Dendritic Cells: A Lesser known Part of the Immune System

Mei Mei Tian

Immunology has long focused on the relationship between lymphocytes and antigens, however their presence in a system does not always lead to effective immunity. To complete the picture, the dendritic cells (DCs) act as initiators and modulators of the immune response[1]. DCs are antigen-presenting cells (APCs), which means that their principle function is to collect and relegate antigens to lymphocytes and antibodies. Found in almost every type of tissue, including lymphatic, blood, and skin, they were first described by Ralph Steinman in the early 1970s. Because their processes closely resembled the dendrites of nerve cells, they were named dendritic cells. At that time, most immunologists considered macrophages as being the immune system's principle type of APC, since they were much more abundant and uniformly distributed in the body. In the early 1990s, the development of new techniques for isolating and growing large number of DCs in cultures led to an explosion of new information about the significance of DCs in the immune system. In short, DCs are crucial to the presentation of peptides and proteins to T- and B-lymphocytes, as well as the induction of T-cell responses resulting in cell-mediated immunity[2].

Among new findings in recent years is the discovery that DCs are not derivative of a single cell type, but a heterogeneous collection of cells that have arisen from distinct, bone marrow-derived haematopoietic lineages[3,4,5,6,7]. Currently there are at least three different pathways that have been identified, each of which has its own distinct progenitors, specific combinations of cytokines that drive developmental events, as well as their own specialized functions[3,4,5,6,7]. CD34+ progenitor cells can enter either the lymphoid-related DC pathway or the myeloid DC pathway. Lymphoid pathways lack a few characteristics commonly found in myeloid pathways, such the absence of defined surface phenotypes CDIlb, CD13, CD14, and CD33 [3]. Recent studies have demonstrated the development of lymphoid DCs from thymic progenitors stimulated with Interleukin 3 (IL-3), as well as from lymphoid precursors in human tonsil treated with CD40 ligand (CD40L)[7,8]. Thus far, they have been attributed to the promotion of negative selection in the thymus and being co-stimulatory for CD4+ and CD8+ T-cells, therefore it has been suggested that this type of DC possesses more of a regulatory effector function rather than a stimulatory function[3].

The other two developmental pathways for DCs are associated with the myeloid lineages. They are special in the sense that their development involves the expression of certain phagocyte-associated features. These DCs are derived from multipotent CD34+ progenitor and peripheral blood mononuclear cells (PBMCs), and are then divided into two subgroups. The most significant characteristic of one of the group is its strong expression of CDla+ and lack of expression for CD14 [3], while the other group of myeloid origin DCs are typical of CD la- and CD14+ [3]. Upon stimulation with a specific combination of colony stimulating factors, lymphoid progenitors give rise to lymphoid DCs; CDla+CD14- myeloid precursors eventually give rise to Langerhans related DCs, and CDla-CD14+ myeloid precursors give rise to interstitial related DCs.

