

Interleukin-15 and Interleukin-15 Receptor α mRNA-engineered Dendritic Cells as Promising Candidates for Dendritic Cell-based Vaccination in Cancer Immunotherapy

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Abstract

Dendritic cell (DC)-based tumor vaccination holds great potential and is intensively being studied in cancer immunotherapy. Although DC vaccination can result in a survival advantage as shown in various cancer types, there is still room for improvement. Therefore, current DC vaccines urge rigorous optimization in order to increase their immune stimulating capacities for induction of antitumor immunity. In this context, strategies where the interleukin (IL)-15 transpresentation mechanism is incorporated, appear to be of great value due to the activating potential of IL-15 towards IL-15R $\beta\gamma$ -expressing cells, such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. In the past 5 years, our research group designed different strategies to generate IL-15-expressing DC with superior T cell and NK cell-activating properties. In this review, we briefly describe the design of our latest DC vaccine, in which DC are genetically engineered to transpresent IL-15 via mRNA electroporation and discuss the capacity of this newly designed DC vaccine to activate NK cells and CTLs. Overall, IL-15-transpresenting DC show the potential to activate antitumor immunity and are promising candidates for DC-based cancer immunotherapy.

Keywords: Interleukin-15; IL-15 receptor α ; IL-15 transpresentation; Dendritic cell-vaccination; NK cells; T cells

Introduction

Already more than four decades ago, dendritic cells (DC) were described as the main orchestrators of the immune system [1]. More specifically, DC can process and present tumor antigens to cytotoxic T lymphocytes (CTLs), in order to eliminate tumors cells. Due to their capacity to induce antigen-specific CTL responses, DC-based vaccines were introduced in clinical trials to treat cancer patients, exactly two decades ago [2,3]. Since then, numerous clinical studies to test the feasibility and efficacy of DC-based cancer vaccines have been performed. In the majority of studies, DC vaccines have been shown to be safe and well tolerated. Moreover, there is a growing body of evidence that DC-based vaccination can be of clinical benefit to cancer patients [2,4,5]. Tumor types with improved survival results following DC vaccination include melanoma, prostate cancer, malignant glioma, renal cell cancer and lung cancer [2,6]. Although these results encourage to continue with antitumor DC vaccination, there is room for improvement to enhance the potency and efficacy of the currently used DC vaccine preparations alone or as part of a combination strategy to further increase the overall survival and the number of responding cancer patients [7-9].

To date, the DC vaccine manufacturing protocol which is most often used in clinical trials involves a one-week, two-step protocol [7,10,11]. The first step is the differentiation of peripheral blood monocytes into immature DC in the presence of interleukin (IL)-4 and granulocyte macrophage colony-stimulating factor (GM-CSF). The second step is carried out in the last two days of the protocol and involves DC maturation in the presence of IL-1 β , IL-6, tumor necrosis factor (TNF)- α and prostaglandin E₂ (PGE₂). A growing body of evidence indicates that these 'gold standard' IL-4 DC might be suboptimal for inducing anticancer immunity [7]. In view of this, we and others have designed new protocols for DC vaccine manufacturing, incorporating IL-15 [12-17]. IL-15 has potent stimulatory effects on both the innate and adaptive components of the antitumor immune

response [10,12,18,19], and is therefore believed to be one of the most promising molecules for antitumor immunotherapy. This is illustrated by its top position in the US National Cancer Institute's ranking of 20 immunotherapeutic drugs with the greatest potential for broad usage in cancer therapy [20]. In this review, we report how IL-15 and/or the α part of the IL-15 receptor has been implemented into a newly designed DC vaccine to optimize its effects on both the innate as well as the adaptive parts of the immune system. We describe the effects of the IL-15 transpresentation mechanism on DC-mediated activation of NK cells and CTLs. Finally, the potential of this optimized DC vaccine in combinatorial antitumor immunotherapy approaches is discussed.

IL-15 transpresentation and generation of IL-15-transpresenting DC by means of mRNA electroporation

In contrast to other cytokines, the IL-15 signal is transmitted via a unique mechanism called transpresentation, which contributes to the unique properties of IL-15 [21-23]. This involves the presentation in *trans* of IL-15 bound to the α moiety of the IL-15 receptor (IL-15R α) to the $\beta\gamma$ chains of the IL-15 receptor on neighboring cells [24-29]. Both NK cells and CTLs express the IL-15R $\beta\gamma$ receptor and are thus receptive for stimulation by the IL-15/IL-15R α complex [13,30-33]. Different IL-15/IL-15R α -targeting immunotherapy approaches have been proposed, including free protein and cell-associated strategies.

