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Review

Gene-Modified Dendritic Cells for Use in Tumor Vaccines

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ABSTRACT

Dendritic cells (DCs) are potent antigen-presenting cells capable of priming activation of naive T cells. Because of their immunostimulatory capacity, immunization with DCs presenting tumor antigens has been proposed as a treatment regimen for cancer. The results from translational research studies and early clinical trials point to the need for improvement of DC-based tumor vaccines before they become a more broadly applicable treatment modality. In this regard, studies suggest that genetic modification of DCs to express tumor antigens and/or immunomodulatory proteins may improve their capacity to promote an antitumor response. Because the DC phenotype is relatively unstable, nonperturbing methods of gene transfer must be employed that do not compromise viability or immunostimulatory capacity. DCs expressing transgenes encoding tumor antigens have been shown to be more potent primers of antitumor immunity both *in vitro* and in animal models of disease; in some measures of immune priming, gene-modified DCs exceeded their soluble antigen-pulsed counterparts. Cytokine gene modification of DCs has improved their capacity to prime tumor antigen-specific T cell responses and promote antitumor immunity *in vivo*. Here, we review the current status of gene-modified DCs in both human and murine studies. Although successful results have been obtained to date in experimental systems, we discuss potential problems that have already arisen and may yet be encountered before gene-modified DCs are more widely applicable for use in human clinical trials.

OVERVIEW SUMMARY

This review discusses the potential benefits and limitations of genetically modified dendritic cells (DCs) for use in therapeutic antitumor vaccines. We discuss the various systems employed for gene transfer to DCs, including the growing consensus that viral vectors represent the most efficient means of transduction. We also describe data supporting the use of DCs modified to express genes encoding tumor antigens and immunomodulatory proteins, such as cytokines, to promote antitumor immunity. Successful preclinical results are described, in which genetically modified DCs are used in *in vitro* immunologic studies with human cells *in vitro* as well as animal tumor models *in vivo*. We point out the benefits and disadvantages of using gene-modified DCs and express our viewpoint on what types of gene-modified DCs may be considered candidates likely to reach the clinic.

INTRODUCTION

SINCE THEIR DISCOVERY more than 25 years ago, dendritic cells (DCs) have emerged as the most potent member of the class of antigen-presenting cells (APCs) (Banchereau and Steinman, 1998). Because of their potent capacity to stimulate T lymphocytes, particularly naive T cells, DCs have been proposed as the basis for vaccines designed for the treatment of cancer (Cohen *et al*., 1994; Schuler and Steinman, 1997). Coupled with our understanding that tumors express antigens (TAA, tumor-associated antigen) capable of being recognized by the immune system, DC-based tumor vaccines have been translated from the laboratory to the clinic (Timmerman and Levy, 1999). The results from early phase I clinical trials support the idea that DCs presenting TAA can initiate an antitumor immune response in certain patients and, in some cases, can lead to partial or complete regression of tumors (Hsu *et al*., 1996; Nestle *et al*., 1998). Although these early results are encouraging, they also

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point to the exigency for modification of DC-based vaccines before they become a commonly used modality for the treatment of cancer. Gene therapy in the setting of DC-based vaccines represents one such modification. The purpose of this review is to bring together data on gene techniques and translational research using gene-modified DCs. By drawing parallels from animal models and *in vitro* assays utilizing human cells, we hope to highlight the promise entailed in creating the next generation of vaccines based on gene-modified DCs.

DC generation and morphology

Because DCs can be derived from multiple cell types using a number of different tissue culture conditions, a brief primer on DC generation and phenotype is necessary before we can begin to address issues of gene transfer. DCs are distinguished by their dendritic, veiled morphology (Fig. 1) and high expression levels of both MHC class I and class II as well as costimulatory and adhesion molecules involved in T cell activation such as CD80/CD86 and ICAM-1 (Hart, 1997; Banchereau and Steinman, 1998). DCs reside in the spleen, lymph nodes, and circulation but can arise from cells such as epidermal Langerhans cells or monocytes, which react to stimuli such as inflammatory cytokines.

