IL-15DCs in the presence of IL-15 and GM-CSF[52]. This cytokine cocktail gives rise to CD1a $^{+}$, HLA-DR $^{+}$, CD14 $^{-}$ DCs, a proportion of which express LC markers, such as E-cadherin, Langerin and CCR6. However, IL-15DCs, which are called LC-like DCs, despite Langerin expression, do not express Birbeck granules [52]. In contrast to immature IL-4-DCs, however, immature IL15-DCs can induce CD8 $^{+}$ T cell proliferation without the help of an exogenous maturation factor such as LPS [53]. These properties may be the result of membrane-bound IL-15, which can only be found within the IL-15DC population[53]. Therefore, IL-15DCs appear to be an efficient stimulator of T cell proliferation, especially CTL priming.

Ex-vivo plasticity of TDCs

In vitro TDC generation may be possible with DC manipulation using one of the following three methods: 1) physiological mediators; 2) pharmacological agents; 3) genetic engineering [54].

Physiological mediators involved in TDC generation can be divided into biological factors and alteration in culture conditions [55, 56]. A variety of cytokines such as anti-inflammatory cytokines (e.g. TGF- β and IL-10) and also costimulatory blockers such as anti- CD40L are included in the group of biological factors [57, 58]. Some cytokines such as IL-10, TGF β 1, and VEGF can prevent DC maturation or function both in vitro and in vivo [57, 59]. Culture of murine bone marrow precursors in low doses of GM-CSF has also been shown to produce large numbers of immature myeloid DCs that induce alloantigen-specific T-cell hyporesponsiveness in vitro [56].

Another method for generating TDCs is with pharmacological agents including anti-inflammatory agents (such as aspirin), cyclic adenosine monophosphate (cAMP) inducers [PGE2, histamine, β 2 agonists, neuropeptides (calcitonin-gene-related peptide, vasoactive intestinal peptide)], vitamin D3, and immunosuppressive drugs (corticosteroids, cyclosporin A, rapamycin, deoxy spargualin, and mycophenolate mofetil (MMF)). These agents inhibit DC activation/maturation or impair in vitro or in vivo IL-12p70 production [60, 61].

Several checkpoints in DC biology such as differentiation, antigen uptake, maturation, and migration provide favorable circumstances for pharmacologic manipulation of their function [60]. Analysis of these checkpoints indicates that the general effect of all the pharmacological agents is on maturation in APCs, possibly via inhibition of the NF- κ B-Rel transcription factor family, which downregulates many cytokines, such as IL-1, IL-6, and

TNF- α , but an increase in IL-10 can be seen at the transcriptional and post-transcriptional level [61,62].

One of the most efficient of pharmacological agents is $1\alpha, 25$ -Dihydroxy vitamin D₃, which affects the differentiation, maturation, and function of monocyte-derived DCs. Moreover, vitamin D₃ promotes the generation of semi-mature DCs, with impaired capacity to mature and to stimulate T cells [63].

The third method for generating TDCs is genetic engineering, in which a variety of genes, such as co-stimulatory molecules and cytokines, can be transferred through viral or nonviral delivery systems or manipulated by selective gene silencing [anti-sense oligodeoxynucleotides (ODNs) and small interfering RNAs (siRNAs)]. In line with this, expression of an inhibitory molecule (through viral or nonviral systems) or suppression of an immunogenic factor (through anti-sense or siRNA) may induce tolerogenic DC [64–66].

Cytokines involved in generating TDCs

When Soumelis et al. investigated the impact of DCs in the pathogenesis of allergic diseases, they discovered that thymic stromal lymphopoietin (TSLP) has the ability to activate CD11c⁺ DCs [67].

TSLP-DCs generated in the presence of GM-CSF and TSLP may induce a strong proliferation of naïve CD4⁺ T cells and also CCL22 and CCL17 chemokine production, both attracting CCR4-expressing Th2 cells[68].

Because these cells induce a Th2, but not a Th1, immune response, they may play an important role in clinical vaccines for autoimmune therapies [69].

It has been shown that IL-3 is an important cytokine for generating DCs from high IL-3R (CD23)-expressing plasmacytoid precursor cells, by inducing their proliferation and survival [70].

DCs generated in the presence of IL-3 and IL-4 exhibit phenotypic and functional properties highly similar to those generated with GM-CSF and IL-4 at the immature stage; the IL-3/IL-4 cytokine cocktail thus gives rise to CD80/DC86^{lo}, CD83⁻, CD14⁻, and CD1a⁻ DCs [70, 71].

