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Dendritic Cell Therapy-A promising approach in Cancer treatment

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ABSTRACT

Dendritic cells (DCs) are the most powerful APCs in the immune system. Both immunity and immune tolerance are controlled by the DCs which are at the centre of the immune system. They have a higher ability to trigger Ag-specific immune responses and promote both adaptive immunity and innate immunity. Therapeutic immunity against cancer can be targeted by DCs. Treatment of tumours in cancer immunotherapy is harnessed by the capacity and specificity of the immune system. Autologous Ag-specific T cells are proliferated *ex vivo* and then re-infused into patients or through vaccination; that is, the provision of an Ag together with an adjuvant to elicit therapeutic T cells *in vivo*. DC vaccine appears to have potential to increase overall survival with minimal complications. However, still certain problems has to be met during vaccination, such as targeting specific or associated Ags, adjuvants, clinical status, and the evaluation of the response.

KEY WORDS: Dendritic Cells, Cancer, Immunity, Vaccine.

1. INTRODUCTION

The dendritic, non-pigmentary cells present in the epidermis of the human skin nerves were regarded as intraepidermal receptors for extra cutaneous signals of the nervous system. Before the immunological importance and function was recognized, these cells were a problem to be solved to dermatologists (Langerhans, 1868). Dendritic cells (DCs) are present in tissues that are uncovered to the external environment, such as the skin (Langerhans cell) and the inner lining of the nose, lungs, stomach and intestines. The peripheral cells of the immune system are represented by the Langerhans cells in the epidermis (Silberberg, 1973). Langerhans cells are the well-studied immature dendritic cells (mDC) subset. DCs are also present in an immature state in the blood. Upon activation, they migrate to the lymph nodes where they interact with T cells and B cells to initiate and process the adaptive immune response.

DCs have been found in adherent cell populations prepared from mouse peripheral lymphoid organs (spleen, lymph node, Peyer's patch). These cells have clearly different morphological characteristics such as large, retractile nucleus contorted in shape, and containing two small nucleoli. The cytoplasm is abundant and arranged in processes of varied length and width containing many large spherical mitochondria. During development, they grow into branched projections, the *dendrites* and hence its name. Though similarity in appearance to neurons is seen, they are largely distinct in function. As they possess large cytoplasmic 'veils', mDCs are also called veiled cells rather than dendrites. The term “dendritic cell” was proposed for this novel cell type (Steinman and Cohn, 1973).

Dendritic Cells: DCs are antigen presenting cells (APCs) of the mammalian immune system. Their main duty is to process antigen (Ag) material and present it on the surface of the cell to the T cells of the immune system. They act as message carriers between the innate and the adaptive immune systems. Under the control of dendritic cells, B and T lymphocytes mediate the immunity. To initiate immune responses, DCs in the periphery capture and process Ags; express lymphocyte co-stimulatory molecules, migrate to lymphoid organs and secrete cytokines. Autoimmune reactions can be minimized by not only activating the lymphocytes but also tolerize T cells to Ags that are innate to the body (self-Ags). DCs are a successful tool for manipulating the immune system. (Banchereau and Steinman, 1998). The Nobel Prize in Physiology/Medicine 2011 was shared, half jointly to Bruce A. Beutler and Jules A. Hoffmann "for their discoveries relating the activation of innate immunity" and the other half to Ralph M. Steinman "for his discovery of DCs and its role in adaptive immunity" (Bruce, Hoffmann and Steinman, 2011).

