Dendritic cell-based vaccines in cancer immunotherapy: an update on clinical and immunological results

W. J. Lesterhuis1, I. J. M. de Vries2, G. J. Adema2 & C. J. A. Punt1

1Department of Medical Oncology and 2Tumor Immunology, University Medical Center St Radboud, Nijmegen, The Netherlands

Introduction

The immunogenicity of tumors has been established. Aspecific stimulation of immunity, such as by cytokine therapy, has been shown to induce durable clinical remissions in metastatic disease [1]. The identification and molecular characterization of tumor-associated antigens in the last 10–15 years has allowed the development of tumor-specific immunotherapy. Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system and have been used for several years as a vaccine in cancer patients with the goal of inducing a tumor-specific T-cell response. Here we review the current status of this approach.

Immunobiology of DCs

DCs reside in peripheral tissues where they act as the sentinels of the immune system, continuously patrolling the environment in search of antigen. At this stage they possess an ‘immature’ phenotype, which is mainly characterized by a low surface expression of MHC class I and II molecules and co-stimulatory molecules [2] (Figure 1). Immature DCs are specialized in the recognition and uptake of antigens. Exogenous antigens are internalized and processed by DCs, and the antigenic peptides are presented in the MHC class II complexes on the cell surface. Endogenous antigens, either self proteins or viral proteins, are cleaved into peptides by proteasomes and assembled into stable MHC class I–peptide complexes in the endoplasmic reticulum, which are subsequently transported to the cell surface. Of importance for DC-based vaccines in cancer immunotherapy is the finding that internalized antigens from exogenous sources, such as apoptotic or necrotic tumor cells, may also be present in MHC class I [3]. Thus, DC may present tumor antigens to both CD4+ and CD8+ T cells.

In the presence of a ‘danger’ signal, derived from tissue damage or microbial products, DCs undergo an activation process called maturation [4]. This maturation process involves a cascade of events that convert the DC into a cell that is exceptionally well equipped for antigen presentation and T-cell activation. During maturation, DCs up-regulate chemokine receptors CCR7 and CD62L, which leads to migration to secondary lymphoid organs. Furthermore, surface expression of MHC class I and II co-stimulatory molecules CD80, CD86, CD40 and CD83 are up-regulated, the latter functioning as a maturation marker for DC [2]. In the lymph nodes, DCs interact with high numbers of T cells (Figure 1). Two signals are essential for T-cell activation: the interaction between the MHC–peptide complex and the T-cell receptor (‘signal 1’), and a co-stimulatory signal from the DC to the T cell (‘signal 2’). A third signal determines the differentiation pathway of the T cell. This ‘signal 3’ is induced by several environmental factors that may skew the differentiation of CD4+ T cells into Th1, Th2 and possibly regulatory T cells [5]. The activated T cells subsequently leave the lymph nodes and circulate through the body in search of antigen.

Factors that determine DC vaccine efficacy

DC subsets

Since DCs constitute only about 0.2% of peripheral blood leukocytes, several ways to generate DCs from precursors have been investigated (Figure 2). Immature DCs cultured from monocytes by adding GM-CSF and IL-4 to the culture medium allow the generation of large quantities of clinical grade DCs [6, 7]. Further maturation may be achieved by adding cytokines such as TNFα, prostaglandin E2, IL-1β, IL-6 or monocyte-conditioned medium [8, 9]. Most clinical studies to date have used this method to generate DCs. The main disadvantage of culturing DCs from monocytes lies in the laborious and costly nature of the culture protocol.

DCs derived from CD34+ precursors in the blood are also used in vaccination protocols. They consist of two distinct populations, one with Langerhans cell-like properties and the other termed interstitial/dermal DCs with properties resembling monocyte-derived DCs [10].

Also, blood DCs can be obtained by several enrichment steps after leukapheresis. However, this usually requires repeated leukaphereses due to the low numbers of blood DCs [11].

Lastly, DCs may be induced in vivo by the administration of DC growth factors. For this purpose Flt3L has been investigated in patients with resectable metastases of colorectal cancer [12]. Increased numbers of DCs were observed in both resected tumor specimens as well as in peripheral blood. These findings demonstrate that in vivo expansion of the blood DC pool in cancer patients is feasible and may be an alternative to the laborious ex vivo culturing procedures.

