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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 phagocyted 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+ T cells that react with the MHC restricted tumor peptides that are derived from mutated proteins, abnormally 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.](image-url)
Studies have indicated that tumors can evade immune responses by effecting DC biology at different stages of their development, maturation and function. 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 CD10 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](image)

**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 T-cells. 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 faced 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+ D11C+ precursors. CD14+ D11C+ 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-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+and 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+ T cells 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](image-url). 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 class I 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 MHC I 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 (Mahanke 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 transduced 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
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](image-url)
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 cell 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 immunosurveillance 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 autoimmune immunity 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 idotype 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)-10 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 chemotheraphy (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 chemotheraphy in patients with glioblastoma. Vaccinated patients receiving subsequent chemotheraphy exhibited significantly longer times to tumor recurrence after chemotheraphy relative to their own previous recurrence times, as well as significantly longer postchemotheraphy recurrence times and survival to patients receiving isolated vaccination or chemotheraphy. These data have significant implications for the development of new protocols combining chemotheraphy with immunotheraphy, indicating an exciting potential for therapeutic synergy with general applicability to many cancers.

II. Conclusion

Variables associated with employing dendritic cell vaccines for tumor immunotheraphy 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 \textit{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.

References


Igna university et al: Dendritic cell-based immunotherapy


Reversal of Tumor Induced Dendritic Cell Paralysis: A Treatment Regimen Against Cancer

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Abstract: Dendritic cells (DC) are the most potent antigens presenting cells with the capacity to stimulate naive T cells and induce primary and secondary immune responses. Due to these features DC have been exploited for vaccine delivery in an attempt to actively immunize cancer patients, but the vaccine-induced immune responses have achieved partial success but are not yet sufficient to attain robust and durable therapeutic effect in cancer patients. The partial failure of current vaccine formulations are explained by the extraordinary complexity of the immune response, which makes the task of exploiting the potential of such a therapeutic approach highly challenging. Overall findings obtained from the clinical observations in human suggest that immune system can be polarized against tumor cells by means of DC mediated vaccine. It is clear that there are complex interactions between tumor cells and DC, through their inhibitory effect on DC tumor cells may negatively regulate priming tumor specific immunity. The complete eradication of tumor is possible only when we gain the thorough understanding of DC biology and its interaction with tumor before we design any vaccine formulations using dendritic cells. This review will summarize the recent advances in understanding the role of DC in the regulation of innate and adaptive anti-tumor immunity, and tumor-induced DC paralysis as a major mechanism by which tumors escape host immune response. Knowledge on these aspects will provide important implications for developing more effective DC mediated vaccination against tumor.

Keywords: Dendritic cells, tumor immune evasion, vascular endothelial growth factor, prostanoids, transforming growth factor-β and Interleukin-10.

INTRODUCTION

Paralysis is a state of function of a system, conventionally referred to neuronal system, whereby the responsiveness to stimuli measured in terms of both receiving the signal and coupling it to the effector functions is impaired. Similarly, the dendritic cells, the most potent antigen presenting cell that elicits the T cell responses against a variety of antigen can also be paralyzed under certain conditions such as tumor metastasis and infectious diseases. Tumors exert systemic impact on all major branches of immune system through various mechanisms. As DC is central to the initiation of immunity, one of those mechanisms is paralyzing the DC unit by altering DC differentiation, maturation and function. Here, we describe the versatility of DC function in both innate and adaptive immune systems and how tumors limit DC functions to evade immune attack.

