

Naturally circulating dendritic cells to vaccinate cancer patients

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Keywords: cancer immunotherapy, dendritic-cell targeting, dendritic-cell vaccination, myeloid dendritic cells, plasmacytoid dendritic cells

Abbreviations: DC, dendritic cell; IFN α , interferon α ; mDC, myeloid dendritic cell; moDC, monocyte-derived dendritic cell; NK, natural killer; pDC, plasmacytoid dendritic cell(s)

Dendritic cell-based immunotherapy is a promising strategy against cancer that appears to be feasible, safe and to induce potent tumor-specific immune responses. The use of naturally circulating dendritic cells (DCs), rather than cultured monocyte-derived DCs, might constitute the next logical step to translate anticancer immune responses into long-lasting clinical benefits.

Monocyte- and CD34-Derived Dendritic Cells

How human CD34⁺ cell progenitors or monocytes can be differentiated in vitro into dendritic cells (DCs) by the combined administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) was first described in 1994. This boosted the enthusiasm for the use of DC vaccination in humans, resulting in the first clinical studies in which monocyte-derived DCs (moDC) were employed as a cellular anticancer vaccine. Since then, numerous—usually small—clinical trials have been performed to demonstrate the efficacy of this approach. After more than one decade, we nowadays know that moDCs can induce anticancer immune responses in a significant number of patients, in particular when both CD4⁺ and CD8⁺ T cells are activated, as recently shown by our DC vaccination studies.¹ Nevertheless, only a limited number of clinical responses have been observed, yet expectations are high as these responses often are long-lasting.

It remains unclear whether DCs differentiated ex vivo from monocytes or

CD34⁺ cell progenitors are optimal for the induction of potent immune responses.² The extensive culture period and the agents that are required to differentiate them into DCs might negatively affect immune functions, in particular their capacity to migrate toward T-cell areas in lymph nodes. Therefore, the interest in naturally circulating DCs has risen, despite the fact that these cells are relatively scarce, constituting only about 0.2–1.5% of peripheral blood leukocytes.

Natural Dendritic-Cell Subsets

Two major populations of naturally circulating DCs can be distinguished in the peripheral blood of humans: myeloid (CD1c⁺) DCs (mDCs) and plasmacytoid DCs (pDCs). These DC subtypes differ in function, localization, and phenotype. On one hand, mDCs mainly migrate to, or reside in, the marginal zone of lymph nodes (a primary entry point for blood-borne antigens) and are thought to recognize and respond to bacterial and fungal antigens. On the other hand, pDCs mainly reside in the T-cell areas of lymph

nodes and seem to be specialized for the recognition of viral antigens.³

Interestingly, we have recently observed that pDCs can take up small but significant amounts of antigens, efficiently cross-present them,⁴ and even exhibit a tumoricidal natural killer (NK) cell-like behavior.⁵ Taken together, these findings suggest that naturally circulating DCs such as pDCs may also be well suited for vaccination studies.

Clinical Studies with Natural Plasmacytoid Dendritic Cells

We have embarked on exploiting pDCs that naturally circulate in the peripheral blood for cancer immunotherapy. Although it is well known that inactive intratumoral pDCs are associated with tumor progression, activated, interferon α (IFN α)-producing pDCs can stimulate NK cells and induce antigen-specific T- and B-cell responses. Our finding that vaccines commonly used for the prophylaxis of infectious diseases could simply be used as adjuvants for stimulating pDCs to secrete significant amounts

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Submitted: 12/21/12; Accepted: 12/28/12

Citation: Bol KF, Tel J, de Vries JM, Figdor CG. Naturally circulating dendritic cells to vaccinate cancer patients. *Oncoimmunology* 2013; 2:e23431; <http://dx.doi.org/10.4161/onci.23431>

