Dendritic cells and antigen trapping technology — A revolution in vaccine/immunotherapy strategy

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Vaccines based on dendritic cells—the immune system’s key responders to foreign invaders—grabbed the spotlight of this decade. Scientists have devised a dozen different ways to make dendritic cell vaccines. They have linked dendritic cells with all kinds of antigens, including peptides derived from gene mutations, tumor/pathogen RNA, viral vectors, and with whole pathogen/tumor lysate. And they are adding cytokines such as granulocyte macrophage colony stimulating factor or interleukin 4 during dendritic cell growth or maturation or at the site of vaccination to try to boost response. We are still learning the best way to generate the dendritic cells, load them with the antigen and send them to the right place in the body, and use of the biological stage of development of dendritic cells that is best suited to stimulate a response. In the present review attempts have been made to present a comprehensive synopsis of the history, development and ramifications of evolving knowledge on dendritic cell biology and the prospects for being developed as a rational immunotherapeutic tool. Further clinical studies are warranted.

Keywords: Cancer, Dendritic cell (DC), Infectious diseases, Immunotherapy, Viral Diseases, Vaccine

Host defense relies on a concerted action of both antigen (Ag)-nonspecific innate immunity and Ag-specific adaptive immunity. Immunology has long been focused on antigens and lymphocytes, but the mere presence of these two parties do not always lead to a perfect immune response. A third party, the dendritic cells (DC) are found to be the key initiator and modulator of the primary immune response after the discovery of a novel cell type in peripheral lymphoid organs of mice by Steinman in 1973¹. Evolutionary pressure has led to the development of adaptive immunity, the key features of which are, (a) the ability to rearrange genes of the immunoglobin family, permitting creation of large diversity of Ag-specific clones and, (b) immunological memory. Yet this highly sophisticated and potent system needs to be instructed and regulated by Ag-presenting cells. Dendritic cells are a fraction of matured and terminally differentiated antigen presenting cells found to be generated from different progenitors with myeloid or lymphoid commitments, with a unique ability to induce primary immune response. DC captures and transfer information from the exogenous antigens to the cells of adaptive immune system. Apart from being highly immunogenic, DC have been shown to play an important role in peripheral tolerance, as well as for the regulation of the type of T cell mediated immune responses and for the long lasting immunological memory. This review for the most part concentrates on the developing strategies of immunotherapy by using the dendritic cells.

Heterogeneity of dendritic cell subsets

Mice

At least two distinct pathways of DC development have been identified in mice. Myeloid precursors give rise to myeloid DC in presence of granulocyte macrophage colony stimulating factor²,³. CD8α⁺ lymphoid DC can also arise from lymphoid precursors⁴,⁸. Transfer of a population of purified lymphoid precursors into irradiated host results development of DC that express CD8α. Lymphoid DC are localized in the T cell rich areas of the peripherinolymphatic sheaths (PALS) in spleen and lymph nodes⁶,⁹⁻¹¹. In contrast myeloid DC are in the marginal zone bridging channels of the spleen⁹⁻¹¹. Both subsets express high levels of CD11c, MHC II and the costimulatory molecules CD86 and CD40. Lymphoid DC make higher level of IL-12 and less
phagocytic than the myeloid DC.

Human

DC heterogeneity in humans is reflected in all the four parameters of anatomical localization, progenitors, functional properties and type of immune response induced12-15.

(a) Anatomical localization— Depending on anatomical localization the DC are classified as the skin epidermal langerhans cells (LC), dermal DC (interstitial DC), splenic marginal DC, T-zone interdigitating cells, germinal-center DC, thymic DC, liver DC and blood DC.

(b) Precursor populations— For instance, in human at least two subsets of DC precursors circulate in the blood, CD14+CD11c+ monocytes and lineage-negative LINnegCD11c+IL3Rα+ precursors DC. The LINnegCD11c+ cells may represent a third precursor, although these cells are more committed because they can spontaneously differentiate into DC when put into culture.

