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Review Paper

Dendritic Cells : Key to Cancer Immunotherapy

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ABSTRACT

Immunity is controlled by a network of professional antigen presenting cells (APCs), the most important of which are known as dendritic cells (DC). Dendritic cells are professional APCs that are designed to activate T cells toward various antigens, such as tumor-associated antigens, due to their potent co-stimulatory activity. They play a crucial role of constantly sampling the microenvironment for 'danger signals', which include inflammatory signals and pathogens. The availability of large numbers of DC, generated either from hematopoietic progenitor cells or monocytes, holds great promise in the development of cancer immunotherapy as well as the treatment of autoimmune diseases and suppressing several viruses. Accordingly, appropriately pulsed or transfected DC may be used for vaccination in the field of infectious diseases or tumor immunotherapy to induce antigen-specific T cell responses. Unlike infectious pathogens, tumors do not induce an effective inflammatory response suitable for optimal activation of DCs, and as a result the immune response is weak and ineffective. The primary purpose of vaccinating individuals with cancer is to overcome this flaw by channeling tumor antigens into DCs and providing the conditions for their optimal maturation into potent immunostimulatory APCs. This article will focus specifically on the use of DCs as vaccines for cancer immunotherapy. We will examine DC biology, preclinical and clinical studies and finally efforts to improve current vaccine formulations.

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Dendritic cells (DCs) are specialized antigen-presenting cells and essential mediators of immunity and tolerance. Studies of immunology and disease have long focused on antigens and lymphocytes (B cells, T cells, NK cells) as the mediators of immune responses. However, accumulating evidence shows that dendritic cells provide vital links between antigens and all types of lymphocytes (1). Dendritic cells are critical and previously missing links in immune system which are referred to as 'professional APCs" due to their principal function of presenting antigens and inducing a primary response in naïve T lymphocytes (2). This group of cells is heterogeneous in terms of cell-surface markers, anatomic location, and function. As sentinels, dendritic cells patrol the body seeking out foreign invaders, no matter these are bacteria viruses, tumor antigens or dangerous toxins. New research shows that dendritic cells are also responsible for a seemingly opposite role in health

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which silences dangerous immune cells and prevents them from attacking materials in the body or the body's own tissues which results in auto-immune diseases (3).

DC morphology and biology

Dendritic cells are stellate or tree-like cells (Greek, dendron, tree) that are found virtually everywhere in the body. Being responsible for the induction of T or T-dependent immunity and tolerance, they are particularly abundant in lymphoid or immune organs, and at the interfaces between our bodies and the environment such as skin and mucous membrane which are the ideal locations to encounter invading pathogens (4). Several morphological characteristics provide DCs with a variety of features to generate an immune response. Dendritic cells are so called because of the presence of numerous membranous processes that resemble the dendrites of neurons. The processes of dendritic cells continually form, bend, and retract, hence lymphocytes can bind to dendritic cells in large numbers. The tentacular shape and constant movement of dendritic cells along with the intracellular structures relating to antigen processing including endosomes and lysosomes, fit precisely with their functions (5).

Discovery of dendritic cells

During the 1970s, most immunologists considered macrophages to be the principal antigen presenting cells in the immune system. They also believed that a group of “accessory cells” which were first thought to be a type of macrophages are responsible for the process of response initiation. But the uncertainty of their identity and function were left behind until 1973 when Ralph Steinman and the late Zanvil A. Cohn first described dendritic cells (5). They found a population of striking dendritic-shaped cells in mouse spleen. When Steinman evaluated this population of cells, obvious resemblance to the well-known macrophages such as great number of membrane enzyme and digestive bodies or lysosomes and Fc receptors was not seen. The cells had poor viability and rapid turnover in contrast to macrophages and showed poor phagocytosis in vivo and in vitro (6). Eventually the researches of Steinman and Cohn led to the novel discovery of dendritic cells and their distinct properties and features. The term "accessory" has since been replaced by the terms "professional" and "co-stimulatory," but the basic concept is unchanged as dendritic cells provide the T cells with needed accessory or co-stimulatory substances, as well as giving them a signal to begin to grow and function. By 1979 Steinman succeeded to increase the number of dendritic cells with a high percent of purity so that it was possible to carry out functional studies (7).

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Further studies revealed their vital stimulatory function since dendritic cells were mostly detected in T-cell rich lymphatic organs which is the most suitable location to initiate immunity (8-10). Subsets of dendritic cells with unique surface markers were also detected in epithelial organs of animals as well as in human blood. The origination of dendritic cells and their development through a pathway shared with macrophages and granulocytes were also discussed. By 1992, Steinman used DC progenitors to generate a large number of dendritic cells to expand cellular and molecular research on DCs (6).

