Editorial

Genetically Modified Dendritic Cell-Based Cancer Vaccines

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Dendritic cells (DC) are professional antigen-presenting cells that play a central role in the induction of T cell-mediated immunity. They are found in most of the non-lymphoid and all lymphoid tissues of the body, where they specialize in capturing and processing antigens. Two functions of APC segregate in time. First, the immature DC internalize and process the antigen. Then, several days later, they express the processed antigen in the form of a peptide bound to major histocompatibility complex (MHC) molecules on the cell surface, migrate to secondary lymphoid organs, differentiate into mature DC, and stimulate T cells. The $CD8^+$ T cells are stimulated in the context of MHC class I molecules, whereas the $CD4^+$ T cells in the context of MHC class II molecules. Dendritic cells activate T cells through a two-signal mechanism. The first (MHC-peptide) signal is antigen-specific and causes T cells to enter the cell cycle. The second (co-stimulatory) signal is antigen-nonspecific and involves a set of co-stimulatory molecules expressed on DC that interact with ligands on the T-cell surface. The second signal induces T-cell cytokine production as well as subsequent T-cell proliferation (Banchereau and Steinman, 1998; Bubeník, 2001).

The unique capacity to present antigens, activate naive T cells, and induce primary cellular immunity make DC attractive vehicles for the delivery of therapeutic cancer vaccines. Dendritic cell-based cancer vaccines were prepared by loading DC *ex vivo* with tumour lysates, apoptotic tumour material, acid-eluted tumour peptides, or tumour associated antigen (TAA)-derived synthetic peptides. To enhance loading of DC with oncoproteins, increase the efficacy of the vaccines, and bypass those antigen-processing and DC-activating pathways that are often deficient (Sedlacek, 1994; Bubeník, 1996; Seliger et al., 2000) in tumour-bearing individuals, genetically modified DC vaccines were designed. The genetical modification involved transfection of DC with polynucleotides coding for oncoproteins, immunostimulatory cytokines, and/or co-stimulatory molecules (Bakker et al., 1995; Boczkowski et al., 1996; Condon et al., 1996), transduction with recombinant viral vectors encoding these molecules (Bubeník, 2001; Jenne et al., 2001), and tumour cell-DC fusion (Gong et al., 1997; Wang et al., 1998; Bubeník, 2001).

Efficient DC-based tumour vaccines were prepared by transfection of DC with tumour-derived DNA or RNA coding for known (as well as unknown) TAA, and with RNA derived using subtraction libraries. Potential advantages of such approaches involve the presentation of previously unknown epitopes of a given TAA in any MHC context, and the ability to include sequences of immunostimulatory cytokines, such as IL-2 and IL-12, or co-stimulatory molecules, such as those of the B7 family. The physical transfection methods, for instance liposome complexes or electroporation, have a relatively low efficiency in DC (Arthur, 1997). The best transfection efficacy was perhaps reported using a combination of *in vitro* transcribed RNA and liposomes, where up to 20% of DC were transduced (Strobel et al., 2000). In contrast, transduction with recombinant retroviruses, adenoviruses, or poxviruses encoding a variety of TAAs has been reported to provide a higher transduction efficacy, reaching even more than 90% with some of the vectors. Several of the viruses may also enhance co-stimulatory molecule expression, antigen presentation and the T cell-stimulatory capacity of transduced DC. Virus vectors may also have disadvantages. Some of the viruses may interfere with the antigen-presentation pathways of the infected DC, inhibit MHC and co-stimulatory molecule expression, interfere with the transport mechanisms of the processed TAA in DC, block the required maturation of DC, and affect the viability of the infected DC, as discussed in detail elsewhere (Jenne et al., 2001). Retroviral vectors have been extensively studied in various gene therapy systems. Their major disadvantage is their inability to infect non-proliferating cells, such as mature DC; however, they can transduce proliferative DC progenitors, and these progenitors retain their capacity to differentiate into DC. In contrast, lentiviral vectors can also infect non-dividing cells, including mature DC. Vaccinia and herpes sim-