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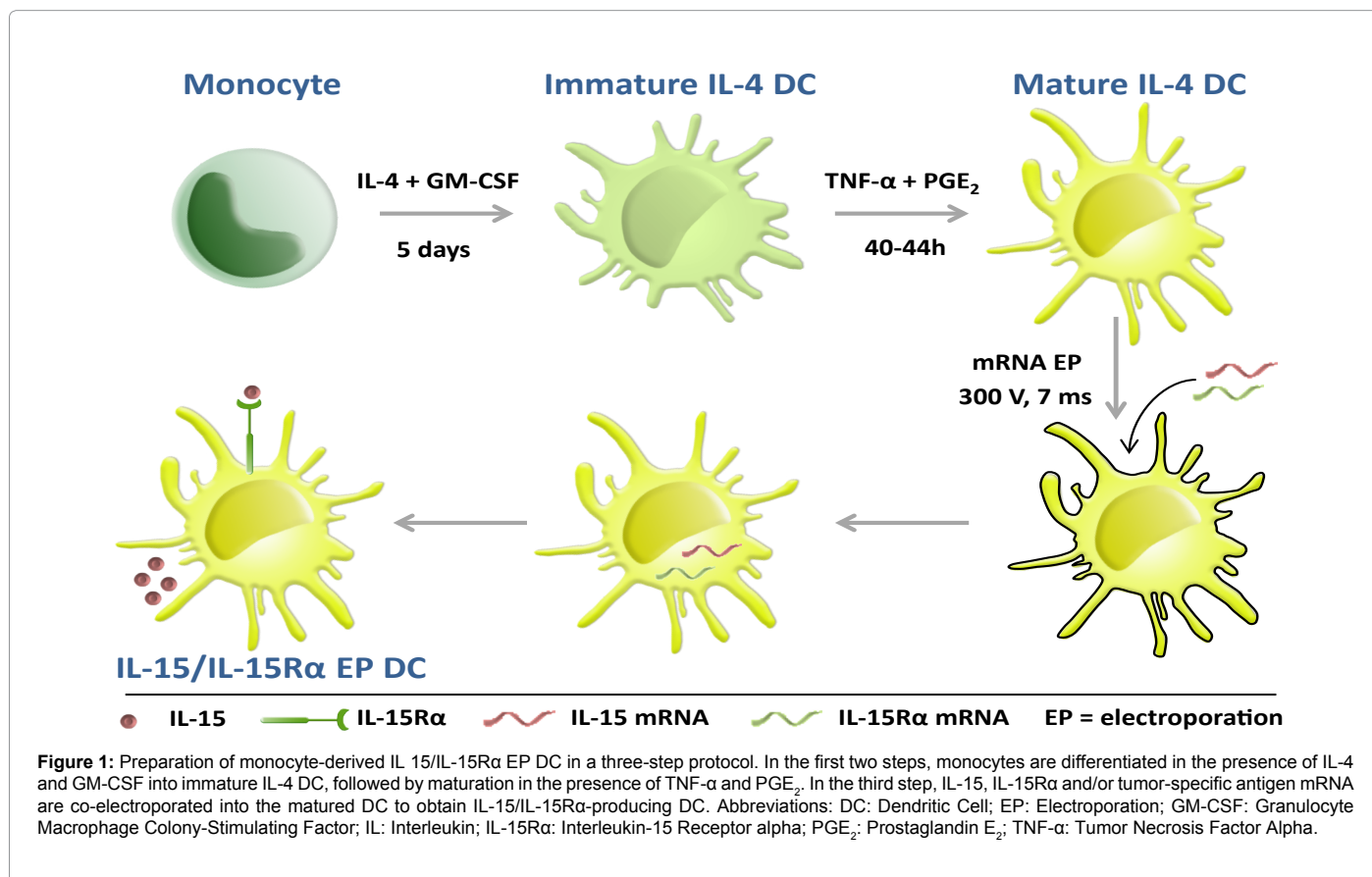
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The group of HC Wong developed a fusion protein of a novel IL-15 mutant with enhanced IL-15 biological activity [34] containing the sushi domain of IL-15Ra. This IL-15 superagonist complex, called ALT-803, improved the IL-15 half-life and resulted in increased numbers of activated CD8⁺ T cells and NK cells [35]. Due to the positive results in preclinical studies and the ability to upscale the production of ALT-803, this IL-15/IL-15Ra fusion complex was implemented into clinical trials in patients with multiple myeloma (NCT02099539), bladder cancer (NCT02138734), hematological malignancies (NCT01885897), non-Hodgkin lymphoma (NCT02384954), non-small cell lung cancer (NCT02523469) and advanced melanoma (NCT01946789). All these clinical studies are designed to obtain the optimal dose level and administration scheme of ALT-803. Although very promising in the treatment of different kinds of cancers, ALT-803 is administered systemically in all these studies, which can dramatically augment the probability of adverse side-effects or autoimmunity. To fully benefit from the advantages of IL-15/IL-15Ra complexes while bypassing systemic side effects, the use of cell carriers to deliver IL-15/IL-15Ra in a more controlled way might be preferable. It is within this context that the DC-based immunotherapy approaches come to the fore. Using the mRNA electroporation technique (detailed in [36]), we genetically engineered DC to express IL-15 and IL-15Ra. These DC, further called IL-15/IL-15Ra EP DC (Figure 1), were generated according to the one-week, two step DC vaccine manufacturing protocol described above with the following two modifications. First, in our protocol, only TNF- α and PGE₂ were used during DC maturation. Second and most importantly, both IL-15 and IL-15Ra were co-transfected by means of mRNA electroporation into the matured IL-4 DC. We showed that IL-15/IL-15Ra is highly expressed on the cell surface of these IL-15/

