RESEARCH ARTICLE

Development and Clinical Evaluation of Dendritic Cell Vaccines for HPV Related Cervical Cancer - a Feasibility Study

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Abstract

Human papillomavirus infection (HPV) and HPV related immune perturbation play important roles in the development of cervical cancer. Since mature dendritic cells (DCs) are potent antigen-presenting cells (APC), they could be primed by HPV antigens against cervical cancers. In this study we were able to generate, maintain and characterize, both phenotypically and functionally, patient specific dendritic cells in vitro. A randomized Phase I trial with three arms - saline control (arm I), unprimed mature DC (arm II) and autologous tumor lysate primed mature DC (arm III) and fourteen patients was conducted. According to WHO criteria, grade 0 or grade one toxicity was observed in three patients. One patient who received tumor lysate primed dendritic cells and later cis-platin chemotherapy showed a complete clinical response of her large metastatic disease and remained disease free for more than 72 months. Our findings indicate that DC vaccines hold promise as adjuvants for cervical cancer treatment and further studies to improve their efficacy need to be conducted.

Keywords: Dendritic cells - immunotherapy - cancer vaccines - cervical cancer - HPV

Introduction

Cancer of the uterine cervix is the most common gynaecological cancer (Saumya Pandey et al., 2012) and the leading cause of mortality from cancer among women worldwide, with more than 80% of the cases occurring in developing countries like India (Ferlay et al., 2010). A causal link has been established between infection with human papillomavirus (HPV) and cervical cancer (Das et al., 2012). A majority of women infected with HPV clear the infection, but in some of them it persists and progresses to cancer. Due to lack of screening, awareness and limited access to treatment in developing countries, a large number of cases of cervical cancer present at an advanced stage.

Cervical cancer patients are now known to have a number of immune system defects ranging from down-regulation of MHC (Major Histo compatibility) class I molecules and abnormal upregulation of MHC class II molecules (Cromme et al., 1994) to transcriptional downregulation of mRNA of anti-tumorigenic cytokines like IFN-γ (Tartour et al., 1998). Hence there is a lack of relevant antitumor response which is essentially mediated by specialised antigen presenting cells called dendritic cells (DC). Some tumors may also secrete inhibitory cytokines like IL-6, which prevent maturation of local dendritic cells (DC) (Srivani and Nagarajan, 2003).

Various strategies have been investigated for stimulating the immune system in cancer patients with advanced disease. Therapeutic vaccination strategies for solid cancers based on tumor associated peptides have failed to elicit significant clinical responses in several trials (Claesson, 2009). In the case of cervical cancer, it has been suggested that the presence of HPV viral peptides could be used as a basis for a ‘vaccine’ in women in whom all other treatment has failed. However the presence of the viral peptides does not make a cancer immunogenic, as found in studies that aimed at studying CD4+ T cell mediated immunity against HPV E2 and E6 epitopes (de Jong et al., 2004).

Dendritic cells (DCs) are the most potent antigen-presenting cells (Banchereau and Steinman, 1998), and the viral peptides present in cervical cancer could be used specifically to prime DCs. But due to the presence of more than 100 different HPV types and lack of knowledge about cross protection, using a HPV specific peptide may not be effective in clearing an existing tumor. Hence, whole-cell antigens might be more effective, as the virus does not affect DCs, and cross-priming is an efficient way of activating DCs. Furthermore, DCs could stimulate a CTL response (Xiang Mai Wu et al., 2014).The objective of the study reported here was to determine the feasibility of generating DC in vitro, evaluating the best strategy for antigen pulsing and then conduct a phase-one trial in advanced, recurrent cervical cancer patients who had...
failed conventional therapy. The tumors needed to be HPV positive and toxicity was the primary endpoint. Secondary endpoint of the study was monitoring immunological responses in vaccinated patients.