Initially, these DCs migrate to and reside in body surfaces and interstitial spaces as immature DCs, incapable of stimulating T-cells. At this stage, they are characterized by having abundant major histocompatibility complex (MHC) II products within their intracellular compartments and they respond rapidly to inflammatory cytokines and microbial products to produce mature T-cell stimulatory DCs with abundant surface MHC II proteins, which present peptides that have been digested from external
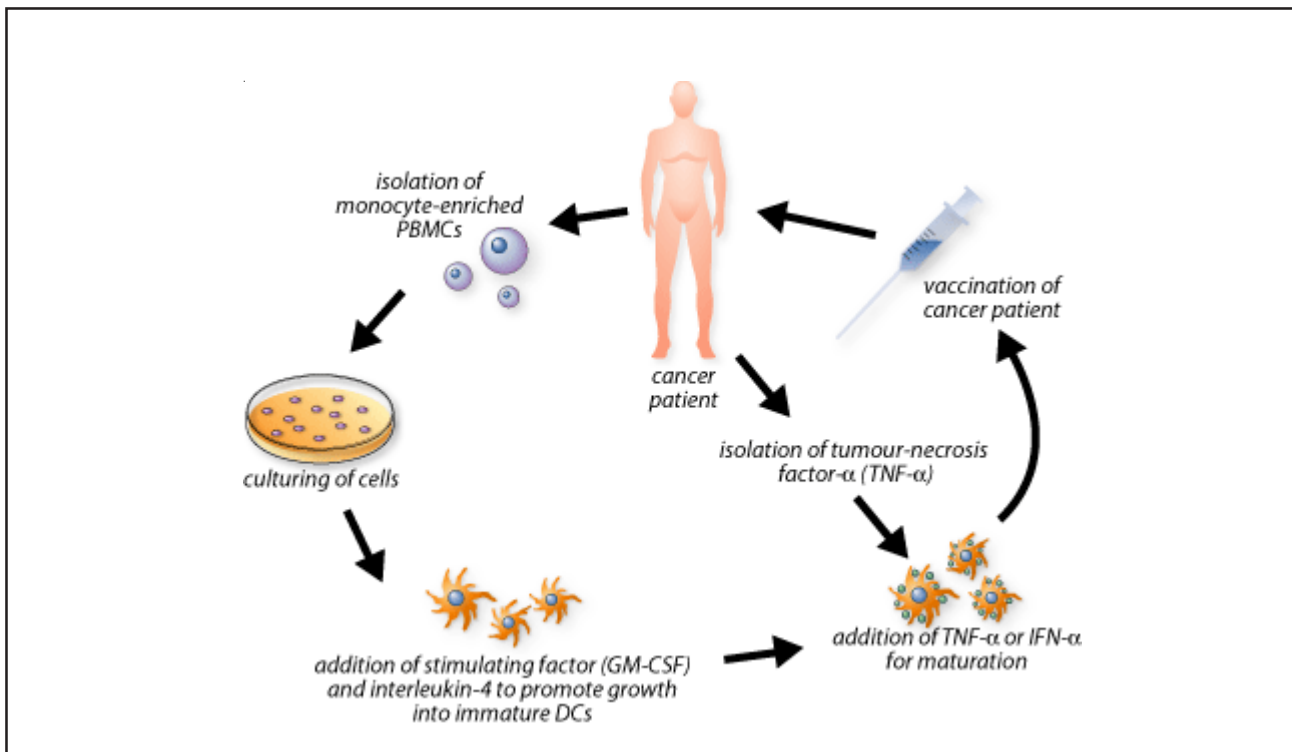
Figure 1. Creation of a vaccine using dendritic cells.

sources[3]. Although they are still not yet well-equipped for T-cell activation, they are extremely efficient at capturing and processing antigens — a key event in the induction of immunity. It is this ability to interact with antigens that enables the induction of the full maturation and mobilization of DCs.

There are a few features that allow for the efficient capturing of antigens by immature DCs. First, their phagocytotic ability allows them to take up particles and microbes[1]. Second, they can form large pinocytic vesicles in which extracellular fluid and solutes can be sampled, in a process called macropinocytosis[9]. Lastly, they express receptors involved in absorptive receptor mediated endocytosis. With respect to pinocytosis and receptor mediated antigen uptake, they are so efficient at antigen presentation that picomolar and nanomolar concentrations of antigen are sufficient, which is much less than the micromolar level of antigen typically used by other type of APCs[1]. However, these abilities decline rapidly after the DC has captured an antigen, and this decline subsequently initiates the signal for the maturation of the DC.

A range of factors, particularly microbial and inflammatory products like whole bacteria, cytokines such as IL-1, GM-CSF, TNF-α, and TGF-β, influence the maturation process of DCs[10]. The most distinguishing characteristic of matured DCs is their display of large extended processes or veils pointing in many directions from the cell body, in addition to their rapid motility compared to immature DCs. Their shape and motility make them especially efficient at capturing antigens and antigen-specific T-cell selection[1].

During the maturation process, MHC class II rich compartments (MIICs) start to be converted to non-lysosomal vesicles and transport their MHC-peptide complexes onto cell surfaces[11,12]. Once fully matured, the DCs migrate to specific lymphoid tissues to present the antigen to naive CD4+ T-cells and CD8+ cytotoxic T-cells and thus activate the T-cells[3]. Once activated by DCs, the T-cells are ready to elicit a primary immune response, such as interacting with B cells for antigen formation, or interacting with macrophages for cytokine release and targeting for lysis[1]. Because DCs are so effective in what they do, only a few DCs are needed to elicit a strong T-cell response both *in vitro* and *in vivo*.

