

Review Article

Dendritic Cells The Tumor Microenvironment and the Challenges for an Effective Antitumor Vaccination

Fabian Benencia,^{1,2,3} Leslee Sprague,¹ John McGinty,³ Michelle Pate,³ and Maria Muccioli²

¹Biomedical Engineering Program, Russ College of Engineering and Technology, Ohio University, Athens, OH 45701-2979, USA

²Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701-2979, USA

³Department of Biomedical Sciences, Heritage College of Osteopathic Medicine, Ohio University, Athens, OH 45701-2979, USA

Correspondence should be addressed to Fabian Benencia, benencia@oucom.ohiou.edu

Received 15 August 2011; Revised 28 October 2011; Accepted 11 November 2011

Academic Editor: Wolfgang Herr

Copyright © 2012 Fabian Benencia et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Many clinical trials have been carried out or are in progress to assess the therapeutic potential of dendritic-cell- (DC-) based vaccines on cancer patients, and recently the first DC-based vaccine for human cancer was approved by the FDA. Herewith, we describe the general characteristics of DCs and different strategies to generate effective antitumor DC vaccines. In recent years, the relevance of the tumor microenvironment in the progression of cancer has been highlighted. It has been shown that the tumor microenvironment is capable of inactivating various components of the immune system responsible for tumor clearance. In particular, the effect of the tumor microenvironment on antigen-presenting cells, such as DCs, does not only render these immune cells unable to induce specific immune responses, but also turns them into promoters of tumor growth. We also describe strategies likely to increase the efficacy of DC vaccines by reprogramming the immunosuppressive nature of the tumor microenvironment.

1. General Characteristics of Dendritic Cells

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) found in peripheral tissues and in immunological organs such as the thymus, bone marrow, spleen, lymph nodes, and Peyer's patches [1–3]. Their function is to scan peripheral tissues where they recognize, take up and process pathogens and present pathogen-derived antigenic peptides in the context of major histocompatibility molecules (MHCs) to naive T lymphocytes at lymphoid organs [4, 5]. Through these processes, DCs form a critical link between innate and adaptive immunity and are essential for the development of antigen-specific immune responses. To understand how DCs function in the development of adaptive immunity and the role of DCs in disease, one must first understand the distinguishing characteristics of innate and adaptive immunity.

Innate immunity is the first response to an immunological challenge, and the onset of an innate immune response is very rapid. Once a foreign pathogen breaches the outer

barrier of the skin and enters the body, several innate immune cells are present to resolve this challenge. Some of the key immune cells that participate in the innate immune response include macrophages, granulocytes, DCs, and natural killer (NK) cells. Macrophages, along with granulocytes and DCs, are all phagocytic cells found in tissues. After taking up a pathogen, these phagocytic cells are able to eliminate it through several mechanisms such as reactive oxygen or nitrogen species. The means by which pathogens are detected by phagocytes is through the expression of conserved pathogen-associated molecular patterns (PAMPs) present on the cell surface of the pathogen. These PAMPs are detected by pattern recognition receptors (PRRs) expressed on the cell surface of the phagocyte. Through pathogen recognition by PRRs, the phagocytes of the innate immune response are able to distinguish between self and foreign (non-self) cells. Some of the main PRRs active in innate immunity include Toll-like receptors (TLRs) and NOD-like receptors (NLRs) [6, 7].

2. DC Activation Process

Immature DCs present in peripheral tissues can detect foreign PAMP-bearing microorganisms through their high expression of cell surface and vesicular PRRs [8]. Following recognition, DCs take up pathogens by phagocytosis and process them into peptide fragments [3]. Since not all pathogens are eliminated by the innate immunity, an adaptive immune response may be needed to target antigenic epitopes associated with the pathogen to resolve the immunological threat completely. Antigenic peptide fragments derived from the processed pathogen are bound and presented on the DC surface by MHC molecules. These MHC molecules can evoke the adaptive immune response by presenting antigenic peptides to naïve T cell receptors [3].

An immature DC that has processed a pathogen will undergo maturation in the presence of proinflammatory cytokines and migrate to lymphoid regions where it can present the antigen peptide to naïve T lymphocytes [3, 4]. The maturation process involves upregulation of MHC class II molecules, costimulatory molecules such as CD40, CD80, CD86, and OX40L, and the chemokine receptor CCR7, while downregulating the expression of the chemokine receptor CCR6. Upon maturation, DCs show a decrease in their phagocytic capability, an augment in their efficacy to present processed antigens in the context of MHC molecules, and consequently an improved capability to activate T cells. Chemokines CCL19 (ELC) and CCL21 (SLC), ligands for CCR7, are constitutively expressed at high levels in lymph nodes [9]. Thus, mature DCs migrate from the sites of antigen capture to T-cell regions of draining lymph nodes, where they contact naïve or memory T cells and initiate a specific immune response [3, 10]. In this manner, DCs form the vital link between innate and adaptive immunity.

3. DCs Subsets in the Mouse

Murine DCs have been broadly divided into myeloid and plasmacytoid populations. The myeloid DCs, currently termed conventional DCs (cDCs), are further subdivided into several subsets present in immune and nonimmune tissues and organs specialized to perform different functions as described below. CD11c has been used as a typical marker of murine cDCs although additional markers have been used to distinguish these cells from other leukocytes such as NK cells and B cells that can also express it. Indeed, all cDC populations (except pre-DCs) are characterized by expressing high levels of CD11c [11, 12]. In the steady state cDCs present in lymphoid organs and tissues originated from bone marrow precursors. As extensively reviewed by Liu and Nussenzweig, 2010 [11], the mouse bone marrow harbors a common DC precursor (CDP) characterized by high expression of CD115 and Flt3, low expression of CD117 (CD117^{lo}), and is negative for lineage markers CD3, NK1.1, B220, TER-119, and Gr-1 (Lin⁻) [13]. This precursor is derived from a common monocyte and DC precursor also present in the bone marrow [11, 13]. The CDP gives rise to a pre-DC circulating precursor (CD11c⁺MHCII⁻SIRPα^{lo}) that rapidly reaches the lymphoid

organs or tissues [11, 14]. As shown in Figure 1, two major DC subpopulations are present in mouse spleen in the steady state, CD11c^{hi}MHCII⁺CD8α⁺CD205⁺SIRPα⁻CD11b⁻ and CD11c^{hi}MHCII⁺CD8α⁻33D1⁺SIRPα⁺CD11b⁺ cells [11, 12]. As determined by elegant studies performed by Dudziak et al. 2007, [15], the CD8α⁺ DC subpopulation is specialized in cross-presentation, primarily presenting peptides associated with MHC-I antigens, while the CD8α⁻ subpopulation is involved in presenting MHC-II-associated peptides. It has been proposed that the CD8α⁺ splenic population exclusively expresses the chemokine receptor XCR1, thus being an excellent marker to investigate this subpopulation in other species [16]. It has been recently reported that this marker is also expressed by lymphatic resident and migratory CD8α⁺ DCs [17], suggesting a common origin for these cells. In addition, it has been shown that the transcription factor *Batf3* is selectively required for the development of CD8α⁺ DC subset [18]. Although cDCs were previously considered to be terminal mature cells, growing evidence has determined that around 5% of spleen DCs are actively dividing at any given time [11, 12, 14, 19].

Similar CD8α⁺ and CD8α⁻ DC populations to the ones observed in mouse spleen are present in the lymph nodes and thymus [11, 12]. In addition a CD11c⁺MHCII^{hi}langerin⁺CD40^{hi} DC migratory subpopulation has been detected at the level of lymph node and tissues [11]. cDC subpopulations have also been characterized as CD11c^{hi}MHC⁺CD103⁺CD11b⁻ or CD11c^{hi}MHC⁺CD103⁻CD11b^{hi} in different organs such as the liver, lung, and kidney [11]. At the level of the intestine, cDCs are populating both Peyer patches (CD11c^{hi}MHC⁺CD103⁺CD11b^{lo}CX3CR1⁻ and CD11c^{hi}MHC⁺CD103⁻CD11b^{hi}CX3CR1⁺) and lamina propria (CD11c^{hi}MHC⁺CD103⁺CD11b⁺CX3CR1⁻) [11]. Finally, the skin presents a particular subtype of DCs, the LCs, which are considered to be derived from a pre-LC precursor. These cells are characterized by expression of CD103⁺CD11b^{lo}langerin⁺ or CD103⁻CD11b^{hi}langerin⁻ (both in the dermis) and CD11c^{hi}CD205^{lo}langerin⁺EpCAM^{hi} (epidermis) [20]. It has been reported that epidermal Langerhans cells and langerin dermal DCs constitute the vast majority of skin DCs, while langerin dermal DCs represent 5% of all skin DCs [20].

Finally, conventional CD8α⁺ DC and CD103⁺ DCs present in different nonimmune tissues express similar *Batf3* requirements, indicating that they might be closely developmentally related [21].

The other main subset of DCs is comprised by plasmacytoid DCs (pDCs). In the mouse, these cells, also derived from the CDP [14], are characterized by the expression of B220, CD45RB, low or null levels of CD11c, and no CD11b [22]. Circulating pDCs have the capability of producing large amounts of type 1 IFN in response to viral infections [22, 23] and so are key mediators of the innate immune response against viruses.

Different protocols have been developed in order to generate murine DC cultures. Usually, these cells are differentiated *in vitro* from bone marrow precursors using GM-CSF alone or in combination with IL-4 [24–26]. The use of

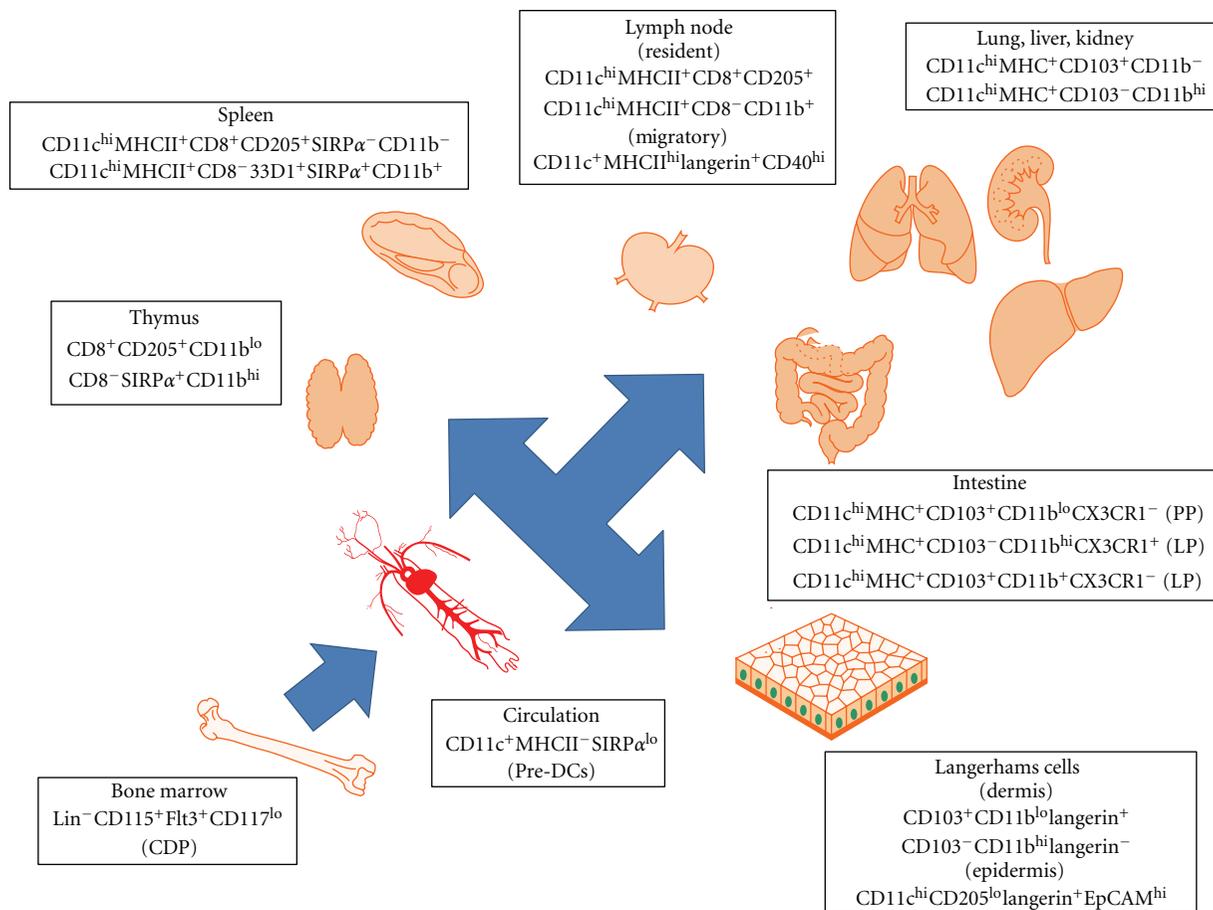


FIGURE 1: Conventional murine DCs in the steady state. Several DC subpopulations have been described in the mouse model colonizing lymphoid organs and other tissues. Figure adapted from Motifolio Biomedical Toolkit Suite.

