Review

Dendritic Cells as Vectors for Immunotherapy of Tumor and Its Application for Gastric Cancer Therapy

Yugang Wu[1], Liang Wang[1, 3] and Yanyun Zhang[2]

Dendritic cells (DCs) are recognized as the most potent antigen-presenting cells (APCs) with the ability to stimulate naïve resting T cells and initiate primary immune responses. DCs are poised to capture antigen (Ag), migrate to draining lymphoid organs, and, after a process of maturation, select Ag-specific lymphocytes to which they present the processed Ag, thereby inducing immune responses. Numerous studies indicated that immunotherapies utilizing DC-presenting tumor-associated antigens can safely be administered to cancer patients and induce significant immunologic and clinical responses. Moreover, it has been demonstrated that DCs are related to clinical stage, invasion, metastasis and prognosis of gastric cancer. DC-based tumor vaccines become a new effective immunoadjuvant therapy for gastric cancer. *Cellular & Molecular Immunology*. 2004; 1(5):351-356.

Key Words: dendritic cell, vaccination, gastric cancer

Introduction

Dendritic cells (DCs) are the professional antigen-presenting cells (APCs), specialized to initiate and regulate immune response (1). 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 (2). DCs have been shown to be a key cell population in the pathway of antigen capture and presentation to T cells, having the unique ability to directly prime naïve $CD4^+$ and $CD8^+$ T cells, through their ability to efficiently uptake, process, and present antigen to major histocompatibility complex (MHC) class I and II molecules, together with costimulatory molecules such as B7 and CD40 (3). The study of DCs has been hampered by two main factors. First, they are present in the blood and tissues in very low amounts. Among peripheral blood mononuclear cells (PBMCs) they account for less than 1%. Second, there are as yet no known DC-specific cell surface antigens with which to aid their positive identification. Despite of these difficulties, in recent years, their importance within the immune system is now generally accepted, and progress is being made towards an understanding of DCs, particularly with respect to their role in antitumor immunity (4). Iaba and coworkers (5) first demonstrated that the injection of DCs, charged with antigen *ex vivo*, could sensitize normal mice to protein antigens. Subsequently, numerous studies in mice showed that DCs loaded with tumor antigens were able to induce protective antitumor responses and produce significant therapeutic immunity to established tumors (6). The immunogenicity of antigens delivered on DCs has now been demonstrated in healthy human volunteers (7). A number of clinical trials have utilized tumor antigen-loaded DCs as vaccines in humans and some clinical and immune responses without any significant toxicity have been observed (8, 9).

Gastric cancer is one of the most common cancers in our country. Despite the efforts to introduce new treatment modalities such as surgery combined with chemotherapy, hyperthermia or radiation therapy, control of gastric cancer at the advanced stage remains difficult. However, the utilization of antitumor T cells as immunoadjuvant therapy for gastric cancer, is extremely appealing. Hoshino, et al. (10) have reported that MHC class I-restricted cytotoxic T lymphocytes (CTLs) from gastric cancer patients could react specifically against autologous tumor cells. It has been demonstrated that tumor-associated-antigen (TAA) can be recognized by gastric cancer specific CTLs (11). Therefore, immunotherapy using anti-gastric cancer CTLs or CTLs recognizing specific TAAs, naturally presented by

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

[2]Health Science Center of Shanhai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200025, China.

[3]Corresponding to: Dr. Liang Wang, Department of General Surgery, the First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China. Tel and Fax: +86-512-653-02888, E-mail: liangwang89@163.com.

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Abbreviations: DC, dendritic cell; APC, antigen-presenting cell; MHC, major histocompatibility complex; CTL, cytotoxic T lymphocyte; PBMC, peripheral blood mononuclear cell; MIP-1α, macrophage inflammation protein-1α; TAA, tumor-associated antigen; IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor; TNF-α, tumor necrosis factor-α.

the tumor cells, could potentially be ideal candidates for immunotherapeutic approaches for gastric cancer. DCs are the professional APCs, and have been demonstrated to critical for the development of tumor-specific immune responses (12). DCs-based vaccination strategies have yielded encouraging results in experimental tumor models and clinical trials (13, 14).