DENDRITIC CELL AS A LINK BETWEEN INNATE AND ADAPTIVE IMMUNITY AGAINST TUMOR

DC in innate immunity to tumor: Strong evidence has been accumulated demonstrating that tumor cells in humans and animals are recognized in general as non-self by the immune system [1-3]. An anti-tumor immune response is initiated when the cells of the innate immune system become alerted to the presence of a growing tumor, at least in part owing to the local tissue disruption that occurs as a result of thestromal remodeling process integral to the basic physiology of solid tumor development [4,5]. The stromal remodeling could produce inflammatory molecules that, together with chemokines may be produced by the tumor cells themselves, summon the cells of the innate immune system to this new source of local danger. The innate response includes soluble factors and several cellular factors, including natural killer (NK) cells, natural killer T (NKT) cells, γδT cells, macrophages, and dendritic cells. The components of innate immunity use pattern recognition receptors and other cell surface molecules to directly detect tumor cells. Cancer cells frequently express families of stress-related genes, such as MICA and MICB, which function as ligands for NKG2D receptors that are expressed by NK cells and other cytotoxic lymphocytes [6,7]. NK cells also monitor for the loss of MHC class I molecules from the surface of tumor cells [8]. It was thought that NK cells, NKT cells, and γδT cells and some soluble factors are the major components of innate effectors playing a role in innate immunity against tumor. Several lines of evidence from recent years suggest that DC have crucial role in regulating these innate effectors against tumor immunity. Here are some evidences to support the notion. The initial study by Fernandez et al., revealed that the adoptive transfer of DC-or fms-like tyrosine kinase 3-ligand (FLT3-L) expansion of DC in mice bearing MHC class-I negative tumors promote NK cell dependent anti-tumor effects [9]. In vitro studies demonstrated that the co-culture of DC and resting NK cells results in a substantial increase in both NK cell cytolytic activity and IFN-γ production in an IL-12 and type I IFN-independent manner [9]. Subsequent in vitro investigations identified that NK cell activation induced by DC requires both cytokine signals and contact between NK cells and DC. Various types of DC,
depending on their activation state, are able to produce cytokines, such as IL-12, IL-15, IL-18, and type I IFN, all demonstrated to act on NK cells [10-12]. NK cells can be triggered for cytolytic activity by both murine and human DC depending on direct cell contact through their expression of cell surface molecules such as CD48, and CD70, which are ligands for the NK cell activating receptors 2B4 and CD27, respectively, or by DC-derived cytokines[12-15]. The co-stimulatory molecules CD80, and CD86, expressed on DC were shown to trigger cytolsis by murine and human NK cells [16-18]. In addition, activation of NK cells by DC is potentially important for the promotion of tumor regression. For example, it was shown that anti-CD40 antibody therapy induces substantial NK cell-mediated anti-tumor and anti-metastatic effects. As CD40 is not expressed by NK cells or by tumor cells used in their study, it was suggested that NK cell activation is mediated by increased cytokine production upon CD40 ligation on DC [19]. It was found that DC/NK cell interaction was bi-directional and complex, as it could result not only in NK cell activation, but also in DC maturation [20,21]. This is evident from another study, where culture of activated human NK cells with immature monocyte derived DC, at low DC/NK ratio (1:5), led to an increase in DC cytokine production [22]. In this context, soluble factors such as TNF-α and IFN-γ, as well as cell-cell contacts are required for NK cell mediated DC activation.

NKT cells represent a cellular population of the innate immune compartment that were shown to protect the host from tumor formation [23]. A role for NKT cells in innate immunity against tumor was implicated when Jα28-/- mice, which lack a large population of invariant NKT cells was found to develop MCA induced sarcoma at higher incidence than wild type counterparts [24]. DC mediated NKT cell activation has been shown in an array of recent studies, which supports a role for both cytokine and cell-cell contact in the enhancement of NKT cell mediated anti-tumor function. For example, tumor antigen-pulsed DC efficiently suppressed the growth of hepatocellular carcinoma in mice; the effect was mediated by enhanced NK cell function [25]. The anti-tumor functions of NK cell ligand alpha-galactosylceramide demonstrate its immuno-potentiating anti-tumor activities in vivo that resemble IL-12 mediated anti-tumor activities. Production of IFN-γ by NKT cells in response to alpha-galactosylceramide required IL-12 production by DC and direct contact between NKT cells through CD40/CD40L interaction [26]. The above findings indicate an important role for DC produced IL-12 in the activation of NKT cells. However, the IFN-γ produced by the NK cells may be able to condition DC for subsequent immune responses, as alpha-galactosylceramide administration in mice activated NKT cells inducing a strong NK activity and cytokine production by CD1-restricted mechanisms. Surprisingly, the alpha-galactosylceramide induced the activation of immunoregulatory cells involved in acquired immunity such as CD69 over-expressing CD4+ T cells and CD8+ T cells, indicating an essential role for the interactions between NKT cells and CD1d expressing DC in the activation of acquired immunity [27]. In addition, soluble antigen presentation by DC to NKT cells induces the differentiation of antigen specific cytotoxic T lymphocytes [28,29]. By activating NK and NKT cells DC might enhance adaptive immunity to tumors in part, the killing of tumor target by NK and NKT cells could provide DC with increased access to tumor antigens. In summary, the cross talk between DC and other innate effectors now regarded as a main stage in the initiation of innate and adaptive immunity to tumors.