Antigen loading of DCs

In order to induce an immune response in cancer patients, DCs should present the relevant tumor antigens. For this purpose
most clinical studies so far have used DCs pulsed with autologous tumor lysate or MHC class I peptides (Figure 2). Tumor lysate has the advantage that the antigen does not have to be identified, but has the disadvantages that sufficient tumor material is needed for preparation of the lysate and that it is more difficult to monitor immune responses as the antigens are not known. Tumor antigen-derived peptides have the advantage that many peptides are commercially available. However, the half-life of MHC–peptide complexes is relatively short and the immune response, if any, is restricted to the epitope(s) used. It has been described, however, that antigen-spreading may occur: killing of tumor cells after vaccination against a single epitope results in release of other tumor antigens. These antigens are subsequently taken up by DCs and presented to T cells, resulting in T-cell responses against antigens that were not included in the vaccine [13]. MHC class II peptides also allow the activation of CD4+ T helper cells [14, 15]. A more recent method of antigen loading concerns the use of transfection of DCs with either whole tumor RNA or RNA encoding a specific cancer antigen [16]. Also, fusion of tumor cells with DCs has been shown to be feasible, resulting in effective antigen presentation [17]. It remains to be established which types of antigen (e.g. tumor differentiation antigens, cancer germline genes, broadly expressed tumor antigens such as TERT [18] or unique tumor antigens) are most suited in terms of the induction of anti-tumor immune responses.

Maturation

In the majority of clinical studies, immature or semi-mature DCs have been used [19]. Studies that have compared the immunogenicity of immature versus mature DCs show that maturation is essential for the induction of immunological responses in cancer patients [20, 21]. Moreover, the use of mature DCs appears to be associated with a better clinical outcome compared to immature DCs [21, 22]. Vaccination with immature DCs may even lead to antigen-specific tolerance [23].

The superiority of mature DCs in inducing T-cell responses is probably not only related to their high expression of HLA and co-stimulatory molecules, but also to their enhanced migratory capacity. Compared with immature DCs, intradermal or intranodal injected mature DCs migrate much better to draining lymph nodes, although this migration process is still rather inefficient. Within the lymph node, mature DCs show a pronounced migration into the T-cell areas where antigen presentation takes place, whereas immature DCs remain at the periphery [24].

Maturation can be achieved by co-culturing the DCs with several stimuli such as cytokines [25], pathogen-associated triggers [4] or endogenous ‘danger signals’ such as heat shock proteins [26]. It is not yet known whether full-blown DC activation enhances the immunogenicity of a DC vaccine. Fully activated DCs may, on the one hand, be superior Th1 inducers, but on the other hand may quickly lose their capacity to produce IL-12, which could be a potential drawback in a vaccination setting [27]. To date, the optimal mode of DC maturation has therefore not been established. In this respect it should be noted that currently used markers to identify mature DCs do not cover all functional properties.

Route of administration

DCs interact with T cells in the peripheral lymphoid organs. Therefore, the migration of antigen-loaded DCs to those areas is essential. Mice models have shown that after intravenous injection most DCs end up in highly vascularized organs, such as the spleen and the lungs [28, 29]. DCs injected subcutaneously or intradermally remain, for the greater part, at the site of injection, although intradermal injection leads to a higher DC yield in draining lymph nodes in mice [30]. We have shown that intranodal injection results in a much higher accumulation of DCs in lymph nodes compared to intradermal vaccination. This concerned not only the injected node, but also subsequent draining nodes [24]. A study in melanoma patients found a small increase in peptide-specific T-cell responses after intranodal injection as compared to intradermal or intravenous injection [31]. Others compared vaccination with blood DCs injected via three different routes in advanced prostate cancer patients: intradermal, intravenous and intralymphatic injection [32]. T-cell responses occurred regardless of the route of delivery. There was, however, a difference in T- and B-cell responses, with an absent IFNγ-secretion in the intravenous group, and less antibody production in the intralymphatic and intradermal groups. This suggests that the type of immune response may depend on the route of DC administration. These authors also showed in mice that intravenous injection of DCs is essential for immune responses against lung melanoma metastases, whereas subcutaneous vaccination is essential for the response against non-visceral metastases [33]. These observations may relate to the interactions between DCs and different types of T cells in lymph nodes and spleen that are differentially involved in controlling tumors at different sites, and/or to different homing patterns of the activated T cells. These results provide a rationale to combine different routes of DC administration.

DC vaccination trials in cancer patients

Melanoma

Melanoma is the most studied cancer type in DC immunotherapy. This is mainly because in melanoma a large variety of tumor-associated antigens have been characterized, consisting of tumor differentiation antigens such as gp100 and tyrosinase and tumor-specific antigens such as MAGE-3 [34].