(c) Function— Precursors of DC correlate with the functional diversity evidenced from in vitro generation of different subtypes of human DC. CD34+ precursors from umbilical cord blood and adult bone marrow cultured in presence of GM-CSF and TNF-α give rise to functional CD1a+CD14+ Langerhans cells. CD34+LinCD10- lymphoid restricted precursors isolated from bone marrow and cultured with cytokines give rise to DC equivalent to murine lymphoid-derived DC. Blood monocytes in presence of cytokines GM-CSF and IL-4, for growth and TNF-α, for maturation give rise to mature DC type 1 (DC1). CD45RAhiCD11cloIL3Rhi plasmacytoid precursors from blood, tonsil and thymus in presence of IL-3 give rise to CD11clo mature DC type 2 (DC2) (Fig. 1). Precursor DC1 cells produce TNF-α and IL-6, whereas the precursor DC2 cells rapidly produce IFN-α and IFN-β in response to the appropriate microbial stimuli. DC1 cells are found to prime T cells to produce a TH1 response which is associated with high production of IL-12 in response to the appropriate stimuli. In contrast DC2 induce the TH2 response.

(d) Outcome of immune response— The final outcome of immune response refers to the induction of tolerance or immunity. Dendritic cells are professional APC that play a key role in initiating effector T cell responses in secondary lymphoid organs. Equally important to the initiation of effector responses, DC also participate in the presentation of self-antigen during the course of central and peripheral T cell tolerance induction via various mechanisms, including activation of T regulatory (Treg) cells, induction of T cell anergy, and TH1/TH2 polarization. The exact mechanisms involved in the decision of a DC to become immunogenic or tolerogenic have not been elucidated, however, upcoming scientific reports suggest that DC function is dependent on their maturation stage16.

Recruitment and maturation: The phenotypic nuances

Newly generated DC precursors migrate, presumably through blood stream, from the bone marrow to non-lymphoid tissues, where they eventually become resident cells. Circulating DC precursors accumulate rapidly (within an hour) at the sites of antigen deposition in response to the production of chemokines upon local inflammation as demonstrated in bronchial epithelium after Ag inhalation17-19. CD34+ hematopoietic cell derived immature DC expresses CCR6, whose ligand, MIP3α,...
appears to be the most powerful chemokine guiding their migration\textsuperscript{20,21}.

Immature DC are very efficient in Ag capture and can use several pathways — (a) macropinocytosis, (b) receptor-mediated endocytosis via C-type lectin receptors (mannose receptor, DEC-205) or Fc\(\gamma\) receptor type I (CD64) and II (CD32), (c) phagocytosis of particles such as latex bead, apoptotic and necrotic cell fragments (involving CD36 and \(\alpha\)v\(\beta3\) or \(\alpha\)v\(\beta5\) integrins), viruses, and bacteria including mycobacteria, as well as intracellular parasites such as \textit{Leishmania major}\textsuperscript{22}.

The antigen or pathogen induces the immature DC to undergo phenotypic and functional changes that culminate in the complete transition from the Ag-capturing cell to APC. DC maturation is intimately linked with their migration from the peripheral tissue to the draining lymphoid organs, and therefore these are the two key events in the life span of DC. Numerous factors induce and regulate DC maturation (Fig. 2). The maturation process is associated with several coordinated cellular events such as (a) loss of endocytic/phagocytic receptors, (b) upregulation of costimulatory molecules CD40, CD80, CD83, CD86, (c) shift in lysosomal compartments with downregulation of CD68 and upregulation of DC-lysosome associated membrane protein (DC-LAMP), (d) changes in MHC II compartments and (e) change in morphology.

Mature DC downregulate CCR6, the receptor for MIP3\(\alpha\), consequently escape from local gradient of chemokines, upregulate the chemokine receptor CCR7 and accordingly acquire responsiveness to MIP3\(\beta\) (ELC, Exodus 3) and 6Ckine [secondary lymphoid tissue chemokine (SLC), Exodus2]. Consequently, maturing DC leave the inflamed tissue and enter the lymph stream, potentially directed by 6 Ckine that is expressed on lymphatic vessel. Maturing DC entering the draining lymph node are driven into the paracortical areas in response to the production of MIP3\(\beta\) and 6Ckine by cells spread over the T cell zone.