However, in response to CD40L, IL-3/IL-4DCs are converted into mDCs secreting less IL-12 and more IL-10, thereby inducing a T cell cytokine secretion pattern shifted toward the Th2 phenotype as compared with IL-4DCs [71].

Quality control of DCs

Clinical-grade DCs

DCs for use in immunotherapy should be generated according to the good manufacturing practice (GMP) guidelines, which is required by the Food and Drug Administration (FDA) as clinical studies progress from Phase I to Phase III [72].

Important issues for manufacturing clinical-grade DCs[73] include: monitoring the quality of antigen-loaded DCs before release for use in a clinical setting; aliquoting batches of DCs into sterile vials, and cryopreserving cells for multiple vaccinations; having a manufacturing center to ensure consistency and quality in the processing steps and final products; having a facility with high-efficiency particulate air (HEPA)-filtered biological safety cabinets; and

the ability to monitor air pressure, incubators, centrifuges, cryopreservation devices, and freezers are important issues for manufacturing clinical-grade DCs[73].

Current release criteria for clinical-grade DC products

Thus far the predefined criteria for release of clinical-grade DC are sterility testing, viability, purity, maturation, stability, and potency [74].

The absence of aerobic or anaerobic microorganisms and of endotoxins in DC products can be confirmed by sterility testing [75].

Viability of DCs following culture is determined by means of light microscope and trypan blue dye or by flow cytometry and propidium iodide (PI) or 7-amino-actinomycin D (7-AAD) [73].

Purity of a DC product is determined by evaluating several surface markers of lymphocytes (CD3⁺, CD19⁺ or CD56/16⁺) and/or monocytes (CD14⁺) by flow cytometry. Therefore, the exact level of purity of DCs remains to be established [76].

When DCs are activated, they may express high levels of maturation markers including MHC molecules, and costimulatory molecules, such as CD80, CD86 and CD83 [77]. Ex vivo activation of DCs can be assessed by up-regulation of surface markers and functional assays, and expression of APM (antigen processing machinery)-associated proteins can be determined by specific antibodies for APM component proteins[76].The viability of DCs at different temperatures or after freezing and thawing cycles can be evaluated by stability assays. Biological functions of DCs are currently measured by the potency assay for IL-12p70 production both in vitro and in vivo[78].

Costimulatory molecules on DCs, which are much higher than on other cells, can be measured by COSTIM bioassay [79].

In addition, there are also other methods available for evaluating DCs loaded with antigen-specific immune responses in vitro such as enzyme-linked immunosorbent spot (ELISPOT) assay, cytokine flow cytometry (CFC) or MHC-tetramer staining [80].

Future Prospects: Application of CD19⁺ IDO⁺ DC in therapy both for autoimmunity and cancer

Recently discovered indoleamine 2, 3-dioxygenase (IDO) ⁺ DCs, which have a morphology similar to plasma cells with CD123⁺CD19⁺ phenotype and constitutive residence in the splenic red pulp, can stimulate Treg cell function [81–83].

The most prominent characteristic of CD19⁺DCs is their unique ability to suppress T cells and activate Tregs by producing large amounts of functional IDO in response to ligation of either CD80/CD86 by cytotoxic T-lymphocyte antigen 4 (CTLA-4) or Toll-like receptor 9 (TLR9) by unmethylated CpG-DNA motifs [84,85].Thus, CD19⁺DCs are key regulators of T cell responses that contribute to the development and maintenance of the immune system at local sites of inflammation [86].It has been shown that splenic IDO-producing cells, which are very similar to CD19⁺DCs, may cause tumor progression in inflamed lymph node (LN) draining sites of tumor growth[87]. An increase in CD19⁺DCs has therefore been seen in tumor microenvironments, which increase Treg cell numbers and promote tumor growth [88].

It has been shown that IDO can stimulate Treg cells and simultaneously prevent the IL-6 production required for Treg cell conversion into Th17-like T cells [83]. In the absence of IDO, but in the presence of other strong pro-inflammatory stimuli such as IFN- γ , Tregs acquire the Th17 phenotype that is associated with autoimmune disorders[89]. There are several inducers such as CpG, soluble CTLA-4 or IFN- γ and inhibitors such as 1-methyl-tryptophan (1-MT)] of IDO expression[85]. 1-MT may cause worsening of disease symptoms and inflammation, as shown in models of inflammatory bowel disease (IBD) [90], and experimental autoimmune encephalomyelitis (EAE) [91].

