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Dendritic cell-based immunotherapy: A promising approach for treatment of cancer

Review Article

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Abbreviations: complete responses, (CRs); delayed-type hypersensitivity, (DTH); dendritic cells, (DC); keyhole limpet hemocyanin, (KLH); myeloid DC, (MDC); partial response, (PR); programmed cell death1, (PD1); tumor associated antigen, (TAA); tumor specific antigens, (TSA)

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Summary

The accumulating evidence in favor of tumor immunosurveillance indicates that immunotherapies may prove effective for the treatment of cancer. Many current approaches against cancer immunotherapy are often limited in their potential to induce effective anti-tumor immune responses. However, recent approach with dendritic cell based therapy proves to be an effective method for induction of anti-tumor immune response. In this review we discuss the effectiveness and complications associated with DC based immunotherapy and new strategies being perused for effective anti cancer response.

I. Introduction

Immunotherapy offers an attractive alternative and also a potential combination therapy to augment conventional chemotherapy and radiotherapy. It aims to exploit body's natural anti-tumor defenses by stimulating immunity and thus leading to tumor regression. Using the body's own protective mechanisms is attractive for a number of reasons, including low toxicity, a high degree of specificity, and the avoidance of cytotoxic drugs. Immunotherapy is generally thought of as conferring either passive or active immunity. Passive immunity involves direct injection of the host with – antibodies, cytotoxic T cells etc. without the involvement of host immune response. Antibody based approaches were the first form of passive immunotherapy to reach fruition as accepted cancer therapies. Monoclonal antibodies such as anti-HER2 (Herceptin) and anti-CD20 (Rituxan), represents some of them in therapeutics (Riethmuller et al, 1993; Weiner et al, 2000). However, there are considerable evidences that suggest that cancer patients have T cells that are capable of attacking tumor (Urban et al, 1992; Boon et al, 1994; Kawakami et al, 1997). This has led to the suggestion that isolating the tumor infiltrating T lymphocytes or whole T cells, activating them in vitro with IL-2, a potent T cell growth factor and reintroduce them into the patients. These approaches have met with some success, albeit short-lived. The expanding research in T cell biology given us broad view of understanding that the infused tumor infiltrating T cells are the mix of all $CD4^+$ and $CD8^+$T Subsets, including Tregs and Th2 cells. Reinfusing the expanded whole T cells together with these Tregs and Th2 may limit the anti-tumor function by their secreted tumor promoting factors. However, infusion of antibody or T cells without the involvement of host-immune system has a shorter half-life *in situ*, resulting in diminished anti-tumor immunity.

Other methods besides passive immunization, such as active immunity where host immune system is directly involved in inducing anti-tumor response have been proposed as ideal therapy for long term efficacy. Active immunity is an endogenous immune response, where the immune system is primed to recognize the antigen/tumor for induction of anti-tumor response. Such therapies offer a unique mechanism of tumor recognition based on the ability of the T cell to distinguish single amino acid differences in any mutated cell protein (tumor specific

antigens, TSA) or self antigens (tumor associated antigen, TAA). The self antigens may differ in density of antigen expression from any compartment of the cell (Urban et al, 1992). Many tumors induce immune tolerance, and the reason for induction of such tolerance is the inefficient presentation of tumor antigen(s) to the immune system. To induce an immune response to tumor antigens the T cells must receive instruction to recognize tumor antigen(s) on tumor cells. Effective antigen presentation requires HLA molecules, but also co-stimulatory molecules, cytokines and chemokines needed for priming naïve T cells. The unique combination of these membranes bound and secreted molecules are characteristic of APCs, of which dendritic cells are the potent one. Many factors appear to be responsible for the unique potency of DCs in activating T cells. These cells express 50-100 fold higher levels of MHC molecules than macrophages, providing more peptide/MHC ligand for T cell receptor engagement. Also, they express extremely high levels of important adhesion and costimulatory molecules critical for T cell activation (Banchereau and Steinman, 1998). Other DC specific genes, such as one encoding a T cell specific chemokine DC-CK1 (Adema et al, 1997), add to the list of features that give DCs their unique prowess in initiating T cell response and boost secondary immune response to foreign antigens. Because of these properties, much attention has been directed toward the use of DCs in vaccine strategies for the treatment of cancer.

A. Dendritic cells in immunity to tumors

Dendritic cells are professional antigen presenting cells and are the most powerful stimulators of naïve T cells (Banchereau et al, 2000; Liu et al, 2001). In the in vivo scenario of tumor bearing animals or cancer patients, the dendritic cells that have phagocytosed tumor cell debris process the material for MHC presentation, upregulate expression of costimulatory molecules and migrate to regional lymph nodes to stimulate tumor specific lymphocytes. This pathway produces $CD4^+$ and $CD8^+$ Tcells that react with the MHC restricted tumor peptides that are derived from mutated proteins, aberrantly expressed gene products and normal differentiated antigens that are produced by the tumor cells. $CD4^+$ T cells can also provide help for the production of antibody responses against tumor associated gene products (**Figure 1**). There is also evidence that infiltration of tumor with dendritic cells has been associated with a better prognosis in different types of malignancies (Hillenbrand et al, 1999; Poindexter et al, 2004; Sandal et al, 2005).

Collectively all these findings show that cancer bearing hosts can frequently mount anti-tumor immune response. However, subsequent progress and development of clinical grade tumors also indicate that the initial immune responses initiated by DC are not enough to preclude disease progression and tumor cells are capable