GM-CSF and/or IL-4 generates high amounts of dendritic cells, capable of stimulating T cells *in vitro* and *in vivo*, which have been extensively used in order to investigate DC: T cell interactions, determine the efficacy of DC-based vaccines, and determine their role in pathological conditions such as infectious diseases or tumor models [27–34]. Alternatively, *in vitro* generated DCs can be obtained from bone marrow progenitors by treatment with fms-related tyrosine kinase 3 ligand (Flt3) and cytokines such as IL-6, stem cell factor, IL3, or insulin-like growth factor [25, 26, 35]. The DC populations generated upon culture of these precursors with Flt3 have been considered to more closely resemble CD8α⁺ splenic DCs, particularly in their capability of producing IL-12 and/or cross-present antigens, although lacking expression of CD8α [36]. Finally, Flt3 can be also used for expansion of murine DCs *in vivo* [35, 37].

4. Murine DC Subsets during Inflammation and Disease

It has been postulated that in the steady state murine DCs only originate from DC precursors, while during inflammatory or pathological settings they might also arise from

monocytes and colonize lymphoid organs or nonimmune tissues [38–42]. In addition, it has also been demonstrated that, upon CD11c depletion, monocytes can contribute to DC repopulation at the level of the intestine [43]. Recent data has challenged this, suggesting that even in the steady state some DC populations can arise from monocytes [44]. In particular, as reported by Jakubzick et al., 2008 [45], in the absence of inflammation CD103⁺ and CD11b^{hi} pulmonary DCs can, respectively, originate from two different monocyte populations characterized by the high or low expression of Ly-6.

Nevertheless, particular DC populations are generated under inflammatory conditions. For example, it has been shown that a DC subset specialized in generating high levels of TNFα and upregulating nitric oxide synthase II is originated from monocytes during bacterial infections [40]. These TNF/iNOS-producing (Tip) DCs are recruited to the spleen via CCR2 signaling and have been shown to mediate the innate immune response against *Listeria monocytogenes*, an intracellular bacterial pathogen [38].

The generation of particular DC populations has also been observed in pathological conditions such as cancer. For example, a DC subset with cytotoxic activity has been described in the last years. This subset, named killer

DC, is characterized by coexpression of B220 and NK1.1 receptors and is able to kill tumor cells, thus preventing tumor growth when used in adoptive therapies [46–49]. These B220⁺CD11c⁺NK1.1⁺ DCs produce large amounts of interferon γ (IFN γ) and are named IFN-producing killer DCs (IKDCs). *In vitro* studies using fusokines (molecules generated by fusing different chemokines) have shown that murine monocytes can be transformed into inducible killer DCs with the capability of inducing apoptosis of tumor cells without losing their antigen presenting capabilities [50]. In addition, treatment of bone marrow precursors with MHC-I peptides in the context of a ligand epitope antigen presentation system (LEAPS) is able to generate yet another DC population characterized by expression of levels of IL-12, thus being able to promote and steer immunity towards a specific T helper-1 (Th1) response [51, 52].

Another subset of DCs described in tumor settings is restricted to the spleen, express CD19, and suppresses T cells responses via indoleamine 2,3-dioxygenase (IDO) expression [53–56]. The expression of IDO in these cells is triggered upon CTLA4-mediated ligation of CD80 or CD86 molecules [53].

Adding to the complexity of DC subsets, it has been shown that some DC populations can change their phenotype under pathological settings. For example, pDCs could acquire cDC characteristics under the influence of viral infection [57]. This DC plasticity was evidenced by pioneering work showing that CD8 α ⁻ DCs can give rise to other splenic DC subpopulations [58].

5. DCs in Humans

Characterization of DC populations in humans is challenging due to their low numbers in circulation (less than 1% of blood mononuclear cells) and limited availability of healthy tissues as opposed to animal models. As in the mouse, human circulating DCs are broadly divided into pDCs and cDCs, characterized by expression of MHC-II and CD11c⁻CD123⁺ (plasmacytoid) or CD11c⁺CD123⁻ (conventional) antigens. cDCs have been further divided into those characterized by the expression of CD16, CD1c (BDCA-1), and CD141 (BDCA-3) [1, 59]. As described in detail by MacDonald et al., 2002 [59], the circulating cDC population was composed by 40%–80% of CD16⁺ DCs, 20% to 50% of BDCA1⁺ DCs, and 2% to 3% of BDCA3⁺ DCs. Much effort has been put into determining the homology of these populations to murine CD8 α ⁺ and CD8 α ⁻ DC populations, although human cDCs do not express this marker. Recent reports indicate that BDCA3⁺ DCs might be the putative homologues of murine CD8 α ⁺ DCs due to their expression of TLR-3, baft3 [60], and XCR1 [16, 17, 61], their capability of producing IL-12 upon stimulation [60], and their higher capability of cross-presenting antigen when compared to CD16⁺ and BDCA1⁺ DCs [60–62]. These DC populations can be also detected in human spleens [60]. On the contrary, these cells do not express TLR9 as their murine putative counterparts [60]. In addition, array analysis clustered together human BDCA3⁺ with mouse CD8 α ⁺ and human BDCA1⁺ with murine CD8 α ⁻ DCs [63].

Three different DC subsets have been described in human skin characterized by expression of CD1a^{high}CD14⁻HLA-DR⁺, CD1a^{dim}CD14⁻HLA-DR⁺ DCs, and CD1a⁻CD14⁺HLA-DR⁺ DCs [64]. CD1a^{high}CD14⁻HLA-DR⁺ Langerhans cells reside in the epidermis, while the other subsets reside in the dermis but contrary to what happens in the mouse they do not express langerin [64].

Recently, 2 skin-derived and 2 resident human cDC subsets were described in skin-draining lymph nodes characterized by the expression of CD1a⁺CD11c^{int} langerin⁺E-cadherin⁺ (skin Langerhans cells); CD1a⁺CD11c^{hi} and variable expression of langerin contrary to what was described above (dermal Langerhans cells); CD14⁻BDCA3/CD141^{hi}CD103⁻ and CD14⁺BDCA3^{lo}CD103⁺ [65].

Finally, in order to generate high amount of DCs for vaccination purposes, these cells have been prepared *ex vivo* from monocytes or CD34⁺ precursors [66–68].

6. DCs and T Cell Responses: The Four Signals

DCs play a multitude of roles in the development of an antigen-specific immune response. Through the expression of both MHC class I and MHC class II molecules, DCs are able to interact with and activate naïve CD8⁺ T cytotoxic and naïve CD4⁺ T helper lymphocytes, respectively [7, 10, 69]. For a naïve T lymphocyte to become an effector cell different signals are required. The first signal comes from the direct interaction of the T cell receptor (TCR) of the naïve T lymphocyte with the peptide bound to the MHC molecule (Signal 1). The second signal required for naïve T cell activation comes from DC: T cell interactions through costimulatory molecules such as CD80 and CD86 on the DC surface with CD28 on the T cell surface (Signal 2). If costimulatory signaling fails to occur, the T lymphocyte will not become activated and T cell anergy will ensue. The third signal derived from DCs, which can lead to a specific immune response, is T-cell differentiation through cytokine signaling (Signal 3). There are multiple T helper subsets, and the differentiation of naïve CD4⁺ T helper cells into activated effector T helper cells is directed by DC-derived cytokines. Recently, it has been proposed that DCs give an additional signal to T cells [70]. This signal 4 instructs T cells to migrate to particular tissues by inducing the expression of specific chemokine receptors and integrins in these cells upon interaction with antigen-pulsed DCs [70].

Effective activation of T cells will depend in the end on the levels of expression and the interplay between positive and negative costimulatory molecules in both DCs and T cells. For example, antigen uptake in the absence of inflammatory signals renders phenotypically immature DCs, expressing low levels of MHC-II and costimulatory molecules. Importantly, antigen presentation in the absence of effective positive costimulation can lead to T-cell anergy and tolerance [71]. These DCs are considered “tolerogenic” in comparison to “immunogenic” DCs capable of inducing potent specific immune responses. Interestingly, DCs can switch from immunogenic to tolerogenic depending on the microenvironment conditions. For example, viral infections

can differentiate pDCs into T-helper-1- (Th1-) inducing DCs [57] while IL-3 can induce Th1-inducing DCs to differentiate into Th-2-inducing ones [72].

7. Properties of the Tumor Microenvironment

Tumors are composed not only by tumor cells, but also by other cellular types such as fibroblasts, endothelial cells, and infiltrating leukocytes that together with extracellular matrix components constitute the microenvironment of the tumor. In recent years the relevance of the tumor microenvironment as a key player in tumor development has been highlighted and the role of its different populations investigated. The protective role of the immune system against tumors has been widely described and tumor-infiltrating lymphocytes, for example, have been associated with improved survival of patients with melanoma, prostate, breast, colorectal, and ovarian carcinomas, among others [73–76]. On the contrary, tumor-associated leukocytes such as regulatory T cells (Treg) or myeloid-derived suppressor cells (MDSCs) can promote tumor growth by inhibiting antitumor immune responses [77, 78]. Indeed, we have previously demonstrated the relevance of the tumor microenvironment in attracting MDSCs by a complement-mediated process [79]. Further, in a tumor setting a subset of spleen DCs with the capability of suppressing T cells responses via indoleamine 2,3-dioxygenase (IDO) expression has been described [53].

In addition to suppressing the immune response, tumor-associated leukocytes can also promote angiogenesis. Leukocyte infiltration can precede the development of a neoplasm, with being chronic inflammation being an important risk factor for the development of cancer [80–82]. Indeed, inflammatory conditions such as those caused by certain types of infections can be involved in the pathogenesis of many human malignancies. For example, gastric carcinomas can arise in a *H. pylori*-induced gastritis environment [81] or hepatitis B virus/hepatitis C virus can induce hepatocellular carcinomas [82]. Also, chronic but noninfective inflammatory conditions as in the case of smoking-related bronchial cancer can induce carcinogenesis [83]. In the same way, chronic pancreatitis is considered a risk factor for the development of pancreatic cancer, and many of the growth factors involved in tissue remodeling and regeneration in chronic pancreatitis are present in pancreatic cancer [84]. In particular, infiltrating inflammatory cells secrete a diverse repertoire of growth factors and proteases that enhance tumor growth by stimulating angiogenesis. We and others have described the capability of antigen presenting cells such as DCs or macrophages, to collaborate with neoangiogenesis in human cancers and in different mouse tumor models [5, 85–89].