Dendritic cells

Ontogeny of DCs

DCs originate from hematopoietic stem cells (4). In mice they have been subdivided to at least two populations depending on their origin, function and localization, myeloid DCs ($CD8\alpha^-$DCs) and lymphoid DCs ($CD8\alpha^+$DCs), which have been distinguished by the expression of CD8α (3). The $CD8\alpha^-$DC subpopulation, which comprises $CD4^-$ and $CD4^+$ subsets, lacks DEC-205, but expresses CD11b, CD11c, and MHC class II on the cell surface, was thought to be myeloid in origin. They are localized in the marginal zone of the spleens and lymph nodes, and can be induced to migrate to the periarteriolar lymphatic sheaths under stimulation with inflammatory signals such as LPS (lipopolysaccharide). $CD8\alpha^+$DC subpopulation 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 (15).

In human peripheral blood there are two distinct types of DC precursors, myeloid monocytes (pre-DC1s) and plasmacytoid DC precursors (pre-DC2s) (16). CD40 ligand (CD40L)-activated myeloid DC1s derived from monocytes produce a large amount of interleukin 12 (IL-12) and preferentially induce Th1 development, whereas CD40L-activated lymphoid DC2s derived from plasmacytoid precursors produce lower amounts of IL-12 and preferentially induce Th2 development (16, 17).

Function of DCs

DCs can induce, sustain and regulate immune responses. Four stages of their development have been delineated: (a) bone marrow progenitor; (b) precursor DCs, which are patrolling through blood, lymphatics as well as lymphoid tissues; (c) tissue-residing immature DCs, which possess high endocytic and phagocytic capacity permitting antigen (Ag)-capture; and (d) mature DCs, present within secondary lymphoid organs, expressing high levels of costimulatory molecules permitting Ag-presentation (18).

Immature DCs

Immature DCs are characterized by high ability of antigen uptake but low T-cell stimulatory capacity. They possess several mechanisms that enable them to capture and present antigen. First, they can take up particles and microbes by phagocytosis (19). Second, they can form large pinocytic vesicles in which extracellular fluid and solutes are sampled, a process called macropinocytosis (20). And third, they express receptors that mediated adsorptive mannose endocytosis, including C-type lectin receptors and DEC-205, as well as Fcα and Fcε receptors (21). Immune DCs also can internalize the peptide loaded heat shock proteins gp96 and Hsp70 though presently unknown mechanisms (22, 23).

Mature DCs

The antigen/pathogen induces the immature DCs to undergo phenotypic and functional changes that culminate in the complete transition from Ag-capturing cell to Ag-presenting DCs. DCs mature is intimately linked with their migration from the peripheral tissue to the draining lymphoid organs (24). Upon maturation, DCs lose endocytic/phagocytic receptors, and increase their ability to migrate. DCs reach the T-cell rich areas in the regional lymph nodes and provide a stable source of antigen. Mature DCs resist the suppressive effects of IL-10, but synthesize high level of IL-12 (25-27) that enhance both innate (NK cells) and acquired (B and T cells) immunity. DCs also express many accessory molecules that interact with receptors on T cells (28, 29) to enhance adhesion and costimulation, for example, LFA (leucocyte function-associated antigen)-3/CD58, ICAM (intercellular adhesion molecule)-1/CD54, B7-2/CD86. All these properties (MHC expression, IL-12 secretion and expression of costimulatory molecules) are upregulated within a day of exposure to many stresses and dangers, including microbial products.

DCs and chemokines

The capacity of DCs to migrate to the sites of inflammation, where they capture the antigens and subsequently migrate to the local lymph nodes, is regulated by the expression of different chemokines and chemokine receptors (30-33). The accumulation of circulating DCs precursors, which express high levels of CCR1, CCR5, CCR6, receptors for macrophage inflammation protein-1α (MIP-1α), MIP-3α, MIP-1β, in the epithelium and in tumors is associated with the production of these chemokines by these cells (34, 35). 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. In contrast, during the maturation process, DCs down-regulate the expression of inflammatory chemokines and their receptors and upregulate the synthesis of constitutive chemokines and the CCR7 receptor. Consequently, maturing DCs acquire the responsiveness to EB-ligand chemokine (ELC) and secondary lymphoid-organ chemokine (SLC) that regulate the trafficking of DCs to the lymphoid vessels (that produce SLC) and secondary lymphoid organs (34-36). Upon contacting with T lymphocytes, DCs receive additional signals provided by the interaction of CD40/CD40L, RANK/TRANCE, 4-IBB/4-IBBL, or OX40/OX40L, which help DCs in terminal maturation.

DCs and tumor immunity

Antitumor function of DCs

Tumor immunity can be initiated by the effectors of innate immunity and further developed by cells of adaptive immunity, with DCs playing a central regulatory role. Several steps are involved: (a) recognition of tumor

molecules by DC precursors, (b) direct and IFN-γ-mediated killing of transformed cells by NK/NK T cells activated by DCs, (c) capture and cross-presentation of released-TAAs by immature DCs, (d) selection and activation of TAA-specific T cells as well as nonspecific effectors including macrophages and eosinophils, and (e) homing of TAA-specific T cells to the tumor site and recognition elements leading to the elimination of tumor cells (24).

