Combination Therapy of Cancer – Escalating the Effect of Dendritic Cell-based Cancer Vaccine in the Tumour Micro-environment

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Abstract

A key factor in initiating and operating the immune system against tumour cells, the dendritic cell (DC) has been regarded as the next possible breakthrough in new cancer therapy. However, the results of more than 15 years of clinical studies with DC vaccine revealed the difficulties fulfilling this expectation. Evidence has disclosed that the DC activation required for proper tumour-specific effector CD4+ and CD8+ T cell stimulation is inhibited in the micro-environment of tumour. Studies have further reported that DC phenotypes in tumour tissue and draining lymph nodes are mostly immature, which results in regulatory immune responses. Also, the existence of MDSCs and TAMs adversely affect both DC function and immune suppression in the cancer-environment. In this review, efforts to overcome the tumour or host-dependent hindering which inhibit the effect of cancer vaccine will be discussed. The combination therapy of cancer with DC vaccine and other immune modulators may improve the clinical efficacy.

Keywords

Dendritic cell (DC) vaccine, combination therapy, tumour micro-environment, antibodies, cytokines, chemotherapeutics, myeloid derived suppressor cells (MDSCs)

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The role of the dendritic cell (DC) is at the centre of the immune system by initiating, progressing and regulating the responses against pathogens, include tumours. After the first successful clinical achievement in DC-based immunotherapy trials in follicular lymphoma and melanoma in the mid-1990s,[1,2] the DC vaccine used to treat patients with cancer such as melanoma, lymphoma and renal cell carcinoma.[3–7] However clinical expectations have not been fulfilled due to an overall clinical response rates of under 10–15 %, the usual response rates observed in various types of immunotherapies.[6–11] The meta analysis performed with 906 prostate and renal cell cancer (RCC) patients in 29 separate DC vaccine clinical trials revealed the objective response rates, 7.7 % in prostate cancer and 12.7 % in RCC.[12] If the stable disease rate was combined as clinical benefit rate (CBR), much better response rate was counted (54 % in prostate cancer and 48 % in RCC). Although the clinical expectation has not been satisfied, the outcomes of many clinical trials with tumour antigen-loaded conventional DCs have provided proof that therapeutic immunity can be elicited.[13–15] And statistically significant effect of DC-mediated cellular immune response and of DC dose on CBR was proved in meta-analysis.[12] The clinical data has helped to establish a standard for properly activated DCs with appropriate form and doses of loading antigens.These activated DCs can migrate to the lymph nodes which then initiate and expand tumour-specific CD4+ and CD8+ T cell responses and later induce meaningful therapeutic responses in patients.

Several mechanisms involved in unsatisfactory anti-tumour responses of DC vaccine in the clinic. Mechanisms include; the presence of suppressive leukocytes like myeloid derived suppressor cells (MDSCs), tumour associated macrophages (TAMs) with or without the presence of constitutive p-STAT3 signalling, immunoediting, abnormal tumour vasculature inhibiting effector T cell entry or tumour cell interaction with the stromal environment.[15–20] On the other hand, in order to improve the DC vaccine clinical efficacy, it is critical to control the therapeutic DC quality and standardise the vaccine design and protocol. Looking at this very view, several investigators have analysed DC vaccine problems in their publications.[4,6,13,15,21–23] One of the efforts is using allogeneic cells, since the DCs isolated from cancer patients express impaired characters for generation of the tumour-specific immunity.[24] Thus, without further discussing about the DC vaccine quality, tumour tissue or host side hindering factors and the possibility of improving antitumour immune-therapeutic efficacy will be discussed in this review.