**The decision-makers: Activating the T cells to shape the immune response**

The ability to prime naïve T cells constitutes a unique and critical function of DC both \textit{in vitro} and \textit{in vivo}. Because virtually all signals to T cells begin at the cell membrane, the efficacy of vaccines and other immunotherapies can be enhanced by the inclusion of ligands that bind to these cell membranes. The enhanced versatility of new vector systems allows the combinatorial construction of immunotherapies that contain elements that target several points in the pathway of T cell activation\textsuperscript{23}.

At the simplest level, T cell activation requires two
signals. Signal 1 is delivered through the T-cell receptor (TCR) after engagement by a peptide-MHC complex. Signal 2, the costimulatory signal, was thought originally to be coming from CD80 (B7-1) interacting with CD28 on T cell. The costimulatory molecules/signals so far identified, fall into three families — the B7 family, the tumor-necrosis factor (TNF) receptor and cytokines. The B7 family currently comprises six members with defined costimulatory activity. The founding members CD80 (B7-1) and CD86 (B7-2), bind to the activating receptor of T cell, CD28, and a counter regulatory inhibitory receptor, cytotoxic T-lymphocyte antigen 4 (CTLA4). Newer B7 family members, B7-H1/PDL1 and B7-DC/PDL2, have been reported to have both costimulatory and inhibitory effect. Their inhibitory activity is probably mediated through the programmed cell death 1 (PD1) receptor, which contains an immunoreceptor tyrosine-based inhibitory motif (ITIM). B7RP-1, which binds to the activating receptor inducible costimulatory molecule (ICOS), seems to be particularly important in T cell-B cell interaction and antibody production. The receptor for B7H3 has not been identified yet. TNF receptor families are upregulated on T cell activation, indicating that the role of this family might be to amplify responses once T cell activation has occurred. T cells can also interact with DC via CD40 ligand-CD40 signaling pathway leading to increased expression of CD80/CD86 and cytokine release (IL-1, TNF, Chemokines and IL-12). Engagement of RANK, a member of TNFR family by its ligand (RANKL/TRANCE) expressed on activated T cells, stimulates the secretion of cytokines like IL-1, IL-6 and IL-12 by DC. Finally cytokines such as IL-12 are very important in determining the direction of T cell development i.e., Th1 versus Th2-as a consequence of T cell stimulation. Once these complex interactions and signaling outcomes are defined, it should be possible to build multiple costimulatory signals into vaccines in an appropriate combinatorial fashion.

**Prospects of DC based vaccination and immunotherapy: Experimental successes and clinical experiences**

DC have been chosen as one of the key factors for vaccine research from late 1980s when it was found that human T cell shows proliferative responses to particulate microbial antigens and are supported by cell populations enriched for dendritic cells. The breakthrough came in mid 90’s when Hsu et. al. successfully vaccinated patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells and in case of inactivated influenza viruses, when presented on dendritic cells in vitro, were shown to elicit human cytolytic CD8+ T cell responses.

The accessibility of DC in the immune system, together with its central role in so many disease processes, makes it highly appropriate for therapeutic manipulations. Until recently, immunotherapies tested clinically have been fairly crude, failing to take advantage of the molecular pathways that regulate immunity. The elucidation of specific molecules that induce DC proliferation and maturation has provided immunologists with an important tool for the engineered vaccines with enhanced therapeutic potency. Recent studies have also shed light on the pathogenic roles of DC in various diseases such as autoimmunity, allergy, transplantation, infection and cancer. This review discusses the ways in which molecular and cell biological insights into immune regulation are being applied to design strategies for both quantitative and qualitative improvement of immunotherapy for diseases.