The role of innate immunity was re-addressed with the discovery of a new class of receptors; involved in the recognition of defined groups of microorganisms was a sophisticated discriminating system that uses a broad innate receptor repertoire to sense the nature of the environmental perturbation (Medzhitov and Janeway, 1997). The type of innate response that follows microbial recognition is dictated by different types of effector responses. The responses of the innate immune could be activated via receptors, which were named pattern recognition receptors (PRR), able to recognize microbial associated molecular patterns (Janeway, 1992). The recognition of the Toll-like receptor (TLR) family members able to bind specific receptors of a large variety of pathogens has provided evidence to this new theory (Medzhitov, 1997). As DCs induce adaptive immune responses, they were called the “natural adjuvants” (Banchereau and Steinman, 1998; Ibrahim, 1995; Steinman, 1991). During infections, the activation and control of both the innate and adaptive immune responses, DCs represent a special class of leukocytes able the immune system attentive (Steinman and Dhodapkar, 2001; Zitvogel, 2002). After Ag uptake, DC efficiently process Ags for their presentation in association with the Major Histocompatibility complex (MHC) molecules. However,

DCs must be completely matured, before priming the adaptive immune response which is initiated by direct exposure to TLR ligands or to other receptors of the innate receptor repertoire. DC activation happens upon interaction with the pathogens that leads to their migration to the T cell-area of lymph nodes where the adaptive immune response can be primed with the Ag-specific cells. The high plasticity of the DC signals determines a particular DC function and finally the type of adaptive immune response depend mostly on the local microenvironment and on the interaction between the DC and the microbial signals. These interactions are complex and very different from one pathogen to another (Fig.1).

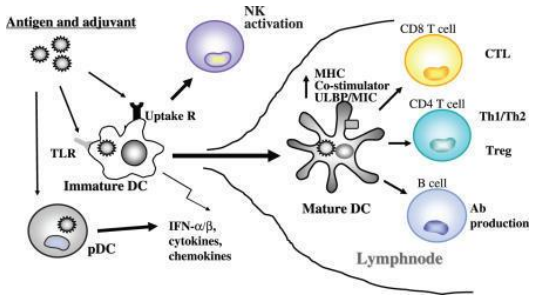

Figure.1. Role of human TLRs in mDC maturation followed by activation of various lymphocytes
(Source: Seya, 2006)

mDCs residing in local tissue undergo maturation upon phagocytosis of exogenous Ag and pattern molecule (namely adjuant). Induce of interferons (IFNs), cytokines and chemokines is by mDC, and allow the upregulation of co-stimulators, natural killer (NK)-activating ligands [UL-16 binding protein (ULBP), MHC class I polypeptide-related sequence (MIC), etc] and MHC, activate a variety of lymphocytes during maturation. The properties of adjuvants determine the maturation events. Induction of CD8+ cytotoxic T-lymphocyes (CTL) by mDCs is switched by adjuvant (Seya, 2006).

Dendritic Cell subtypes: Haematopoietic precursors of DC originate from bone marrow. Different subtypes were identified on the basis of expression of specific markers and tissue distribution (Ardavin, 2003; Ardavin, 2001; Shortman and Liu, 2002). Origin of human DC subtypes was possible from *in vitro* studies. Blood monocyte culturing may result in DCs. mDC, expressing low levels of CD86 and MHC class II can be differentiated from monocytes in the presence of granulocute macrophage colony stimulating factor (GM-CSF) and interlukein (IL)-4. Maturity of DC phenotype showing high levels of MHC class II and costimulatory molecules happen by incubating with inflammatory products (Sallusto and Lanzavecchia, 1994). CD11c CD45RA+ CD123+ found in blood and lymphoid tissue are a second human DC subtype, IFN-producing plasmacytoid DC (Kadowaki and Liu, 2002).

Plasticity of Dendritic Cells: Induction of the immunological response happens by two major immunotherapeutic strategies:

- Use of DCs for the activation of the immune system is active immunotherapy;
- Use of Ag-specific T lymphocytes or components of the immune system (anti-tumour antibodies) is passive immunotherapy.

