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Cancer immunotherapy via dendritic cells

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Abstract

Cancer immunotherapy attempts to harness the power and specificity of the immune system to treat tumours. The molecular identification of human cancer-specific antigens has allowed the development of antigen-specific immunotherapy. In one approach, autologous antigen-specific T cells are expanded *ex vivo* and then re-infused into patients. Another approach is through vaccination; that is, the provision of an antigen together with an adjuvant to elicit therapeutic T cells *in vivo*. Owing to their properties, dendritic cells (DCs) are often called 'nature's adjuvants' and thus have become the natural agents for antigen delivery. After four decades of research, it is now clear that DCs are at the centre of the immune system owing to their ability to control both immune tolerance and immunity. Thus, DCs are an essential target in efforts to generate therapeutic immunity against cancer.

Immunity results from a complex interplay between the innate immune system (which is antigen-nonspecific) and the adaptive immune system (which is antigen-specific). The cells and molecules of the innate immune system use non-clonal recognition receptors, including lectins, Toll-like receptors (TLRs), NOD-like receptors (NLRs) and helicases. B cells and T cells of the adaptive immune system use clonal receptors that recognize antigens, or their derived peptides, in a highly specific manner. In 2011, the Nobel Prize for Medicine or Physiology was awarded for the discovery of molecular and cellular sensors of microbes. Jules Hoffman and Bruce Beutler received the award for their seminal contributions to the discovery of TLRs in the 1990s. Ralph Steinman received the award for the discovery in 1973 of DCs, a rare cell type that is one of the key cellular sensors of microbes. DCs are linked to their environment through a wealth of molecular sensors that allow them to capture invading microbes and to transmit the resulting information to lymphocytes. Thus, DCs provide an essential link between the innate and adaptive immune responses.

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Competing interests statement
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DATABASES
National Cancer Institute Drug Dictionary:
http://www.cancer.gov/drugdictionary
imiquimod | rituximab | sipuleucel-T

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The immune system has the potential to eliminate neoplastic cells. Perhaps the most compelling evidence of tumour immunosurveillance in humans is provided by paraneoplastic diseases, which are neurological disorders that are a consequence of an anti-tumour immune response. Onconeural antigens, which are normally expressed on neurons, can also be expressed in breast cancer cells[1]. Some patients with paraneoplastic disease develop a strong antigen-specific $CD8^+$ T cell-mediated response that controls tumour expansion but that concomitantly results in autoimmune cerebellar degeneration[2], which causes a severe neurological disease. However, tumour cells themselves are poor antigen-presenting cells (APCs), which raises the question of how such potent immunity can be generated. Mouse models demonstrate that the generation of protective anti-tumour immunity depends on the presentation of tumour antigens by dendritic cells (DCs)[3,4].

Vaccination strategies involving DCs have been developed owing to the special properties of these cells in coordinating innate and adaptive immune responses. The aim of DC vaccination is to induce tumour-specific effector T cells that can reduce the tumour mass specifically and that can induce immunological memory to control tumour relapse. In this process, the first step is to provide DCs with tumour-specific antigens. This can be achieved either by culturing *ex vivo* DCs that have been derived from patients with an adjuvant (that induces DC maturation) and the tumour-specific antigen, and then injecting these cells back into the patient, or by inducing DCs to take up the tumour-specific antigen *in vivo*. To improve the therapeutic use of DC vaccination strategies it is important to understand the biology of DCs and how they regulate the innate and the adaptive immune systems — particularly in the context of the tumour microenvironment. This Review discusses our current knowledge of DC biology, and the progress and future challenges of DC vaccination strategies.

Basics of DC biology

DCs are bone marrow-derived cells that are seeded in all tissues (reviewed in REFS 5–7). DCs are poised to sample the environment and to transmit the gathered information to cells of the adaptive immune system (T cells and B cells)[5–7]. DCs initiate an immune response by presenting the captured antigen, which is in the form of peptide–major histocompatibility complex (MHC) molecule complexes, to naive (that is, antigen inexperienced) T cells in lymphoid tissues[5–7]. When compared with other APCs, such as macrophages, DCs are extremely efficient and can elicit very low numbers of T cells to respond[7–9], thus explaining their nickname of ‘professional APCs’. Mice and humans have two major subsets of DCs: myeloid DCs (mDCs; also known as conventional DCs and classical DCs) and plasmacytoid DCs (pDCs). We focus our discussion on human DC subsets and refer readers to excellent reviews on mouse DCs[10–13].

In peripheral tissues, DCs capture antigens through several complementary mechanisms[14]. Antigen-loaded DCs then migrate into the draining lymph nodes through the afferent lymphatics (FIG. 1). Meanwhile, they process the proteins into peptides that bind to both MHC class I molecules and MHC class II molecules. Lipid antigens are processed differently and are loaded onto non-classical MHC molecules of the CD1 family[15]. Antigens can also directly reach lymph node-resident DCs through the lymph[16] (FIG. 1). Distinct T cell responses are generated depending on whether antigen is captured by DCs in peripheral tissues or directly in lymph nodes[16].

Non-activated (immature) DCs can present self-antigens to T cells[17–19], which leads to immune tolerance either through T cell deletion or through the differentiation of regulatory or suppressor T cells. By contrast, activated (mature), antigen-loaded DCs can launch the differentiation of antigen-specific T cells into effector T cells with unique functions and

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cytokine profiles (FIG. 1). Lymph node-resident DCs that acquired antigen directly from the lymph are the first to present peptides to naive $CD4^+$ T cells, which results in T cell priming and interleukin-2 (IL-2) production, which in turn facilitates T cell proliferation and clonal expansion. Subsequently, tissue-resident DCs that captured antigen in peripheral tissues migrate into the lymph node and present peptides to the already activated $CD4^+$ T cells, which facilitates the generation of effector T cells.

On interaction with DCs, naive $CD4^+$ T cells and $CD8^+$ T cells can differentiate into antigen-specific effector T cells with different functions. $CD4^+$ T cells can become T helper 1 (T_H1) cells, T_H2 cells, T_H17 cells or T follicular helper (T_{FH}) cells that help B cells to differentiate into antibody-secreting cells, as well as regulatory T (T_{Reg}) cells that downregulate the functions of other lymphocytes. Naive $CD8^+$ T cells can give rise to effector cytotoxic T lymphocytes (CTLs). The type of T cell response — for example, $CD4^+$ helper T cells or $CD8^+$ CTLs — is at least partly linked to the subset of DCs that presents the antigen[5]. DCs can also interact with cells of the innate immune system, including natural killer (NK) cells, phagocytes and mast cells[5–7].

DCs also have an important role in controlling humoral immunity. They do so both directly by interacting with B cells and indirectly by inducing the expansion and differentiation of $CD4^+$ helper T cells[20,21]. The mechanism by which DCs route antigens into a non-degrading recycling compartment that results in the presentation of unprocessed antigens to B cells is poorly characterized[22,23]. These key properties of DCs, which allow the activation of both arms of the adaptive immune system (that is, cellular and humoral) and which launch the immune response, render DCs the central candidates for antigen delivery and therapeutic vaccination against cancer.

DC plasticity

In addition to discovering DCs, Ralph Steinman and colleagues demonstrated that DCs are able to mature; that is, they acquire novel functions following microbe encounters (reviewed in REF. 14. Normally, DCs in peripheral tissues are immature. These immature DCs have the ability to efficiently capture antigens; they can accumulate MHC class II molecules in the late endosome-lysosomal compartment; they can express low levels of co-stimulatory molecules; they can express a unique set of chemokine receptors (for example, CCR7) that allow their migration to lymphoid tissues; and they have a limited capacity for secreting cytokines[14]. Such immature DCs induce immune tolerance either through T cell deletion or through the expansion of regulatory or suppressor T cells[24].

