

What is a dendritic cell?

Dendritic cells are antigen-presenting cells (APCs) which play a critical role in the regulation of the adaptive immune response. Dendritic cells (DCs) are unique APCs and have been referred to as “professional” APCs, since the principal function of DCs is to present antigens, and because only DCs have the ability to induce a primary immune response in resting naïve T lymphocytes. To perform this function, DCs are capable of capturing antigens, processing them, and presenting them on the cell surface along with appropriate costimulation molecules. DCs also play a role in the maintenance of B cell function and recall responses. Thus, DCs are critical in the establishment of immunological memory.

Background:

DCs were first described by Ralph Steinman nearly thirty years ago. He found a population of striking dendritic-shaped cells in the spleen. Shortly thereafter it became clear that DCs existed in all lymphoid and most non-lymphoid tissues. During the 1970s, most immunologists considered macrophages to be the principal APC in the immune system. Compared to DCs, macrophages were more abundant, were uniformly distributed throughout the body, and were known to have antigen presenting capabilities. Since DCs are so rare, initial studies were difficult and it was not until the 1980s that it became widely accepted that DCs were “professional APCs”.

While the rarity of DCs was a hindrance to studying them, in the 1990’s researchers learned how to generate large numbers of DCs from CD34+ bone marrow precursors or from CD14+ monocytes *in vitro*. The ability to expand functional DCs *in vitro* has increased researchers ability to study DCs to understand mechanisms of DC interactions with the immune system and to begin to test DC therapy regimens in the clinic.

How is a dendritic cell detected?

Mature DCs are defined by key morphological features and by the presence or absence of various molecules on the cell surface. The key morphological characteristic of DCs is the presence of numerous membrane processes that extend out from the main cell body (similar to dendrites on neurons). An additional morphological feature of DCs is that they contain abundant intracellular structures relating to antigen processing including endosomes, lysosomes, and Birbeck granules of Langerhans cells of the epidermis.

An obstacle to the detection of DCs is that a single cell surface marker exclusively expressed on DCs has not yet been identified. Therefore, a combination of the presence and absence of various surface markers has been used to identify DCs. These include the presence of large amounts of class II MHC antigens and the absence of various lineage markers such as CD3 (T cell), CD14 (monocyte), CD19 (B cell), CD56 (natural killer cell) and CD66b (granulocyte). DCs also express a variety of adhesion molecules including CD11a (LFA-1), CD11c, CD50 (ICAM-2), CD54 (ICAM-1), CD58 (LFA-3), and CD102 (ICAM-3). DCs also express costimulatory molecules including CD80 (B7.1), and CD86 (B7.2), which are upregulated during DC activation. CD86 tends to be a marker of early DC maturation, while CD80 only appears in mature DC. Two additional markers of mature DC in humans are CD83 and CMRF-44. CD83 also stains activated B cells, and CMRF-44 will also stain macrophages and monocytes.

With such a variety of surface markers available for identifying DCs, yet none which specifically stain DCs only, the neophyte to detecting DCs may become bewildered at the prospect of where to start when attempting to identify DCs. In practice, however, the identification of DCs by surface phenotyping may be accomplished by simply demonstrating a high level of MHC class II or a costimulatory molecule such as CD80 and the absence of lineage markers. Thus, a two-color analysis by flow cytometry using a cocktail of lineage markers in one color and a positive marker for DC in the other can be sufficient to identify DCs. Of course, depending on the purpose of identification, the use of additional markers/colors may be required as well.

Recently, subsets of DC were recognized based on their function in immune responses. DC1 cells, also called myeloid DCs, express different Toll-like receptors (TLR)-2, -3, -4, and -7. After encountering different natural ligands or pathogens for these TLRs in the blood, DC1 cells become activated and mature into antigen-presenting cells (APC) that can secrete Th-1 or Th-2 cytokines and prime naive T cells for a proper immune response. DC2 cells, also called plasmacytoid DC, only express TLR7 and TLR9 receptors and are the principle producers of interferon-alpha after encountering invading viruses. These two types of DCs play important role on linking the innate and adaptive immunity through their unique expression patterns of TLRs and cytokine production.

What is the function of dendritic cells?

The function of DCs falls broadly into three categories, each of which involve antigen presentation. The first category of DCs function is antigen presentation and activation of T cells. 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. The third category of DCs, known as follicular DCs, appear to work to maintain immune memory in tandem with B cells.

T cell activation:

DCs process and present antigen to activate both CD4+ and CD8+ T cells. This appears to be the most prominent role for DCs, since *only* DCs are capable of activating naïve T cells. Immature DCs originate in the bone marrow and migrate throughout the body. Once there, the immature DCs lay dormant waiting to interact with invading pathogens or other foreign bodies. At this point, the primary function of the immature DC is to capture antigens. In the case of a wound accompanied by inflammation, DCs are attracted to the area of inflammation and stimulated to capture and internally process antigens. So, the function of an immature DC is to find and capture foreign bodies and antigens. Once captured, the antigen is processed either by an exogenous or endosomal pathway, or by endogenous or proteosomal pathway. For MHC class I presentation to stimulate CD8+ cytotoxic T cells, 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 newly synthesized MHC class I molecules for presentation on the cell surface. For MHC class II presentation to stimulate CD4+ T helper cells, antigen is taken up by phagocytosis or receptor-mediated endocytosis to endosomes where some proteolysis occurs. The peptides enter a vesicle containing MHC class II where they bind and are transported to the cell surface. A key component on DCs in addition to antigen capture, processing and presentation is the presence of costimulatory molecules. DCs maintain on their cell surface costimulatory molecules including members of the B7 family, TNF family and intracellular adhesion molecules which are critical to the activation of T cells and for the proper homing of DCs before and after antigen capture.