Studies by using DCs in anti-tumour immunotherapy on animal models have observed triggering of the cell humoral response in some and immune response in others when DCs generated *ex-vivo* and activated with specific tumour Ags. Other studies resulted in better prognosis for the disease in classical therapy combined with DCs. For the determination of DC efficacy in different types of cancer treatment, different clinical protocols were developed. The problem faced in DC culture is to obtain a homogeneous DC population is to obtain high purity initial monocyte population (Lewis, 2006). In comparison to standard vaccines used for disease prevention, vaccines based on DCs used for anti-tumour treatment are developed exclusively to induce the immunological system to react vigorously against disorder (Durrant and Ramage, 2005; Wack and Rappuoli, 2005).

A standard method for the isolation of a monocyte population from mononuclear cells was modified involving a second Percoll gradient after the Ficoll gradient, result in the enhanced monocyte population of high purity (> 90%). Increase in the expression of the DC1a+ molecule was found similar in both induced with tumour necrosis factor (TNF)-α or prostaglandin E (PGE_1) and those obtained after induction with TNF-α + PGE_1 or lipopolysaccharide (LPS). DC maturation can be triggered by LPS, because of its high toxicity, cannot be used in clinical assays. Analysis by protein profiling showed the expression of the co-stimulatory molecules (DC80, DC40 and DC83), the combined activity of TNF-α + PGE_1 is more efficient when compared to each of these agents alone. These results explain the fact that TNF-α, PGE_1 and LPS induce DC maturation by different mechanisms. The stimulus used dictates the maturation of DCs (Pereira, 2005).

DCs may activate and differentiate native CD4+ T cells into Th1 or Th2 phenotypes when bacterial LPS or allergen is present depending on microenvironment. The combined effect of Ags with TLR ligands such as LPS

enhances DCs activities compared to Ag alone. However, it is not clear to which direction CD4+ T cells will be polarized into in such combinations. The response of CD4+ T cell to DCs loaded with ovalbumin (OVA) or endotoxin depleted ovalbumin (OVA_{ED}) vs. LPS-stimulated DCs. Upon exposure to Ags, DCs became matured as indicated by up-regulation of MHC and costimulatory molecules and increased secretion of IL-10 and IL-12. Interestingly, the levels of CD40 expression, and IL-10 and IL-12 productions appeared to be correlated with LPS levels. LPS-stimulated DCs induced nativeCD4+ T cells to produce more IFNγ while OVA_{ED}-stimulated DCs induced T cells to produce more IL-4. Addition of LPS into OVA_{ED}-stimulated DCs during T cell activation compromised the production of IL-4. These results suggest that DC maturation can be polarized into type 1 (LPS-stimulated) or type 2 (Allergen-stimulated) DCs influenced by the nature of Ags. Types 1 DCs promote Th1 while Type 2DCs promotes Th2 immune responses. The complexity of the mechanism of DC maturation depends on the different stimuli and can modulate the plasticity of these cells *in vitro* or *ex-vivo*. This plasticity of DCs provides flexibility for applications in the development of DC-based vaccines (Paiboon, 2016).

Dendritic Cell vaccination against Cancer: Numerous clinical studies to evaluate therapeutic vaccination in cancer during the past two decades assess the desired properties of vaccine-elicited $CD8^+$ T cells associated with the rejection of cancer (Appay, 2008). These include:

- Expression of high T-cell receptor (TCR) affinity and T cell avidity for peptide MHCs on tumour cells (Appay, 2008),
- High amounts of granzymes and perforin (Appay, 2008),
- The expression of surface molecules (e.g., CXCR3) into the tumour leading to T cell trafficking (Mullins, 2004); persistence in the tumour site e.g., integrins CD103 (Le Floc'h, 2007) and CD49a (Sandoval, 2013), and
- Less expression of inhibitory molecules e.g., cytotoxic T-lymphocyte antigen (CTLA)-4 (Peggs, 2009) or PD-1 (Freeman, 2006) and high expression of costimulatory molecules (e.g., CD137 (Wilcox, 2002).