An important step in the application of DCs in therapeutic settings was the ability to obtain them in large numbers in highly purified form from donors. In both rodents and humans, DCs can be generated from bone marrow or peripheral blood cells; in the case of peripheral blood both circulating $CD34^+$ hematopoietic progenitor cells and $CD14^+$ adherent monocytes were found to give rise to DCs under defined tissue culture conditions. On culture in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4), the resulting cells display the veiled morphology characteristic of DCs and express T cell stimulatory molecules such as MHC I/II, CD80/86, and ICAM-1 (Bender *et al*., 1996; Romani *et al*., 1996; Talmor *et al*., 1998). In humans, these cells represent "immature DCs," defined in part by their high antigen uptake. Further culture of these cells in monocyte conditioned medium (MCM) or with the addition of tumor necrosis factor α (TNF-α), lipopolysaccharide, type I interferons (IFNs), or IL-1β can increase the expression of T cell stimulatory receptors and induce expression of DC maturation markers such as CD83 (Bender *et al*., 1996; Luft *et al*., 1998). These "mature DCs" are more potent T cell stimulators than immature DCs but have reduced phagocytic and endocytic activities (Banchereau and Steinman, 1998). Mature DCs are thought to be better for use in immunization regimens than their immature counterparts because of their increased immunostimulatory capacity, stability of their phenotype, and increased trafficking to lymphoid organs. In the mouse the distinction between immature and mature DCs is not clear-cut due, in part, to lack of a selectable marker for maturation. However, murine bone marrow-derived DCs (BMDCs) cultured in GM-CSF plus IL-4 are functionally more mature (as measured by phagocytic/endocytic activities and T-cell stimulatory capacity) than DCs derived from GM-CSF cultures (Talmor *et al*., 1998; Labeur *et al*., 1999). The addition of CD40L or TRANCE, both ligands for TNF receptor family members, has also been shown to promote the maturation and survival of DCs (Wong *et al*., 1997; Kuniyoshi *et al*., 1999). Taken together, it appears that multiple modes of DC maturation are capable of resulting in a cell with high immunostimulatory capacity. Whether these different sources of DC progenitors and different protocols for DC generation result in similarly effective antigen-presenting cells is a matter still open to debate but should be kept in mind by the reader of both clinical and translational research reports.

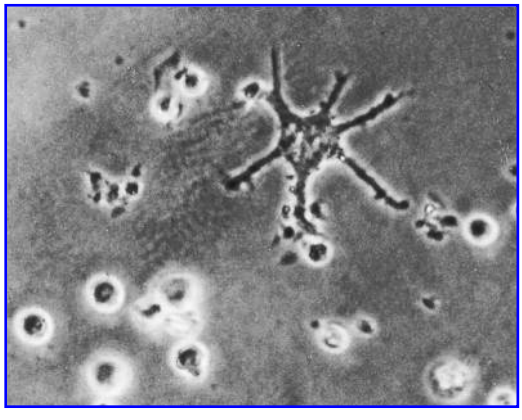

FIG. 1. Photomicrograph of a murine dendritic cell. Typical morphology shows a stellate appearance with multiple cell processes. The DC was isolated from spleen as described in Fields *et al*. (1998).

GENETIC MODIFICATION OF DENDRITIC CELLS

Broadly defined, the target genes transferred into DCs fall into two categories: TAA and immunomodulatory proteins such as cytokines. In the case of DCs expressing TAA, gene constructs encoding whole proteins and cytotoxic T lymphocyte (CTL)-specific epitopes have been utilized. There are a number of potential advantages to using TAA gene-modified DCs. DCs expressing TAA via a transgene should, in theory, present antigen for a longer period than peptide or tumor lysate-pulsed DCs, in which the duration of expression is restricted by the half-life of the peptide–MHC complex created during antigen pulsing. Plasmid DNA is relatively stable and easy to generate, making it a more suitable source of antigen than whole tumor RNA and eliminating the possible transformation of DCs by oncogenes or tumor suppressor genes encoded in tumor DNA. However, TAA gene-transfected DCs present only the antigen encoded by the transgene, resulting in an antigenic restriction not present when tumor lysate or RNA/DNA is used as the antigen source. Because DCs are potent APCs, use of viral gene vectors could result in priming of antiviral immunity, particularly CTLs, resulting in elimination of transfected DCs during subsequent rounds of immunization. Furthermore, the relative instability of the DC phenotype necessitates a method of transfection that does not compromise viability or negatively affect DC functions (Fields *et al*., 1998). The challenge to the broader use of gene-modified DCs in vaccine applications then is twofold: determine the most efficient means of transfecting DCs

and the optimal target genes to be transfected. In this regard, DCs transfected with genes encoding cytokines or T cell costimulatory molecules represent APCs that are potentially more immunostimulatory or longer lived than unmodified DCs.

Efficient gene transfer into DCs via viral vectors

Published reports of gene-modified DCs have used a variety of gene transfer vehicles including cationic lipids, viral vectors, and plasmid-coated gold particles (Table 1). Gene transfer efficiencies, when reported, ranged from 5% efficiency to greater than 95% transgene-expressing cells. The variation in gene transfer efficiencies may be due, in part, to the transfection vector system employed but may also be due to modifications in gene transfer protocols. Several laboratories have attempted to determine which gene transfer system(s) are optimal for transfecting DCs efficiently. The growing consensus is that viral vectors represent a more efficient and productive means compared with physical or chemical methods. While the majority of published reports detailing virus-mediated transduction efficiency and its effect on DC phenotype have utilized adenovirus, DCs or their progenitors have been transduced with similar efficiencies in studies using retrovirus (Aicher *et al*., 1997), poxvirus (Brown *et al*., 1999), and herpesvirus (Coffin *et al*., 1998).