However, why tumors may escape immune surveillance? Current concepts explaining lack of effective tumor immunity in a majority of cases include: (a) immunological tolerance, either central against self-tissue differentiation antigens or peripheral against tumor-specific mutated antigens; (b) immunological ignorance, where tumor is invisible to the immune system; and (c) tumor related factors that prevent tumor recognition and elimination by activated T cells, for instance no expression of CTL-restriction elements (37).

Whichever model in tumor immunity is favored, key issue is antigen presentation. DCs represent attractive vectors for tumor immunotherapy because of their unique properties including high antigen capture and presenting capacity resulting in extremely efficient inducting and maintenance of immune responses.

DCs as vectors for immunotherapy of tumor

The first clinical studies were performed primarily in melanoma patients using DCs pulsed with peptides or loaded with tumor cell lysates (38). These studies revealed that such vaccines can be applied safely without significant side effects and induce antigen-specific CTL responses *in vivo*. There are some different strategies that are now being applied in clinical trials to deliver antigens to DCs.

(a) Firstly, DCs can be pulsed with synthetic peptides or proteins derived from known TAAs such as melanoma-associated antigen-derived epitopes 1 (MAGE 1), MAGE 3, MUC1 (mucin 1, transmembrane), HER-2/neu, tyrosinase, carcinoembryonic antigen (CEA), or Melan-A (melanoma antigen)/MART (melanoma antigen recognized by T cells) (39-43). 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. However, the application of antigenic peptides is limited to use in patients who express a defined specific HLA haplotype. Moreover, using MHC class I-restricted peptides ignores the important role of MHC class II-restricted Th cells in initiating and maintaining an effective immune response (44). It has been demonstrated that patients with advanced breast and ovarian cancers 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 (45). The DC vaccinations were performed subcutaneously with no side effects. In 5 of 10 patients, antigen-specific CTLs could be detected in the peripheral blood using both intracellular TNF-α staining and ^{51}Cr-release assays.

(b) Another strategy is to use full-length native or recombinant proteins as antigens, 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 immunodominant epitopes including both MHC class I- and class II-restricted peptide antigens.

(c) An alternative approach 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. DCs can be transduced with recombinant viruses such as retroviral or adenoviral vectors, transfected with DNA or RNA coding for a specific tumor antigen or whole tumor RNA (46, 47). DCs pulsed with tumor-derived RNA elicit tumor-specific CTL responses thus offering an interesting alternative to ensure responses against unique, patient-specific TAA. Some promising results have reported in patients using this approach. For example, CEA-RNA transfection of DCs was used in the evaluation of colorectal and lung carcinomas (48).

(d) Other approaches utilizing whole tumors as a source of antigen have been developed using DCs loaded with tumor lysates, dying tumor cells (apoptotic bodies, necrotic cells), or fused with tumor cells (49-51). This technique does not require the definition of the TAA or MHC haplotype of the patient and has the potential for broad clinical applications. This approach has been used in patients with renal cell cancer, for instance, Kugler A, et al. (52) used allogeneic monocyte-derived mature DCs fused to autologous tumor cells in an electric field. After subcutaneous vaccinations and a mean follow-up of 13 months, significant clinical responses could be induced in 7 of 17 patients. In four patients completely rejecting all metastatic tumor lesions, two had a tumor regression of more than 50%, and one presented a mixed response.

(e) Furthermore, a strategy to augment the antigen-presenting function of DCs is their genetic modification to express immunostimulatory cytokines such as IL-7 and IL-12 or costimulatory molecules. The transduction of DCs with an adenoviral vector coding for CD40L, a molecule preferentially expressed on activated $CD4^+$T cells, was shown to efficiently induce a protective and antigen-specific immunity in mice when injected into the tumor that was mediated by $CD8^+$ antigen-specific CTLs (53, 54). Kikuchi, et al. (55) demonstrated that genetically modified CD40L-expressing DCs were able to take up, process and present antigens from a complex mixture of bacterial proteins and establish a protective B-cell-mediated immunity independent of $CD4^+$T cells. Expression of CD40L on DCs may activate and protect DCs from spontaneous and Fas/FasL-induced apoptosis and antagonize the inhibitory effects on the function of DCs mediated by anti-inflammatory cytokines such as IL-10.