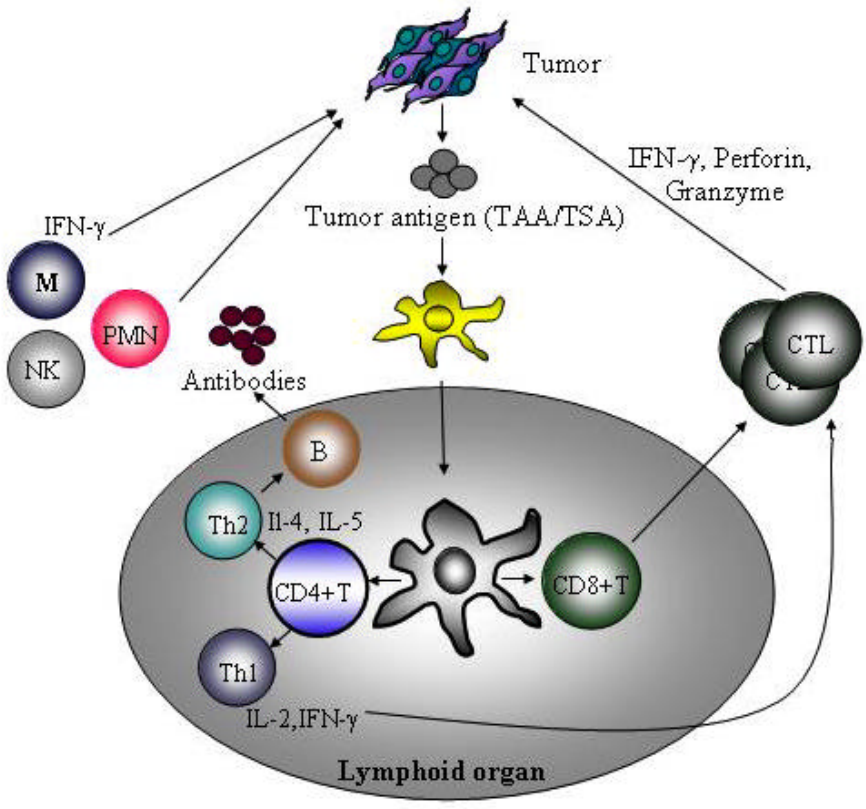

Figure 1. DC play a central role in the elicitation and maintenance of anti-tumor immune response. DC acquire, process and present tumor-associated or tumor-specific antigens and present the epitopes to both $CD4^+$ and $CD8^+$ T cells. The $CD8^+$ T cells exert IFN-γ-dependent and independent anti-tumor cytotoxic activity. The $CD4^+$ T cells help B cells to form antibody and also secrete inflammatory cytokines that cause inflammation into the tumor tissue.

of evading host immune-responses. Studies have indicated that tumors can evade immune responses by effecting DC biology at different stages of their development, maturation and function (**Figure 2**). Gabrilovich and colleagues, 1996 reported ineffective CTL induction in a murine mutant p53 fibrosarcoma model associated with defects in DC function. Supernatants from tumor cells suppressed DC maturation, ultimately attributed to an effect of VEGF (Gabrilovich et al, 1996). Inhibition of the differentiation of dendritic cells from $CD34^+$ progenitors by tumor cells: role of IL-6 and M-CSF (Menetrier-Caux et al, 1998). STAT-3 activation in tumor cells induces the elaboration of multiple factors that inhibit dendritic cell differentiation, one of which is VEGF (Gabrilovich et al, 1996; Niu et al, 2002). Metastatic melanoma secreted IL-10 that down regulates CD1on dendritic cell in tumor lesions (Gerlini et al, 2004). Increased level of IL-10 in serum from patients with hepatocellular carcinoma correlate with profound numerical deficiencies and immature phenotype of circulating DC subsets (Beckebaum et al, 2004). Patients with squamous cell carcinoma of the Head and Neck show alterations in the frequency of dendritic cell subsets in the peripheral circulation (Hoffman et al, 2002). Dendritic cell function is also suppressed by cyclooxygenase-2 from tumors (Sharma et al, 2003). Decreased antigen presentation by dendritic cells in patients with breast cancer have been also reported (Gabrilovich et al, 1997). Tumor infiltrating dendritic cells have been reported to be defective in antigen presentation inducible expression of B7 (Chaux et al, 1997).

B. Advantages of DC therapy

DC have been cultured *in vitro* for treating cancer patients. A key advantage of differentiating dendritic cells *in vitro* is that the precursor-DC are removed from immunosuppressive tumor environment. Next advantage of DC culture *in vitro* is that the high endocytic capacity of DC can be exploited for efficient loading with antigen of choice, such as protein, peptide, tumor lysate etc (Mayordomo et al, 1995; Holtl et al, 2002; Shibagaki et al, 2002). DC can also take up and express RNA (encoding tumor antigen) or with recent development in DNA transfer technology viral vectors can be reliably transfer transgene for intracellular expression (Boczkowski et al, 1996; Jenne et al, 2001). The advantage of loading DCs *in vitro* using these approaches is the ability to concentrate often limited supplies of antigens into DC. It has also been reported that DC can be activated matured with different immuno-stimulatory microbial adjuvants such as CpG, LPS, etc prior to *in vivo* delivery for effective induction of anti cancer immune response (Atkins et al, 2003; Okamoto et al, 2003; Pulendran, 2004).

C. Immunotherapeutic potential of dendritic cells

To date DC based therapy has produced promising results in both basic research and clinical trials. DC generated in vitro from bone marrow progenitor's stimulated allogenic T cell response. DCs pulsed with tumor lysate, tumor protein extracts, and synthetic peptide tumor epitopes or DCs fused with irradiated tumor cells could generate protective immunity to subsequent tumor challenge in animal models.

A number of DC cancer vaccine trials have been reported so far. Hsu and colleagues, 1996 reported the first DC vaccine trial for the treatment of cancer in patients with follicular B cell lymphomas. Using tumor specific

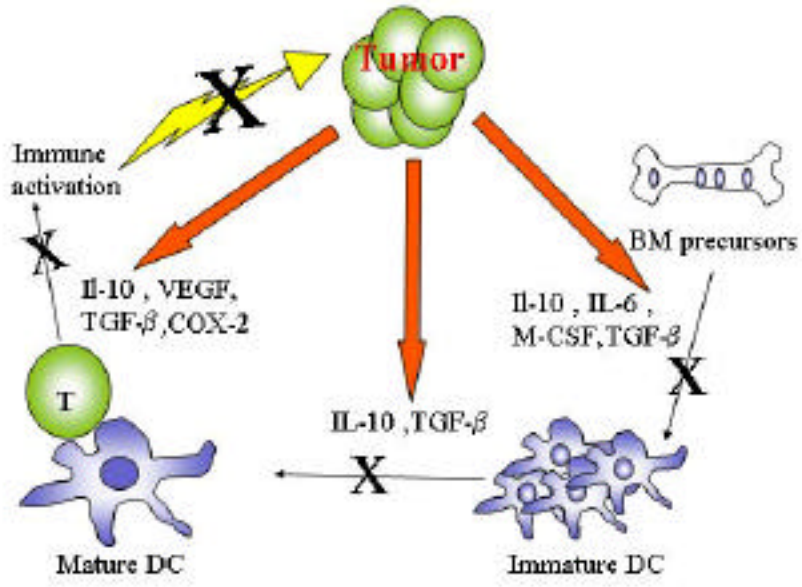

Figure 2. Tumors can evade the host immune response from dendritic cell mediated initial stage of immune recognition and activation by their secreted suppressive factors. To evade host immunity tumors use several strategies to hinder normal DC differentiation, maturation and function. For example, the tumor associated cytokines IL-6, M-CSF, IL-10, VEGF, TGF-β and COX-2 (Cyclooxygenase-2) inhibit DC differentiation, maturation and function, preventing activation of potentially protective anti-tumor immunity.