In the first reported study in advanced melanoma patients, monocyte-derived DCs loaded with peptides or tumor lysate were injected intranodally [35]. Out of 16 patients, two complete remissions and two partial remissions were observed. The results of further trials with DCs cultured in the presence of maturation stimuli are summarized in Table 1.

Several studies have used mature monocyte-derived peptide-pulsed DCs injected via different routes in advanced stage IV melanoma patients, using different culture protocols
<table>
<thead>
<tr>
<th>Tumor</th>
<th>Culture method</th>
<th>Dose/schedule</th>
<th>Route</th>
<th>No. patients&lt;sup&gt;a&lt;/sup&gt;</th>
<th>T-cell response&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Clinical response</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>IL-4/GM-CSF/FCS moDC</td>
<td>10⁶/1 week × 5–10</td>
<td>i.n.</td>
<td>16</td>
<td>n.d.</td>
<td>2 CR, 3 PR</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>IL-4/GM-CSF/IL-1β/IL-6/TNFα/PEG2 moDC</td>
<td>3–12 × 10⁶/2 weeks × 5</td>
<td>s.c. + i.d. + i.v</td>
<td>13</td>
<td>11</td>
<td>–</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>IL-4/GM-CSF/IL-1β/IL-6/TNFα/PEG2 moDC</td>
<td>2–6 × 10⁶/2 weeks × 5</td>
<td>s.c.</td>
<td>30</td>
<td>13</td>
<td>8 SD, 1 CR</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Calcium-ionophore/IL-2/IL-12 moDC</td>
<td>5–50 × 10⁴/2 weeks × 4</td>
<td>i.v./i.d./i.n.</td>
<td>28</td>
<td>13</td>
<td>3 PR</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>IL-4/GM-CSF/IL-1β/IL-6/TNFα/PEG2 moDC</td>
<td>8–12 × 10⁶/2 weeks × 6</td>
<td>i.n.</td>
<td>11</td>
<td>5</td>
<td>3 SD</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>IL-4/GM-CSF/MCM/TNFα/PEG2 moDC</td>
<td>10–30 × 10⁶/2 weeks × 3</td>
<td>i.d. + i.v.</td>
<td>10</td>
<td>2</td>
<td>4 SD, 1 PR</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>CD34 +/Flt3L/GM-CSF/TNFα</td>
<td>0.1–1.0 × 10⁶/2 weeks × 4</td>
<td>s.c.</td>
<td>18</td>
<td>16</td>
<td>3 CR, 3 PR, 3 SD</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>CD34 +/IL-3/IL-6/SCF/IL-4/GM-CSF/TNFα</td>
<td>5–50 × 10⁶/2 weeks × 4</td>
<td>i.v.</td>
<td>14</td>
<td>2</td>
<td>6 SD, 1 PR</td>
<td>36</td>
</tr>
<tr>
<td>NHL</td>
<td>Blood DC + Id</td>
<td>2–17 × 10⁶/4 weeks × 4</td>
<td>i.v.</td>
<td>10</td>
<td>8</td>
<td>2 CR, 1 PR, 1 molR</td>
<td>11, 37</td>
</tr>
<tr>
<td>NHL after CT</td>
<td>Blood DC + Id/Id-KLH</td>
<td>4–10 × 10⁶/4 weeks × 4</td>
<td>i.v.</td>
<td>25</td>
<td>9</td>
<td>18 PF, 4 CR of RD</td>
<td>37</td>
</tr>
<tr>
<td>MM</td>
<td>IL-4/GM-CSF/IL-1β/TNFα moDC</td>
<td>20 × 10⁶/2 weeks × 3</td>
<td>s.c.</td>
<td>5</td>
<td>4</td>
<td>3 SD, 1 decrease M protein</td>
<td>38</td>
</tr>
<tr>
<td>MM after SCT</td>
<td>Blood DC + Id</td>
<td>2–8 × 10⁶/4 weeks × 2</td>
<td>i.v.</td>
<td>12</td>
<td>2</td>
<td>9 PF</td>
<td>39</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma</td>
<td>IL-4/GM-CSF/IL-1β/IL-6/TNFα moDC</td>
<td>1 × 10⁶ × median 9.5</td>
<td>i.n.</td>
<td>10</td>
<td>3</td>
<td>4 PR, 1 CR</td>
<td>43</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Flt3 expanded enriched blood DC</td>
<td>10⁷–10⁶/4 weeks × 2</td>
<td>i.v.</td>
<td>12</td>
<td>7</td>
<td>2 CR, 2 SD, 1 MR</td>
<td>45</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>IL-4/GM-CSF/RNA transfected</td>
<td>10–50 × 10⁶/2 weeks × 3</td>
<td>i.v. + i.d.</td>
<td>13</td>
<td>13</td>
<td>–</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Blood DC</td>
<td>0.3–40 × 10⁶/4 weeks × 2</td>
<td>i.v./i.l./i.d.</td>
<td>21</td>
<td>21</td>
<td>–</td>
<td>32</td>
</tr>
<tr>
<td>RCC</td>
<td>IL-4/GM-CSF/IL-1β/IL-6/TNFα/PEG2 moDC</td>
<td>5–10 × 10⁶/2 weeks × 3–13</td>
<td>i.v./i.d.</td>
<td>35</td>
<td>5</td>
<td>2 CR, 1 PR</td>
<td>51</td>
</tr>
<tr>
<td>Breast and Ovarian cancer</td>
<td>IL-4/GM-CSF/TNFα moDC</td>
<td>2–17 × 10⁶/2 weeks × 3</td>
<td>s.c.</td>
<td>10</td>
<td>5</td>
<td>1 SD, 1 CR</td>
<td>54</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>IL-4/GM-CSF/TNFα moDC</td>
<td>Dose not given × 4</td>
<td>i.n.</td>
<td>16</td>
<td>9</td>
<td>2 PR</td>
<td>55</td>
</tr>
</tbody>
</table>

