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# Differentiation of stem and progenitor cells in activated gene-engineered dendritic cells with anti-malignant properties

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Studies on the biology of dendritic cells (DCs) are mainly focused on their role as immune activators and modulators. In their appropriate cultivation and/or modifications, they have shown abilities for an enhanced expression of specific effective molecules. These properties have characterized them as promising candidates for construction of novel safe vaccines and gene-engineering products on their basis. In this aspect, in the last years the attention is directed to development of new safe therapeutic methods and techniques with DCs.

*Key words*: dendritic cells, stem/progenitor cells, recombinant viral vectors.

## Introduction

Dendritic cells (DCs) have been found to play a pivotal role in the processess of immune response initiation and modulation, mainly as powerful antigen-presenting cells (APCs) [5, 9, 10, 21, 26—31, 33—36, 38, 42]. On the other hand, they have been found to participate in the maintenance of peripheral tolerance, and their capacity to induce anti-nuclear auto-immune response has been proven in experimental models [12].

### *Biological properties of dendritic cells and their role in generation of adequate immune response*

In the light of the unique properties of DCs, they have been proposed as powerful immunomodulation agents, including in the composition of novel vaccines and gene-engineering products for treatment of malignant disorders [1, 2, 6, 8, 9, 12, 15—17, 19, 23, 24, 26, 28, 29, 33, 37—40, 44, 45, 48]. Complex mechanisms, which include molecular, genetic and cellular components, such as *Wnt-*, *BMP-* and *Notch/*

*Delta*-signalling pathways, have been found to underlie differentiation and functions of stem and progenitor cells [5, 7—10, 13, 21, 26, 29, 31, 34, 35, 42]. By use of polymerase chain reaction in real time (RT-PCR), an ability for initiation of erythroid (β-globin) and/or myeloid (myeloperoxidase) gene expression programs by the same cell prior to exclusive commitment to the erythroid and/or, respectively, myeloid lineages for it, has been shown [21, 29]. On the other hand, protein BCL-6 has also been detectable in inter- and intra-follicular CD4+ T-lymphocytes, but not in other follicular components, including B-lymphoid cells, plasma cells, monocytes/macrophages and DCs [5, 10, 13, 17, 21, 26, 29, 31, 34, 35, 43].

### *Origin of dendritic cells by differentiation of myeloid precursors in the presence of specific antigens*

According to many literature data, granulocyte-macrophage colony-stimulating factor (GM-CSF) mobilizes CD34+ bone-marrow progenitor cells both *in vitro* and *in vivo* with an increased frequency and generation of DCs with anti-malignant properties [8, 9, 13, 35, 37, 39]. Similarly, in the addition of GM-CSF plus tumor necrosis factor-α (TNF-α), induced development of DCs from purified CD34+ cells of bone marrow, cord blood and peripheral blood, has been observed. The critical role of TNF-α for the differentiation of DCs has been supported by the demonstration that this cytokine induces the expression of molecule CD40 in CD34+ cells. Besides that, CD34+/CD40+ cells have been found to express only myeloid markers, significantly increase allo-antigen presenting function, compared with total CD34+ cells, and have also given rise to DCs' number. Capable of modulating differentiation of DCs from these bipotent CD34+/CD40+ cells during the later stages of their cultivation, has also been shown to be cytokine interleukin-4 (IL-4) [38].

The possibility of generating or expanding tolerant DCs *ex vivo* has been found to open novel therapeutic perspectives. Their *in vitro* and/or *ex vivo*-maturation has been characterized as a critical step in the induction of T-cell responses and it has been proven to depend on the activation of transcription factors from the family of Nuclear Factor-kappaB (NF-κB) [29]. It has also been suggested that kinetic and the quality of DCs' activation is controlled by cytokine IL-10, which has been characterized as alternative promising pathway of their differentiation [25]. On the other hand, DCs, differentiated in the presence of vaso-active intestinal peptide (VIP), have shown impaired allogeneic haplotype-specific responses to donor CD4+ T-lymphocytes in mice, and have been found to induce generation of regulatory T-cells in the graft [10, 21]. As a critical component for optimal function of DCs, has been characterized the TNF supper-family member lymphotoxin-αβ (LTαβ), independently of its described role in maintaining of the lymphoid tissue organization [30]. In the absence of LTαβ on antigen-specific T-cells, DCs' dysfunction *in vivo* could be rescued via CD40 or LTβ receptor stimulation, respectively, which has suggested a possibility for eventual cooperation of these pathways. It has also been indicated that DCs, induced by ligand Flt3, are well positioned to regulate the qualitative nature of intestinal immune responsiveness, depending on the presence or absence of appropriate inflammatory signals [37]. In this way, a potential use of ligand Flt3 as a mucosal vaccine adjuvant in conjunction with the inflammatory mediator IL-1 has been suggested.