**DC as innate effectors against tumor:** Since DC can produce TNF-α, express membrane FasL and a high level of inducible nitric oxide synthase, the effector functions in innate immune systems [30-33], these cells may contribute to the innate defense against malignancies. DC can infiltrate solid tumors, but their T cell stimulatory ability is often suppressed [34,35]. However, the density of DC infiltration may be associated with enhanced patient survival in certain tumors [36,37]. In addition several studies in mouse tumor models indicate that fusion of DC without loading specific antigens can also decrease growth of tumors to a certain degree [38-40]. These observations lead to investigations into direct inhibition of tumor growth by DC. Here are some evidences to support the tumoricidal activity of DC without the participation of other innate effectors. Human PBMC-derived DC were shown to inhibit the growth of a wide spectrum of tumor cell lines of different tissue origin in a cell-cell contact dependent manner [41]. The addition of LPS pretreatment induced DC to secrete soluble factors that could inhibit tumor growth. In contrast to the cytostatic activity in the previous study, peripheral blood DC were shown to lyse a number of breast cancer lines in vitro, and this activity could be neutralized by the addition of anti-TNF-α antibodies [42]. DC mediated cytotoxicity was augmented by the addition of maturation or activation factors such as IFN-α, IL-15 and LPS. Peripheral blood myeloid DC was also shown to induce apoptosis in lymphoma, ovarian, prostate and melanoma cell lines [43,44]. Significantly, plasmacytoid DC did not share this property. This cytolytic activity was found to be mediated by the DC expressed TRAIL (TNF related apoptosis inducing ligand) [45]. In addition, it was observed that activated human umbilical cord blood DC can serve as cytotoxic cells against hematological tumor cells without damaging the normal hematopoietic progenitor cells [46]. Another study by Yang et al., demonstrated that the immature DC could exert a significant cytotoxicity towards autologous and allogeneic ovarian tumor cells. This cytotoxicity was independent of Ca2+ and could be inhibited by anti-FasL monoclonal antibody, indicating the involvement of Fas/FasL pathway in the cytotoxic mechanism [47]. Finally, a recent report described a new subset of CD11C+ myeloid DC that express high levels of FCγRII and FCγRIII, could efficiently lyse HER-2 neu expressing breast and colon cancer cells in the presence of herceptin, an antibody directed against this tumor associated antigen. This antibody dependent cell mediated cytotoxicity (ADCC) could be inhibited by blocking antibody against FC receptors and antibody against TNF-α [48]. Taken together, these results suggest that in addition to their predominant role as immune regulatory cells, DC could serve as innate effector cells in tumor immunity.

**DC in adaptive immunity to tumor:** It is well established that the immune system recognizes tumor associated antigen (TAA) and tumor specific antigens (TSA). It has been proven that effector mechanisms of the adaptive immune system are able to eliminate tumor cells in vitro and in vivo, however require activation by antigen presenting cells, and it is the DC, which is pivotal for this process. The DC that
Reversal of Tumor Induced Dendritic Cell Paralysis

Dendritic cells play a central role in the regulation of innate and adaptive immunity to tumors. They have been recruited to the tumor site become activated either by exposure to the cytokine milieu created during the ongoing attack on the tumor by innate immunity or by interacting with tumor infiltrating NK cells. The activated DC can acquire tumor antigens directly by ingestion of tumor cell debris or potentially through indirect mechanisms involving transfer of tumor cell derived heat shock protein/tumor antigen complexes. Activated antigen bearing mature DC then migrate to the draining lymphnode, where they may receive the antigens from the B cells (49) and present peptides to naïve T cells, thereby inducing cellular immune response that involves both CD4+ T helper (Th1) cells and cytolytic CD8+ T cells. DC are famous for their T cell stimulatory properties, are now known to have major effects on B cell growth and immunoglobulin secretion. DC can directly activate both naïve and memory B cells (50-52). Dendritic cell type-2 (DC2) secretes IL-6, a cytokine known to promote plasma cell differentiation (53). IL-12 produced by activated DC, supports the differentiation of CD40 activated B cells to plasma cells (54,55). These results suggest the DC mediated direct activation of naïve B cells during the initiation of the immune response and the involvement of DC in the development of humoral immune response (Fig. 1). Collectively, these findings indicate that DC offers a great potential to successfully fight cancer. However, the development of clinically evident tumors indicates that these innate and adaptive responses are not always sufficient to preclude disease progression, as tumor cells manage to escape immune recognition and elimination. Therefore, to rescue such a potential we need to have a better knowledge of mechanism by which tumor cells can avoid or inhibit the immune reaction in order to tip the balance in favor of tumor bearing hosts.