8. Characteristics of Tumor-Associated DCs

DCs are conspicuous members of the microenvironment of several types of cancer [86, 90–93]. Tumor-associated cytokines such as vascular endothelial growth factor (VEGF),

interleukin- (IL-) 10, and prostaglandin E-2 (PGE2) can profoundly affect the nature of DCs [94]. Several reports indicated that tumor-associated DCs (TA-DCs) are immunosuppressive, incapable of inducing specific immune responses, or can induce regulatory T cell expansion. In particular, DCs showing low levels of costimulatory molecules have been detected in tumors expressing high levels of VEGF [95]. But besides an immune “paralysis,” we and others have shown that TA-DCs, or leukocyte expressing DC markers, are able to produce angiogenic factors and can promote angiogenic processes in the tumor microenvironment [79, 86, 93, 96].

Tumors require blood supply for expansive growth. With increasing distance from vessels, hypoxic tumor cells produce angiogenic factors that induce the formation of neovessels [97–99]. Until recently, angiogenesis, or sprouting of endothelial cells from existing vessels, was the only accepted mechanism of tumor vascularization. Recent studies have suggested that vasculogenesis, or recruitment of endothelial progenitors that differentiate into endothelial cells, might contribute to the formation of tumor neovessels [100]. Endothelial cell progenitors were first identified by expression of the hematopoietic stem cell antigens, CD34 and flk-1, and other hematopoietic stem cell antigens, such as CD133 (AC133) [100]. Several populations of hematopoietic cells assume an endothelial phenotype when cultured under proangiogenic conditions. These include CD34⁺, Sca1⁺, CD133⁺, and CD14⁺ cells. In particular, the capability a CD34⁻ monocytes to differentiate into endothelial-like cells *in vitro* has been reported [101–103]. Further, different studies have demonstrated that monocytes or monocyte-like cells can also function as endothelial cell progenitors and incorporate into growing vasculature in experimental models [104–106]. For example it has been recently shown that monocytes, under the influence of proteins present in the tumor microenvironment such as pleiotrophin or M-CSF, transdifferentiate into endothelial cells that incorporate into tumor blood vessels [107]. In addition, interaction of monocytes with extracellular matrix components such as fibronectin might also contribute to the monocyte-endothelial cell transdifferentiation process [108].

We and others have shown that DCs cultured in the presence of tumor factors can undergo an endothelialization process characterized by the loss of CD14/CD45 and displayed endothelial markers such as CD31, CD34, von Willebrand factor, vascular-endothelial-growth-factor-receptor- (VEGFR-) 2, and VE-Cadherin [85, 109–112]. Furthermore, as we and others have shown, DCs can display other characteristics of endothelial cells such as LDL uptake, lectin binding, and formation of cord-like structures in 3D gels [85, 109, 110] and are able to assemble into vascular structures *in vitro* and *in vivo*, [85, 109, 110]. Although this evidence suggests that DCs can transdifferentiate into endothelial cells, the capability of these cells of acting as *bonafide* endothelial cells is debatable. For example, we have shown that tumor-associated DC precursors purified from mouse or human ovarian carcinomas are able to participate in the generation of neovessels *in vivo* [85, 88]. A follow-up study by Huarte et al., 2008 [113], demonstrated that these cells localize at the pericyte level *in vivo* in a mouse

model of ovarian carcinoma, acting as a scaffold for the generation of neovessels. Indeed, it has been shown that DCs have the capability of intimately interacting with endothelial cells and help to stabilize newly expanded vasculature at the level of lymph nodes [114]. Thus, it is tempting to speculate that in some tumor settings, this pericyte-like function of DCs might help shape the characteristics of tumor endothelium. In addition, DCs can also contribute to angiogenesis by producing factors that promote growth of *bonafide* endothelial cells [115].

9. Dendritic Cells as a Source of Angiogenic Factors

We have recently shown that myeloid DCs are able to produce a gamut of angiogenic molecules *in vitro* such as matrix metalloproteases, VEGF, angiogenin, heparanase, and basis fibroblast growth factors [116]. We have also previously shown that DC precursors participate in tumor progression and angiogenesis in a mouse model of ovarian cancer [85]. For those studies, we used the *ID8-Defb29/Vegf-A* mouse model of ovarian carcinoma. ID8 is a cell line derived from spontaneous *in vitro* malignant transformation of C57BL/6 mouse ovarian surface epithelial cells that we engineered to express mouse β -defensin 29 and VEGF-A. Our published data support that this model mimics the pathophysiology of human ovarian cancer which expresses both β -defensins and levels of VEGF-A similar to our model. *ID8-Defb29/Vegf-A* tumor cells are able to generate solid tumor or ascites when injected into syngeneic C57BL/6 mice subcutaneously or via the intraperitoneal route respectively. In this tumor model, immature DCs contribute to ovarian cancer progression by acquiring a proangiogenic phenotype in response to VEGF via VEGF-R2 [88, 115, 117]. Further, it has been shown that depletion of TA-DCs *in vivo* reduces tumor growth and decreases angiogenesis in this mouse model of ovarian cancer [113, 118]. In the same way, data from the late Dr. J. Folkman's lab [119] highlighted the contribution of DCs to angiogenesis in a murine model of endometriosis and in the peritoneal Lewis lung carcinoma tumor model. Similar to what we observed in our model, they showed that these proangiogenic DCs have an immature phenotype, and express VEGF-R2.

Taking into account all these data, it becomes clear that tumors have the capability to attract and reprogram the biology of DCs, inducing them to exert immunosuppressive or angiogenic functions.

10. Dendritic Cells and Antitumor Therapy

Considerable effort has been made in order to develop strategies for using DCs to induce tumor-specific immunity, including nearly 100 clinical trials designed to evaluate their safety or efficacy in humans [120]. The goal of DC-based vaccination for antitumor therapy is to stimulate robust and long lasting specific CD4 and CD8 T cell responses [121]. To accomplish this, several studies have been performed in order to generate DCs with the capability of inducing robust T cell

responses. For vaccination studies, DCs have been generated from bone marrow precursors in the mouse and mostly from monocytes in humans as described above. Different steps in the antigen presentation process have been evaluated such as antigen loading, DC maturation, and delivery route and dose scheme.

Assayed methods of loading DCs with tumor-associated antigens in the mouse model included pulsing the cells with peptides derived from tumor antigens [122], whole tumor lysates [123], apoptotic or necrotic cells [124] alone or conjugated with toll-like receptor ligands [125], or antigens coated with antibodies to target them to DCs via Fc γ receptors [126]. We have showed that inducing the expression of danger signals in tumor cells by means of replication-deficient or replication-restricted virus appears to be an efficient method to pulse DCs for vaccination purposes [124]. In addition, other strategies include encapsulating peptides in biodegradable polymers that are phagocytized by DCs [127], preparing DCs fused with tumor cells [128], or pulsing DCs with RNA encoding tumor antigens [129]. In recent years, the use of lentiviruses to induce stable transduction of DCs has also been successfully evaluated [130–132]. These vectors have the advantage of infecting nondividing cells, thus being excellent tools to express different molecules in DCs. Moreover, hematopoietic stem cells have been transduced with lentiviruses and then differentiated into antigen-expressing DCs [133].

Similar studies have been performed using human DCs. Among other strategies, these cells have been pulsed *in vitro* with apoptotic or necrotic cells [134, 135], with nucleic acids encoding tumor antigens [136, 137], or fused with tumor cells [138] or pulsing DCs. An alternate method for loading DCs with tumor antigen involves the insertion of full-length antigens by genetic modification using viral systems. Vector-transferred recombinant antigens synthesized in the cytosol of the cells may enter the degradation process of intracellular molecules, yielding peptides that can be directly presented by MHC-I molecules. Several viral vectors have been used to transduce human DCs [139] including recombinant adenoviruses [140–142], poxviruses [143], and retroviruses [139]. Lentiviruses have also been used to induce stable transduction of human hematopoietic stem cells or DCs [144, 145].

This information regarding DC pulsing have been translated to the human, where clinical trials have involved, among others, DCs pulsed with peptides [146], whole-tumor lysates [147], with RNA encoding tumor antigens [27, 148, 149], or fused with tumor cells [150, 151].

In order to improve DC-based vaccines for human therapy, different methods to induce DC maturation and optimization of antigen processing and presentation have also been proposed [121, 152]. The most widely used maturation protocol for human monocyte-derived DCs employs the combination of IL-6, tumor-necrosis-factor- (TNF-) α , IL-1 β , and PGE-2. Although these *ex vivo* matured DCs have the capability to migrate towards lymph nodes, PGE-2 has been shown to induce the production of IL-10 and VEGF, which can be harmful in a tumor setting. Moreover, these maturation stimuli have been shown to generate mature DCs

capable of expanding regulatory T cells *in vitro* and *in vivo* [153, 154]. Alternative protocols using different TLR ligands have been extensively studied [155–157].

In recent years a different maturation cocktail has been tested on human DCs. This cocktail, named the α DC1 cocktail, is composed of a combination of cytokines and TLR ligands (IL-1 β /TNF α /IFN α /IFN γ /poly-I:C) [156, 158, 159]. The α DC1 cocktail has been suggested as a better option for maturation since treated DCs show higher migratory responses to SLC, a CCR7 ligand constitutively produced by lymph nodes, and produce higher levels of IL-12p70 as compared to DCs matured with TNF- α , IL-1 β , and PGE-2 [155]. But some data argues that this cocktail does not induce better T cell activation [160]. Other proposed maturation strategies for human DC vaccines involve activating tumor antigen-pulsed DCs with CD40 ligand before injection [161].

As previously reviewed in detail [162, 163], clinical trials with DC vaccines have used different methods of antigen pulsing, maturation status of the cells, route of administration, and dose scheme. The use of so many different strategies makes it difficult to interpret in detail the causes for the success or failure of the vaccinations, and argues for a consensus regarding DC preparation, maturation, and route and dose scheme for DC-based vaccinations.

11. The First FDA-Approved Antitumor DC Vaccine and the Challenges for Improvement

Recently, the first autologous cellular vaccine for antitumor therapies (Sipuleucel-T) has been approved by the Federal and Drug Administration (FDA) for the treatment of asymptomatic or minimally symptomatic metastatic castrate-resistant (hormone refractory) prostate cancer. In order to generate the vaccine, the patients are subjected to apheresis and the cells are cultured for 36–44 h in a media containing a synthetic protein generated by the fusion of prostatic acid phosphatase and GM-CSF [164]. Then, this preparation containing at least 50 million CD54⁺ antigen-presenting cells is infused back into the patient. In all, the process may consist of three cycles of apheresis, pulsing stimulation, and reinfusions [164]. Sipuleucel-T therapy has shown mild to moderate, short-term, reversible adverse events in patients with no evidence of a treatment-related increase in autoimmune complications or secondary malignancies [165]. The treatment generates an increase in patient survival. In particular, it has been reported in a clinical trial that the median survival in treated prostate cancer patients was 4.1 months longer (25.8 months) than in the placebo group (21.7 months) [166]. Taking into account the promising but rather modest increase in the patients' survival, it becomes clear that efforts must be done in order to improve the efficacy of DC-based vaccines. A recent clinical trial in human ovarian carcinoma shows that DC vaccine therapy induced an increase in the antitumor immune response in treated patients [167]. Interestingly, an impaired immune response against an unrelated vaccine antigen in the same

patients highlights the immunosuppressive status induced by tumors [167]. Thus, in order to increase the efficacy of these vaccines it might be interesting to block at the same time the deleterious influence of the tumor microenvironment. The role of the tumor microenvironment is also relevant, taking into account that most human DC vaccines are generated from monocytes, which, as described above, have a high plasticity and can change their phenotype in response to tumor factors.