**Engineered dendritic cells: Way to customized vaccines**

The most common target of active immunotherapy strategies is the enhancement or modulation of the function of antigen-presenting cells (APC). This strategy is based on the concept that the quantitative and qualitative characteristics of a T cell response to an antigen depend on the signals that the T cells receive from the APC. DC are the most potent type of APC and virtually all stages of DC development and function can be modulated by engineered vaccines. GM-CSF and other cytokines, such as FLT3 ligand and IL-4 that are mitogenic or co-mitogenic, induce an intermediate stage of DC differentiation that is characterized by the efficient uptake and processing of antigen. DC maturation is dependent on binding of two classes of receptors present on DC, the Toll like receptor (TLR) and Tumor necrosis factor receptor (TNFR) families. TLRs are pattern recognition receptors, which bind common chemical moieties that are expressed by pathogens and known as pathogen associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) and unmethylated CpG DNA sequences. DC have been shown to be responsible for the capacity of CpG...
oligodeoxynucleotides to act as an adjuvant for vaccine against Leishmania major in mice. The discovery of specific receptors on DC that are responsible for receptor mediated endocytosis has enabled the modification of antigens, so that they can be more efficiently bound to these DC uptake receptors. Indeed, antigen-GM-CSF fusion protein is an example of potential DC targeting because GM-CSF not only stimulates DC proliferation, but might also act to target the antigen to endosomal compartments by binding to the GM-CSF receptor. Fusion of antigen and immunoglobin Fc region enhanced the Fc receptor (FcR)-mediated antigen uptake by APC. DNA vaccine encoding an E7-HSP70 fusion protein was 30-fold more effective than the wild type E7 protein in generating T cell response.

The two best characterized endogenous DC maturation factors of the TNF family are TNF-α and CD40 ligand (CD40L). In vivo ligation of CD40 enhances priming against the endogenous tumor antigen and promotes CD8+ T cell effector function in SV40 T antigen transgenic mice.

The ability to culture DC ex vivo has led to numerous studies using ex vivo antigen loaded DC as vaccine against tumor and infectious diseases. The incorporation of GM-CSF, IL-12 or its encoding gene into recombinant protein, DNA or viral vaccines has also been shown to significantly enhance immunization. Even Dendritic cells engineered to express the FLT3 ligand stimulate type I immune response, and induce enhanced cytotoxic T and natural killer cell cytotoxicities and anti-tumor immunity.

Human telomerase reverse transcriptase (hTERT) is the catalytic component of a functional telomerase complex, which is important in maintaining cell immortality. In most normal human adult cells, the expression of telomerase is very low and/or transient. In contrast, almost 90% of human tumors express a relatively high level of telomerase implying the possibility of using hTERT as a universal candidate tumor antigen. Human monocyte-derived dendritic cells (DC) lack telomerase activity. Similar to other normal somatic cells, DC express the RNA (hTR) component but not the catalytic component, hTERT. Telomerase activity could be reconstituted using either lipid-mediated transfection of the hTERT plasmid DNA or transfection of an E1, E3-deleted adenoviral vector containing the hTERT gene. AdhTERT-transfected DC were able to generate CTL responses and these CTLs primed against hTERT exhibiting killing of telomerase positive but not telomerase negative tumor lines of diverse tissue origins. This study revealed the fact that hTERT gene, particularly as delivered via the recombinant adenovirus, may be useful as a vaccine to induce specific T-cell-mediated tumor immunity in cancer patients. Use of adenovirus and retrovirus as vector for the transduction of DC during vaccine engineering has been favoured in several recent studies. Immunization of retrovirus-transfected DC induces specific cytotoxic T lymphocytes for two distinct malarial peptides presented by Kd molecule. DC transfected with an adenovirus vector encoding IL-12 is a potent vaccine for invasive pulmonary aspergillosis, a fungal infection. DC are uniquely able to decode the fungus-associated information and translate it in qualitatively different T helper (Th) immune responses, in vitro and in vivo. DC sense fungi in a morphotype-specific manner, through the engagement of distinct recognition receptors ultimately affecting cytokine production and costimulation. Adaptive transfer of different types of DC activates protective and non-protective Th cells as well as regulatory T cells and affects the outcome of the infections. DC transfected with fungal RNA also restore antifungal resistance in hematopoietic transplantation. Thus, the remarkable functional plasticity of DC in response to fungi can be exploited for the deliberate targeting of cells and pathways of cell-mediated immunity in response to DC based fungal vaccines.