idiotype immunoglobulin pulsed DCs in patients with follicular lymphoma, Timmerman and colleagues, 2002, reported 2 long–lasting complete responses (CRs) and 1 partial response (PR) among 10 patients with measurable disease in the pilot phase of study. Next to lymphoma, clinical trial reports made a considerable success in patients with multiple myeloma. Clinical trials of peptide loaded DCs have been reported in patients with cancer, including melanoma, with encouraging immune response, and possible clinical responses detected. Patients with advanced breast and ovarian cancer have been treated with DCs loaded with peptide from HER-2/neu or MUC1 peptide specific IFN-γ producing CTL were detected in 5 of 10 patients. Holtl and colleagues, 2002 reported a trial of 35 patients with metastatic renal cell carcinoma who received monthly injections of autologous, mature monocyte derived DCs loaded with tumor lysates. Of 27 evaluable patients, 2 had objective CR, 1 had PR, and 7 had stable disease. Objective responses and and disease stabilization were long lasting, ranging from 6 months to 3 years. Yu and colleagues, 2001 reported first time a trial of 10 patients with malignant glioma who received three injections 2 weeks apart with autologous DCs pulsed with tumor lysates. Six of 10 patients demonstrated robust systemic cytotoxicity as demonstrated by IFN-γ expression by peripheral blood mononuclear cells in response to tumor lysate after vaccination. Using HLA-restricted tetramer staining, they identified a significant expansion in $CD8^+$ antigen-specific T-cell clones against one or more of tumor-associated antigens MAGE-1, gp100, and HER-2 after DC vaccination in four of nine patients. The median survival for patients with recurrent glioblastoma multiforme in this study (n = 8) was 133 weeks. In another study Heiser and colleagues, 2002 reported the efficacy of autologous dendritic cells transfected with RNA encoding prostate specific antigen stimulate CTL responses against metastatic prostate tumors. In 13 study subjects, escalating doses of PSA mRNA-transfected DCs were administered with no evidence of dose-limiting toxicity or adverse effects, including autoimmunity. Induction of PSA-specific T cell responses was consistently detected in all patients, suggesting in vivo bioactivity of the vaccine. Vaccination was further associated with a significant decrease in the log slope PSA in six of seven subjects; three patients that could be analyzed exhibited a transient molecular clearance of circulating tumor cells. Maier colleagues, 2003 reported the vaccination of patients with cutaneous T cell lymphoma by monocytes derived dendritic cells. The patients were treated with intranodal injection dendritic cells pulsed with tumor lysate protein and keyhole limpet hemocyanin (KLH). Tumor specific delayed-type hypersensitivity (DTH) reactions developed in 8 of 8 patients challenged with tumor-lysate pulsed DCs and in 3 of 8 patients challenged with tumor lysate alone. Three of 5 patients showed significant tumor-lysate specific increase of in vitro peripheral blood lymphocyte proliferation coinciding with increased interferon-alpha (IFN-α) production. Five of 10 (50%) patients had objective responses. Four patients had partial responses (PRs). One patient had a complete response (CR) for 19 months that is ongoing. The remaining 5 patients had progressive disease. In the 5 responder patients, 6.8 +/-1.4 vaccinations were necessary to induce an objective clinical response. Response was associated with low tumor burden. A peptide based DC vaccine was used by Svane and colleagues, 2004, who demonstrated how wild type p53 derived HLA-A2 binding peptides are able to activate human T cells in patients with advanced breast cancer. In this phase I pilot study, the toxicity and efficacy of autologous dendritic cells loaded with a cocktail of three wild-type and three modified p53 peptides are analyzed in six HLA-A2+ patients with advanced breast cancer. Vaccinations were well tolerated and no toxicity was observed. Disease stabilization was seen in two of six patients, one patient had a transient regression of a single lymph node and one had a mixed response. ELISpot analysis showed that the p53-peptide loaded DCs were able to induce specific T cell responses against modified and unmodified p53 peptides in three patients.

D. Promises and pitfalls

A central goal of immunotherapy is to activate tumor antigen specific Tcells. To enhance T cell responses to tumors, DCs have been investigated for their ability to prime $CD4^+$ and $CD8^+$T cells. Established techniques for growing DCs in culture *ex vivo* have allowed development of DC based vaccines. In light of promising preclinical results, clinical trials for many tumor types have been initiated using *ex vivo* generated DC vaccines. Although these trials showed overall that immune responses could be generated against tumor antigens, but limited success have been achieved by using these protocols (Ridgway, 2003). These results underscore the potentials for improvement of DC based immunotherapy for cancer prevention. Similarly, different improved vaccination strategies can be adopted for increasing efficiency of DC vaccination.

E. DC generation

Currently the major sources of human DC for immunotherapy are (1) blood derived DC obtained through a modified gradient method (Zhang et al, 2002). The use of DC directly from the peripheral blood is complicated by the low percentage of them in blood. The most frequently described method for obtaining DCs remain *ex vivo* generation from peripheral blood precursors such as (2) generation from $CD34^+$ progenitor cells using complex cytokine cocktails including SCF, IL-3, IL-6, GM-CSF, TNF-α and IL-4 (Palucka et al, 2003; Di Nicola et al, 2004; Paczesny et al; 2004). (3) Differentiating DCs from leukapheresis derived monocytes with GM-CSF and IL-4 (Thurner et al, 1999). All three types of DC preparation can stimulate antigen-specific T cell responses in human subjects and have been associated with clinical responses in cancer patients. No direct comparisons between different methods of DC generation and vaccination efficiency have been performed in clinical trials yet.