Included are studies in which DCs cultured in the presence of maturing stimuli were used.

<sup>a</sup>The number of patients that have received at least one vaccination.

<sup>b</sup>Number of patients in which peptide/tumor-specific T-cell responses were detected.

NHL, non-Hodgkin’s lymphoma; CT, chemotherapy; MM, multiple myeloma; SCT stem cell transplantation; RCC, renal cell carcinoma; moDC, monocyte-derived DC; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; TNFα, tumor necrosis factor alpha; PGE2, prostaglandin E2; MCM, monocyte-conditioned medium; CD34+, CD34+ progenitor derived DC; Id, idiotype; i.n., intranodal; s.c., subcutaneous; i.d. intradermal; i.v. intravenous; i.l., intralymphatic; n.d. not documented; CR, complete response; PR partial response; SD, stable disease; molR, molecular response; PF, progression-free; RD, residual disease; MR mixed response.
In all these studies antigen-specific T cells were detected in peripheral blood or in biopsies of delayed type hypersensitivity (DTH) reactions, and clinical responses were observed. A correlation between clinical outcome and antigen-specific T-cell response was found in a minority of these studies. This may relate to the fact that most studies monitor T-cell responses in peripheral blood. We have focused on the detection of T cells in DTH biopsies and found a correlation between the presence of antigen-specific DTH-infiltrating T cells and clinical response [21].

An interesting observation was made in advanced melanoma patients vaccinated with monocyte-derived DC that were pulsed with a single melanoma peptide: the only clinically responding patient showed evidence of spreading of T-cell reactivity against other antigens as well, indicating that determinant spreading is of importance for the induction of clinical responses [13].

The first clinical results using autologous antigen-loaded DCs concerned four patients with follicular non-Hodgkin’s lymphoma [11]. Blood DCs were enriched from peripheral blood mononuclear cells and loaded with idiotype after several enrichment steps. A follow-up of this trial was published in which patients in first remission after cytoreductive chemotherapy were also included [37]. Some patients received DCs that were loaded with idiotype immunoglobulin coupled to keyhole limpet haemocyanin (KLH), a protein providing a specific CD4+ T-cell help. Most patients developed anti-idiotype T-cell responses and some objective clinical responses were observed. Interestingly, booster injections of idiotype coupled to KLH induced clinical responses in patients that initially progressed on DC therapy.

Several studies have focused on the application of idiotype-pulsed monocyte-derived DC in multiple myeloma patients [38–42]. Clinical and immunological responses were infrequent, which may relate to the use of immature DCs in most studies.

An interesting observation was made in two patients with cutaneous T-cell lymphoma: after disease progression upon discontinuation of vaccination, a second clinical remission was achieved after revaccination [43].

**Hematologic malignancies**

Malignancies of B-cell origin express monoclonal immunoglobulins carrying unique tumor-specific antigenic determinants in the variable regions, called idiotypes [37]. These idiotypes can be isolated from B-cell malignancies and subsequently be used as antigens for the induction of CD4+ and CD8+ T cells. In contrast to the antigens used in solid tumors, these antigens are thus not only tumor-specific but also patient-specific.

