

# Dendritic cells and their role in tumor immunosurveillance

Marius Strioga<sup>1</sup>, Virgil Schijns<sup>2,3</sup>, Daniel J Powell Jr<sup>4</sup>,  
Vita Pasukoniene<sup>1</sup>, Neringa Dobrovolskiene<sup>1,5</sup>  
and Jaroslav Michalek<sup>6,7</sup>

Innate Immunity  
19(1) 98–111  
© The Author(s) 2012  
Reprints and permissions:  
sagepub.co.uk/journalsPermissions.nav  
DOI: 10.1177/1753425912449549  
ini.sagepub.com



## Abstract

Dendritic cells (DCs) comprise a heterogeneous population of cells that play a key role in initiating, directing and regulating adaptive immune responses, including those critically involved in tumor immunosurveillance. As a riposte to the central role of DCs in the generation of antitumor immune responses, tumors have developed various mechanisms which impair the immunostimulatory functions of DCs or even instruct them to actively contribute to tumor growth and progression. In the first part of this review we discuss general aspects of DC biology, including their origin, subtypes, immature and mature states, and functional plasticity which ensures a delicate balance between active immune response and immune tolerance. In the second part of the review we discuss the complex interactions between DCs and the tumor microenvironment, and point out the challenges faced by DCs during the recognition of tumor Ags. We also discuss the role of DCs in tumor angiogenesis and vasculogenesis.

## Keywords

Dendritic cell biology, immunogenic and tolerogenic dendritic cells, killer dendritic cells, tumor immunosurveillance, immunosuppression, vascular leukocytes

Date received: 5 February 2012; revised: 28 March 2012; accepted: 5 April 2012

## Introduction

Numerous studies have demonstrated that the immune system plays a significant role in the control of tumor development and progression (reviewed in Vesely et al.<sup>1</sup> and Schreiber et al.<sup>2</sup>). The components of both innate and adaptive immunity are involved in fighting tumor cells; however, current data suggest that the pivotal role in antitumor immune response belongs to T cell-mediated responses.<sup>3–6</sup> It is well known that naïve and central memory T cells can be appropriately activated only when they receive at least two activation signals.<sup>7,8</sup> The first is delivered through the interaction of TCR with the peptide-loaded MHC molecules (i.e. MHC-I for CD8<sup>+</sup> T cells and MHC-II for CD4<sup>+</sup> T cells)<sup>7,8</sup> or (glyco)lipid-loaded non-polymorphic CD1 family molecules, mainly CD1d [for the activation of natural killer T (NKT) cells].<sup>9</sup> The second (co-stimulatory) signal is delivered through the interaction of various other molecules; the best described (but in no way the only) co-stimulus essential for adequate immunogenic T cell activation involves the interaction of CD28 molecules on the surface of a T cell with co-stimulatory molecules CD80 (B7.1) and CD86 (B7.2) on the surface

of professional APC (pAPC).<sup>7–9</sup> In the absence of co-stimulation, T cell activation induced through the engagement of TCR alone will, in the majority of cases, lead to T cell tolerance,<sup>10,11</sup> which may manifest as: (i) T cell anergy (a long-term, hyporesponsive state characterized by an active inhibition of TCR signaling,

<sup>1</sup>Department of Immunology, Center of Oncosurgery, Institute of Oncology, Vilnius University, Vilnius, Lithuania

<sup>2</sup>Immune Intervention, Cell Biology and Immunology group, Wageningen University, Wageningen, the Netherlands

<sup>3</sup>Epitopoeitics Research Corporation (ERC), Namur, Belgium

<sup>4</sup>Ovarian Cancer Research Center, Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA

<sup>5</sup>Department of Immunology, State Research Institute Center for Innovative Medicine, Vilnius, Lithuania

<sup>6</sup>Advanced Cell Immunotherapy Unit, Department of Pharmacology, Medical Faculty, Masaryk University, Brno, Czech Republic

<sup>7</sup>CEO, Cellthera Ltd, Brno, Czech Republic

## Corresponding author:

Marius Strioga, Department of Immunology, Center of Oncosurgery, Institute of Oncology, Vilnius University, P. Baublio Str. 3b-321, LT-08406, Vilnius, Lithuania.  
Email: marius.strioga@vuoi.lt

and expression of IL-2 and inflammatory cytokines);<sup>10,12,13</sup> (ii) peripheral T cell clonal deletion, which is more characteristic of CD8<sup>+</sup> T cells, particularly if the TCR stimulus is weak;<sup>14,15</sup> and (iii) generation of induced regulatory T (iTreg) cells, especially in the presence of immunosuppressive cytokines, such as TGF- $\beta$ <sup>16</sup> and IL-10.<sup>17</sup>

The essential co-stimulatory molecules are expressed mainly on the surface of pAPCs, including dendritic cells (DCs), monocytes/macrophages and B cells. However, more than 30 years ago it was found that DCs are at least two orders of magnitude more potent than other pAPCs in presenting Ags to T cells in order to elicit Ag-specific T cell responses.<sup>18</sup> It is generally accepted that only DCs are able to initiate primary immune responses, i.e. to activate naïve T cells efficiently, whilst macrophages and B cells function as APCs in the expansion of pre-activated T cells that are already responding to Ag stimulation; they are also involved in activation of memory T cells.<sup>19,20</sup>

The delivery of Ag-specific signal 1 and co-stimulatory signal 2 by DCs ensures optimal activation and clonal expansion of naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells; however, these two signals are not sufficient for the induction of functional polarization of the activated CD4<sup>+</sup> Th0 cells, i.e. their differentiation into distinct Th1, Th2, Th17, or Th9 effectors, which are needed for fighting particular pathology (e.g. fungal infection versus cancer), which requires different defense mechanisms.<sup>21–23</sup> Current data reveal that a third signal is obligatory for driving the selective Th-cell response. Only mature DCs may provide a full-scale polarizing signal 3 which is delivered both by cytokines (such as IL-12p70) and DC surface molecules (such as Delta and Jagged which are ligands of Notch receptors on T cells).<sup>21,22,24</sup> It was shown that signal 3 is also required for the generation of functionally efficient CD8<sup>+</sup> T lymphocytes (CTLs).<sup>25–27</sup> The nature of the third signal depends highly on the nature and combination of danger signals, their duration, and the order of delivery to DCs.<sup>21,22,24,28</sup> Moreover, there is evidence that only directly activated DCs are able to produce signal 3 molecules, i.e. DCs should receive a direct signal from a pathogen or tumor cell through triggering various pattern recognition receptors (PRRs) rather than be indirectly activated by pro-inflammatory factors secreted by other cells.<sup>26,29</sup> Furthermore, to be effective the signal 3 must be provided to the T cell by the presenting DC rather than by various cytokines secreted by non-presenting DCs or other cells in the microenvironment.<sup>26,29,30</sup>

In relatively rare cases both MHC-I and MHC-II-class molecules, as well as co-stimulatory molecules (such as CD80, CD86), may be expressed on tumor cells,<sup>31</sup> enabling them to activate T cells directly.<sup>32</sup> However, tumor cells would hardly be able to deliver

the crucial polarizing signal 3 to T cells. Therefore, generally, tumor cells should be uptaken and processed (loaded onto MHC-I and MHC-II class or CD1 family molecules) by immature DCs in the periphery. Following Ag uptake, immature DCs should undergo maturation and migrate through the afferent lymphatics to the draining lymph nodes where they “find” and activate cognate naïve and central memory T cells (reviewed in Petersen et al.<sup>33</sup>).

## General characteristics of DCs

DCs are a heterogenous group of cells of innate immunity that originate from bone marrow CD34<sup>+</sup> hematopoietic precursors and are the most potent pAPCs that play a central role in the delicate regulation (active immunity versus immune tolerance) of naïve and memory adaptive immune responses.<sup>34</sup>

DCs are defined by their distinct (but not entirely unique) phenotype.

- DCs lack expression of lineage-specific markers (CD3, CD19, CD20, CD14, CD16, CD56) and are defined as lineage-negative (Lin<sup>-</sup>). There are some exceptions, such as a subset of human dermal DCs, which express CD14,<sup>35</sup> or a subset of human circulating DCs expressing CD16.<sup>36</sup> These DC subsets are briefly described below.
- DCs express both MHC-I and MHC-II class molecules.<sup>37</sup> The ability to constitutively express and, upon activation, upregulate surface MHC-II molecules is a very important feature of DCs (and also other pAPCs). Almost all other nucleated cells in the body express MHC-I and only under certain conditions (mainly upon persistent IFN- $\gamma$  stimulation) may express MHC-II class molecules;<sup>38</sup> however, these cells lack other important pAPC features, which will be described later. Therefore, in general, only pAPCs are able to activate CD4<sup>+</sup> Th cells (their TCR recognizes MHC-II-loaded peptides), which are pivotal for the generation of both cellular and humoral immune responses.
- DCs express various co-stimulatory molecules, such as CD80, CD86, OX-40L (CD252), 4-1BBL (CD137L), and CD70, which are essential for optimal immunogenic activation of T cells and induction of both effector and memory T cell responses.<sup>8,39</sup>

MHC-I and MHC-II class and various co-stimulatory molecules are also expressed on the surface of monocytes/macrophages and B lymphocytes, which are also defined as pAPCs. However, with their unique capability to initiate effective primary immune responses DCs are the most potent APCs, as discussed above.

It should be noted that DCs are also involved in Ag presentation to B cells and their activation.<sup>35,40</sup> During processing of Ags, engulfed as immune complexes through the inhibitory FcγRIIB (CD32b) receptor, DCs can direct these Ags to non-degradative recycling compartments thus preserving them from proteolytic degradation and enabling their presentation in intact (three-dimensional) form to B cells, whose receptor (BCR) recognizes unprocessed Ags.<sup>40</sup> Moreover, human dermal DCs expressing CD14 can induce naïve Th0 cells to differentiate into T-follicular helper (Tfh)-like cells, which are specialized in B-cell help.<sup>35</sup>

Endothelial cells (ECs) also express both MHC-I and MHC-II class molecules, as well as co-stimulatory molecules, and secrete various cytokines after activation with inflammatory cytokines. However, ECs are not migratory (i.e. they lack a very important feature of pAPCs) and they are also not able to induce primary immune responses (although they can activate circulating memory T cells and induce secondary immune responses); therefore, they are termed non-professional APCs (reviewed in Choi et al.<sup>41</sup>).