Dendritic Cells in Cancer Patients

DCs are lymphocytes in the immune system which control overall immunity by interacting with other immune cells, including T cell, B cell and natural killer (NK) cells.[6,25–26] DCs themselves are a complicated system consisting of various anatomic localisations, subsets and functions that are correlated with one another. DCs control the immune system, not only in stimulatory but also in regulatory immunity as professional APC.[23,25] In cancer tissues or cancer-draining lymph nodes, DCs are found as resting, non-activated and immature cells.[27–31] Tumour-induced immunosuppressive milieu generally causes a decrease in the numbers of conventional myeloid DCs in patients.[27] In rodent models, immature myeloid DCs promote the expansion of regulatory T cells (Treg) in tumour-draining lymph

nodes, which are associated with tumour progression in a TGF-β dependent fashion. Immunosuppressive factors, mostly pro-inflammatory molecules from the tumour micro-environment, target endogenous DCs in patients, resulting in dysfunction and impaired development of tumour-specific effector lymphocytes.[32–33] Typical inflammatory mediators of tumour-induced DC dysfunctions include; IL-10, TGF-, VEGF, IL-6 and prostanoids such as PGE2-634-37. These mediators are produced from either the tumour itself or the infiltrated host factors including MDSCs and TAMs. In this milieu, DCs are having trouble maturing, expressing the co-stimulatory molecules needed for T cell activation, and producing the cytokines needed to support tumour specific effector T cell activation and survival.[38-41] Tumour-related malfunctions of DCs are noted in patients with ovarian, breast, melanoma, renal cell, prostate carcinoma[42–45] and in the blood of head and neck, lung and breast cancer patients.[38,46] The major intracellular signalling pathway required for DC activation and final maturation in the immunosuppressive milieu of the tumour micro-environment is STAT3.[47] Oncogene or cytokine-induced over-expression of the STAT3 protein in tumour cells up-regulates the expression of several immunosuppressive cytokines, including IL-10 and TGF-β, and suppresses Th1 cell immune responses.[16,48–49] STAT3 expression from cancer cells leads to STAT3 production by a variety of leukocytes, including DCs. STAT3 expression in tumour-associated DCs causes reduced expression of co-stimulatory and MHC II molecules, and correlates with an accumulation of immature DCs, which may induce T_{reg},[50] an inhibitor of effector T cell function. Anti-tumour effects of the STAT3 inhibitor, cucurbitacin I was observed in mice.[51–52] Although dysfunctional tumour-associated DCs may support immune suppression and promote oncogenesis, it may be possible to evoke therapeutic antitumour activity in these DCs by molecularly defined triggers of DC maturation, causing induction of tumour-specific effector T cells.

Inflammatory Nature of Tumour Micro-environment

The development of about 15–20 % of malignancies worldwide are known to be related to chronic inflammation, including oesophageal, gastric, hepatic, pancreatic and colorectal cancer.[53] Inflammatory mediators produced by the tumour cell can create an inflammatory micro-environment and cause both leukocyte recruitment and angiogenesis.[54–56] Also, these inflammatory milieus can help tumour cell survival, motility and chemotaxis. For example, breast cancer cells are known to produce the inflammatory chemokines CCL2 and CCL5, which are poorly expressed in normal breast cells. These chemokines recruit TAMS and inhibit potential antitumour effector T cells.[57] In other words, the immunosuppressive tumour micro-environment is created by the inflammatory nature of tumours and an infiltration of assorted leukocytes, in particular MDSCs and TAMs. This infiltration leads to the suppression of the DC-induced effectors, CD4+ and CD8+ T cell responses and the induction of T_{reg}.[27]