Use of DC in vaccination strategies against infections

Infectious disease is one of the leading causes of human death. Particularly in the developing world, malaria, infectious diarrhea, tuberculosis, leishmaniasis and many more illness are still existing and contributing to significant suffering as well as mortality. For a time infectious diseases were thought to be under control but at present appearance of AIDS and other infectious diseases including leishmaniasis is the major threat for our survival. Ahuja et al. successfully transferred adoptively the autologous DC engineered to secrete IL-12 (IL-12 transfected) and pulsed ex vivo with soluble protozoan parasite L. donovani antigens (SLDA) in a mouse model.
Following vaccination it protects the mice from infection as evidenced from parasite load in liver and spleen and also from cytokine profile. By current estimates, leishmaniasis affects people in 88 countries, with 350 million at risk of contracting the disease and approximately 2 million new cases each year (www.who.int/emc/diseases/leish/leisdis1.html). The devastating impact of this disease is exemplified by the epidemic of visceral leishmaniasis that occurred in the 1990s in Sudan. Of all the parasitic diseases, leishmaniasis is considered the most likely to succumb to vaccination. There are concerns about whether the same vaccine will work for all leishmaniasis. New discovery of vaccines against leishmaniasis still draws the attention of science. There are concerns about the cost-effective production of vaccines against visceral leishmaniasis because the leishmaniasis is the primary threat for the developing and under developed tropical countries. The present authors, being the members of Dr. Bandyopadhyay’s group for the first time, tried to formulate an anti-leishmanial immunotherapy strategy that is really useful for poor countries. The experimental strategy was successfully developed in murine model. The cheaper DC-based immunotherapy combined with antimony based chemotherapy cures established murine visceral leishmaniasis. Bone marrow derived DC differentiated after treatment with GM-CSF and IL-4 and pulsed with SLDA in the presence of TNF-α produced significant amounts of IL-12 (276 ± 40 pg/ml vs 76 ± 22 pg/ml by unpulsed DC), and their administration in infected mice significantly reduced splenic and hepatic parasite burden (by 96.6 ± 3.5 and 90.9 ± 4.2% respectively, compared with PBS-treated infected controls (P<0.0005 for each comparison). This difference in two organs may be attributed to the DC subtypes and/or CCR7-mediated migration of DC, which is dependent on the expression of CCL21 and CCL19 chemokines by stromal cells within the T cell areas of secondary lymphoid organs. The parasite burden in both the organs also decreased significantly following administration of unpulsed DC (P<0.05). SLDA-pulsed or unpulsed MΦ reduced parasite burden in both organs only marginally, and the differences were not statistically significant. In the mouse model of visceral leishmaniasis, the optimum antileishmanial effect by Sb is achieved by a high dose of Sb (~500 mg/kg body weight), and only a marginal effect is seen at 50 mg/kg body weight. Treatment with Sb (50 mg/kg body weight) alone reduced parasite burden by 39.6±13.5% (P<0.05) and 51.7 ± 13.3% (P<0.01) in spleen and liver, respectively. Strikingly, complete clearance of parasite burden from spleen and liver was achieved when infected mice received both SLDA-pulsed DC and Sb but not in all other experimental groups. The repetitive in vitro stimulation by SLDA-pulsed DC has confirmed the fact that CD4+IFNγ+ T cells effectively emerged in higher percentage, when the splenocytes were taken from L. donovani infected mice (13.62%) compared to uninfected mice (6.1%). Accumulation of IFN-γ mRNA has been detected in lymph node cells of L. donovani infected mice administrated with SLDA-pulsed DC, but the expression of IFN-γ mRNA was not further enhanced when infected mice received combined therapy with SLDA-pulsed DC plus Sb. Our data on combined therapy may have the following explanations. Sb may have potentiated the antileishmanial activity of DC-based therapy induced IFN-γ by enhancing IFN-γ signaling. Alternatively, Sb, by its ability to potentiate the production of reactive oxygen species and to induce programmed cell death in Leishmania, may provide additional antileishmanial activity over DC-based therapy induced IFN-γ.