However, these methods of DC generation in vitro are time-consuming and laced with different regulatory concerns. Recently, to overcome these limitations of in vitro DC generation, attempts have been made to generate

DC in vivo by using various cytokines and their combination. Prominent among them are the use of FLT-3 ligand (Fong et al, 2000, 2001; Marroquin et al, 2002) GM-CSF and IL-4 (Roth et al, 2000), etc. Various animal model studies of in vivo DC generation and tumor immunotherapy has indicated that transient anti tumor response can be induced in such models (Chen et al, 1997; Lynch et al, 1997; Basak et al, 2002; Bjorck et al, 2002). Some of these studies are undergoing clinical trials in cancer patients for various diseases. These studies have opened up a new frontier in vivo DC mediated immunotherapy not only for cancer immunotherapy but also for various diseases. However, these studies need further evaluation for subset of DC induction by such method, strategies for effective in vivo antigen loading etc.

F. Choice of DC for immunotherapy

The different methods of DC generation result in different types of DC both *in vitro* as well as *in vivo* that differ in their markers and functions (Liu et al, 2001). Choosing the ideal DC for use in therapeutic purpose has been complicated by the diversity of DC and moreover, it will be critical to consider the function of distinct DC subsets, and induction of appropriate maturation and migration. If the antigen is loaded onto a different DC subset and/or fails to induce its maturation, the DC may not induce protective immunity, and possibly it may cause the induction of tolerance (Steinman et al, 2003). Humans DC subsets can be broadly subdivided into two distinct types of DC subsets that are identified *in vivo* on the basis of their ability for cytokine production, surface marker expression and induction of T cell response (Banchereau et al, 2000; Steinman, 2003). The subsets include the traditionally described myeloid–derived DC1 and the more recent described plasmacytoid-DC2 (**Figure 3**). Recently, considerable interest has been directed toward identifying the type of T cell response induced by these different DC subsets. The tolerogenic role of DCs could compromise vaccine efficacy. One mechanism contributing to immunologic unresponsiveness toward tumors may be presentation of tumor antigens by tolerogenic host DCs. Studies in mice and humans have shown that tolerogenic DC exerts its suppressive activity in many ways.In humans, a subset of monocyte derived DCs has been described that expresses indoleamine 2, 3 dioxygenase (IDO), inhibits T cell proliferation, and induces T cell death. IDO mediated suppressor activity was found in fully mature as well as immature DCs. Large number of IDO-DCs can be found in tumor draining lymph nodes, suggesting that they may be involved in immunologic unresponsiveness seen in cancer patients (Munnet al, 2002). DC STAT3 actively may be critical to the induction of antigen specific T cell tolerance. Stat3 is activated by tyrosine phosphorylation following DC exposure to IL-10 and other factors produced by tumor cells, and forced

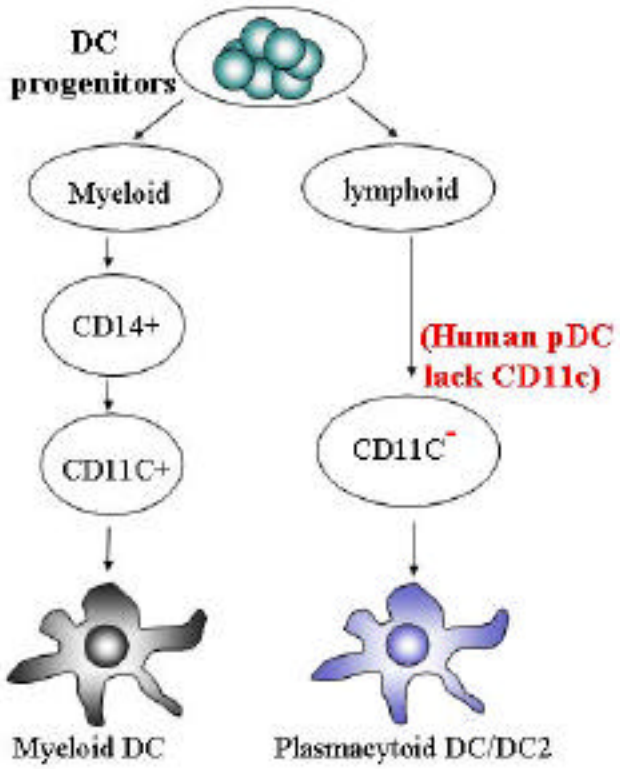

Figure 3. The family of Human DC displays considerable heterogeneity .DC may derive from two potential lineages: myeloid and lymphoid. Myeloid progenitors give rise to two main precursors, $CD14^-$ $D11C^+$ precursors and $CD14^+$ $CD11C^+$ precursors. $CD14^+$ $CD11C^+$ cells differentiate in the presence of GM-CSF and IL-4 into interstitial DC, which corresponds to dermal DCs *in vivo*. $CD14^-$ $CD11C^+$ precursors yield DC of Langerhans cell type in response to GM-CSF and IL-4. The second major subset of DC with a presumed lymphoid origin is $CD14^-$ $CD11C^+$ $IL\text{-}3R^+$ DC precursor called PDC2, plasmacytoid T cells. These cells depend on IL-3 as survival factor.

expression of activated Stat3 in DCs can result in impaired antigen specific T cell responses (Nefedova et al, 2004; 2005).

DC1 subsets polarize T-cells toward the Th1 functions and DC2 polarize DC toward Th2 functions. It has been also reported that DC1 induces the differentiation of naïve $CD8^+$ T cells into CTL whereas DC2 induces a population of $CD8^+$ T regulatory cells that are anergic, non-cytolytic and capable of inhibiting primary T cell responses through the production of IL-10 (Gilliet et al, 2002). DC2 are also responsible for IFN-α production when stimulated with pathogens and ligands for toll receptors (Colonna et al, 2004).

Thus, it may be more appropriate to choose the source of DC by the type of T cell response desired for anti-tumor responses, that is mostly Th1 type of immune response for effective cancer immunotherapy. There is a need to determine optimal conditions for expansion of DC that specifically promote anti-tumor T cell response and to devise methods for selectively removing undesirable DC subsets for effective cancer immunotherapy.

G. Approaches for antigen preparation and DC loading

The optimal strategy for tumor antigen delivery to DCs remains one of the important aspects that clearly deserves further exploration. Antigen can be delivered to DCs in the form of MHC restricted peptides, protein, tumor derived antigen mixtures or through transfection with genetic materials, each of which greatly influence the efficacy of T cell activation by dendritic cells (**Figure 4**). Ample evidences indicate that $CD4^+$T cells, particularly IFN-γ producing Th1 cells are another critical component of an effective anti tumor immune response as Th1 (1) help to initiate antigen specific $CD8^+$T cells by expressing CD40L and activating DCs via CD40 (Bennett et al, 1998). (2), that amplifies and sustain $CD8^+$ T cell function by secreting cytokines such as IL-2 (Hung et al, 1998). (3). Help in the formation and retaining memory $CD8^+$ T cells (Shedlock et al, 2003; Sun et al, 2003) Thus, a DC vaccine should incorporate antigens targeting both $CD4^+$nd $CD8^+$ T cells.

