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Review

Dendritic cells: A novel therapeutic modality

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Introduction

Dendritic cells (DC) are bone marrow derived professional antigen presenting cells (APC). Since the recognition of DC in lymphoid organs in 1973 [1] remarkable progress has been made in the understanding of their origin and their important role in the immune system.

DC comprise a heterogeneous population, with different properties and characteristics. Langerhans cells, the first DC described, are widely distributed in human skin, oesophagus, cervix and buccal epithelia. Other DC have been reported in the dermis of the skin and in the interstitium of all tissues with exception of the brain (interstitial or tissue DC). They may also be found in the afferent lymph (veiled DC), in the cortical zones of the lymph nodes and in the spleen (interdigitating DC) [2]. The primary function of DC is to act as sentinels between the outside world and the body. For this purpose they possess a high capacity for antigen uptake and processing. Following antigen uptake and in the presence of appropriate 'danger signals', DC migrate rapidly to T-cell areas in the lymph nodes where they can initiate an immune response [3]. In contrast to other members of the APC family (macrophages, B cells) DC are capable of inducing primary immune responses by the activation of naïve T cells [4].

Escape from immune surveillance by cytotoxic T cells (CTL) is a fundamental feature of tumours and contributes to their uncontrolled growth. Although tumour cells express tumour antigens, antigen presentation by tumour cells to T cells is an ineffective process. The number of antigenic peptide containing major histocompatibility complex (MHC) molecules on tumour cells and the chance that their recognition by sparse antigen specific T cells will occur is very low. Moreover, tumour cells mostly lack co-stimulatory surface molecules necessary for stimulation and clonal expansion of T cells. Indeed, antigen presentation in the absence of co-stimulatory signals can lead to T-cell anergy rather than to T-cell activation. The central role of DC in the initiation of immune responses and new methods for the *ex vivo* expansion of DC creates possibilities for the development of novel immunotherapeutic strategies against tumours and other diseases. This makes the *ex vivo* generation of DC an interesting tool in the development of vaccines for cancer patients.

This review outlines recent progress in the understanding of the place of DC within the haematopoietic lineage, their role in antitumour immunity and new experimental approaches for the application of DC in the immunotherapy of cancer patients.

Origin

More and more facts about the nature of the precursor and the growth factors necessary for the development of DC are emerging. DC originate from haematopoietic stem cells. This was first demonstrated in the mouse and the rat. In mouse bone marrow a common MHC class II-negative progenitor for granulocytes, macrophages and dendritic cells was demonstrated in cultures supplemented with granulocyte-macrophage colon-stimulating factor (GM-CSF) [5]. In experiments with human peripheral blood and bone marrow, stimulated by leukocyte conditioned medium, colonies containing pure DC and DC mixed with macrophages were observed [6]. These reports strongly suggested the existence of a common myeloid progenitor for granulocytes, monocytes and DC, which was later identified as a pluripotent CD34+ stem cell [7].

In vitro three sources of DC have been identified: a myeloid CD34+ progenitor cell, a lymphoid precursor and peripheral blood monocytes [8, 9]. Different cytokines are necessary for the development of DC subsets from these progenitors. For example, lymphoid DC can develop in the absence of GM-CSF [10], but they can mature from CD34+ progenitors in the presence of CD40-ligand (CD40) [11]. The functional properties of lymphoid DC may be different in comparison to myeloid DC [9]. Resident lymphoid DC have been suggested to be instrumental in maintaining peripheral tolerance

whereas migratory myeloid DC instigate immune responses upon encountering foreign antigens [12]. In this review we will further focus on the myeloid DC.

The cytokines that support the proliferation and differentiation of myeloid DC precursors *in vitro* are GM-CSF, interleukin-4 (IL-4) and tumour necrosis factor-α (TNF-α) [13–15]. In the mouse GM-CSF alone is sufficient for the generation of DC from their precursors. In humans GM-CSF alone increases both the number of monocytes, macrophages and DC. Addition of TNF-α to GM-CSF increases the proportion of DC, probably by the inhibition of granulocyte differentiation. Addition of stem cell factor (SCF or cKit-ligand) or Flt3-ligand, both factors capable of stimulating expansion of early CD34+ progenitors, to the culture medium can increase the yield of DC generated from haemotopoietic stem cells [16]. The combination of GM-CSF and TNF-α is widely used to generate DC from CD34+ stem cells derived from bone marrow, umbilical cord blood or peripheral blood [17–19]. For the generation of DC from peripheral blood monocytes the combination of interleukin 4 (IL-4) and GM-CSF is used [14]. Under these conditions monocytes develop into a DC population without further proliferation. *In vitro* IL-4 suppresses the acquisition of macrophage properties by the CD14+ precursor. It is still unclear whether interconversion between macrophages and DC via the CD14+ monocyte takes place *in vivo*.

