

## Advances in immunotherapy using dendritic cells

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**Abstract:** The immune system is the host natural defence against cancer. Cancers are caused by progressive growth of the progeny of a single transformed host cell. The immune system is generally not able to mount immune responses to “self-antigens”, due to various mechanisms of immunological tolerance that are in place. This means that despite possessing a natural defence against tumours, many of the cancer patients may not be able to mount an effective immune response to fight the tumours. Dendritic cells (DC) are highly specialised in antigen presenting that can initiate and stimulate immune responses. These cells have the ability to stimulate naïve T cell proliferation and perform specific stimulatory and tolerogenic functions respectively. When the DC are activated by antigens, these cells undergoes further maturation and migrate to secondary lymphoid tissues, present antigen to T cells and finally induce an immune response. The ability of the DC to activate naïve and primed T-lymphocytes makes these cells a good candidate to be explored as a potential immunotherapeutic agent that can modulate anti-tumour immune responses in the affected host.

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### Background

Dendritic cells (DC) belong to a very unique class of cells. These cells represent a small population of bone marrow-derived leucocytes that gained its name by the dendrite morphology.<sup>1</sup> These cells were first described in 1973 by Steinman and Cohn.<sup>2</sup> Dendritic cells constitute about 0.1-1% leucocytes of human, murine or other non-primate animals. These cells are well-known for their highly heterogeneous populations that are reflected by their variations in terms of precursor populations, anatomical locations, functions and the final outcome of immune responses.<sup>1,3</sup>

Dendritic cells are widely accepted as the only antigen presenting cells (APC) that are capable of

eliciting primary and boosting secondary immune responses.<sup>1,3</sup> According to Lanzavecchia *et al.* (1996), the DC are the sentinels of the immune system.<sup>4</sup> These cells play important roles in inducing immune responses. Dendritic cells play an important role in activating T-lymphocytes<sup>4</sup> and also in the maintenance of immunological tolerance.<sup>5,6</sup> The ability of DC to activate antigen-specific T-cells responses has opened up new approaches for cancer therapy.

### Life-cycle of dendritic cells

Dendritic cells are derived from the myeloid precursor in the bone-marrow. The newly developed DC will exit the bone marrow and circulate in the blood as immature cells. Following this, these cells will form an extensive network of interstitial DC in several non-lymphoid organs. As soon as these cells encounter any danger signals i.e. any form of injury to the host, these DC will undergo maturation and activation and rapidly migrate to the lymph nodes.<sup>1</sup> The DC will usually home to the para-cortex areas of the lymph nodes, which is the residence of naïve T-lymphocytes. These cells will then interact with the antigen-specific T-lymphocyte to initiate antigen-specific primary immune response or stimulate a memory immune response. The DC can also be found in the follicular areas of the lymph nodes, which is the home of naïve B-lymphocytes. These cells are known as the follicular dendritic cells (FDC). It has been suggested that the DC will die in the lymph nodes after completing their antigen presentation function, as they do not appear to exit the lymph node after performing this function.<sup>1</sup>

### Types of dendritic cells

There are many types of DC, depending on the tissues that these cells are isolated from such as Langerhan cells in the epidermal layer of the skin, dermal or interstitial DC (intDC), splenic marginal DC, T-zone interdigitating cells, germinal-centre DC, thymic DC, liver DC and blood DC.<sup>1</sup> The different types of DC can be identified based on the different cell surface molecules

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that these cells express. However, their maturation and functional differences have yet to be established. The wide distribution of DC allows these cells to work together and to generate a complete and extensive immune network to protect host from infection as well as prevent invasion by pathogens or foreign agents. The diverse roles of the DC in the immune system is evident by the ability of DC precursors to respond to different antigens and their ability to secrete large amounts of pro-inflammatory or antiviral cytokines either to modulate T-cell responses or to directly remove the antigens.<sup>1</sup> This means that DC are not only responsible for the generation of adaptive immune responses but these cells also play a major role in linking the main two arms (*innate and adaptive*) of the immune system for efficient removal of pathogens.

