Generation of dendritic cell-tumor cell hybrids by electrofusion for clinical vaccine application

INTRODUCTION

Dendritic cells (DCs) are highly potent antigen-presenting cells that show tremendous promise as adjuvants for cancer vaccine immunotherapy. In particular, fusion hybrids generated from DCs and tumor cells produce immunization against whole-cell tumor-associated antigens (TAAs). There are several methods for generating the DC-tumor cell hybrids, but electrofusion is increasingly used for its simplicity, high reproducibility and efficiency. This paper reports on the efficiency of electrofusion generated DC-tumor cell hybrids for several tumor cell lines and reports on the T cell immune response induced by these DC-tumor cell hybrids.

RESULTS

1. Electrofusion was used to produce DC-tumor cell hybrids for four tumor cell lines: A549, Colo 829, MCF-7 and DC-U251. Multinuclear cells were immediately visible (Figure 2).
2. FACS analysis determined 8-13% fusion efficiency across all tumor cell lines (Table 1).
3. Fusion hybrids were stable at 48 hour postfusion (Figure 5A) and after freeze/thaw (Figure 5B) with similar numbers of hybrids (~10 %).
4. Hybrid DC-Colo 829 cells induced a robust T-cell response compared to mixed cells or Colo 829 cells alone (Figure 6). This response was enhanced after incubating hybrids in a cocktail that promotes DCs to mature.

CONCLUSIONS

Electrofusion was used to produce fusion hybrids of dendritic cells and tumor cell lines at ~ 10% efficiency. The fusion hybrids were stable and could induce immunological response to antigen.

METHODS

Dendritic cells and cell culture

DCs were generated from leukapheresis product obtained as peripheral blood mononuclear cell (PBMC) separations from healthy donors after written informed consent, and study approval by the University of Arizona Institutional Review Board. Large-scale production of DCs was produced using standard methods in gas-permeable hydrophobic bags (Baxter, Glendale, CA, USA) and frozen in liquid nitrogen at 2.5x107 cells/ml in 0.9% NaCl (Baxter) containing 10% DMSO (Sigma, St Louis, MO, USA), 4% human serum albumin (Baxter). Cells thawed at 4–8 weeks postfreezing were >90% viable. The A549 human lung cancer, U251 human glioma, MCF-7 human breast cancer, and Colo 829 human melanoma cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The A549, Colo 829, and MCF-7 cell lines were maintained in RPMI 1640 medium (Gibco, Grand Island, NY, USA) containing 10% heat-inactivated fetal bovine serum (FBS; Omega Scientific, Tarzana, CA, USA). The U251 cell line was maintained in Dulbecco's modified essential medium (DMEM; Fisher, Tustin, CA, USA) containing 10% heat-inactivated fetal bovine serum, 1% glutamine, 1% penicillin-streptomycin, 1% sodium pyruvate, and 1% MEM nonessential amino acids (Gibco). For clonogenic assays of irradiated Colo 829 cells, control cells and populations receiving 25, 50, and 200 Gy of irradiation were plated in duplicate at 2·106 cells in 75-cm2 tissue culture flasks. Cultures were maintained for 2 weeks and fed every 3 days with RPMI 1640 containing 10% FBS. Colonies were stained using Diμ-Quik (VWR, West Chester, PA, USA) and counted.