Cytokines important for the generation of DC *in vivo* still need to be identified. In transgenic mice with excessive levels of GM-CSF there was a 50% increase in DC in thymus and spleen and a threefold increase in lymph node DC [20]. GM-CSF surprisingly does not, however, appear to be the most important growth factor for DC *in vivo*. In GM-CSF null mice and GM-CSF receptor null mice normal DC of all phenotypes were present in all lymphoid organs with only a small decrease in DC levels [20]. The one exception was lymph nodes of GM-CSF receptor null mice, which showed a threefold, decrease in DC [20].

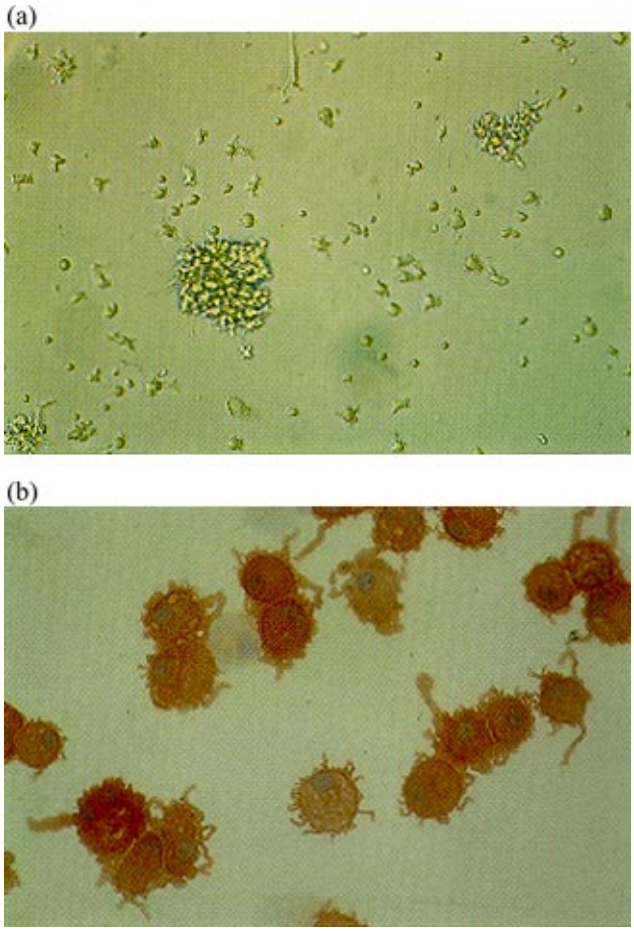

Figure 1. Morphology of monocyte derived DC. The adherent fraction of peripheral blood mononuclear cells of a normal donor was cultured for seven days with GM-CSF and IL-4. (a) Phase contrast microscopy at 100× magnification of the cells in a culture disk on day 5 shows DC in clusters or as individual cells with the typical long cytoplasmatic veils. (b) CD86 staining of the cultured monocyte derived DC at 400× magnification.

Morphology

DC are large cells with an irregular shape [21]. Constant formation and retraction of long cytoplasmatic processes or veils (>10 μm) is responsible for the characteristic appearance of DC (Figure 1). In peripheral tissues this characteristic enhances the efficacy of antigen uptake and in the lymph nodes it increases the likelihood of DC-T cell interaction. DC have a lobulated nucleus, a large Golgi apparatus and many multi-vesicular bodies. The large Golgi apparatus is necessary for the synthesis of high amounts of MHC class II molecules, co-stimulatory molecules and cytokines. Birbeck granules, rod-shaped intra-cytoplasmic organelles, are found in Langerhans cells and DC generated *in vitro* from CD34+ progenitor cells, but not in DC generated from monocytes [22]. The function of these granules is not clear. The multi-vesicular bodies comprise endosomes, lysosomes and MHC class II enriched compartments (MIIC) [23], all of which are involved in the processing and presentation of exogenous antigens. These endosomes are excreted from the cell as exosomes, which are also capable of stimulating an antigen specific T-cell response [24]. In the absence of specific DC lineage markers these morphological features have long been considered as the hallmarks of the quintessential DC.