**Materials and Methods**

**Evaluation of antigenic sources for pulsing**

Dendritic cells were generated in vitro for evaluating different antigen priming strategies. After obtaining institutional ethical committee clearance and an informed consent, 30-ml blood and punch biopsy samples were collected from cervical cancer patients before they underwent treatment. PBMC separated using a Ficoll Paque plus density gradient (GE Healthcare, Amersham, UK) were washed and plated with serum-free AIM-V medium (Gibco BRL, Grand Island, New York, USA) for monocyte enrichment at 37°C in 5% CO₂ for 2 h. After 2 h the non-adherent fraction was removed and fresh medium containing IL-4 and GM-CSF (Sigma Aldrich, St Louis, Missouri, USA) was added at 100 ng/ml concentration each, and cultured for 7 days at 37°C in 5% CO₂. Medium containing cytokines was replenished on the third and fifth day. On the seventh day, immature DC floating were transferred in fresh AIM-V medium to a six-well plate for antigen loading and maturation.

Antigen loading was evaluated using three strategies: tumor lysate, cervical cancer cell line lysate and tumor RNA. Cervical tissue samples were obtained by punch biopsy and minced with a surgical scalpel blade and then digested with a cocktail of enzymes- collagenase (0.07%) and hyaluronidase (0.1%) in HBSS (both from Sigma) at 37°C/10% CO₂. Cell lysate was prepared by alternating cycles of freezing with liquid nitrogen and thawing in a water bath at 56°C. A trypan blue exclusion test was done to confirm cell death. Tumor lysate was added to wells containing immature DC at ratio of 1:0.5, 1:1 and 1:3 (DC: tumor cell ratio) in order to determine the optimal priming ratio.

For priming with cell line cocktail lysate, the cervical cancer cell lines HeLa, SiHa and C33A were chosen. For RNA pulsing, total RNA was isolated from a punch biopsy obtained in RNAlater® (Ambion, Austin, Texas, USA). For RNA extraction, Trizol reagent (Invitrogen, San Diego, California, USA) was used according to manufacturer’s instructions. The final pellet was dissolved in RNA Secure® (Ambion) and added to the immature DC at a concentration of 5 μg /ml.

After 4 h of exposure to antigen, IL-1β and TNF-α (100ng/ml-both from Invitrogen, USA) were added to the cells which were incubated for 3 days at 37°C under 5% CO₂. A fraction of the cells (1x10⁵) were phenotyped by flow cytometry and the remaining cells were assessed for their functional activity in a mixed lymphocyte reaction (MLR).

**Flow cytometry**

Immature and mature DCs were labelled with the primary antibodies HLA-DR, DQ, DR (clone CR3/43) dilution 1:75, CD86 (clone BU63) dilution 1:25 and FITC-CD14 (clone TUK4) 4 µl (all from Dako, Glostrup, Denmark) for 30 min. After incubation, the cells were washed with 2% BSA-PBS, and FITC anti-mouse rabbit secondary antibodies (also from Dako) were added to the control tube and unstained primary antibody containing tubes alone, washed and finally fixed in 0.5% paraformaldehyde. Acquisition and analysis were done on a BD FACS Calibur flow cytometer using the Cell Quest Pro software.

**Mixed lymphocyte proliferation assay**

Graded numbers of (0-10 000) irradiated (24 Gy) mature dendritic cells were added to 2X10⁵ cells of T cells from allogeneic donors in a 96-well tissue culture plate in triplicates. Stimulation of responding T cells was determined after 5 days of co-culture. Cell Titer 96 aqueous one-solution proliferation assay (Promega, Madison, Wisconsin, USA) was done following the manufacturer’s instructions. Readings were taken at 492 nm to determine the percentage cell proliferation, with wells containing no DC as baseline. Positive control cells were stimulated with phytohemagglutinin (Gibco) at a concentration of 5μg/µl on the second day. Unstimulated cells were used for calculating the percentage proliferation.

**Patient recruitment for the Phase I trial**

The clinical trial was done from 2004-2008, at a time when the registration for the clinical trials in India was not mandatory. It became mandatory from June 2009 and by then the study had been completed. Fourteen patients were enrolled in the study after an informed consent had been obtained, and the trial design was approved by the Institutional Ethical Committee. The schema for recruitment in the clinical trial is given in Figure 1. The eligibility criteria were a histologically confirmed diagnosis of cervical cancer with HPV presence detected by polymerase chain reaction (PCR) using MY09 and MY11 consensus primers and local or distant recurrent malignancy after initial radical treatment, a Karnofsky score ≥70, normal baseline haematological parameters (within 1 week of first vaccination) - hemoglobin>9.9 g/dl total granulocyte count>1000/µl, platelet count>60

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**Figure 1. Schema for Recruitment of Patients for the Phase I Clinical Trial**
Dendritic cell vaccine generation and phenotyping by flow cytometry

From patients recruited for the randomized Phase I trial, 75ml of blood was drawn and processed as done for the in vitro studies. They were matured on day 7 and 72 h and was considered positive only if the reaction had a diameter of more than 5 mm in any one dimension.