DCs promptly respond to environmental signals and differentiate into mature DCs that can efficiently launch immune responses. Maturation is associated with the downregulation of antigen-capture activity, the increased expression of surface MHC class II molecules and co-stimulatory molecules, the ability to secrete cytokines[14], as well as the acquisition of CCR7, which allows migration of the DC into the draining lymph node[14]. The ligation of the co-stimulatory receptor CD40 (also known as TNFRSF5) is an essential signal for the differentiation of immature DCs into fully mature DCs that are able to launch adaptive T cell-mediated immunity[25,26]. However, DC maturation alone does not result in a unique DC phenotype (FIG. 2). Instead, the different signals that are provided by different microbes either directly or through the surrounding immune cells induce DCs to acquire distinct phenotypes that eventually contribute to different immune responses. Indeed, DC maturation varies according to different microbes because microbes express different pathogen-associated molecular patterns (PAMPs) that trigger distinct DC molecular sensors, which are called pattern recognition receptors (PPRs) (reviewed in REFS 6,27,28). Strikingly, although most microbes activate DCs, a few can block DC maturation through various pathways (reviewed in REFS 6,27,28). Tissue-localized DCs can also be polarized into

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distinct phenotypes by the products released from surrounding immune cells that respond to injury. For example, γδ-T cells and NK cells release interferon-γ (IFNγ), mast cells release pre-formed IL-4 and TNF, pDCs secrete IFNα, stromal cells secrete IL-15 and thymic stromal lymphopoietin (TSLP), and so on (reviewed in REFS 29,30). These cytokines induce the differentiation of progenitor cells or of precursor cells such as monocytes into distinct inflammatory DCs that yield unique types of T cells (FIG. 2).

It is now accepted that the adjuvant component of vaccines primarily acts by triggering DC maturation. As described above with microbes, different adjuvants activate DCs through distinct molecular pathways, leading to various types of T cell responses[31,32]. For example, alum mediates its adjuvant effects partly through the activation of the DC inflammasome[33], as well as through the sensing of lipids in the plasma membrane of DCs[34].

The recent progress that has been made in genomics and systems immunology[35] is expected to uncover the pathways that determine the type of immune responses that DCs elicit. Approaches that combine transcriptional profiling, genetic and small-molecule screening together with phosphoproteomics have unravelled molecules that regulate TLR signalling in DCs[36]. These strategies will help us to understand the molecular paths of DC maturation and thereby enable the discovery of novel adjuvants. The crucial issues related to cancer that need to be overcome include the lack of necessary DC co-stimulation signals in the tumour microenvironment and the presence of inappropriate signals that lead to DCs that are unable to induce protective anti-tumour immune responses.

DC subsets

Subsets of human DCs in the blood can be distinguished by the differential expression of three cell-surface molecules: CD303 (also known as BDCA2 and CLEC4C), CD1C (also known as BDCA1) and CD141 (also known as BDCA3 and thrombomodulin)[37] (FIG. 3). $CD303^+$ pDCs represent a front line of anti-viral immunity owing to their ability to secrete large amounts of IFNα in response to virus encounters[38]. Their presynthesized stores of MHC class I molecules[39] may allow a rapid initial $CD8^+$ T cell response to viral infections. pDC-derived IFNα may also promote the immunogenic maturation of other subsets of DCs, thus helping to activate novel T cell clones. Furthermore, activated pDCs can induce the maturation of activated B cells into plasma cells through both cytokines and surface signalling[40,41]. In their resting state, pDCs are considered to have an important role in immune tolerance, including in oral tolerance[42].

Two subsets of mDCs in the blood can be distinguished by reciprocal expression of CD1C and CD141. Human $CD141^+$ DCs share with mouse $CD8^+$ DCs the high capacity to capture exogenous antigens for presentation on MHC class I molecules (known as cross-presentation). $CD141^+$ DCs express XCR1, which is the receptor for the chemokine XCL1 (also known as lymphotactin) that is produced by NK cells and activated $CD8^+$ T cells[43,44]. Thus, mouse $CD8^+$ DCs and human $CD141^+$ DCs are equipped for the generation of $CD8^+$ T cell-mediated immune responses. In mice, gene ablation studies have shown that the $CD8^+$ subset of DCs have an important role in crosspresentation[11]. However, other human DCs, such as epidermal Langerhans cells[45], also cross-present antigens. Whether $CD141^+$ blood mDCs are related to subsets of DCs in peripheral tissues remains to be determined. The unique functions of $CD1C^+$ DCs also continue to be analysed.

The human skin hosts two main subsets of mDCs: epidermal Langerhans cells and dermal interstitial DCs (dermal DCs)[46] (FIG. 3). The dermal DCs can be further subdivided into $CD1a^+$ DCs and $CD14^+$ DCs[46,47]. Human $CD14^+$ DCs can directly help activated B cells, as well as induce naive T cells to differentiate into cells with the properties of T_{FH} cells[45,48]. $CD14^+$ DCs may thus be specialized for the development of humoral responses[45,49].

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Langerhans cells are more efficient in cross-presenting peptides from protein antigens to $CD8^+$ T cells and can prime the differentiation of $CD8^+$ T cells into effector CTLs.

The development and homeostasis of tissue-resident DCs subsets in steady state conditions (that is, when there is no infection or activation of the immune system) is dependent on the activation of the receptor tyrosine kinase FLT3 and of the macrophage colony-stimulating factor 1 receptor (M-CSFR; also known as CSF1R)[12]. However, inflammatory processes such as those initiated by microbial invasion substantially alter the populations of DC subsets. The origin of DCs that are recruited to sites of inflammation is still under investigation, although it is clear that monocytes can give rise to inflammatory DCs *in vivo*[50]. Human studies depend on the *in vitro* exposure of monocytes to different cytokine combinations based on granulocyte–macrophage colony-stimulating factor (GM-CSF; also known as CSF2) (FIG. 3). Together with IL-4 (REF. 51), IFNα[52], TNF[53] or IL-15 (REF. 54) GM-CSF can induce the differentiation of inflammatory DCs that can activate T cells. The cytokine combination is crucial as it results in DCs with different phenotypes and functions. Several of these combinations have been administered as vaccines to cancer patients. Tolerogenic DCs can be generated by culturing monocytes with IL-10 (REF. 55) or with vitamin A[56] or vitamin D3 (REF. 57). The activation of DCs through E-cadherin-mediated signalling[58] also induces tolerogenic DCs.

We still need to establish how these distinct subsets of DCs are related, how they contribute to disease pathogenesis and how they can be used to design efficient vaccines.

Molecules expressed by DCs

DCs sense their environment through both surface and intracellular receptors, which comprise several families, including cell surface C-type lectins (CLRs), surface and intracellular TLRs, and intracellular helicases[59]. The helicases are a very large family of molecules, including retinoic acidinducible gene I (RIGI), which recognize nucleic acids. Activation of these helicases can differentially modulate DC functions, which thus yields distinct immune responses[60,61]. Some of the CLRs, which are receptors for both PAMPs and endogenous ligands, have signalling motifs in their cytoplasmic regions that deliver either activation or suppression signals[62]. For example, macrophage-inducible C-type lectin (MINCLE; also known as CLEC4E) detects small nuclear ribonucleoproteins released from damaged cells, whereas CLEC9A detects a ligand that is exposed on necrotic cells that has not yet been identified[63]. NLRs, such as NLRP1 (also known as NALP1), NLRP3 (also known as NALP3), NLRC4 (also known as IPAF) and NAIP5 (also known as BIRC1E), are components of the inflammasome, which cleaves substrates such as pro-IL-1β and pro-IL-18 to generate the active cytokines. The RNA helicase complex, DDX1–DDX21–DHX36, is a cytosolic sensor of double-stranded RNA that uses the TRIF pathway to activate type I IFN responses in mDCs[59]. Thus, DCs are endowed with a complex set of molecules that helps them to respond to the bewildering complexity of the external molecular and cellular world.