TUMOR IMMUNE EVASION

Tumor evasion strategies can either be pre-existing or arise through outgrowth of escape mutants, as shown in Fig. 2. There are a number of mechanisms by which tumor may actively escape/suppress immune responses. Since, both the innate and adaptive components of the immune system function in cancer immunosurveillance network, tumors most likely would have to circumvent both arms of immunity in order to achieve progressive tumor growth. Some of these mechanisms target immune anti-tumor effector cells. First of all, the tumor escape can result from changes that occur directly at the level of the tumor. For example, loss of antigen expression, loss of MHC components and shedding NKG2D ligands represents some of the mechanism by which a tumor may go unnoticed by the immune effectors cells such as NK cells and Cytotoxic T lymphocytes (56-60). During the progressive growth phase, tumors may become more immune activating for a variety of reasons. Tumors can damage surrounding tissue or trigger a stress response causing upregulation of stress factors, such as heat shock proteins and death by necrosis or apoptosis (61-63). In addition, as tumors grow progressively, dysregulated genetic–epigenetic events lead to the expression of large number of neo-antigens (64). Collect-
tively, all these events make tumors more accessible to the activated innate and adaptive effectors. In this occasion tumor cells have additional mechanisms to escape from these immune effectors, the process employs a simple deletion of those effectors. The limited number of T cells and NK cells detectable in tumor site suggests that mechanisms acting in down modulating anti-tumor immune response are likely to be at work in tumor. Mechanisms that have been implicated in the depletion of tumor reactive T cells include secretion of the immunosuppressive cytokines IL-10 and TGF-β and the expression of apoptosis inducing Fas ligand, resulting in apoptosis of tumor reactive T cells [65-70]. In addition, it has been recently demonstrated that expression by tumor cells of human B7-H1, a member of the B7 family of costimulatory molecules, leads to induction of T cell apoptosis [71]. Another recently described receptor, RCAS1, which is expressed by tumors has been shown to induce apoptosis in T and NK cells [72]. Additionally, the exploitation of IDO expression, an important enzyme in pregnancy-related immunosuppression by cancer cells known to induce apoptosis of tumor reactive T cells [73,74]. Other mechanism of down regulation of tumor reactive effector cells responses against tumors might be the presence of regulatory T cells within the tumor [75-77]. It appears to be an additional mechanism to suppress immune response when the tumors can hide/avoid notice by immune effectors. Thus, tumor cells may stop the anti-tumor immune responses in one particular step, which may be the initial stage of immune recognition and activation by dendritic cells. Given the central role of DC in regulating the innate and adaptive immunity to tumors, tumors have developed strategies to successfully evade DC-mediated initial stage of immune recognition and activation by interfering with all the three phases, namely, generation from hematopoietic progenitors, maturation and their effector functions.

TUMOR-INDUCED DENDRITIC CELL PARALYSIS

Tumor induced suppression of DC can be mediated by tumor cells directly, through the secretion of soluble immunosuppressive factors or indirectly, through the induction of host suppressor cells by the tumor. This review focuses on the effect of tumor secreted soluble factors such as VEGF, TGF-β, IL-10 and others in various stages of dendritic cell life cycle, as shown in Fig. 3.

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**Fig. (2).** Overview of Tumor immune evasion.
VEGF

