Review Article

Dendritic cell, the immunotherapeutic cell for cancer

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Dendritic cells play an important role in the development of effective cancer vaccines. These cells have the potential to present tumour-specific antigens and thereby induce an immune response. Various studies involving clinical trials have investigated the efficacy of administering antigen-loaded dendritic cells for cancer therapy. In order to design such experiments it is important to consider specific antigens, which initiate either a CD4+ or CD8+ response or both. The present review discusses the unique properties of dendritic cells as an immunotherapeutic cell for cancer.

Key words Dendritic cell - immunotherapy - peptide - transfection - vaccine

The failure of conventional treatment for many forms of cancer has opened the doors for novel, experimental therapies. The first clinical trial with dendritic cell (DC) was started in the early 1990s though Steinman and Cohn discovered this cell in 1973. The potency of the DC to present and sensitize the T cells has led to the development of effective vaccines. The first step in the induction of an effective anti-tumour therapy/vaccine is to identify a specific tumour antigen from an ever-growing list of tumour antigens. If a tumour antigen is presented to an antigen-presenting cell (APC), a more efficient and effective anti-tumour response is generated. Thus a critical target of vaccines would be APC, the most immunologically potent being the dendritic cell.

DCs represent a heterogeneous cell population both phenotypically and functionally, residing mostly in peripheral tissues where they represent 1-2 per cent of the total cell number. DCs may be derived from either a pro-myeloid or a pro-lymphoid cell. In humans, two distinct subsets based on differential phenotype and function are, CD11c(-) plasmacytoid DCs (PDCs) and CD11c(+) myeloid DCs (MDCs). Myeloid DCs can be further subdivided into two fractions, one which is capable of differentiating into Langerhans cells (MDC1), and another, which is lacking this ability (MDC2).

Morphologically, mature DCs are large cells with elongated and stellated processes. They express high levels of major histocompatibility complex (MHC) I and II, CD11 a, b, c, CD40, CD54, CD58, CD80, CD83, CD86. The most typical markers at present are MHC I, II and co-stimulatory markers such as CD80, CD86.

DCs are mainly localised in tissues and represent only a small proportion of less than 0.5 per cent of peripheral blood leukocytes. For therapeutic purposes, large numbers of DCs can be generated either from proliferating CD34+ bone-marrow precursor cells which differentiate under different cytokines like IL-2, IL-6, IL-7, IL-13, IL-15, hepatocyte growth factor (HGF), stem cell factor (SCF), granulocyte macrophage-colony stimulating factor (GM-CSF) or G-CSF, transforming growth factor-b (TGF-b) and tumour necrosis factor-a (TNF-a) or from non-proliferating peripheral CD14+ cells (monocytes). Usually, CD34+ precursors are mobilized from bone marrow by G-CSF or GM-CSF and isolated by leucopheresis to obtain large number of peripheral cells for therapeutic purposes. The presence of FLT3-ligand (FLT3-L), SCF, and the differentiating growth factors GM-CSF and TNF-a leads to a sustained proliferation of CD34+ cells for long periods. These cells seem to be more efficient in the activation of tumour-
specific cytotoxic T lymphocytes (CTLs) than CD14+ derived DCs. Protocols for the generation of large number of monocyte-derived DCs exist since 1994 for both experimental and therapeutic purposes; different culture conditions have been reported.

Briefly, the most common way of generation of DCs from CD14+ cells (monocytes) includes isolation of peripheral blood lymphocytes from buffy coats or from patients’ blood, by Ficoll-Hypaque® density-centrifugation. The isolated adherent cells are cultured in RPMI 1640 with serum, GM-CSF and IL-4. Maturation of DCs has been achieved by using different cytokine cocktails. Addition of cytokines like TNF-alpha or IL-6 or IL-1beta or PGF2 and TNF-alpha or by culturing in monocyte conditioned medium elicits the required maturation signal.

Mature DCs are more potent in inducing Th1 and CTL responses in vitro. Immature DCs are not stable in vitro. They differentiate themselves to macrophages if the medium lacks GM-CSF and IL-4. After a week of culture, 25 to 50 per cent of the starting population differentiates into DCs which can be used for therapeutic purposes.