In direct comparisons of adenoviral vectors with physical methods of transfection such as liposomes, electroporation and $CaPO_4$, viral vectors have resulted in consistently higher levels of gene transfer efficiency and expression and transfection efficiencies (90–100 versus 5–10%) (Arthur *et al*., 1997; Dietz and Vuk-Pavlovic, 1998; Zhong *et al*., 1999). In fact, most physical methods of transfection were toxic to DCs, resulting in loss of phenotype and substantial cell death (Arthur *et al*., 1997). Even under optimal conditions when electroporation resulted in 15% transfection efficiency, viability of the DC was less than 60% (Van Tendeloo *et al*., 1998), and transfection via electroporation occurred in $CD34^+$ HSC-derived but not in monocyte-derived DCs. However, it should be noted that substantial protein production after gene gun transfection has been achieved, although no determination of transfection efficiency was determined (Tuting *et al*., 1998). Adenovirus titers of up to 1000–10,000 multiplicities of infection (MOIs) could be well tolerated by DCs as opposed to the M202 and M207 human melanoma cell lines, which demonstrated 40–100% cell death at the same titers (Arthur *et al*., 1997). Generally, no alterations in DC phenotype after adenoviral infection have been noted (Arthur *et al*., 1997; Zhong *et al*., 1999), although there are some reports of increases in T cell stimulatory molecules, such as MHC class I, CD80, and CD86, suggesting that adenovirus may initiate DC maturation (Kaplan *et al*., 1999; Rea *et al*., 1999). Transgene expression in adenovirus-infected human DCs could persist for greater than 1 week (Arthur *et al*., 1997) as opposed to gene gun-mediated transfection, which resulted in only transient expression lasting less than 72 hr (Tuting *et al*., 1997). When transgene expression from viral vectors was lost rapidly, addition of DC survival factors such as TRANCE could prolong reporter gene expression (although it is not known whether this effect is due to maintenance of the transgene or prolonged survival of the DCs) (Zhong *et al*., 1999). Adenovirus-transfected DCs were either as effective (Arthur *et al*., 1997; Zhong *et al*., 1999) stimulators of the mixed lymphocyte reaction (MLR) or more effective (Kaplan *et al*., 1999; Rea *et al*., 1999) than uninfected control DCs; increased stimulatory capacity accompanied increased expression of T cell stimulatory molecules. It appears that adenovirus has been equally capable of mediating gene transfer into human or mouse

TABLE 1. GENE TRANSFER TECHNIQUES EMPLOYED IN DENDRITIC CELL MODIFICATION

Transfection vector	*DC source*	*Comments*	*References*
Virus			
Adenovirus	Human PBMCs Mouse bone marrow	>90% transduction efficiency (MOI >100)	Arthur *et al.* (1997); Zhong *et al.* (1999)
Poxvirus	Human PBMCs Mouse bone marrow	Antiviral immunity does not affect *in vivo* efficacy	Kim *et al.* (1997); Brossart *et al.* (1997); Brown *et al.* (1999)
Retrovirus	Human $CD34^+$ HSCs Mouse bone marrow	Postinfection DC maturation required	Aicher *et al.* (1997) Specht *et al.* (1997)
Herpesvirus	Human PBMCs	Multiple gene transduction achieved	Coffin *et al.* (1998)
CD40-targeted adenovirus	Human PBMCs	CD40 targeting resulted in DC maturation	Tillman *et al.* (1999)
Liposome-modified adenovirus	Human PBMCs	Efficient transduction at MOI <100	Dietz and Vuk-Pavlovic (1998)
Physical methods			
$CaPO_4$, liposomes	Human PBMCs	>10% transfection efficiency	Arthur *et al.* (1997); Zhong *et al.* (1999)
Electroporation	Human PBMCs	15% transfection efficiency, low DC viability	Van Tendeloo *et al.* (1998)
Biolistic device	Human PBMCs Mouse bone marrow	Transient expression (<72 hr) 5–10% transfection efficiency	Tuting *et al.* (1997) Tuting *et al.* (1998)

Abbreviations: PBMCs, Peripheral blood mononuclear cells; HSCs, hematopoietic stem cells; DC, dendritic cells.

DCs (or DC lines), although no comparative studies have yet been reported (Arthur *et al*., 1997; Brossart *et al*., 1997; Ribas *et al*., 1997; Dietz and Vuk-Pavlovic, 1998; Sonderbye *et al*., 1998; Kaplan *et al*., 1999; Zhong *et al*., 1999).