H. Peptides and proteins

Several approaches have been developed to arm DCs with tumor antigen for use in experimental animal model and clinical trials. The most widely used being incubation of DCs with MHC restricted peptides; which can directly bind to MHC molecules on cell surface. A broad array of tumor specific peptides presented by different HLA class I and class II molecules recognized by $CD8^+$ and $CD4^+$ T cells had been identified. These defined tumor peptides can be readily synthesized and used to load onto ex-vivo generated DCs. Vaccination with peptide pulsed DCs has been shown to induce both peptide specific $CD8^+$ and $CD4^+$ Tcells in healthy volunteers and even in advanced cancer patients (Mayordomo et al, 1995; Celluzzi et al, 1996; Schuler-Thurner et al, 2002). Although straightforward and technically easy, peptide based approach has some major limitations. The choice of peptides is restricted to the HLA typing of the patient, at least for HLA class I peptides, which are less promiscuous binders than HLA class II peptides. Vaccination with peptide pulsed DCs should only induce a T cell response directed against a limited number of tumor antigens, which may not be sufficient to effectively combat the tumor. In this scenario, the tumor might escape the immune response directed against a small array of peptides and emergence of antigen-loss tumor cell variants may occur. Using MHC I–restricted peptides ignores the role of MHC-II-restricted T helper cells in initiating and sustaining an immune response. DCs loaded with a mixture of peptides may induce responses only to immunodominant T cell epitopes,

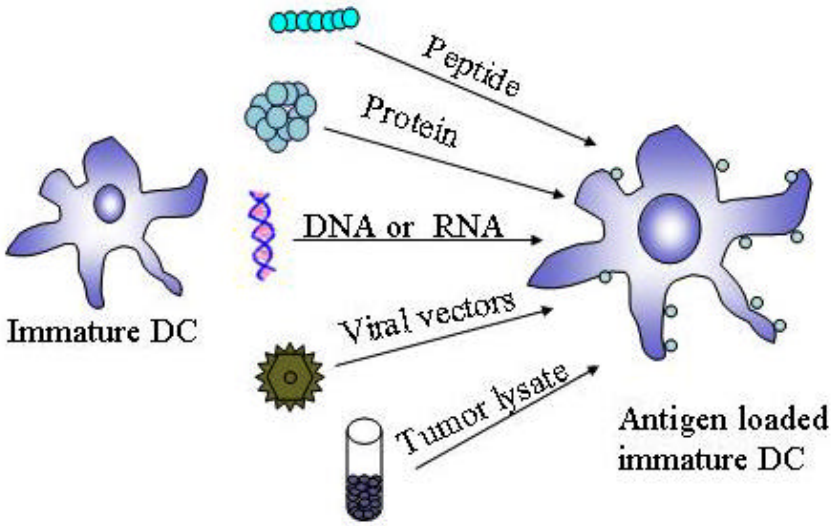

Figure 4. To date, several approaches have been used to load DCs with tumor antigens for use in clinical trials. DC may be loaded with peptide, recombinant protein or purified proteins, tumor lysates. It can also be transfected with RNA, plasmid vector encoding tumor antigens, or transduced with non-replicating recombinant viral vectors.

than compromising the ability to mount a broad T cell immune response that limit the risk of tumor strategies to elicit simultaneous $CD4^+$ and $CD8^+$ T cell response. Use of longer peptides provided that they contain both class1 and class II epitopes could be useful. Recent report by Millard and colleagues, 2003 suggested that DC KLH loading together with MHC I peptide induced a strong cytotoxic T lymphocyte response against the peptide. Such a concomitant presentation of KLH and peptide by the same DC strongly augmented the peptide specific CTL response, as compared to the response induced by DC pulsed with the peptide alone. The use of optimized peptide and KLH loaded DC may improve the efficacy of therapeutic anti-tumor peptide vaccination. Although DCs can be loaded with peptides, the half-life such peptide MHC complex is relatively short. Substitution of favorable key peptide residues enhances affinity of MHC-Peptides or stability of the T cell receptor of a T cell specific for MHC-Peptide complexes, and this enhancement has correlated with improved T cell responses and anti-tumor activity both in vivo and in vitro. In addition Wang and colleagues, 2002 demonstrated that TAT mediated delivery of T cell peptides into DC results in prolonged antigen presentation and enhanced T cell responses. These results suggest that TAT-mediated peptide delivery can enhance the efficacy of DC mediated cancer immunotherapy.

Protein may offer some advantages over peptide antigen since they may contain more than one antigenic epitopes, including MHC class II T- helper epitope, and they may avoid the need for MHC restriction. Under normal circumstances, addition of intact soluble proteins to DC would be expected to result in entry of the proteins into MHC II processing pathway, which allow for presentation of antigenic epitopes to $CD4^+$ T cells. Although DC may also present exogenous antigens on MHC I molecules, which can lead to the activation $CD8^+$T cells, this occurs inefficiently. To overcome this problem there are number of approaches are being developed, including transferring gene that result in antigen processing in the MHC1 pathway of DC to activate $CD8^+$T cells. Conjugating certain transporters peptides onto full-length proteins allow these to translocate across cell membranes and into the MHC class I pathway. Targeting protein antigens to Fc receptors on DCs using antibody complexes has been shown to activate both $CD4^+$ and $CD8^+$ T lymphocytes in vivo and *in vitro* (Regnault et al, 1999). Cross presentation can also be enhanced by targeting DC surface receptors such as DEC-205 (Mahnke et al, 2000). In addition the application of sterically stabilized liposomes encapsulated protein loading of DC offers a novel effective, safe vaccine approach if a combination of $CD4^+$ and $CD8^+$ T cell responses is desired (Ignatius et al, 2000). Several methods exist for production of proteins in large amount *in vitro* by cell culture techniques. However manufacturing of clinical grade proteins by GMP facilities are monitored by stringent regulatory procedures.