Functional stages and phenotypes

DC, as a distinct leukocyte population, are defined more by the lack than by the presence of selectively expressed cell surface antigens. Maturational changes of DC are associated with functional changes and alteration in surface antigen expression. The two main functional stages are the immature and the mature DC. The immature or tissue DC's main function is antigen uptake and processing whilst the mature DC's main function is antigen presentation and T-lymphocyte activation (Table 1).

Table 1. Phenotype of dendritic cells.

Kind of cell surface antigen [85–90]	Immature DC	Mature DC
Products of MHC		
MHC class I and II	++	+++
CD1a	+	++
Receptors for antigen-uptake		
Mannose receptor	+++	−
CD64 (FcγRI)	+/−	−
CD32 (FcγRII)	+	+/−
CD16 (FcγRIII)	−	−
Integrins and adhesins		
CD54 (ICAM-1)	+	++
CD58 (LFA-3)	++	++
CD11a (LFA-1)	++	++
CD11b (Mac-1)	++	+
CD11c (gp150.95)	+	+
CD29 (β1 integrin)	+/−	++
Lineage restricted markers		
Monocyte/macrophage		
CD68	+	+
CD14 (LPS receptor)	−	−
CD115 (M-CSF receptor)	−	−
Myeloid		
CD33	+	+
Lymphoid		
CD8	−	−
CD4	+	−
CD2	+	−
CD3	−	−
B lymphocytes		
CD19–22	−	−
NK cells		−
CD56	−	
CD57	−	−
Co-stimulatory molecules		
CD80 (B7.1)	+/−	++
CD86 (B7.2)	+	++
CD40	+	++
DC restricted molecules		
CD83	−	+
S100	+	++
CMRF-44	+	+++

−: negative; +/−: variable; +: positive; ++: strong positive; +++: very strong positive.

DC progenitors, comprising 0.1%–1% of the peripheral blood mononuclear cells (PBMC), are spread via the blood into peripheral tissues, where they develop to a stage referred to as an immature or tissue DC. Tissue DC are characterised by a low motility and high capacity of antigen uptake and processing. The mechanisms of antigen capture are macropinocytosis, receptor mediated endocytosis and phagocytosis. Macropinocytosis endows immature DC with the ability to take up large volumes of fluid containing soluble antigens. Immature DC express receptors involved in antigen uptake (Fc-receptor, mannose receptor) [25]. Receptor mediated antigen uptake allows an extra capacity for antigen capture with some degree of selectivity for non-self molecules [23]. The mannose receptor contains several carbohydrate binding domains and mediates endocytosis or phagocytosis of antigens that express mannose or fructose residues. Antigen capture by DC is so efficient that only picomolar and nanomolar concentrations of antigen are needed for antigen presentation [23]. Internalised antigens enter the endocytic pathway of the cell for processing and class II restricted presentation. DC are also capable of presentation of exogenous antigen on MHC class I molecules [26, 27]. Following antigen uptake and recognition of potential 'danger', DC are mobilised and they migrate via afferent lymph vessels to the draining lymph nodes or via blood to the spleen [4]. Mobilisation of DC is accompanied by maturation. Progression into the mature stage requires signalling and this can be achieved *in vitro* using live bacteria, lipopolysaccharide (LPS) or inflammatory cytokines such as TNF-α or IL-1β [28]. P-glycoprotein, a transmembrane glycoprotein capable of pumping chemotherapeutic agents out of the cell and inducing of drug-resistance [29], has recently been shown to have a physiological function mediating the mobilisation of DC [30]. Migration of DC could be blocked by anti MDR-1-antibodies and MDR-1 antagonists in an *in vitro* skin model [30].

Maturation of DC is accompanied by a decrease of receptors associated with antigen uptake and an increase in expression of receptors associated with antigen presentation. Mature DC therefore express a high level of class I and II MHC structures, adhesion molecules, and co-stimulatory molecules for T-lymphocyte stimulation [17, 31]. Vice versa, T cells may also play an important role in activating DC and thus further enhancing the T-cell stimulatory capacity of DC [32, 33]. Ligation of CD40 on DC and CD40-ligand on T cells induces DC maturation (upregulation of adhesion and co-stimulatory molecules) and production of IL-12 by DC, a key cytokine for the generation of T helper (Th) 1 and CTL responses [34–37]. The mature stage of DC ends in cell growth arrest and finally cell death by apoptosis in the draining lymph node [38, 39]. IL-10 increases the apoptosis rate of DC [40] while CD40-ligand inhibits Fas-mediated and spontaneous apoptosis in DC [33]. DC apoptosis in lymph nodes may serve to prevent overstimulation of the immune response. The estimated *in vivo* life span of DC varies from three days to four weeks [41].