12. Reprogramming the Tumor Microenvironment to Enhance DC Vaccination Efficacy

In general, although several reports indicate that DC vaccines are able to induce immune responses in cancer patients, they have only rarely resulted in objective clinical responses based on the response evaluation criteria in solid tumors (RECISTs) and no indication or evidence has been obtained that DC vaccines represent a method of stimulating protective immunity in cancer patients that is superior to other vaccination strategies [121]. One of the main reasons why DC vaccines have been suboptimal in clinical trials might be the inhibitory effect of the tumor microenvironment. As described above, the tumor microenvironment is highly immunosuppressive due the presence of soluble factors such as VEGF or IL-10 and immunosuppressive cell populations such as MDSCs and Treg. This can affect the efficacy of DC vaccination at different levels. First, there could be a direct effect of soluble factors on DC-based vaccines, impairing their immune capabilities. Indeed, it has been shown in a clinical trial that *ex vivo* matured DCs, loaded with tumor antigen, could be trapped by the tumor microenvironment, thus rendering the immunization completely ineffective [168]. Although this argues for an intranodal immunization with DC vaccines, factors produced by the tumor microenvironment can affect distal tissues [169]. For example, VEGF, which is produced by several tumors, can modify the immunological profile of lymph nodes, and the generation of immune precursors at primary and secondary lymphoid organs [95, 170]. Thus, not only TA-DCs might be affected by the tumor microenvironment. In addition, tumor-induced Treg and MDSCs can directly impair the antitumor properties of T effector cells induced by DC vaccines. Taking into account this, in the last years different strategies have been proposed in order to improve the efficacy of DC vaccines by reprogramming the immunosuppressive status of the tumor microenvironment.

The most common strategy has involved the combination of DC vaccination with Treg depletion. To accomplish this, Tregs have been depleted *in vivo* by antibody therapy with anti-CD25, a molecule expressed at high levels by Tregs. In the mouse, promising results were obtained in different tumor models, where it was reported that Treg depletion enhanced the efficacy of the vaccination [171–173]. In clinical trials, it has been shown that depletion of Tregs

before vaccination with DCs pulsed with carcinoembryonic antigen (CEA), enhanced specific T-cell immunity against CEA in patients having a metastatic cancer expressing CEA, as defined by immunohistochemical analysis or elevated CEA in peripheral blood [174]. In addition, depletion of Tregs also enhanced antitumor immunity in patients harboring metastatic renal cell carcinomas [175]. In both trials, Tregs were depleted by using denileukin diftitox, a fusion between the active domain of diphtheria toxin and IL-2 that binds cells expressing high levels of CD25. Upon internalization, this molecule leads to cell death due to blockade of protein synthesis. On the other hand, Jacobs et al., 2010 [176], demonstrated in a phase I/II study in metastatic melanoma that depletion of Tregs with daclizumab, a humanized antibody directed against CD25, did not enhance antitumor immunity in treated patients. These data point out that the efficacy of this combinatorial therapy might depend on the type of cancer and the strategies used to deplete the Treg population prior to vaccination.

A novel proposed combinatorial strategy assayed in the mouse model involved adding the use of CTLA-4 blockade to Treg depletion in the context of DC-based vaccination [177]. In this study, CTLA-4 blockade and depletion of Treg cells improved the potency of DC vaccination in a mouse model colon carcinoma expressing both CEA and HLA-A2 antigens.

Other proposed strategies involve elimination of tumor cells by radiotherapy, chemotherapy, antibody therapy, or viral oncolytic therapy in combination with DC vaccination as determined in animal models of lymphoma and prostate cancer, among others [178–181]. This aims to decrease the deleterious effect of tumor cell products while generating an inflammatory milieu that can enhance the efficacy of the vaccination. TA-DCs are usually described as immature cells with low expression of costimulatory molecule and, incapable of inducing robust antitumor immune responses [182–185]. In this way, elimination of cancer cells will not only generate tumor antigen that can be acquired by resident DCs, but also abrogate the immunosuppressive milieu generated by molecules produced by the same cancer cells. In this new milieu, DCs that have acquired tumor antigen might be able to turn into mature DCs, thus being able to induce effective immune responses.

Interestingly, depletion of TA-DCs from the tumor microenvironment of ovarian cancer has been shown to boost antitumor immune responses in the mouse model [113]. Thus, depletion of these cells prior to DC-based vaccination may induce effective antitumor immune responses. Finally, albeit generating a specific immune response, vaccination efforts may fail due to the incapability of T cells to reach their targets. Indeed, it has been previously shown that differential expression of endothelin receptor B in murine tumor endothelial cells determines the capability of T cells of infiltrating tumors [186]. Thus, future strategies designed to reprogram the tumor microenvironment to enhance the efficacy of DC vaccination might include blocking endothelin receptor in endothelial cells to facilitate cytotoxic T cell infiltration into the tumors.

13. Reprogramming DCs In Situ to Induce Better Antitumor Immunity

As described above, DCs are present in the microenvironment of different tumors, but they are usually cells with impaired capability of inducing antitumor effector T cells. A tantalizing strategy would be to reprogram these cells *in vivo*, transforming them into effective antigen-presenting cells. Different strategies are being developed and have been assayed in the mouse model in order to specifically target DCs *in situ*. For example, targeted delivery of antigens to DCs via specific molecules expressed on the surface of these cells has been investigated. Targeting ovalbumin to CD205 and 33D1 molecules on the surface of DCs *in vivo* helped identify the antigen presenting properties of CD8 α^+ and CD8 α^- DC subpopulations of splenic DCs [15]. Building on these studies, effective immunization procedures have been obtained by using antibody-tumor antigen fusion proteins targeting DCs via CD205 [187] or CD11c [188]. In addition, antibodies specific for DC molecules have been used to coat liposomes or nanoparticles in order to deliver antigens and inflammatory compounds to DCs *in situ* in the mouse model [189] or to target human DCs [190]. Other strategies involve generating DC vaccines that express tumor antigens under a specific DC promoter, such as CD11c variant [191], or engineering antigen-carrying lentiviral vectors capable of selectively binding to DCs [192].

In the context of a murine ovarian cancer model, pioneering research has been performed by the Conejo-Garcia group [193] in order to reprogram DCs *in situ*. By using a mouse model of ovarian cancer, this group was able to demonstrate that *in situ* activation of TA-DCs can induce a potent antitumor immune response, creating a *de facto* vaccine with these cells. In order to accomplish this, they reprogrammed TA-DCs by administration of linear polyethyleneimine nanoparticles encapsulating nonviral siRNA. These particles were avidly engulfed by TA-DCs, activating them through TLR5 and inducing a potent antitumor immune response. This strategy has the advantage of using the TA-DCs, which might already harbor tumor antigens [194]. If translatable to humans, this will avoid costly *ex vivo* preparation and pulsing of the patient's DCs. This *in situ* reprogramming of TA-DCs will benefit by combinatorial therapies destined to abrogate the immunosuppressive properties of the tumor microenvironment, such as using Treg depletion therapies.

14. Summary and Outlook for Future Development

Herewith we described that DCs comprise a population of leukocytes with the capability of inducing specific immune responses. These cells have the ability to capture antigens and select and activate T cells capable of recognizing and orchestrating an attack against the microbes or cells that harbor the same antigen. This property had made DCs ideal candidates for cellular vaccine therapies. DCs are divided into different subsets extensively investigated in the mouse

model. In recent years a similar complexity has started to unravel in humans. This heterogeneity is subjacent to a characteristic that seems to be a hallmark of these cells: their plasticity. It has been shown that these cells can modify their phenotype in response to microenvironmental factors. This characteristic seems to be exploited by tumors that not only repress the maturation of these cells, thus abrogating specific antitumor immune responses, but also transform them into promoters of angiogenesis. In the mouse model, it has been shown that DC-based vaccines can effectively induce antitumor immune responses. In humans, a cellular DC-based vaccine has been recently approved by the FDA for treatment of prostate cancer. In order to build on this promising scenario, combinatorial therapies destined to abrogate the deleterious influence of the tumor microenvironment are being investigated. This will render more powerful DC vaccines with the capability of generating a robust and long-lasting antitumor immune response.