Vaccination and dosage

The DCs that were found to be sterile for more than 72 h microbiologically were frozen in a mixture containing 85% autologous serum (heat-inactivated), 10% DMSO and 5% glucose. On the day of vaccination, the cells washed three times with saline and given to each patient intra-dermally. Patients received three vaccinations, one every 14 days. A maximum of 1x10^6 cells were given at each vaccination.

Clinical response and toxicity assessment

Although a clinical response was not expected, patients were evaluated through clinical examination before and after each vaccination and during subsequent follow up. X-ray or ultrasound was done when considered necessary. Adverse events were recorded using WHO common toxicity criteria.

DTH skin test

Lymphocytes cryopreserved from the patients before vaccination and after the third vaccination were rested overnight, pulsed with tumor lysate at a 1:1 ratio and incubated at 37°C /5% CO₂ for 6h. After an hour, Golgi stop solution (Pharmingen, San Diego CA) was added to arrest cytokine secretion. The cells were stained with anti-CD3-PE, fixed and permeabilized with Cytofix-Cytoperm solution (Pharmingen), and stained with anti IFN-γ-FITC antibody. Isotype-matched controls were run and analysis was done on a BD FACS Calibur machine using Cell Quest Pro software.

Immunohistochemistry

Paraffin embedded sections of 10µm thickness from biopsy samples taken before and after the third vaccination were stained with antibodies against CD8 (C8/144B clone M7103 at 1/25 dilution), CD45RO (OPD4 clone M0834 at 1/50 dilution), CD20 (L26 clone M0755 at 1/200dilution), and stained with antibodies against CD8 (C8/144B clone M0852 at 1/10 dilution; (DAKO corp., Glostrup, Denmark).

Antinuclear antibody ELISA

Plasma collected after Ficoll Paque (GE Life sciences) was stored at -70°C and used for evaluating any autoimmunity post vaccination. An ANA Detect kit (ORG 600) was used and an ELISA was done according to the manufacturer’s instructions.

Results

Phenotypic characterization of DC generated in vitro

Our in vitro study results showed that immature DCs generated from monocytes showed increased HLA-DR expression, slightly increased CD86 levels but almost no CD14 expression. Mature DCs when compared to immature ones showed significant increase in HLA, CD3, DR (+) but almost no CD14 (-) expression. Mature DCs when compared to immature ones showed significant increase in HLA class II expression in the cell periphery along with that of CD86 co-stimulatory molecules.

Antigen loading strategies may influence functional capacity of DC

Mixed lymphocyte reaction with allogeneic donor PBMCs revealed that the cervical cancer cell line cocktail lysate (prepared from HeLa, SiHa and C33a) primed DCs showed increased stimulatory capacity but not significantly higher than tumor lysate primed DC (paired two tailed test, p value=0.6) or RNA primed DC (p value=0.9) at 10,000 DC concentration. We observed in...
our experiments that mature DC, irrespective of antigen loading strategy, definitely had better ability to stimulate PBMC compared to immature DC (Figure 3). Although not strictly significant, the autologous tumor lysate primed DC (p value=0.08) but not the autologous RNA (p=0.7) primed DC were better stimulators of PBMC when compared to unprimed immature DC at 10,000 DC concentration. Proliferation responses were significantly higher (Figure 4) for lysates added in a tumor cell: DC ratio of 3:1 than 0.5:1, (paired two-tailed t test, p= 0.01). Hence this ratio was determined to be ideal for culturing and maturing DCs in vitro for use in the phase I clinical trial. As the Institutional human ethics committee did not permit the usage of cell line lysate, autologous tumor lysate priming was chosen as the antigen loading strategy.