IL-15Ra mRNA-electroporated DC [37]. In addition to local delivery of IL-15, these IL-15/IL-15Ra-expressing DC have two important advantages, as compared to non-transient systemic protein delivery, resulting from the mRNA electroporation technique. From a Good Manufacturing Practices (GMP) perspective, it is easier to obtain clinical grade IL-15 mRNA than protein, so mRNA-based transfection (e.g. through electroporation) with IL-15 mRNA in DC circumvents the obstacle of the scarcely available clinical grade protein IL-15. Biologically, a transient effect of IL-15/IL-15Ra complexes is in favor of NK cell activation as compared to prolonged stimulation by IL-15/IL-15Ra complexes [38]. In conclusion, our IL-15 and IL-15Ra mRNA transfected DC are promising candidates to improve current DC-based vaccination strategies.

IL-15/IL-15Ra effects on NK cells

In antitumor immunotherapy, DC vaccines are still primarily designed to induce effective adaptive (CTL-mediated) responses, disregarding the antitumor potential of the innate immune system (primarily NK cells). Nevertheless, bidirectional crosstalk between DC and NK cells results in enhanced activation of both cell types and increases their antitumor activity [39-41]. In addition, since NK cells express the $\beta\gamma$ -moiety of the IL-15 receptor, it has been acknowledged that IL-15 can be an important mediator in DC-mediated NK-cell activation [12,23,42,43]. This has driven us to examine the NK-cell activating properties of our IL-15/IL-15Ra EP DC with respect to phenotypical activation, NK cell-mediated killing of tumor cells and production of both proinflammatory and lytic effector cytokines (Figure 2A). To demonstrate the contribution of the IL-15 transpresentation mechanism, the effects of IL-15/IL-15Ra EP DC were compared with



those of mock transfected DC (Mock EP DC) and DC transfected only with IL-15 but without IL-15R α (IL-15 EP DC) [37].

In a first series of experiments, IL-15 and/or IL-15R α -engineered DC were cocultured with autologous NK cells to check for changes in the expression of activation markers and natural cytotoxicity receptors on the NK cells. Although NK cells showed a clear increase of NKP30, NKp44, NKp46, NKG2D, CD69 and CD56 after coculture with IL-15 EP DC, the enhancement was more pronounced when IL-15/IL-15R α EP DC were used for NK-cell priming. Next, we investigated whether these DC activated NK cells were capable of killing tumor cells, which is pivotal in an antitumor DC therapy setting. In these experiments, DC-primed NK cells were cocultured with both NK cell-sensitive and resistant tumor cells, the latter being Daudi cells (Burkitt's lymphoma cell line). In both cases, IL-15 obtained from either IL-15 EP DC or IL-15/IL-15R α EP DC resulted in increased NK cell-mediated killing of the tumor cells, with a statistically significant superior effect when IL-15 was presented by IL-15R α as compared to IL-15 EP DC. The fact that IL-15R α is desirable for appropriate NK cell-priming by DC [42] and that transcellular IL-15 presentation by IL-15R α is the key mechanism in the induction of NK cell-mediated killing [43-45], ratify the usefulness of our IL-15/IL-15R α mRNA-electroporated DC, especially in malignancies that are susceptible to NK cell-mediated killing. From a mechanistic point of view, we found that the increased tumor cell killing was linked to increased granzyme B and perforin secretion by NK cells, indicating that these lytic effector molecules play a principal role in the observed NK cell-mediated killing of tumor cells. In addition, transcellular presentation of IL-15 to NK cells leads to enhanced IFN- γ secretion, promoting the differentiation of T helper 1 cells, which is in favor of generating antitumor immunity [23,42,43]. Overall, our data