I. DNA and RNA

Loading DC with genetic material permits delivery of full-length antigens and has the advantage of easier manufacture than full-length protein. Although DC may be loaded with DNA, the efficiency of transfection is low and viral vectors are generally used to deliver DNA (Jenne et al, 2001). An alternative is to load DC with mRNA encoding tumor antigens or derived from tumor, either as naked genetic material or with liposomes or electroporation (Heiser et al, 2001; Muller et al, 2003; Nencioni et al, 2003). Although DCs can be loaded with mRNA, obtainable and amplifiable from small specimen of tumor, this may lead to autoimmune diseases.

J. Viral vectors

Several different types of viral vectors have been developed for delivering genes to DC. Recent strategies have focused on retroviruses, lentiviruses, and adenoviruses as the main viral vectors for antigen delivery to DC. Recent studies with retroviruses found that they can successfully transduce proliferating $CD34^+$ progenitors prior to differentiation to DC (Jenne et al, 2001). Lentiviral vectors represent a possible advance over retroviruses because they can transduce dividing and nondividing cells with the efficiency of 90% moreover those transuded DCs maintained their characteristic phenotype and allostimulatory capacity (Chinnasamy et al, 2000; Dyall et al, 2001; He et al, 2005). Adenoviral vectors have also shown to transfer genes to DC, and these now entering clinical trials due to greater and faster virus entry and to an increased transgene expression, especially following DC maturation with 100% potential, and no cytopathic effects on the infected DCs (Dietz et al, 1998). Pox virus vectors such as avipox and vaccinia are also suitable for transduction of DCs; however infection is followed by a significant decrease in viability of immature DCs, which undergoes apoptosis. Furthermore, infected immature DCs show a block in maturation, impairing their T cell stimulatory properties (Jenne et al, 2000). The major drawback in using virus infected DCs is the induction of antiviral cellular and humoral immune responses in patients, which may impair the desired induction of anti-tumor response and the destruction of subsequently administered DCs. In this regard modified virus lacking viral genome components have been developed. To achieve these goals “gutless” adenoviral vectors lacking viral genome has been developed that may facilitate lowering of anti viral immune response (Basak et al, 2004; Harui et al, 2004). To overcome similar problems of viral vector based antigen deliver to DC, further basic research involving viral vectors and DC interaction needs to be evaluated.

K. Tumor cell lysates

To optimize the anti-tumor effects of DC based immunotherapy it is tempting to allow the DCs to present the whole antigenic spectrum of a given tumor. Tumor cell lysates are good source of whole tumor antigens (Strome et al, 2002). These tumor lysates can be loaded on DC effectively for induction of an anti-tumor T cell response directed against a broad array of tumor antigens. Thus the probability of tumor escaping by loss of antigen(s) can be

reduced. The use of tumor lysate as antigenic source has several advantages, which include mimicking the physiologic processes by which a growing tumor induces an immune response *in vivo*. Tumor lysates circumvent the need for molecular characterization of the tumor antigen(s) for effective immunization. The approach of using tumor lysates pulsed onto DC would offer the potential advantage augmenting a broader T cell immune response to tumor–associated antigens that would not be obtained by pulsing DC with a single or perhaps several defined tumor peptides. Several concerns have been raised regarding this approach. First, it is often difficult to obtain sufficient quantities of autologous tumor material from patients. The use of allogenic tumor cell lines may present an alternative to overcome this problem and even amplify the immune response by activation of alloreactive T cells. Second, immunizing with DCs loaded with whole tumor cell preparations bears the potential risk of inducing autoimmunity against self antigens expressed on tumor.

L. DC-Tumor cell fusion

Another approach for delivering the full complement of tumor antigens to DC is to produce fusions of tumor and DC .The concept behind this approach is to use autologous tumor cells with DCs, thereby allowing for the co expression of all relevant tumor antigens and DC molecules within the same cell. Preclinical data has demonstrated that DC fused with tumor cells are potent inducers of tumor specific immune responses (Wang et al, 1998; Siders et al, 2003). A similar approach of fusing autologous tumor and allogenic dendritic cells has been used to vaccinate patients with advanced renal cell carcinoma, and this trial met with some success (Kugler et al, 2000, Kikuchi et al, 2001, 2004). DC may be fused with autologous, HLA matched, or unmatched tumor cells and appear to stimulate CTL activity in autologous T cells (Koido et al, 2001). One of the main limitations for the clinical use of an approach of this type, besides the need of primary tumor, is the efficiency with which fusions can be achieved between DCs and tumor cells in the absence of selection.

M. Maturation of antigen-loaded DC

The immunization of patients with antigen loaded immature DCs can result in tolerance or suppression of antigen specific response (Dhodapkar et al, 2001). This has led to the suggestion that DCs should be loaded with antigen in the presence of maturation signals or it can be transduced with genes that encode maturation signals. An important issue regarding ex vivo antigen loaded DC is the degree of maturation that is induced in vitro and its relevance to the homing and function of loaded DCs after re-injection. At present, the maturation protocols used for the DC therapy are quite variable and range from the use of monocyte conditioned medium to various defined agents, such as TNF-α, IL-1β, soluble CD40L and prostaglandins (Jonuleit et al, 1997; Reddy et al, 1997; Scandella et al, 2002). However, the processes leading to DC maturation, using PGE2 need further investigation. Because recent data suggest that PGE2 may be necessary to determine DC responsiveness to MIP3β, which attract them to the afferent lymph nodes from the injection site. This requirement apply to monocyte-derived DCs, whereas circulating CD1+DCs may not need this prostaglandin in order to migrate. In light of this evidence, addition of PGE2 to the culture medium before DC injection may help improve vaccination efficacy, especially when DCs are generated from monocytes. On the other hand PGE2 inhibits the secretion of IL-12 by DCs(Kalinski et al, 1998, Spisek et al, 2001), and induce regulatory T cells (Akasaki et al, 2004, Sharma et al, 2005, Baratelli et al, 2005) and is therefore likely to decrease the efficacy of Th1 priming in vivo. Hence so far, it is possible to construct arguments both for and against the inclusion of PGE2 in DC-based anticancer therapies on the basis of in vitro results, but extremely difficult to predict whether the presence of PGE2 during DC maturation will increase or decrease the efficacy of anti-tumor therapy in vivo. In addition, dendritic cells can be activated and matured by some danger signals such as Uric acid (Shi et al, 2003), Bradykinin (Aliberti et al, 2003) and heat shock proteins (Binder et al, 2000; Manjili et al, 2005). The important of using mature DC rather than immature DCs have a greater potential to migrate to the T cell areas of draining lymph nodes (De Vries et al, 2003). The sequence of antigen loading and maturation is also an important aspect of effective tumor antigen presentation (**Figure 5**). For example, if protein or messenger RNA is