References

- [1] R. Bonasio and U. H. von Andrian, "Generation, migration and function of circulating dendritic cells," *Current Opinion in Immunology*, vol. 18, no. 4, pp. 503–511, 2006.
- [2] A. Lanzavecchia and F. Sallusto, "The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics," *Current Opinion in Immunology*, vol. 13, no. 3, pp. 291–298, 2001.
- [3] J. Banchereau, F. Briere, C. Caux et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.
- [4] J. M. Timmerman and R. Levy, "Dendritic cell vaccines for cancer immunotherapy," *Annual Review of Medicine*, vol. 50, pp. 507–529, 1999.
- [5] E. Riboldi, T. Musso, E. Moroni et al., "Cutting edge: proangiogenic properties of alternatively activated dendritic cells," *Journal of Immunology*, vol. 175, no. 5, pp. 2788–2792, 2005.
- [6] M. Feldmann and R. N. Maini, "Discovery of TNF- α as a therapeutic target in rheumatoid arthritis: preclinical and clinical studies," *Joint Bone Spine*, vol. 69, no. 1, pp. 12–18, 2002.
- [7] M. L. Kapsenberg, "Dendritic-cell control of pathogen-driven T-cell polarization," *Nature Reviews Immunology*, vol. 3, no. 12, pp. 984–993, 2003.
- [8] T. M. Doherty, E. A. Fisher, and M. Ardit, "TLR signaling and trapped vascular dendritic cells in the development of atherosclerosis," *Trends in Immunology*, vol. 27, no. 5, pp. 222–227, 2006.
- [9] N. Sánchez-Sánchez, L. Riol-Blanco, G. De La Rosa et al., "Chemokine receptor CCR7 induces intracellular signaling that inhibits apoptosis of mature dendritic cells," *Blood*, vol. 104, no. 3, pp. 619–625, 2004.
- [10] N. Kadowaki, "Dendritic cells—a conductor of T cell differentiation," *Allergy International*, vol. 56, no. 3, pp. 193–199, 2007.
- [11] K. Liu and M. C. Nussenzweig, "Origin and development of dendritic cells," *Immunological Reviews*, vol. 234, no. 1, pp. 45–54, 2010.
- [12] K. Shortman and W. R. Heath, "The CD8 $^+$ dendritic cell subset," *Immunological Reviews*, vol. 234, no. 1, pp. 18–31, 2010.
- [13] K. Liu, G. D. Victora, T. A. Schwickert et al., "In vivo analysis of dendritic cell development and homeostasis," *Science*, vol. 324, no. 5925, pp. 392–397, 2009.
- [14] K. Liu, C. Waskow, X. Liu, K. Yao, J. Hoh, and M. Nussenzweig, "Origin of dendritic cells in peripheral lymphoid organs of mice," *Nature Immunology*, vol. 8, no. 6, pp. 578–583, 2007.
- [15] D. Dudziak, A. O. Kamphorst, G. F. Heidkamp et al., "Differential antigen processing by dendritic cell subsets in vivo," *Science*, vol. 315, no. 5808, pp. 107–111, 2007.
- [16] K. Crozat, R. Guiton, V. Contreras et al., "The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8 α^+ dendritic cells," *Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1283–1292, 2010.
- [17] K. Crozat, S. Tamoutounour, T.-P. Vu Manh et al., "Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the CD8 α^+ type," *Journal of Immunology*, vol. 187, no. 9, pp. 4411–4415, 2011.
- [18] K. Hildner, B. T. Edelson, W. E. Purtha et al., "Batf3 deficiency reveals a critical role for CD8 α^+ dendritic cells in cytotoxic T cell immunity," *Science*, vol. 322, no. 5904, pp. 1097–1100, 2008.
- [19] C. Waskow, K. Liu, G. Darrasse-Jèze et al., "The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues," *Nature Immunology*, vol. 9, no. 6, pp. 676–683, 2008.
- [20] K. Nagao, F. Ginhoux, W. W. Leitner et al., "Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 9, pp. 3312–3317, 2009.
- [21] B. T. Edelson, K. C. Wumesh, R. Juang et al., "Peripheral CD103 $^+$ dendritic cells form a unified subset developmentally related to CD8 α^+ conventional dendritic cells," *Journal of Experimental Medicine*, vol. 207, no. 4, pp. 823–836, 2010.
- [22] Y. J. Liu, "IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors," *Annual Review of Immunology*, vol. 23, pp. 275–306, 2005.
- [23] G. Penna, M. Vulcano, S. Sozzani, and L. Adorini, "Differential migration behavior and chemokine production by myeloid and plasmacytoid dendritic cells," *Human Immunology*, vol. 63, no. 12, pp. 1164–1171, 2002.
- [24] M. B. Lutz, N. Kukutsch, A. L. J. Ogilvie et al., "An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow," *Journal of Immunological Methods*, vol. 223, no. 1, pp. 77–92, 1999.
- [25] K. Inaba, M. Inaba, N. Romani et al., "Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor," *Journal of Experimental Medicine*, vol. 176, no. 6, pp. 1693–1702, 1992.
- [26] C. Masurier, C. Pioche-Durieu, B. M. Colombo et al., "Immunophenotypical and functional heterogeneity of dendritic cells generated from murine bone marrow cultured with different cytokine combinations: implications for antitumoral cell therapy," *Immunology*, vol. 96, no. 4, pp. 569–577, 1999.
- [27] E. Gilboa and J. Vieweg, "Cancer immunotherapy with mRNA-transfected dendritic cells," *Immunological Reviews*, vol. 199, pp. 251–263, 2004.
- [28] A. Grolleau-Julius, L. Abernathy, E. Harning, and R. L. Yung, "Mechanisms of murine dendritic cell antitumor dysfunction in aging," *Cancer Immunology, Immunotherapy*, vol. 58, no. 12, pp. 1935–1939, 2009.

- [29] P. A. MacAry, C. T. Too, and X. Dai, "Targeting tumours by adoptive transfer of immune cells," *Clinical and Experimental Pharmacology and Physiology*, vol. 33, no. 5-6, pp. 569-574, 2006.
- [30] N. R. Bianco, S. H. Kim, A. E. Morelli, and P. D. Robbins, "Modulation of the immune response using dendritic cell-derived exosomes," *Methods in Molecular Biology*, vol. 380, pp. 443-455, 2007.
- [31] C. Murdoch, M. Muthana, S. B. Coffelt, and C. E. Lewis, "The role of myeloid cells in the promotion of tumour angiogenesis," *Nature Reviews Cancer*, vol. 8, no. 8, pp. 618-631, 2008.
- [32] J. C. Simon, H. Hara, R. W. Denfeld, and S. Martin, "UVB-irradiated dendritic cells induce nonproliferating, regulatory type T cells," *Skin Pharmacology and Applied Skin Physiology*, vol. 15, no. 5, pp. 330-334, 2002.
- [33] S. Yamagami, T. Usui, S. Amano, and N. Ebihara, "Bone marrow-derived cells in mouse and human cornea," *Cornea*, vol. 24, no. 8, pp. S71-S74, 2005.
- [34] U. Yrliid, M. Svensson, C. Johansson, and M. J. Wick, "Salmonella infection of bone marrow-derived macrophages and dendritic cells: influence on antigen presentation and initiating an immune response," *FEMS Immunology and Medical Microbiology*, vol. 27, no. 4, pp. 313-320, 2000.
- [35] E. Maraskovsky, K. Brasel, M. Teepe et al., "Dramatic increase in the number of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified," *Journal of Experimental Medicine*, vol. 184, no. 5, pp. 1953-1962, 1996.
- [36] P. Brawand, D. R. Fitzpatrick, B. W. Greenfield, K. Brasel, C. R. Maliszewski, and T. De Smedt, "Murine plasmacytoid pre-dendritic cells generated from Flt3 ligand-supplemented bone marrow cultures are immature APCs," *Journal of Immunology*, vol. 169, no. 12, pp. 6711-6719, 2002.
- [37] T. Hieronymus, T. C. Gust, R. D. Kirsch et al., "Progressive and controlled development of mouse dendritic cells from Flt3⁺ CD11b⁺ progenitors in vitro," *Journal of Immunology*, vol. 174, no. 5, pp. 2552-2562, 2005.
- [38] N. V. Serbina, T. P. Salazar-Mather, C. A. Biron, W. A. Kuziel, and E. G. Pamer, "TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection," *Immunity*, vol. 19, no. 1, pp. 59-70, 2003.
- [39] P. M. Domínguez and C. Ardavin, "Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation," *Immunological Reviews*, vol. 234, no. 1, pp. 90-104, 2010.
- [40] D. Engel, U. Dobrindt, A. Tittel et al., "Tumor necrosis factor alpha- and inducible nitric oxide synthase-producing dendritic cells are rapidly recruited to the bladder in urinary tract infection but are dispensable for bacterial clearance," *Infection and Immunity*, vol. 74, no. 11, pp. 6100-6107, 2006.
- [41] H. Nakano, K. L. Lin, M. Yanagita et al., "Blood-derived inflammatory dendritic cells in lymph nodes stimulate acute T helper type 1 immune responses," *Nature Immunology*, vol. 10, no. 4, pp. 394-402, 2009.
- [42] K. Shortman and S. H. Naik, "Steady-state and inflammatory dendritic-cell development," *Nature Reviews Immunology*, vol. 7, no. 1, pp. 19-30, 2007.
- [43] C. Varol, L. Landsman, D. K. Fogg et al., "Monocytes give rise to mucosal, but not splenic, conventional dendritic cells," *Journal of Experimental Medicine*, vol. 204, no. 1, pp. 171-180, 2007.
- [44] L. Landsman, C. Varol, and S. Jung, "Distinct differentiation potential of blood monocyte subsets in the lung," *Journal of Immunology*, vol. 178, no. 4, pp. 2000-2007, 2007.
- [45] C. Jakubzick, F. Tacke, F. Ginhoux et al., "Blood monocyte subsets differentially give rise to CD103⁺ and CD103⁻ pulmonary dendritic cell populations," *Journal of Immunology*, vol. 180, no. 5, pp. 3019-3027, 2008.
- [46] J. Taieb, N. Chaput, C. Ménard et al., "A novel dendritic cell subset involved in tumor immunosurveillance," *Nature Medicine*, vol. 12, no. 2, pp. 214-219, 2006.
- [47] C. Chauvin, J. M. Philippeau, C. Hémond et al., "Killer dendritic cells link innate and adaptive immunity against established osteosarcoma in rats," *Cancer Research*, vol. 68, no. 22, pp. 9433-9440, 2008.
- [48] C. W. Chan, E. Crafton, H. N. Fan et al., "Interferon-producing killer dendritic cells provide a link between innate and adaptive immunity," *Nature Medicine*, vol. 12, no. 2, pp. 207-213, 2006.
- [49] Q. Jiang, H. Wei, and Z. Tian, "IFN-producing killer dendritic cells contribute to the inhibitory effect of poly I:C on the progression of murine melanoma," *Journal of Immunotherapy*, vol. 31, no. 6, pp. 555-562, 2008.
- [50] P. Williams, M. Bouchentouf, M. Rafei et al., "A dendritic cell population generated by a fusion of GM-CSF and IL-21 induces tumor-antigen-specific immunity," *Journal of Immunology*, vol. 185, no. 12, pp. 7358-7366, 2010.
- [51] P. R. Taylor, G. K. Koski, C. C. Paustian et al., "J-LEAPS vaccines initiate murine Th1 responses by activating dendritic cells," *Vaccine*, vol. 28, no. 34, pp. 5533-5542, 2010.
- [52] P. R. Taylor, C. C. Paustian, G. K. Koski, D. H. Zimmerman, and K. S. Rosenthal, "Maturation of dendritic cell precursors into IL12-producing DCs by J-LEAPS immunogens," *Cellular Immunology*, vol. 262, no. 1, pp. 1-5, 2010.
- [53] B. Baban, A. M. Hansen, P. R. Chandler et al., "A minor population of splenic dendritic cells expressing CD19 mediates IDO-dependent T cell suppression via type I IFN signaling following B7 ligation," *International Immunology*, vol. 17, no. 7, pp. 909-919, 2005.
- [54] A. L. Mellor, B. Baban, P. R. Chandler, A. Manlapat, D. J. Kahler, and D. H. Munn, "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," *Journal of Immunology*, vol. 175, no. 9, pp. 5601-5605, 2005.
- [55] A. L. Mellor, P. Chandler, B. Baban et al., "Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase," *International Immunology*, vol. 16, no. 10, pp. 1391-1401, 2004.
- [56] A. L. Mellor and D. H. Munn, "IDO expression by dendritic cells: tolerance and tryptophan catabolism," *Nature Reviews Immunology*, vol. 4, no. 10, pp. 762-774, 2004.
- [57] S. S. Diebold, M. Montoya, H. Unger et al., "Viral infection switches non-plasmacytoid dendritic cells into high interferon producers," *Nature*, vol. 424, no. 6946, pp. 324-328, 2003.
- [58] K. Shortman and Y. J. Liu, "Mouse and human dendritic cell subtypes," *Nature Reviews Immunology*, vol. 2, no. 3, pp. 151-161, 2002.
- [59] K. P. A. MacDonald, D. J. Munster, G. J. Clark, A. Dzionic, J. Schmitz, and D. N. J. Hart, "Characterization of human blood dendritic cell subsets," *Blood*, vol. 100, no. 13, pp. 4512-4520, 2002.