show that IL-15/IL-15R α EP DC are excellent stimulators of autologous NK cells, in favor of improving current DC vaccination strategies.

IL-15/IL-15R α effects on CTLs

As principal effector killer cells of the adaptive immune system, CTLs are still the primary targets of DC-based antitumor vaccinations. Despite more studies show a clear induction of CTL immunity in cancer patients, durable clinical responses after DC vaccination could be improved [2]. Mechanisms that can augment the amount of CTLs, increase their antitumor activity and enhance their *in vivo* long-term survival are highly favorable in the battle against cancer. IL-15 transpresented by IL-15R α has already been demonstrated to expand tumor-specific CD8⁺ CTLs and to promote their interferon (IFN)- γ synthesis and cytotoxicity in a metastatic and autochthonous liver cancer mouse model [46]. Moreover, this IL-15 transpresentation mechanism induced robust IL 12 and IFN- γ production, as seen in the plasma of tumor-bearing mice, and reduced the expression of co-inhibitory molecules on DC [46]. According to these results, integrating the IL-15 transpresentation mechanism into a DC vaccine, such as in our IL-15/IL-15R α EP DC, could be advantageous to induce adaptive immunity (Figure 2B). This has led to the start of a next series of experiments, whereby the potential of the IL-15/IL-15R α EP DC to increase specific CTL-mediated antitumor responses will be investigated.

Since DC can be loaded with different kinds of tumor antigens and since the expansion and survival effects of IL-15 on CTLs are antigen-independent [47], our DC vaccine can be used against a broad range of tumor types. Our preferred antigen-loading strategy is mRNA electroporation for different reasons. First, mRNA transfection results in a superior cytoplasmic expression efficiency, is easier to execute as