## Types and origin of DCs

There are several subtypes of DCs which differ in their phenotype, localization, migration pathways, and function, i.e. they may differ in effects on innate and adaptive immunity.<sup>42</sup> All these subtypes can be grouped into two major DC subsets: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), with the former being more prevalent.<sup>43,44</sup>

### Myeloid DCs

mDCs (also called conventional or classical DCs) originate from common myeloid progenitor in the bone marrow and are distinguished by their expression of CD11c (α<sub>x</sub> integrin).<sup>45</sup> They are released from the bone marrow into the blood as precursor DCs (pre-DCs), which circulate for a very short period (up to 1 h)<sup>46</sup> and migrate into various tissues where they differentiate into immature mDCs.<sup>47</sup> Based on tissue localization and migratory capacity, mDCs can be divided into lymphoid tissue-resident mDCs and migratory mDCs. The former permanently settle in the lymphoid organs and encounter soluble Ags that enter these sites. The latter are found in the peripheral tissues (skin, gut, liver, kidney, lung, aorta), lymphoid organs (mainly in the lymph nodes and spleen), and blood (in very small quantities, constituting <1% of human PBMCs).<sup>48</sup> Migratory mDCs constantly recirculate between these compartments, even in the absence of danger.<sup>49</sup>

After encountering an Ag and receiving essential co-stimuli tissue migratory mDCs mature and migrate to the draining lymph nodes via afferent lymphatics

where they present the epitopes of the processed Ags to T cells. However, there is evidence that in some situations (depending on the pathogen, as well as the route and site of infection) murine migratory mDCs may present Ags to T cells indirectly, i.e. migratory mDCs capture Ags in the periphery and after migrating to the lymph nodes transfer them (e.g. through direct cell-cell interaction and/or exosomes) to the lymphoid-resident DCs which, in turn, present these Ags to T cells.<sup>50,51</sup> There is evidence that the activation of CD8<sup>+</sup> may be mainly performed by lymphoid-resident DCs,<sup>50</sup> whereas both migratory and lymphoid-resident DCs are required for efficient CD4<sup>+</sup> T cell priming.<sup>51</sup> The exact role of various mDC subsets in Ag presentation to T cells in distinct infections (and possibly cancer as well) seems to be very complex and remains to be fully elucidated.

Three phenotypically and functionally distinct subsets of CD11c<sup>+</sup> mDCs were identified in the human blood, including CD141<sup>+</sup> (human blood dendritic cell Ag (BDCA)-3<sup>+</sup>), CD1c<sup>+</sup> (BDCA-1<sup>+</sup>), and CD16<sup>+</sup> mDCs.<sup>36,52</sup>

CD141<sup>+</sup> mDC subset is the rarest and constitutes only about 3% of human circulating CD11c<sup>+</sup> mDCs.<sup>52,53</sup> These cells were also identified in human bone marrow, tonsils, lymph nodes, and spleen.<sup>53</sup> CD141<sup>+</sup> mDCs exhibit a very strong capacity to cross-present extracellular protein Ags with MHC-I class molecules, express high levels of TLR3, and, upon activation, secrete high amounts of IL-12p70, IFN-β, and CXCL10. CD141<sup>+</sup> mDCs seem to be preferentially specialized in inducing Th1 polarization and the generation of CD8<sup>+</sup> cytotoxic T lymphocyte (CTL) responses, presumably both in an IL-12p70 secretion-dependent and secretion-independent manner.<sup>53</sup>

CD1c<sup>+</sup> mDCs comprise about 35% of human circulating CD11c<sup>+</sup> mDCs.<sup>52</sup> Their functional specialization is less well described. Current data suggest that these mDCs efficiently present Ags to CD4<sup>+</sup> T cells; however, they seem to be intrinsically poor inducers of Th1 type polarization and CTL responses.<sup>43</sup> In addition, human CD1c<sup>+</sup> mDCs were shown to exhibit strong chemoattractant activity owing to their high secretion of IL-8 (CXCL8).<sup>53</sup>

The CD16<sup>+</sup> mDC subset is the most abundant, comprising about 60% of human circulating CD11c<sup>+</sup> mDCs.<sup>52</sup> These DCs seem to be involved in Ab-dependent cell cytotoxicity and the early encounter of microbial pathogens. They are principally activated by microbial rather than inflammatory cytokine stimuli.<sup>36</sup> However, based on global transcriptomic analysis, genome-wide comparative gene expression profiling, and their hierarchical clustering, some authors argue the inclusion of these Lin<sup>+</sup>HLA-DR<sup>+</sup>CD14<sup>neg/low</sup>CD16<sup>+</sup> cells into the DC family, proposing that they constitute a rare subset of monocytes.<sup>54,55</sup>

With regard to human tissue migratory mDCs, the ones present in the skin are probably the best

characterized. There are at least three CD11c<sup>+</sup> mDCs subsets in the human skin: epidermal Langerhans cells (LCs, CD1a<sup>+</sup>CD207<sup>+</sup>E-Cadherin<sup>+</sup>) and dermal interstitial DCs, including CD14<sup>+</sup> and CD1a<sup>+</sup> DCs.<sup>35</sup> Current data suggest that human LCs preferentially activate cell-mediated adaptive immunity and induce potent CTL responses, although data from mice studies regarding the ability of LCs to induce CTL responses are conflicting.<sup>35</sup>

Human dermal CD14<sup>+</sup> DCs are preferentially involved in the induction of Th0-cell differentiation into Tfh-like cells, but not typical Th2 cells. Tfh-like cells induce robust B-cell proliferation, immunoglobulin (Ig) isotype switch toward IgG and IgA, and differentiation into plasma cells capable of producing large amounts of Igs. Dermal CD14<sup>+</sup> DCs are poor inducers of CTL differentiation; therefore, they seem to be specialized in the development of humoral rather than cellular adaptive immune responses.<sup>35</sup>

Human dermal CD1a<sup>+</sup> DCs seem to be functionally intermediate between LCs and dermal CD14<sup>+</sup> DCs.<sup>35,56</sup> Their role in skin immunity is not fully understood.

### Plasmacytoid DCs

Plasmacytoid DCs (pDCs) originate in the bone marrow from both common myeloid progenitor and common lymphoid progenitor, however their myeloid origin predominates.<sup>48</sup>

Human pDCs are characterized by:<sup>57,58</sup> (i) round shape, i.e. secretory plasmacytoid (plasma cell-like) morphology; (ii) the lack of expression of CD11c; (iii) Lin<sup>-</sup> phenotype; (iv) expression of CD4 and CD45RA on some pDCs; (v) expression of IL-3 receptor  $\alpha$  chain (IL-3R $\alpha$ , CD123), BDCA-4 (neuropilin-1, CD304), and ILT3 (CD85k), and (vi) the expression of the highly pDC-specific surface markers ILT7 (CD85g) and BDCA-2 (CD303). The latter two markers are expressed only on resting pDCs and are downregulated upon their activation.<sup>57</sup>

Based on the expression of surface CD2, human pDCs are subdivided into two functionally distinct subsets, namely CD2<sup>high</sup>, which shows prevalent expression of myeloid-related genes, and CD2<sup>low</sup>.<sup>59</sup> The former exhibits high expression of intracellular lysozyme, the ability to form very tight clusters with other cells (a property that may enable thorough screening for and subsequent capturing of Ags from the surface of cancer or virally-infected cells), and is a more potent inducer of allogeneic naïve T cell proliferation than the latter.<sup>59</sup>

pDCs (both in humans and mice) mainly reside in lymphoid organs (bone marrow, thymus, spleen, lymph nodes, tonsils) and blood;<sup>58</sup> however, in fact, they are present in all non-lymphoid peripheral organs, albeit at low numbers,<sup>60,61</sup> with the exception of murine liver, where relatively high pDC numbers are detected.<sup>62</sup>

pDCs are the main cellular producers of type I IFNs (IFN- $\alpha/\beta$ ) in response to viral, bacterial and self nucleic acids. Their activation is mainly induced by ligation of TLR7 (which recognizes single-stranded viral RNA) and TLR9 (recognizing unmethylated CpG sites in DNA).<sup>58,59</sup> pDCs respond to these stimuli very rapidly and are up to 1000-fold potent type I IFN producers than other cell types; moreover, IFN- $\alpha$  secretion in response to unmethylated CpG stimulus *in vivo* is mediated exclusively by pDCs.<sup>58</sup>

Activated pDCs may undergo typical mDC maturation and act as pAPCs capable of presenting Ags to CD4<sup>+</sup> and CD8<sup>+</sup> T cells; however, the manner and efficacy of Ag presentation by pDCs seems to be distinct from that described for mDCs and they may be specialized at presenting a very specific category of Ags (e.g. viral) rather than serving as a multipurpose APC-like mDCs (reviewed in Villadangos and Young<sup>63</sup>). pDCs have a specialized early endosomal compartment containing pre-synthesized vesicular stores of MHC-I class molecules which are “waiting” for extracellular Ag loading and enable rapid cross-presentation of these Ags to memory CD8<sup>+</sup> T cells in a proteasome-independent manner.<sup>64</sup> Additionally, in contrast to mDCs, pDCs are able to continuously present protein Ags with MHC-II class molecules owing to sustained MHC-II class molecule synthesis and MHC class II-peptide complex surface expression long after pDC activation.<sup>65</sup>

