Application note

A Simple Method for Delivery and Antigen Presentation of a Malaria Protein in Human Dendritic Cells

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BioPORTER™ Transfection reagent

Introduction

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that play critical roles in the initiation and modulation of immune responses. Because of their potent antigen presenting ability, dendritic cells offer a promising strategy for eliciting immunity against tumors or pathogens of interest when they are loaded with target antigens. Historically, transfecting dendritic cells with DNA or RNA has proved to be problematic and most researchers can achieve only very low transfection efficiencies using retroviral delivery methods. This article demonstrates successful delivery and subsequent presentation of a malaria antigen in human dendritic cells using BioPORTER®, a novel cationic lipid-based protein delivery reagent.[1]

Materials & Methods

Dendritic Cells and T Cells

Peripheral blood mononuclear cells (PBMC's) were isolated from a human volunteer immunized with radiation-attenuated sporozoites from *P. falciparum*, the infectious agent responsible for malaria. The PBMC's were cultured with GM-CSF/IL-4 for 6 days to generate dendritic cells, which were used as APCs in an IFN-γ ELIspot assay. Autologous PBMC populations were used as a source of responder cells.

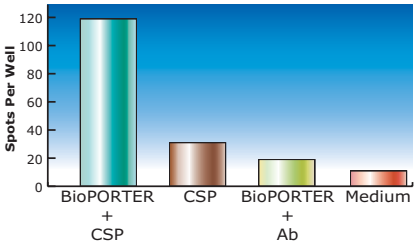
Antigen Delivery.

A total of 10 µg of recombinant *P. falciparum* circumsporozoite protein (PfCSP) expressed in *Saccharomyces cerevisiae* was diluted to a final volume of 40 µl in serum-free medium (OptiMEM). The protein solution was added to one pre-coated vial containing BioPORTER reagent (BioPORTER QuikEase™ Kit, GTS) and incubated at room temperature for 5 min. The BioPORTER /protein mixture was adjusted to a final volume of 0.5 ml in serum-free medium (OptiMEM). Then, 125 µl of the BioPORTER /protein mixture was added to 1 x 10^5 cultured dendritic cells, in a 12 ml snap-cap polypropylene tube, and incubated for 4 hrs at 37°C. Cells were washed twice with PBS and resuspended in standard cell culture medium (RPMI 1640 supplemented with 10% FCS).

IFN-γ ELIspot assay

PfCSP protein transduced dendritic cells were transferred to standard ELIspot plates precoated with anti-IFN-γ mAb. Autologous PBMCs were added to each well, at a target cell:effector cell (DC:PBMC) ratio of 1:10 (50,0000 DC/500,000 PBMCs and 25,000 DC/250,000 PBMCs). Cells were cultured for 36 hours at a temperature of 37°C in an atmosphere of 5% CO_2. Plates were then processed as per a

Figure 1. Cellular Immunity Assay



Dendritic cells were generated from PBMCs of a volunteer immunized with radiation-attenuated *P. falciparum* sporozoites, for use as target cells. Recombinant *P. falciparum* CSP protein was delivered to the dendritic cells using BioPORTER. Autologous PBMC populations were added to the protein-transduced dendritic cells and cultures processed as per a standard ELIspot assay. A total of 120 PfCSP antigen-specific T-cells out of 250,000 input PBMCs were detected, translating to 480 SFCs per million PBMCs.

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standard ELIspot assay for evaluation of the number of PfCSP-specific IFN-γ secreting cells. The number of spots corresponding to IFN-γ producing cells was determined visually using a stereomicroscope (KS ELIspot, Zeiss). Results were expressed as the number of IFN-γ-secreting cells per 10^6 PBMCs.

Result and Discussion.

ELIspot assay results are presented in Figure 1. A total of 120 antigen specific T-cells were detected in the 250,000 input PBMCs, translating to 480 spot forming colonies (SFCs) per million PBMC's, when antigen specific target cells were generated by delivering PfCSP to the cells in the presence of BioPORTER reagent. In contrast, the signal was barely above background when the dendritic cells were treated with either recombinant CSP alone, BioPORTER + control mouse IgG, or culture medium alone. These data confirm results from additional studies demonstrating efficient delivery of fluorescent goat IgG into dendritic cells using BioPORTER reagent (Figure 2). Based on these results, we conclude that BioPORTER is an effective reagent for efficiently delivering proteins into dendritic cells, and that BioPORTER-mediated antigen delivery to dendritic cells results in functional antigen presentation. These properties of BioPORTER reagent make it a valuable tool for studying antigen presentation in difficult-to-transfect dendritic cells.

Reference

1. Zelphati, O. *et al.* (2001) *J. Biol. Chem.* **276**: 35103-35110.

Acknowledgments

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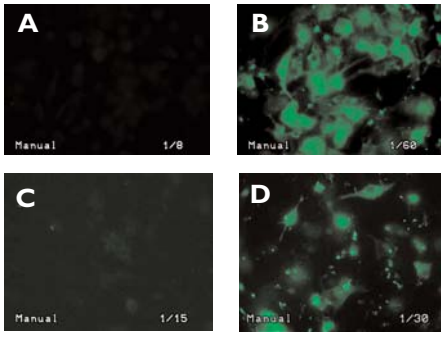
Figure 2. Delivery of fluorescent antibody to human and mouse dendritic cells



A fluorescein labeled goat IgG antibody was delivered to human dendrtitic cells (A&B), or mouse bone marrow dendritic cells (C&D) either with BioPORTER (B&D) or without BioPORTER (A&C). For both cell types the results show that BioPORTER can very efficiently deliver antibodies to most of the cells in the culture. (Courtesy Gene Therapy Systems)