- [60] L. F. Poulin, M. Salio, E. Griessinger et al., "Characterization of human DNGR-1⁺ BDCA3⁺ leukocytes as putative equivalents of mouse CD8 α ⁺ dendritic cells," *Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1261–1271, 2010.
- [61] A. Bachem, S. Güttler, E. Hartung et al., "Superior antigen cross-presentation and XCR1 expression define human CD11c⁺CD141⁺ cells as homologues of mouse CD8⁺ dendritic cells," *Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1273–1281, 2010.
- [62] S. L. Jongbloed, A. J. Kassianos, K. J. McDonald et al., "Human CD141⁺ (BDCA-3)⁺ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens," *Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1247–1260, 2010.
- [63] S. H. Robbins, T. Walzer, D. Dembélé et al., "Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling," *Genome Biology*, vol. 9, no. 1, article no. R17, 2008.
- [64] E. Klechevsky, R. Morita, M. Liu et al., "Functional specializations of human epidermal Langerhans cells and CD14⁺ dermal dendritic cells," *Immunity*, vol. 29, no. 3, pp. 497–510, 2008.
- [65] R. Van De Ven, M. F. C. M. Van Den Hout, J. J. Lindenberg et al., "Characterization of four conventional dendritic cell subsets in human skin-draining lymph nodes in relation to T-cell activation," *Blood*, vol. 118, no. 9, pp. 2502–2510, 2011.
- [66] E. West, R. Morgan, K. Scott et al., "Clinical grade OK432-activated dendritic cells: in vitro characterization and tracking during intralymphatic delivery," *Journal of Immunotherapy*, vol. 32, no. 1, pp. 66–78, 2009.
- [67] J. C. Gluckman, B. Canque, F. Chapuis, and M. Rosenzweig, "In vitro generation of human dendritic cells and cell therapy," *Cytokines, Cellular and Molecular Therapy*, vol. 3, no. 3, pp. 187–196, 1997.
- [68] S. Balan, V. P. Kale, and L. S. Limaye, "A large number of mature and functional dendritic cells can be efficiently generated from umbilical cord blood-derived mononuclear cells by a simple two-step culture method," *Transfusion*, vol. 50, no. 11, pp. 2413–2423, 2010.
- [69] M. D. Cahalan and I. Parker, "Close encounters of the first and second kind: T-DC and T-B interactions in the lymph node," *Seminars in Immunology*, vol. 17, no. 6, pp. 442–451, 2005.
- [70] P. Kalinski, "Dendritic cells in immunotherapy of established cancer: Roles of signals 1, 2, 3 and 4," *Current Opinion in Investigational Drugs*, vol. 10, no. 6, pp. 526–535, 2009.
- [71] M. B. Lutz and G. Schuler, "Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity?" *Trends in Immunology*, vol. 23, no. 9, pp. 445–449, 2002.
- [72] Y.-J. Liu, H. Kanzler, V. Soumelis, and M. Gilliet, "Dendritic cell lineage, plasticity and cross-regulation," *Nature Immunology*, vol. 2, no. 7, pp. 585–589, 2001.
- [73] D. G. DeNardo and L. M. Coussens, "Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression," *Breast Cancer Research*, vol. 9, no. 4, p. 212, 2007.
- [74] J. E. Talmadge, M. Donkor, and E. Scholar, "Inflammatory cell infiltration of tumors: Jekyll or Hyde," *Cancer and Metastasis Reviews*, vol. 26, no. 3-4, pp. 373–400, 2007.
- [75] M. Waldner, C. C. Schimanski, and M. F. Neurath, "Colon cancer and the immune system: the role of tumor invading T cells," *World Journal of Gastroenterology*, vol. 12, no. 45, pp. 7233–7238, 2006.
- [76] J. R. Conejo-Garcia, F. Benencia, M. C. Courreges et al., "Letal, a tumor-associated NKG2D immunoreceptor ligand, induces activation and expansion of effector immune cells," *Cancer Biology and Therapy*, vol. 2, no. 4, pp. 446–451, 2003.
- [77] T. Condamine and D. I. Gabrilovich, "Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function," *Trends in Immunology*, vol. 32, no. 1, pp. 19–25, 2011.
- [78] M. W. Teng, D. S. Ritchie, P. Neeson, and M. J. Smyth, "Biology and clinical observations of regulatory T cells in cancer immunology," *Current topics in microbiology and immunology*, vol. 344, pp. 61–95, 2011.
- [79] M. M. Markiewski, R. A. DeAngelis, F. Benencia et al., "Modulation of the antitumor immune response by complement," *Nature Immunology*, vol. 9, no. 11, pp. 1225–1235, 2008.
- [80] C. Rüegg, "Leukocytes, inflammation, and angiogenesis in cancer: fatal attractions," *Journal of Leukocyte Biology*, vol. 80, no. 4, pp. 682–684, 2006.
- [81] R. M. Peek Jr. and J. E. Crabtree, "Helicobacter infection and gastric neoplasia," *Journal of Pathology*, vol. 208, no. 2, pp. 233–248, 2006.
- [82] E. Szabó, C. Páska, P. Kaposi Novák, Z. Schaff, and A. Kiss, "Similarities and Differences in Hepatitis B and C Virus Induced Hepatocarcinogenesis," *Pathology and Oncology Research*, vol. 10, no. 1, pp. 5–11, 2004.
- [83] M. D. Williams and A. B. Sandler, "The epidemiology of lung cancer," *Cancer treatment and research*, vol. 105, pp. 31–52, 2001.
- [84] N. Jura, H. Archer, and D. Bar-Sagi, "Chronic pancreatitis, pancreatic adenocarcinoma and the black box in-between," *Cell Research*, vol. 15, no. 1, pp. 72–77, 2005.
- [85] J. R. Conejo-Garcia, F. Benencia, M. C. Courreges et al., "Tumor-infiltrating dendritic cell precursors recruited by a β -defensin contribute to vasculogenesis under the influence of Vegf-A," *Nature Medicine*, vol. 10, no. 9, pp. 950–958, 2004.
- [86] T. J. Curiel, P. Cheng, P. Mottram et al., "Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer," *Cancer Research*, vol. 64, no. 16, pp. 5535–5538, 2004.
- [87] D. Ribatti, "The paracrine role of tie-2-expressing monocytes in tumor angiogenesis," *Stem Cells and Development*, vol. 18, no. 5, pp. 703–706, 2009.
- [88] J. R. Conejo-Garcia, R. J. Buckanovich, F. Benencia et al., "Vascular leukocytes contribute to tumor vascularization," *Blood*, vol. 105, no. 2, pp. 679–681, 2005.
- [89] P. J. Gough, I. G. Gomez, P. T. Wille, and E. W. Raines, "Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice," *Journal of Clinical Investigation*, vol. 116, no. 1, pp. 59–69, 2006.
- [90] R. B. Baleeiro, L. B. Anselmo, F. A. Soares et al., "High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor- α in lung cancer," *Cancer Immunology, Immunotherapy*, vol. 57, no. 9, pp. 1335–1345, 2008.
- [91] M. R. Shurin, G. V. Shurin, A. Lokshin et al., "Intratumoral cytokines/chemokines/growth factors and tumor infiltrating dendritic cells: friends or enemies?" *Cancer and Metastasis Reviews*, vol. 25, no. 3, pp. 333–356, 2006.
- [92] T. L. Whiteside, "The role of immune cells in the tumor microenvironment," *Cancer Treatment and Research*, vol. 130, pp. 103–124, 2006.

- [93] A. Mantovani, S. Sozzani, M. Locati et al., "Infiltration of tumours by macrophages and dendritic cells: tumour-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes," *Novartis Foundation Symposium*, vol. 256, pp. 137–145, 2004.
- [94] Q. Liu, C. Zhang, A. Sun, Y. Zheng, L. Wang, and X. Cao, "Tumor-educated CD11bhighIalow regulatory dendritic cells suppress T cell response through arginase I," *Journal of Immunology*, vol. 182, no. 10, pp. 6207–6216, 2009.
- [95] D. I. Gabrilovich, T. Ishida, S. Nadaf, J. E. Ohm, and D. P. Carbone, "Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function," *Clinical Cancer Research*, vol. 5, no. 10, pp. 2963–2970, 1999.
- [96] O. Fainaru, N. Almog, C. W. Yung et al., "Tumor growth and angiogenesis are dependent on the presence of immature dendritic cells," *FASEB Journal*, vol. 24, no. 5, pp. 1411–1418, 2010.
- [97] S. Patan, "Vasculogenesis and angiogenesis as mechanisms of vascular network formation, growth and remodeling," *Journal of Neuro-Oncology*, vol. 50, no. 1-2, pp. 1–15, 2000.
- [98] M. Papetti and I. M. Herman, "Mechanisms of normal and tumor-derived angiogenesis," *American Journal of Physiology*, vol. 282, no. 5, pp. C947–C970, 2002.
- [99] V. Djonov, O. Baum, and P. H. Burri, "Vascular remodeling by intussusceptive angiogenesis," *Cell and Tissue Research*, vol. 314, no. 1, pp. 107–117, 2003.
- [100] A. S. Bailey and W. H. Fleming, "Converging roads: evidence for an adult hemangioblast," *Experimental Hematology*, vol. 31, no. 11, pp. 987–993, 2003.
- [101] B. Fernandez Pujol, F. C. Lucibello, U. M. Gehling et al., "Endothelial-like cells derived from human CD14 positive monocytes," *Differentiation*, vol. 65, no. 5, pp. 287–300, 2000.
- [102] A. Schmeisser, C. D. Garlich, H. Zhang et al., "Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel® under angiogenic conditions," *Cardiovascular Research*, vol. 49, no. 3, pp. 671–680, 2001.
- [103] L. Bellik, C. Musilli, M. C. Vinci, F. Ledda, and A. Parenti, "Human mature endothelial cells modulate peripheral blood mononuclear cell differentiation toward an endothelial phenotype," *Experimental Cell Research*, vol. 314, no. 16, pp. 2965–2974, 2008.
- [104] J. Rehman, J. Li, C. M. Orschell, and K. L. March, "Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors," *Circulation*, vol. 107, no. 8, pp. 1164–1169, 2003.
- [105] C. E. Lewis, M. De Palma, and L. Naldini, "Tie2-expressing monocytes and tumor angiogenesis: regulation by hypoxia and angiopoietin-2," *Cancer Research*, vol. 67, no. 18, pp. 8429–8432, 2007.
- [106] M. Kuwana, Y. Okazaki, H. Kodama, T. Satoh, Y. Kawakami, and Y. Ikeda, "Endothelial differentiation potential of human monocyte-derived multipotential cells," *Stem Cells*, vol. 24, no. 12, pp. 2733–2743, 2006.
- [107] H. Chen, R. A. Campbell, Y. Chang et al., "Pleiotrophin produced by multiple myeloma induces transdifferentiation of monocytes into vascular endothelial cells: a novel mechanism of tumor-induced vasculogenesis," *Blood*, vol. 113, no. 9, pp. 1992–2002, 2009.
- [108] B. Li, A. Pozzi, and P. P. Young, "TNF α accelerates monocyte to endothelial transdifferentiation in tumors by the induction of integrin α 5 expression and adhesion to fibronectin," *Molecular Cancer Research*, vol. 9, no. 6, pp. 702–711, 2011.
- [109] B. F. Pujol, F. C. Lucibello, M. Zuzarte, P. Lütjens, R. Müller, and K. Havemann, "Dendritic cells derived from peripheral monocytes express endothelial markers and in the presence of angiogenic growth factors differentiate into endothelial-like cells," *European Journal of Cell Biology*, vol. 80, no. 1, pp. 99–110, 2001.
- [110] E. Gottfried, M. Kreutz, S. Haffner et al., "Differentiation of human tumour-associated dendritic cells into endothelial-like cells: an alternative pathway of tumour angiogenesis," *Scandinavian Journal of Immunology*, vol. 65, no. 4, pp. 329–335, 2007.
- [111] J. Lu, K. Liu, J. Zhao et al., "VEGF-A not Ang2 mediates endothelial-like differentiation of immature DCs by ERK1/2 signaling in the microenvironment of human colon adenocarcinoma," *International Journal of Oncology*, vol. 38, no. 6, pp. 1579–1588, 2011.
- [112] J. Lu, J. Zhao, K. Liu et al., "MAPK/ERK1/2 signaling mediates endothelial-like differentiation of immature DCs in the microenvironment of esophageal squamous cell carcinoma," *Cellular and Molecular Life Sciences*, vol. 67, no. 12, pp. 2091–2106, 2010.
- [113] E. Huarte, J. R. Cubillos-Ruiz, Y. C. Nesbeth et al., "Depletion of dendritic cells delays ovarian cancer progression by boosting antitumor immunity," *Cancer Research*, vol. 68, no. 18, pp. 7684–7691, 2008.
- [114] T. C. Tzeng, S. Chyou, S. Tian et al., "CD11chi dendritic cells regulate the re-establishment of vascular quiescence and stabilization after immune stimulation of lymph nodes," *Journal of Immunology*, vol. 184, no. 8, pp. 4247–4257, 2010.
- [115] S. Sozzani, M. Rusnati, E. Riboldi, S. Mitola, and M. Presta, "Dendritic cell-endothelial cell cross-talk in angiogenesis," *Trends in Immunology*, vol. 28, no. 9, pp. 385–392, 2007.
- [116] L. Sprague, M. Muccioli, M. Pate et al., "The interplay between surfaces and soluble factors define the immunologic and angiogenic properties of myeloid dendritic cells," *BMC Immunology*, vol. 12, article 35, 2011.
- [117] G. Coukos, F. Benencia, R. J. Buckanovich, and J. R. Conejo-Garcia, "The role of dendritic cell precursors in tumour vasculogenesis," *British Journal of Cancer*, vol. 92, no. 7, pp. 1182–1187, 2005.
- [118] S. P. Bak, J. J. Walters, M. Takeya, J. R. Conejo-Garcia, and B. L. Berwin, "Scavenger receptor-A-targeted leukocyte depletion inhibits peritoneal ovarian tumor progression," *Cancer Research*, vol. 67, no. 10, pp. 4783–4789, 2007.
- [119] O. Fainaru, A. Adini, O. Benny et al., "Dendritic cells support angiogenesis and promote lesion growth in a murine model of endometriosis," *FASEB Journal*, vol. 22, no. 2, pp. 522–529, 2008.
- [120] D. Ridgway, "The first 1000 dendritic cell vaccinees," *Cancer Investigation*, vol. 21, no. 6, pp. 873–886, 2003.
- [121] E. Gilboa, "DC-based cancer vaccines," *Journal of Clinical Investigation*, vol. 117, no. 5, pp. 1195–1203, 2007.
- [122] S. Yamaguchi, T. Tatsumi, T. Takehara et al., "Dendritic cell-based vaccines suppress metastatic liver tumor via activation of local innate and acquired immunity," *Cancer Immunology, Immunotherapy*, vol. 57, no. 12, pp. 1861–1869, 2008.
- [123] P. Hatfield, A. E. Merrick, E. West et al., "Optimization of dendritic cell loading with tumor cell lysates for cancer immunotherapy," *Journal of Immunotherapy*, vol. 31, no. 7, pp. 620–632, 2008.