Importantly, the acquisition of pAPC properties by pDCs is associated with the loss of capacity to produce type I IFNs.<sup>66</sup> It was suggested that initial pDC activation induces rapid and abundant secretion of type I IFNs followed by differentiation into mDCs (reviewed in Reizis et al.<sup>58</sup>). There is some *in vivo* data to support this notion both in mice<sup>67</sup> and humans;<sup>68</sup> however, firm *in vivo* evidence is needed to unequivocally confirm this conception. Furthermore, in some cases (depending on the type of stimulus) pDC may selectively exert either pAPC activity or the production of type I IFNs.<sup>69</sup>

### Killer (cytotoxic) DCs

Recently, it was shown that besides their APC function, some human and rodent DC subsets (both myeloid and plasmacytoid) may exhibit cytotoxic activity. These DCs were named killer DCs (KDCs). In fact, human and rodent KDCs differ in their phenotype, heterogeneity and functional activity (reviewed in<sup>70</sup>); some murine KDC subsets (e.g. IFN- $\gamma$ -secreting KDCs, IKDCs) originally attributed to KDCs may actually be NK cells at an intermediate stage of differentiation and with high functional plasticity.<sup>71</sup> In this review we are more focused on the human system and do not discuss rodent KDC in more detail. Briefly, native human KDCs (hKDCs) were isolated from peripheral blood and detected in tumor tissue (namely in



basal cell carcinoma treated with TLR7/8 agonist imiquimod<sup>72</sup>). However, because of the paucity of DCs *in vivo*, most studies investigated the killing activity of *ex vivo*-generated hKDCs, derived mainly from peripheral blood monocytes. It was shown that hKDCs kill cancer cells (both freshly isolated and various cancer cell lines) and endothelial cells, whereas other normal cells seem to be resistant to hKDC cytotoxic effect for still undefined reasons.<sup>73</sup> Most likely tumor cells express some receptors that are not expressed on normal cells. There is conflicting data with regard to: (i) the requirement of activation stimuli (e.g. by type I IFNs, IFN- $\gamma$ , CD40L, viruses, etc.) for the induction or augmentation of hKDCs killing activity or, conversely, the inhibitory effects of these stimuli on the cytotoxic activity of KDC; and (ii) the mechanisms that KDCs use for their killing activity [various data support TNF- $\alpha$ , FasL, TNF-related apoptosis-inducing ligand (TRAIL)-dependent versus independent pathways].<sup>74</sup> A very recent study suggested that peroxynitrites may be the primary molecules responsible for hKDC tumoricidal function.<sup>73</sup> This study also showed that immature hKDCs generated from peripheral blood monocytes of cancer patients with advanced disease and healthy donors exhibited very similar phenotype and cytotoxic activity following LPS-induced maturation and activation. These data imply that the tumor microenvironment and its systemic effects have no negative impact on the cytotoxic potential of *ex vivo*-generated monocyte-derived hKDCs. Moreover, following the killing and subsequent uptake of necrotic cancer cell fragments, hKDCs differentiated into fully mature pAPCs capable of inducing proliferation of autologous T cells and activation of tumor Ag-specific CTLs. These findings indicate that *ex vivo*-generated hKDCs may have strong tumoricidal activity and therefore may be used for designing improved DC-based vaccines.<sup>75</sup> However, it is unknown whether such KDCs are generated *in vivo* in cancer patients, as the tumor microenvironment may severely impair the functional activity of DCs (as will be discussed later in this review). Furthermore, current evidence suggests that mainly immature DCs are endowed with cytotoxic activity.<sup>74,75</sup> Therefore, if KDCs are naturally generated in cancer patients and kill and subsequently engulf tumor cells but do not undergo maturation (owing to the suppressive effect of tumor milieu), they will present tumor Ags to T cells in a tolerogenic manner and will induce T cell tolerance rather than active antitumor immune response.

### Origin and lifespan of DCs

The relationship between monocytes/macrophages and DCs has been a matter of debate. It is well known that monocytes cultured *in vitro* with particular cytokines (e.g. a mixture of GM-CSF and IL-4) can differentiate into cells which share many phenotypic and functional characteristics of DCs. Moreover, functional activity of these

monocyte-derived DCs depends on the cytokines, used as differentiation stimuli.<sup>76</sup> A very similar phenomenon of monocyte differentiation into DCs was also observed *in vivo*, e.g. during *Listeria monocytogenes* infection in mice.<sup>77</sup> Therefore, for a long time it was believed that all DCs originate from monocytes *in vivo*; however, recent data indicate that, generally, monocytes are not precursors for either lymphoid or non-lymphoid tissue DCs in the steady state.<sup>47</sup> Therefore, DCs compose a distinct lineage of leukocytes and only their distinct subsets, for example CD11b<sup>+</sup>CD103<sup>-</sup> mDCs in mice, are of monocyte origin (reviewed in Liu et al.<sup>48</sup>).

LCs seem to be a distinct subset of mDCs in terms of their origin, as they have some peculiar features like self-renewal capacity *in situ*, independent of bone marrow-derived DC precursors.<sup>48,78</sup> It was suggested that LCs derive from local progenitors of myeloid origin which home the skin prior to birth.<sup>48</sup>

For a long time it was believed that DCs are terminally-differentiated cells and are not able to proliferate; however, more recent data indicate that mDCs,<sup>46</sup> but most likely not pDCs,<sup>79</sup> can proliferate *in situ*. Fms-like tyrosine kinase-3 ligand (Flt3L) is essential for their proliferative activity.<sup>80</sup> In contrast to pDCs, which are thought to be long-lived cells,<sup>79</sup> mDCs divide *in situ* for 10–14 d before being replaced by newly-generated pre-DCs.<sup>48</sup>

### Immature and mature DCs

DCs exist in two stages—immature and mature—which differ in their morphology, phenotype, and functional activity. In the steady state immature DCs constantly screen their environment, like sentinels. Upon encountering an Ag, they capture it by phagocytosis, macropinocytosis, or receptor-mediated (clathrin-dependent or clathrin-independent) endocytosis.<sup>81</sup> DCs may also uptake tumor Ags by “nibbling”<sup>82</sup> or trogocytosis,<sup>83</sup> or they may engulf tumor-derived exosomes.<sup>84</sup> High endocytic activity (Ag uptake and sequestration) is a hallmark of immature DCs.<sup>85</sup>

Once inside the DC, protein Ags are processed into peptides which are complexed with both MHC-I and MHC-II class molecules and presented on the surface of DC.<sup>85</sup> It is well known that exogenous protein Ags are presented mainly with MHC-II class molecules by pAPCs, whereas MHC-I class molecules present mainly intracellular Ags. However, some DC subsets (e.g. human LCs<sup>35</sup> or circulating CD141<sup>+</sup> DCs<sup>53</sup>), but generally not monocytes/macrophages and B cells, are capable of presenting exogenous protein Ags with MHC-I class molecules through an alternate process called cross-presentation.<sup>86</sup>

MHC-loaded antigenic peptides interact with TCR; however, as was mentioned above, various co-stimulatory signals are needed for the optimal activation of naïve and central memory T cells, and initiation of an adaptive immune response. Immature DCs express only low or intermediate levels of these

molecules on their surface.<sup>37,85</sup> Therefore, after encountering an Ag DCs should upregulate the expression of co-stimulatory molecules, apart from MHC-I and MHC-II molecules, through the process called maturation.

### Induction and characterization of DC maturation

Various signals are required for the induction of DC maturation. These signals are provided by:

- recognition of foreign, “non-self” evolutionary conserved microbial structures, known as pathogen-associated molecular patterns (PAMPs);<sup>87</sup>
- recognition of various endogenous intracellular host molecules, collectively designated as damage-associated molecular patterns (DAMPs).<sup>88,89</sup> Normally, the latter should not be outside the cell at high concentrations [e.g. uric acid, ATP, high mobility group box (HMGB)-1 protein, self DNA and RNA, etc.], or should not be expressed on the cell surface [e.g. calreticulin, heat shock protein (Hsp)70, Hsp90]. However, these “hidden” self-molecules may be abundantly released extracellularly or translocated from intracellular compartments to the cell surface by dying cells, although it depends on the type of cell death, as will be discussed later. These ectopically exposed “self” molecules are recognized as DAMPs by the components of innate immunity.<sup>88</sup> It is believed that receptors for DAMPs and PAMPs are often shared;<sup>89</sup>
- recognition of altered “self” molecules expressed by tumor cells. If these molecules (tumor Ags) acquire immunogenic (i.e. sufficiently “non-self”) alterations they may also act as DAMPs;<sup>90</sup>
- various inflammatory molecules released by other activated immune cells, e.g. NK, NKT cells.<sup>91</sup>

DC maturation is characterized by several typical features:<sup>37,85,92–94</sup> (i) high expression of CD83 molecules, which serve as a selective marker for DC maturation; (ii) diminished endocytic/phagocytic activity—it is generally accepted that all forms of endocytosis are downregulated in mature DCs; however, recent data challenge this notion showing that only non-specific forms of endocytosis (constitutive macropinocytosis and phagocytosis) are dramatically dampened, whilst receptor-mediated endocytosis and phagocytosis seem to be retained following mDC maturation;<sup>95</sup> (iii) upregulation of surface expression of both MHC-I and MHC-II class molecules; (iv) increased expression of various co-stimulatory molecules, chemokine receptors (e.g. CCR7), and adhesion molecules [e.g. intercellular adhesion molecule-1 (ICAM-1)]; (v) secretion of various cytokines (their profile depends on maturation-inducing signals, as well as on the subtype of DC); and (vi) enhanced

migratory potential, which directs mature DCs from the periphery to the secondary lymphoid organs via afferent lymphatic vessels.