**Phase I trial patient characteristics**

In all, nine patients were recruited for the three arm trial and five patients were recruited for the two arm trial after they were found to fit the eligibility criteria and provided an informed consent. Age range of the patients was 27-70 years (median=49 yrs.). All patients except one received standard treatment at Cancer Institute (WIA), Chennai, India. Patient 7 alone received the same at the Govt. General Hospital, Chennai, for cervical cancer. Other patient characteristics are summarized in Table 1. Two patients (3 and 11) had lung metastasis while Patient 10 had a left external iliac vessel metastasis.

**DC vaccine yield, phenotype and dosage**

Immature DC yield was found to depend on individual monocyte counts. But in arm III patients’ tumor cell percentage influenced mature DC numbers. A maximum of 7.6×10^6 primed DCs could be generated in one arm III patient after pulsing with tumor lysate but this was not possible in all patients as tumor cell numbers and ratio of pulsing (3 tumor cells: 1 DC) limited the number of antigen primed DCs that could be generated. Tumor cell percentage was determined by a qualified cytologist from cytopsin preparations after tissue digestion. This percentage was used to calculate the actual no. of tumor cells that could go to priming DCs in arm III and it varied from 20% to 86% (median-43%). All the patients who enrolled for the trial were previously treated and hence viable number of cells was also a limiting factor for generating mature lysate pulsed DC.

DC phenotype was consistent with our in vitro studies as indicated in Table 1. Following DC preparation and maturation with or without antigen loading, they were subject to microbiological tests of sterility including gram staining and cultures for a period of 72-96 h and were administered after being certified sterile by the Dept. of Microbiology, Cancer Institute (WIA). The dosage and arm of vaccination of each patient are also indicated in Table 1.

**Clinical Outcome, toxicity and DTH response**

The vaccine was well tolerated in all patients. WHO toxicity criteria were used for evaluation and accordingly patients had only grade 0 or grade 1 toxicity. Nevertheless, any other symptoms experienced even if they were unrelated to vaccination, were also recorded and patients were managed accordingly. The DTH responses developed are summarized in Table 1. Table 2 summarizes the grade and type of toxicity seen. The tests were done in 11/14 patients. In patients 10, 13 and 14, DTH could not be done. Patient 10 developed mild icterus prior to DTH. Liver function tests revealed that she had mildly elevated levels of bilirubin and alkaline phosphatase (ALP). This patient had elevated ALP levels even at the time of recruitment but the levels were lower than twice the upper limit and was recruited as she fit the eligibility criteria. On further observation, her icterus subsided but her ALP level did not return to normal and hence DTH testing was not done. Patient 10 had a pre-existing hydronephrosis saw
Table 1. Patient Characteristics, Arm of Vaccination, DC Phenotype and DTH Reaction to the Dendritic Cell Vaccine

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Stage</th>
<th>Arm of vaccine</th>
<th>HLA-class</th>
<th>CD86 status</th>
<th>DTH with 1×10⁶ cells (minimum)</th>
<th>DTH with 1×10³ cells (minimum)</th>
<th>DTH with 1×10⁴ cells (minimum)</th>
<th>DTH with 1×10⁵ cells (minimum)</th>
<th>DTH with 1×10⁶ cells (minimum)</th>
<th>DTH with 1×10⁷ cells (minimum)</th>
<th>DTH with 1×10⁸ cells (minimum)</th>
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<td>2</td>
<td>50</td>
<td>II A</td>
<td>II</td>
<td>(+)</td>
<td>1×10⁶ cells in the I dose only</td>
<td>0.6×10⁶ cells in the I I I vaccination</td>
<td>1×10⁶ cells in the I I I vaccination</td>
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<td>I I B</td>
<td>I I</td>
<td>(+)</td>
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<td>II</td>
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<td>(+)</td>
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<td>1×10⁶ cells in the I I I vaccination</td>
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<td>II</td>
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*Due to disease progression (++) – >75% expression, (++) – >50-75% expression; Original stage at presentation. Patient 5 and 10 alone had adenosquamous carcinoma while the others had squamous cell carcinoma