Vascular endothelial growth factor (VEGF) is an angiogenic cytokine that plays an important role in tumor growth and progression [78]. An extensive literature exists linking VEGF with cancer. In humans VEGF mRNA or protein expression has been identified in most of the tumors including primary gliomas, colon cancer, pulmonary adenocarcinoma, renal cell carcinoma, non-small cell lung carcinoma (NSCLC), breast cancer, and ovarian cancer [79-82]. Survival of patients with VEGF positive tumors was significantly less than patients with VEGF negative tumors. For example, in one study of NSCLC, patients with low VEGF levels had a median survival time of 151 months, whereas those with high VEGF expression had a mean survival time of only 8 months [83]. Like wise, increased levels of VEGF in the plasma of patients with cancer have been shown to correlate with an adverse prognosis [84-86]. Recent evidence suggests an alternate, albeit indirect, role of VEGF on host immune response to tumors [87]. VEGF appears to diminish host immunity by altering the function of major antigen-presenting cells such as dendritic cells. It was the first tumor derived factor shown to inhibit DC differentiation. Expanding research in tumor immunology provided us enough evidence that VEGF not only affect DC differentiation but also can inhibit DC maturation and its function. The involvement of VEGF in tumor induced defects of DC differentiation was indicated by in vitro experiments in which neutralizing VEGF specific antibody abrogated the negative effect of tumor cell conditioned medium on the differentiation of DC from hemopoietic progenitors [88]. The findings were then confirmed by further studies, in which the in vivo infusion of recombinant VEGF resulted in a dramatic inhibition of DC development, associated with an increase in the production of B cells and immature Gr-1+ myeloid cells [89,90]. Recent study demonstrated the dual role of VEGF in the pathogenesis of human head and neck cancer linking angiogenesis and immune tolerance; Multivariate analysis demonstrated that VEGF correlated with microvessel density, disease progression, and reduced number of local and peripheral mature DC [91]. The ability of mature DC to stimulate allogeneic T cells and produce IL-12 was suppressed by the addition of VEGF in a dose dependent manner and a lesser expression of costimulatory molecules (CD80 and CD86) was seen in DC treated with VEGF. Consistent with this, DC incubated with tumor supernatant differentiated into immature DC and did not develop full allostimulatory activity [92]. It has been shown that VEGFR1 is the primary mediator of the VEGF inhibition of DC maturation, where as VEGFR2 tyrosine kinase signaling is essential for early hemopoietic differentiation, but only marginally affects final DC maturation and thus VEGFR1 signaling was sufficient for blocking NF-κB activation in bone marrow progenitor cells [93,94]. VEGF significantly inhibited TNF-α induced activation of NF-κB, the transcription factor that regulates many genes involved in immune responses and that is particularly important for DC differentiation and maturation. Lastly, an in vivo study demonstrated that treatment of established tumors with anti-VEGF antibodies significantly improved the number and function of lymphnode and spleen DC in tumor bearing animals [88]. Similarly, data indicate that antibodies to VEGF are able to substantially correct DC defects in patients with metastatic lung carcinoma [95].

TGF-β

Transforming growth factor-β has been implicated in oncogenesis since the time of its discovery almost 25 years ago. The multifunctional activities of TGF-β endow it with both tumor suppressor and tumor promoting activities, depending on the stage of carcinogenesis and the responsiveness of the tumor cell [96]. Negative regulation of the cellular proliferation by TGF-β has been shown to constitute a tumor suppressor pathway [97]. However, reduction or alteration of TGF-β signaling in tumor cells as they progress
through the stages of tumorigenesis is often accompanied by increased secretion and activation of the ligand, which functions both in a paracrine fashion through its effects on accessory cells and as recently appreciated, in an autocrine manner on the tumor cells to promote tumorigenesis and increase metastasis [98,99]. In addition, both experiments using animal models and clinical data clearly showing that TGF-β has tumor promoting activity by allowing tumors to evade immune surveillance. TGF-β is one of the most potent immunosuppressive cytokines yet characterized. It is capable of affecting the proliferation, activation and differentiation of cells participating in innate and acquired immune response. The proliferation of Thymocytes, T cells, B cells, natural killer cells, monocytes and macrophages is inhibited by TGF-β [99-102]. Experiments in which highly immunogenic tumor cells become less immunogenic and more proliferative when transfected with an expression vector for TGF-β, has led to the hypothesis that TGF-β production by tumor cells may be important in determining whether the tumor disappears or grows progressively [103]. Supporting the concept that advanced tumors secrete substantial amounts of TGF-β that can contribute to a generally suppressed immune response are data showing that plasma levels of TGF-β often correlate with disease progression and decrease following surgical resection of the tumor [104]. TGF-β mediated suppression of anti-tumor immune responses were found to be associated with decreased functions of NK cell and CTL [105,106]. The observation that in line with the recent study showed that RNAi inhibition of TGF-β expression in glioma cells restores the NK and CTL activity in vivo, which is associated with decreased tumor growth [107]. Although NK cells and cytotoxic T lymphocytes are the major innate and adaptive effectors, respectively, both require activation by dendritic cells. TGF-β has been demonstrated to impair dendritic cell functions in vitro and in vivo [108]. TGF-β inhibits upregulation of critical costimulatory molecules on the surface of DC and reduces the antigen-presenting capacity [109,110]. In recent years, more correlative clinical data have supported the inhibitory role of TGF-β in the observed defects in cancer [111]. Increased serum TGF-β in human colorectal cancer correlates with reduced circulating DC and increased colonic Langerhans cell infiltration [112]. TGF-β exposure inhibits the ability of DC to present antigen, stimulate T lymphocytes, and migrate to draining lymph nodes. In addition to direct inhibitory effect of TGF-β on effector T lymphocytes, inhibitory effects of TGF-β at the level of DC may critically contribute to previously characterized effects of TGF-β.