Given that DCs are critical to the initiation of immune responses, especially T cell responses, it is possible that future immunotherapy for treatment of autoimmune diseases and organ transplantation can be done by injection of “CD19⁺ DCs plus an IDO inducer” and, on the other hand, for treatment of tumors “CD19⁺DCs plus IDO inhibitors” might be used (Fig. 2) [92]. CD19⁺ DCs may therefore provide novel opportunities for immunotherapy of cancers and auto immune diseases.

Conclusion

Here, we have discussed the different cell sources and cytokine cocktails for generation of both immunogenic and tolerogenic DCs, as well as quality control of manufactured DCs. To date, DC therapies are either tested in the treatment of several types of cancers and infectious diseases to induce robust immune responses [9, 16, 17], or in patients with post-GVHD, and in animal models of autoimmune diseases [18, 19]. Immunotherapies with DCs are nontoxic and safe, but because of the complexity of DC production processes and the lack of international criteria for quality control and release of therapeutic DC products, their clinical efficacy has not yet been confirmed [93]. Currently, DC products cannot be compared one with the other, because they are prepared in various laboratories with different nonstandard methods. To standardize DC production, several issues must be considered, including the selection of culture media, the use of semi-automatic culture apparatuses for clinical-scale production, composition of cytokine cocktails used for DC maturation, and standardization of assays for quality control [73].

In addition, predefined criteria for sterility, viability, purity, maturation, and stability must be established to characterize DC products for therapy[74]. The most significant advance in future successful evaluations of DC-based vaccines will therefore be obtained by an established quality control program.

In future studies we hope to see the perfect approach of producing clinical-grade DC vaccines for treatment of both cancer and autoimmune diseases.

Acknowledgments

We are very grateful to K. Regan for editing the manuscript.

References

1. Langerhans P. Über die Nerven der Menschlichen Haut. Virchows Arch. 1868; 44:325–337.
2. 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; 137:1142–1162. [PubMed: 4573839]
3. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature. 2007; 449:419–427. [PubMed: 17898760]
4. Sato K, Fujita S. Dendritic cells: nature and classification. Allergol Int. 2007; 56:183–191. [PubMed: 17646733]