### “Licensing” of mature DCs

Even DC maturation is not sufficient for adequate immunogenic activation of T cells, especially in the context of CD8<sup>+</sup> T cell activation. An additional process, called DC “licensing”, is required. It involves the interaction of mature DCs with activated CD4<sup>+</sup> T cells<sup>96</sup> or NKT cells<sup>97</sup> via CD40 on the surface of DC and CD40L on the surface of T cell. This interaction leads to the final step in DC maturation, further enhancing the Ag-presenting capacity of DCs by: (i) augmentation of co-stimulatory molecule expression; (ii) enhancement of cytokine secretion; and (iii) promotion of cross-presentation of exogenous protein Ags with MHC-I class molecules.<sup>98</sup>

Currently, it is strongly believed that only licensed DCs are capable of activating naïve CD8<sup>+</sup> T cells efficiently. Hence, without this interaction the activated “helpless” primary CTLs may function as effector cells, but with a limited lifespan, as they undergo activation-induced cell death (AICD) following subsequent stimulation by the cognate Ag. The AICD is mediated by the secretion of TRAIL, which triggers apoptosis in both “helpless” CD8<sup>+</sup> T cells and bystander-activated T cells.<sup>99</sup>

### Tolerogenic/regulatory DCs

It is important to note that immature DCs are capable of presenting Ags to naïve T cells. However, due to the lack of co-stimulation and low expression of MHC-I and MHC-II class molecules they induce suboptimal T cell priming resulting in T cell tolerance which is characterized by anergy or depletion of Ag-specific T cells, or by their conversion into iTreg subsets.<sup>100</sup> In fact, all immature DCs, irrespective of their tissue origin are tolerogenic *per se*.<sup>101</sup> In addition, some immature DCs may exhibit immunosuppressive/regulatory phenotype and activity. For example, immature tumor-infiltrating myeloid DCs (mTIDCs) co-expressing programmed death-1 (PD-1) and PD-L1 (B7-H1, CD274) were recently identified in mouse ovarian carcinoma.<sup>102</sup> These mTIDCs show low expression of co-stimulatory molecules, low secretion of inflammatory cytokines, and poor response to danger signals. Accumulation of these cells at the tumor site is associated with both a decreased number of tumor-infiltrating T cells and suppression of tumor Ag-specific effector activity.<sup>102</sup>

It should be emphasized that during the maturation process DCs may undergo an alternative maturation program and establish a stable semi-mature or “mature tolerogenic” state, which is characterized by:<sup>103–108</sup> (i) relatively high (intermediate) expression

of CD83, co-stimulatory, and MHC-II class molecules ("mature-like" phenotype), i.e. their expression is higher than on immature DCs but still lower than on fully mature DCs; (ii) lower production of pro-inflammatory cytokines, such as IL-12p70; (iii) higher secretion of immunosuppressive cytokines, such as IL-10 and TGF- $\beta$ ; and/or (iv) increased expression of inhibitory molecules, such as immunoglobulin-like transcript ILT3 and ILT4 on the surface of the DC.

The generation of mature immunogenic versus tolerogenic myeloid and plasmacytoid DCs depends on the microenvironment (*in vitro* and *in vivo*) in which the maturation takes place, both in health and disease.<sup>100,109,110</sup> For example, the generation of semimature tolerogenic DCs was induced by culturing immature DCs from healthy donors with the plasma of ductal pancreatic adenocarcinoma patients<sup>107</sup> or supernatants of various tumor cells lines.<sup>105</sup>

It should be also noted that there are DC subsets with intrinsic "natural" tolerogenic potential. Gregori et al. identified a stable mDC-10 subset which comprises only 0.2% of human PBMC and is characterized by a mature phenotype (CD83<sup>+</sup>, CD86<sup>+</sup>, HLA-DR<sup>+</sup>); expression of CD14, CD16, and the tolerogenic molecules HLA-G and ILT4; spontaneous secretion of immunosuppressive cytokine IL-10; low secretion of pro-inflammatory cytokine IL-12; and generation of induced regulatory Tr1 cells.<sup>111</sup>

### Recognition of tumor cells— a challenging task for DCs

pAPCs easily recognize various microbial PAMPs, which have distinct evolutionary conserve structure and are inherently foreign for humans.<sup>112</sup> By contrast, tumor cells are transformed own cells and the majority of Ags expressed on their surface are normal. Thus, they are recognized as "self" and not "worthy" of inducing an immune response.

It is known that owing to genetic instability and a high mutation rate in tumor cells, various altered (mutant) protein molecules may harbor potentially immunogenic peptides (epitopes).<sup>113</sup> Although it was demonstrated that immature DCs are able to recognize various tumor glycoprotein Ags, for example, aberrantly glycosylated carcinoembryonic Ag (CEA) on colorectal cancer cells,<sup>114</sup> it should be borne in mind that even such highly altered tumor cell surface glycoproteins are distributed between abundant unaltered normal self-Ags, which act like a camouflage on the cancer cell surface. Furthermore, various tumor glycoprotein Ags expressed on the surface of cancer cells may have very subtle structural changes, i.e. only slightly altered glycosylation. Therefore, they may be weakly "non-self"<sup>90</sup> and not easily recognized by the components of non-specific innate immunity, including pAPCs. In general, it appears that the recognition of

live tumor cells and their subsequent phagocytosis or "nibbling" of plasma membrane and cytosol is really a very difficult task for DCs.

Tumors always contain apoptotic and necrotic cells as a result of hypoxia or nutrient deprivation, and these dying tumor cells are more easily recognized by DCs.<sup>33</sup> There are also autophagic cells and tumor cells undergoing mitotic crisis (mitotic catastrophe, M2-type mortality) or pyroptosis,<sup>115</sup> which are not discussed here in detail. As reviewed by Kono and Rock<sup>88</sup>, Green et al.,<sup>116</sup> and Griffith and Ferguson<sup>117</sup> recognition and uptake of necrotic cells and unremoved apoptotic bodies undergoing rupture (secondary necrosis) generally leads to inflammation, because various DAMPs are abundantly released during necrotic cell death. These DAMPs and their induced inflammatory milieu may trigger DC maturation. The uptake of apoptotic cells, which is a normal process during natural tissue turnover, is performed mainly by macrophages,<sup>118</sup> and, to a lesser extent, by immature DCs and neighboring cells. Most importantly the uptake of apoptotic cells is an immunologically silent process; moreover, it induces pAPC tolerance rather than their activation in order to avoid autoimmunity.<sup>88,116,117</sup> Indeed, it was shown *in vitro* that recognition of surface phosphatidylserine (PS), an early marker of apoptotic cell death, renders DCs resistant to activation stimuli,<sup>119</sup> for example by suppressing TLR signaling.<sup>120</sup> Similarly, ingestion of apoptotic cells by macrophages and, most likely, by DCs induces secretion of immunosuppressive substances, such as TGF- $\beta$ , prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), platelet-activating factor (PAF), IL-10, which in autocrine and paracrine action inhibit the release of pro-inflammatory cytokines, such as IL-12, TNF- $\alpha$ , or IL-1.<sup>121–123</sup> Finally, apoptotic cells themselves can secrete immunosuppressive cytokines, such as TGF- $\beta$ .<sup>124</sup> Therefore, in general, it appears that recognition of apoptotic tumor cells by innate immunity results in the secretion of various immunosuppressive cytokines and generation of tolerogenic DCs which, in turn, contribute to the establishment of the immunosuppressive microenvironment at the tumor site.

Obviously, the numbers of apoptotic (tolerogenic) and necrotic (potentially immunogenic) cells varies among tumors. Moreover, various immunosuppressive factors released by apoptotic tumor cells and pAPCs that have phagocytosed them, as well as by live tumor cells (as will be discussed below), may interfere with the activation and immunogenic maturation of DCs that have encountered tumor cells undergoing immunogenic necrotic cell death. Therefore, despite the fact that DCs more easily recognize dying tumor cells, this may not lead to the generation of immunogenic DCs capable of inducing an effective antitumor immune response.

It is important to note that under certain conditions tumor cells may undergo an immunogenic apoptotic

cell death, a recently described subtype of apoptosis, which is characterized by:<sup>115,125–127</sup> (i) translocation of calreticulin (CR) from the endoplasmic reticulum to the cell surface (in this ectopic position it acts as an “eat me” signal)—importantly, this translocation of CR occurs at an early pre-apoptotic stage, prior to the exposure of phosphatidylserine (PS; an inhibitor of DC maturation) on the outer layer of the plasma membrane; and (ii) exposure of various other DAMPs, which may be released extracellularly (such as ATP, HMGB1, uric acid) or ectopically translocated to the cell surface (e.g. Hsp90). These DAMPs are pivotal for DC activation, as the sole relocation of CR to the cell surface is not sufficient, albeit absolutely required, for the induction of immunogenic DC maturation. Other additive tumor-derived factors that induce DC maturation during immunogenic cancer cell death are yet to be identified.<sup>127,128</sup>

It should be emphasized that immunogenic apoptosis occurs only under specific cellular stress, including: (i) exposure to some anti-neoplastic agents, such as anthracyclines (e.g. doxorubicine, daunorubicine) and oxaliplatin, but not cisplatin or topoisomerase inhibitors, such as etoposide; (ii) ultraviolet C (UVC) radiation; (iii)  $\gamma$ -radiation;<sup>125–128</sup> (iv) effect of some targeted cancer therapy agents, such as epidermal growth factor receptor antagonists<sup>129</sup> or the protease inhibitor, bortezomib;<sup>130</sup> and (v) photodynamic therapy.<sup>131</sup> Therefore, immunogenic apoptosis of tumor cells does not occur naturally and is not involved in eliciting natural anti-tumor immune responses; however, it may play a critical role in determining the therapeutic activity of various chemotherapeutical and radiation therapy regimens.

## DCs and the tumor microenvironment

There is some *in vivo* evidence that both tumor-infiltrating mDCs<sup>132–134</sup> and pDCs<sup>135</sup> are able to recognize tumor Ags, fully mature in the tumor microenvironment, and act as effective pAPCs inducing specific antitumor immune response. However, tumor-infiltrating DCs (TIDCs), both myeloid and plasmacytoid, often have impaired functions, even if they recognize tumor Ags. For example, TIDCs may remain immature<sup>136,137</sup> or may acquire expression of various immunosuppressive molecules.<sup>138–140</sup> Therefore, they are inefficient Ag presenters, but potent inducers of T cell tolerance, T cell death or conversion into iTreg cells.<sup>141,142</sup> Plasmacytoid TIDCs may exhibit an altered secretion of type I IFNs, which are very important players in antitumor immune response.<sup>143</sup> This alteration in pDC functional activity may result from tumor-induced decrease of TLR9 expression<sup>144</sup> or active signaling through ILT7 whose ligand BST2 (bone marrow stromal cell Ag-2) may be expressed on tumor cells.<sup>145,146</sup> Collectively, these data suggest that

TIDCs may act as inducers of immune tolerance to tumor Ags and promote tumor development rather than stimulate an antitumor immune response.