**Gastrointestinal malignancies**

In colorectal cancer the most widely used antigen for loading on DCs is carcinoembryonic antigen (CEA) [44].
Patients with CEA-expressing malignancies have been treated with Flt3L expanded, in vitro-enriched blood DCs, pulsed with a modified CEA peptide. Antigen-specific immune responses and objective clinical responses were observed [45]. Interestingly, a correlation between clinical response and the magnitude of the expansion of tetramer positive cytotoxic T cells in peripheral blood was found.

Monocyte-derived DCs transfected with RNA encoding tumor antigens is an alternative method of antigen loading in DC-based immunotherapy. Specific T-cell reactivity was demonstrated in a patient with advanced CEA-expressing DC-based immunotherapy. Specific T-cell reactivity was demonstrated in a patient with advanced CEA-expressing DCs transfected with CEA RNA [46].

In a study using immature DCs pulsed with MAGE-3, an antigen which is expressed in a variety of gastrointestinal tumors, antigen-specific T-cell reactivity in peripheral blood and minor tumor regressions were observed [47].

Urological malignancies

In renal cell carcinoma studies with immature tumor-lysatelpulsed DCs have not resulted in either immunological or clinical responses [48–50]. One study used TNFα/PGE2 matured monocyte-derived DCs that were either loaded with autologous tumor lysate or tumor lysate from an allogeneic tumor cell line [51]. Clinical responses were restricted to patients who were vaccinated with autologous lysate-pulsed DCs.

Patients with metastatic prostate cancer have been vaccinated with recombinant prostatic acid phosphatase-pulsed mature blood DCs resulting in proliferative T-cell responses against the protein; no clinical responses occurred [32].

The immunogenicity of DCs transfected with whole tumor RNA or RNA encoding prostate-specific antigen has been investigated in patients with metastatic renal cancer and prostate cancer [52, 53]. Due to the prostate-specific antigen transfection the DCs underwent phenotypical changes with up-regulation of CD83 and CD86. In most patients antigen-specific IFNγ-producing T-cell responses were detected, which support the immunogenicity of this mode of antigen loading.

Other malignancies

MUC-1 and HER-2/neu peptide-pulsed monocyte-derived DCs have been used in breast and ovarian cancer patients resulting in peptide-specific cytotoxic T-cell responses in peripheral blood and some long-term clinical responses [54].

Epstein–Barr virus peptide-pulsed monocyte-derived DCs in nasopharyngeal carcinoma have resulted in antigen-specific T-cell responses in peripheral blood up to 3 months after vaccination, and partial responses were noted [55].

Other tumor types that have been investigated using DC vaccines include hepatocellular carcinoma, pancreatic tumors, adrenal carcinoma, cholangiocarcinoma, parathyroid carcinoma, non-small-cell lung cancer, head and neck cancer, sarcoma, bladder cancer, glioma and pediatric malignancies [56–60]. In all these studies DCs were cultured without maturating stimuli. Although some specific T-cell responses and clinical responses were seen in these studies, results may be improved using mature DCs.

Recent developments

New developments in DC therapy soon to be tested in clinical studies include the targeting of DCs in vivo via DC-specific molecules which may circumvent the problem of laborious in vitro culturing and antigen-loading protocols [61]. Furthermore, the depletion of CD4+CD25+ regulatory T cells which have the ability to suppress T-cell-mediated immunity may be a way of enhancing the potency of the DC vaccine-induced immune responses [62]. Lastly, plasmacytoid DCs may be an attractive DC subtype in vaccination of cancer patients as they have the capacity to infiltrate tumors, induce primary T-cell responses and secrete large amounts of type I interferons [63].

Conclusions

Generation of large quantities of DCs for clinical use is feasible, and this applies to monocyte-derived DCs, CD34 progenitor-derived DCs and blood DCs. Vaccination with DCs appears to be safe, and immunological responses as well as durable clinical remissions have been observed. More importantly, in several trials a correlation between immunological and clinical outcome has been demonstrated. These studies have greatly enhanced our knowledge of the induction of antitumor immunity in cancer patients. Mature DCs are superior to immature DCs in terms of the induction of specific T-cell responses. For reasons of comparison, details on culture methods as well as phenotypical data of the administered DCs should be provided in scientific publications [19]. Release criteria of the vaccine should at least contain purity, viability and mature phenotype. Current questions regarding the optimal DC subset, culture method, mode of maturation, route of administration, antigen loading and role of adjuvants should be addressed in small-scale studies with extensive immunomonitoring as the primary parameter for efficacy. Given these questions, as well as the theoretical options to further improve DC vaccines, it is too early to put DC vaccines to the test of randomized comparison in phase III trials.

References


