Preparing clinical-grade myeloid dendritic cells by electroporation-mediated transfection of in vitro amplified tumor-derived mRNA and safety testing in stage IV malignant melanoma

INTRODUCTION

Dendritic cells (DCs) are increasingly used in vaccine research as immunotherapy for cancer and other diseases. DCs can be transfected with DNA or RNA to produce anti-tumor antigens that break tolerance to tumors and induce tumor-specific therapeutic immunity. While there are several ways to transfect DCs, electroporation significantly enhances transfection efficiency. This paper describes the optimization of parameters for electroporation-mediated transfection (electrotransfection) of myeloid dendritic cells (DCs) with in vitro expanded RNA isolated from tumor tissue to produce clinical-grade DC vaccines.

RESULTS

1. Operating parameters were optimized for electroporation-mediated transfection of eGFP mRNA into normal immature dendritic cells (IDCs) (Figure 2). The optimal settings were 1.0-kV/cm pulses of 150-µs duration for 10 µg RNA /10^6 cells.
2. Electrotransfection of patient DCs with mRNA isolated from tumor tissue shows strong expression of CD83 and CD86 surface markers.

CONCLUSIONS

Standardized preparation of viable clinical-grade DCs transfected with tumor-derived and in vitro amplified mRNA is feasible and their administration is safe. Electroporation-mediated transfection was optimized for maximal efficiency and cell viability.

METHODS

Overall scheme of dendritic cell vaccine preparation

The overall scheme of DC vaccine preparation was to separate immature dendritic cells (IDCs) from autologous CD14-positive cells and isolate the total RNA from autologous tumor tissue. RNA was reversely transcribed to obtain cDNA and amplified using cDNA as template incorporating a T7 RNA promoter. Amplified cDNA was in vitro transcribed and loaded into IDCs by electroporation. The DCs were subsequently matured in the presence of inflammatory cytokines and cryopreserved as single aliquots prior to use.

Dendritic cell electrotransfection with RNA

For experiments aimed at optimizing electroporation conditions, a cDNA encoding the enhanced green fluorescent protein (eGFP) gene and containing a T7 promoter and polyadenylation signal suitable for DC transfection was prepared using standard methods. For transfection into DCs, mRNA was dissolved in water at 1.0 µg/mL. Electrotransfection parameters were optimized by monitoring transfection efficiency and DC viability as a function of electrode separation, pulse amplitude and length and amplified mRNA concentration in the medium (Figure 2). In all experiments we employed a PA-4000 PulseAgile square-wave generator and the proprietary cGMP-grade low-conductivity (80 µS/cm) Cytoporation Medium Formula R medium (both Cyto Pulse Sciences, Glen Burnie, MD). We transfected IDCs with mRNA encoding the eGFP gene, matured the cells for 48 hours and measured transfection efficiency (by eGFP fluorescence) and viability (by exclusion of 7-amino-actinomycin D, 7-AAD; Pharmingen, San Diego, CA) following transfection in electroporation cuvettes with a 4-mm electrode separation (400 µL). The mRNA concentration was varied between 4.0 µg/mL and 25 µg/mL, pulse amplitude between 0.5 kV/cm and 2.5 kV/cm and pulse width between 0.05 ms and 0.45 µs. From the dependence of transfection efficiency and viability on mRNA concentration (Figure 2A), we optimized the effect of pulse amplitude for mRNA concentration in the 20–25 µg/mL range (Figure 2B). Because the pulse of 1.0 kV/cm resulted in acceptable transfection efficiency and reasonable viability, we further studied the effect of pulse width at 20–25 µg/mL RNA and 1.0 kV/cm (Figure 2C).

Electrotransfection of patient DCs with mRNA isolated from tumor tissue

Immature DCs manufactured from the blood of melanoma patients were electrotransfected with mRNA isolated from autologous tumor tissue and in vitro amplified. The cells were prepared as above except that the IDCs were washed, suspended in the Cytoporation Formula R Medium (Cyto Pulse Sciences, Inc., Glen Burnie, MD) at a density of 1 x 10^7/mL in the presence of 20–50 µg/mL of autologous mRNA. The cell suspension was transferred to sterile, disposable electroporation cuvettes with a 4-mm electrode gap (Molecular BioProducts, San Diego, CA). The cells were subjected to two square 400-V pulses of 50 µs each from the PA-4000 PulseAgile generator. Following electroporation the cells were rested in X-VIVO 15 medium containing HABS, GM-CSF and IL-4 as above at 37°C in humidified 5% CO_2 for one hour. Subsequently the cells were washed once and suspended in the maturation medium containing 1100 IU/mL TNF- and 1.0 µg/mL PGE2 for two more days. MDCs were collected, assayed for compliance with release criteria.