These properties of DCs can be harnessed for the benefit of tumour immunotherapy through several means. One strategy is to target the cell-surface molecules of DCs with antibodies. This could be used to modulate DC function using naked antibodies — for example, by inducing their activation through CD40 — or to link activation signalling with antigen delivery to DCs *in vivo* using antibodies linked with antigens (known as *in vivo* DC targeting)[64]. Another strategy is to exploit DC receptors for *in vivo* capture of dead tumour cells. Regarding this exploitation of DC receptors, immunogenic cancer cell death, which is triggered by some forms of chemotherapy, allows the exposure of ligands for DC sensors and results in the capture of dead cells by DCs *in situ*[65]. These DCs can then present the captured antigens to T cells, which thereby elicits immune responses.

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An important molecular component of DCs that can be targeted for immunotherapy includes receptors that recognize microbes. Indeed, the activation of DCs is likely to contribute to therapeutic effects that are associated with the nonspecific activation of antitumour immunity by PAMPs[66,67], which was pioneered with Coley's toxin. Since then, the Bacillus Calmette-Guerin (BCG) vaccine has been used for the local therapy of bladder carcinoma[67]. Medicinal chemists have made progress in identifying chemicals that activate immune responses. Imiquimod, which is a ligand for TLR7 and TLR8, is used for the treatment of superficial basal cell carcinoma[67]. Several other TLR ligands are currently being tested in clinical trials, including CpG oligodeoxynucleotides (ODNs)[68] (which target TLR9) and polyinosinic-polycytidylic acid (polyI:C), which — depending on the size of this PAMP — targets either TLR3 or helicases[69].

DCs in cancer immunotherapy

Mouse models of cancer demonstrate that DCs can capture tumour antigens that are released from tumour cells, either alive or dying, and cross-present these antigens to T cells in tumour-draining lymph nodes. This results in the generation of tumour-specific CTLs that contribute to tumour rejection[3,4]. Thus, DCs represent important targets for therapeutic interventions in cancer.

DCs in the tumour microenvironment

DCs are found in most tumours in humans and mice. DCs can sample tumour antigens through the capture of dying tumour cells and through the 'nibbling' of live tumour cells (reviewed in REF. 70).

Dying tumour cells mobilize at least three types of signal when interacting with DCs and other phagocytes, including 'find me', 'eat me' and 'do not eat me' signals (reviewed in REF. 71) (FIG. 4). There are currently four sets of molecules that are known to be released by apoptotic cells that function as 'find me' signals: lipid lysophosphatidylcholine (LPC), sphingosine 1-phosphate (S1P), CX3CL1 (also known as fractalkine), and the nucleotides ATP and UTP[71]. The eat me signals are membrane-bound and serve as markers for phagocytes for recognizing and internalizing dying cells. These signals include phosphatidylserine, alterations in cell-surface charge, αvβ5 integrin and CD36. The eat me signals also include molecules such as milk fat globule-EGF factor 8 (MFG-E8; also known as lactadherin), which bridge the phosphatidylserine of apoptotic cells with the integrin αvβ3 of DCs[71]. Opsonizing antibodies can promote the capture of dying tumour cells through Fc receptors and complement component receptors that are expressed on DCs. The do not eat me signals serve as negative regulators for the capture of cancer cells by DCs and other phagocytes. These signals include lactoferrin and CD47, the interaction of which with signal-regulatory protein-α (SIRPα; also known as SHPS1) on phagocytes provides inhibitory signals that prevent phagocytosis. Accordingly, combining a CD47-blocking antibody with rituximab (a CD20 antibody that depletes B cells) results in enhanced phagocytosis of dead lymphoma cells and improved tumour eradication in mice[72].

Tumours can prevent antigen presentation and the establishment of tumour-specific immune responses through a variety of mechanisms. By switching the differentiation of monocytes to macrophages, which is mediated by the interplay of IL-6 and macrophage colony-stimulating factor (M-CSF; also known as CSF1)[73], rather than DCs, tumours can prevent the priming of tumour-specific T cells by DCs. Alternatively, the tumour glycoproteins carcinoembryonic antigen (CEA; also known as CEACAM5) and mucin 1 (MUC1) that are endocytosed by DCs can be confined to early endosomes, which thus prevents efficient processing and presentation to T cells[74].

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Tumours also interfere with DC maturation. First, they can inhibit DC maturation through the secretion of IL-10 (REFS 75,76), which leads to antigen-specific anergy. Second, tumour-derived factors can alter the maturation of mDCs and so yield cells that indirectly promote tumour growth ('pro-tumour' DCs) (FIG. 5). For example, TSLP, which is produced by tumour cells, induces DCs to express OX40 ligand (OX40L; also known as TNFSF4), which directs the generation of T_H2 cells. These skewed $CD4^+$ T cells accelerate breast tumour development through the secretion of IL-4 and IL-13 (REF. 77). These cytokines prevent tumour cell apoptosis and indirectly promote the proliferation of tumour cells by stimulating tumour-associated macrophages to secrete epidermal growth factor (EGF). A similar pathway operates in pancreatic cancer[78], and studies in mice corroborate the pro-tumorigenic effect of T_H2 cells[79].

The mDCs are not the only culprit in tumour progression, as pDCs that infiltrate breast carcinomas produce little IFNα on ligation of TLRs[80]. This inhibition results from the ligation of ILT7 (also known as LILRA4) on pDCs with bone marrow stromal antigen 2 (BST2) on tumour cells[80]. These pDCs induce naive $CD4^+$ T cells to differentiate into IL-10-producing T cells with immunosuppressive functions. The inhibition of IFNα secretion might also affect the generation of effector CTLs, as DCs require type I IFN signalling to cross-present tumour antigens[3,4]. Whether this mechanism explains why pDCs are associated with poor prognosis[81] remains to be determined. Finally, DCs can have direct pro-tumour effects: mDCs directly promote the survival and clonogenicity of multiple myeloma tumour cells[82,83]. In ovarian cancer, pDCs contribute to tumour angiogenesis by secreting pro-angiogenic cytokines[84,85].

Therefore, understanding the functions of DCs in the tumour bed might represent a rich field of investigation. Ultimately, rewiring the pro-tumour DCs into anti-tumour DCs might represent a novel approach to cancer immunotherapy (BOX1).

Box 1

Endogenous vaccination and combination therapies

The classical cancer chemotherapies seem to engage the immune system, and part of their efficacy might result from this. For example, chemotherapeutic agents, such as anthracyclines and oxaliplatin, induce tumour cells to undergo apoptosis, which is associated with cell surface exposure of calreticulin. The expression of calreticulin on the tumour cell surface might contribute to the capture of apoptotic bodies by dendritic cells (DCs) and thus might elicit tumour-specific $CD8^+$ T cell-mediated immune responses. These T cells might contribute to the elimination of tumour cells[138] that have not responded to chemotherapy.