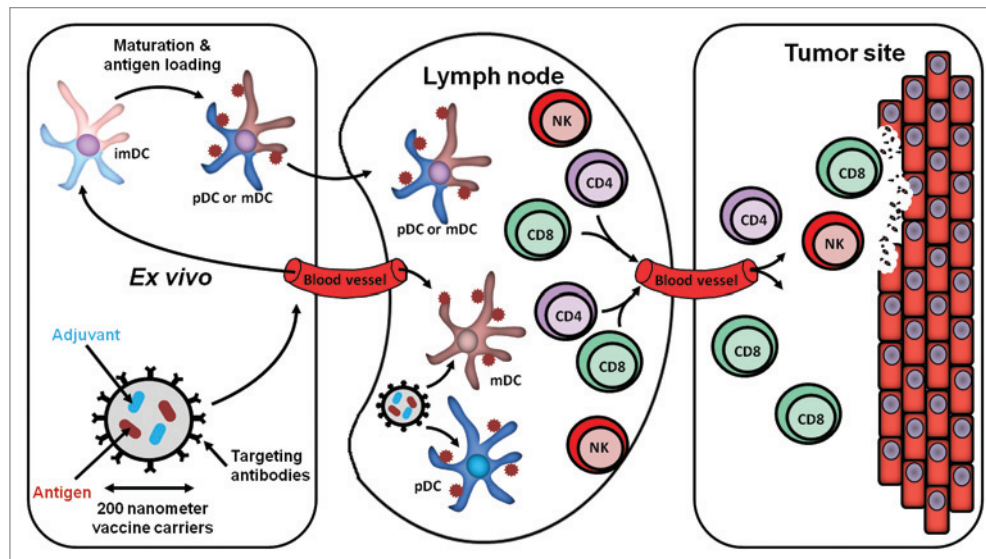


Figure 1. Induction of tumor antigen-specific T cells with naturally circulating dendritic cells. Exploiting naturally circulating dendritic cells (DCs) can either be performed by isolating plasmacytoid DCs or myeloid DCs and stimulating them ex vivo with adjuvant(s) and antigens or by targeting them in vivo, by means of nanoparticles that carry antigens and adjuvant(s) and are coated with antibodies against DC-specific cell surface receptors. Within lymph nodes, DCs present antigens to T cells and initiate immune responses. Activated tumor antigen-specific T cells proliferate and migrate out of the lymph node toward the tumor site (where the antigen is located). CD4, CD4⁺ T helper cell; CD8, CD8⁺ cytotoxic T cell; imDC, immature dendritic cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; NK, natural killer cell.

of IFN α has certainly expedited the use of naturally circulating DCs in the clinic.⁶

About three years ago, we initiated the first human study exploiting pDCs to treat advanced (Stage IV) melanoma patients. Designed as a safety study, we treated 15 patients in three cohorts with dose escalating pDC-based vaccines, ranging from 0.3 to 3 million cells per injection. As the number of cells was low and no data existed on their migratory capacity in vivo, we decided to perform intranodal injections, in order to maximize the possibility to elicit an immune response. Although the trial was not designed to measure clinical efficacy, we obtained some surprising clinical results.⁷ We observed a consistent improvement in clinical responses when these freshly isolated, naturally circulating DCs were compared with conventional moDCs. Indeed, 7 out of 15 Stage IV melanoma patients are still alive two years after

the initiation of treatment. Interestingly, we obtained similar results even with a 10-fold lower dose of cells, demonstrating the potency of natural pDCs. Interestingly, Celli et al. have recently demonstrated that as few as one hundred DCs are needed to elicit a T-cell response, suggesting that perhaps only small amounts of DCs are needed for the induction of antitumor responses in patients.⁸

Conclusions

Altogether, our recent findings indicate that it is worthwhile to further explore the potential of pDCs and other naturally circulating DCs for cancer immunotherapy. It will be interesting to see if also other DC subsets, such as mDCs are equally, or even more, effective than pDCs in this setting.

Another strategy of exploiting these cells for immunotherapy would be to

target specific naturally circulating DC subsets in vivo. This would prevent isolation, laborious culturing, and antigen loading ex vivo (Fig. 1). Early studies have demonstrated that antigen-antibody conjugates can target DCs in vivo, resulting in efficient antigen presentation.⁹ However, if antibody-antigen conjugates are not accompanied by adjuvants, tolerance rather than immune responses might be induced. Therefore, several investigators have embarked on the development of nanoparticles that are coated with antibodies to target naturally circulating DCs and loaded with both antigens and adjuvant(s).¹⁰ Preliminary clinical studies based on the targeting of naturally circulating DCs are underway.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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