5. Adorini L, Penna G. Dendritic cell tolerogenicity: a key mechanism in immunomodulation by vitamin D receptor agonists. *Hum Immunol.* 2009; 70:345–352. [PubMed: 19405173]
6. Jacobs B, Wuttke M, Papewalis C, Seissler J, Schott M. Dendritic cell subtypes and in vitro generation of dendritic cells. *Horm Metab Res.* 2008; 40:99–107. [PubMed: 18283627]
7. Traver D, Akashi K, Manz M, Merad M, Miyamoto T, Engleman EG, Weissman IL. Development of CD8alpha positive dendritic cells from a common myeloid progenitor. *Science.* 2000; 290:2152–2154. [PubMed: 11118150]
8. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol.* 2003; 3:984–993. [PubMed: 14647480]
9. Manicassamy S, Pulendran B. Dendritic cell control of tolerogenic responses. *Immunol Rev.* 2011; 241:206–227. [PubMed: 21488899]
10. Mahnke K, Schmitt E, Bonifaz L, Enk AH, Jonuleit H. Immature, but not inactive: The tolerogenic function of immature dendritic cells. *Immunol Cell Biol.* 2002; 80:477–483. [PubMed: 12225384]
11. Lutz MB, Schuler G. Immature, semi-mature and fully mature dendritic cells: Which signals induce tolerance or immunity? *Trends Immunol.* 2002; 23:445–449. [PubMed: 12200066]
12. Yamazaki S, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K, Steinman RM. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J Exp Med.* 2003; 198:235–247. [PubMed: 12874257]
13. Wu J, Horuzsko A. Expression and function of immunoglobulin-like transcripts on tolerogenic dendritic cells. *Hum Immunol.* 2009; 70:353–356. [PubMed: 19405174]
14. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol.* 2003; 21:685–711. [PubMed: 12615891]
15. Matta BM, Castellana A, Thomson AW. Tolerogenic plasmacytoid DC. *Eur J Immunol.* 2010; 40:2667–2676. [PubMed: 20821731]
16. Connolly NC, Whiteside TL, Wilson C, Kondragunta V, Rinaldo CR, Riddler SA. Therapeutic immunization with HIV-1 peptide-loaded dendritic cells is safe and immunogenic in HIV-1-infected individuals. *Clin Vaccine Immunol.* 2008; 15:284–292. [PubMed: 17942609]
17. Gilboa E. DC-based cancer vaccines. *J Clin Invest.* 2007; 117:1195–1203. [PubMed: 17476349]
18. Ezzelarab M, Thomson AW. Tolerogenic dendritic cells and their role in transplantation. *Semin Immunol.* 2011 Jul 6. In press.
19. Lo J, Clare-Salzler MJ. Dendritic cell subsets and type I diabetes: focus upon DC-based therapy. *Autoimmune Rev.* 2006; 5:419–423.
20. Jähnisch H, Füßel S, Kiessling A, et al. Dendritic cell-based immunotherapy for prostate cancer. *Clin Dev Immunol.* 2010; 2010:517493. [PubMed: 21076523]
21. Aarntzen EH, Figdor CG, Adema GJ, Punt CJ, de Vries IJ. Dendritic cell vaccination and immune monitoring. *Cancer Immunol Immunother.* 2008; 57:1559–1568. [PubMed: 18618110]
22. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. *J Exp Med.* 2002; 195:327–333. [PubMed: 11828007]
23. Ueno H, Tcherepanova I, Reygrobellet O, Laughner E, Ventura C, Palucka AK, Banchereau J. Dendritic cell subsets generated from CD34⁺ hematopoietic progenitors can be transfected with mRNA and induce antigen-specific cytotoxic T cell responses. *Journal of Immunological Methods.* 2004; 285:171–180. [PubMed: 14980432]
24. Mallon DF, Buck A, Reece JC, Crowe SM, Cameron PU. Monocyte-derived dendritic cells as a model for the study of HIV-1 infection: Productive infection and phenotypic changes during culture in human serum. *Immunol Cell Biol.* 1999; 77:442–450. [PubMed: 10540211]
25. Ozkurt ZN, Yegin ZA, Suyani E, Aki SZ, Acar K, Yagci M, Sucak GT. Factors affecting stem cell mobilization for autologous hematopoietic stem cell transplantation. *J Clin Apher.* 2010; 25:280–286. [PubMed: 20623783]
26. Chen CH, Wu TC. Experimental vaccine strategies for cancer immunotherapy. *J Biomed Sci.* 1998; 5:231–252. [PubMed: 9691216]