Toxoplasma gondii can cause severe sequelae in the fetus when the mother acquires the infection during pregnancy, as well as life-threatening neuropathy in immunocompromised patients, in particular those with AIDS. T. gondii antigen-pulsed-dendritic cell-derived exosomes induce a protective immune response against T. gondii infection. Adoptive transfer of T. gondii-pulsed DC-derived exosomes to the spleen elicited a strong systemic Th1-modulated Toxoplasma-specific immune response in vivo, and conferred good protection against infection. These findings support the possibility that DC-derived exosomes can be used for T. gondii immunoprophylaxis and for immunoprophylaxis against many other pathogens.

Burkholderia pseudomallei, the causative agent of melioidosis, is a gram-negative bacterium which can cause either chronic infections or acute lethal sepsis in infected individuals. The disease is endemic in Southeast Asia and northern Australia. Upon virulent challenge with B. pseudomallei strains K96243, NCTC 4845 or 576, animals immunized with
dendritic cells that were pulsed with heat-killed K96243 and matured in the presence of CpG 1826 showed significant levels of protection\textsuperscript{45}.  

**DC in anti-viral immunotherapy**  
Control of a viral infection \textit{in vivo} requires a rapid and efficient cytotoxic T lymphocyte response. Lentivirus mediated introduction of antigen in DC confers a protective antiviral immunity \textit{in vivo} in a lymphocytic choriomeningitis virus model. Therefore, lentiviral vectors may be excellent vaccine candidates for viral infections\textsuperscript{46}. Induction of protective immunity to bacteria \textit{Listeria monocytogenes} was observed in case of DC retroviral transfected with a cytotoxic T lymphocyte epitope minigene\textsuperscript{47}. In a preliminary investigation on therapeutic DC based vaccine, 18 chronically HIV-1 infected and untreated individuals showing stable viral loads at least for 6 months were immunized with autologous monocyte derived DC loaded with autologous aldrithiol-2-inactivated HIV-1. Plasma viral load levels were decreased by 80% (median) over the first 112 day following immunization. Prolonged suppression of viral load of more than 90% was seen in 8 individuals for the last 1 year. The suppression of viral load was positively correlated with HIV-1 specific IL-2 or IFN-\textgamma expressing CD4+ T cells and with HIV-1 gag specific perforin expressing CD8+ effector cells, suggesting that a robust virus-specific CD4+ T helper (T_{H1}) response is required for inducing and maintaining virus specific CD8+ effector to contain HIV-1 \textit{in vivo}. The results suggest that inactivated whole virus pulsed DC vaccines could be a promising strategy for treating people with chronic HIV-1 infection\textsuperscript{48}.  

A majority of hepatitis C virus (HCV)-infected individuals becomes chronically infected, which can result in liver cirrhosis and hepatocellular carcinoma. Patients with chronic HCV are unable to prime and maintain vigorous T-cell responses. Hepatitis C virus (HCV) specific T cell responses have been suggested to play a significant role in viral clearance. Recombinant adenoviral vectors containing HCV derived Core and NS3 genes were used to endogenously express HCV Core and NS3 proteins in human DC. These HCV-antigen expressing DC were used to prime and stimulate autologous T cells obtained from uninfected healthy donors. The DC expressing HCV Core or NS3 antigens were able to stimulate T cells to produce various cytokines and proliferate in HCV Ag-dependent manner\textsuperscript{49-52}. Relative to mice inoculated with non-transfected dendritic cells, splenocytes derived from mice immunized with either hepatitis C virus Core-transfected or non-structural 5-transfected dendritic cells showed 3 to 5-fold greater antigen-specific cytotoxic T lymphocyte activity. These findings suggest that vaccination with protein-transfected dendritic cells may constitute an important antiviral strategy for hepatitis C virus\textsuperscript{53}.  