DC in malignant disease

In head and neck cancer, nasopharyngeal tumours, bladder, lung, oesophageal, cervix and gastric carcinoma DC infiltration of the primary tumour has been associated with significantly prolonged survival and a reduced incidence of metastatic disease [42–50].

In patients with breast cancer a reduction in DC function has been demonstrated [51]. A decreased number and potency of DC might in part explain the observed general immune suppression and deficiency in cellular immune responses against the tumour in cancer patients. The molecular mechanism of this DC dysfunction probably reflects a defective maturation of the DC, caused by soluble substances (e.g., IL10, vascular endo-

thelium growth factor (VEGF) and transforming growth factor β (TGF-β) released by tumour cells [52–54].

In patients with metastatic malignant melanoma DC isolated from regressing metastases showed a potent T-cell stimulatory capacity whereas a depressed T-cell activation and downregulation of co-stimulatory molecules was observed in DC from progressive lesions [55]. From these data it would appear that the tumour-dependent cytokine environment is responsible for DC-mediated tolerance or anti-tumour immune response induction. *In vivo* modulation of this environment by tumour reduction and/or activation and differentiation of DC might diminish the tolerance induction and enhance the immune response. Recently we observed a higher percentage of draining lymph node DC in locally advanced breast cancer patients treated with neo-adjuvant chemotherapy in combination with GM-CSF in comparison with stage I–II breast cancer patients and patients treated with neo-adjuvant chemotherapy without GM-CSF (Luykx et al., manuscript in preparation).

DC vaccination and immunotherapy

Another way of circumventing the immune suppressive conditions in cancer patients is by the *ex vivo* generation of autologous DC, followed by tumour-antigen loading and re-injection into the patient: the so-called DC vaccination.

In animal studies this approach has resulted in protection against tumours and also a reduction in the size of established tumours [56–61]. Based on these promising results in murine models clinical trials are being initiated all over the world.

Methods for in vitro *generation of DC for vaccination purposes*

Several methods are employed for the generation of human DC *in vitro* from haematopoietic precursor cells in peripheral blood. One approach utilises proliferating CD34+ precursors harvested from bone marrow, cord blood or peripheral blood in combination with GM-CSF and TNF-α [14, 17, 18]. Peripheral blood under normal conditions contains only 0.1% of CD34+ cells and therefore mobilisation with granulocyte colony-stimulating factor (G-CSF) and leukapheresis is required to increase the yield of CD34+ cells [17, 62]. Another approach makes use of non-proliferating peripheral blood CD14+ cells or monocytes [14, 63]. Using this culture method 3–8 × 10^6 DC can be obtained from 40 ml of blood. The yield of DC from cultured CD34+ cells varies from 1 × 10^6 from 500 ml of peripheral blood to 1.7 × 10^6 per single ml of normal adult bone marrow dependent on the cytokine mixture used [16, 62]. Monocyte derived DC need GM-CSF, IL-4 and serum. The type of serum that is used varies among laboratories and consists of either foetal calf serum (FCS), human pooled serum (HPS), or autologous serum or plasma. FCS should be avoided in therapeutic use because of the potential risk of immunogenicity and infection. In clinical trials FCS can be avoided since it is also possible to generate DC in its absence [14, 17, 63, 64].

A unique type of DC can be derived from a malignant clone of Philadelphia chromosome-positive chronic myelogenous leukemia (CML) cells *in vitro* [65]. These malignant DC are capable of antileukemic autologous T-cell response induction without the necessity for additional exogenous antigen [65]. Phase I studies with CML derived DC vaccination are ongoing.

Antigen loading of DC

Many tumour cells express tumour-associated antigens (TAA), which can be recognised by specific cytotoxic T lymphocytes. TAA, such as differentiation antigens, protein products of oncogenes and antigens of viral origin, are attractive candidates for use in tumour vaccines [66–69].

Clinical use of DC as tumour vaccines requires loading of DC with TAA for induction of CTL response and tumour specific immunity. *In vitro* generated autologous DC from cancer patients can be pulsed with peptides, proteins or mRNA, fused with carcinoma cells [59] or transfected with genes that encode for the TAA.