The requirement for high viral titers (MOI >100) to achieve efficient gene transfer suggests that DCs lack the adenovirus attachment factor Coxsackie–adenovirus receptor (CAR) or the α_v integrins, $\alpha_v\beta_3$ or $\alpha_v\beta_5$, used by the virus for fusion with its target cell (Wickham *et al*., 1993; Bergelson *et al*., 1997, 1998). Indeed, monocyte-derived DCs cultured in GM-CSF and IL-4 are deficient in CAR but do express the α_v integrin (Rea *et al*., 1999; Tillman *et al*., 1999). Instead, DCs may incorporate virus through phagocytic activity. This is consistent with the observation that gene transfer efficiency and expression levels (as measured by green fluorescent protein [GFP] fluorescence) were greater in immature DCs than in DCs induced to mature by lipopolysaccharide (LPS) or MCM (Zhong *et al*., 1999). This had led some investigators to use adenovirus to enhance uptake of conjugated DNA particles or to augment adenovirus infection with antibodies or liposomes. Successful gene transfer to both mouse and human DCs has resulted using adenovirus to enhance the uptake of DNA bound to chemical linkers such as poly-L-lysine (Mulders *et al*., 1998), modified receptor proteins such as transferrin (Curiel-Lewandrowski *et al*., 1999) and modified chemical linkers targeted to DC receptors such as mannosylated polyethylenimine (Diebold *et al*., 1999a,b). These systems, which used the virus to aid in DNA/conjugate uptake, were purported to be less toxic than using high-titer adenovirus and would result in a reduced induction of antiadenovirus immunity. Other investigators have attempted to modify adenoviral delivery to DCs by additional strategies. In this regard, adenovirus targeted to CD40 or β_1 integrins on human DCs by a bispecific antibody led to increased reporter gene (luciferase) activity compared with adenovirus alone, even at relatively low MOI (<100) (Tillman *et al*., 1999). Besides improving gene transfer efficiency, addition of CD40 antibody with the adenovirus had the additional benefit of delivering a possible DC maturation signal; both IL-12 production and allo-MLR stimulation were increased in CD40-targeted adenovirus DC preparations (Tillman *et al*., 1999). Improved gene transfer efficiency was also seen when adenovirus were admixed with liposomes (Dietz and Vuk-Pavlovic, 1998; Tillman *et al*., 1999). It is not entirely clear why Tillman *et al*. (1999) and Dietz and Vuk-Pavlovic (1998) reported low transduction efficiencies with unmodified adenovirus whereas Zhong *et al*. (1999) and Arthur *et al*. (1997), among others, reported productive efficiencies approaching 95–100% of human DCs. Concerns over initiation of antiadenovirus immunity were generally not supported by *in vivo* murine data. While adenovirus-infected human DCs could prime an antiviral CTL response after several rounds of *in vitro* stimulation (Butterfield *et al*., 1998; Perez-Diez *et al*., 1998), mice immunized with adenovirus-infected DCs did not develop significant antiviral CTLs (Brossart *et al*., 1997; Kaplan *et al*., 1999). Furthermore, adenoviral immune mice could still be protected from tumor challenge by immunization with DCs infected by adenovirus encoding TAA transgenes (Kaplan *et al*., 1999). A paucity of antiadenoviral immunity after immunization with adeno-infected DCs has also been reported by Wan *et al*. (1997). While these results raise concerns that mice may not represent a suitable model for studying *in vivo* effects of adenovirus-infected DCs (possibly because of a reduced ability to mount an antiviral response compared with humans), these disparate results may also represent differences arising from the priming of CTLs *in vitro* versus *in vivo*. Collectively, it appears that adenovirus (and possibly other viral vectors) represents an efficient means for inserting transgenes into DCs without detrimental effects to its phenotype or function.

Tumor antigen gene-modified DCs capable of priming antitumor immunity

The feasibility of gene modification of DCs to express TAA was first reported by Alijagic *et al*. (1995). Using peripheral blood monocytes cultured in GM-CSF and IL-4, these investigators were able to transfect the resulting DCs with either a chloramphenicol acetyl transferase (CAT) reporter gene or a gene encoding human tyrosinase, a melanoma TAA. Gene transfection (via cationic lipids) resulted in a fivefold increase in CAT activity, while tyrosinase-transfected DCs elicited T cell clustering and TNF-α release in an antigen-specific CTL clone similar to that achieved using peptide-pulsed DCs (Alijagic *et al*., 1995). However, neither transfection efficiency nor the effect of transfection on DC phenotype and function (other than activation of a long-term T cell clone) was determined.

Another approach to engineering DCs to express TAA was demonstrated by Reeves *et al*. (1996), who utilized retroviral transduction to deliver the MART-1 melanoma TAA. Since retrovirus most efficiently introduces genes into actively cycling cells, $CD34^+$ hematopoietic stem progenitor cells (HSCs) as cycling DC precursors were chosen as targets. Using murine CD86 as a marker for viral transfection, 20% transfection efficiency, as determined by flow cytometric analysis of murine CD86-expressing cells, resulted from infection of HSCs. MART-1-expressing DCs were able to stimulate MART-1-specific tumor-infiltrating lymphocytes (TILs) *in vitro*. These DCs could generate a MART-1-specific CTL response from autologous peripheral blood mononuclear cells (PBMCs), suggesting that like protein or peptide-pulsed DCs, genetically modified DCs could initiate a specific antitumor T cell response.