There is some evidence that the immune system might also partly contribute to the beneficial effects of radiotherapy[139]. Less surprisingly, there is now strong evidence that antibody therapy — for example, anti-CD20 and anti-HER2 agents[140] — involve the adaptive immune system beyond the elicitation of antibody-dependent cell-mediated cytotoxicity (ADCC). Indeed, antibodies against HER2 can enhance cross-presentation of tumour antigens, leading to a break in immune tolerance against this antigen[140]. Accordingly, patients responding to trastuzumab (a HER2 antibody) showed enhanced $CD8^+$ T cell-mediated immune responses to HER2 (REF. 140). The use of 'armed' antibodies against cancer cells[141] is a promising strategy to improve antibody therapy. These antibodies can be fused with effector molecules, such as toxins or radionuclides, which enables the therapeutic modulation of the tumour microenvironment.

The negative immunomodulatory cues of the tumour microenvironment can be counteracted by a series of therapeutic modalities. These include antibodies that

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neutralize cytokines, such as interleukin-10 (IL-10)[142], IL-13 (REF. 143) and transforming growth factor-β (TGFβ)[144,145]. Antibodies targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA4)[146,147] and programmed cell death 1 ligand 1 (PDL1)[148–150], which block co-inhibitory signalling of the immune checkpoint in lymphocytes represent important contributors to cancer vaccines. Likewise, antibodies[151,152] that promote co-stimulation of effector T cells, such as those that target CD137 (REFS 153,154), should be tested in combination with vaccination. Just as oncologists currently use different combinations of cytostatic drugs and targeted therapies to treat cancer patients, we foresee the development of clinical protocols combining DC vaccines with individualized adjuvant therapies. This development, however, is almost entirely dependent on partnership and collaboration between academia and industry. Indeed, it has been hampered not by the lack of ideas and candidate targets and vaccines in academia but mainly by the lack of access to drugs available in industry.

Therapeutic vaccination via DCs

The goal of cancer vaccinologists is to elicit tumour-specific $CD8^+$ T cell-mediated immune responses that will be sufficiently robust and long-lasting to generate durable tumour regression and/or eradication. This goal is encouraged by clinical studies showing that the infusion of autologous tumour-specific $CD8^+$ T cells can eventually lead to the rejection of large metastatic tumours in patients[86–88]. The aim is to identify vaccination protocols that will result in the generation of potent T cell responses *in vivo*. Ideally, vaccine-elicited $CD8^+$ T cells should be of high avidity and able to recognize peptide–MHC class I complexes on tumour cells; be able to express high levels of granzyme and perforin — molecules that are essential for cytotoxic activity against cancer cells; be able to enter the tumour microenvironment; and be able to overcome immunomodulatory mechanisms that are present in the tumour[45,89]. At least four components of the immune response are necessary for this ideal response to happen: the presence of appropriate DCs; the quality of induced $CD4^+$ T helper cells; the elimination and/or non-activation of T_{Reg} cells; and the breakdown of the immunosuppressive tumour microenvironment. Several of these elements have been discussed above and in recent excellent reviews[90,91]. Here, we briefly elaborate on the fate of $CD8^+$ T cells in the context of DC vaccination.

Naive $CD8^+$ T cells initiate a CTL differentiation programme on encountering DCs that present tumour-derived peptides[92]. A complex system of signals drive the subsequent $CD8^+$ T cell expansion and differentiation, which includes co-stimulatory pathways mediated by CD80 (REF. 93), CD70 and 4-1BB (also known as TNFRSF9)[94], as well as DC-derived cytokines such as IL-12 and IL-15 (REFS 90,91,95). The quality of $CD8^+$ T cell differentiation is further regulated by $CD4^+$ T cells[96], as they influence the differentiation and expansion of tumour antigen-specific CTLs[97] and the induction of long-term memory $CD8^+$ T cells[98]. $CD4^+$ T cells also contribute to the eradication of the tumours through the activation of macrophages at tumour sites[99], and they actively kill tumour cells[100]. Furthermore, the expression of CD103 (also known as integrin αE) on CTLs mediates the adherence of these cells to E-cadherin, which seems to be an important pathway in tumour cell lysis and tumour rejection[101].

Unfortunately, $CD4^+$ T cells can also suppress CTL differentiation. Thus, T_{Reg} cells can inhibit CTLs through the secretion of IL-10 (REF. 102), and/or transforming growth factor-β (TGFβ)[103]. T_{Reg} cells can also compete with $CD8^+$ T cells for IL-2 through constitutive expression of CD25 (also known as IL2Rα)[104]. T_H2 cells can inhibit the generation of CTLs by secreting IL-4, which instead leads to the generation of a subpopulation of CTLs, termed

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T_C2 cells, which have limited killing capacity owing to their low expression of granzymes and perforin[105].

Antigen-specific CTLs must also traffic into the tumour bed, the details of which are not clearly understood[89,106]. Deregulation of chemokine homeostasis might prevent CTLs from entering the tumour bed; indeed, tumours might actively repel the infiltration of CTLs[101,107]. Finally, the tumour-infiltrating myeloid-derived suppressor cells[108] and T_{Reg} cells[109] might inhibit CTL functions.

Ex vivo-generated DC vaccines

The therapeutic use of cancer vaccines has recently been revived owing to a series of clinical trials that have yielded encouraging clinical outcomes. First, treatment of metastatic prostate cancer with sipuleucel-T (also known as APC 8015), which is a cellular product based on enriched blood APCs that are briefly cultured with a fusion protein of prostatic acid phosphatase (PAP) and GM-CSF, resulted in an approximately 4-month-prolonged median survival in Phase III trials[110,111]. Sipuleucel-T has been approved by the US Food and Drug Administration (FDA) for the treatment of metastatic prostate cancer, thereby paving the clinical development and regulatory path for the next generation of cellular immunotherapy products. Second, a Phase III trial in metastatic melanoma that tested peptide vaccine in combination with high-dose IL-2 versus IL-2 alone showed significant improvement in overall response rate and progression-free survival in patients who received the vaccine[112]. Third, a Phase III trial in patients with follicular lymphoma showed that an idiotype vaccine therapy significantly prolongs the duration of chemotherapy-induced remission[113]. Furthermore, a randomized Phase II trial of a poxvirus-based vaccine targeting prostate-specific antigen (PSA) (PROSTVAC) in men with metastatic castration-resistant prostate cancer showed an improved overall survival in patients when compared with patients receiving control vectors (an observed difference in median survival of 8.5 months)[114].

DCs that are generated *ex vivo* by culturing haematopoietic progenitor cells or monocytes with cytokine combinations have been tested as therapeutic vaccines in cancer patients for more than a decade[29]. These studies concluded that DC-based vaccines are safe and can induce the expansion of circulating $CD4^+$ T cells and $CD8^+$ T cells that are specific for tumour antigens (TABLE 1). Objective clinical responses have been observed in some patients. The clinical response takes time to build up but remissions can be very long-lasting (TABLE 1) (reviewed in REFS 115,116).

The selection of tumour antigens for loading the DCs is an important parameter. Candidate tumour antigens include unique (mutated) antigens and shared nonmutated self-antigens[117–120]. To generate broadly applicable vaccines, non-mutated self-antigens have often been selected. These, however, have potential shortcomings: the range of high-avidity clones might be depleted through negative selection[89,121], and the existing memory T cells often include T_{Reg} cells.