Unfractionated tumour-derived peptides, acid-eluted peptides from tumour cells or cell lines, tumour cell derived mRNA and synthetic peptides can all be used for the pulsing of DC [70, 71]. Addition of peptides to the culture medium during the maturation of DC results in an effective loading of these peptides into MHC class I complexes. Tumour derived peptides will consist of a mixture of TAA, present in the distinct tumour, and might, therefore, be effective even when expression of certain antigens is lost [72, 73]. A disadvantage might be the risk of generating auto-immune responses. For the use of synthetic TAA no tumour tissue is necessary and the purity of the product might enhance the effectiveness of the vaccine. An important limitation for the use of tumour-derived peptides as a source of antigen might be the availability of sufficient tumour necessary for the isolation of antigen. In contrast, antigen loading of DC with nucleic acids requires just a small amount of tumour cells [71]. The technique of isolating the mRNA content of cells, which can then be amplified using RT-PCR, is nowadays well established. The corresponding cDNA libraries of mRNA offer a virtually unlimited source of tumour antigens. Another disadvantage of peptide pulsed DC vaccines is the dissociation of peptides from MHC molecules and MHC epitopes. In contrast to pulsing, transduction of DC with genetic material encoding TAA can result in a stable expression of antigens and a long-term antigen presentation [74, 75].

Transduction of DC can be achieved by non-viral vectors [76], retroviral vectors [64, 74, 75, 77] and by adenoviral vectors [57, 78, 79]. An advantage of gene transfer to DC is the fact that endogenous expression leads to MHC I presentation. Class I restricted presen-

tation leads to generation of CTL against the protein coded by the gene. The most efficient method for gene transfer to DC still has to be determined [80]. The combination of adenovirus-mediated gene transfer and cationic liposomes or a polycationic amino acid compound [81], seems to be a highly efficient method for gene transfer into DC. A more potent immune response could be induced by co-expression of transgenes for cytokines such as GM-CSF or IL-12, to overcome *in vivo* immune suppression, leading to a more efficient activation and maturation of DC and subsequent stimulation of Th1 and CTL response.

Clinical studies

At the moment only a few pilot studies with DC vaccination have been published [73, 82–84]. Gjertsen et al. [83] showed specific T-cell responses against mutant-*ras* in vaccinated pancreatic carcinoma patients. Vaccination was performed with mutant-*ras* peptide pulsed DC. This study demonstrates the exquisite sensitivity of the immune system, since mutant-*ras* has only one amino acid different from the wild type protein and yet cytotoxic T cells could be generated capable of killing cells expressing the mutant ras protein but not those with the wild type protein. Hsu et al. [82] investigated the ability of autologous monocyte-derived DC pulsed with tumour-specific idiotype protein and showed a measurable anti-tumour cellular response in all four vaccinated patients with B-cell lymphoma. One patient developed a complete remission, one patient a partial remission and in the third patient all evidence of disease disappeared. Murphy et al. [84] performed a phase I study in patients with metastatic prostate cancer, who received either prostate-specific membrane antigen peptide (PSMA), alone, autologous DC alone or PSMA pulsed DC. No significant toxicity was observed and in some patients who received PMSA pulsed DC a decrease in the serum PSA level was detectable. Vaccination of 16 melanoma patients with peptide- or tumour lysate-pulsed DC was studied by Nestle et al. [73]. Autologous monocyte-derived DC were pulsed with tumour-lysate or a cocktail of TAA peptides known to be recognised by CTL. DC were injected directly into an uninvolved inguinal lymph node. The vaccination was well tolerated and no physical signs of auto-immunity were detected. Anti-tumour immunity *in vivo*, assessed as by delayed-type hypersensitivity (DTH) reactivity, toward a helper antigen (KLH: keyhole limpet hemocyanin) was induced in all patients and toward TAA peptide pulsed DC in 11 patients. Objective responses were evident in five patients and consisted of two complete and three partial responses.

Conclusion

This review considers some aspects of the origin, physiology and function of a relatively recently discovered cell: the dendritic cell. Its professional antigen presenting properties makes it an interesting tool for cancer immunotherapy. Over the coming years clinical studies will address many questions concerning the best DC culture methods, the optimum means of antigen loading on DC, the most important TAA, and the most efficacious vaccination schedules and dosages. The results of the aforementioned clinical trials are very promising and further studies will be necessary to demonstrate the effectiveness and impact on survival of this approach. Survival benefit will be expected especially in patients with minimal residual disease. DC-based therapies are creating a major change in the prospects for cancer immunotherapy. The results of DC vaccination trials are awaited with high expectation.

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