Table 2. Toxicity Observed Following DC Vaccinations

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Post I vaccination</th>
<th>Post II vaccination</th>
<th>Post III vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Grade 0: itching at the site of vaccination</td>
<td>Grade 0: itching at the site of vaccination</td>
<td>NONE</td>
</tr>
<tr>
<td>7</td>
<td>NONE</td>
<td>Grade 1: fever, chills and rigor due to urinary tract infection</td>
<td>Grade 0: vague abdominal discomfort and vomiting</td>
</tr>
<tr>
<td>10</td>
<td>NONE</td>
<td>NONE</td>
<td>Grade 1: 99F fever. The patient had higher ALP levels at the time of recruitment but lower than twice the upper limit so she fit the eligibility criteria. The levels persistently remained high throughout the study</td>
</tr>
</tbody>
</table>

*Only three patients developed adverse reactions to the DC vaccination. The nature and grade of toxicity is described below. The other patients did not develop any such symptoms following vaccination.

Figure 5. X-ray Images of a Regressing Lung Metastasis in Patient 3. a) Lung metastatic lesion detected at patient enrolment (indicated by black arrow); b) and c) show progression of lesion after vaccination (5c. Was taken a year after vaccination); d) Shows complete regression of lesion three years after vaccination and 1.5 years after cis-platin chemotherapy

progression of her cancer and deterioration of her renal condition because of which DTH testing could not be done. Patient 14 dropped out of the trial just after receiving the first dose of vaccine.

Most of the arm I and II patients did not show any appreciable change in their disease status and were found to have disease progression during follow up. Patient 9 (arm III) had stable disease at follow up while Patient 3 (also in arm III) who was vaccinated in November 2004, had achieved local disease control at the primary site during her six month review after the third dose but showed progression of her lung metastatic lesion. She was advised symptomatic treatment in view of the progression of her lung metastasis in 2005. In Dec 2007, when updating the records we called the patient’s relative telephonically to inquire about the patient’s condition and were informed that she was fine. She was asked to be reviewed at the Institute. The patient presented to the Institute two years after her last follow up and clinically and radiologically she was found to be free of disease in the right lung (Figure 5). On enquiry, she revealed that she had received four injections of chemotherapy.
with cis-platin between March and June 2006, a year and a half after the third dose. Examination of the cervix showed vaginal adhesions and she was considered to have achieved complete clinical remission. She continues to be disease free nearly eight years after treatment with cis-platin.

Two patients in arm II whose DC were not primed with tumor antigen responded with a positive DTH reaction. These patients responded to even the smaller tumor lysate dose (104). The survival status of some of the patients who were lost to follow up was determined by either letters written to them several times or telephonically while we could not ascertain the status of others.

**DC vaccination improved proliferation response and IFN-gamma secretion post vaccination**

One patient (Patient 3) showed improved proliferation of lymphocytes after the third vaccination (Figure 6) but the increase however was not significant (paired two tailed t test =0.06). No significant proliferation responses were seen in any of the other patients. Comparing arm II and arm III patients’ responses also did not show significant difference. Secretion of IFN-γ was higher in post vaccination samples of 3/4 arm III patients and 2/5 arm II patients (Table 3). Although no significant difference between patients of the two arms could be ascertained, we observed that patients 9 and 12 of arm III who did not show a DTH positivity showed an IFN-γ response and patient 7

### Table 3. IFN-Gamma Secretion in Vaccinated Patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Pre vaccination (%)</th>
<th>Post third vaccination (%)</th>
</tr>
</thead>
<tbody>
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<td>Patient 1</td>
<td>32</td>
<td>17.6</td>
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<tr>
<td>Patient 2</td>
<td>12</td>
<td>47.6</td>
</tr>
<tr>
<td>Patient 3</td>
<td>17.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Patient 4</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Patient 5</td>
<td>30.2</td>
<td>19.4</td>
</tr>
<tr>
<td>Patient 6</td>
<td>19.2</td>
<td>9.9</td>
</tr>
<tr>
<td>Patient 7</td>
<td>23.7</td>
<td>7</td>
</tr>
<tr>
<td>Patient 8</td>
<td>4.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Patient 9</td>
<td>18.6</td>
<td>26.9</td>
</tr>
<tr>
<td>Patient 10</td>
<td>19.8</td>
<td>29.8</td>
</tr>
<tr>
<td>Patient 11</td>
<td>5.4</td>
<td>28.1</td>
</tr>
<tr>
<td>Patient 12</td>
<td>4.2</td>
<td>19.3</td>
</tr>
</tbody>
</table>

*Percentage of CD3+ cells secreting IFN-gamma before and after vaccination in each patient.