#### Brief history and epidemiology of cancer

The awareness of cancer has been documented since a few thousand years ago. It has been reported that the term “cancer” was used in ancient times to describe this disease where this word was found to be written on Egyptian papyrus between 3000-1500 BC, which incidentally was reported to be the oldest description of human breast tumour.<sup>7</sup> Hence, cancer cannot be regarded to be a modern disease. Today, the most common cancer worldwide in males and females are lung cancer<sup>8</sup> and breast cancer<sup>8</sup> respectively. The incidence of breast cancer worldwide has increased since the 1960s and the number of newly diagnosed cancer patients is escalating at an alarming rate. So, the finding that breast cancer makes up the largest number of cancer-related deaths in women<sup>8</sup> was not at all surprising. The scenario is not different in Malaysia. In 2002, a total of 26,089 cases of cancer were diagnosed among the residents in Peninsular Malaysia.<sup>9</sup> Of these cases, 11,815 were males and 14,275 were females. The number of new cases of women with breast cancers was found to be 4,339 in the year 2002.<sup>9</sup> In contrast, there were only 3,825 new cases of women diagnosed with breast in the year 2000.<sup>9</sup> These findings meant that Malaysian women had a 1 in 19 chance of developing breast cancer.

The battle against cancer is not a new one. In the last few decades, scientists and clinicians have worked very hard to understand the pathogenesis of cancer so that they can use the information to help cancer patients to fight this deadly disease. The discovery of anaesthesia greatly facilitated the surgical removal of small and localised tumours.<sup>7</sup> However, the advantages of surgical interventions were found to be limited to early stage cancer patients with small and localised tumours. Another milestone in cancer therapy was when radiotherapy was used to remove residual tumour cells that were not completely removed during surgery.<sup>10</sup>

The discovery of cytotoxic drugs that can kill rapidly dividing cells, like tumour cells provided another therapeutic approach to fight cancer i.e. chemotherapy.<sup>10</sup> The chemotherapy approach can work synergistically with the surgical intervention and/or radiotherapy to fight cancer as the cytotoxic drugs have the ability to destroy cancer cells that have spread beyond the reach of a surgeon or radiotherapist. The disadvantage of using the cytotoxic drugs is that these drugs are not able to distinguish between rapidly dividing normal cells and cancer cells. In addition, some cancer patients can develop multi-drug resistance. Another therapeutic approach was to use hormone therapy. This treatment approach had a favourable effect in only treating cancers due to hormone disorders.<sup>10</sup> This means that the hormone therapy approach only has limited applicability.

Currently, there are several new therapeutic approaches used for treating cancers such as stem cell transplantation<sup>11-14</sup> and immunotherapy using dendritic cells (DC) or T-cells to combat cancer.<sup>15-17</sup> The stem cell transplantation approach appears to be effective in treating leukaemia, lymphoma and other haematological disorders.<sup>18-20</sup> The overall high cost and difficulties in finding suitable HLA-matched donors means that this approach cannot be made readily available to many patients. Another drawback of this approach is the graft-versus-host disease (GVHD), which is the major cause of mortality in many patients who have undergone transplantation. Combination therapy using high doses

of cytotoxic drugs and stem cells transplantation in the treatment of breast cancer has also tested.<sup>21</sup> However, this approach remains controversial due to the use of high-doses of the chemotherapeutic drugs.

There have been significant advances made in cancer therapies during the last decades. However, one of the main challenges of current treatment strategies is the spread of tumours i.e. tumour metastasis. Tumour metastasis is still the most common cause of death from cancer.

### Dendritic cells in cancer immunotherapy

The immune system plays an important role in detecting and destroying cancer cells.<sup>22,23</sup> However, many cancers fail to activate host immune response and most can evade recognition by the lymphocytes. So, the question that one can ask is “*if one of the functions of the immune system is to detect and destroy cancer cells, then why do humans have cancer?*” The relatively simple answer is that a cancer cell is actually a transformed host cell whose cell cycle regulatory mechanisms have gone awry, allowing it to proliferate and differentiate in an uncontrolled manner. Cancers are caused by progressive growth of the progeny of a single transformed cell. Lymphocytes are selected during their development based on their inability to mount immune responses to host antigens.<sup>24</sup> This means that the lymphocytes generally do not make immune response to “self” proteins or antigens. So, it will be rather difficult to induce the activation of the host immune system against tumours. The main goal in cancer therapy is to remove and destroy “all” malignant cells without harming the patient. Although surgical intervention, radiotherapy and chemotherapy can make great impact on reducing tumour load, it is usually not possible to remove and destroy all the tumour cells. There are bound to be some residual tumour cells that can reinitiate onset of tumour and metastases. Therefore it is important to not only retard the growth and metastasis of primary tumour but to have strategies that will prevent these tumours from recurring once the initial mass is removed. The best

way would be to activate the host immune response against the tumour so that there would be continuous surveillance that can prevent the residual tumour cells from regaining their aggressive growth and spread.