MDSCs in the Tumour Micro-environment

The mechanisms which chronic inflammation promotes the onset and development of tumours are differentiated into non-immunological and immunological ways.[58] The non-immune mechanisms include: 1) the production of reactive oxygen species which cause DNA mutation, 2) the production of pro-angiogenic factors, like VEGF which promote tumour neo-vascularisation 3) the production of matrix metalloproteases which facilitate invasion and metastasis.[59–61] The predominant immune mechanism is the disturbance of myelopoiesis and haemopoiesis, which causes a deficiency in APCs and in dysfunctional cell-mediated anti-tumour immunity. One of the important parameter in this deficiency is MDSC.[27] In individuals with an established tumour, MDSCs are known to prevent the efficacy of cancer vaccines.[62] In most patients and experimental mice tumour settings, the accumulation of MDSCs in the blood, lymph nodes, bone marrow and tumour sites is observed. These cells are known to inhibit both adoptive and innate immunity. MDSC induction and recruitment into the tumour site is mediated by tumour-secreted and host-secreted factors, many of which are pro-inflammatory molecules. Thus it may be said that inflammation promotes the accumulation of MDSCs, which down-regulate immune surveillance and anti-tumour immunity, thereby facilitating tumour growth.[58] Recently, the clinical perspective of MDSCs in cancer patients are reviewed elsewhere.[63] Identification of MDSCs in cancer patients and experimental mice were analysed by the activity of T cell suppression. In mice, MDSCs are characterised as $Gr1^{+}CD11b^{+}$ expressing cells. Gr1 includes Ly6C, a macrophage marker and Ly6G, a neutrophil marker. CD11b is the characteristic marker of macrophage.[58] In some subsets of MDSCs, several markers are ascribed, including the IL-4 and IL-13 receptor alpha chain (IL-4Ra),[64,65] F4/80, a macrophage marker,[64,66,67] *c-fms*(CD115),[67] and CD80.[68] Among the MDSCs, mononuclear cells are defined as 'monocytic' $CD11b^{+}Ly6G^{+/-}Ly6C^{high}$, whereas 'granulocytic/ neutrophil-like' multi-lobed nuclei possessing cells are characterised by $CD11b^{+}Ly6G^{+}Ly6C^{low}$.[66,69,70] Immunosuppressive substances produced from MDSCs include arginase, inducible NO synthase, and/or ROS.[71–75] Unlike mice, MDSC characterisation in cancer patients is complicated but typically characterised by the phenotype $CD11b^{+}CD33^{+}CD34^{+}$CD14-HLA-DR- with various expressions of CD15 and other markers. Recent findings have identified $CD14^{+}HLA\text{-}DR^{/low}$ as a new MDSC subtype in melanoma and hepatoma patients.[76,79] It is known that different tumours induce different subtypes of MDSCs in cancer patients.[76,77] Along with heterogeneity characterised by the surface phenotype, internal markers, morphology and suppressive substances in both mice and humans, MDSCs suppressed multiple immune effectors include; inhibition of CD4+ and CD8+ T cell functions,[80–83] induction of Treg by secreting TGF-β, IL-10 or arginase,[67] interaction with NKT cells to enhance tumour growth by suppressing antitumour immunity.[84]

Improvement of Clinical Efficacy of the Dendritic Cells Vaccine

Considering the inflammatory tumour micro-environment and dysfunctional DCs with suppressed-immunity in cancer patients, it is not surprising to see recent reports indicating that the cancer vaccine induced tumour-specific T cells is not necessarily associated with the induction of functional cytotoxic T lymphocytes, but instead leading to the undesirable activation and expansion of regulatory T cells.[15] Tumour antigen-induced immune responses are weak or ineffective, because unlike infectious pathogens, tumours do not induce the strong enough inflammatory responses for the optimal activation of DCs. Thus, the primary purpose of a cancer vaccine is to overcome this defect by educating DCs with a stronger antigenic signal and providing optimal conditions for the maturation into potent immune-stimulatory APCs.[23] In the immunosuppressive milieu of cancer patients, sufficient numbers of properly activated tumour-specific Th1 cells and CTLs are not generated despite ample expression of tumour-associated antigens in cancers. The effects of therapeutic cancer vaccines, including DC based therapy, can be enhanced by combination with the methods that overcome the immune-suppression associated

with tumour cells. Then the generation of large numbers of high avidity antigen-specific effector CD8+ T cell can be expected. Such therapies target either tumour cells, T_{regs} or DC and even effector cell itself then over-ride the immune-suppressive milieu of the tumour-bearing host.[13] Among potential combination methods, therapeutic antibodies are differentiated into two functional groups: one is the antibody which is blocking the suppressive cytokines (IL-10, IL-13, TGF-b, VEGF) or inhibitory co-stimulatory molecules (PD-1, CTLA-4), the other is the agonistic antibody which is further promoting co-stimulation between the antigen presenting cells and activated effector T cells such as anti-CD137 (4-1BB signal). The author's experience[85] with DC vaccine and agonistic anti 4-1BB Ab combination against mouse liver metastatic colon cancer confirmed the synergistic anti-tumour effect.