In an attempt to improve the efficiency of transgene-expressing DCs, pox and adenoviral vectors capable of directly infecting DCs have been used to express TAA. Infection with either a fowlpox or vaccinia viral vector encoding MART-1 resulted in 50–75% MART-1-expressing DCs (Kim *et al*., 1997). A single stimulation of peripheral blood lymphocytes (PBLs) from some melanoma patients with MART-1 transgene-expressing DCs but not with MART-1 peptide-pulsed DCs resulted in antigen-specific IFN-γ production and CTL generation. Full processing of the whole MART-1 protein and presentation by the DCs was confirmed by generation of CTLs against multiple MART-1 epitopes. Other investigators using an adenoviral vector encoding full-length MART-1 also reported the generation of MART-1-specific CTLs by virally infected DCs (Butterfield *et al*., 1998; Perez-Diez *et al*., 1998). MART-1-expressing DCs (but not DCs infected with empty vector) stimulated IFN-γ production by $CD4^+$ and $CD8^+$ T cells from many melanoma patients and healthy donors as measured by bulk enzyme-linked immunosorbent assay (ELISA) and intracellular cytokine staining (Perez-Diez *et al*., 1998). Interestingly, no IL-4-producing T cells arose from coculture of

MART-1-expressing DCs with bulk PBLs, suggesting a bias towards generation of a helper T cell type 1 (Th1) response by TAA gene-modified DCs (Perez-Diez *et al*., 1998). TAA-specific CTLs or IFN-γ production could not be elicited by virally infected DC-stimulated peripheral blood in all melanoma patients. Also of note, adenovirus-specif ic CTLs and IFN-γ production were present in PBL cultures after repeated stimulation with adeno-infected DCs, raising a concern about the efficacy of virally infected DCs *in vivo* (see above) (Butterfield *et al*., 1998; Perez-Diez *et al*., 1998). With those caveats in mind, however, these data suggest that DCs expressing a full-length TAA protein were capable of processing and presenting TAA via both the MHC class I and class II pathways and could elicit superior T cell stimulation compared with peptide-pulsed DCs.

Mouse tumor models have been used to test the *in vivo* therapeutic efficacy of TAA gene-modified DCs. Early efforts centered on tumor lines altered to express a model "tumor" antigen such as β-galactosidase (β-Gal) or ovalbumin (OVA) and DCs expressing β-Gal or OVA transgenes (Brossart *et al*., 1997; Song *et al*., 1997; Specht *et al*., 1997; Wan *et al*., 1997; McArthur and Mulligan, 1998; De Veerman *et al*., 1999). Both retroviral (Specht *et al*., 1997; De Veerman *et al*., 1999) and adenoviral (Brossart *et al*., 1997; Song *et al*., 1997; Wan *et al*., 1997; McArthur and Mulligan, 1998) transduction systems have been used to insert genes encoding OVA or β-Gal into DCs derived from bone marrow or splenic precursors or immortalized DC lines. The resulting DCs generally showed no alterations in phenotype as measured by flow cytometric analysis of T cell stimulatory molecules such as MHC I/II, CD80/CD86, and ICAM-1 (De Veerman *et al*., 1999). Genetically modified DCs were able to activate antigen-specific T cell lines *in vitro* (Brossart *et al*., 1997; De Veerman *et al*., 1999), generate CTLs *in vivo* (Brossart *et al*., 1997; Song *et al*., 1997; Specht *et al*., 1997; Wan *et al*., 1997), and promote antitumor immunity in both naive and tumor-bearing mice (Brossart *et al*., 1997; Song *et al*., 1997; Specht *et al*., 1997; Wan *et al*., 1997; De Veerman *et al*., 1999). In some studies peptide-pulsed and gene-modified DCs were compared for their ability to initiate T cell activation (both *in vitro* and *in vivo*) and antitumor immunity (Brossart *et al*., 1997; Specht *et al*., 1997). DCs infected with either an adenovirus or vaccinia virus encoding a CTL epitope of OVA were equivalent to OVA protein or peptide-pulsed DCs in their ability to activate an OVA-specific $CD8^+$ T cell line, prime OVA-specific CTLs from naive mice, and generate CTLs on immunization in naive mice (Brossart *et al*., 1997). More importantly, virally (either adenovirus or vaccinia) transduced DCs generated protective antitumor immunity in both naive mice or mice previously infected with the corresponding virus, suggesting that antiviral immunity does not affect the efficacy of transfected DCs, at least in mice. Using DCs generated from retrovirally transduced bone marrow precursors, Specht *et al*. (1997) showed that genetically engineered DCs could promote therapeutic antitumor immunity in a metastatic model of disease. Treatment of day 3 lung metastases of a β-Gal-expressing CT26 colon carcinoma cell line was equally effective in reducing tumor load using either peptide-pulsed or gene-transduced DCs; however, gene-transduced DCs were more potent primers of β-Gal CTLs *in vivo*. In another interesting mouse model, NFSA murine fibrosarcoma cells engineered to express the MART-1 human melanoma antigen were rejected in mice immunized with DCs transfected with a MART-1 gene-encoding adenovirus (Ribas *et al*., 1997). While these results are suggestive, tumor cells expressing novel antigens are rendered more immunogenic compared with the parental cell line, which poses the question of whether these results are due not to potent antigen presentation by DCs but rather arise from the artificial nature of the tumor model per se.