IL-10

IL-10 is an immunoregulatory cytokine with numerous effects, such as down regulation of proinflammatory cytokine, chemokines, and costimulatory molecules. Several mechanisms have been proposed for the IL-10 mediated inhibition of anti-tumor immune response. IL-10 produced by some tumor cells is associated with the inhibition of immune cells activity, including DC which are crucial for the induction of anti-tumor immune response. IL-10 exerts its suppressive effects on DC in all stages of its life cycle such as differentiation from its progenitors, maturation and function. Culture of human monocytes with GM-CSF and IL-4 for 6 days induces a population of immature DC that can be activated by LPS, CD40-Ligand, or TNF-α to mature into highly efficient APC that induce differentiation of naïve T cells to Th cells. Addition of IL-10 during culture with GM-CSF and IL-4 or the activation step inhibits generation and maturation of monocytes derived DC. Instead as observed with monocyte cultures in the absence of GM-CSF and IL-4, IL-10 induced differentiation of these immature DC into macrophages like cells that expressed reduced levels of costimulatory molecules and MHC II [113-116]. The same inhibitory effect was observed in patients and animals with IL-10 secreting tumors of various types. In particular, direct ex vivo flow cytometric analysis of various DC sub populations in peripheral blood from hepatocellular carcinoma patients revealed an immature phenotype and a substantial reduction of circulating DC that was associated with increased IL-10 concentration in serum and tumor progression [117]. According to the current knowledge, the net outcome of T cell responses seems to be significantly influenced by the maturation state of DC [118,119]. IL-10 produced by tumor cells not only reduced the differentiation of dendritic cells, but also inhibited DC function, which is associated with maturation state of DC. Patients with myeloma exhibited functionally defective DC due to IL-10 secretion by the tumor [120]. The inhibition of the IL-10 release from the tumor cells resulted in the enhanced expressions of MHC class I, MHC class II and B7.1 molecules on dendritic cells. The treatment also resulted in the rejection of tumor graft and strong CTL activity in vivo. DC expressed CD1 molecules mediates primary immune responses to lipids and glycolipids which have been shown to be expressed by various tumors. Glycolipid over expressing metastatic melanoma secreted IL-10 down regulates CD1 molecules on DC in metastatic tumor lesions [121]. Furthermore, it is reported that tumor derived IL-10 inhibits CD40 expression on DC[122].

PROSTANOIDS

Elevated expression of cyclooxygenase-2 (COX-2) in pre-neoplastic and neoplastic cells leads to increased synthesis of prostanoids in tumors [123-126]. PGE2, the most abundant prostanoid detected in the majority of epithelial malignancies, has been shown to promote cell survival, stimulate cell proliferation and motility, induce angiogenesis and suppress immune surveillance. PGE2, synthesized by cyclooxygenase-2 over-expressing tumor, is known to contribute to cellular immune suppression in cancer patients [127-129]. Recent reports provided convincing data to support the notion that COX-2 over-expressing tumors escape from the host immune response by suppressing the differentiation and function of dendritic cells, thereby preventing initial immune recognition and activation. Human tumor derived supernatants profoundly hampered the in vitro DC differentiation from CD14(+) plastic adherent monocytes or CD34(+) precursors [130]. COX-1 and COX-2 regulated prostanoids present in the primary tumor derived supernatants were found to be solely responsible for the observed hampered differentiation of monocyte derived dendritic cells. It has been observed that reduced dendritic cell function is related to COX-2 overexpression and PGE2 secretion in patients with breast cancer [131]. DC from these patients showed significantly reduced expression of costimulatory molecules and demonstrated reduced phagocytic ability, and reduced antigen presentation to T cells.
DC with exogenous PGE2 induced the production of large amounts of IL-10 and less IL-12 from BM-DC [132-134]. The suppressive effect of PGE2 was found to be mediated through the DC expressed E prostanoid receptors (EPR) such as EP(2)R and EP(4)R [135,136]. Consistent with this observation is the fact that COX-2 over-expressing glioma leads to over-production of IL-10 but decreased production of IL-12 p70 by mature DC [137]. These DC induced Tr1 response characterized by robust secretion of IL-10 and TGF-β inhibition of the functions of the admixed lymphocytes. Selective COX-2 inhibition in COX-2 over-expressing gliomas at the time of phagocytic uptake by DC abrogated this regulatory response and instead elicited Th1 response. Furthermore, it was observed that the specific inhibition of COX-2 restores anti-tumor reactivity by altering the balance of IL-10 and IL-12 synthesis [138].