27. Luft T, Pang KC, Thomas E, Bradley CJ, Savoia H, Trapani J, Cebon J. Serum-free culture model for studying the differentiation of human dendritic cells from adult CD34⁺ progenitor cells. *Exp Hematol*. 1998; 26:489–500. [PubMed: 9620282]
28. Lim FT, Kanhai HH, Falkenburg JH. Characterization of the human CD34⁺ hematopoietic progenitor cell compartment during the second trimester of pregnancy. *Haematologica*. 2005; 90:173–179. [PubMed: 15710568]
29. Felzmann T, Witt V, Wimmer D, Ressmann G, Wagner D, Paul P, Hüttner K, Fritsch G. Monocyte enrichment from leukapheresis products for the generation of DCs by plastic adherence, or by positive or negative selection. *Cytotherapy*. 2003; 5:391–398. [PubMed: 14578101]
30. Weekx SF, Van Bockstaele DR, Plum J, et al. CD34⁺⁺ CD38[–] and CD34⁺ CD38⁺ human hematopoietic progenitors from fetal liver, cord blood, and adult bone marrow respond differently to hematopoietic cytokines depending on the ontogenic source. *Exp Hematol*. 1998; 26:1034–1042. [PubMed: 9766443]
31. Tseng SY, Nishimoto KP, Silk KM, et al. Generation of immunogenic dendritic cells from human embryonic stem cells without serum and feeder cells. *Regen Med*. 2009; 4:513–526. [PubMed: 19580370]
32. Michiels A, Tuyaeerts S, Bonehill A, Heirman C, Corthals J, Thielemans K. Delivery of tumor-antigen-encoding mRNA into dendritic cells for vaccination. *Methods Mol Biol*. 2008; 423:155–163. [PubMed: 18370196]
33. Berger TG, Feuerstein B, Strasser E, Hirsch U, Schreiner D, Schuler G, Schuler-Thurner B. Large-scale generation of mature monocyte-derived dendritic cells for clinical application in cell factories. *J Immunol Methods*. 2002; 268:131–140. [PubMed: 12215381]
34. Babatz J, Röhlig C, Oelschlägel U, Zhao S, Ehninger G, Schmitz M, Bornhäuser M. Large-scale immunomagnetic selection of CD14⁺ monocytes to generate dendritic cells for cancer immunotherapy: a phase I study. *J Hematother Stem Cell Res*. 2003; 12:515–523. [PubMed: 14594508]
35. Romani N, Gruner S, Brang D, et al. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med*. 1994; 180:83–93. [PubMed: 8006603]
36. Cobb A, Roberts LK, Palucka AK, et al. Development of a HIV-1 lipopeptide antigen pulsed therapeutic dendritic cell vaccine. *J Immunol Methods*. 2011; 365:27–37. [PubMed: 21093448]
37. Conti L, Gessani S. GM-CSF in the generation of dendritic cells from human blood monocyte precursors: recent advances. *Immunobiology*. 2008; 213(9–10):859–870. [PubMed: 18926300]
38. Cardone M, Varano B, Puddu P, Belardelli F, Gessani S. Role of the cytokine environment and cytokine receptor expression on the generation of functionally distinct dendritic cells from human monocytes. *Eur J Immunol*. 2008; 38:750–762. [PubMed: 18236400]
39. 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; 179:1109–1118. [PubMed: 8145033]
40. Zhou LJ, Tedder TF. Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *J Immunol*. 1995; 154:3821–3835. [PubMed: 7706722]
41. Romani N, Reider D, Heuer M, et al. Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *J Immunol Meth*. 1996; 196:137–151.
42. O'Doherty U, Steinman RM, Peng M, et al. Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte conditioned medium. *J Exp Med*. 1993; 178:1067–1076. [PubMed: 8102389]
43. Jonuleit H, Kühn U, Müller G, Steinbrink K, et al. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *Eur J Immunol*. 1997; 27:3135–3142. [PubMed: 9464798]
44. Scandella E, Men Y, Gillessen S, Forster R, Groettrup M. Prostaglandin E2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells. *Blood*. 2002; 100:1354–1361. [PubMed: 12149218]

45. Morelli AE, Thomson AW. Dendritic cells under the spell of prostaglandins. *Trends Immunol.* 2003; 24:108–111. [PubMed: 12615202]
46. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med.* 2005; 10:909–915. [PubMed: 15340416]
47. Mailliard RB, Wankowicz-Kalinska A, et al. Alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. *Cancer Res.* 2004; 64:5934–5937. [PubMed: 15342370]
48. Santini SM, Lapenta C, Logozzi M, Parlato S, Spada M, Di Pucchio T, Belardelli F. Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. *J Exp Med.* 2000; 191:1777–1788. [PubMed: 10811870]
49. Della Bella S, Nicola S, Riva A, Biasin M, Clerici M, Villa ML. Functional repertoire of dendritic cells generated in granulocyte macrophage colony stimulating factor and interferon-alpha. *J Leukoc Biol.* 2004; 75:106–116. [PubMed: 14525963]
50. Lapenta C, Santini SM, Logozzi M, et al. Potent immune response against HIV-1 and protection from virus challenge in hu-PBL-SCID mice immunized with inactivated virus-pulsed dendritic cells generated in the presence of IFN-alpha. *J Exp Med.* 2003; 198:361–367. [PubMed: 12874266]
51. Miyazaki A, et al. TNF-alpha drives human CD14+ monocytes to differentiate into CD70+ dendritic cells evoking Th1 and Th17 responses. *J Immunol.* 2007; 179:1449–1457. [PubMed: 17641010]
52. Mohamadzadeh M, Berard F, Essert G, et al. Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. *J Exp Med.* 2001; 194:1013–1020. [PubMed: 11581322]
53. Dubsky P, Saito H, Leogier M, Dantin C, Connolly JE, Banchereau J, Palucka AK. IL-15-induced human DC efficiently prime melanoma-specific naïve CD8+ T cells to differentiate into CTL. *Eur J Immunol.* 2007; 37:1678–1690. [PubMed: 17492620]
54. Morelli AE, Thomson AW. Dendritic cells: regulators of alloimmunity and opportunities for tolerance induction. *Immunol Rev.* 2003; 196:125–146. [PubMed: 14617202]
55. Rutella S, Danese S, Leone G. Tolerogenic dendritic cells: cytokine modulation comes of age. *Blood.* 2006; 108:1435–1440. [PubMed: 16684955]
56. Lutz MB, Suri RM, Niimi M, et al. Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 is maturation resistant and prolongs allograft survival in vivo. *Eur J Immunol.* 2000; 30:1813–1822. [PubMed: 10940870]
57. Steinbrink K, Wölfl M, Jonuleit H, Knop J, Enk AH. Induction of tolerance by IL-10-treated dendritic cells. *J Immunol.* 1997; 159:4772–4780. [PubMed: 9366401]
58. Lu L, Li W, Zhong C, Qian S, Fung JJ, Thomson AW, Starzl TE. Increased apoptosis of immunoreactive host cells and augmented donor leukocyte chimerism, not sustained inhibition of B7 molecule expression are associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb. *Transplantation.* 1999; 68:747–757. [PubMed: 10515374]
59. Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, Gabrilovich DI. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor kappa B activation in hemopoietic progenitor cells. *J Immunol.* 1998; 160:1224–1232. [PubMed: 9570538]
60. Hackstein H, Thomson AW. Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. *Nat Rev Immunol.* 2004; 4:24–34. [PubMed: 14704765]
61. Hackstein H, et al. Rapamycin inhibits IL-4-induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. *Blood.* 2003; 101:4457–4463. [PubMed: 12531798]
62. Hu J, Wan Y. Tolerogenic dendritic cells and their potential applications. *Immunology.* 2011; 132:307–314. [PubMed: 21208205]
63. Taher YA, van Esch BC, Hofman GA, Henricks PA, van Oosterhout AJ. 1alpha,25-dihydroxyvitamin D3 potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF-beta. *J Immunol.* 2008; 180:5211–5221. [PubMed: 18390702]