The idea that the immune system could control

cancer has been proposed and toyed with for over two centuries since the first evidence of cancer regression following non-specific immunostimulation by bacterial components[13]. An extreme example of the link between failed immunity and cancer is cervical carcinoma where infection by the human papilloma virus is associated with 89 percent of all cases[14]. Many tumours induce immune tolerance, thus in order for the full effect of immunotherapy to take effect this tolerance must be broken. This is where the dendritic cells come into the picture as the vehicle to present the tumour-associated antigen to the immune system.

Under normal conditions, induction of effective tumour immunity can be described as a four-step process involving presentation and recognition of tumour-associated antigens (TAAgs) in tissues; activation and trafficking of DCs to regional tumour-draining lymph nodes, followed by activation of TAAg-specific cytotoxic T lymphocytes (CTLs) and, lastly, migration of CTLs to the tumour site which eventually leads to the induction of cancer cell death. This is what commonly referred to as "immune surveillance"[15,16,17].

Escape from this immune surveillance is believed to be a fundamental biological feature of malignant disease in humans, which contributes to uncontrolled tumour growth, and the eventual death of the host[18].

The connection between tumours and poor immune response have been well documented in animal models, and to some extend in humans as well. A study done by Radmyar *et al.* demonstrated that a substantial amount of DCs could be obtained from the peripheral blood of patients with renal cell carcinoma, that possessed the normal expression of DC-associated molecules, but that lacked T-cells, B cells and monocyte markers[19].

In 1997, a study by Gabriolovich *et al.* evaluated the T-cell responses to defined antigens in breast cancer patients. It was found that the advanced breast cancer patients showed defects in response to tetanus toxoid and influenza virus, suggesting that reduced DC function could be a major cause for the observed defects in the patients' cellular immunity[20]. Ninomiya *et al.* showed that DCs from patients with hepatocellular carcinoma had a significantly lower capacity to stimulate T-cell proliferation compared to DCs isolated from patients with liver cirrhosis or normal controls[21]. Almand *et al.* showed that defective DC function in patients with head and neck cancer was the result of a decrease in the number of competent DCs and the accumulation of immature cells[22]. All of these studies imply to some degree an important relationship between the proper functioning of DCs and immunity in patients with cancer.

Given their central role in controlling immunity, DCs are the logical focus for much clinical research that centres around T-cells, including transplantation, allergy, autoimmune disease, resistance to infection and to tumours, immunodeficiency, and vaccines[1]. What makes them a good candidate for anti-cancer therapy is their ability to migrate through tissues and infiltrate into tumours, as well as their capacity to activate naive T-cells in regional lymph nodes and their differentiation into CTLs, and lastly their role as APCs and their capacity to process and present a wide range of different antigens (also known as Ags) simultaneously[1]. The simultaneous presentation of a wide range of different Ags allows for the induction of a broad repertoire of anti-tumour immune responses to occur[3].

In animals this strategy of using tumour antigen-bearing DCs has been shown to lead to protection against tumours and even a reduction in the size of established tumours[23,24,25]. The current application of DCs in animal tumour models involves the *in vitro* isolation of DCs, loading them with tumour Ags and eventually injecting them into animals as anti-cancer vaccines. This can also be used therapeutically to induce regression of pre-existing tumours[26]. The range of objects with which DCs are capable of loading is not limited solely to tumour antigens; loading with tumour lysates, tumour Ag-derived peptides, synthetic MHC class I restricted peptides and whole proteins have all been demonstrated to generate tumour-specific immune responses and anti-tumour activities[27,28,29,30].

In recent years, advances in the understanding of dendritic cell function and immunity have made the DC-based anti-cancer therapy in humans possible. The first successful case using autologous *ex-vivo* processed DCs to treat malignancy was reported in 1996[31]. Although things like the mode of antigen delivery of DCs, the method of DC manufacturing, as well as the target sites themselves, may vary among the clinical trials, the common theme uniting them all

is that DCs are used to deliver tumour associated antigens. Various systems have been incorporated into delivering TAAgs to the DCs, such as using defined peptides of known sequences, using retroviral and adenoviral vectors, tumour cell-derived RNA, and even fusing DCs with tumour cells[32,33]. Unfortunately, nothing is ever perfect. Before DC-based vaccines can be formally recognized as a good supplement to anti-cancer therapy, a few obstacles still need to be overcome. The first obstacle is to have a sufficient amount of DCs since they are notorious for their scarcity in the body. Naturally, it would be beneficial to find alternative ways for obtaining a reasonable amount of DCs. A common method for accumulating DCs currently used in many research fields is the culturing of CD14+ monocyte-enriched PBMCs *in vitro*, such that media supplements like granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) can be added[3]. This method allows for the production of a large number of cells that are both morphologically and phenotypically very similar to the DCs that are naturally produced in the body[34,35]. However, these cytokine-generated DCs cannot mature on their own, they require the addition of maturation factors *in vitro*, such as tumour necrosis factor-α (TNF-α) or interferon-α (DFN-α) in order for them to prime Ag-specific T-cell responses in vitro and *in vivo*[36,37]. The maturation step is important in that without it DC phenotypes would tend to revert to that of the monocyte and thus be unable to induce strong immunity.