**GANGLIOSIDES**

One class of molecules with a potential to interfere with anti-tumor immune response is the gangliosides [139-141]. Gangliosides consist of an oligosaccharide core with an altered sialic acid(s) and a ceramide and are found primarily in the outer leaflet of the cell membrane. Many tumors such as neuroblastoma (NB), medulloblastoma, and renal cell carcinoma are known to over express and shed gangliosides into the circulation. For example, analysis of serum obtained from patients with neuroblastoma showed a 50-100 fold increase in the levels of GD2 ganglioside [142]. Approximately, 100 different gangliosides have been detected in different tissues; among them GD2, GD3, and GM3 are known to have important role in tumor progression. Evidence suggests that exogenous or tumor derived gangliosides are able to inhibit function of immune cells involved in adaptive and innate anti-tumor immune response and promote tumor growth [143,144]. For instance, addition of tumor-derived gangliosides to the tumor inoculum enhances tumor formation in mice [145]. Gangliosides, predominantly GD2, isolated from the human neuroblastoma cells inhibited murine cellular responses in vivo. Recent reports suggest an additional role of gangliosides in the regulation of dendritic cell generation and their function [146]. Shurin et al, first reported that the addition of NB derived GD2 and GM3 gangliosides to murine and human CD34+ hematopoietic precursor cells results in a significant decrease in CD83+ and CD86+ expressing cells at the end of the culture. In presence of gangliosides DC precursors lose their ability to differentiate into DC and activate T cells. It has been shown that co-incubation of human CD34+ progenitor cells with human neuroblastoma cells resulted in a significant inhibition of dendropoiesis in vitro up to 90% [147]. Gangliosides, purified from human melanoma tumors inhibited the generation of DC at concentrations close to those detected in the sera from melanoma patients. Furthermore, it has been demonstrated that mouse bone marrow cells treated in vitro with gangliosides, derived from T cell lymphoma, undergo apoptotic cell death [148]. The major ganglioside produced by this tumor is GD3. The anti-GD3 antibody protected bone marrow cells from tumor-induced apoptosis.

**IL-6**

Interleukin-6 is implicated as playing a major pathophysiological role in many malignancies, including hematopoietic and non-hematopoietic origin [149-151]. Which is evident from the observation cancer cells both make and respond to IL-6; down regulation of the IL-6 receptor by treatment with retinoic acid, a cytotoxic IL-6 fusion protein resulted in death of cancer cells [152,153]. In addition, anti-IL-6 administration induced regression of human PC-3 prostate cancer cell tumors in athymic (nu/nu) mice [154]. Besides being a growth/survival factor, IL-6 promotes tumor growth in part by suppressing the host immune response by means of interfering with dendritic cell differentiation and maturation. The real role of IL-6 in dendritic cell development has been realized by an observation in which in vitro culture of IL-6-/− bone marrow cells yielded 10 times more DC when cultured with GM-CSF in comparison to their wild type counterpart. The difference persisted even when IL-6-/− and WT bone marrow cells were cultured together [155]. The absolute number of circulating precursors of myeloid and plasmacytoid DC was significantly lower in multiple myeloma patients than in healthy individuals, which is associated with increased serum IL-6 in cancer patients [156]. IL-6, together with M-CSF has been reported to be involved in tumor mediated regulation of DC differentiation. For example, renal carcinoma derived IL-6 together with M-CSF inhibit the differentiation of DC from CD34+ progenitor cells. Both neutralizing IL-6 and M-CSF specific antibodies abrogated the negative effect of renal cell carcinoma conditioned medium on DC differentiation [157]. In Addition to their suppressive role on DC development, IL-6 has profound effects on DC maturation. DC derived from multiple myeloma patients showed significantly lower expression of HLA-DR, CD40, and CD80 antigens and impaired induction of T cell proliferation. Remarkably, they were not capable of presenting the patient specific tumor idiotype to autologous T cells [158]. In addition, a recent study clearly demonstrated an in vivo evidence for the IL-6 mediated inhibition of dendritic cell maturation [159]. The list of tumor derived factors that modulate DC differentiation and function is not complete and is constantly growing. There are also data that indicate the potential involvement of tumor derived lactic acid in DC differentiation and the modulation of DC phenotype [160].