To that end, studies have addressed the question of whether DCs genetically engineered to express naturally occurring TAA are capable of eliciting antitumor immunity *in vivo*. Tuting *et al*. (1997) inserted a plasmid encoding wild-type p53 peptide into bone marrow-derived DCs via a particle bombardment or "gene gun" technique. This biolistic approach yielded a 5–10% transfection efficiency and transgene expression lasting less than 72 hr (Tuting *et al*., 1997). Nevertheless, immunization of naive mice with p53-transfected DCs resulted in protective immunity from a CMS4 sarcoma, which is known to overexpress p53 (Tuting *et al*., 1997). However, CMS4 is a chemically induced tumor, which is generally more immunogenic than naturally arising tumors found in humans. In another mouse model, immunization of naive mice with DCs transduced with an adenovirus encoding human p53 resulted in protection of $>70\%$ of mice challenged with tumors expressing human p53 or those expressing mutated murine p53 (Ishida *et al*., 1999). Another report has shown that DCs infected with adenoviral vectors encoding different TAA expressed by the murine melanoma line B16 could elicit antitumor immunity to this naturally occurring and poorly immunogenic tumor model (Kaplan *et al*., 1999). In this study, DCs infected by adenovirus showed a 95% transfection efficiency and no alterations in phenotype, with the exception of an increase in MHC class I expression. When expressed by DCs, target genes encoding CTL epitopes of tyrosinase-related protein 2 (TRP-2), or human gp100 (hgp100), but not mouse gp100, elicited nearly complete protective immunity in immunized mice subsequently challenged with viable tumor cells. Immunization with TRP-2- and hgp100-expressing DCs could also slow the growth of recently implanted tumors; combination of both TAA-expressing DCs further enhanced the antitumor effect. Although encouraging, established tumors could not be completely eliminated after vaccination with TAA-expressing DCs even though treatment began 4 days after tumor challenge, before a palpable tumor arises (Kaplan *et al*., 1999). This limitation may be due to the fact that only CTL epitopes were encoded by the transgene and that the DCs could not fully initiate $CD4^+$ T cell activation in the absence of helper epitopes, a requisite for antitumor immunity (Toes *et al*., 1999). These translational studies extend the *in vitro* data using genetically modified human DCs to stimulate antitumor immunity to well-characterize d animal models. The results to date suggest that TAAs are suitable transgenes for use in immunotherapy protocols (Table 2). However, since most of these models utilize artificially generated tumor antigens or treated mice with small tumor loads (and did not result in curative treatment), further modification of the system will need to be carried out to improve therapeutic results.

Cytokine gene-modified DCs

Besides genetic modification to express TAA, DCs have been successfully gene modified to express immunomodulatory

TABLE 2. TUMOR ANTIGEN GENE-MODIFIED DCS

Species	*Antigen (transfection vector)*	*Comments*	*References*
Human	Tyrosinase (liposomes)	Transfected Dc-activated Ag-specific T cell line	Alijagic *et al.* (1995)
	MART-1 (viral vectors)	Superior to peptide-pulsed DCs in eliciting CTLs Primed $CD4^+$ and $CD8^+$ T cell response Primed antiadenoviral $CD8^+$ T cell response	Kim *et al.* (1997); Butterfield *et al.* (1998); Perez-Diez *et al.* (1998)
	Multiple melanoma TAA (gene gun)	Improved priming of $CD8^+$ T cell response by cotransduction with IL-12 or IFN-α	Tuting *et al.* (1998)
Mouse	Ovalbumin, β-galactosidase, human MART-1 (viral vectors)	Immunization promoted protection and therapeutic antitumor immunity Highly immunogenic model "tumor" antigen	Brossart *et al.* (1997); Ribas *et al.* (1997); Specht *et al.* (1997)[a]
	Human p53 (adenovirus)	Protected mice from challenge with human p53-expressing tumors and mutant murine p53 tumors	Tuting *et al.* (1997)
	Murine p53 (gene gun)	Protected mice from challenge with CMS4 sarcoma	Ishida *et al.* (1999)
	Tyrosinase-related protein 2, human gp100 (adenovirus)	Inhibited growth of established B16 melanoma	Kaplan *et al.* (1999)

[a]See text for complete references.

proteins such as cytokines and chemokines (Table 3). One disadvantage in selecting TAA as a target gene for modification of DCs is the tissue and MHC haplotype restriction of whole tumor proteins and CTL epitopes, respectively. But DCs modified to express genes encoding T cell stimulatory cytokines, for example, may be potentially used as adjuvants to treat any number of tumors, so long as a source of TAA is available. Cytokine gene-modified DCs represent a potentially more potent vaccine than similarly gene-modified tumor cells since the former are both APC and cytokine factories while the latter require host APC function (Tepper and Mulé, 1994).