- [124] M. C. Courrèges, F. Benencia, J. R. Conejo-García, L. Zhang, and G. Coukos, "Preparation of apoptotic tumor cells with replication-incompetent HSV augments the efficacy of dendritic cell vaccines," *Cancer Gene Therapy*, vol. 13, no. 2, pp. 182–193, 2006.
- [125] H. Shirota and D. M. Klinman, "CpG-conjugated apoptotic tumor cells elicit potent tumor-specific immunity," *Cancer Immunology, Immunotherapy*, vol. 60, no. 5, pp. 659–669, 2011.
- [126] S. Pilon-Thomas, M. Verhaegen, L. Kuhn, A. Riker, and J. J. Mulé, "Induction of anti-tumor immunity by vaccination with dendritic cells pulsed with anti-CD44 IgG opsonized tumor cells," *Cancer Immunology, Immunotherapy*, vol. 55, no. 10, pp. 1238–1246, 2006.
- [127] W. Ma, T. Smith, V. Bogin et al., "Enhanced presentation of MHC class Ia, Ib and class II-restricted peptides encapsulated in biodegradable nanoparticles: a promising strategy for tumor immunotherapy," *Journal of Translational Medicine*, vol. 9, article 34, 2011.
- [128] F. Xu, Y.-J. Ye, W. Liu, M. Kong, Y. He, and S. Wang, "Dendritic cell/tumor hybrids enhances therapeutic efficacy against colorectal cancer liver metastasis in SCID mice," *Scandinavian Journal of Gastroenterology*, vol. 45, no. 6, pp. 707–713, 2010.
- [129] F. Benencia, M. C. Courrèges, and G. Coukos, "Whole tumor antigen vaccination using dendritic cells: comparison of RNA electroporation and pulsing with UV-irradiated tumor cells," *Journal of Translational Medicine*, vol. 6, article 21, 2008.
- [130] H. G. Yang, B. L. Hu, L. Xiao, and P. Wang, "Dendritic cell-directed lentivector vaccine induces antigen-specific immune responses against murine melanoma," *Cancer Gene Therapy*, vol. 18, no. 5, pp. 370–380, 2011.
- [131] Y. Liu, L. H. Butterfield, X. Fu et al., "Lentivirally engineered dendritic cells activate AFP-specific T cells which inhibit hepatocellular carcinoma growth in vitro and in vivo," *International Journal of Oncology*, vol. 39, no. 1, pp. 245–253, 2011.
- [132] T. C. Felizardo, J. C. M. Wang, R. A. J. McGray et al., "Differential immune responses mediated by adenovirus- and lentivirus-transduced DCs in a HER-2/neu overexpressing tumor model," *Gene Therapy*, vol. 18, no. 10, pp. 986–995, 2011.
- [133] Y. Cui, E. Kelleher, E. Straley et al., "Immunotherapy of established tumors using bone marrow transplantation with antigen gene-modified hematopoietic stem cells," *Nature Medicine*, vol. 9, no. 7, pp. 952–958, 2003.
- [134] L. Kacani, M. Wurm, I. Schwentner, J. Andrlé, H. Schennach, and G. M. Sprinzl, "Maturation of dendritic cells in the presence of living, apoptotic and necrotic tumour cells derived from squamous cell carcinoma of head and neck," *Oral Oncology*, vol. 41, no. 1, pp. 17–24, 2005.
- [135] D. Brusa, S. Garetto, G. Chiorino et al., "Post-apoptotic tumors are more palatable to dendritic cells and enhance their antigen cross-presentation activity," *Vaccine*, vol. 26, no. 50, pp. 6422–6432, 2008.
- [136] A. Bonehill, C. Heirman, S. Tuyaerts et al., "Messenger RNA-electroporated dendritic cells presenting MAGE-A3 simultaneously in HLA class I and class II molecules," *Journal of Immunology*, vol. 172, no. 11, pp. 6649–6657, 2004.
- [137] L. Sun, B. Kong, X. Sheng, J. J.-C. Sheu, and I.-M. Shih, "Dendritic cells transduced with Rsf-1/HBXAP gene generate specific cytotoxic T lymphocytes against ovarian cancer in vitro," *Biochemical and Biophysical Research Communications*, vol. 394, no. 3, pp. 633–638, 2010.
- [138] Y. Zhang, B. Ma, Y. Zhou et al., "Dendritic cells fused with allogeneic breast cancer cell line induce tumor antigen-specific CTL responses against autologous breast cancer cells," *Breast Cancer Research and Treatment*, vol. 105, no. 3, pp. 277–286, 2007.
- [139] M. Lotem, Y. Zhao, J. Riley et al., "Presentation of tumor antigens by dendritic cells genetically modified with viral and nonviral vectors," *Journal of Immunotherapy*, vol. 29, no. 6, pp. 616–627, 2006.
- [140] G. P. Linette, S. Shankara, S. Longerich et al., "In vitro priming with adenovirus/gp100 antigen-transduced dendritic cells reveals the epitope specificity of HLA-A*0201-restricted CD8⁺ T cells in patients with melanoma," *Journal of Immunology*, vol. 164, no. 6, pp. 3402–3412, 2000.
- [141] A. B. Dietz and S. Vuk-Pavlović, "High efficiency adenovirus-mediated gene transfer to human dendritic cells," *Blood*, vol. 91, no. 2, pp. 392–398, 1998.
- [142] M. Miyazawa, M. Iwahashi, T. Ojima et al., "Dendritic cells adenovirally-transduced with full-length mesothelin cDNA elicit mesothelin-specific cytotoxicity against pancreatic cancer cell lines in vitro," *Cancer Letters*, vol. 305, no. 1, pp. 32–39, 2011.
- [143] C. Bonini, S. P. Lee, S. R. Riddell, and P. D. Greenberg, "Targeting antigen in mature dendritic cells for simultaneous stimulation of CD4⁺ and CD8⁺ T cells," *Journal of Immunology*, vol. 166, no. 8, pp. 5250–5257, 2001.
- [144] Y. Cui, J. Golob, E. Kelleher, Z. Ye, D. Pardoll, and L. Cheng, "Targeting transgene expression to antigen-presenting cells derived from lentivirus-transduced engrafting human hematopoietic stem/progenitor cells," *Blood*, vol. 99, no. 2, pp. 399–408, 2002.
- [145] G. Lizée, M. I. Gonzales, and S. L. Topalian, "Lentivirus vector-mediated expression of tumor-associated epitopes by human antigen presenting cells," *Human Gene Therapy*, vol. 15, no. 4, pp. 393–404, 2004.
- [146] J. Wierdecky, M. R. Müller, S. Wirths et al., "Immunologic and clinical responses after vaccinations with peptide-pulsed dendritic cells in metastatic renal cancer patients," *Cancer Research*, vol. 66, no. 11, pp. 5910–5918, 2006.
- [147] E. Ovali, T. Dikmen, M. Sonmez et al., "Active immunotherapy for cancer patients using tumor lysate pulsed dendritic cell vaccine: a safety study," *Journal of Experimental and Clinical Cancer Research*, vol. 26, no. 2, pp. 209–214, 2007.
- [148] E. M. I. Suso, S. Dueland, A.-M. Rasmussen et al., "hTERT mRNA dendritic cell vaccination: complete response in a pancreatic cancer patient associated with response against several hTERT epitopes," *Cancer Immunology, Immunotherapy*, vol. 60, no. 6, pp. 809–818, 2011.
- [149] A. Van Driessche, A. L. R. Van De Velde, G. Nijs et al., "Clinical-grade manufacturing of autologous mature mRNA-electroporated dendritic cells and safety testing in acute myeloid leukemia patients in a phase I dose-escalation clinical trial," *Cytotherapy*, vol. 11, no. 5, pp. 653–668, 2009.
- [150] Y. Akasaki, T. Kikuchi, M. Irie et al., "Cotransfection of poly(I: C) and siRNA of IL-10 into fusions of dendritic and glioma cells enhances antitumor T helper type 1 induction in patients with glioma," *Journal of Immunotherapy*, vol. 34, no. 2, pp. 121–128, 2011.
- [151] J. Rosenblatt, B. Vasir, L. Uhl et al., "Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma," *Blood*, vol. 117, no. 2, pp. 393–402, 2011.
- [152] T. H. Han, P. Jin, J. Ren, S. Slezak, F. M. Marincola, and D. F. Stroncek, "Evaluation of 3 clinical dendritic cell maturation