The immunogenic versus tolerogenic or tumor growth-facilitating potential of TIDCs seems to be very flexible, as DCs show high functional plasticity and their functional activity may be modulated by tumor microenvironment.<sup>109,147</sup> Tumor-derived factors that may influence tolerogenic versus immunogenic polarization of TIDCs include the lack of danger signals required for DC activation and maturation (as discussed above), and/or exposure of TIDCs to various local immunosuppressants, including cytokines (such as IL-6, IL-10, IL-13, TGF $\beta$ ), enzymes (e.g. indoleamine 2,3-dioxygenase, arginase), growth factors [e.g. GM-CSF, vascular endothelial growth factor (VEGF)], and other factors (e.g. some liver X receptor ligands, gangliosides, PGE<sub>2</sub>).<sup>109,147–149</sup> These immunosuppressive factors may be secreted by a variety of tumors (their parenchyma, stroma, or both), including tumor-infiltrating immune cells, in particular myeloid-derived suppressor cells (MDSCs)<sup>150</sup> and tumor-associated macrophages.<sup>151</sup>

Importantly, many studies demonstrated that the effect of the tumor on DC functional alterations is systemic rather than localized to tumor tissue and may be restored by either removing the tumor or by isolating DC precursors from tumor-bearing host and culturing them *ex vivo* under normal conditions, i.e. in the absence of tumor-associated changes in microenvironment.<sup>107,109</sup> However, even the removal of the tumor is not always associated with the restoration of DC function and this sustained negative effect may be explained, at least in part, by continual secretion of various suppressive inflammatory factors by tumor-adjacent tissues.<sup>107</sup>

Cancer may be associated not only with impaired functions of DCs, but also with their reduced numbers, especially in advanced disease stages.<sup>107,109,147,152</sup> This situation may recover after removal of the tumor.<sup>109,147,152</sup> The decrease of DC numbers in cancer patients was shown to be a consequence of general tumor-induced immunosuppression rather than increased migration of DCs to the tumor site.<sup>152</sup>

## DCs in tumor neovessel formation

Besides numerical alterations and impaired APC activity of DCs in cancer, TIDCs may participate in the formation of tumor neovessels. There is evidence that immature DCs have inherent pro-angiogenic properties and are involved in promoting angiogenesis through the secretion of various angiogenic factors, such as VEGF-A and basic fibroblast growth factor (bFGF) in several pathologies, including cancer.<sup>153,154</sup> Tumors take advantage of this property by recruiting immature mDCs. The ability of the tumor to maintain mTIDCs



in an immature state enables them to induce and sustain angiogenesis that is pivotal for tumor progression.<sup>154</sup> Recent *in vitro* data indicate that the pro-angiogenic potential of DCs may be promoted by particular pathological conditions (including those associated with cancer), such as the presence of specific soluble factors and/or certain composition and organization of extracellular matrix.<sup>155</sup>

In their review, Coukos et al.<sup>156</sup> discuss intriguing data showing that immature myeloid DCs, derived from monocytes or bone marrow CD34<sup>+</sup> precursors, may undergo endothelial transdifferentiation (“endothelization”) when cultured in the presence of angiogenic factors or in media conditioned by cancer cells expressing high levels of VEGF-A, which is critical for the transdifferentiation process. These immature DCs acquire expression of endothelial markers (such as von Willebrand factor, VEGF receptor-2, VE-cadherin, CD31), whilst retaining the expression of typical immature mDC markers (low expression levels of MHC-II, CD80, CD86).<sup>157</sup> Moreover, in angiogenic conditions these cells were shown to behave as endothelial-like cells, i.e. single cells oriented with the same polarity progressively aligned and formed string-like structures, which further grew longitudinally and finally forming cord-like structures in three-dimensional gels. In addition, the cells created intercellular junctions, organizing themselves around a lumen. Importantly, when these immature DCs expressing both endothelial and DC markers were placed in a pro-inflammatory milieu containing LPS, TNF- $\alpha$  and CpG, they differentiated into typical pAPCs, capable of inducing specific T cell responses.<sup>156,157</sup> Owing to their dual nature (DC and endothelial-like phenotype and functional activity) these cells were named vascular leukocytes (VLCs). Importantly, cells with a phenotype and functional activity very similar to VLCs were detected by immunofluorescence and isolated by sorting from samples of human ovarian carcinoma.<sup>158</sup> Their ability to form perfusable blood vessels was shown *in vivo* when human VLCs were xenotransplanted in Matrigel plugs into severe combined immunodeficiency (SCID) mice. Based on these results it was proposed that VLCs, generated from immature TIDCs following high production of VEGF by the tumor, may participate both in angiogenesis (formation of new blood vessels from the pre-existing ones) and vasculogenesis (*de novo* formation of blood vessels).<sup>156,158</sup>

It was later found that in the center of mouse ovarian carcinoma immature mTIDCs with dual phenotype are distributed in both pericytic-like and perivascular patterns (pericytic VLCs), whilst endothelial VLCs are primarily clustered in the tumor periphery at the area of tumor growth. The former secrete VEGF-A, FGF, and IL-8 which are crucial for angiogenesis, whilst the latter may also play a role in vasculogenesis.<sup>159</sup> Therefore, it appears that VLCs may acquire endothelial- and

pericytic-like attributes in different tumor areas. However, there is no direct evidence to date that they transdifferentiate into *bona fide* endothelial cells or pericytes. Based on these results a more recent “scaffold” model explaining the role of immature mTIDCs/VLCs in tumor vascularization suggests that, initially, endothelial-like VLCs may form tubular structures in the extracellular matrix, which are permeated by blood owing to the leaky nature of pre-existing tumor vessels. Sprouted endothelial cells brought by the blood flow subsequently line the tubules, displacing VLCs to the outer layers, i.e. to the pericytic location, as the vessel matures.<sup>159</sup>

In conclusion, it is evident that VLCs play a critical role in tumor angiogenesis and possibly vasculogenesis through paracrine effects, as well as direct structural support for the survival, stabilization, and branching of new blood vessels rather than direct formation of neovessels by themselves.<sup>158,159</sup> Therefore, in a peculiar tumor-associated milieu immature mTIDCs may be involved in tumor neovascularization. Hence, if TIDCs are not driven to initiate an immune response they may become tolerogenic or acquire a pro-angiogenic state and are therefore forced to support tumor growth and progression.<sup>155,156</sup>

### Conclusion: DCs need help to elicit an efficient antitumor immune response

All the peculiarities discussed above indicate that the recognition of tumor Ags and induction of immunogenic antitumor T cell responses are rather complicated tasks for DCs. Therefore, various strategies facilitating the introduction of tumor Ags to DCs have been developed in order to stimulate a robust and long-lasting Ag-specific antitumor immune response for the reduction or elimination of tumor cells, or for prevention of recurrences and metastases.<sup>160</sup> These strategies are defined as specific active tumor immunotherapy or therapeutic cancer vaccination and can be divided in two major approaches.<sup>160–162</sup>

- DCs may be targeted *in vivo*, e.g. as a result of administration of irradiated autologous or heterologous tumor cells (which may be additionally pre-treated with haptens to augment their immunogenicity or transfected with genes, coding immunostimulatory molecules), tumor Ag-derived peptides, intact tumor proteins, plasmid DNA encoding tumor Ags, etc.;
- DCs may be isolated from the cancer patient and manipulated (matured, loaded with tumor Ags, using various approaches) *ex vivo* and then injected back into the patient. A novel, cell-free approach employs DC-derived exosomes rather than intact DCs for therapeutic cancer vaccination.<sup>163</sup> In most cases “natural” DCs are not isolated from cancer

patients for *ex vivo* manipulations; instead, DCs are generated from the peripheral blood monocytes or marrow-derived CD34<sup>+</sup> precursor cells.

Although it is evident that DCs need help to induce an effective antitumor immune response in cancer patients, this help should be rational and well-designed. Indeed, current therapeutic cancer vaccination strategies still show relatively limited clinical efficacy.<sup>164</sup> It is crucial to verify the most potent protocols for the generation of immunogenic DCs as there are data that some current DC manipulation protocols may result in the generation of cancer vaccines with low immunogenic, or even potentially, tolerogenic activity.<sup>165,166</sup> Furthermore, parameters of tumor micro-environment and antitumor immune response should be evaluated<sup>4-6,167</sup> and in certain cases manipulated (e.g. MDSCs and Tregs should be disarmed or depleted)<sup>150,168</sup> in order to achieve a full-scale clinical advantage of methods that resuscitate the function of DCs in cancer patients.

## Funding

The publication of this article was partly sponsored by the International Consortium for Cell Therapy and Immunotherapy grant MSMT OPVK No. CZ.1.07/2.3.00/20.0012 and Ministry of Health of the Czech Republic grant IGA No. NT-11137.