In murine models of melanoma and sarcoma, IL-12 gene-modified DCs injected directly into tumors induced a profound antitumor response (Nishioka *et al*., 1999). While the amount of tumor growth inhibition correlated with IL-12 production by the DCs, the effect was dependent on DC stimulation of tumor-specific immunity because IL-12 gene-modified fibroblasts injected intratumorally had little effect on tumor growth (Nishioka *et al*., 1999). Tuting *et al*. (1998) reported that IL-12 gene-modified DCs could boost priming of TAA-specific $CD8^+$ CTLs *in vitro*. In this study, DCs were genetically modified to express different melanoma TAAs as well as either IL-12 or IFN-α. IL-12 gene modification has also been shown to boost $CD4^+$ T cell responses in infectious disease models. $CD34^+$ DCs derived from peripheral blood and retrovirally transduced with genes to produce either IL-12 or IFN-γ could augment $CD4^+$ T cell-mediated cytokine production in response to bacterial antigens (Ahuja *et al*., 1998). Furthermore, immunization of naive mice with IL-12 gene-modified DCs loaded with soluble antigens from *Leishmania donovani* provided better protection from leishmaniasis than antigen-loaded unmodified DCs (Ahuja *et al*., 1999). In both the mouse and human studies, IL-12 gene transfer led to DC maturation, as measured by increased MHC class II and costimulatory molecule expression (Tuting *et al*., 1998; Nishioka *et al*., 1999). Together, IL-12 gene modification of DCs could augment priming to antigens delivered to DCs in a variety of manners and that these DC could represent powerful adjuvants for activation of both $CD4^+$ helper T cells and $CD8^+$ CTLs.

Additional cytokine genes have been introduced into DCs with the intent of increasing adjuvanticity. Retroviral infection of PBMCs with an IL-7 gene construct followed by maturation with GM-CSF plus IL-4 resulted in a DC population with increased stimulatory capacity compared with untransfected cells (Westermann *et al*., 1998). Presumably, the increased proliferation seen in allogeneic and autologous MLR was due to IL-7 enhancement of T cell proliferation. However, MLR assays do not address how cytokine expression of DCs affects antigen-

TABLE 3. CYTOKINE GENE-MODIFIED DENDRITIC CELLS

Cytokine	*Use of gene-modified DCs (species)*	*References*
GM-CSF	Immunization for treatment of established tumors (mice)	Cao *et al.* (1999); Curiel-Lewandrowski *et al.* (1999)
Lymphotactin	Immunization for treatment of established tumors (mice)	Cao *et al.* (1999); Zhang *et al.* (1999)
IL-12	*In vitro* priming of human T cells	Ahuja *et al.* (1998); Tuting *et al.* (1998)
	Intratumoral injection (mice)	Nishioka *et al.* (1999)
	Immunization against leishmaniasis (mice)	Ahuja *et al.* (1999)
IL-7	*In vitro* stimulation of MLR in human PBLs	Westermann *et al.* (1998)
	Intratumoral injection (mice)	Miller *et al.* (2000)

TABLE 4. COMPARISONS OF TAA GENE-MODIFIED DCS WITH TAA PEPTIDE-PULSED DCS

Source of DCs	*Tumor antigen*	*Results*	*Reference*
Adherent human PBMCs cultured in GM-CSF + IL-4	Tyrosinase: full-length gene versus tyrosinase peptide (Ty-2)	Equal aggregation of DCs with Ty-2-specific T cell line	Alijagic *et al.* (1995)
Adherent human PBMCs cultured in GM-CSF + IL-4	MART-1: full-length cDNA versus MART-1_{27-35} peptide	Increased IFN-γ production by CTLs raised by gene-modified DCs	Kim *et al.* (1997)
Murine bone marrow cells cultured in GM-CSF + IL-4	Ovalbumin: $OVA_{257-264}$ cDNA versus $OVA_{257-264}$ peptide	Equivalent protective tumor immunity; equivalent CTL generation *in vivo*	Brossart *et al.* (1997)
Murine bone marrow cells cultured in GM-CSF + IL-4	β-Galactosidase: full-length LacZ cDNA versus β-$Gal_{876-884}$ peptide	Equivalent protective tumor immunity; superior generation of IFN-γ and CTLs *in vitro* by gene-modified DCs	Specht *et al.* (1997)