**REVERSAL OF TUMOR-INDUCED DENDRITIC CELL PARALYSIS AND THEIR THERAPEUTIC RELEVANCE**

It is becoming clear that the immune response may be hindered by the hurdles created by the tumor to the immune system. As noted, TGF-β, VEGF, IL-10 and other tumor derived factors can inhibit DC generation, maturation and their function. Demonstration of these acquired defects in DC in tumor bearing animals and cancer patients suggested a rationale for using ex vivo generated DC as anti-tumor vaccine. DC expanded and activated in vitro, may be effective by virtue of their number and the concentration of antigen. The introduction of cultured DC, activated and loaded with antigen in the absence of inhibitory tumor associated factors, can be re-infused into the patient to generate responding T cells in vivo. When loaded with the appropriate antigen, these antigens loaded DC form a vaccine that may be more potent than simple introduction of the antigen into a tumor bearing host. The effectiveness of DC based vaccine against cancer has been reviewed elsewhere in detail [161-163].
However, the promise of harnessing the immune system for a therapeutic effect has remained largely unfulfilled. This failure is likely to be the result of an unreceptive environment for the reintroduced DC. Therefore, manipulations aimed at restoring the functions of re-infused DC may provide novel immunotherapeutic strategies against cancer. This could be achieved by the concomitant neutralization of tumor induced DC suppressor together with DC vaccine. Recent studies documented the impressive efficacy of such therapies that are directed against tumor secreted soluble factors together with DC vaccine. One of the factors that compromise the efficiency of immunotherapy could be the capacity of DC to migrate from the injected area to the T cell areas of the draining lymph node. This is crucial for T cell priming and is attenuated by tumor secreted factors. One such factor is TGF-β. Recently, a study demonstrated that TGF-β inhibits antigen presenting function and anti-tumor activity of dendritic cell vaccines by preventing DC migration to the T cell areas. Neutralization of TGF-β using the TGF-β neutralizing antibody enhanced the ability of DC vaccines to inhibit the growth of established murine mammary tumors. The anti-tumor effect was most evident when antisense TGF-β expressing tumor cell lysates were treated with DC plus anti-TGF-β monoclonal antibody [110]. In addition, another observation demonstrated a novel combination of anti-angiogenesis and immunotherapy based on the dual role of VEGF. Therapy with tumor peptide pulsed DC alone resulted in considerable slowing of tumor growth only during the period of treatment, and tumor growth resumed after the end of the therapy. Combined treatment with peptide pulsed DC and anti-VEGF antibody resulted in a prolonged anti-tumor effect, which was associated with the induction of significant tumor peptide specific CTL response [88]. Reversal of the tumor-induced DC paralysis was also observed when tumor infiltrating DC were injected together with anti-IL-10R antibody [164]. Likewise, the following results clearly explain the potential of combination therapy, in which the bone marrow DC were cultured in either tumor supernatant (TSN) or TSN from COX-2 inhibited tumor. After culture, DC were pulsed with tumor specific peptides, and their ability to generate anti-tumor immune response was assessed following injection into established murine lung cancer. DC cultured in TSN failed to generate antitumor immune responses and caused immuno-suppressing effects that correlated with enhanced tumor growth. However, genetic or pharmacological inhibition of COX-2 expression restored DC function and effective anti-tumor immune responses [132]. In summary these studies demonstrates the potential usefulness of a combined therapeutic approach to eliminate immuno-suppressive tumor derived factors to improve the effectiveness of DC-based vaccines (Fig. 4).

CONCLUSION

DC are showing promise as potential adjuvant for immunotherapy of cancer. However, the initial enthusiasm for DC based vaccine is being tempered by clinical results not meeting expectations. The insensitivity of established tumors to DC therapy is likely because of the re-exposure of infused DC to the tumor secreted immuno-suppressive factors that are inimical to the optimal induction of anti-tumor immune response. Knowledge of the particular evasion strategies that tumors employ is necessary to design DC based vaccine that specifically targets the ability of tumors to escape immunity.

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Fig. (4). Illustration of a novel immunotherapeutic approach to overcome tumor induced DC suppression.
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