The process of DC isolation is also of special concern, because the most widely used density-based isolation is limited directly by the low frequency of DC precursors in the blood (about 1 percent of PBMCs)[38]. The most popular technique to combat this problem is the use of leukopheresis, for which blood is drawn out from one arm and passed through a machine that automatically removes many white cells, and then returns the remaining blood back to the other arm[38]. This has been proven to be much more efficient in isolating sufficient numbers of DCs for therapeutic vaccination in humans. The degree of activation of these DCs is also important; inactivation would lead to appropriate antigens and co-stimulatory molecules, required to activate T-cells, not being expressed.

Lastly, the effectiveness of the DC-based vaccination depends on the route of administration. DCs could be administered generally by systemic injection, or more specifically by being injected into the relevant lymphoid organs. There has yet to be any conclusive evidence to show the benefit of one over the other.

The research surrounding DC-based vaccination against cancer is still in its infancy; much more still need to be learned. The results from various clinical trials over the years have produced many exciting findings and the outlook is quite promising for this field of research.

At the present time, the choices for suitable candidate Ags are limited by the fact that only few TAAgs have been identified and proven suitable for the loading and priming of DCs, but with the new advances in gene mapping and isolation, the number of suitable candidates are continually expanding[39,40]. Lately, there has been suggestion of potential benefits in administering DC activators in combination with DC vaccination, which may enhance as well as magnify the ability of DCs in eliciting strong T-cell responses. Ultimately, there has to be some consensus between the researchers with regard to optimal approach in assessing immune responses in patients undergoing these therapies, as well as with respect to the vaccination itself. All in all, the future looks bright for the use of DCs as an effective therapy to fight cancer.

References

1. Banchereau, J and Steinman, RM. Dendritic cells and the control of immunity. *Nature* **392**, 245-252 (1998).

2. Satthapom, S and Eremin, 0. Dendritic cells (I): biological functions. *J. R. Coll. Surg.Edinb.* **46**, 9-20 (2001).

3. Caux, C *et al*. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic pathways in response to GM-CSF and TNF-alpha. *Adv. Exp. Med. Biol.* **417**, 21-25 (1997).

4. Cella, M, Sallusto, F and Lanzavecchia, A. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol.* **9**, 10-16 (1997).

5. Hart, DN. Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* **15**, 409-419 (1997).

6. Reid, CD. The dendritic cell lineage in haemopoiesis. *Br. J. Haematol.* **96**, 217-223 (1997).

7. Shortman, K and Caux, C. Dendritic cell development: multiple pathways to nature's adjuvants. *Stem Cells* **15**, 409-419 (1997) .

8. Grouard, G. *et al*. The enigmatic plasmacytoid T-cells development into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J. Exp. Med.* **185**, 1101-1111 (1997).

9. Sallusto, F and Lanjavecchia, A. Dendritic cells used macropinocytosis and the mannose receptor to concentrate antigen to the MHC class n compartment: down- regulation by cytokines and bacterial products. *J. Exp. Med.* **182**, 389-400 (1995).

10. Buelens, C. *et al*.. Human dendritic cell responses to lipopolysaccharide and CD40 ligation are differentially regulated by EL-10. *Eur. J. Immunol.* **27**, 1848-1852 (1997).

11. Pierre, P. *et al*. Developmental regulation of MHC class n transport in mouse dendritic cells. *Nature* **388**, 787-792 (1997).

12. Cella, M. *et al*. Inflammatory stimuli induce accumulation of MHC class n complexes on dendritic cells. *Nature* **388**, 782-787 (1997).

13. Sinkovics, JG and Horvath, JC. Vaccination against human cancer. *Int. J. Oncol.* **16**, 81-96 (2000) .

14. Pisani, P. *et al*. Cancer and infection: estimates of the attributable fraction in 1990. *Cancer Epidermiol. Biomarkers Prev.* **6**, 387-400 (1997).