### *Development of novel therapeutic strategies with dendritic cells*

In the last years, the development of novel therapeutic strategies with DCs has become extensively investigated [1—7, 9—11, 14—26, 28, 30, 36 - 48]. So genetically modified DCs have been widely tested in pre-clinical studies, included as anti-malig-

nant agents. After such application of DCs, peptide-specific responses by cytotoxic T-lymphocytes (CTLs), improvement in performance status, decrease in malignancy markers levels, regression of malignancies, and, at the same time, no toxic side effects have been accounted [33]. Because isolated DCs, loaded with malignant antigens *ex vivo* and administered as effective cellular vaccines, have induced protective and therapeutic anti-malignant immunity in experimental animals with induced malignant disorders, adjuvant treatment of malignancies at high risk for recurrence after operation, as well as methods for targeting malignant antigens to DCs *in vivo*, have been explored [38]. On the other hand, appropriate modifications of DCs to express malignancy-specific antigens by *in vitro* and/or *ex vivo*-transfer of genes, coding respective antibodies, has been suggested [1, 2, 6, 9, 11, 13—15, 17, 18, 22, 23, 25, 27, 28, 32, 36, 37, 40, 42, 44 - 48]. Therefore, exploitation of the antigen-presenting properties of DCs offers promise for the development of effective anti-malignant immunotherapy. For this aim, different therapeutic strategies of DCs, have been developed.

### *Development of novel therapeutic strategies with hybrid vaccine constructs, received by fusion of dendritic cells with malignant cells*

As alternative method for delivery into DCs, their fusion with malignant cells has been utilized, as well as the hybrid cells-based vaccines have shown high therapeutic activity, even in patients with malignant diseases and disorders [3, 17, 19, 20, 22, 26, 27, 40—42, 44—47]. The immunization with such hybrid conjugates, derived by fusion between DCs and malignant cells, has significantly increased the production of Th1 cytokine-producing cells, the number of antigen-specific CD8+ T-cells, as well as the anti-malignant immunity. The observed anti-malignant immunity, induced by vaccination with DCs/malignant cells hybrid fusion products has reacted differently to injected malignant cells and autochthonous malignancies [46]. It has also been shown that immunization with such fusion cells induces rejection of metastases. Hybrid cells, obtained by fusion between DCs and malignant cells, have been found to express major histo-compatibility complex (MHC) molecules, both class I and class II-restricted malignancy-associated epitopes and might, therefore, be useful for the induction of specific malignancy-reactive CD8+ and CD4+ T-lymphocytes both *in vitro* and *in vivo*, by human vaccination trials [30]. The observed greatly reduced number of established pulmonary metastases, both with and without *in vivo*-administration of *IL-2*-adoptive transfer of T-lymphocytes, derived from *B16/DC* vaccine-primed lymph nodes into *B16* tumor-bearing mice, has suggested a role of malignant cells/DCs hybrids as effective cellular vaccines for eliciting T-cell-mediated anti-malignant immunity [19]. It has also been demonstrated an enhanced immunization with received by fusion of DCs with mouse 4TOO plasmacytoma cells *FC/4TOO* hybrid cells, plus anti-malignant immunity of IL-12 [20]. Findings, according which fusions of ovarian cancer cells to autogenic or allogeneic DCs induce cytolytic T-cell activity and lysis of autologous malignant cells, mediated by MHC molecules class I-restricted mechanism, have suggested that the fusions are probably functional, when are generated by autolgenic and/or allogeneic transplantation of DCs [30]. It has also been demonstrated that sequential stimulation with DCs/breast carcinoma cells fusion hybrids results in a marked expansion of activated malignancy-specific T-lymphocytes, which has suggested these fusion cells are probably effective APCs, which stimulate inhibitory T-cells that limit vaccine efficacy [39, 44]. Similarly, hybrids, derived by fusion of spleen DCs from C57BL/6 mice with *B16* melanoma cells, have expressed MHC-

molecule B7, as well as *B16* tumor marker M562, and have been characterized as an attractive strategy for immunotherapy of malignancies [9]. On the other hand, the results, according to which the *ex vivo*-exposure of DCs on the presence of cytokine transforming growth factor-beta (TGF-β) hasn't appeared to lessen the efficacy of DCs vaccines, have suggested that this cytokine, derived from malignant cells, has probably reduced the their efficacy via *in vivo*-mechanism, and the neutralization of produced by the fusion cells TGF-β might enhance it [24, 27, 40]. An increase in the immunogenic potential of DCs/malignant cells fusion cell-based vaccines has been observed in heat-treated malignant cells [26].