64. Crooke ST. Progress in antisense technology. *Ann Rev Med.* 2004; 55:61–95. [PubMed: 14746510]
65. Fjose A, Ellingsen S, Wargelius A, Seo HC. RNA interference: mechanisms and applications. *Biotechnol Annu Rev.* 2001; 7:31–57. [PubMed: 11686048]
66. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature.* 2001; 411:494–498. [PubMed: 11373684]
67. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol.* 2002; 3:673–680. [PubMed: 12055625]
68. Reche PA, Soumelis V, Gorman DM, et al. Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. *J Immunol.* 2001; 167:336–343. [PubMed: 11418668]
69. Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. Reciprocal control of T helper cell and dendritic cell differentiation. *Science.* 1999; 283:1183–1186. [PubMed: 10024247]
70. Buelens C, Bartholomé EJ, Amraoui Z, et al. Interleukin-3 and interferon beta cooperate to induce differentiation of monocytes into dendritic cells with potent helper T-cell stimulatory properties. *Blood.* 2002; 99:993–998. [PubMed: 11807004]
71. Ebner S, Hofer S, Nguyen VA, et al. A novel role for IL-3: human monocytes cultured in the presence of IL-3 and IL-4 differentiate into dendritic cells that produce less IL-12 and shift Th cell responses toward a Th2 cytokine pattern. *J. Immunol.* 2002; 168:6199–6207. [PubMed: 12055233]
72. Nicolette CA, Healey D, Tcherepanova I, et al. Dendritic cells for active immunotherapy: Optimizing design and manufacture in order to develop commercially and clinically viable products. *Vaccine.* 2007; 25:B47–B60. [PubMed: 17669561]
73. Giordano R, Lazzari L, Rebulla P. Clinical grade cell manipulation. *Vox Sang.* 2004 Aug; 87:65–72. [PubMed: 15355496]
74. Whiteside TL. Evaluation of Dendritic Cell Products Generated for Human Therapy and Post-Treatment Immune Monitoring. *BioPharm International.* 2008; 21:42–67.
75. Soncin S, Lo Cicero V, Astori G, Soldati G, Gola M, Sürder D, Moccetti T. A practical approach for the validation of sterility, endotoxin and potency testing of bone marrow mononucleated cells used in cardiac regeneration in compliance with good manufacturing practice. *J Transl Med.* 2009; 7:78. [PubMed: 19737416]
76. Meyer-Wentrup F, Burdach S. Efficacy of dendritic cell generation for clinical use: recovery and purity of monocytes and mature dendritic cells after immunomagnetic sorting or adherence selection of CD14+ starting populations. *J Hematother Stem Cell Res.* 2003; 12:289–299. [PubMed: 12857370]
77. Ishri RK, Menzies S, Hersey P, Halliday GM. Rapid downregulation of antigen processing enzymes in ex vivo generated human monocyte derived dendritic cells occur endogenously in extended cultures. *Immunol Cell Biol.* 2004; 82:239–246. [PubMed: 15186253]
78. Butterfield LH, Gooding W, Whiteside TL. Development of a potency assay for human dendritic cells: IL-12p70 production. *J Immunother.* 2008; 31:89–100. [PubMed: 18157016]
79. Shankar G, Fourrier MS, Grevenkamp MA, Lodge PA. Validation of the COSTIM bioassay for dendritic cell potency. *J Pharm Biomed Anal.* 2004; 36:285–294. [PubMed: 15496321]
80. Whiteside TL. Immune monitoring of clinical trials with biotherapies. *Adv Clin Chem.* 2008; 45:75–97. [PubMed: 18429494]
81. Baban B, Chandler PR, Johnson BA, et al. Physiologic control of IDO competence in splenic dendritic cells. *J Immunol.* 2011; 187:2329–2335. [PubMed: 21813777]
82. Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat Rev Immunol.* 2007; 7:610–621. [PubMed: 17627284]
83. Munn DH, Sharma MD, Hou D, et al. Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J Clin Invest.* 1994; 114:280–290. [PubMed: 15254595]
84. Mellor AL, Chandler P, Baban B, et al. Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2, 3 dioxygenase. *Int Immunol.* 2004; 16:1391–1401. [PubMed: 15351783]

