Immunology Letters 155 (2013) 6–10

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Novel perspectives on dendritic cell-based immunotherapy of cancer

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ARTICLE INFO

Article history:
Available online 26 September 2013

Keywords:
Dendritic cells
Cancer vaccines
Immunotherapy
Lymph
Cell interactions

ABSTRACT

Advances in immunobiology knowledge as well as in cell culture processes that generate large numbers of purified and functionally mature dendritic cells (DCs) have raised the possibility that DCs might represent promising clinical agents to generate effective immune responses against cancer.

Here, we discuss the present pitfalls of dendritic cell vaccines for the treatment of human cancer with regard to the most recent knowledge in the biology of DCs. In particular, we highlight the relevance of improving our current understanding of DC trafficking, functions and interactions with other cells of innate immunity for the development of more effective cancer vaccines.

1. Introduction

The capability of dendritic cells (DCs) to generate antitumor immune response *in vivo* has been documented in many animal models. In these experiments DCs were usually isolated *in vitro* and loaded with tumor antigens; these antigen-bearing DCs were then injected into syngeneic animals as cancer vaccine [1].

DCs loaded with tumor lysate or tumor antigen-derived peptides or whole protein have all been demonstrated to generate tumor-specific immune responses and antitumor activity. In addition, antigen-loaded DCs can be used therapeutically to induce regression of pre-existing tumors. These observations have established the rationale for evaluating tumor antigen-bearing DCs as therapeutic vaccines in different human cancers.

Various clinical trials using DCs loaded with tumor antigens are already underway. Despite the fact that encouraging clinical and biological results have been obtained, only some of the treated patients in these trials have experienced tumor regression, suggesting that the nature of DCs, DC dose, route of administration, choice of antigen, or method of antigen loading may have been limiting factors. In addition, tumor heterogeneity in terms of HLA expression and related antigens may justify variability in responses.

One of the main critics to this immuno-therapeutic approach regards the understanding of why the administration of a small number of antigen pulsed DCs is supposed to induce antigen-specific T cell responses (and tumor regression) in patients in whom both tumor antigen(s) and DCs are already largely available. An obvious answer could be that tumor-associated DCs might be impaired in their capacity to take up and process antigens, become activated and differentiate into mature DCs able to migrate to lymphoid organs and present antigens to naive T cells. If defective maturation of DCs is, indeed, a common occurrence in cancer patients, the development of methods that induce their physiological maturation *in vivo* may represent an appealing solution to this problem. In the absence of this possibility, administration of DCs derived from circulating precursors, which have been induced to mature *ex vivo* and armed with appropriate tumor antigens, may prove useful in the treatment of cancer patients. However, differentiation of DCs from hematopoietic precursors requires extensive *in vitro* manipulations with labor-intensive cell processing in expensive facilities. This is currently a major impediment for many cancer centers intending to employ therapeutic DC vaccines. To avoid this hindrance, alternative approaches aimed to directly isolate DCs from patients should be explored.

The issues indicated below represent some of the present main challenges for the optimization of DC-based immunotherapy and, for each of them, the current knowledge is discussed.

2. Source of dendritic cells

Different kinds of DC preparations have been demonstrated to stimulate antigen-specific T-cell responses in human subjects. Although no direct comparison has been performed in clinical trials, all kinds of DCs tested so far have shown to induce some clinical responses in cancer patients.

The most common approach in the use of DCs for vaccines is to prepare large numbers of autologous DCs *ex vivo*, load them

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http://dx.doi.org/10.1016/j.imlet.2013.09.021