Using mutated antigens might avoid these drawbacks. Cancer vaccines that are designed to elicit strong immune responses against these mutated antigens will require a fully personalized approach. This represented a considerable challenge a few years ago. Fortunately, however, the development of RNA sequencing (RNA–Seq) technologies will allow us to determine the complete range of mutated antigens from the primary tumour and metastases of a patient, thereby allowing us to tailor therapeutic vaccines to the patient's tumour.

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Targeting antigen to DCs in vivo

Antigens can be directly delivered to DCs *in vivo* using chimeric proteins that are comprised of an antibody that is specific for a DC receptor fused to a selected antigen. Ralph Steinman and colleagues demonstrated that the specific targeting of antigens to DCs *in vivo* elicits potent antigen-specific $CD4^+$ and $CD8^+$ T cell-mediated immunity[122–124]. The induction of immunity also requires the provision of DC maturation signals[122–124]. Otherwise, this strategy induces antigen-specific tolerance — a finding of substantial value in the context of autoimmunity. The use of vaccines that target surface molecules that are expressed on different subsets of DCs allowed Steinman and Nussenzweig to demonstrate that distinct subsets of DCs elicit distinct immune responses[64]. This confirmed earlier studies that were carried out *in vitro* or *in vivo* with purified DC subsets[31,32]. In this regard, $CD8^+$ DCs that express the cell-surface protein CD205 (also known as Ly-75) present delivered antigens in the context of both MHC class I and MHC class II molecules; whereas, $CD8^-$ DCs, which express the DC marker antigen recognized by the monoclonal antibody 33D1, are specialized for antigen presentation on MHC class II molecules[64]. Furthermore, targeting antigen to these distinct receptors on DCs leads to the generation of T cell-mediated responses through independent pathways. Thus, DCs expressing CD205 generate a T_H1 cell-mediated immune response in an IL-12-independent, CD70-dependent mechanism; whereas, $CD8^-33D1^+$ DCs generate T_H1 cell-mediated immune responses through the classic IL-12 pathway[125].

DC targeting is not purely confined to the delivery of an antigenic cargo, as the engagement of some surface molecules, such as dendritic cell-associated C-type lectin 1 (dectin 1; also known as CLEC7A), dendritic cell-specific ICAM3-grabbing non-integrin 1 (DCSIGN; also known as CD209) and CD40, by targeting antibodies also provides activation signals. It remains important to determine whether the activation signals might actually polarize the DCs in the desired manner. For example, engaging dendritic cell asialoglycoprotein receptor (DCASGPR; also known as ASGPR1)[126] induces DCs to secrete IL-10, which polarizes T cells into IL-10-secreting suppressor T cells, which in turn might negatively affect tumour-specific effector T cells. Thus, the challenge will be to match the DC surface target and the selected adjuvant with the desired immune outcome, all in the context of an altered immune system. This will undoubtedly require considerable studies over the coming years.

Immune and clinical efficacy

Most Phase I and II studies have stumbled on two crucial issues: how to assess the clinical efficacy of cancer immunotherapy; and how to define the correlates (biomarkers) of clinical efficacy[127]. Initially, the Response Evaluation Criteria In Solid Tumours (RECIST) had been designed to assess chemotherapy-based trials and were considered to be crucial for assessing clinical efficacy in vaccine and immunomodulation trials[127,128]. However, this has recently been challenged. A randomized Phase III clinical trial testing anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA4) in patients with stage IV melanoma, showed a twofold improved overall survival in patients who received the drug[129]. No early tumour shrinkage was observed in treated patients, which reflects the slow build-up of anti-tumour immunity. In fact, in the early phase of treatment, tumours might increase in size and new lesions might appear; these are findings that normally call for removing the patients from the trial. However, the enlarged tumour size might instead reflect the inflammatory process that is associated with active immune responses and lymphocyte infiltration.

Thus, overall survival might be the only objective parameter of clinical efficacy. However, surrogate markers are needed, because clinical trials that are based on overall survival might be exceedingly long and costly, which could result in the loss of potentially valuable treatments. Several cancer vaccine studies have suggested that the therapeutic vaccination

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outcome — success or failure — correlates with the vaccine-induced expansion of antigen-specific effector T cells[130,131]. However, the quality, more than the quantity, of the antigen-specific immune response remains one of the key parameters of efficacy. A better understanding of how effective vaccines stimulate protective immune responses[132,133] might contribute to a better selection of immune parameters that indicate the efficacy of cancer vaccines. Systems immunology approaches that combine transcriptional profiling with multi-parameter flow cytometry, proteomics and transcriptomics might enable the identification of novel biomarkers of immune efficacy[35,134].

Final remarks

Nearly 40 years after their discovery, the importance of DCs has been recognized by the award to Ralph Steinman of the Nobel Prize for Medicine or Physiology in 2011. However, it has not been a smooth road, and many in the scientific community were sceptical at the time DCs were discovered. Ralph, however, pursued his studies of DCs relentlessly. He used to tell his laboratory members and colleagues “just do the experiment”! Ultimately, the data have not only erased the scepticism but have also created a new field in immunobiology[135]. It is impossible not to draw parallels with the cancer immunotherapy field, which was initially met with scepticism and which now, finally, is coming of age[136,137]. Our views on the potential role of DCs in cancer immunotherapy have expanded remarkably, moving from the early trials with *ex vivo* DC vaccines to a whole new array of therapeutic options that include rewiring DC molecular pathways (FIG. 6). Just as immunotherapy is moving to the forefront of cancer therapy, DC-based therapy is moving to the forefront of cancer immunotherapy.

Acknowledgments

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Glossary

Innate immune system	Comprises the cells and molecules that defend the host from infection by other organisms in a nonspecific manner. That is, cells of the innate immune system recognize and respond to pathogens in a generic way and, unlike the adaptive immune system, do not confer immune memory
Adaptive immune system	The part of the immune system that is mediated by antigen-specific lymphocytes and antibodies; it is highly antigen-specific and includes the development of immunological memory
Paraneoplastic diseases	Neurological diseases induced as a result of tumour burden; generally caused by the release of tumour-derived hormones, peptides and/or cytokines, or by the misguided destruction of normal tissue by immune cells targeted against malignant cells. Most commonly present with cancers of the lung, breast, ovaries or lymphatic system (lymphoma)

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$CD8^+$ T cell	A subgroup of T lymphocytes that recognize their targets by binding to antigen that is associated with major histocompatibility class I molecules, which are present on the surface of nearly every cell. They can give rise to cytotoxic T lymphocytes, which can kill virally infected cells and tumour cells
Antigen-presenting cells	(APCs). Cells that display foreign antigen complexes with major histocompatibility complex molecules to T cells
Adjuvant	An agent mixed with an antigen that enhances the immune response to that antigen on vaccination
Macrophages	Specialized monocyte-derived phagocytic cells that can capture and degrade invading microbes
Afferent lymphatics	Vessels that enter the periphery of the lymph node and bring cells and particles from the tissue to the lymph node
$CD4^+$ T cells	Also known as helper T cells. A subgroup of T lymphocytes that regulate other immune cells and that are essential in B cell antibody class switching, as well as in the activation and growth of cytotoxic T cells. These cells recognize antigen that is associated with major histocompatibility complex class II molecules
Regulatory T (T_{Reg}) cells	A subset of $CD4^+$ T cells that maintain self-tolerance. They can express high levels of CD25 and the forkhead transcription factor FOXP3. They can secrete cytokines, such as IL-10 and TGFβ, which inhibit other T cells
Natural killer (NK) cells	Innate immune system lymphocytes that are able to kill virally infected cells and tumour cells, particularly cells that lack the expression of major histocompatibility complex class I molecules (the presence of which inhibits NK cell cytotoxicity)
Phagocytes	White blood cells that are able to ingest foreign particles, microbes and dying cells
Mast cells	Tissue-resident cells that contain histamine and heparin-rich granules and that mediate allergy and anaphylaxis. They are also important in the defence against pathogens
Humoral immunity	A component of the adaptive immune system that is mediated by secreted antibodies. Antibodies are secreted from B cells that have differentiated into plasma cells
Opsonizing antibodies	A subclass of antibodies that can bind to a pathogen or particle and at the same time bind to an Fc receptor on a phagocyte, thereby facilitating phagocytosis and pathogen clearance
Idiotype	Individual antigenic determinants from the variable regions of the immunoglobulin heavy and light chains are referred to as idiotopes; the sum of the individual idiotopes is referred to as the idiotype
Memory T cells	A subgroup of antigen-specific T cells that persist long after an infection has resolved. They quickly expand to large numbers of effector T cells on re-exposure to cognate antigen, thus providing immunological ‘memory’