85. Mellor AL, Baban B, Chandler PR, Manlapat A, Kahler DJ, Munn DH. Cutting edge: CpG oligonucleotides induce splenic CD19+ dendritic cells to acquire potent indoleamine 2,3-dioxygenase-dependent T cell regulatory functions via IFN Type 1 signaling. *J. Immunol.* 2005; 175:5601–5605. [PubMed: 16237046]
86. Mellor AL, Munn DH. Creating immune privilege: Active local suppression that benefits friends, but protects foes. *Nat Rev Immunol.* 2008; 8:74–80. [PubMed: 18064049]
87. Muller AJ, Sharma MD, Chandler PR, et al. Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc Natl Acad Sci USA.* 2008; 105:17073–17078. [PubMed: 18952840]
88. Sharma MD, et al. Chronic inflammation that facilitates tumor progression creates local nodes activate mature Tregs via indoleamine 2,3-dioxygenase. *J Clin Invest.* 2007; 117:2570–2582. [PubMed: 17710230]
89. Sørensen RB, Hadrup SR, Svane IM, Hjortsø MC, Thor Straten P, Andersen MH. Indoleamine 2, 3-dioxygenase specific cytotoxic T cells as immune regulators. *Blood.* 2011; 117:2200–2210. [PubMed: 21079151]
90. Gurtner GJ, Newberry RD, Schloemann SR, McDonald KG, Stenson WF. Inhibition of indoleamine 2,3-dioxygenase augments tri nitro benzene sulfonic acid colitis in mice. *Gastroenterology.* 2003; 125:1762–1773. [PubMed: 14724829]
91. Kwidzinski E, Bunse J, Aktas O, et al. Indoleamine 2, 3-dioxygenase is expressed in the CNS and downregulates autoimmune inflammation. *FASEB J.* 2005; 19:1347–1349. [PubMed: 15939737]
92. Adorini L, Lombardi G, Vasquez YR. *Dendritic Cells Handbook of Experimental Pharmacology.* 2009; Volume 188(II):165–190.
93. Osada T, Clay TM, Woo CY, Morse MA, Lysterly HK. Dendritic cell-based immunotherapy. *Int Rev Immunol.* 2007; 25:377–413. [PubMed: 17169781]

Figure 1.

Application of CD19⁺ IDO⁺ DCs in autoimmune, transplantation and cancer therapy. CD19⁺ IDO⁺ DCs may be used for treatment of both autoimmune diseases and cancers after stimulation with IDO inducers (such as CpG, soluble CTLA-4 and IFN- γ) and IDO inhibitors (such as 1-MT), respectively.