**Figure 6. Proliferation Assay Using Autologous PBMC of Vaccinated Patients.** Fifty thousand PBMC obtained before and after each dose of vaccination were monocyte depleted and stimulated with tumor lysate for 5 days (ratio 1:1). Unstimulated cells were taken as the baseline to determine percentage of proliferation which was measured using a colorimetric assay. Patient 3 showed robust proliferation responses post vaccination although the response was not strictly significant (p=0.06)

**Figure 7. Anti-Nuclear Antibody ELISA.** Anti-nuclear antibody ELISA was done using plasma samples from 9 patients obtained before vaccination (PRE VAC), after the second (POST II) and the third vaccination (POST III) as indicated above. Negative, cut off and positive controls are indicated above. Values were considered positive only if they were above the cut off control

(arm II) who had a robust DTH response did not produce higher levels of the cytokine, post vaccination. Patient thirteen could not be evaluated for immune response due to lack of tumor cells for the assays. Punch biopsy samples could not be obtained from all patients after the vaccine administration. Only three patients consented and hence immunohistochemistry was performed in those patients alone. Patient 9 who did not show any infiltration of CD8+ cells in the pre vaccination biopsy showed 30% CD8+ T cell infiltration in the post vaccination biopsy sample which was considered significant. But post vaccination biopsy samples could be obtained only from Patient 1, 2 and 9 hence the levels could not be compared evenly. Other markers like CD19, 56 and 45RO did not show any increase.

**DC vaccination did not generate auto-immune responses in vaccinated patients**

None of the nine patients evaluated had any elevation of auto antibody levels after the third vaccination although patient 1, who was in arm I (saline only) showed slight elevation of ANA levels (Figure 7) in the plasma obtained before the second dose. Although this value was slightly above the cut-off, it quickly dropped to well below the cut-off in the day 35 sample.

**Discussion**

This was the first study in India to use dendritic cells as therapeutic vaccines against HPV induced cervical cancer in a phase I clinical trial, with toxicity as its primary endpoint. Dendritic cell based therapeutic approach has now been applied as an adjuvant for the treatment of several types of cancers such as lymphomas (Hsu et al 1996), myelomas (Curti et al., 2007), brain cancers (Arden et al., 2012) and pediatric malignancies (Dohnal et al., 2007) with some degree of success. Since no uniform standardised protocol has emerged, we developed a protocol and initiated a phase I trial. This trial was approved by the Institutional Ethical Committee and the Drug Controller General of India.

Fourteen patients enrolled in the trial and eleven of them received DC vaccinations. Of the eleven patients, six received DC matured with inflammatory cytokines alone while five received antigen primed mature DC. Three
other patients received saline only. A lower dose of DC was initially given but was later increased to one million DC, injected three times, at an interval of two weeks. One patient received a single dose of antigen loaded DCs and then opted out of the trial for reasons unknown.

A previous study showed that antigen loaded and cryopreserved mature DC were as potent as freshly loaded and matured DC (Thumann et al., 2003) providing an alternative to frequent blood sample collection for DC cultures. Since this approach was more practical, we used cryopreserved DCs for the study after appropriate testing for sterility.

Although different routes of vaccination exist, when given intranodally, the most frequent side effects so far reported to DC vaccines are systemic flu-like symptoms with fever or painful swelling of the injected lymph node whereas with intradermal injection only itching and erythema were the most commonly observed reactions that regressed within 48-72h in most cases. Additionally previous studies involving DCs and other vaccines have shown that the intradermal route is more physiologic with the injected cells migrating efficiently to the regional nodes (Reinhard et al., 2002, PATH, WHO review Dec-2010). Hence the intradermal route was chosen for our study. Itching and mild erythema were observed in two patients of our study but the others did not have any such complaints.