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At a glance

- The molecular identification of human cancer antigens has allowed the development of antigen-specific immunotherapy. In one approach, autologous antigen-specific T cells are expanded *ex vivo* and then re-infused into patients. Another approach is through vaccination; that is, the provision of an antigen together with an adjuvant to elicit therapeutic T cells *in vivo*. Cancer vaccines aim to induce tumour-specific effector T cells that can reduce the tumour mass and to induce tumour-specific memory T cells that can control tumour relapse.
- Owing to their properties, dendritic cells (DCs) are often called 'nature's adjuvants' and thus have become the natural targets for antigen delivery. DCs provide an essential link between the innate and the adaptive immune responses. DCs are at the centre of the immune system owing to their ability to control both tolerance and immune responses. These key properties of DCs render them the central candidates for antigen delivery and vaccination, including therapeutic vaccination against cancer.
- The immune system has the potential to eliminate neoplastic cells. However, tumour cells alone are poor antigen-presenting cells (APCs). Studies with mouse models demonstrate that the generation of protective anti-tumour immune responses depends on the presentation of tumour antigens by DCs. When compared with other APCs, such as macrophages, DCs are extremely efficient at antigen presentation and inducing T cell immunity, thus explaining their nickname of 'professional APCs'.
- Mice and humans have distinct functional subsets of DCs that generate different types of immune response. DCs are also able to mature; that is, to acquire novel functions following microbe encounters. Under steady state conditions, DCs in peripheral tissues are 'immature'. These immature DCs induce tolerance either through T cell deletion or through inducing the expansion of regulatory and/or suppressor T cells. DCs promptly respond to environmental signals and differentiate into mature DCs that can efficiently launch immune responses. It is now accepted that the adjuvant component of vaccines primarily acts by triggering DC maturation.
- DCs are important targets for therapeutic interventions in cancer. Two themes of research are growing: first, how cancer cells alter DC physiology; and second, how we can build on the powerful properties of DCs to generate novel cancer immunotherapies (including vaccines).

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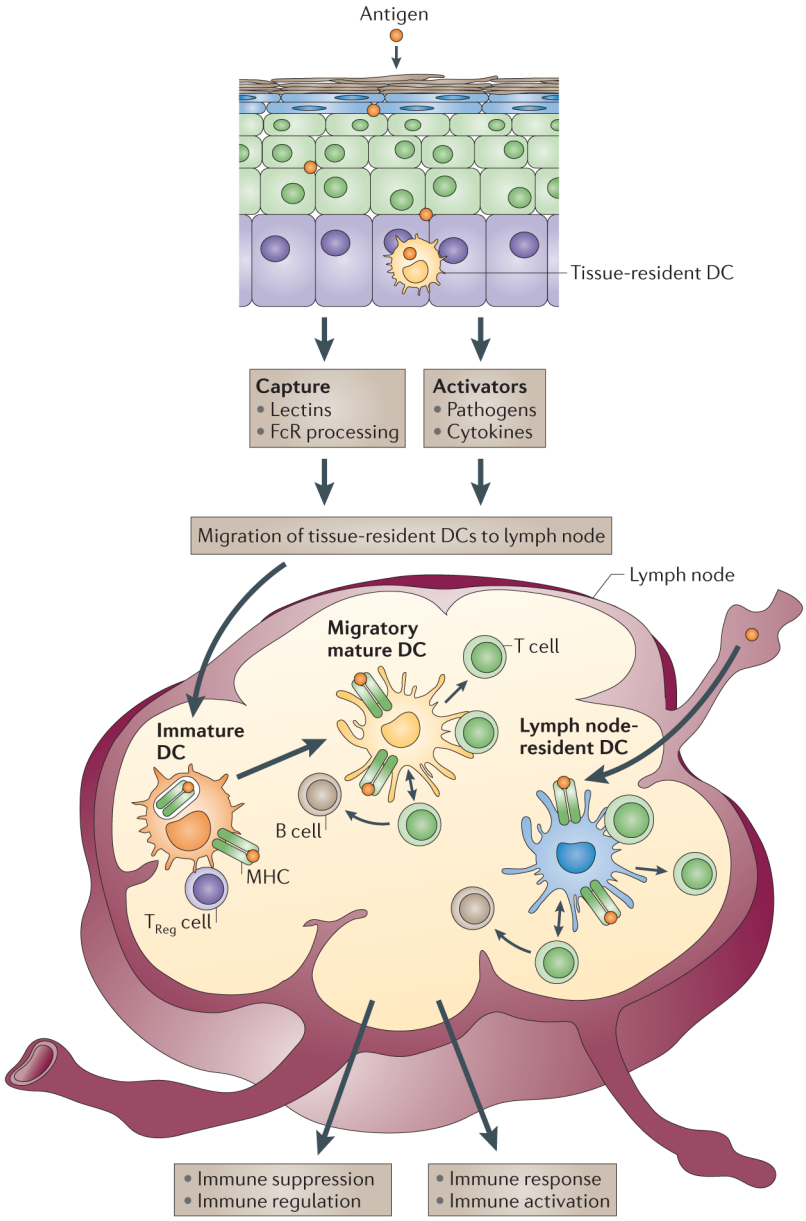

Figure 1. Launching the immune response

Antigens can reach lymph nodes through two pathways: via lymphatics, where the antigen is captured by lymph node-resident dendritic cells (DCs), or via tissue-resident DCs. These immature DCs capture antigens, and DC activation triggers their migration towards secondary lymphoid organs and their maturation. DCs display antigens in the context of classical major histocompatibility (MHC) class I and MHC class II molecules or in the context of non-classical CD1 molecules, which allow the selection of rare antigen-specific T lymphocytes. Activated T cells drive DCs towards their terminal maturation, which induces further expansion and differentiation of T lymphocytes into effector T cells. If DCs do not receive maturation signals, they will remain immature and antigen presentation will lead to immune regulation and/or suppression. T_{Reg} cell, regulatory T cell.