Immune tolerance:

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. The primary mechanism for

central tolerance in T cells is the induction of T cell death. DCs are found in abundance in the thymus, where newly produced T cells are educated to become functional CD4+ T cells and CD8+ T cells and undergo selection to eliminate immunity against 'self'. Low affinity reactive T cells are positively selected and allowed to survive and reach the periphery. This does not involve DC function. T cells that respond to DCs carrying self-peptides are destroyed in the thymus by negative selection. This process involves T cells which recognize MHC/peptides with high avidity. DCs contribute to this negative selection process along with thymic epithelial cells. The mechanisms of peripheral tolerance include T cell death, anergy, and active suppression by T regulatory cells. DCs could contribute by inducing apoptosis in T cells and by producing IL-10, a cytokine that stimulates T cells and induces T regulatory cells. DCs might also contribute to tolerance by inducing anergy in responder T cells.

B cell stimulation/function:

DCs can contribute to the stimulation of B cells, both in the lymph node T cell areas and in germinal centers. DCs produce a number of cytokines and factors which are critical to the activation and differentiation of B cells. The follicular DCs (FDCs), which are found in germinal centers of lymph nodes, appear to be important in the maintenance of B cell memory. FDCs are not involved in the initial antibody response to foreign antigens. However, after the initial antibody response begins, the FDCs form numerous complexes of antigen and antibody. FDCs are believed to serve as both a 'reservoir' for antibody and as a source of continued stimulation for B cells. The B cells can in turn take up the antigen from FDCs and present it to T cells. The reservoir of antigen and antibody complexes on FDCs is believed to be able to last a very long time, perhaps up to months or years.

Origins and nomenclature:

There is little argument that DCs are derived from hematopoietic stem cells; however, DCs can originate from both lymphoid and myeloid lineages. In humans, myeloid lineage DCs are considered the "classical" DC. These cells originate from myeloid committed CD34+ progenitor cells, and monocytes can be driven to become DCs in the presence of GM-CSF and TNF α \pm IL-4. When this type of DC matures, it is known as an interstitial DC. In addition to being able to activate naïve CD4 and CD8 T cells, interstitial DCs can induce differentiation of naïve B cells to antibody secreting plasma cells. Interstitial DCs are assumed to migrate to the lymphoid follicles and become follicular DCs. Myeloid committed CD34+ cells which are CD14 negative can mature to what is called a Langerhans DC, in the presence of TGF- β . Langerhans DC are also capable of activating naïve T cells, but not B cells. Langerhans DC are those that capture antigen, migrate to lymphoid tissues and present antigen to T cells. The lymphoid DCs -- the DC subset that originates from CD34+ cells committed to the lymphoid lineage, are CD11c- and are driven to become DCs by IL-3. These DCs are referred to as Plasmacytoid DCs, and they have the capacity to produce IFN- α and reside in the T cell compartment of lymphoid tissues.

Dendritic cell generation:

DCs can be generated by culturing CD34+ cells in the presence of various 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 \pm 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, these 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 responses. Additional information of the generation of DCs and the cytokines involved is presented in a review article by Syme and Gluck.

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 10-30 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.

Dendritic cell immunotherapy:

There is a great deal of interest in how DCs might be exploited as a form of immunotherapy. DCs are being studied as adjuvants for vaccines or as a direct therapy to induce immunity against cancer. That DCs may prove useful in cancer has been most often studied in animal models. DCs loaded with tumor lysates, tumor antigen-derived peptides, MHC class I restricted peptides, or whole protein have all been shown to generate anti-cancer immune responses and activity, including in some cases the ability to induce complete regression of existing tumor. Thus, there is a great desire to test these strategies and use tumor-antigen bearing DCs as a vaccine in humans. Human clinical trials are ongoing in several institutions to use DCs to induce immunity to antigens against breast cancer, lung cancer, melanoma, prostate and renal cell cancers.

While efforts to isolate DCs, expand them *in vitro*, coat them with antigens, and infuse them into patients are ongoing, it is going to be important to understand which antigens or peptides are going to be the most useful to use on the DCs for immunotherapy. Therefore, research to determine which antigens are most immunogenic and useful in the immune system's fight against cancer needs to continue. Since each cancer is heterogeneous, it is likely that immunity against multiple antigens may be required for complete, effective immunity against cancer. In the future, it may be possible to screen each cancer and determine which "cocktail" of antigens is required to induce immunity in that particular patient. How the antigen is loaded onto the DCs also needs to be optimized. It may be useful for the DCs to be transfected with RNA or DNA to produce the appropriate antigen and to present it on the surface. Finally, it may be that DC immunotherapy will work better when administered in combination with cytokines or other molecules which activate DCs.

In summary, DCs are being actively pursued as a means to induce immunity in human patients. Since DCs are potent regulators of the immune system, much research is being done to try to understand how DCs can be harnessed to induce immunity. While our understanding of DCs has evolved tremendously in the 30 years since they were first described, the use of DCs as a form of immunotherapy is in its infancy. We are poised to learn whether and how DCs will be useful as a tool in our arsenal to exploit human immunity against disease.

Further reading:

The source of much of the material contained in this educational note and additional information can be found in the following reviews:

Lipscomb M.F., Masten B.J., Dendritic cells: Immune regulators in health and disease. *Physiol. Rev.* 82:97-130, 2002.

Fong L., Engleman E.G., Dendritic cells in cancer immunotherapy. *Ann. Rev. Immunol.* 18:245-273, 2000.

Syme R., Gluck S. Generation of dendritic cells: role of cytokines and potential clinical applications. *Transfusion and Apheresis Science* 24:117-124, 2001.

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