## References

- Vesely MD, Kershaw MH, Schreiber RD and Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol* 2011; 29: 235–271.
- Schreiber RD, Old LJ and Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011; 331: 1565–1570.
- Shankaran V, Ikeda H, Bruce AT, et al. IFN- $\gamma$  and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001; 410: 1107–1111.
- Sato E, Olson SH, Ahn J, et al. Intraepithelial CD8<sup>+</sup> tumor-infiltrating lymphocytes and a high CD8<sup>+</sup>/regulatory T-cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci USA* 2005; 102: 18538–18543.
- Tosolini M, Kirilovsky A, Mlecnik B, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, Th2, Treg, Th17) in patients with colorectal cancer. *Cancer Res* 2011; 71: 1263–1271.
- Galon J, Pages F, Marincola F, et al. The immune score as a new possible approach for the classification of cancer. *J Transl Med* 2012; 10: 1.
- Bernard A, Lamy L and Alberti I. The two-signal model of T-cell activation after 30 years. *Transplantation* 2002; 73: S31–S35.
- Boesteanu AC and Katsikis PD. Memory T-cells need CD28 costimulation to remember. *Semin Immunol* 2009; 21: 69–77.
- van den Heuvel MJ, Garg N, Van Kaer L and Haeryfar SM. NKT cell costimulation: experimental progress and therapeutic promise. *Trends Mol Med* 2011; 17: 65–77.
- Gimmi CD, Freeman GJ, Gribben JG, et al. Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc Natl Acad Sci USA* 1993; 90: 6586–6590.
- Hernandez J, Aung S, Redmond WL and Sherman LA. Phenotypic and functional analysis of CD8<sup>+</sup> T cells undergoing peripheral deletion in response to cross-presentation of self-antigen. *J Exp Med* 2001; 194: 707–717.
- Chappert P and Schwartz RH. Induction of T cell anergy: integration of environmental cues and infectious tolerance. *Curr Opin Immunol* 2010; 22: 552–559.
- Hoyne GF. Mechanisms that regulate peripheral immune responses to control organ-specific autoimmunity. *Clin Dev Immunol* 2011; 2011: 294968.
- Redmond WL, Marincek BC and Sherman LA. Distinct requirements for deletion versus anergy during CD8 T cell peripheral tolerance in vivo. *J Immunol* 2005; 174: 2046–2053.
- Sirivivasan M and Frauwirth KA. Peripheral tolerance in CD8<sup>+</sup> T cells. *Cytokine* 2009; 46: 147–159.
- Kretschmer K, Apostolou I, Hawiger D, et al. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol* 2005; 6: 1219–1227.
- Levings MK, Gregori S, Tresoldi E, et al. Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not CD25+CD4+ Tr cells. *Blood* 2005; 105: 1162–1169.
- Nussenzweig MC, Steinman RM, Gutchinov B and Cohn ZA. Dendritic cells are accessory cells for the development of anti-trinitrophenyl cytotoxic T lymphocytes. *J Exp Med* 1980; 152: 1070–1084.
- Stagg AJ and Knight SC. *Antigen-presenting Cells*. Chichester: eLS John Wiley & Sons Ltd, 2001. DOI: 101038/npg.els.0000903.
- Fürnrohr BG, Sheriff A, Munoz L, et al. Signals, receptors, and cytokines involved in the immunomodulatory and anti-inflammatory properties of apoptotic cells. *Signal Transduct* 2005; 5: 356–365.
- Kaiko GE, Horvat JC, Beagley KW and Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* 2008; 123: 326–338.
- Kaliński P, Hilkens CM, Wierenga EA and Kapsenberg ML. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol Today* 1999; 20: 561–567.
- Ma CS, Tangye SG and Deenick EK. Human Th9 cells: inflammatory cytokines modulate IL-9 production through the induction of IL-21. *Immunol Cell Biol* 2010; 88: 621–623.
- Amsen D, Blander JM, Lee GR, et al. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* 2004; 117: 515–526.
- Curtsinger JM, Lins DC, Johnson CM and Mescher MF. Signal 3 tolerant CD8 T cells degranulate in response to antigen but lack granzyme B to mediate cytotoxicity. *J Immunol* 2005; 175: 4392–4399.
- Kratky W, Reis e Sousa C, Oxenius A and Spörri R. Direct activation of antigen-presenting cells is required for CD8<sup>+</sup> T-cell priming and tumor vaccination. *Proc Natl Acad Sci USA* 2011; 108: 17414–17419.
- Watchmaker PB, Berk E, Muthuswamy R, et al. Independent regulation of chemokine responsiveness and cytolytic function versus CD8<sup>+</sup> T cell expansion by dendritic cells. *J Immunol* 2010; 184: 591–597.
- Macagno A, Napolitani G, Lanzavecchia A and Sallusto F. Duration, combination and timing: the signal integration model of dendritic cell activation. *Trends Immunol* 2007; 28: 227–233.
- Spörri R and Reis e Sousa C. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4<sup>+</sup> T-cell populations lacking helper function. *Nat Immunol* 2005; 6: 163–170.
- Joffre O, Nolte MA, Spörri R and Reis e Sousa C. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. *Immunol Rev* 2009; 227: 234–247.
- Chang CS, Chang JH, Hsu NC, et al. Expression of CD80 and CD86 costimulatory molecules are potential markers for better survival in nasopharyngeal carcinoma. *BMC Cancer* 2007; 7: 88.

32. Ochsenbein AF, Sierro S, Odermatt B, et al. Roles of tumour localization, second signals and cross priming in cytotoxic T-cell induction. *Nature* 2001; 411: 1058–1064.
33. Petersen TR, Dickgreber N and Hermans IF. Tumor antigen presentation by dendritic cells. *Crit Rev Immunol* 2010; 30: 345–386.
34. Steinman RM and Banchereau J. Taking dendritic cells into medicine. *Nature* 2007; 449: 419–426.
35. Klechevsky E, Morita R, Liu MC, et al. Functional specializations of human epidermal Langerhans cells and CD14<sup>+</sup> dermal dendritic cells. *Immunity* 2008; 29: 497–510.
36. Piccioli D, Tavarini S, Borgogni E, et al. Functional specialization of human circulating CD16 and CD1c myeloid dendritic-cell subsets. *Blood* 2007; 109: 5371–5379.
37. Saththaporn S and Eremis O. Dendritic cells (I): biological functions. *J R Coll Surg Edinb* 2001; 46: 9–19.
38. Giroux M, Schmidt M and Descoteaux A. IFN- $\gamma$ -induced MHC class II expression: transactivation of class II transactivator promoter IV by IFN regulatory factor-1 is regulated by protein kinase C- $\alpha$ . *J Immunol* 2003; 171: 4187–4194.
39. Croft M. Costimulation of T cells by OX40, 4-1BB, and CD27. *Cytokine Growth Factor Rev* 2003; 14: 265–273.
40. Bergtold A, Desai DD, Gavhane A and Clynes R. Cell surface recycling of internalized antigen permits dendritic cell priming of B cells. *Immunity* 2005; 23: 503–514.
41. Choi J, Enis DR, Koh KP, et al. T lymphocyte-endothelial cell interactions. *Annu Rev Immunol* 2004; 22: 683–709.
42. Ueno H, Schmitt N, Klechevsky E, et al. Harnessing human dendritic cell subsets for medicine. *Immunol Rev* 2010; 234: 199–212.
43. Ueda Y, Hagihara M, Okamoto A, et al. Frequencies of dendritic cells (myeloid DC and plasmacytoid DC) and their ratio reduced in pregnant women: comparison with umbilical cord blood and normal healthy adults. *Hum Immunol* 2003; 64: 1144–1151.
44. Masten BJ, Olson GK, Tarleton CA, et al. Characterization of myeloid and plasmacytoid dendritic cells in human lung. *J Immunol* 2006; 177: 7784–7793.
45. Osugi Y, Vuckovic S and Hart DNJ. Myeloid blood CD11c<sup>+</sup> dendritic cells and monocyte-derived dendritic cells differ in their ability to stimulate T lymphocytes. *Blood* 2002; 100: 2858–2866.
46. del Hoyo GM, Martin P, Vargas HH, et al. Characterization of a common precursor population for dendritic cells. *Nature* 2002; 415: 1043–1047.
47. Liu K, Victoria GD, Schwickert TA, et al. In vivo analysis of dendritic cell development and homeostasis. *Science* 2009; 324: 392–397.
48. Liu K and Nussenzweig MC. Origin and development of dendritic cells. *Immunol Rev* 2010; 234: 45–54.
49. Bonasio R and von Andrian UH. Generation, migration and function of circulating dendritic cells. *Curr Opin Immunol* 2006; 18: 503–511.
50. Allan RS, Waithman J, Bedoui S, et al. Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity* 2006; 25: 153–162.
51. Allenspach EJ, Lemos MP, Porrett PM, et al. Migratory and lymphoid-resident dendritic cells cooperate to efficiently prime naive CD4 T cells. *Immunity* 2008; 29: 795–806.
52. MacDonald KPA, Munster DJ, Clark GJ, et al. Characterization of human blood dendritic cell subsets. *Blood* 2002; 100: 4512–4520.
53. Jongbloed SL, Kassianos AJ, McDonald KJ, et al. Human CD141<sup>+</sup> (BDCA-3)<sup>+</sup> dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* 2010; 207: 1247–1260.
54. Robbins SH, Walzer T, Dembele D, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* 2008; 9: R17.
55. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* 2010; 116: E74–E80.
56. Palucka K, Banchereau J and Mellman I. Designing vaccines based on biology of human dendritic cell subsets. *Immunity* 2010; 33: 464–478.
57. Cao W. Molecular characterization of human plasmacytoid dendritic cells. *J Clin Immunol* 2009; 29: 257–264.
58. Reizis B, Bunin A, Ghosh HS, et al. Plasmacytoid dendritic cells: recent progress and open questions. *Annu Rev Immunol* 2011; 29: 163–183.
59. Matsui T, Connolly JE, Michnevit M, et al. CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions. *J Immunol* 2009; 182: 6815–6823.
60. Geissmann F, Manz MG, Jung S, et al. Development of monocytes, macrophages, and dendritic cells. *Science* 2010; 330: 656–661.
61. Swiecki M and Colonna M. Accumulation of plasmacytoid DC: roles in disease pathogenesis and targets for immunotherapy. *Eur J Immunol* 2010; 40: 2094–2098.
62. Pillarisetty VG, Shah AB, Miller G, et al. Liver dendritic cells are less immunogenic than spleen dendritic cells because of differences in subtype composition. *J Immunol* 2004; 172: 1009–1017.
63. Villadangos JA and Young L. Antigen-presentation properties of plasmacytoid dendritic cells. *Immunity* 2008; 29: 352–361.
64. Di Pucchio T, Chatterjee B, Smed-Sorensen A, et al. Direct proteasome-independent cross-presentation of viral antigen by plasmacytoid dendritic cells on major histocompatibility complex class I. *Nat Immunol* 2008; 9: 551–557.
65. Young LJ, Wilson NS, Schnorrer P, et al. Differential MHC class II synthesis and ubiquitination confers distinct antigen-presenting properties on conventional and plasmacytoid dendritic cells. *Nat Immunol* 2008; 9: 1244–1252.
66. Sozzani S, Vermi W, Del Prete A and Facchetti F. Trafficking properties of plasmacytoid dendritic cells in health and disease. *Trends Immunol* 2010; 31: 270–277.
67. Liou LY, Blasius AL, Welch MJ, et al. In vivo conversion of BM plasmacytoid DC into CD11b<sup>+</sup> conventional DC during virus infection. *Eur J Immunol* 2008; 38: 3388–3394.
68. Lindstedt M, Lundberg K and Borrebaeck CAK. Gene family clustering identifies functionally associated subsets of human in vivo blood and tonsillar dendritic cells. *J Immunol* 2005; 175: 4839–4846.
69. Kerkmann M, Rothenfusser S, Hornung V, et al. Activation with CpG-A and CpG-B oligonucleotides reveals two distinct regulatory pathways of type I IFN synthesis in human plasmacytoid dendritic cells. *J Immunol* 2003; 170: 4465–4474.
70. Wesa AK and Storkus WJ. Killer dendritic cells: mechanisms of action and therapeutic implications. *Cell Death Differ* 2008; 15: 51–57.
71. Giumont-Desrochers F, Boucher G, Dong Z, et al. Redefining interferon-producing killer dendritic cells as a novel intermediate in NK cell differentiation. *Blood* 2012; 119: 4349–4357.
72. Stary G, Bangert C, Tauber M, et al. Tumoricidal activity of TLR7/8-activated inflammatory dendritic cells. *J Exp Med* 2007; 204: 1441–1451.
73. Janjic BM, Lu GW, Pimenov A, et al. Innate direct anticancer effector function of human immature dendritic cells. I. Involvement of an apoptosis-inducing pathway. *J Immunol* 2002; 168: 1823–1830.
74. Larmonier N, Fraszczak J, Lakomy D, et al. Killer dendritic cells and their potential for cancer immunotherapy. *Cancer Immunol Immunother* 2010; 59: 1–11.
75. Lakomy D, Janikashvili N, Fraszczak J, et al. Cytotoxic dendritic cells generated from cancer patients. *J Immunol* 2011; 187: 2775–2782.