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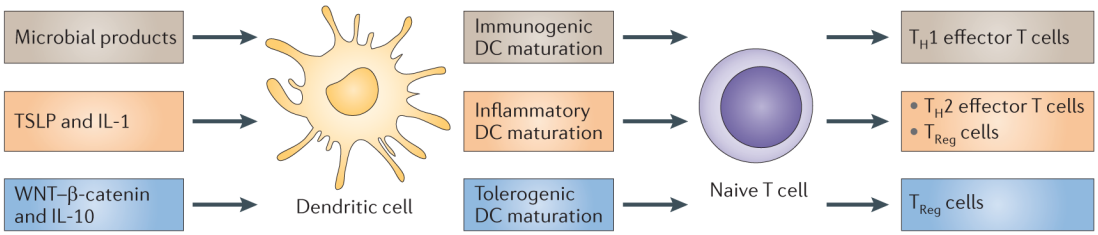

Figure 2. DC maturation

Dendritic cell (DC) maturation is a simple concept that is rendered complex by the likelihood that not all mature (or activated) DCs are equivalently immunogenic. For example, under steady state conditions, particularly in lymphoid tissue, DC populations that display at least some of the features of mature DCs (for example, elevated expression of surface co-stimulatory molecules) can be found despite the absence of overt inflammation or infection. The functional importance of these cells is unknown but it is not unreasonable to suspect that tolerogenic DCs may have to acquire the capacity to present antigen, migrate and interact with T cells (characteristics of mature DCs) in order to induce antigen-specific regulatory T (T_{Reg}) cells or to induce anergy or T cell apoptosis at high efficiency. The priming of T_{Reg} cells either in the thymus or in the periphery may require activation by endogenous mediators, such as thymic stromal lymphopoietin (TSLP) or WNT, respectively[155,156]. Whether these mediators induce morphologically recognizable maturation *in vivo* is likely but not known. However, it is clear that resting or immature DCs can or must be activated in some way to induce T cell tolerance; thus, it is inaccurate to assume that the relevant steady state DCs are 'immature' or resting. IL, interleukin; T_H, T helper.

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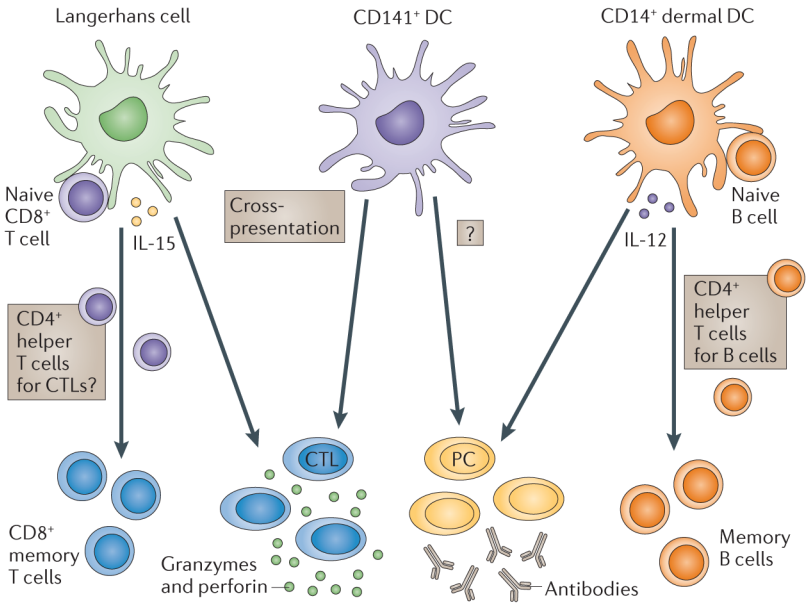

Figure 3. Subsets of DCs

The two arms of the adaptive immune response — humoral and cellular — are regulated by different subsets of dendritic cells (DCs) in humans. Humoral immunity is preferentially regulated by $CD14^+$ dermal DCs, which produce interleukin-12 (IL-12). IL-12, in turn, acts directly on B cells and promotes the development of T follicular helper (T_{FH}) cells. Cellular immune responses in the skin are preferentially regulated by Langerhans cells. Among the candidate mediators is IL-15. It is also possible that Langerhans cells can preferentially activate a dedicated subset of $CD4^+$ T cells that are specialized to help $CD8^+$ cytotoxic T lymphocytes (CTLs). Given their capacity to cross-present antigens to $CD8^+$ T cells, $CD141^+$ DCs might be involved in the development of CTL-mediated responses. $CD141^+$ DCs might also be involved in the development of humoral responses through IL-12 secretion. PC, plasma cell.

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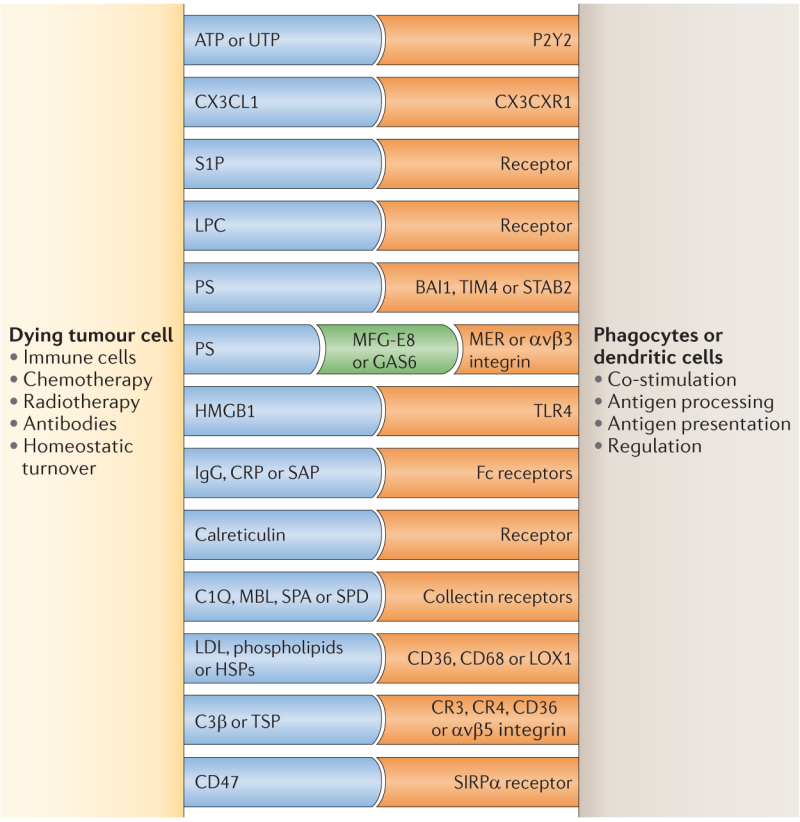

Figure 4. DC interaction with tumour cells: antigen capture

The figure illustrates some phagocyte surface receptors and their putative ligands that are implicated in the recognition of dying tumour cells. Receptors shown in blue represent molecules expressed by dying tumour cells, receptors shown in orange represent molecules expressed by dendritic cells (DCs) and the receptors shown in green function as a 'bridge' between the two cells. BAI1, brain-specific angiogenesis inhibitor 1; C1Q, complement C1q subcomponent; C3β, complement C3β; CRP, cysteine-rich protein; GAS6, growth arrest-specific protein 6; HMGB1, high mobility group protein B1; HSP, heat shock protein; LDL, low-density lipoprotein; LOX1, lectin-like oxidized LDL receptor 1; LPC, lipid lysophosphatidylcholine; MBL, mannose binding lectin; MFG-E8, milk fat globule-EGF factor 8 (also known as lactadherin); P2Y2, P2Y purinoceptor 2; PS, phosphatidylserine; S1P, sphingosine 1-phosphate; SAP, sphingolipid activator protein 1; SIRPα, signal-regulatory protein-α; STAB2, stabilin 2; TIM4, T cell membrane protein 4; TLR4, Toll-like receptor 4; TSP, thrombospondin.