Peripheral blood monocytes represent the most commonly utilized source for generation of DCs *in vitro*. When cultured for 6 to 7 days in the presence of GM-CSF and IL-4, monocytes have been shown to differentiate into immature DCs, making it possible to obtain large numbers of DCs for vaccine production. These DCs require additional maturation stimuli, such as TNF-α, monocyte-conditioned medium, CD40 ligation, or activated lymphocytes, to increase their stimulatory capacity, as detected by mixed leukocyte reactions and induction of antigen-specific CTLs. Activation of DCs with monocyte-

conditioned medium or a cytokine cocktail consisting of TNF-α, IL-1, IL-6, and prostaglandin E_2 was demonstrated to be superior to other stimuli with regard to the yield of DCs, their immunostimulatory capacity, and migration and induction of an irreversible maturation status of DCs (56).

Application of DCs for gastric cancer

DCs and gastric cancer

According to the investigation of gastric cancer from 1990 to 1992, the mortality of patients with gastric cancer is 25.2/100,000, and is the highest among all malignant tumors in our country (57). Although the effect of therapy for gastric cancer has been made great progress, it is still difficult for us to treat gastric cancer at the advanced stage, particularly as same time having metastasis to other organs and far lymph nodes. At present radical surgery represents the standard method of therapy, and adjuvant therapy such as chemotherapy and radiation therapy have been applied widely, but control of gastric cancer at the advanced stage remains difficult. Therefore we need to introduce a new adjuvant therapeutic method in order to ameliorate prognosis of patients with gastric cancer, improve the 5-year survival rates of patients with gastric cancer. Now the utilization of antitumor T cells, as immunoadjuvant therapy for gastric cancer, is extremely appealing. Sumiya Ishigami, et al. (58) examined 169 patients with gastric cancer by immunohistochemical staining of CD57 and S-100-protein and found that DCs infiltrated in the tissue of gastric caner, but which cannot play an important role due to lacking Th cells in the tumor microenvironment (59). In addition, the poorer differentiation of gastric cancer, the lower amount DCs infiltration in the tumor tissue. Patients with many DCs infiltration had lower lymph node metastases and lymphatic invasion than patients with fewer DCs infiltration. The 5-year survival rates of patients with many DCs infiltration were 78%, better than that of patients with fewer DCs infiltration (58). According to the function of DCs described above, we know that DCs are related to clinical stage, invasion, metastasis and prognosis of gastric cancer (60). Therefore, it will be feasible that DC-based tumor vaccines become a new effective immunoadjuvant therapy for gastric cancer, which can decrease the incidence and recurrence rates after operation for gastric cancer.

Clinical trials

Galetto, et al. (61) gained DCs derived from adherent blood mononuclear cells of five patients with gastric cancer, which were exposed to apoptotic autologous tumor (AAT) cells and cultured for 24 h with monocyte-conditioned medium to achieve full DCs maturation. Tumor-specific response was evaluated as single-cell cytokine release in an enzyme-linked immunospot (ELISPOT) and cytotoxicity in a cold target inhibition ^{51}Cr-release assay. Data showed that T-cell memory against gastric cancer antigens could be triggered by tumor-loaded autologous DCs.

Koji Kono, et al. (62) reported that tumor vaccination therapy with DCs pulsed with HER-2/neu-peptides may be a potential candidate for the novel treatment of gastric cancer patients. Nine gastric cancer patients with recurrent or unresectable tumor are enrolled in the clinical trial. Their tumors were proved to overexpress HER-2/neu by immunohistochemistry. Vaccinations with DCs pulsed with HER-2 (p369) peptide were performed at 2-week intervals. DCs administered intradermally in a single site at a supraclavicular location. In 3 of 9 patients, the tumor markers (CEA or CA19-9) were decreased after vaccination. Two had a tumor regression of more than 50%, and two presented a mixed response. The vaccines can be applied safely without significant side effects.

Conclusions

The studies of DCs for origin, differentiation and function have made great progress since the discovery of DCs. Now we may obtain many DCs possessing better function by the new isolated and cultured methods conveniently. It will be more ease for us to produce vaccines based on DCs for the clinical trial and treatment. However, the complexity of DCs system brings about a lot of problems, which need to be solved in the future, particularly about antitumor immunity. Therefore it will be a long time when DCs-based tumor vaccines are applied for clinical treatment of gastric cancer broadly. In a word, DCs-based vaccines as a new effective immunotherapeutic approach for gastric cancer, though which cannot replace the surgery and other approaches of adjuvant therapy, undoubtedly will be a good prospect.

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