***Development of novel therapeutic strategies with dendritic cells, transduced by recombinant viral vectors, coding malignant antigens***

DCs have shown a possibility to be genetically engineered to express constitutively respective genes of interest, coding immune-modulating cytokines, antibodies and/or antigens, derived from transformed cells or other pathogens [1, 2, 6, 11, 13—15, 18, 22, 25, 28, 32, 37, 48]. In laboratory conditions, human DCs, transduced with recombinant *adenoviral* vectors, have shown inhibition of a mixed leukocyte culture, reduced cell surface expression of co-stimulatory molecules CD80/CD86, as well as inability for production of the potent allo-stimulatory cytokine IL-12 [11, 14, 15, 17, 25, 32, 37, 48]. In investigation on the *in vivo*-properties of the so modified DCs, skin transplantation of experimental mice with non-obese diabetes, combined with severe immunodeficiency (NOD/SCID), reconstituted via intraperitoneal injection with allogeneic mononuclear cells (MNCs) mixed with autologous to the skin donor DCs, transduced with either recombinant *adenoviral* gene construct *AdV/IL-10* or *AdV/MX-17*, a reduced skin graft rejection, characterized by reduced mononuclear cell infiltration and less destruction of derma—epidermis junctions, in comparison with the animals with inoculation of DCs, has been observed [10]. *Adenovirus*-transduced immature DCs have shown ability to differentiate in the presence of lipopolysaccharide (LPS) or a monocyte-/macrophage-conditioned medium to express the surface markers of mature DCs, such as CD25, CD83, high levels of molecules CD86 and HLA-DR, as well as to secrete IL-12. Their ability to induce T-lymphocytes' growth has also been enhanced. It has also been suggested that *adenoviruses* probably have mediated minor effects on the phenotype of DCs, which, however, could be seen only when a sufficient number of particles enter in each cell [37]. Recombinant *adenoviral* vectors have also been found to transduce effectively DCs and direct the generation of specific CTLs, which would be a potent strategy in the immunotherapy of Hodgkin's lymphoma [14]. According to the results from other study, transduction of DCs with recombinant vectors with insertion of gene *mTRP-2* (encoding tyrosinase-related protein-2, respectively), provides a potential therapeutic strategy for the management of melanoma, especially in the early stage of that disease [25]. So modified DCs have also shown high stimulatory activity in both allogenic and autogenic mixed lymphocyte reaction. Similarly, mouse DCs, infected with recombinant *fowlpox virus* (*rFWPV*) vector, have stimulated a powerful, MHC class I-restricted immune response against the recombinant antigen [5]. These data have also supported the efficiency of the recombinant viral vectors in studies on the biologic properties of DCs, including the expression of specific antigens for active immune therapy.

### *Development of combined therapeutic strategies with dendritic cells*

The fact that an increased Th1 cytokine production and stronger anti-malignant effect haven't been observed in mice, depleted of gamma-interferon (IFN-γ), has also supported the maintenance of DCs/malignant cells conjugates as potent anti-malignant vaccines, as well as the cytokine *IL-18* [42, 44]. These data could be additionally administrated by gene transfection of cells for enhancement of the immunity, which is probably mediated mainly by IFN-γ.

### *Development of combined therapeutic strategies with gene-engineered dendritic cells*

For further increase of the potency of the vaccine, a combined variation of both technologies has been applied, in which *IL-18*-transfected DCs have been used to prepare DCs/malignant cells conjugates [27, 36, 40, 42, 44—48]. It has also been indicated that *GM-CSF* gene-modified DCs might lead to the generation of hybrid vaccines with potentially increased therapeutic efficacy [9]. Although the observed elicited anti-malignant effect with participation of both CD4+ and CD8+ T-lymphocytes by the hybrid vaccine *IL-18DC-E.G7*, derived by fusion between gene-engineered DCs, transduced with recombinant *adenoviral* vectors, carrying genes for enzyme β-galactosidase (*AdlacZ*) and/or for cytokine IL-18 (*AdIL18*), respectively, and *E.G7* malignant cells, derived from *EL4* cells, transfected with *cDNA*, carrying gene for chicken egg albumin, it has been largely blocked by anti-IFN-γ antibodies [23].

### *Development of combined therapeutic strategies with gene-engineered malignant cells*

Results from experiments for immunization with fusion hybrids, derived by fusion of DCs with *IL-12* gene-transferred malignant cells, have shown an ability to elicit a previously enhanced anti-malignant effect in experimental therapeutic models [27, 40—44]. Such novel *IL-12*-producing fusion cell vaccine has been characterized as a promising intervention for future immune therapy of malignant diseases [36].

### *Development of combined therapeutic strategies with gene-engineered both malignant and dendritic cells*

In immunization of mice with gene-engineered *DCRMAT/J558-IL-4* fusion hybrids, an elicited stronger *J558* tumor-specific CTLs immune response has been induced, in comparison of hybrid vaccine *DCRMAT/J558 in vivo* [27]. Similar results have been observed in immunization of C57BL/6 mice with gene-engineered *DC/J558-IL-4* hybrids, and gene-engineered fusion hybrid vaccine constructs have been characterized as an attractive strategy for immunotherapy of malignancies [27, 44, 45, 47].

## Conclusion

Dendritic cells (DCs) have been characterized as hopeful vehicles for appropriate modulation of the immune response, including in composition of vaccine constructs and gene-engineered products with anti-malignant activity. They have also shown

abilities for enhanced expression of specific molecules in appropriate conditions of cultivation and/or by appropriate modifications. These properties characterize them as promising candidates for construction of novel and safe therapeutic products on their basis, by use of new technologies, as their fusion with malignant cells; transduction with recombinant viral vectors, as well as a combined variation, in which malignant cell, DC or both components of the hybrid fusion vaccine might be genetically transduced.

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