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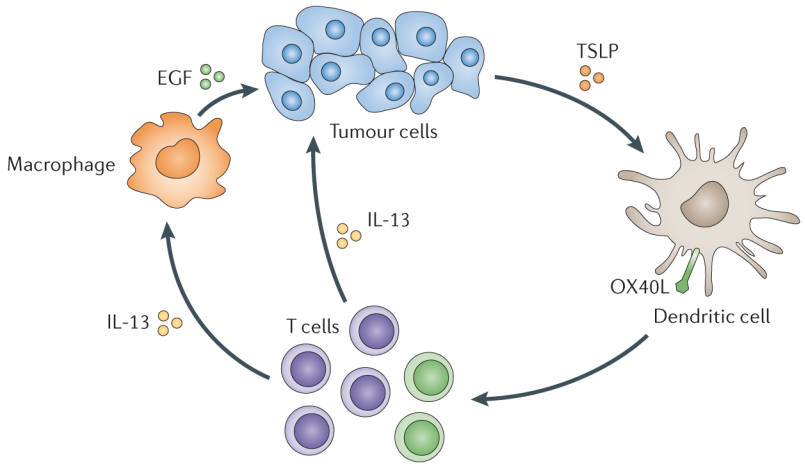

Figure 5. The interaction of DCs with tumour cells: modulation of DC maturation

Cancer cells attract immature dendritic cells (DCs), possibly through chemokines such as CCL20 or CXCL12. DCs can then be exposed to cancer-derived factors — for example, thymic stromal lymphopoietin (TSLP) — which skews their maturation towards T helper 2 (T_H2)-type inflammation. In this environment, T_H2 cells promote tumour development either directly or via macrophages. EGF, epidermal growth factor; IL-13, interleukin-13; OX40L, OX40 ligand.

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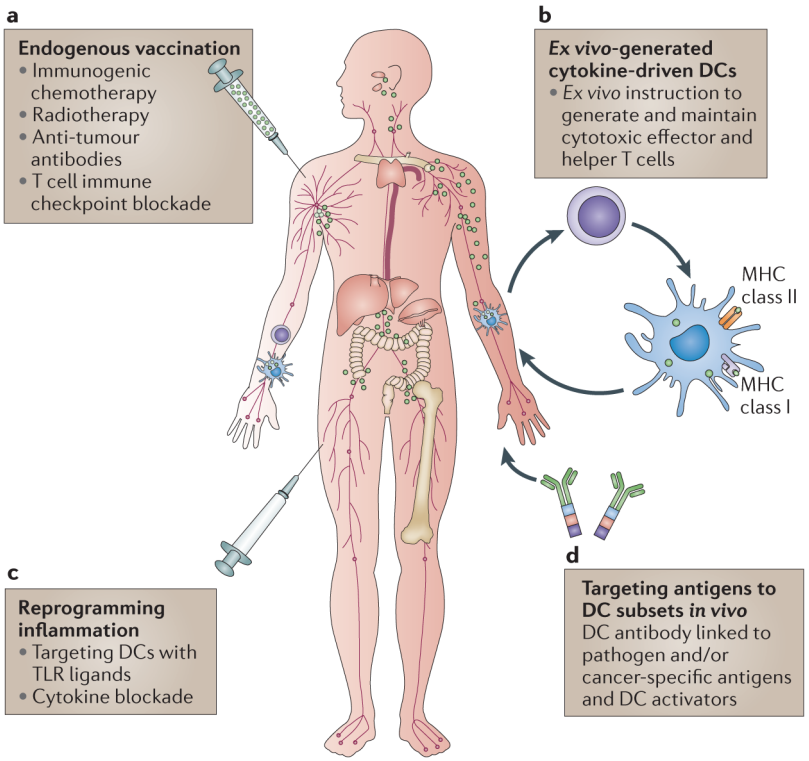

Figure 6. DCs and cancer immunotherapy

a | Random targeting of dendritic cells (DCs) in 'endogenous' vaccination results from *in vivo* antigen release owing to immunogenic cell death in response to chemotherapy, radiotherapy and immunomodulation approaches that are targeted at T cells. **b** | Vaccines can be based on *ex vivo*-generated tumour antigen-loaded DCs that are injected back into patients. **c** | Specific *in vivo* DC targeting with DC antibodies fused with antigens and with DC activators is shown. **d** | Targeting DCs in the tumour microenvironment to reprogramme pro-tumour inflammation towards tumour rejection is shown. MHC, major histocompatibility complex; TLR, Toll-like receptor.

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Table 1

Examples of clinical trials testing vaccination with *ex vivo* DCs

Vaccine and antigen	Indication	Key observations	Refs
GM-CSF–IL-4 DCs with or without HLA-A*0201-restricted peptides or peptides alone	Metastatic prostate cancer	One of the first studies that tested the immunogenicity of DCs	157
GM-CSF–IL-4 DCs with peptides, tumour lysates or autologous tumour-eluted peptides	Stage IV melanoma, renal cell carcinoma and malignant glioma	• Loading DCs with complex antigen preparations • Objective clinical responses	158–160
Blood DCs and idiotype antigens	Multiple myeloma	• Immunogenicity of DCs • Tumour regression	161,162
Mature GM-CSF–IL-4 DCs and peptides	Stage IV melanoma	• Well-controlled and validated vaccine manufacture process • Testing mature DCs • Immunogenicity • Objective clinical responses	163
$CD34^+$ HPC-derived DCs and peptides	Stage IV melanoma	• One of the first studies to test $CD34^+$ HPC-derived DCs • Loading vaccines with a mixture of well-defined peptides • Durable immune responses in long-term survivors • Objective clinical responses	164,165
FLT3 ligand-expanded blood DCs and altered peptides	Advanced CEA^+ cancer	• Immunogenicity • Objective clinical responses	166
Immature GM-CSF–IL-4 DCs	Healthy volunteers	Antigen-specific inhibition of effector T cell function after injection of immature DCs	167
GM-CSF–IL-4 DCs and tumour lysates	Refractory paediatric solid tumours	• Immunogenicity • Objective clinical responses	168
Mature cryopreserved GM-CSF–IL-4 DCs	Stage IV melanoma	Immunogenicity	169
DCs loaded with autologous tumour RNA	Colon cancer	• Feasibility • Immunogenicity	170
DCs loaded with killed allogeneic tumour cells	Stage IV melanoma	• Immunogenicity • Durable objective clinical responses • Long-term survival	171,172
Monocyte-derived DCs loaded with the NK T cell ligand α-galactosylceramide	Advanced cancer	Adjuvant effect of NK cell activation on $CD8^+$ T cell-mediated immune response	173
Monocyte-derived DCs	Melanoma	*In vivo* identification of antigen-specific immune response by PET imaging in patients	174

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Vaccine and antigen	Indication	Key observations	Refs
		Route of DC administration affects T cell activation, with intra-dermal administration showing better responses than intra-nodal administration	175
Comparative study of $CD34^+$ HPC-derived Langerhans cells versus monocyte-derived DCs	Melanoma	Langerhans cell-based vaccines stimulated significantly greater tyrosinase-HLA-A*0201 tetramer reactivity than the monocyte-derived DC vaccines	176
Type 1-polarized monocyte-derived DCs	Glioma	Combination of DC vaccination with polyICLC to trigger systemic inflammation driven by type I interferon family members	177

CEA, carcinoembryonic antigen; DC, dendritic cell; IL-4, interleukin-4; GM-CSF, granulocyte–macrophage colony-stimulating factor; HLA, human leukocyte antigen; HPC, haematopoietic progenitor cell; NK cell, natural killer cell; PET, positron emission tomography; polyICLC, polyinosinic–polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose.