Review

Dendritic Cell as Therapeutic Vaccines against Tumors and Its Role in Therapy for Hepatocellular Carcinoma

Kang Sun[1, 2], Liang Wang[1] and Yanyun Zhang[2, 3]

Dendritic cells (DCs) are the most potent professional antigen-presenting cells, and capable of stimulating naïve T cells and driving primary immune responses. DCs are poised to capture antigen, migrate to draining lymphoid organs, and after a process of maturation, select antigen-specific lymphocytes to which they present the processed antigen, thereby inducing immune responses. The development of protocols for the *ex vivo* generation of DCs may provide a rationale for designing and developing DC-based vaccination for the treatment of tumors. There are now several strategies being applied to upload antigens to DCs and manipulate DC vaccines. DC vaccines are able to induce therapeutic and protective antitumor immunity. Numerous studies indicated that hepatocellular carcinoma (HCC) immunotherapies utilizing DC-presenting tumor-associated antigens could stimulate an antitumour T cell response leading to clinical benefit without any significant toxicity. DC-based tumor vaccines have become a novel immunoadjuvant therapy for HCC. *Cellular & Molecular Immunology*. 2006;3(3):197-203.

Key Words: dendritic cell, vaccination, hepatocellular carcinoma

Introduction

Dendritic cells (DCs) firstly were visualized as Langerhans cells (LCs) in the skin in 1868 by Paul Langerhans, then Ralph Steinman and Cohn identified DCs from mouse spleen in 1973 (1). DCs are the most potent antigen-presenting cells (APCs) with the unique ability to initiate and maintain primary immune responses when pulsed with antigens (2-4). Owing to their unique capacity to regulate T cell immunity, DCs are increasingly used as adjuvants for vaccination, which present essential component of any vaccination strategy. The goal of vaccination approaches in human cancer is to induce tumor-specific, long-lasting immune response that leads to tumor elimination. The induction of tumor immunity can be viewed as a three-step process that includes: 1) presentation of tumor associated antigens (TAAs); 2) selection and activation of TAA-specific T cells as well as non-antigen-specific effectors; 3) homing of TAA-specific T cells to the tumor site and recognition of restriction. DCs are important in inducing cellular and humoral immunity and can also activate natural killer (NK) cells and natural killer T (NKT) cells (5, 6). In addition, adaptive immune responses which are induced, coordinated and regulated by DCs seem more significant in tumor immunity (3, 7). So, DCs can conduct all of the elements of the immune orchestra, and they are therefore a fundamental target and tool for vaccination.

Inaba and coworkers first demonstrated that the injection of DCs, charged with antigen *ex vivo*, could sensitize normal mice to protein antigens (8). Subsequently, numerous studies in mice showed that DCs loaded with tumor antigen are able to induce protective antitumor responses and produce therapeutic immunity to established tumors (9). The immunogenicity of antigens delivered by DCs has been shown in patients with cancers (10). A number of clinical trials have utilized tumor antigen-loaded DCs as vaccines in human and some clinical and immune responses without any significant toxicity have been observed (11-13).

Hepatocellular carcinoma (HCC) is a major malignancy in Asian countries such as China and Japan, and usually causes death within a few weeks or months of detection. Chronic viral hepatitis patients, especially hepatitis B or C patients, often fall victims to liver cirrhosis and subsequent HCC (14, 15). As the efficacy of current therapeutic regimens such as surgery, chemotherapy and radiotherapy is limited, tumors tend to relapse or metastasize easily. Therefore, immunotherapy becomes the important means for treating HCC. It is reported that Hsp70-peptide complexes derived from human HCC cells can serve as a potent tumor

[1]Department of General Surgery, the First Affiliated Hospital of Soochow University, Suzhou 215006, China;

[2]Shanghai Institute of Immunology, Medical School of Shanghai Jiaotong University, Shanghai 200025, China;

[3]Corresponding to: Dr. Yanyun Zhang, Shanghai Institute of Immunology, Medical School of Shanghai Jiaotong University, 225 South Chongqing Rd, Shanghai 200025, China. Tel & Fax: +86-21-6385-2705, E-mail: yyzhang @sibs.ac.cn.

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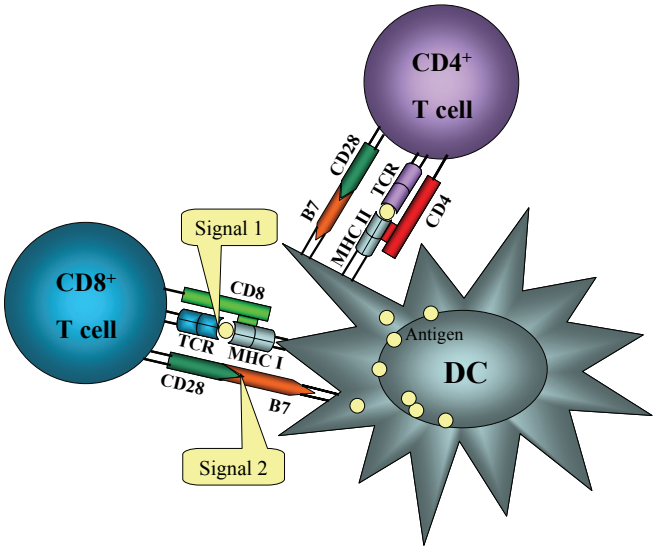

Figure 1. Generation of an adaptive immune response requires two independent signals. Binding of T-cell receptor (TCR) to the antigen- MHC complex on the DC delivers a signal (Signal 1) that can induce activation and expansion of T cells when the co-stimulatory signal (Signal 2) is given by binding of CD28 to B7 molecules.

antigen source for pulsing DCs. The pulsed DCs are effective in activating specific T-cell responses against HCC cells (16). It has been demonstrated that DCs are critical for the development of tumor-specific immune responses, and DC-based vaccination strategies have yielded encouraging results in clinical trials (17).

Biological characters of DCs

Murine and human DC subsets

In 1990s, culture systems were discovered that produced large amounts of mouse and human DCs, thereby accelerating the study of DCs and making their clinical use feasible (18).

Depending on origin, function, and localization，murine DCs could be subdivided into at least two populations: myeloid and lymphoid DCs, which have been distinguished by the expression of CD8α (7). The $CD8\alpha^-$ DC subpopulation, which comprises $CD4^-$ and $CD4^+$ subsets, lacks DEC-205, but expresses CD11b, CD11c, and major histocompatability complex (MHC) class II on the cell surface, was thought to be myeloid in origin, are localized in the marginal zone of the spleen and lymph nodes, and can be induced to migrate to the periarteriolar lymphatic sheaths under stimulation with inflammatory signals such as lipopolysaccharide (LPS). $CD8\alpha^+$ DC lacking myeloid marks such as CD11b and bearing CD11c, DEC-205, MHC class II, was thought to be lymphoid in origin. They are present in all mouse lymphoid organs in the T-cell rich areas of the periarteriolar lymphatic sheaths and constitute the major population in the thymus (19).

In human peripheral blood there are two distinct types of DC precursors, which are myeloid monocytes (pre-DC1s) and plasmacytoid DC precursors (pre-DC2s) (20). CD40 ligand (CD40L)-activated myeloid DC1s derived from monocytes produce a large amount of interleukin (IL)-12 and preferentially induce T helper (Th) 1 development, whereas CD40L-activated lymphoid DC2s derived from plasmacytoid precursors produce lower amounts of IL-12 and preferentially induce Th2 development (20, 21). Human DC are characterized by the surface expression of high amounts of MHC class II molecules and the absence of lineage markers such as CD14, CD3, CD19, CD20, CD24, and CD56. The DC phenotype varies with different stages of maturation and differentiation. CD1a is preferentially expressed on human immature myeloid DCs (21).

Antigen-presenting function of DC

In terms of function, the unique and most critical function of DCs is their ability to prime naïve T cell to proteins that require processing into peptides (22, 23). They are the principal stimulators of primary immune responses (24, 25). DCs process internalized antigen and load peptide fragments onto MHC proteins. Peptides associated with MHC proteins are transported to the surface of the cell and externalized for presentation to other immune cells. In addition to presenting antigen, mature DCs express co-stimulatory molecules (CD40, CD80 and CD86) necessary for T cell activation. Generation of an adaptive immune response requires both T cell receptor recognition of the MHC-associated antigen (Signal 1) and interaction of DC co-stimulatory molecules with corresponding T cell ligands (Signal 2) (Figure 1) (26).

DC maturation

Functional maturation culminates with DC residing in T cell-rich areas of lymphoid tissues presenting peptide antigens acquired in the periphery in the context of MHC to passing T cells (27). *In vivo*, the maturation of DCs is closely linked to their migration from the peripheral tissue to the draining lymphoid organs. It is associated with several coordinated events, including the following: (a) loss of endocytic and/or phagocytic receptors; (b) changes in morphology; (c) changes in MHC class II compartments; (d) upregulation of expression of co-stimulatory molecules such as CD40, CD58, CD80 and CD86; (e) a shift in lysosomal compartments, with downregulation of expression of CD68 and upregulation of expression of lysosomal-associated membrane protein 3 (LAMP3); (f) expressing several adhesion molecules including CD2, CD11a, CD54, CD58, and the integrins β1 and β2 (2, 7, 28).

DC migration

Migration is a multistep process that involves the adhesion with endothelial cells and the interaction with physical obstacles. The capacity of DCs to migrate to the sites of inflammation, where they capture the antigen and subsequently migrate to the local lymph nodes, is regulated by the expression of different chemokines and chemokine

receptors (29-31). The accumulation of circulating DCs precursors, which express high levels of CCR6, a receptor for macrophage inflammatory protein 3α (MIP-3α), in the epithelium and in tumors is associated with the production of MIP-3α by these cells. MIP-3α appears to be the most potent chemokine regulating their migration into tissues (32). Immature DCs can produce inflammatory chemokines, including MIP-1α, monocyte chemoattractant protein 1 (MCP-1), MCP-2, and MCP-4 and express receptors for inflammatory chemokines, such as CCR1, CCR2, CCR5, CCR6, and CXCR1 (33). In contrast, maturing DCs acquire the responsiveness to Epstein-Barr virus-induced molecule 1 ligand chemokines (ELC) (MIP-3β) and secondary lymhoid tissue chemokines (SLC) that regulate the trafficking of DCs to the lymphoid vessels and secondary lymphoid organs. DCs entering the lymph node are further directed by the ELC and SLC chemokine gradients to the paracortical T-cell areas and produce chemokines such as DC-CK-1 and MDC, which chemoattract naïve and memory T cells (30, 31) .

DCs vaccines and tumor immunity

The characteristics of DC vaccines

Earlier studies in mice showed that the immune system can recognize and reject tumors (34). In humans, the incidence of some cancers is increased in immunodeficient patients and is increased with age, owing to immunosenescence (35, 36). These observations support the scientific rationale for immunotherapy for cancer. The most attractive strategy is vaccination, which is expected to induce both therapeutic T-cell immunity and protective T-cell immunity (37). Therapeutic cancer vaccines are now being developed that using DC expansion and focusing strategies. Fundamental principles permit this approach: (a) DCs are the most potent APCs; (b) DCs are important for the induction of tumor-specific immune response, as they have a unique capacity to activate a broad range of immune effector cells including T cells, B cells, NK cells and NKT cells; (c) DCs are capable of inducing cell-mediated immunity through the promotion of Th1 responses; (d) DCs directly stimulate cytotoxic T lymphocytes (CTLs); (e) cell-mediated immunity is essential for tumor defense (38). Given these characteristics, many investigators have hypothesized that DCs can be used to immunize patients against their own cancers.

Manipulation of DCs

Functions of DCs are related to their complexity involving their type, maturation stage and the optimal maturation stimuli. In clinic, the most popular way to generate DCs is to culture blood monocytes with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4, which yield a uniform population of immature DCs devoid of LCs. This contrast with hematopoietic stem cells that, when cultured with GM-CSF and tumor necrosis factor-α (TNF-α), yield preparations that include both LCs and interstitial DCs as well as a third population of cells also with DC function. While GM-CSF/IL-4-induced DCs require additional maturation factors, $CD34^-$ DCs do not, as they are generated in the presence of TNF-α, a DC activation factor. Indeed, immature DCs are weak immunogens and can be tolerogenic. Injection of immature DCs does not lead to significant immune responses, but leads to the inhibition of $CD8^+$ T cell immunity to viral peptide with the appearance of peptide-specific IL-10 producing T cells in healthy volunteers (39, 40). In contrast, mature DCs induce functionally superior $CD8^+$ T cells and polarize $CD4^+$ T cells towards interferon γ (IFN-γ) production (41). Thus, maturation is a critical parameter for the use of DCs in active immunization of patients. As the optimal type of DCs for vaccination still remains to be determined, numerous researches should be done to assess the immunological and clinical efficacy of various DC vaccines (42).

Strategies for delivering tumor antigens to DCs

The nature of the TAA and the optimal methods for DC loading are likely to constitute the most crucial parameter that needs to be analyzed. In clinical trials, there are some different strategies that are now being applied to deliver antigens to DCs.

Loading MHC class I and class II molecules at the cell surface of DCs with peptides derived from defined antigens is the most commonly used strategy for DC-based vaccination (43, 44). The first clinical studies were performed primarily in melanoma patients using DCs pulsed with peptides (45). Brossart and his colleagues demonstrated that patients with advanced breast and ovarian cancer could be efficiently vaccinated with autologous mature monocyte-derived DCs produced *in vitro* with GM-CSF, IL-4, and TNF-α and pulsed with HER-2/neu or MUC1-derived peptides even after high-dose chemotherapy (46). These studies revealed such vaccines can induce antigen-specific CTL responses successfully *in vivo*. Some of these peptide epitopes have been modified using single amino acid substitutions in the anchor sites of the peptide to give stronger binding affinity for the MHC class I molecules, thus increasing their ability to induce CTL responses. Although this technique is important for proof-of-principle studies, the application of antigen peptides has limitations: (a) peptide restriction to a given HLA type; (b) induction of CTL responses only; (c) limitation of the induced responses to defined TAA. Indeed, a common drawback to DC-based immunotherapy protocols of today is that it remains to be determined whether, or which of, the defined TAA peptides represent rejection antigens *in vivo*.

Another strategy is to use full-length native or recombinant proteins as an antigen, thus allowing the induction of immune responses against different epitopes that could be potentially restricted by multiple HLA alleles. Furthermore, the antigen-processing and presenting machinery would direct responses to important and immuno-dominant epitopes including both MHC class I and II restricted peptide antigens (47).

The third most commonly used antigen delivery method is RNA transfection of DCs, which has the potential of giving access of a broad spectrum of tumor epitopes to the

MHC class I presentation pathway. A recent trial in metastatic renal cell cancer using immature DCs transfected with RNA derived from renal cancer demonstrated that the majority of evaluable subjects had expansion of some tumor-specific T cells against a broad range of targets (48, 49).

An alternative strategy involves the gene-based delivery of TAA to DCs that does not require prior knowledge of the patients MHC type or the relevant T-cell peptide epitope. Tumor derived antigens may also be delivered to DCs by fusing DCs and tumor cells. An intriguing example is a recently published study in metastatic renal cancer where in 4 patients vaccination with allogeneic DC-autologous tumor hybrids led to resolution of all metastatic tumor lesions (50).

Route of administration

The route of administration in immunization protocols is an important consideration. In the case of human, *in vitro*-generated, antigen loaded, human DC injected intravenously (*i.v.*) localized to the lungs and then redistributed to the liver, spleen, and bone marrow, but were not detected in lymph nodes or tumors (51). A small percentage of DCs injected intradermal (*i.d.*), however, migrated rapidly to the regional lymphatics in some individuals. No lymph node activity was detected after subcutaneous (*s.c.*) injection (52). In a clinical trial for prostate cancer, induction of IFN-γ production, indicative of Th1 type immunity, was seen only with *i.d.* and intralymphatic (*i.l.*) routes of administration, and not the *i.v.* route (53). The evidence thus points to the superiority of both *i.d.* and *s.c.* routes of administration over the *i.v.* route, with *i.d.* appearing better than *s.c.* for directing lymph node migration. Intranodal (*i.n.*) and *i.l.* administrations may also be justified, but are notably more difficult (53).

Host-related factors

In clinic, the stage of disease can affect the efficacy of any cancer vaccine. Even the best immune response might ultimately succumb to an overwhelming tumour, so to realistically judge the clinical efficacy. It is important that trials now are carried out in patients with less advanced disease. Indeed, when disease is limited, it might be the best time for therapeutic vaccination with DCs, or any other immunotherapy. However, the lack of measurable disease in such studies makes it difficult to assess objective clinical responses, which are the common parameters in measuring vaccine efficacy, even in Phase I clinical trials.

DC-based vaccines for HCC therapy

DC and HCC

HCC is one of the major malignancies worldwide (54-56). Highest risk areas are in East Asia especially in China, Central Africa and some countries of West Africa. Surgical resection and liver transplantation are regarded as the only potentially curative therapies. Screening programs based on abdominal ultrasound (US), computer tomography (CT) and serum α-fetoprotein (AFP) determination have improved HCC detection in high risk patients at a relative early potentially curable stage. However, due to advanced or decompensated liver cirrhosis, co-morbidity and multicentricity of the tumor lesions, 70%-80% of HCC patients are inoperable at the time of diagnosis (57). Although transarterial chemoembolization (TACE), radiofrequency thermal ablation (RFTA), laser and microwaves therapies (58) applied widely in clinic, the control of HCC at the advanced stage remains difficult. Therefore we need to introduce a novel therapeutic strategy in order to ameliorate therapy for HCC, improve the 5-year survival rate of patients with HCC.

According to research in recent years, HCC patients had some immunity dysfunctions, including innate and adaptive immune responses (59). Nakamoto et al. showed that the number of DCs was decreased or DCs displayed low function (14). DCs function was also suppressed in patients with HCC with hepatitis B and C virus infections (60). Decreased function of DCs might allow the development of tumor or the tumor itself might suppress the function of DCs (61). DCs are considered unique APCs for their highly efficient capability of priming naïve T cells *via* direct cell-cell interactions and cytokine production, stimulating the propagation of naïve T cells, and playing a critical role in innate and adaptive immunity. They have been considered as one of the most potent regulators of the immunological mechanism, and may be effective for the prevention of carcinogenesis, as well as for the treatment of already developed cancers (62, 63). So the utilization of DC-based tumor vaccines, as immuno-adjuvant therapy for HCC, is extremely appealing.

Clinic trials

Recent years, several studies have been performed testing infusions of DCs. In each case, the DCs were pulsed with HCC or HCC lysate to prepare a patient specific vaccine to activate an immune response to any and all antigens in HCC, both tumor-specific and tumor-associated.

An example of successful vaccination using immatured DCs loaded with HCC lysate has been reported by Andrew Ladhams and coworkers (59). Two patients with metstatic HCC were treated with immature DCs, grown in GM-CSF and IL-4, the most common and straightforward method for generation of DCs. Of the two patients, one had slowed tumor growth compared with their pretreatment status. In general, immature DCs are more effective at antigen acquisition than at T-cell stimulation. Therefore, immature DCs are optimal for mixing with tumor or tumor lysate to allow uptaking and processing of large amounts of tumor antigens. However, more recent data indicate that potent T-cell activation is the result of a maturation step of DCs (after antigen uptake), which reduces phagocytosis activity and upregulates T-cell activation cell surface molecules, improves trafficking to lymph nodes, and increases production of T-cell-activating cytokines such as IL-12. However, the degree of maturation required for optimal T-cell activation and the extent to which maturation may occur *in vivo* after injection of DCs are unknown.

Whether DC-based vaccination therapy can be effective as treatment of primary HCC was demonstrated in a recent report. Iwashita (64) used DCs pretreated with GM-CSF

and IL-4, then loaded with HCC lysate, stimulated with TNF-α, and mixed with keyhole limpet hemocyanin (KLH) before injection. The KLH was added as a foreign protein that activates non-antigen-specific Th cells and serves as a marker of successful immunization by subsequent DTH testing. In this trial, ten subjects with unresectable HCC were treated, positive DTH responses to the KLH (indicating successful vaccination) developed in seven patients, and one subject had a mixed tumor response.

Is there any toxicity effect on the liver with DC-based vaccination? It was shown that vaccination of HCC patients with monocyte-derived DCs pulsed with tumor-eluted peptides was safe. Liver fuction tests showed no difference before and after DC vaccination. The first 14 patients underwent pulsed therapy with five courses of DC vaccination intravenously at weekly intervals. The other 17 patients underwent monthly boost vaccinations after the initial pulsed therapy. Among the 31 patients, 4 (12.9%) exhibited partial response to DC vaccination, 17 patients (54.8%) had stable disease and 10 patients (32.3%) had progressive disease (65). It seems that pulsed DC vaccination followed by boosters can provide better clinical survival for advanced HCC patients without any significant toxicity.

Future challenges

Although several studies had indicated that the administration of DC-based HCC vaccines has antitumor activity, the prospects for notable success in the immunotherapy of human HCC are currently impossible because there are fundamental questions in immunology and hepatology that need more precise answers: 1) What types of DCs should be used and how should these be presented to the host for induction of cancer immunity? 2) How are we to obtain adequate amounts of immunogenic tumor antigens? 3) How do we obtain insights about the hepatic immunological microenvironment in HCC patients? Further understanding of the role of the immunological microenvironment of the liver in DC maturation is critically important (42, 66). The development of successful DC-based therapy will mainly depend on the proper understanding of the hepatic microenvironment in patients with HCC, which is difficult to access, and may be highly variable among patients with HCC.

Summary

DC is an attractive target for therapeutic manipulation of the immune system to enhance insufficient immune responses in cancer. It will be more convenient for us to produce DC-based vaccines for the clinical trial and treatment because of matured DCs isolation and cultivation methods. However, the complexity of the DC system requires rational manipulation of DCs to achieve protective or therapeutic immunity. So, further research is needed to analyse the immune responses induced in patients by distinct *ex vivo*-generated DC subsets which are activated through different pathways. These *ex vivo* strategies should help to identify the parameters for *in viv*o targeting of DCs, which is the critical step in the development of DC-based vaccination. As to DC-based vaccines against tumors may be too inefficient to significantly impact the advanced stages of HCC, it will take a long time to be applied for clinical treatment of HCC extensively. In the long run, with the more study of DC system and liver microenvironment, DC-based vaccines will become one of the most effective therapeutic methods for HCC.

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