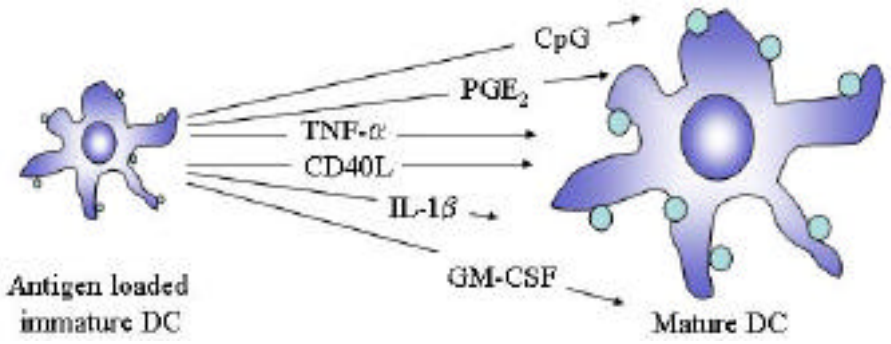

Figure 5. Maturation of DC in vitro. The current standard method of inducing DC maturation prior to injection is by adding cocktails of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and GM-CSF. In addition CD40L or TLR ligands such as CPG and LPS also can be used for inducing DC maturation.

used for loading DC, only the immature DC are good at antigen uptake and they should be matured after efficient loading. Contrary to this, if peptides are loaded, which requires no processing before antigen presentation by DC, the DC can be mature first and then load to optimize the number of MHC molecules on the surface. Life span of antigen bearing DCs in lymphoid organs/tissues may also be an important key for determining the outcome of protective T cell response, most likely by regulating the availability of antigen for these cells. Recent findings provide direct evidence that the survival genes such as Bcl2 and bcl-XL are required for the promotion of DC survival by TLR ligands and T cell costimulatory molecules (in particular CPG and CD40L) by activating NF- κB family proteins (Hon et al, 2004; Hou et al, 2004). Thus choosing a maturation signal, which can induce both maturation and increased life span of DCs may lead to effective T call response against tumor antigen.

N. Dose, frequency and route of DC administration

One of the most important limiting factors for the effective use of DC based vaccines is the ability of the injected DCs to reach secondary lymphoid organs to elicit T cell responses. Different studies have used different routes of delivery for immunotherapy. Intravenous, intra-dermal, subcutaneous, intra-nodal, and intra-tumoral injections of DCs have been evaluated. Studies in humans indicate that intravenous injected DCs may preferentially localize to the lungs and afterwards, to spleen and liver (Mackensen et al, 1999). Conversely intra-dermal injection may result in DC migration to the afferent lymph nodes. A comparative study by Fong and colleagues, 2001 suggests that Th1 immune response are more likely induced by intra-dermal injection than by other delivery methods. However, significant immune responses also have been noticed in studies that made use of subcutaneous and intravenous injections (Smith et al, 1999). Route of administration may also directly affect the nature of T cell priming. Skin injections may be required to induce immunity to cutaneous tumors, whereas intravenous injections may be less effective at Th1 induction but more effective at induction of humoral immunity. Injection into lymph nodes or lymphatics has also been attempted (Maier et al, 2003), to increase DC homing to lymphatics because only 5% or fewer DCs may migrate to draining nodes following subcutaneous injection. However, this mode of delivery often necessitates an ultrasonographic visualization of the lymph nodes to deliver the injection, and may lead to the damage of the lymph node. Direct injection of DC into tumors has also been investigated (Triozzi et al, 2000; Mazzolini et al, 2005). The number of injected DCs into tumors may be equally important for induction of anti tumor response. High DC:T cell ratios polarize helper responses toward Th1 type in vitro and give rise to higher affinity T cells (Gett et al, 2003). In particular when DCs are pulsed with different peptides and injected separately into the skin, the number of DCs finally reaching the draining lymph node may simply to be too low to effectively induce T cell response. However in previous studies the number of injected DCs varied from 4 to 40 million cells per vaccination without striking differences being observed. The schedule and time duration of DC vaccination must be determined, as frequent T cell stimulation may lead to activation induced cell death, whereas activated cytotoxic T lymphocytes can kill antigen loaded dendritic cells that may diminish immune response (Ronchese et al, 2001). In fact, it is still unclear whether the anti- tumor immunity elicited by vaccination would last forever, in the absence of subsequent injections. These questions must be taken into account in the planning phase of DC based vaccination trials.

O. Incorporating combinatorial strategies with DC therapy

Although a number of the newer generation vaccines can effectively transfer antigen to and activate dendritic cells *in vivo*, T cell tolerance remains a major barrier that is difficult to overcome by therapeutic vaccinations. Preclinical models demonstrated that for poorly immunogenic tumors, therapeutic vaccine alone are ineffective at curing animals with a significant tumor burden, particularly once tolerance has been established. Combination of cancer vaccines administered in conjunction with inhibitors of immunologic checkpoints and agonists for Toll like receptors or T cell costimulatory pathway can overcome tolerance and generate significant anti-tumor immune responses even in cases of metastatic cancer. One of the most promising examples is the blockade of CTLA-4 inhibitory pathway (Leach et al 1996). CTLA-4 binds to B7 at 10 fold higher affinity than does CD28 (Von Boehmer et al, 2005). Occupancy of CTLA-4 appears to directly counter the effect of CD28 on T cell activation and lymphokines induction (Lee et al, 1998). Blockade of CTLA-4 has been shown to improve tumor immunosurveillence and amplify the effects of cancer vaccines in animals and recent clinical trial in melanomas (Hodi et al, 2003). However in vivo CTLA-4 blockade predictably had effects beyond the antitumor response causing significant autoimmunity (Phan et al, 2003). Although the vaccine and CTLA-4 combination approach induced autoimmune disease, the autoimmunity was confined to the tissue from which the tumor vaccine was derived (Van Elsas et al, 1999). Thus, the treatment of mice with B16 melanoma-GMCSF vaccine plus anti CTLA-4 antibody resulted exclusively in vitiligo–patchy de-pigmentation due to an auto immune response restricted to melanocytes, but no other signs of autoimmunity. These findings show that there is a hierarchy of tolerance induction, in which tolerance to tissue–specific antigens might be maintained by less stringently than tolerance to more–ubiquitous self-antigens. Hsu and colleagues, 2002, have shown that CTLA-4 blockade maximizes anti tumor T cell activation by dendritic cells by presenting idiotype protein. These studies suggest that safe and effective disruption of checkpoint signals could yield substantial therapeutic benefit. The dissection of signaling pathways in T cells has revealed several additional potential targets for inhibitors of immunological checkpoints. The membrane molecule programmed cell death1 (PD1), expression of