- protocols containing lipopolysaccharide and interferon- γ ," *Journal of Immunotherapy*, vol. 32, no. 4, pp. 399–407, 2009.
- [153] D. K. Banerjee, M. V. Dhodapkar, E. Matayeva, R. M. Steinman, and K. M. Dhodapkar, "Expansion of FOXP3high regulatory T cells by human dendritic cells (DCs) in vitro and after injection of cytokine-matured DCs in myeloma patients," *Blood*, vol. 108, no. 8, pp. 2655–2661, 2006.
- [154] R. Muthuswamy, J. Urban, J. J. Lee, T. A. Reinhart, D. Bartlett, and P. Kalinski, "Ability of mature dendritic cells to interact with regulatory T cells is imprinted during maturation," *Cancer Research*, vol. 68, no. 14, pp. 5972–5978, 2008.
- [155] R. B. Mailliard, A. Wankowicz-Kalinska, Q. Cai et al., " α -type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity," *Cancer Research*, vol. 64, no. 17, pp. 5934–5937, 2004.
- [156] K. Gustafsson, K. Junevik, O. Werlenius, S. Holmgren, A. Karlsson-Parra, and P.-O. Andersson, "Tumour-loaded α -type 1-polarized Dendritic cells from patients with chronic lymphocytic leukaemia produce a superior NK⁻, NKT⁻ and CD8⁺ T cell-attracting chemokine profile," *Scandinavian Journal of Immunology*, vol. 74, no. 3, pp. 318–326, 2011.
- [157] W. Van Den Ancker, M. M. Van Luijn, J. M. Ruben et al., "Targeting Toll-like receptor 7/8 enhances uptake of apoptotic leukemic cells by monocyte-derived dendritic cells but interferes with subsequent cytokine-induced maturation," *Cancer Immunology, Immunotherapy*, vol. 60, no. 1, pp. 37–47, 2011.
- [158] E. Wieckowski, G. S. Chatta, R. M. Mailliard 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*, vol. 71, no. 2, pp. 125–133, 2011.
- [159] K. Gustafsson, M. Ingelsten, L. Bergqvist, J. Nyström, B. Andersson, and A. Karlsson-Parra, "Recruitment and activation of natural killer cells in vitro by a human dendritic cell vaccine," *Cancer Research*, vol. 68, no. 14, pp. 5965–5971, 2008.
- [160] R. Trepiakas, A. E. Pedersen, Ö. Met, M. H. Hansen, A. Berntsen, and I. M. Svane, "Comparison of α -Type-1 polarizing and standard dendritic cell cytokine cocktail for maturation of therapeutic monocyte-derived dendritic cell preparations from cancer patients," *Vaccine*, vol. 26, no. 23, pp. 2824–2832, 2008.
- [161] R. J. Barth Jr., D. A. Fisher, P. K. Wallace et al., "A randomized trial of ex vivo CD40L activation of a dendritic cell vaccine in colorectal cancer patients: tumor-specific immune responses are associated with improved survival," *Clinical Cancer Research*, vol. 16, no. 22, pp. 5548–5556, 2010.
- [162] L. D. Cranmer, K. T. Trevor, and E. M. Hersh, "Clinical applications of dendritic cell vaccination in the treatment of cancer," *Cancer Immunology, Immunotherapy*, vol. 53, no. 4, pp. 275–306, 2004.
- [163] W. J. Lesterhuis, I. J.M. de Vries, G. J. Adema, and C. J.A. Punt, "Dendritic cell-based vaccines in cancer immunotherapy: an update on clinical and immunological results," *Annals of Oncology*, vol. 15, supplement 4, pp. 145–151, 2004.
- [164] E. Carballido and M. Fishman, "Sipuleucel-T: prototype for development of anti-tumor vaccines," *Current Oncology Reports*, vol. 13, no. 2, pp. 112–119, 2011.
- [165] S. J. Hall, L. Klotz, A. J. Pantuck et al., "Integrated safety data from 4 randomized, double-blind, controlled trials of autologous cellular immunotherapy with sipuleucel-T in patients with prostate cancer," *Journal of Urology*, vol. 186, no. 3, pp. 877–881, 2011.
- [166] P. W. Kantoff, C. S. Higano, N. D. Shore et al., "Sipuleucel-T immunotherapy for castration-resistant prostate cancer," *New England Journal of Medicine*, vol. 363, no. 5, pp. 411–422, 2010.
- [167] C. S. Chu, J. Boyer, D. S. Schullery et al., "Phase I/II randomized trial of dendritic cell vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer in first or second remission," *Cancer Immunology, Immunotherapy*. In press.
- [168] E. Feijóo, C. Alfaro, G. Mazzolini et al., "Dendritic cells delivered inside human carcinomas are sequestered by interleukin-8," *International Journal of Cancer*, vol. 116, no. 2, pp. 275–281, 2005.
- [169] S. S. McAllister, A. M. Gifford, A. L. Greiner et al., "Systemic endocrine instigation of indolent tumor growth requires osteopontin," *Cell*, vol. 133, no. 6, pp. 994–1005, 2008.
- [170] D. I. Gabrilovich, H. L. Chen, K. R. Girgis et al., "Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells," *Nature Medicine*, vol. 2, no. 10, pp. 1096–1103, 1996.
- [171] W. Maes, G. G. Rosas, B. Verbinnen et al., "DC vaccination with anti-CD25 treatment leads to long-term immunity against experimental glioma," *Neuro-Oncology*, vol. 11, no. 5, pp. 529–542, 2009.
- [172] S. J. Prasad, K. J. Farrand, S. A. Matthews, J. H. Chang, R. S. McHugh, and F. Ronchese, "Dendritic cells loaded with stressed tumor cells elicit long-lasting protective tumor immunity in mice depleted of CD4⁺CD25⁺ regulatory T cells," *Journal of Immunology*, vol. 174, no. 1, pp. 90–98, 2005.
- [173] S. Delluc, P. Hachem, S. Rusakiewicz et al., "Dramatic efficacy improvement of a DC-based vaccine against AML by CD25 T cell depletion allowing the induction of a long-lasting T cell response," *Cancer Immunology, Immunotherapy*, vol. 58, no. 10, pp. 1669–1677, 2009.
- [174] M. A. Morse, A. C. Hobeika, T. Osada et al., "Depletion of human regulatory T cells specifically enhances antigen-specific immune responses to cancer vaccines," *Blood*, vol. 112, no. 3, pp. 610–618, 2008.
- [175] J. Dannull, S. Nair, Z. Su et al., "Enhancing the immunostimulatory function of dendritic cells by transfection with mRNA encoding OX40 ligand," *Blood*, vol. 105, no. 8, pp. 3206–3213, 2005.
- [176] J. F. M. Jacobs, C. J. A. Punt, W. J. Lesterhuis et al., "Dendritic cell vaccination in combination with anti-CD25 monoclonal antibody treatment: a phase I/II study in metastatic melanoma patients," *Clinical Cancer Research*, vol. 16, no. 20, pp. 5067–5078, 2010.
- [177] A. Saha and S. K. Chatterjee, "Combination of CTL-associated antigen-4 blockade and depletion of CD25⁺ regulatory T cells enhance tumour immunity of dendritic cell-based vaccine in a mouse model of colon cancer," *Scandinavian Journal of Immunology*, vol. 71, no. 2, pp. 70–82, 2010.
- [178] Z. Gadri, T. Kukulansky, E. Bar-Or, J. Haimovich, and N. Hollander, "Synergistic effect of dendritic cell vaccination and anti-CD20 antibody treatment in the therapy of murine lymphoma," *Journal of Immunotherapy*, vol. 32, no. 4, pp. 333–340, 2009.
- [179] G. Driessens, L. Nuttin, A. Gras et al., "Development of a successful antitumor therapeutic model combining in vivo dendritic cell vaccination with tumor irradiation and intratumoral GM-CSF delivery," *Cancer Immunology, Immunotherapy*, vol. 60, no. 2, pp. 273–281, 2011.

- [180] Q. He, J. Li, W. Yin et al., "Low-dose paclitaxel enhances the anti-tumor efficacy of GM-CSF surface-modified whole-tumor-cell vaccine in mouse model of prostate cancer," *Cancer Immunology, Immunotherapy*, vol. 60, no. 5, pp. 715–730, 2011.
- [181] S.-N. Zhang, I.-K. Choi, J.-H. Huang, J.-Y. Yoo, K.-J. Choi, and C.-O. Yun, "Optimizing DC vaccination by combination with oncolytic adenovirus coexpressing IL-12 and GM-CSF," *Molecular Therapy*, vol. 19, no. 8, pp. 1558–1568, 2011.
- [182] B. Almand, J. R. Resser, B. Lindman et al., "Clinical significance of defective dendritic cell differentiation in cancer," *Clinical Cancer Research*, vol. 6, no. 5, pp. 1755–1766, 2000.
- [183] A. P. Vicari, C. Chiodoni, C. Vaure et al., "Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody," *Journal of Experimental Medicine*, vol. 196, no. 4, pp. 541–549, 2002.
- [184] W. Vermi, R. Bonecchi, F. Facchetti et al., "Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas," *Journal of Pathology*, vol. 200, no. 2, pp. 255–268, 2003.
- [185] C. Ugolini, F. Basolo, A. Proietti et al., "Lymphocyte and immature dendritic cell infiltrates in differentiated, poorly differentiated, and undifferentiated thyroid carcinoma," *Thyroid*, vol. 17, no. 5, pp. 389–393, 2007.
- [186] R. J. Buckanovich, A. Facciabene, S. Kim et al., "Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy," *Nature Medicine*, vol. 14, no. 1, pp. 28–36, 2008.
- [187] B. Wang, J. M. Y. Kuroiwa, L. Z. He, A. Charalambous, T. Keler, and R. M. Steinman, "The human cancer antigen mesothelin is more efficiently presented to the mouse immune system when targeted to the DEC-205/CD205 receptor on dendritic cells," *Annals of the New York Academy of Sciences*, vol. 1174, pp. 6–17, 2009.
- [188] H. Wei, S. Wang, D. Zhang et al., "Targeted delivery of tumor antigens to activated dendritic cells via CD11c molecules induces potent antitumor immunity in mice," *Clinical Cancer Research*, vol. 15, no. 14, pp. 4612–4621, 2009.
- [189] A. Faham and J. G. Altin, "Antigen-containing liposomes engrafted with flagellin-related peptides are effective vaccines that can induce potent antitumor immunity and immunotherapeutic effect," *Journal of Immunology*, vol. 185, no. 3, pp. 1744–1754, 2010.
- [190] L. J. Cruz, P. J. Tacken, R. Fokkink et al., "Targeted PLGA nano- but not microparticles specifically deliver antigen to human dendritic cells via DC-SIGN in vitro," *Journal of Controlled Release*, vol. 144, no. 2, pp. 118–126, 2010.
- [191] J. Ni, B. Nolte, A. Arnold, P. Fournier, and V. Schirmmacher, "Targeting anti-tumor DNA vaccines to dendritic cells via a short CD11c promoter sequence," *Vaccine*, vol. 27, no. 40, pp. 5480–5487, 2009.
- [192] B. Hu, B. Dai, and P. Wang, "Vaccines delivered by integration-deficient lentiviral vectors targeting dendritic cells induces strong antigen-specific immunity," *Vaccine*, vol. 28, no. 41, pp. 6675–6683, 2010.
- [193] J. R. Cubillos-Ruiz, X. Engle, U. K. Scarlett et al., "Polyethylenimine-based siRNA nanocomplexes reprogram tumor-associated dendritic cells via TLR5 to elicit therapeutic antitumor immunity," *Journal of Clinical Investigation*, vol. 119, no. 8, pp. 2231–2244, 2009.
- [194] F. Benencia, M. C. Courrèges, N. W. Fraser, and G. Coukos, "Herpes virus oncolytic therapy reverses tumor immune dysfunction and facilitates tumor antigen presentation," *Cancer Biology and Therapy*, vol. 7, no. 8, pp. 1194–1205, 2008.



Hindawi
Submit your manuscripts at
<http://www.hindawi.com>