Dendritic Cells Generation and Function

Currently there are two known major subsets of dendritic cells in humans: Firstly lymphoid dendritic cells derived from plasmacytoid cells in blood, and myeloid dendritic cells, the second group arise from myeloid precursor cells e.g., peripheral blood monocytes or CD34+ progenitors from bone marrow (11). In humans, myeloid lineage are considered the "classical" DC. Alternatively DCs can be generated by culturing CD34+ cells in the presence of various appropriate cytokines. One approach which has been taken involves depleting the CD34+ cells of differentiated precursors and then culturing the cells in the presence of GM-CSF and IL-4 ± TNF-α. CD34+ cells can be obtained from bone marrow, cord blood or G-CSF mobilized peripheral blood. Another approach is to generate DC-like cells by culturing CD14+ monocyte-enriched PBMC. In the presence of GM-CSF and IL-4, there cultures give rise to large numbers of DC like cells. These monocyte-derived DCs need additional conditioning in vitro with either TNF-α or monocyte-conditioned media to be able to fully function as a DC capable of priming antigen-specific T cell response. A novel approach to expand DCs in vivo is presented in a review by Fong and Engleman. They saw an increase in circulating DCs by 10-3- fold in a clinical protocol using Flt3-ligand. These cells could be harvested with a leukapheresis procedure and used for later immunotherapy. Other molecules may also prove useful for the in vivo expansion and mobilization of DCs (12). Monocyte-derived DCs generated in vitro are phenotypically and functionally similar to DCs present in blood. The important point is that they can be generated in significant numbers, which is a necessity for clinical studies. In vitro generation of dendritic cells has facilitated clinical vaccine studies using DCs pulsed with antigen; which can be formulated as peptides, proteins, cell lysates, apoptotic tumor cells, DNA and RNA. Dendritic cells develop further or mature as they capture, process antigens, and migrate under the influence of other chemical messengers to tissues such as spleen and lymph nodes. There they attract and stimulate T and B cells to produce strong immune responses. Dendritic cells as professional APCs, are specialized to uptake antigens and convert them into MHC-peptide complexes recognized by lymphocytes thanks to their receptors. However, dendritic cells are capable of doing more than presenting antigens to T cells. The function of DCs is divided into three main categories, each of which include antigen presentation. The first category of DC function is antigen presentation and activation of T cells. DCs process and present antigen to activate

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both CD4+ and CD8+ T cells. Only DCs are capable of activating naïve T cells hence, this appears to be the most crucial role for DCs. Immature DCs originate in the bone marrow and migrate throughout the body, Once the immature DCs interact with invading pathogens or other foreign bodies they carry out the primary function of capturing the antigens. When exogenous and endogenous antigens are captured, they can be processed by DCs and presented in the context of either major histocompatibility complex (MHC) class I or II molecules. For MHC class I presentation to stimulate CD8+ cytotoxic T cells through endosomal pathway, the antigen or protein is taken up by phagocytosis or receptor mediated endocytosis into the cytosol. The antigens are further degraded in the cytosol via proteasome and enter the endoplasmic reticulum where peptides bind to lately synthesized MHC class I molecules to be presented on the cell surface. For MHC class II presentation through proteosomal pathway to stimulate CD4+ T helper cells, antigen is taken up by phagocytosis or receptor -mediated endocytosis to endosomes where some proteolysis happens (13,14). The peptides enter a vesicle containing MHC class II where they bind and are transported to the cell surface. DCs also maintain costimulatory molecules on their cell surface including members of the B7 family, TNF family and intracellular adhesion molecules which are vital to the activation of T. The second category of DC function is not as well established, but it has been suggested that a different class of DCs exist with the function of inducing and maintaining immune tolerance.(15-19) Dendritic cells not only activate T lymphocytes, but also tolerize T cells to antigens that are innate to the body (self-antigens), thereby minimizing autoimmune reactions. Tolerance is the inability of the immune system to respond to specific antigens. Central tolerance occurs in the thymus for T cells and the bone marrow for B cells. T cells that respond to DCs carrying self-peptide are destroyed in the thymus by negative selection, preventing the occurrence of autoimmune diseases. The third category of DCs, known as follicular DCs, appear to work to maintain immune memory with the help of B lymphocytes. DCs produce a number of cytokines and factors which are critical to the activation and differentiation of B cells. The follicular DCs appear to be important in the maintenance of B cell memory (20).