Selection of tumour antigen for pulsing of dendritic cells: Antigens are either tumour-specific or tumour-associated antigens (TAA). They are used for pulsing in the form of peptides, tumour extracts, apoptotic bodies or nucleic acids. Single immuno-reactive peptide has been used to a cocktail of peptides or tumour lysates. Tumour lysates deliver an entire gamut of antigenic peptides resulting in a multivalent immune response. The possible occurrence of antigen-loss cell variants within the same tumour may restrict the applicability of tumour-associated antigens. The disadvantages of using DCs pulsed with synthetic TAAs include the uncertainty regarding longevity of antigen presentation, and restriction imposed by the patients' haplotype. The selection of the peptide used for vaccination depends on the type of tumour, the HLA type of the patient and successful induction of CTL-response in vitro or in vivo, etc. The right choice would be the antigen, which would be efficiently processed and expressed on the cell surface of a DC and elicit the activation of CTLs, which in turn would lead to a significant antitumour response. Analysis of tumour-associated antigens has determined immunodominant peptides for some HLA types. Several groups have shown that human DCs when pulsed with synthetic peptides in vitro, can elicit strong antigen-specific CTL response. In some cancers like pancreatic carcinoma, allogeneic tumour cells have been used as a source of antigens. Though more than 200 tumour-associated epitopes recognise T cells, most of them do not elicit a strong immune response.

Pulsing of DCs with small peptides is the easiest method of delivering antigen to immune cells. The first clinical study involved injecting monocyte derived DCs pulsed with idiotype protein derived from B cell lymphoma. Since then there have been many clinical studies using peptide pulsed DCs. In the first study, 16 patients with melanoma were injected with monocyte-derived DCs pulsed with tumour lysate of or a cocktail of melanoma antigens consisting of MART, tyrosinase and GP 100. Other methods using nucleic acids or genes are also being tried out.

Nucleic acids in the form of DNA or RNA have been increasingly used to transfet DCs. DNA vaccination has become an attractive strategy since it induces both cellular and humoral immunity but it has a limited potency to induce immune response. Using adenovirus-MART and alpha-fetoprotein constructs, DCs were transduced effectively and strong CTL response was reported. Others have investigated the potential of RNA to deliver antigen to DCs.

Capture, processing, and presentation of an antigen by DC: In general, immature DCs take up and process the antigen whereas mature DCs neither have the antigen capturing ability nor the processing mechanism. Antigens are internalised, processed on either a MHC class I or II pathway and presented as a peptide-MHC complex. Antigenic peptides (about 8-10 amino acids) are normally loaded directly on to the MHC-I molecule on the cell surface whereas proteins from tumour lysate are internalized and then presented with the MHC II-molecule on the cell surface. Cross-presentation is achieved when tumour lysates are used for pulsing or co-culturing. This implies that pulsing with tumour lysate activates both CD4+ and CD8+ T-cells by the conventional MHC-II and MHC-I pathways, respectively. Macropinocytosis is the method of choice for most of the soluble antigens or it could be a receptor
mediated phenomenon using mannose receptors, FcγRI and FcγRII or by phagocytosis. Any of these methods results in efficient capture of the antigens.

**Introducing genes into dendritic cells:** Unfortunately, the advantages of peptide vaccines are to some extent diminished by their inherent lack of immunogenicity. The immune system in most species has evolved through time to fight life threatening infectious agents, it should not be surprising that vaccines consisting of aseptic, endotoxin-free peptides are likely to be ignored and ineffective at inducing T cell immunity. The use of gene transfer techniques might prove to be more effective and specific methods to generate tumour-specific T-cells. In order to enhance the tumour response, DCs are modified by introducing genes like cytokine genes interleukin-7, GM-CSF, interleukin-12, interferon-gamma and interferon-alpha or genes coding for tumour antigen by viral or non-viral methods. Cytokine genes in most instances enhance tumour immunogenicity.