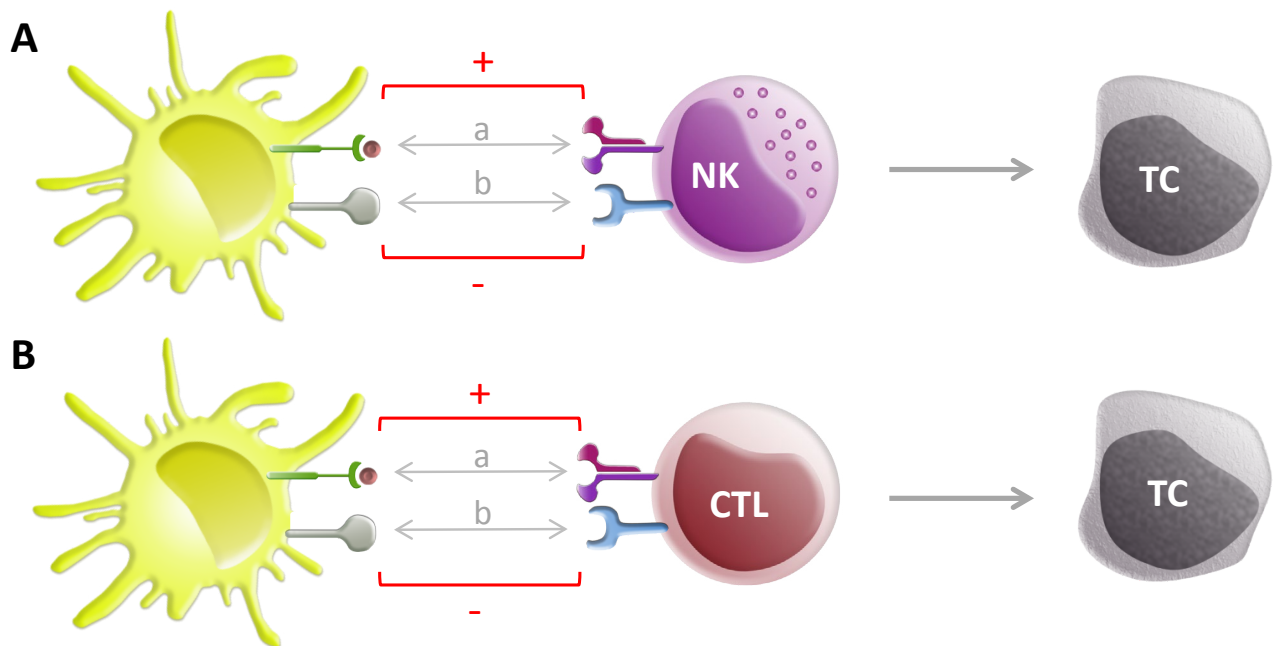


Figure 2: IL-15/IL-15R α EP DC can activate both NK cells (A) and CTL (B) in order to eliminate tumor cells. The interaction between IL-15/IL-15R α -complexes and IL-15R $\beta\gamma$ -expressing cells can further activate the latter (a). Interaction between the immune checkpoint receptor PD-1 and its ligand PD-L1 has an inhibitory effect on the activation of CTLs and NK cells (b). Abbreviations: CTL: Cytotoxic T Lymphocytes; IL-15: Interleukin-15; IL-15R α : Interleukin-15 Receptor Alpha; NK: Natural Killer Cell; PD-1: Programmed Cell Death Protein 1 Receptor; PD-L1: Programmed Death-Ligand 1; TC: Tumor Cell.

compared to viral transduction protocols and has a beneficial clinical safety profile (due to a strictly transient expression and inability to integrate into the host genome) [48]. Secondly, mRNA electroporation results in the presentation of multiple T cell epitopes without the need for prior knowledge of the patient's HLA type. Lastly, tumor antigen mRNA can be co-electroporated simultaneously with other translational mRNAs coding for immune-stimulating proteins, avoiding additional manipulations. Due to the clear CTL-activating effect of IL-15/IL-15R α complexes, in addition to activation of NK cells, IL-15/IL-15R α EP DC are strong candidates for the improvement of current DC vaccination strategies.

Conclusions and Future Perspectives

Although DC vaccination can result in a survival advantage of cancer patients, there is a general agreement among cancer researchers that the true clinical benefit of DC based cancer immunotherapy has not been attained yet [10]. In order to improve the clinical outcome of DC based immunotherapies, an improvement of the current DC vaccine preparations is urgently required. Together with the launch of DC vaccination, IL-15 was discovered and identified as a stimulus of both innate and adaptive antitumor immune effector cells. To date, IL-15 has become one of the most promising molecules for antitumor immunotherapy illustrated by its top position in the US National Cancer Institute's ranking of 20 immunotherapeutic drugs with the greatest potential for broad usage in cancer therapy [20]. IL-15 owes this nomination partly to its unique transpresentation mechanism, whereby IL-15R α transpresents IL-15 to neighboring cells. We believe there is a momentum to integrate this superior IL-15 transpresentation mechanism in current DC vaccines. This integration has already led to an acknowledged increased activation of autologous NK cells *in vitro*, including enhanced NK cell-mediated killing of otherwise NK cell-resistant tumor cells. On top, IL-15/IL-15R α EP DC has the potential to improve activation of antigen-specific antitumor T cells, which is favorable in the combat against cancer.

However, the tumor immunosuppressive environment could impede the powerful effects of our IL-15/IL-15R α EP DC. To overcome this hurdle, we suggest to combine our immunostimulatory DC vaccine with strategies which counteract the mechanisms used by tumors to evade immune control, such as B7/cytotoxic T lymphocyte associated protein 4 (CTLA-4) and programmed death ligand 1 (PD-L1)/programmed cell death protein 1 (PD-1) interactions [22,23]. In recent years, the use of PD-L1, PD-1 and CTLA-4 blocking antibodies gained momentum, which already led to FDA approval of ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1) and nivolumab (anti-PD-1) for the treatment of advanced melanoma and lung cancer patients [49-51]. As an alternative, the use of silencing RNAs (siRNAs) to block PD-L1 and PD-L2, expressed on DC, has been suggested [52,53]. In this context, the immunostimulatory capacity of DC can be boosted further, which might be beneficial for the antitumor effects of our IL-15/IL-15R α EP DC. Altogether, the use of IL-15/IL-15R α EP DC is an appealing strategy in the optimization of current DC-based vaccination strategies.

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