which is induced after T cell activation, is a CTLA-4 like inhibitory molecule that decreases cytokine responses in T cells and might enhance their activation induced cell death (Zha et al, 2003). PD1 is a receptor for two of the newer B7 family members, B7-H1/PDL1 and B7-DC/PDL2 can co-stimulate enhanced cytokine production by naïve T cells, it is probable that PD1 is a counter–regulatory inhibitory receptor paired with an as yet unidentified costimulatory receptor on naïve T cells (Greenwald et al, 2004). Dong and colleagues, 2002 reported that the B7-H1 is expressed in many human cancers and promotes apoptotic death of activated tumor antigen specific T cells. Another study by Curiel and colleagues, 2003 suggest that B7-H1 expression is up regulated on myeloid DC (MDC) from tumor bearing patients, blockade of B7-H1 enhanced MDC mediated T cell activation and was accompanied by down regulation of T cell interleukin (IL)-I0 and up regulation of IL-2 and IFN-γ.

Regulatory T cells suppress T cell responses in both Antigen-specific and non-specific manner, in part through membrane bound TGF-β and IL-10 secretion and provide another mechanism for compromising the development of effective tumor immune response (Berencsi et al, 2002; Nishikama et al, 2005). Such cells are induced by antigens, especially in the absence of inflammatory signals, particularly in the presence of TGF-β and have been detected in increased frequency in some cancer patients (Ormandy et al, 2005). Thus depletion of Treg *in vivo* leads to effective anti tumor T cell response in murine models resulting in effective anti-tumor T cell responses (Shimizu et al, 1999). However activated effector CD8 and CD4 Tcells also express CD25, depletion of these cells during acute phase of the anti-tumor T cell response may severely limit the application of this approach. Thus defining alternative molecules that permit selective targeting of Treg cells for depletion, such as GITR, should uncover greater anti tumor activity. In a ground breaking study by Peng and colleagues, 2005, suggested that activation of TLR signaling using ligand TLR8 can reverse the Treg cell function. This effect was independent of dendritic cells but required functional TLR8-MyD88-IRAK4 signaling in Treg cells. Adoptive transfer of TLR8 ligand stimulated Treg cells into tumor bearing mice enhanced anti-tumor immunity. These results suggest that TLR8 signaling could play a critical role in controlling immune responses to cancer. Although the development of immune based therapies for various cancers heralded with much hope and optimism objective clinical improvements in most vaccinated cancer patients have not been realized. To broaden the search for vaccine induced benefits, couples of investigators are being involved in studying the synergy of vaccines with conventional chemotherapy (Emens et al, 2005; Lake and Robinson, 2005). The approach of using combined chemotherapy and immunotherapy shown to induce better immunity resulted in complete eradication of tumors in mouse models. In a recent study by Wheeler and colleagues, 2005 examined the synergy of vaccines with conventional chemotherapy in patients with glioblastoma. Vaccinated patients receiving subsequent chemotherapy exhibited significantly longer times to tumor recurrence after chemotherapy relative to their own previous recurrence times, as well as significantly longer postchemotherapy recurrence times and survival to patients receiving isolated vaccination or chemotherapy. These data have significant implications for the development of new protocols combining chemotherapy with immunotherapy, indicating an exciting potential for therapeutic synergy with general applicability to many cancers.

II. Conclusion

Variables associated with employing dendritic cell vaccines for tumor immunotherapy are numerous (**Figure 6**). To achieve effective anti-cancer immune response, we

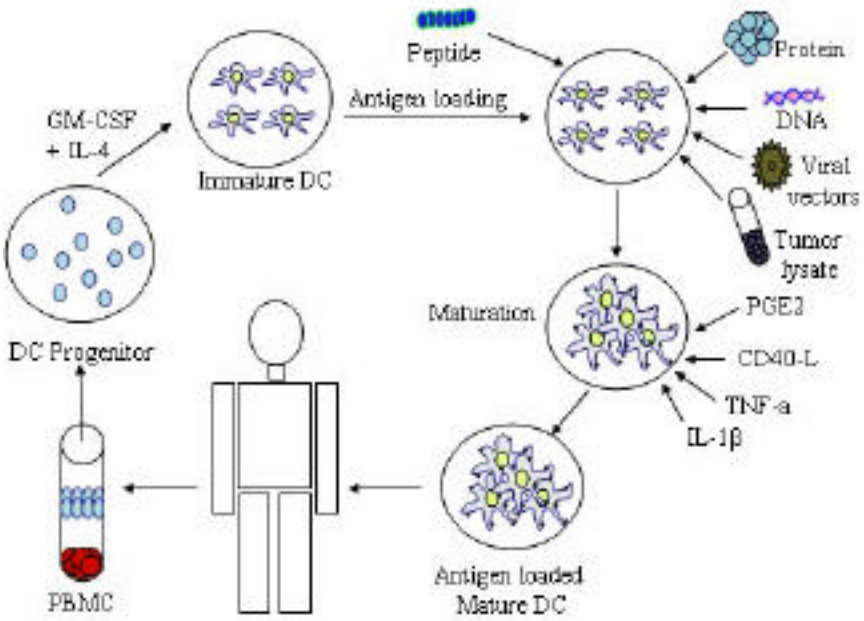

Figure 6. Summary of the DC-based anti-cancer therapy. DCs can be generated from the PBMC progenitors using GM-CSF and IL-4. The resultant immature DCs can be used for loading with tumor antigens, which are then matured with suitable maturation signal and then re-infused back into the patient.

must consider the use of the best lineage of dendritic cell for antigen delivery. These DC once identified, the next question is should we use DC directly isolated from the peripheral blood or generate them *ex vivo* from precursors or even better we should induce then in vivo? Next, how do we load the antigen? Maturation and/or activation are the other factors to consider, as data suggest immature dendritic cells may give a diminished immune response. Furthermore, the route of DC vaccine administration is always a question with tumor vaccines, as there are advantages and disadvantages to all of the available routes. Perhaps most importantly, we need to understand how best to evaluate the immune and clinical response to dendritic cell vaccines to permit efficient development of this strategy for effective immunotherapy against cancer.

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