Electrofusion and culture of DC-tumor cell hybrids

DCs were thawed and washed twice in AIM-V medium. Tumor cells (~70% confluent) were detached using a 0.02% EDTA solution (Sigma, St Louis, MO, USA), washed in phosphate-bu_ered saline (PBS) and irradiated (50 Gy). In some cases, cells were prestained with individual membrane dyes for detection of fusion hybrids by FACS analysis. DCs were pre-stained for 30 min with 2.25 mM of the ''red'' fluorescent CMFTR CellTracker dye (Molecular Probes, Eugene, OR, USA), and tumor cells were pre-stained for 30 min with 1.25 mM of the ''green'' fluorescent CMFDA CellTracker dye (Molecular Probes, Eugene, OR, USA). The DCs and tumor cells were then mixed at a 1:1 ratio, pelleted, and washed three times in Cytofusion Medium C, an isotonic sorbitol solution containing magnesium and calcium (Cyto Pulse Sciences, Columbia, MD, USA). The cells were resuspended in 3 ml of Cytofusion Medium C (2 x107 cells/ml, 6 x 107 cells total) and placed in a 6-ml coaxial electrofusion chamber (4-mm gap distance) (Cyto Pulse Sciences, Columbia, MD, USA). Electrofusion was performed using a PA-101 generator (Cyto Pulse Sciences). Cells were fused by first aligning using an alternating current at 100 V/cm (30 s) and then 187.5 V/cm (10 s) followed by four direct current pulses at 2,000 V/cm (40 ms each, 0.125-s pulse intervals, 0.8-MHz). After the fusion pulses, an alternating current at 112.5 V/cm (55 s) was applied to help maintain cell contact. Following fusion, the cells were incubated in the chamber for 30 min at room temperature. An equal volume of RPMI 1640 medium was then added, and the cells were incubated for a further 15 min. Mixed populations were treated identically but without experiencing electrofusion. Cell populations were then either immediately analyzed for hybrid formation or placed in culture for later analysis. Populations were cultured at 1 x 106 cells/ml in RPMI 1640 containing 10% FBS, GM-CSF (500 IU/ml), and IL-13 (50 ng/ml). Mature DC–hybrid cultures were generated by a further 24-h culture in a cocktail mixture containing GM-CSF (500 IU/ml), IL-13 (50 ng/ml), 10 ng/ml TNF-a (R&D Systems, Minneapolis, MN, USA), 10 ng/ml IL-1μ (R&D Systems), 15 ng/ml IL-6 (R&D Systems), 1 mg/ml PGE2 (Sigma), and 25 μg/ml poly I:C (Sigma). The percentage of multinucleated cells was determined by cytocentrifugation followed by dye staining with Diμ-Quik (VWR, West Chester, PA, USA) and counting of multinucleated cells. The percentages of heterologous hybrids were determined by FACS analysis. Cell viabilities were assessed by dye exclusion staining using trypan blue (Sigma).

Generation of dendritic cell-tumor cell hybrids by electrofusion for clinical vaccine application (continued)

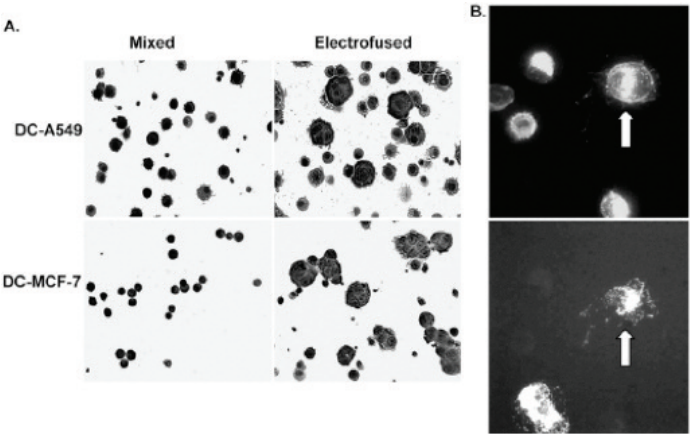

Fig. 2 *Photomicrographs of mixed and electrofused populations. A) Di_-Quik dye staining of mixed and fused DC-tumor cell populations. Mixed and electrofused populations were cytocentrifuged and dye stained. B) Dual immunofluorescent staining of DC-A549 hybrid cell product. Electrofused cells were cytocentrifuged and immune stained using an anti-HLA-DR-PE monoclonal antibody (upper panel) and an anticytokeratin-FITC monoclonal antibody (lower panel). Arrow indicates a large, fused DC-tumor hybrid cell positive for both HLA-DR and epithelial keratin filaments.*

Table 1 Fusion efficiency of DC-tumor cell hybrids. Percentages were determined by FACS analysis at 45 min postfusion.

DC-Tumor Cell Fusion	Average % Hybrid	Range (%)
DC-A549 (n=10)	9	8-11
DC-Colo 829 (n=10)	8	6-15
DC-MCF-7 (n=3)	10	9-13
DC-U251 (n=2)	9.5	9-10