Some studies using immunotherapeutic adjuvants report that prior to inducing tumor rejection, there is immune cell infiltration leading to an increase in tumor size appearing as disease progression (Marchand et al., 1995). Even in our study we initially saw an increase in the responder’s metastatic lesion and even though she received chemotherapy, this patient’s disease free status nearly eight years later points to an immune system with active surveillance against tumor antigens.

DC vaccine trials especially those in glioma patients report massive intra tumoral infiltration of CD8+ T cells in several patients who received multiple DC vaccines (Liu et al., 2005). As we could not obtain a post vaccination biopsy from all our patients, it was not possible to ascertain the infiltration status in most of them especially, the patient who responded, but a biopsy from another patient who received lysate primed DC showed significant infiltration post vaccination.

Some biological agents that are used in immunotherapy may need longer time or booster doses for sustained and productive benefits. Dendritic cell vaccines may as well fall in this category when used against tumors. A study using DCs against glioma had shown that cytotoxic T cell activity may develop even twelve weeks (Yu et al., 2001) after vaccination while recently another reported CD8+ T cell and IFN-γ responses in melanoma patients after they were given multiple DC vaccines loaded with a cocktail of synthetic peptides including MART-1 (Melanoma antigen recognized by autologous T cells-1) (Okoshieta et al., 2012). Ardon et al. (2012) also noted that DC-based tumor vaccines in booster doses improved the six month progression free and median overall survival of newly diagnosed glioma patients who underwent surgery and concomitant chemoradiotherapy. For sustained stimulation of the immune system through multiple vaccinations, leukapheresis may be the best strategy. This process may yield large numbers of DCs for booster doses but since we did not get Institutional ethical committee clearance for the procedure we were only able to generate DC that were sufficient for three vaccination doses.

In a previous study (de Vries et al., 2003), patients with advanced melanoma were treated using both immature and mature DC with KLH as the adjuvant. They noted that none of the patients who received immature DC showed a positive DTH response while almost all the patients injected with mature DC developed a positive DTH which confirmed that DC maturation is a pre-requisite for vaccination. Although following vaccination, we did not expect any specific immune reaction especially to the unprimed mature DC two of the patients who responded to tumor lysates in DTH test were in arm II and respond to even the lower dose. This affirms the fact that even DC matured in the absence of antigen with cytokines alone may be active stimulators of the immune system.

Immunotherapeutic approaches especially DC vaccines have been used in combination with surgery and chemotherapy as a treatment modality in glioblastoma with standard treatment succeeded by vaccination. But a study conducted in 2004 (Wheeler et al., 2004) showed that vaccination or chemotherapy alone could not elicit significant tumor regressions whereas chemotherapy following DC vaccination significantly improved the mean survival of patients (p=0.04). Although most of our patients did not show any objective clinical response, one of the patients in arm III (patient 3) with a prominent lung metastasis, showed an excellent response to cis-platin chemotherapy given nearly a year after the third DC vaccine dose. She continues to be disease free for nearly 8 years now. Reports of an excellent complete response in metastatic cervical cancer are rare. A review on chemotherapy for metastatic and/or recurrent cervical cancer (Scatchard et al., 2012) found that in two randomized control studies, the median survival of patients after single agent cis-platin chemotherapy for metastatic disease was 17 (Alberts et al., 1987) and 13 (Cadron et al., 2005) months respectively. It is therefore interesting to speculate as to whether the DC vaccine had a contributory role in this patient achieving a sustained disease free status. The hypothesis put forward is that the mature antigen loaded DC vaccine induces an immune response as shown by the proliferation assay and DTH response. However, the immune response is unable to prevent the disease progression. The use of chemotherapy kills cells and releases tumor antigens acting as a booster dose. This could help mount a robust memory response in addition to the chemotherapeutic effect, effectively eliminating the tumor and extending a sustained anti tumor immune surveillance. This model therefore could have implications with regard to sequencing of treatment.

We plan to conduct a phase II trial in the near future with tumor lysate primed dendritic cells as adjuvants for HPV induced cervical cancer patients of stage IIIB along with concurrent chemo radiation as standard treatment.
Acknowledgements

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References