In the past decade, there have been numerous studies that have helped immunologists to better understand the concept of immune surveillance and develop an appreciation of the mechanisms by which tumours can escape the host immune system. Studies have shown that antibodies and T-lymphocytes that identify tumour antigens can be isolated from patients with cancer.<sup>22,25</sup> It is clear that the immune system is capable of recognising tumour cells. Several cellular immunotherapy studies have shown that it is possible to stimulate anti-tumour activity in the patient either through the use of DC vaccines, autologous and/or allogeneic lymphocytes.<sup>22,24</sup> The aim of these immunotherapeutic strategies is to harness potent immunological weapons to destroy cancer cells.<sup>26</sup> These findings have contributed to the development of promising new strategies against cancer.

Dendritic cells play an important role in activating T-lymphocytes as these cells are found to be highly specialised in presenting antigen to T-lymphocytes.<sup>4,28</sup> The DC play a key role in mediating anti-tumour immune responses and these cells are also the most potent antigen-presenting cell (APC).<sup>28,29</sup> Although DC have been identified to be the sentinels of immune system, their role in combating cancer was not thought to be prominent due to the low numbers of these cells that are present in peripheral blood. The recent advances in cell culture techniques have allowed large scale propagation of DC from several sources including peripheral blood mononuclear cells. This has paved the way for using these cells in a clinical setting for cancer immunotherapy. In recent years, several studies have shown that DC vaccines i.e. tumour antigen-pulsed DC are capable of inducing antigen-specific T-helper (T<sub>H</sub>) and cytotoxic T-lymphocytes (CTL) responses to tumour.<sup>26</sup> These CTL were capable of fighting tumours when these cells were appropriately activated by allogeneic DC that can present tumour peptides on

their major histocompatibility complex (MHC) proteins found on their cell surface.<sup>30,32</sup> Although, only some of the antigens expressed by tumour cells may be tumour-specific, the host immune system will be able to use these antigens to distinguish the tumour cells from the normal cells.<sup>32-34</sup> These findings suggest that tumour-specific immune responses can be generated if the tumour-specific peptides are appropriately presented by DC and used to activate the antigen-specific T-lymphocytes. These observations form the basis of clinical trials involving the use of DC as immunological adjuvants or therapeutic vaccines in cancer immunotherapy.

The first pilot study using the DC-vaccine approach was conducted in non-Hodgkin's lymphoma patients in 1996.<sup>35</sup> The results from this study<sup>35</sup> and a larger scale study on the same tumour,<sup>17</sup> showed that DC vaccine was effective in producing host anti-tumour specific immune responses. However, a similar clinical trial conducted in multiple myeloma patients who developed minimal residue disease following high-dose chemotherapy showed variable results.<sup>36</sup> To date, there are several clinical trials have been conducted using the DC vaccine approach. These include malignancy<sup>37</sup> and solid tumours such as colon cancer,<sup>38,39</sup> prostate cancer,<sup>40</sup> non-small cell lung cancer<sup>41</sup> and melanoma.<sup>16,42</sup> The most promising results observed for solid tumours were in the melanoma DC immunotherapy clinical trial.<sup>16</sup> The use of this approach to treat other solid tumours such as breast cancer and colon cancer are still in the early stages of clinical application.

Despite the existence of evidence to support the use of DC vaccines to generate anti-tumour responses, the progress of the DC therapeutic approach is hampered due to several reasons such as (i) impaired tumour-infiltration by lymphocytes due to inhibitory cytokines<sup>43</sup>, (ii) aberrant Fas-ligand expression<sup>44</sup> as well as (iii) altered expression of MHC molecules on tumours.<sup>22,45</sup> We have recently demonstrated in an immunocompetent mouse model of breast cancer that it is possible to enhance the anti-tumour effect of DC vaccines through the use of adjuvant therapy in the form of tocotrienols.<sup>15</sup>

Despite the rapid progress in the field of cancer immunotherapy, there is still a lot of room for more studies that will provide a better understanding on how to harness the potential of the host immune system against tumours. After all, fighting tumours is one of the physiological functions of the immune system.

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