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Abbreviations: DC – dendritic cells, MHC – major histocompatibility complex, TAA – tumour-associated antigen.

plex virus-based vectors have the ability to package large genetic insertions, allowing simultaneous expression of multiple TAA genes together with immunoenhancing genes such as IL-2, IL-12 or B7. Adenovirus-mediated delivery of TAA, immunostimulatory cytokines, and/or co-stimulatory molecules into DC is mediated by an integrin expressed preferentially in immature DC and securing an extremely high transduction rate. Similarly as retroviral vectors, also adenoviral vectors do not disturb maturation of DC precursors after transduction, which, together with the safety of replication-deficient adenovirus strains, makes them valuable for gene delivery into DC. An alternative method which can generate efficient TAA-presenting DC-based tumour vaccines is fusion of DC with tumour cells. Multiple known and unknown TAA expressed on the surface of hybrid cells may secure more efficient tumour immunity than immunization with DC pulsed with known oncoproteins or their immunodominant peptides. This hypothesis has been repeatedly tested by fusion of murine DC with syngeneic experimental tumours such as MC38 adenocarcinoma, B16 melanoma, P815 mastocytoma, and by fusion of human ovarian or renal carcinoma cells with human DC.

A direct evidence has been obtained in a variety of tumour systems that viral delivery and non-viral delivery of TAA genes into tumour cells, as well as hybridization of DC with tumour cells, can provide us with efficient tumour vaccines. These vaccines were able to elicit primary cytotoxic T lymphocyte (CTL) responses *in vitro* and prophylactic or therapeutic tumour protection *in vivo* (Hart, 1997; Bubeník, 2001). Co-insertion of immunostimulatory cytokine genes, particularly IL-2 and IL-12, similarly as insertion of genes coding for co-stimulatory molecules, has substantially increased the efficacy of the vaccines in experimental tumour models (Bubeník, 2001). Human TAA such as Gp100, CA-125, MAGE-1 and -3, CEA, MART-1, HPV16 E7 and MUC-1 expressed in genetically engineered DC were able to generate TAA-specific CTL in cultures of leukocytes from normal donors. Despite the encouraging results obtained *in vivo* in experimental models with genetically engineered, DC-based cancer vaccines, a definite clinical benefit was demonstrated rather episodically. Treatment of 26 patients with DC plus CEA RNA resulted in only one complete/partial response, two minimal responses and four cases of stable disease (Salgaller et al., 1999). Kugler et al. (2000) have repeatedly vaccinated 17 metastatic renal cell carcinoma patients with tumour cell x DC hybrids and followed the patients for a mean time of 13 months. The DC used were derived from normal allogeneic donors, and TNF-α was used in the last 2–7 days of culture before vaccination to differentiate mature DC. Four patients demonstrated a prolonged complete response, two partial responses, two had stable disease, and nine had progressive disease. The $CD8^+$ T lymphocytes were shown to migrate to the site of DC vaccination and 65% of patients developed positive delayed-type hypersensitivity reactions. These controlled clinical trials have shown that DC-based immune therapy of cancer is a safe and, in a proportion of cancer patients, also effective modality of treatment. However, further research of DC-based tumour vaccines is urgently needed. This research should focus on optimization of therapeutic protocols, particularly with regard to the genetic modification of DC with polynucleotides, preferentially RNA, coding for TAA and immunodominant peptides of TAA, insertion of genes for immunostimulatory cytokines such as IL-12 or IL-2, delivery of genes for various activating and co-stimulatory molecules, and utilization of genes encoding cytokines with anti-apoptotic effects on DCs. In this respect, transduction of DC with viral vectors and vaccination with tumour cell-DC hybrids also seem to be promising. In the future, studies should focus on the treatment of minimal residual tumour disease and identification of *in vitro* methods to be used for immune monitoring prior and during the DC-based vaccine therapy, with the final aim to find the individuals who will benefit from the DC vaccination. Correlation between the DC-based vaccine efficacy and the patient´s disease characteristics (type of the tumour, its stage, grading, invasiveness) should also be established.

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