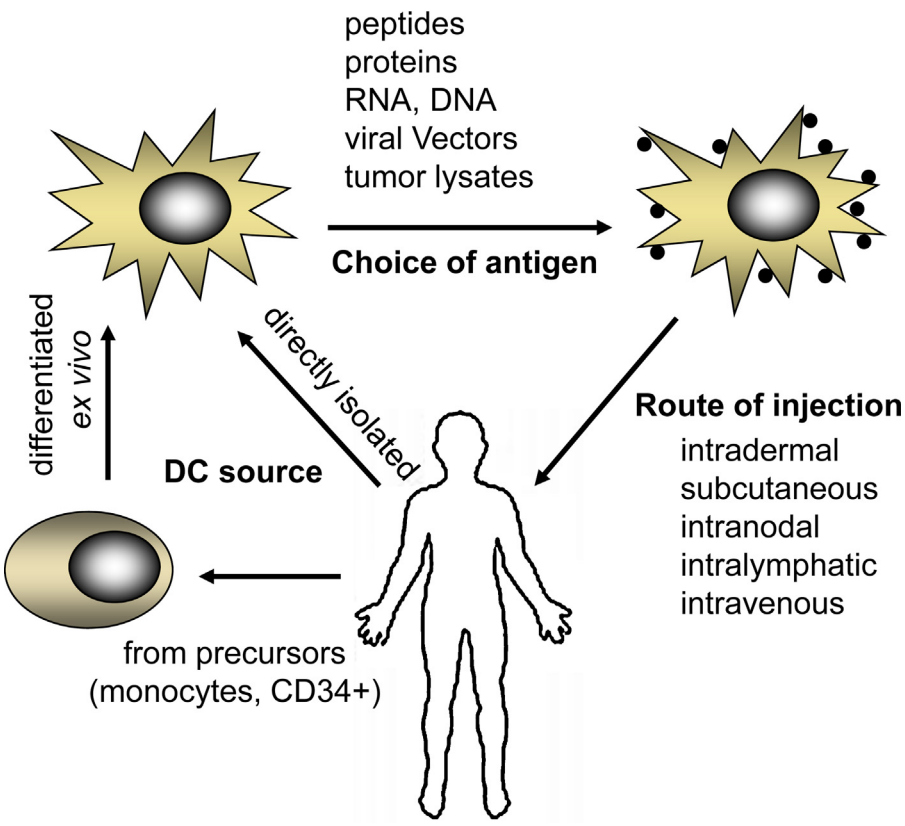

Fig. 1. Issues still needing optimization for the use of DCs in cancer vaccines. *DC source:* DCs can be derived from hematopoietic precursors *in vitro* or directly isolated from the human body, for example, from peripheral blood upon treatment with cytokines able to mobilize DCs (Flt3L) or from seroma fluid, which contains a large number of afferent lymph DCs not yet sufficiently explored (see also Fig, 2). *Choice of antigen:* different options are available for DC loading and the choice is strictly related also to the *Route of injection*. Peptide loading is an effective option but it does not fit with subcutaneous/dermal administration (see text), whereas the intranodal/lymphatic route should be further investigated.

with antigens, and inject them back into the subject (Fig. 1) [2,3]. Three general methods have been described, involving, respectively (i) differentiating DCs from leukapheresis-derived monocytes cultured with GM-CSF and IL-4 (or other Th2 cytokines) [4]; (ii) differentiation of $CD34^+$ hematopoietic progenitor cells in the presence of GM-CSF and TNFα [5–7] with or without an *in vitro* expansion of $CD34^+$ cells with specific growth factors such as Flt3L or stem cell factor or (iii) directly isolating DCs from leukapheresis products [7] frequently with the aid of commercially available closed systems that use immunomagnetic beads.

Eventually, all DC preparations are usually activated *ex vivo* before administration to the patient. Most frequently, DC maturation on monocyte-derived DCs is induced by the addition of a "cytokine cocktail" of IL-1β, IL-6, TNFα, and PGE2 [8]. Different authors have reported that DCs activated by this cocktail are impaired in their production of IL-12. Nevertheless, they have been shown to be capable of priming tumor-specific cytotoxic T cells [9,10]. The identification of other factors, possibly related to pathogen-associated-molecular patterns, capable of increasing the capability of DCs to induce T cell activation, might lead to more effective anti-tumor responses *in vivo*.

As mentioned above, to benefit from DC vaccines on a large scale, other approaches aimed to directly isolate DCs from the human body should be explored. Indeed, the yields of both plasmacytoid and classic myeloid DCs purified from blood can be significantly enhanced by stimulating patients with Flt3L prior to leukapheresis [11]. Also, in many animal models, afferent lymph has been shown to contain a large number of *veiled* cells with characteristics of DCs. These cells migrate continuously from periphery to secondary lymphoid organs in steady state and might represent an abundant and physiologic source of antigen presenting cells. Recently, we have reported that seroma, an accrual of fluid reported after surgical procedures such as axillary lymph node dissection, is associated with an accumulation of afferent lymph drained from upstream tissues during the interval of time needed for lymphatic vessels to re-anastomose with the efferent ducts after the removal of lymph nodes [12]. Further supporting this hypothesis, we reported the presence, in seroma fluids, of a large amount of antigen presenting cells, which represent DCs migrating from peripheral tissues to secondary lymphoid organs *via* afferent lymph [13]. Two subsets of DCs have been identified in seroma fluids, one expressing CD14 and lower amounts of HLA-DR and the other expressing CD1a and different markers of maturation, including higher levels of co-stimulatory molecules. Remarkably, $HLA\text{-}DR^{dim}CD14^+$ DCs can convert spontaneously into the more mature $HLA\text{-}DR^{bright}CD1a^+$ DCs. Axillary lymph node dissection is usually performed in patients with higher risk of recurrence after breast cancer surgery. These patients often require multiple needle-aspirations of seroma fluid (mainly represented by afferent lymph), and each seroma collection on average contains $71 \pm 108 \times 10^6$ mononuclear cells (MNC) in a mean volume of 360 ± 147 ml. Among these MNC, 8.5% are DCs (range between 3.5 and 22%) [13]. Therefore, although the functional characterization of these lymph DCs has not yet been completed, a possible exploitation of this source of abundant human DCs for the treatment of breast cancer is conceivable (Fig. 2). Afferent lymph DCs from seroma fluids might represent a "ready to use" useful tool for minimizing DC culture manipulations (currently necessary for obtaining an amount of DCs suitable for clinical purposes), which require expensive procedures and facilities.