The induction of such $CD8^+$ T cells by the immune system components include:

- The binding of Ag by appropriate APCs (Joffre, 2012; Lizée, 2013);
- The expression of $CD4^+$T cells producing cytokines helping $CD8^+$ T cell multiplication and differentiation, e.g., IL-21 (Spolski and Leonard, 2008).

Numerous ways of therapeutic vaccination against cancer are currently being pursued (Finn, 2008). Searching for "cancer vaccines" at http://www.clinicaltrials.gov as of Sep, 2016 yields total cancer studies taken up were 56857. Out of which studies concerned to cancer vaccines was 281 and DC Tumour studies was 12. A commonality in these studies, and a crucial step in vaccination, is the efficient presentation of cancer Ags to T cells. Yield of improved therapeutic vaccines may happen because DCs are the efficient APCs (Banchereau and Steinman, 1998); their diversity exploited in means of both plasticity and subsets.

Tumour-derived peptides via MHC class I are presented by CTL differentiation program upon encounter with DCs initiated by Native $CD8^+$ T cells. This is assisted by costimulation intervened by CD80, CD70, and 4-1BB and by DC-derived cytokines such as IL-15. XCR1 chemokine secreted by DCs provides the interaction with native $CD8^+$ T cells. Transforming growth factor (TGF)-β expressed by DCs is critical for $CD8^+$ T cells to express CD103 and acquire a mucosal phenotype. $CD8^+$ T cell differentiation, especially generation of memory, is relied on the quality of $CD4^+$ T cell help. The latter one is partially dependent on the IL-12 secreted by DCs. $CD4^+$ T cells producing IFN-γ and/or IL-21 can help $CD8^+$ T cell expansion and differentiation. Regulatory T-lymphocytes (T_{reg}) cells might play a critical role during the selection of high-avidity $CD8^+$ T cells. This might be leading to the crosstalk between DCs and $CD4^+$ T cells where $CD4^+$ T cells control DC functions. There, T_{reg} cells can suppress DCs via IL-10 production and also regulate the production of chemokines, thereby limiting the interactions between DCs and low-avidity T cells. $CD4^+$ T cells can also provide DC maturation signals via CD40.

Dendritic cells preparation: DCs could be derived from peripheral blood or bone marrow. Peripheral blood mononuclear cells (PBMCs) are the main stream to generate DCs by stimulating GM-CSF and IL-4 (Gustafson, 2008). TNF-α and IL-1β were added by some investigators before DC maturation (Yamanaka, 2009), and IL-1β, TNF-α, IFN-α and IFN-β were applied to generate αDC1 which could initiate more effective anti-tumour immune response (Okada, 2007; Fujita, 2009). For patients without sufficient tumour tissues for tumour Ags, transfection with mRNA or DNA into DCs to express more cytokines and co-stimulatory molecules might be feasible (Baral, 2014).

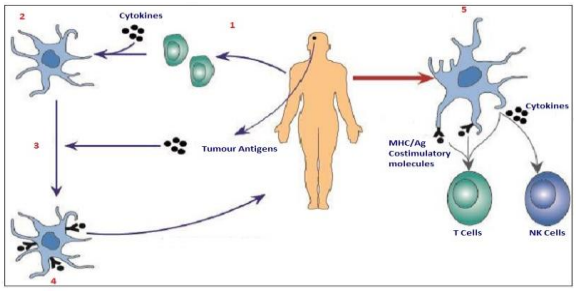

Figure.2. DCs-based immunotherapeutic strategies (Source: Chao, 2015)

- to harvest peripheral blood mononuclear cells,
- to generate mDCs with cytokine stimulation,
- to mature DCs by loading tumour Ags,
- to transfer activated APCs back to the patients,
- to stimulate robust anti-tumour immune effector cells such as NK cells and T cells.