Viral methods use attenuated forms of virus. Non-viral methods like nucleofection, electroporation, lipofection and gene-gun method have been tried to induce a better gene inoculation and to achieve a better T cell response. Viral vectors can disturb the function of DCs as antigen presenting cells. They induce death, interfere with antigen presentation, or affect maturation of DCs. The use of viral vectors to present an antigen will lead into a phenomenon known as immunological dominance. In such situations, other antigenic peptides like viral antigens mask or suppress the response to the tumour-specific antigen or else the CTLs may recognize and kill the DCs expressing both viral and specific tumour antigens. Therefore, a viral vector, which has a very limited or no viral protein expression will be the vector of choice. Regardless of these problems, viral vectors which are replicative defective like E1, E3-deleted, replication-deficient recombinant adenoviruses, have been used extensively in immunotherapy. Adenoviral vectors seem to provide a most efficient transfection. For retroviral vectors, a lesser transfection efficiency has been reported since they only infect dividing cells. There are reports on the use of other viral vectors like adeno-associated viruses, herpes simplex virus, influenza virus, fowl pox virus, lentivirus, avipoxvirus or vaccinia virus for transfection. Recombinant adenoviruses provide a less efficient immune response. The immunogenicity to adenoviruses has been a major obstacle for its application for long-term genetic modifications. However, for immunotherapy, short-lived genetic transduction could suffice for T cell priming. Adenoviral transfection involves creating AdV-poly-L-lysine (PLL)-DNA complexes. Our studies showed gene transfer efficiency up to 80 per cent (unpublished observations).

Nucleofection of DCs provides a non-viral method of introducing genes. DCs were transfected by nucleofection using the electroporation system nucleofector from Amaxa Biosystems GmbH (Cologne, Germany) under various conditions. This technique combines special electrical parameters and cell type specific solutions to deliver the DNA directly to the cell nucleus under mild conditions. An efficiency of 40 per cent expression was achieved using this technique. For a short-term T cell priming use of this technique would be useful.

**Clinical trials:** Results of phase I and II clinical studies proved that DCs could be pulsed with tumour lysate, and immune response against metastatic renal carcinoma could be achieved. Fifteen patients were treated with a median of 3.95x10^6 DCs administered and ultrasound-guided into a lymph node or into adjacent tissue. Seven patients remained with progressive disease, 7 showed stable disease (SD), and one patient displayed a partial response (PR). CD3+CD4+ and CD3+CD28+cells as well as the proliferation rate of peripheral blood lymphocytes (PBL) increased significantly in the blood of patients during therapy.

**In vitro** data and results of a clinical phase I/II trial using DC tumour fusions in patients with progressive metastatic renal cell carcinoma demonstrated an increase in cytotoxicity of peripheral blood lymphocytes against renal cell carcinoma cells during treatment. DC precursor cells were obtained from the peripheral blood mononuclear cells of healthy donors and were fused with either allogeneic (8 patients) or autologous (4 patients) renal tumour cells. In total, 12 patients with progressive metastatic renal cell carcinoma were treated with an average of 2.8x10^7 tumour cells fused with 1.8x10^7 DC each administered on days 0, 28, and 56 intradermally. Fusion efficacy for the tumour cells used was 14.3±7.8 per cent. Cell viability was 59.8±6.8 per cent after fusion and irradiation. No adverse effects were observed and
no difference in clinical outcome between the allogeneic and the autologous treatment was found. Eight patients remained in a progressive disease state and four in a stable disease state. The lack of adverse effects together with positive immunologic signs justifies further investigation of this novel therapeutic approach.


**Future prospects:** The unique ability of DCs to induce and sustain primary immune responses makes them optimal candidates for vaccination protocols in cancer. DCs definitely are the nature's adjuvant for immune resistance and play a key role in immunity. Vaccine design extends beyond the identification of antigens. Vaccines with DC may harness the immunological mechanisms that lead to a strong and lasting immunity. There is concern regarding development of unwanted immunity in cancer immunotherapy. Normal tissues expressing the tumour antigen may be harmed immunologically. Preventing immune escape, for example preventing immunosuppressive cytokines, and preventing recurrence of tumour lesions are important in successful immunotherapy. With new methods of immunomonitoring and improved protocols of cancer immunotherapy, dendritic cell therapy most likely, will have an edge over the other modes of therapy.

**References**


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