Immunization with Ad-S-transfected [Recombinant adenovirus expressing hepatitis B surface antigen (HBsAg) (Ad-S)] DC effectively induced HBsAg-specific CTLs \textit{in vivo} and down-regulated the circulating HBsAg and HBV DNA in HBV transgenic mice. Furthermore, these efficacies were stronger than that of HBsAg-pulsed DC and plasmid DNA. Thus, DC transfected with recombinant adenovirus may be a promising candidate for an effective CTL-based therapeutic vaccine against HBV\textsuperscript{54}. The DC from chronic hepatitis B patients had a lower expression of costimulatory molecules CD80, CD86 and impaired allogeneic mixed lymphocyte reaction capacity compared to those from normal controls. However, the impaired DC function could be restored partially by a cytokine cocktail supplementation \textit{in vitro}. Mature DC loaded with HBsAg or HBcAg showed a greater capacity for autologous T cell proliferation and antigen-specific IFN-\textgamma production than immature DC. Moreover, as a DC-loading antigen, HBcAg was more immunogenic than HBsAg. The impaired function of DC in patients with CHB may be restored by supplementation \textit{in vitro} with a cocktail of cytokines, and therapeutic DC vaccines might be effective to treat CHB infection in humans\textsuperscript{55}.  

**DC in anti-tumor immunotherapy**  
The supremacy of DC based anti-tumor vaccination strategies can be judged clearly from a single injection of CD34+ progenitor-derived dendritic cell vaccine leading to induction of T-cell immunity in patients with stage IV melanoma\textsuperscript{56}. Treatment of malignant glioma is difficult and discouraging. Even after resection and maximal adjuvant therapy, the prognosis remains poor. Yu \textit{et al.}\textsuperscript{57} successfully proved the idea that vaccination of malignant glioma patients with peptide-pulsed DC elicits systemic
cytotoxicity and intracranial T-cell infiltration. The same group demonstrated that an active immunotherapy strategy with tumor lysate pulsed autologous DC could be possible for generating antigen-specific cytotoxicity in phase I study in brain tumor patients\textsuperscript{58}. Results of such a phase I study utilizing monocyte-derived DC pulsed with tumor RNA in children and young adults with brain cancer showed that DC-RNA vaccines (Fig. 3) were both safe and feasible in children with tumors of the central nervous system with a single leukapheresis\textsuperscript{59}.

Immunoglobulin produced by myeloma cells is clone-specific and carries an idiotype which may be a suitable tumor-specific antigen for immune targeting. Advances in DC technology suggest an opportunity for using this potent antigen presentation system to deliver myeloma Id to the autologous host to elicit anti-tumor immune responses. Murine dendritic cells pulsed with an anti-idiotype antibody induce antigen-specific protective anti-tumor immunity\textsuperscript{60}.

Heat-shock protein-based anticancer immunotherapy is an idea mooted in early 90’s. But the use of dendritic cells for this purpose is just new and novel. Heat-treated CT-26 (HSCT) cells showed a higher HSP70 protein expression. Pulsing with heat shocked CT-26 cell lysate elevated the co-stimulatory and MHC-II molecule expression on bone marrow derived DC as well as IL-12 p70 secretion. The frequency of IFN-γ secreting CTLs induced by HSCT-26 DC was significantly more than that induced by non heat treated CT-26 lysate pulsed-DC. Heat-shocked tumor cell lysate-pulsed DC can evoke anti-tumor immune response in vivo effectively and serve as a novel DC-based tumor vaccine\textsuperscript{61}. The mice vaccinated with HSP105-pulsed BM-DC were markedly protected from the growth of subcutaneous tumors, accompanied by a massive infiltration of both CD4+ T cells and CD8+ T cells into the tumors. Both CD4+ T cells and CD8+ T cells play a crucial role in anti-tumor immunity. In this study both CD4+ T cells and CD8+ T cells specific to HSP105 were induced by stimulation with HSP105-pulsed DC. As a result, vaccination of mice with BM-DC pulsed with HSP105 could elicit a stronger tumor rejection compared to DNA vaccination\textsuperscript{62}.