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DC-based vaccines

Development of practical procedures to prepare sufficient numbers of functional DCs in culture from the peripheral blood precursors, paved the way for clinical trials to evaluate various DC-based strategies in patients with malignant diseases. Vaccination strategies involving DCs as the most potent antigen-presenting cells have been developed owing to the special properties of these cells in initiation and maintenance of innate and adaptive immune responses. By contributing to the generation and proliferation of tumor-specific cytotoxic T lymphocytes and helper T cells, DC play a critical role in the induction of protective and therapeutic anti-tumor immunity with tumor antigen-loaded DC have been reported in murine tumor models and in trials in tumor-bearing patients. The aim of DC vaccination is to induce tumor-specific effector T cells that can reduce the tumor mass specifically and that can induce immunological memory to control tumor relapse. In this process, the first step is to provide DCs with tumor-specific antigens. This can be achieved either by culturing ex vivo DCs that have been derived from patients with an adjuvant and the tumor-specific antigen, and then injecting these cells back into the patient, or by inducing DCs to take up the tumor-specific antigen in vivo.

The goal of cancer vaccinologists is to elicit tumor-specific CD8+ T cell-mediated immune responses that will be sufficiently robust and long-lasting to generate durable tumor regression and/or eradication. This goal is encouraged by clinical studies showing that the infusion of autologous tumor-specific CD8+ T cells can eventually lead to the rejection of large metastatic tumors in patients (21-23). The aim is to identify vaccination protocols that will result in the generation of potent T cell responses in vivo. Ideally, vaccine-elicited CD8+ T cells should be of high avidity and able to recognize peptide–MHC class I complexes on tumor cells; be able to express high levels of granzyme and perforin — molecules that are essential for cytotoxic activity against cancer cells; be able to enter the tumor microenvironment; and be able to overcome immunomodulatory mechanisms that are present in the tumor (24-26). At least four components of the immune response are necessary for this ideal response to happen: the presence of appropriate DCs; the quality of induced CD4+ T helper cells; the elimination and/or non-activation of TReg cells; and the breakdown of the immunosuppressive tumor microenvironment (27).

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Optimization of the treatment parameters, including the DC source, DC maturation, choice of antigens, Ag-loading methods, sires of injection, doing, and appropriate anti-tumor immunological monitoring, will be required to further improve the efficacy of this approach.

General approaches

There are two general approaches for preparing and loading tumor antigens. One uses a single tumor-associated Ag for the induction of a single antigen-specific monoclonal CTL, and the other uses whole tumor cells or their derivatives for the induction of multiple antigen-specific polyclonal CTLs. Variations in the former approach include the use of DCs pulsed with MHC class I-restricted tumor Ag-derived peptides. Check the ref 10-11. DCs transfected with cDNA encoding a particular tumor-associated Ag (28) and tumor-peptide pulsed DC-derived exosomes (29). Although clinical trials using peptide-pulsed DCs have demonstrated immunological and clinical responses in cancers such as malignant melanoma (25,30) and prostate cancer (24) the application of specific peptides is limited to patients who express class I MHCs restricted to peptides associated with those tumors. Peptide-based immunotherapies also fail to utilize MHC class II and CD40L-mediated CD4+ T cell helper responses. Various methods have been reported for generating DC-based vaccines using whole tumor cells or their derivatives including DCs pulsed with tumor-associated native or recombinant proteins or DCs transfected with whole tumor cell-derived RNA (3). In clinical trials, some investigators reported the efficacy of DCs pulsed with whole tumor lysates (13, 25) or stressed tumor cells (necrotic or apoptotic) (7, 26), which are more practical to obtain than isolated proteins or RNA. DC-tumor cell fusion hybrids (DC/TC hybrids) have been shown to demonstrate superior efficacy for the treatment of murine tumor models (14,15) It is important that the hybrids express multiple tumor antigens in the context of MHC class I and/or class II molecules as well as co-stimulatory molecules essential for T-cell activation. Moreover DC/TC hybrids have been shown to be efficacious for the treatment of both melanoma and renal cell carcinoma in Phase I/II clinical trials (31). Strict comparative analysis that optimize methods for Ag-loading whole tumor cells or their derivatives have been conducted for melanoma, thymoma or acute myeloid leukemia (32-36).

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Future Perspective

Since the first trial of DC vaccines for cancer was published in 1996, over 100 clinical studies have been reported, but there is little consensus as to the optimal manufacturing strategy (25) Turnis and Rooney (2012) speculate that in the future, DC vaccines will not be a sole mode of therapy, but a weapon in a complementary arsenal.

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Conclusion

Effective therapies may require combinations of genetically modified DCs with additional treatments such as modulation of angiogenic pathways, depletion of soluble inhibitors, blockade of regulatory cells or adoptive transfer of T cells, themselves genetically modified to resist T-cell-directed tumor immune evasion strategies. Many unanswered questions hamper the development of DC-based vaccines, including the source of DC preparation and protocols for DC generation, activation and loading with tumor antigens, source of tumor antigens and route of vaccine administration and methods of immunomonitoring. Fortunately, in spite of the many obstacles, DC vaccines continue to hold promise for cancer therapy.