Preparing clinical-grade myeloid dendritic cells by electroporation-mediated transfection of in vitro amplified tumor-derived mRNA and safety testing in stage IV malignant melanoma (continued)

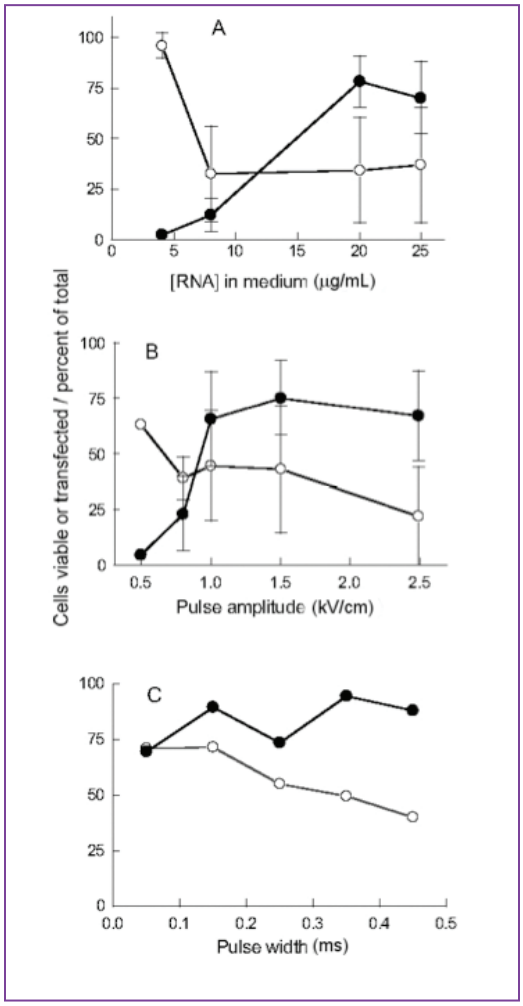

Fig. 2 *Identifying conditions for electrotransfection of immature dendritic cells. Normal IDCs were electro-transfected with in vitro transcribed eGFP-mRNA. Following electrotransfection, the cells were matured for 48 hours when viability (open symbols) and transfection efficiency (closed symbols) were quantified (by 7-AAD exclusion and eGFP fluorescence, respectively). Shown are the data from the final iteration in the analysis where mRNA concentration varied from 4.0 µg/mL to 25 µg/mL (A), pulse amplitude from 0.5 kV/cm to 2.5 kV/cm (B) and pulse width from 0.05 µs to 0.45 µs (C). Symbols denote mean values of measurements in cells from three or more individuals ± standard deviation (except in panel C that is an example of an entire experiment conducted with cells from one individual).*

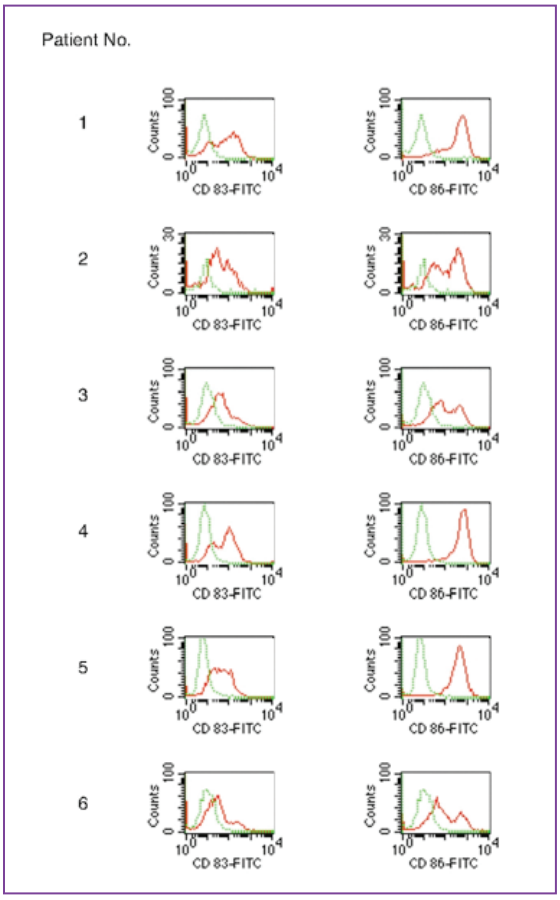

Fig. 5 *Expression of CD83 (left) and CD86 (right) by patients' RNA-transfected DCs (red) used for vaccination. Isotype controls are shown in green.*

REFERENCE

Markovic, SN, Dietz, AB, Greiner, CW, Maas, ML, Butler, GW, Padley, DJ, Bulur, PA, Allred, JB, Creagan, ET, Ingle, JN, Gastineau, and Vuk-Pavlovic, S. (2006) Preparing clinical-grade myeloid dendritic cells by electroporation-mediated transfection of in vitro amplified tumor-derived mRNA and safety testing in stage IV malignant melanoma. J Transl. Med 2006, 4:35-48. doi:10.1186/1479-5876-4-35

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