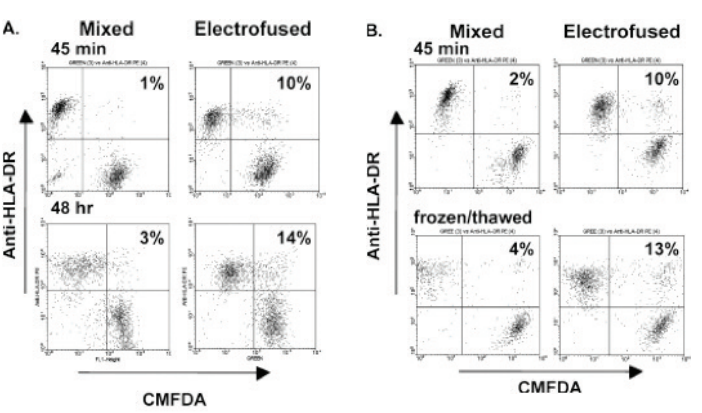

Fig. 5 *A FACS analysis of DC–Colo 829 populations at 45 min and 48 h postfusion. Colo 829 cells were pre-stained with CMFDA dye prior to electrofusion. The DC marker, HLA-DR, was detected by immune staining postfusion. Mixed and electrofused populations were analyzed at 45 min postfusion and after 48 h of culture. Cell populations were cultured in RPMI 1640 containing 10% FCS. B Analysis of DC-tumor hybrids after freeze/thaw. FACS analysis of mixed and electrofused populations was performed at 45 min postfusion and after freezing for 1 week and thawing. Colo 829 cells were pre-stained with CMFDA prior to fusion. The DC marker, HLA-DR, was detected by immune staining postfusion*

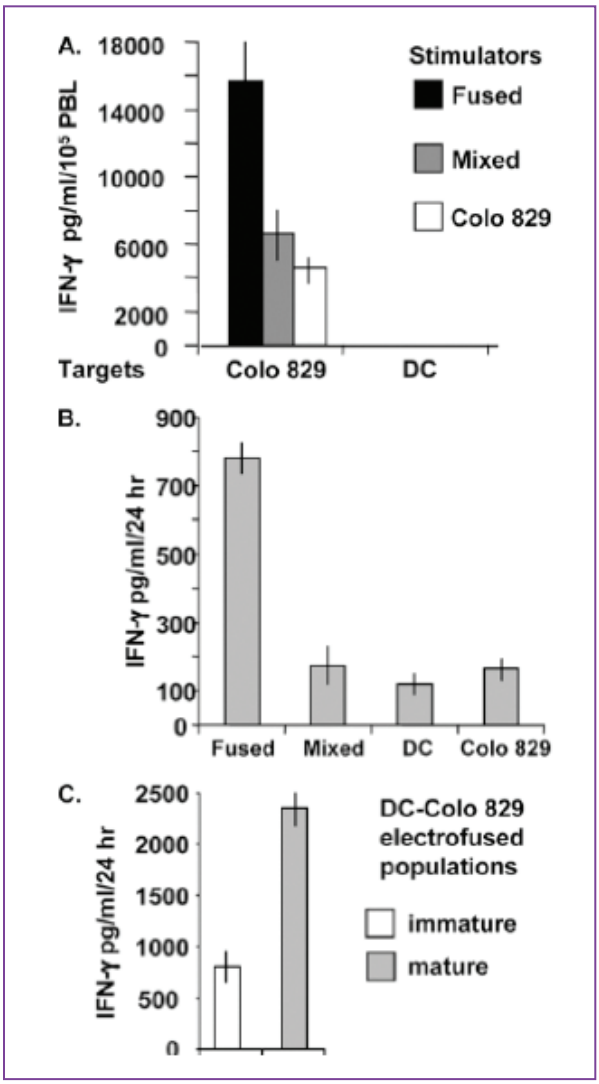

Fig. 6A–C *Induction of T-cell responses against DC–Colo 829 hybrid populations. A Autologous PBMC responses. Autologous PBMCs (105 cells) were stimulated weekly for 3 weeks in the presence of DC–Colo 829 electrofused cells, mixed DC–Colo 829 cells, or Colo 829 cells only (104 cells). The stimulated PBMC cultures were tested against Colo 829 tumor cells or DCs for activation-induced IFN-c release (ratio 105 PBMCs to 104 target cells). Supernatants were analyzed by IFN-c ELISA at 24 h postincubation. B Induction of gp100 antigen-specific H3.1 CTL response by electrofused DC–Colo 829 hybrid populations. H3.1 CTLs (1·105 cells) were cocultured with the indicated targets (104 cells). The electrofused population indicated 8% DC–Colo829 fusion hybrids as determined by FACS analysis (data not shown). After 24 h, supernatants were removed and tested for IFN-c production by ELISA. C Comparison of gp100 antigen-specific H3.1 CTL responses induced by immature or mature DC–Colo829 hybrid populations. H3.1 CTLs (1·105 cells) were cultured with 1·104 cells from either an immature hybrid DC–Colo 829 population or the same population exposed to maturation agents for 24 h prior to coculture with H3.1 CTLs. Supernatants were removed after 24 h and tested for IFN-c production by ELISA. For all samples, mean values and standard deviations of triplicate samples are shown*

REFERENCE

Trevor, KT, et al. (2004) Generation of dendritic cell-tumor cell hybrids by electrofusion for clinical vaccine applications. Cancer Immunol Immunother 53: 705-714. DOI 10.1007/s00262-004-0512-1

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