Another candidate, cytokine has complicated effects. Cytokines work on the DCs as well as lymphocytes and tumour cells but also secreted by those cells and manipulate the immune responses. Combinatorial administration of cytokines like IL-2, IL-12, GM-CSF with DC vaccine improved the anti-tumour effect. Those cytokines may promote the DC vaccine activity by inducing the survival, migration, activity of lymphocytes as well as DCs. Reported clinical study with DC vaccine combined with IL-2 and IFN-α for RCC observed the improved anti-tumour effects.[86] The possible mechanism of IL-2 might the correction of the T cell receptor signalling defects in cancer patients. IFN-α suggested to induce the MHC molecule and tumour-associated antigen expression, thus enhanced the tumour immunogenicity.[86]

The clinical and immunological result of combination therapy of RCC and breast cancer with CD34+-haematopoietic stem cell derived DC vaccine and IL-2 was reported by author.[87] Clinical response was observed in one RCC patient as stable disease. However, DC-vaccine related antigen-specific immune responses including peripheral blood lymphocyte proliferation and the number of IFN-γ secreting cells were induced in six patients without clear correlation with clinical responses. Also NK activity was induced significantly in six patients after vaccination. DC vaccine-related decrease of TGF-β level or increase of IL-12p70 level and decline of $CD4^+CD25^+$ T cells were observed in three patients. However only in the RCC patient whose disease stabilised, concomitant immune alterations including induction of IFN-γ secreting T cell and reduction of $CD4^+CD25^+$ T cell were correlated with clinical responses. However, it has been reported that the expansion of T_{reg} in DC vaccine and IL-2 combination protocol.[88,89] Although it is not conclusive whether the induced Treg directly inhibit the anti-tumour efficacy of DC vaccine, the immune suppressive condition needs to be controlled to achieve the better clinical anti-tumour results.

Generally chemotherapeutics kill the rapid proliferating cells including tumour cells as well as bone marrow stem cells which are the cause of immune-suppression in treated patients. High dose cyclophosphamide, a chemotherapeutics, inhibits T cell function and anthracyclines affect the macrophages. On the other hand, low dose cyclophosphamide induces the immunity. Doxorubicin did not inhibit but induce macrophage-related anti-tumour activity *in vivo*. This data[90–91] provides the immunological rationale for testing immune-modulating doses of chemotherapy in combination with tumour vaccines in patients with cancer. Limited number of recent papers including the reports from Zitvogel group[92] determined the contribution of conventional chemotherapeutics in anti-tumour immunity. Chemotherapeutics like anthracyclines or radiotherapy can induce the immunological death of tumour cell and/or stimulate the immune system as a side effect. Thus the anti-tumour immunity can be induced and activated by the DC antigen uptake of dead tumour cells.

Reports of the author[93] proved the chemical-induced immunogenic tumour cell death and the increased DC uptake of them. This immunogenic dead tumour cells also secrete the cytokines that may convert the tumour micro-environment from the immune-suppressive to immune-stimulatory.These data suggest the new combinatorial protocol for cancer immunotherapy with DC vaccine and chemotherapeutics.[94-96] Drugs other than conventional chemotherapeutics, non-steroid anti-inflammatory agents like COX-2 inhibitors are also considered as a helper of anti-cancer activity. By altering inflammatory condition of tumour micro-environment, COX-2 inhibitor may improve the anti-tumour immunity of infiltrated effector T cells.[97] Conclusively, correcting the immunological balance in the tumour micro-environment from suppression to a tumour-rejecting condition may be the key factor in succeeding with a DC vaccine clinical trial. ■

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