Antigen Loading: For Ag loading, peptide-pulsing, transfection/transduction, and protein-pulsing continue to be used, as well as tumour lysate loading. The simple step approach has many rounds of freezing (in a dry ice/ethanol bath at 80° C) and thawing (by37°waterbath). This method can break open cells by manner mimicking necrosis and allow subsequent tumour protein isolation. However, there are other approaches. Upon exposure to ultra violet and gamma irradiation, tumour cell has shown to mimic apoptosis, which delivers different signals to DC than necrotic cells. For DC vaccine loading, tumour treatment with hypo chlorous acid before lysate purification was tested (Chiang, 2013), and this method of oxidation and rapid necrosis may be superior (Fig.2).

The changes in tumour Ag expression when tumours are cultured in conditions of hypoxia (5% instead of 20%) may be specifically mimicking *in situ* hypoxic tumour oxygen levels (Olin, 2010) is another important element. Such improved Ag preparation approaches may yield improved clinical outcomes.

Route of delivery: Based on data demonstrating that DC vaccines delivered intradermally show very low level (<2%) migration to lymph nodes (based most often on 111In-labeling (Morse, 1999; DeVries, 2003), and that ultrasound guided intra-nodal delivery has a risk of the vaccine being injected into fat instead of a cellular area, other delivery routes were tested. The results showed variation in the induction of T cell response between mice and humans, and in patients, and all the methods have proven to be immunogenic. Identification of superior routes of delivery remain unknown without higher rates of objective clinical responses. There was no perfect correlation between phenotypic measures, like CCR7 level on the DC surface and subsequent migration so far (Quillien, 2005). It was assumed that the maturation cocktail used impacts migration (Quillien, 2005). Magnetic resonance imaging (MRI)-based DC vaccine labels were tested (Helfer, 2010) and prolonged, semi-continuous intra lymphatic delivery of DC was tested (Kalinski, 2011), continued efforts attracting DC migration *in vivo* and optimizing delivery routes may yield more potent DC vaccines. A few such DC trials are underway.

DC vaccine can be delivered subcutaneously or intramuscularly after maturation. However, the post-injective lymphatic return rate (LRR) is always limited. So, ultrasound-guided intranodal injection (Okada, 2011) and injection through Ommaya reservoir connected to tumour area or ventricles was also tried (Yamanaka, 2005; Wheeler, 2010; Pellegatta, 2010). Severe complications like edema after the administration of DCs was noticed. There is no guideline for the course of vaccine; it was deemed that both low quantity and limited dosages were likely to limit DCs' effect (Okada, 2009).

Combination with adjuvants or other therapies: Not only the tumour Ag, but also the specific adjuvant, in conjunction with other treatment procedures as well, should be considered during the design and evaluation of tumour vaccine. Traditionally Bacillus Calmette Guerin (BCG) and Freund's adjuvant were used as adjuvants. New effective and targeted adjuvants, such as cytokines (IL-12), MF59, cholera toxin B (CTB), AS04 and TLR agonist (CpG oligonucleotides, Imiquimod and poly I:C), have demonstrated some efficiency and safety (Maughan, 2015). Development of adjuvants is a major issue in tumour vaccine strategy to improve immunogenicity. The main concern is that there are no effective universal adjuvants for tumour immunization protocols. Adjuvants can enhance specific type of immune responses albeit cellular or humoral of poorly immunogenic Ags. DCs could elicit cellular immune reponse (Draube, 2011), induce tumour-specific cytotoxic T cells and also enhance NK cell immunity (Lion, 2012). Therefore adjuvants that can boost specific type of cell- mediated vaccine responses would be more helpful. This may also require temporal events that reflect tumour status and vaccine effectiveness during the course of immunotherapy. Chemotherapy and radiotherapy are the main adjuvant treatment modalities for high grade gliomas after surgical resection. Immunotherapy has good synergism with chemotherapy and radiotherapy, and has speculated that apoptotic tumour cells after adjuvant therapies might provide abundant tumour Ags to DCs (Prins,

2011). CD8+T cells could increase significantly in glioblastoma multiformae (GBM) after treatment with the combo of DC vaccine and temozomide (Ardon, 2010). As for the time frame to administrate DC vaccines, vaccine was recommended first, since ionizing radiation will not only kill the tumour cells, but also may impair the immune responses (Walker, 2008; Chang, 2011).