15. Sogn, GA. Tumour immunology: the glass is half full. *Immunity* **9**, 757-763 (1998).

16. Pardoll, DM. Cancer vaccines. *Nat. Med.* **4**, 525-531 (1998).

17. Pawelec, G. *et al*.. Escape from host-anti-tumor immunity. *Crit. Rev. Oncog.* **8**, 111-141 (1997).

18. Chaux, P. *et al*. Tumor-infiltrating dendritic cells are defective in their antigen- presenting function and inducible B7 expression in rats. *Int. J. Cancer* **72**, 619-624 (1997).

19. Radmayr, C. *et al*. Dendritic antigen-presenting cells from peripheral blood of renal-cell-carcinoma patients. *Int. J. Cancer* **63**, 627-633 (1995).

20. Gabrilovich, DI. *et al*. Decreased antigen presentation by dendritic cells in patients with breast cancer, *Clin. Cancer Res.* **3**, 483-490 (1997).

21. Ninomiya, J. *et al*. Dendritic cells with immature phenotype and defective function in the peripheral blood from patients with hepatocellular carcinoma. *J. Hepatol.* **31**, 323-331 (1999) .

22. Almand, B. *et al*. Clinical significance of defective dendritic cell differentiation in cancer, *Clin. Cancer. Res.* **6**, 1755-1766 (2000).

23. Specht, JM. *et al*. Dendritic cells retrovirally transduced with a model tumor antigen gene are therapeutically effective against established pulmonary metastases. *J. Exp. Med.* **186**, 1213-1221 (1997).

24. Song, W. *et al*. Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model tumour antigen induce protective and therapeutic anti-tumour immunity. *J. Exp. Med.* **186**, 1247-1256 (1997).

25. Schuler, G. and Steimman, RM. Dendritic cells are adjuvants for immune- mediated resistance to tumors. *J. Exp. Med.* **186**, 1183-1187 (1997).

26. Gong, J. *et al*. Induction of anti-tumor activity by immunization with fusions of dendritic and carcinoma cells. *Nat. Med.* **3**, 558-561 (1997).

27. Flamand, V. *et al*. Murine dendritic cells pulsed in vitro with tumour antigen induce tumour resistance in vivo. *Eur. J. Immunol.* **24**, 605-610 (1994).

28. Mayordomo, JI. *et al*. Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic anti-tumour immunity. *Nat. Med.* **1**, 1297-1302 (1995).

29. Mayordomo, JI. *et al*. Bone marrow-derived dendritic cells serve as potent adjuvants for peptide-based anti-tumour vaccines. *Stem Cells* **15**, 94-103 (1997).

30. Fields, RC, Shimizu, K and Mule, JJ. Murine dendritic cells pulsed with whole tumour lysates mediate potent anti-tumour immune responses in vitro and in vivo. *Proc. Nati. Acad. Sci. USA* **95**, 9482-9487 (1998).

31. Hsu, FJ. *et al*. Vaccination of patients with B-cell lymphoma using autologues antigen-pulsed dendritic cells. *Nat. Med.* **2**, 52-58 (1996).

32. Bell, D. Young, JW and Banchereau, J. Dendritic cells. *Adv. Immunol.* **72**, 255-324 (1999).

33. Timmerman, JM and Levy, R. Dendritic cell vaccines for cancer immunotherapy. *Annu. Rev. Med.* **50**, 507-529 (1999).

34. Romani, N. *et al*. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* **180**, 83-93 (1994).

35. Palucka, KA. *et al*. Dendritic cells as the terminal stage of monocyte differentiation. *J. Immunol.* **160**, 4587-4595 (1998).

36. Caux, C. *et al*. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. *Nature* **360**, 258-261 (1992).

37. Luft, T. *et al*. Type I IFNs enhance the terminal differentiation of dendritic cells. *J. Immunol.* **161**, 1947-1953 (1998).

38. Markowicz, S. Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. *J. Clin. Invest.* **85**, 955-961 (1990).

39. Ribas, A. *et al*. Genetic immunization for the melanoma antigen MART-1/melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res.* **57**, 2865-2869 (1997).

40. Ribas, A. *et al*. Generation of T-cell immunity to a murine melanoma using MART-1 engineered dendritic cells. *J. Immunol.* **23**, 59-66 (2000).