76. Kalantari T, Kamali-Sarvestani E, Ciric B, et al. Generation of immunogenic and tolerogenic clinical-grade dendritic cells. *Immunol Res* 2011; 51: 153–160.
77. Serbina NV, Salazar-Mather TP, Biron CA, et al. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* 2003; 19: 59–70.
78. Hemmerling J, Wegner-Kops J, von Stebut E, et al. Human epidermal Langerhans cells replenish skin xenografts and are depleted by alloreactive T cells in vivo. *J Immunol* 2011; 187: 1142–1149.
79. O’Keeffe M, Hochrein H, Vremec D, et al. Mouse plasmacytoid cells: Long-lived cells, heterogeneous in surface phenotype and function, that differentiate into CD8<sup>+</sup> dendritic cells only after microbial stimulus. *J Exp Med* 2002; 196: 1307–1319.
80. Waskow C, Liu K, Darrasse-Jeze G, et al. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol* 2008; 9: 676–683.
81. Doherty GJ and McMahon HT. Mechanisms of endocytosis. *Annu Rev Biochem* 2009; 78: 857–902.
82. Harshyne LA, Zimmer MI, Watkins SC and Barratt-Boyes SM. A role for class A scavenger receptor in dendritic cell nibbling from live cells. *J Immunol* 2003; 170: 2302–2309.
83. Zhang QJ, Li XL, Wang D, et al. Trogocytosis of MHC-I/peptide complexes derived from tumors and infected cells enhances dendritic cell cross-priming and promotes adaptive T-cell responses. *PLoS ONE* 2008; 3: e3097.
84. Yang CJ and Robbins PD. The roles of tumor-derived exosomes in cancer pathogenesis. *Clin Dev Immunol* 2011; 2011: 842–849.
85. Trombetta ES and Mellman I. Cell biology of antigen processing in vitro and in vivo. *Annu Rev Immunol* 2005; 23: 975–1028.
86. Flinsenberg TWH, Compeer EB, Boelens JJ and Boes M. antigen cross-presentation: extending recent laboratory findings to therapeutic intervention. *Clin Exp Immunol* 2011; 165: 8–18.
87. Kumar H, Kawai T and Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol* 2011; 30: 16–34.
88. Kono H and Rock KL. How dying cells alert the immune system to danger. *Nat Rev Immunol* 2008; 8: 279–289.
89. Rubartelli A and Lotze MT. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol* 2007; 28: 429–436.
90. Schreiber TH, Raez L, Rosenblatt JD and Podack ER. Tumor immunogenicity and responsiveness to cancer vaccine therapy: the state of the art. *Semin Immunol* 2010; 22: 105–112.
91. Munz C, Steinman RM and Fujii SI. Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *J Exp Med* 2005; 202: 203–207.
92. Benvenuti F, Lagaudriere-Gesbert C, Grandjean I, et al. Dendritic cell maturation controls adhesion, synapse formation, and the duration of the interactions with naive T lymphocytes. *J Immunol* 2004; 172: 292–301.
93. Guernonprez P, Valladeau J, Zitvogel L, et al. antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 2002; 20: 621–667.
94. Prazma CM and Tedder TF. Dendritic cell CD83: A therapeutic target or innocent bystander? *Immunol Lett* 2008; 115: 1–8.
95. Platt CD, Ma JK, Chalouni C, et al. Mature dendritic cells use endocytic receptors to capture and present antigens. *Proc Natl Acad Sci USA* 2010; 107: 4287–4292.
96. Ridge JP, Di Rosa F and Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4<sup>+</sup> T-helper and a T-killer cell. *Nature* 1998; 393: 474–478.
97. Fujii SI, Liu K, Smith C, et al. The linkage of innate to adaptive immunity via maturing dendritic cells in vivo requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J Exp Med* 2004; 199: 1607–1618.
98. Elgueta R, Benson MJ, de Vries VC, et al. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev* 2009; 229: 152–172.
99. Janssen EM, Droin NM, Lemmens EE, et al. CD4<sup>+</sup> T-cell help controls CD8<sup>+</sup> T-cell memory via TRAIL-mediated activation-induced cell death. *Nature* 2005; 434: 88–93.
100. Manicassamy S and Pulendran B. Dendritic cell control of tolerogenic responses. *Immunol Rev* 2011; 241: 206–227.
101. Steinman RM, Hawiger D, Liu K, et al. Dendritic cell function in vivo during the steady state: A role in peripheral tolerance. *Ann NY Acad Sci* 2003; 15–25.
102. Krempski J, Karyampudi L, Behrens MD, et al. Tumor-infiltrating programmed death receptor-1<sup>+</sup> dendritic cells mediate immune suppression in ovarian cancer. *J Immunol* 2011; 186: 6905–6913.
103. Manavalan JS, Rossi PC, Vlad G, et al. High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. *Transpl Immunol* 2003; 11: 245–258.
104. Azzaoui I, Yahia SA, Chang Y, et al. CCL18 differentiates dendritic cells in tolerogenic cells able to prime regulatory T cells in healthy subjects. *Blood* 2011; 118: 3549–3558.
105. Kuang DM, Zhao Q, Xu J, et al. Tumor-educated tolerogenic dendritic cells induce CD3 $\epsilon$  down-regulation and apoptosis of T-cells through oxygen-dependent pathways. *J Immunol* 2008; 181: 3089–3098.
106. Stoop JN, Harry RA, von Delwig A, et al. Therapeutic effect of tolerogenic dendritic cells in established collagen-induced arthritis is associated with a reduction in Th17 responses. *Arthritis Rheum* 2010; 62: 3656–3665.
107. Tjomsland V, Spangeus A, Sandstrom P, et al. Semi mature blood dendritic cells exist in patients with ductal pancreatic adenocarcinoma owing to inflammatory factors released from the tumor. *PLoS ONE* 2010; 5: e13441.
108. Lutz MB and Schuler G. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol* 2002; 23: 445–449.
109. Bennaceur K, Chapman JA, Touraine JL and Portoukalian J. Immunosuppressive networks in the tumour environment and their effect in dendritic cells. *Biochim Biophys Acta* 2009; 1795: 16–24.
110. Steinbrink K, Mahnke K, Grabbe S, et al. Myeloid dendritic cell: From sentinel of immunity to key player of peripheral tolerance? *Hum Immunol* 2009; 70: 289–293.
111. Gregori S, Tomasoni D, Pacciani V, et al. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. *Blood* 2010; 116: 935–944.
112. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; 18: 767–811.
113. Segal NH, Parsons DW, Peggs KS, et al. Epitope landscape in breast and colorectal cancer. *Cancer Res* 2008; 68: 889–892.
114. van Gisbergen KPJM, Aarnoudse CA, Meijer GA, et al. Dendritic cells recognize tumor-specific glycosylation of carcinoembryonic antigen on colorectal cancer cells through dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin. *Cancer Res* 2005; 65: 5935–5944.
115. Tesniere A, Apetoh L, Ghiringhelli F, et al. Immunogenic cancer cell death: a key-lock paradigm. *Curr Opin Immunol* 2008; 20: 504–511.
116. Green DR, Ferguson T, Zitvogel L and Kroemer G. Immunogenic and tolerogenic cell death. *Nat Rev Immunol* 2009; 9: 353–363.
117. Griffith TS and Ferguson TA. Cell death in the maintenance and abrogation of tolerance: the five Ws of dying cells. *Immunity* 2011; 35: 456–466.
118. Geske FJ, Monks J, Lehman L and Fadok VA. The role of the macrophage in apoptosis: hunter, gatherer, and regulator. *Int J Hematol* 2002; 76: 16–26.
119. Chen X, Doffek K, Sugg SL and Shilyansky J. Phosphatidylserine regulates the maturation of human dendritic cells. *J Immunol* 2004; 173: 2985–2994.