DC fused with tumor cells may also be effective for immune response induction. A pilot clinical trial using dendritomas, purified hybrids from the fusion of dendritic cells and tumor cells combined with a low dose of IL-2 was conducted in metastatic melanoma patients in order to determine its safety and potential immunological and clinical responses. Eight of nine patients demonstrated immunologic reactions by increased IFN-γ expressing T cells\textsuperscript{63}.

DC can capture antigen from apoptotic tumor cells and induce MHC class I and class II restricted responses. Dendritic cells loaded with apoptotic tumor cells induce stronger T cell responses than dendritic cell-tumor hybrids in B-CLL. But endocyted apoptotic tumor cells induced a significantly stronger T cell response than DC hybrids and as such should be a better candidate for vaccine production\textsuperscript{64}. Autologous DC transfected with total renal tumor

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Fig. 3 — Ways of DC-based vaccination. Immature DC are efficient in phagocytosis compared to matured DC. Peptide eluted from cell lysate of tumors or other pathogens like Leishmania sp., Toxoplasma sp. are successfully tried for vaccination. RNA/DNA from bacteria, virus or fungi are good agent for DC pulsed vaccination. Immature DC engulfs apoptic bodies more efficiently and proved as best adjuvant against tumor cells. TNF-α, CD40L, IL-10, IL-6 etc. are used as maturation stimuli to mature the DC before administration.
RNA have been shown to be potent stimulators of CTLs and anti-tumor immunity in vitro. A Phase I trial revealed that the tumor-related mortality of the study subjects was unexpectedly low with only 3 of 10 patients dying from disease after a mean follow-up of 19.8 months. These data provide a scientific rationale for continued clinical investigation of this polyvalent vaccine strategy in the treatment of metastatic renal cell carcinoma and potentially, for other cancers (Fig. 4).

**Provenge**: DC vaccine from bench to bedside

Provenge® is a therapeutic vaccine composed of autologous antigen presenting cells cultured ex vivo with a recombinant fusion antigen consisting of prostatic acid phosphatase (PAP) linked to granulocyte macrophage colony stimulating factor. The drug is in the final stage of clinical evaluation in men with androgen-independent prostate cancer. Results from a completed phase 3, double-blind placebo controlled trial suggest that in men with Gleason ≤7 tumors, Provenge® delays disease progression, delays the onset of disease related pain and results in a survival advantage when compared to patients who had been randomized to placebo.

**Epilogue**

The quest for the ideal immunotherapeutic approach has for long been the desired objective in the treatment of cancer and other infectious diseases (Table 1). DC used as an adjuvant in the immunotherapy for patients with malignant glioma seems to be promising. As with DC vaccination trials for other malignancies, DC immunotherapy of glioma proved to be biologically safe and without major side effects. Nevertheless, its efficacy remains to be more closely examined in randomized and controlled clinical trials. The development of advanced methods for genetic manipulation and transduction of DC prior to vaccination in humans may considerably increase...
the clinical benefits from this type of biological treatment.

Dendritic cells, being recognized to have the unique capability of initiating a strong primary immune response, has long been identified to be an adept machinery for vaccination against different nonself pathogenic antigens and modified-self tumor antigens. Pathogen-specific immunotherapies or tumor-targeting vaccination strategies can be far more strengthened by use of a potent natural adjuvant like dendritic cells. Moreover, characterization of the tolerogenic properties of few dendritic cell subsets can open up new avenues in the form of tolerizing-immunotherapy against autoimmune disorders or post-transplantation graft versus host diseases. The investigations in these new fields are still in a nascent state of development. Nevertheless, scads of cerebration have already gone into it and the literature reporting the myriad stories of success and pitfalls have enriched the understanding in a great way.

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References

1. Steinman R M & Cohn Z A, Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology,


29 Mohamadzadeh M & Luftig R, Dendritic cells: In the forefront of immunopathogenesis and vaccine development