Evaluation of curative effect: No standard was proposed yet to assess the activity of DC vaccine. Overall survival and progression free survival (PFS) are often adopted to evaluate the effect. Interestingly, overall survival seems to have more chance to be prolonged by vaccine than PFS. Age, tumour invasiveness status and the degree of tumour resection have been proven to be independent prognostic predictors (De Vleeschouwer, 2008). The bias of patients' selection with potential good prognosis will significantly sway the survival time. Maximal tumour resection still dominates other considerations, and obviously DC vaccines are inept to big tumour burdens. So the strategy is to remove all clinically evident tumour, and then to vaccinate to inhibit subclinical micro-metastatic disease. DCs vaccine seems effective on the improvement of overall survival. In the two trials reported consecutively, the median overall survival of GBM patients received DCs vaccine primed with acid-eluted tumour peptides was much longer than the control patients (Yu, 2001, 2004). In other studies, the improvement of overall survival in pediatric patients with high grade gliomas treated with DCs vaccine was not observed, while an effective responses with an significant increase of overall survival was observed in adult patients (Liau, 2005) and Hilko, (Ardon, 2010; Ardon, 2010), and the survival rate after five years were 18.8% for newly diagnosed high grade gliomas (Chang, 2011). In the randomized clinical trial (RCT) study, most patients in the experiment arm received salvage therapy. As a group, the numbers of enrolled patients and RCT studies were so limited, phase II/III RCT and long-term evaluation are urgently needed (Cho, 2012), and the results showed that the vaccinated patients got an impressive improvement of overall survival. Overall, DCs based vaccine appears to benefit certain high grade glioma patients. As for the immunological index, tumour infiltrating lymphocytes (TILs) is one objective indicator, which has been verified in several clinical trials (Liau, 2000; Yu, 2001; Okada, 2007; Chang, 2011; Walker, 2008). Delayed type hypersensitivity (DTH) was observed in a number of clinical trials (Yamanaka, 2003; Rutkowski, 2004; De Vleeschouwer, 2004; Yamanaka, 2005; De Vleeschouwer, 2008). It was found that the PFS and overall survival of the patients with the elevation of IFN-γ (more than 1.5 times in serum) were longer than those without IFN-γ elevation after vaccination. Other factors such as the dosage of vaccine, regulatory T cells, and the ratio of Th1/Th2 showed no correlations with the prognosis (Walker, 2008; Prins, 2011). Melanoma antigen family-A (MAGE)-1 specific CTL as a marker was used to address the Ag specific immune response of the patients (Yu, 2004).

Evaluation of side effect: DC vaccine for high grade gliomas seems to be well tolerated across all variations in all clinical trials. The adverse events associated with immunotherapy were evaluated by the response evaluation criteria in solid tumours (RECSIT). Skin itch, erythema on the injection location, headache, fever, and lymphopenia were observed in some cases. Notably, seizure was found in the study with a frequency of 16.6% (Chang, 2011). Since there is no comparison with other treatment modalities, no statistical analysis or definite conclusion could be drawn. By and large, no evidence of toxicity or severe side effect was observed.

2. CONCLUSION

DC vaccine is a promising approach in cancer therapy. DC generation protocol, route of Ag loading onto DCs, type and dose of tumour Ags, DC maturation status, DC migration potency, amount of cells administrated, number of injections, time of injections, and the route of vaccine administration can be optimized to improve clinical efficacy of DC vaccines as it is observed in more recent studies. Co-administration of agents that promote DCs survival and their immune stimulatory function may be beneficial. Furthermore, combinational therapy and modulation of the immunosuppressive environment of the tumour, which suppresses antitumour activities of DCs, can increase DC vaccine efficacy.

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