120. Rothlin CV, Ghosh S, Zuniga EI, et al. TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell* 2007; 131: 1124–1136.
121. Fadok VA, Bratton DL, Konowal A, et al. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF- $\beta$ , PGE<sub>2</sub>, and PAF. *J Clin Invest* 1998; 101: 890–898.
122. Voll RE, Herrmann M, Roth EA, et al. Immunosuppressive effects of apoptotic cells. *Nature* 1997; 390: 350–351.
123. Girkontaite I, Urbonaviciute V, Maseda D, et al. Apoptotic cells selectively suppress the Th1 cytokine interferon  $\gamma$  in stimulated human peripheral blood mononuclear cells and shift the Th1/Th2 balance towards Th2. *Autoimmunity* 2007; 40: 327–330.
124. Chen WJ, Frank ME, Jin WW and Wahl SM. TGF- $\beta$  released by apoptotic T cells contributes to an immunosuppressive milieu. *Immunity* 2001; 14: 715–725.
125. Ma YT, Kepp O, Ghiringhelli F, et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. *Semin Immunol* 2010; 22: 113–124.
126. Zitvogel L, Kepp O and Kroemer G. Decoding cell death signals in inflammation and immunity. *Cell* 2010; 140: 798–804.
127. Zitvogel L, Kepp O and Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nat Rev Clin Oncol* 2011; 8: 151–160.
128. Hannani D, Sistigu A, Kepp O, et al. Prerequisites for the anti-tumor vaccine-like effect of chemotherapy and radiotherapy. *Cancer J* 2011; 17: 351–358.
129. Garrido G, Rabasa A, Sánchez B, et al. Induction of immunogenic apoptosis by blockade of epidermal growth factor receptor activation with a specific antibody. *J Immunol* 2011; 187: 4954–4966.
130. Spisek R, Charalambous A, Mazumder A, et al. Bortezomib enhances dendritic cell (DC)-mediated induction of immunity to human myeloma via exposure of cell surface heat shock protein 90 on dying tumor cells: therapeutic implications. *Blood* 2007; 109: 4839–4845.
131. Garg AD, Krysko DV, Vandenabeele P and Agostinis P. DAMPs and PDT-mediated photo-oxidative stress: exploring the unknown. *Photochem Photobiol Sci* 2011; 10: 670–680.
132. Schwaab T, Weiss JE, Schned AR and Barth Jr RJ. Dendritic cell infiltration in colon cancer. *J Immunother* 2001; 24: 130–137.
133. Iwamoto M, Shinohara H, Miyamoto A, et al. Prognostic value of tumor-infiltrating dendritic cells expressing CD83 in human breast carcinomas. *Int J Cancer* 2003; 104: 92–97.
134. Daud AI, Mirza N, Lenox B, et al. Phenotypic and functional analysis of dendritic cells and clinical outcome in patients with high-risk melanoma treated with adjuvant granulocyte macrophage colony-stimulating factor. *J Clin Oncol* 2008; 26: 3235–3241.
135. Salio M, Cella M, Vermi W, et al. Plasmacytoid dendritic cells prime IFN- $\gamma$ -secreting melanoma-specific CD8 lymphocytes and are found in primary melanoma lesions. *Eur J Immunol* 2003; 33: 1052–1062.
136. Stoitzner P, Green LK, Jung JY, et al. Inefficient presentation of tumor-derived antigen by tumor-infiltrating dendritic cells. *Cancer Immunol Immunother* 2008; 57: 1665–1673.
137. Perrot I, Blanchard D, Freymond N, et al. Dendritic cells infiltrating human non-small cell lung cancer are blocked at immature stage. *J Immunol* 2007; 178: 2763–2769.
138. Dumitriu IE, Dunbar DR, Howie SE, et al. Human dendritic cells produce TGF- $\beta$ 1 under the influence of lung carcinoma cells and prime the differentiation of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells. *J Immunol* 2009; 182: 2795–2807.
139. Norian LA, Rodriguez PC, O'Mara LA, et al. Tumor-infiltrating regulatory dendritic cells inhibit CD8<sup>+</sup> T cell function via L-arginine metabolism. *Cancer Res* 2009; 69: 3086–3094.
140. Munn DH, Sharma MD and Mellor AL. Ligation of B7-1/B7-2 by human CD4<sup>+</sup> T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. *J Immunol* 2004; 172: 4100–4110.
141. Enk AH, Jonuleit H, Saloga J and Knop J. Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. *Int J Cancer* 1997; 73: 309–316.
142. Wei S, Kryczek I, Zou LH, et al. Plasmacytoid dendritic cells induce CD8<sup>+</sup> regulatory T cells in human ovarian carcinoma. *Cancer Res* 2005; 65: 5020–5026.
143. Dunn GP, Bruce AT, Sheehan KCF, et al. A critical function for type I interferons in cancer immunoediting. *Nat Immunol* 2005; 6: 722–729.
144. Hartmann E, Wollenberg B, Rothenfusser S, et al. Identification and functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer. *Cancer Res* 2003; 63: 6478–6487.
145. Cai DQ, Cao J, Li Z, et al. Up-regulation of bone marrow stromal protein 2 (BST2) in breast cancer with bone metastasis. *BMC Cancer* 2009; 9: 102.
146. Vermi W, Soncini M, Melocchi L, et al. Plasmacytoid dendritic cells and cancer. *J Leukoc Biol* 2011; 90: 681–690.
147. Chaput N, Conforti R, Viaud S, et al. The Janus face of dendritic cells in cancer. *Oncogene* 2008; 27: 5920–5931.
148. Aspod C, Pedroza-Gonzalez A, Gallegos M, et al. Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4<sup>+</sup> T cells that facilitate tumor development. *J Exp Med* 2007; 204: 1037–1047.
149. Bellone G, Carbone A, Smirne C, et al. Cooperative induction of a tolerogenic dendritic cell phenotype by cytokines secreted by pancreatic carcinoma cells. *J Immunol* 2006; 177: 3448–3460.
150. Apetoh L, Végran F, Ladoire S and Ghiringhelli F. Restoration of antitumor immunity through selective inhibition of myeloid derived suppressor cells by anticancer therapies. *Curr Mol Med* 2011; 11: 365–372.
151. Solinas G, Germano G, Mantovani A and Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *J Leukoc Biol* 2009; 86: 1065–1073.
152. Pinzon-Charry A, Ho CSK, Maxwell T, et al. Numerical and functional defects of blood dendritic cells in early- and late-stage breast cancer. *Br J Cancer* 2007; 97: 1251–1259.
153. Curiel TJ, Cheng P, Mottram P, et al. Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer. *Cancer Res* 2004; 64: 5535–5538.
154. Fainaru O, Almog N, Yung CW, et al. Tumor growth and angiogenesis are dependent on the presence of immature dendritic cells. *FASEB J* 2010; 24: 1411–1418.
155. Sprague L, Muccioli M, Pate M, et al. The interplay between surfaces and soluble factors define the immunologic and angiogenic properties of myeloid dendritic cells. *BMC Immunol* 2011; 12: 35.
156. Coukos G, Benencia F, Buckanovich RJ and Conejo-Garcia JR. The role of dendritic cell precursors in tumour vasculogenesis. *Br J Cancer* 2005; 92: 1182–1187.
157. Conejo-Garcia JR, Benencia F, Courreges MC, et al. Tumor-infiltrating dendritic cell precursors recruited by a  $\beta$ -defensin contribute to vasculogenesis under the influence of Vegf-A. *Nat Med* 2004; 10: 950–958.
158. Conejo-Garcia JR, Buckanovich RJ, Benencia F, et al. Vascular leukocytes contribute to tumor vascularization. *Blood* 2005; 105: 679–681.
159. Huarte E, Cubillos-Ruiz JR, Nesbeth YC, et al. Depletion of dendritic cells delays ovarian cancer progression by boosting antitumor immunity. *Cancer Res* 2008; 68: 7684–7691.
160. Nierkens S and Janssen EM. Harnessing dendritic cells for tumor antigen presentation. *Cancers* 2011; 3: 2195–2213.

161. Srinivasan R and Van Epps DE. Specific active immunotherapy of cancer: potential and perspectives. *Rev Recent Clin Trials* 2006; 1: 283–292.
162. Sabado RL and Bhardwaj N. Directing dendritic cell immunotherapy towards successful cancer treatment. *Immunotherapy* 2010; 2: 37–56.
163. Viaud S, Thery C, Ploix S, et al. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? *Cancer Res* 2010; 70: 1281–1285.
164. Baxevanis CN, Perez SA and Papamichail M. Developing effective cancer vaccines. *Eur J Cancer* 2011; 47: S364–S365.
165. Gustafsson K, Junevik K, Werlenius O, et al. Tumour-loaded  $\alpha$ -type 1-polarized dendritic cells from patients with chronic lymphocytic leukaemia produce a superior NK-, NKT- and CD8<sup>+</sup> T cell-attracting chemokine profile. *Scand J Immunol* 2011; 74: 318–326.
166. Castiello L, Sabatino M, Jin P, et al. Monocyte-derived DC maturation strategies and related pathways: a transcriptional view. *Cancer Immunol Immunother* 2011; 60: 457–466.
167. Gajewski TF, Louahed J and Brichard VG. Gene signature in melanoma associated with clinical activity: a potential clue to unlock cancer immunotherapy. *Cancer J* 2010; 16: 399–403.
168. Cubillos-Ruiz JR, Rutkowski M and Conejo-Garcia JR. Blocking ovarian cancer progression by targeting tumor micro-environmental leukocytes. *Cell Cycle* 2010; 9: 260–268.