specific or, more specifically, tumor antigen-specific T cell activation. Further experimentation using antigen-specific CTL assays and *in vivo* tests of therapeutic efficacy will be necessary in order to determine if IL-7 gene modification represents a significant improvement on DC-based vaccines. In this regard, one report suggests that IL-7 gene-modified DCs can mediate tumor regression through direct intratumoral injection (Miller *et al*., 2000). Two reports suggest that modification of DCs with another cytokine cDNA, GM-CSF, can increase therapeutic antitumor immunity *in vivo* as compared with unmodified DCs (Cao *et al*., 1999; Curiel-Lewandrowski *et al*., 1999). However, the level of increased antitumor immunity varied between the two studies. In a protective immunity model of a squamous cell carcinoma, 20% of mice immunized with lysate-pulsed, GM-CSF gene-modified DCs (derived from GM-CSF-cultured bone marrow cells) developed a tumor as opposed to 100% of mice treated with unmodified but lysate-pulsed DCs (Curiel-Lewandrowski *et al*., 1999). It was postulated that increased survival of and migration by GM-CSF gene-modified DCs were responsible for the increased antitumor immunity (Curiel-Lewandrowski *et al*., 1999). However, in the B16 mouse melanoma model, GM-CSF-expressing splenic DCs fused with tumor cells were only slightly (but not significantly) more effective than unmodified DC/tumor fusions in both protecting mice from tumor challenge and increasing survival of mice with established metastatic disease (Cao *et al*., 1999). It is unlikely that this discrepancy is due to reported differences in GM-CSF production as Cao *et al*. (1999) reported GM-CSF levels in DC supernatants three to five times the levels reported by Curiel-Lewandrowski *et al*. (1999). More likely, the difference resides in the tissue source of DCs because bone marrow-derived DCs were shown previously to be more potent APCs than splenic DCs and have increased antigen-processing capacity (Fields *et al*., 1998). However, differences in tumor models and antigen loading may also explain these contradictory results, which may have potential significance for the design of clinical protocols. Finally, DCs genetically engineered to secrete a T cell chemotactic factor, lymphotactin, and subsequently loaded with a peptide of the Mut1 TAA were found to be potent stimulators of antitumor immunity against the 3LL Lewis lung carcinoma (Cao *et al*., 1998). In this study, lymphotactin gene-modified DCs were significantly more potent than unmodified DCs. When tumor RNA was used as the source of antigen, lymphotactin-secreting DCs were also superior to untransfected DCs in the induction of antitumor immunity to the B16 melanoma (Zhang *et al*., 1999), suggesting that this chemokine could be applicable to a range of tumors and antigen sources. Because of the emerging evidence that chemokines play an important role in the priming of naive T cells by DCs (Cyster, 1999), it is likely that future reports will describe the modification of DCs with genes encoding other chemokines.

CONCLUSION

A number of studies have indicated that genetic modification of DCs can improve their immunostimulatory capacity and provide an efficient means for antigen delivery to T cells. Animal tumor model data suggest that tumor antigen- and cytokine gene-transfected DCs are equally capable and, in many cases, superior APCs relative to unmodified DCs. It is premature at this point to render a decision on whether TAA gene-modified DCs represent a significant improvement in the development of DC-based cancer vaccines since only four studies to date have directly compared the efficacy of TAA gene-transduced DCs with TAA peptide-pulsed counterparts (Table 4). The cumulative results of these studies provide inconclusive evidence to support the hypothesis that TAA gene-expressing DCs are superior APCs relative to TAA peptide-pulsed DCs. Furthermore, tumor lysate- or tumor RNA-pulsed DCs should be compared with TAA gene-modified DCs in order to determine if presentation of a single TAA (via transgene expression) can induce the same level of antitumor immunity as DCs expressing multiple TAAs. However, the results using cytokine gene-modified DCs show, with few exceptions, a significant enhancement of T cell priming and antitumor immunity. An important issue that must still be addressed centers on the multitude of tissue culture protocols employed and different cellular sources from

which DC can be generated. Future work in this field should involve determining optimal conditions and maturation factors for gene-modified DCs. An early indication of this effort comes from a comparison of the timing of antigen pulsing and CD40 maturation in peptide and tumor RNA-loaded DCs (Morse *et al*., 1998). The effect of CD40-mediated DC maturation on T cell stimulation was found to be most effective when it occurred after peptide pulsing but before loading with tumor RNA. It remains to be seen what temporal restrictions apply to gene modification of DCs with regard to DC maturation protocols. Finally, the question of which target genes are ideally suited for the clinic remains to be fully addressed. Because cytokine production by DCs may enhance antitumor immunity against tumors, it is conceivable that genes encoding cytokines IL-7 and IL-12 or the chemokine lymphotactin may be the first to be used in experimental DC-based vaccines in humans. However, it is also likely that additional cytokines will be shown to augment DC priming of an antitumor response. Another, as yet untested, avenue of research into gene-modified DCs involves expression of T cell adhesion/costimulatory molecules such as B7 or ICAM-1 or DC survival receptors such as CD40 or TRANCE-R. Just as the initial discovery of DC-mediated antitumor immunity prompted excitement for future therapeutics, the early success of gene-modified DCs has engendered another round of optimism.

NOTE ADDED IN PROOF

Since the initial submission of this article, several reports have described the use of genetically modified DC. Using retrovirally transduced murine DC encoding full-length OVA protein or a class I MHC restricted epitope of OVA, Schnell *et al.* (2000) provide evidence for a role of $CD4^+$ T cells in the generation of antitumor immunity against OVA-expressing tumors but not for the initial generation of OVA-specific CTL. Immunization of mice with DC genetically modified to express either full-length human gp100 (Wan *et al.*, 1999) or human MART-1 (Ribas *et al.*, 2000) can protect mice from a lethal challenge of B16 melanoma cells (Wan *et al*., 1999). MART-1 gene-modified human DC were equivalent to protein loaded counterparts in their ability to stimulate MART-1 specific CTL from melanoma patients (Philip *et al*., 2000). However, Osterroth *et al*. (2000) report that idiotype protein-pulsed DCs were far superior to idiotype gene-modified DCs (via Semlike forest virus) in inducing antigen-specific CTL from PBMC. Finally, Jonuleit *et al.* (2000) report that infection of $CD83^+$ human DC with adenoviral vectors leads to suppression of the allostimulatory capacity of DC.

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