Transfection of murine dendritic cell line (JAWS II) by a nonviral transfection reagent

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Dendritic cells are the most potent antigen-presenting cells that initiate and modulate the host immune system. Based on their immunostimulatory activity, a variety of strategies have been developed to use dendritic cells as vaccines and immunotherapeutic agents against infection and cancer. Genetically modified dendritic cells are useful for immunotherapeutic purposes because of their sustained activity in vivo. However, transfection of dendritic cells with plasmid DNA has been very difficult. While the viral transfection is associated with nonspecific activation of dendritic cells, commonly used nonviral transfection reagents have a low efficiency of transfection. Here we describe an improved, simple, less time-consuming transfection protocol using the nonviral nonliposomal lipid polymer, TransIT-TKO[®] transfection reagent, for transfecting murine dendritic cells (JAWS II) with the gene that encodes *Coccidioides immitis* antigen 2 (Ag2). The JAWS II cells were cotransfected with pHYG-enhanced green fluorescent protein (EGFP) and pVR1012-*C. immitis* Ag2 plasmid DNAs using TransIT-TKO reagent. We reproducibly obtained 30%–50% transfection efficiency. The transfected cells maintained their immature phenotype and were functionally active. In addition, the flexibility of this agent for expressing multiple antigens (GFP and *C. immitis* Ag2) offers an advantage of delivering multiple immunogens.

INTRODUCTION

Dendritic cells are unique antigen-presenting immune cells that play an important role in regulating the host immune responses (1,2). Immature dendritic cells have a unique capability of sensing the maturation signals of infection or inflammation and inducing primary immune responses (3). These cells can internalize and process bacterial, fungal, viral, and other particulate antigens for major histocompatibility complex (MHC) classes I and II antigen presentation to T cells. Following an encounter with an antigen, dendritic cells mature into antigen-presenting cells, up-regulate their chemokine and co-stimulatory receptors, and migrate to secondary lymphoid organs where they can prime naive T cells and naive and memory B cells and activate natural killer cells to induce specific immune responses (4–6). Because of these unique characteristics, dendritic cells have gained considerable interest in the field of vaccine development and immunotherapy. Ex vivo activation and maturation of dendritic cells have been achieved by incubating the

cells with viable (7–11) or killed infectious agents or tumor cells and derived antigens (11,12). It has been noted that genetic transfection is a highly effective means for induction of immature dendritic cells with sustained immunomodulatory activity (13,14). However, transfection of dendritic cells with plasmid DNA using nonviral reagents has been very difficult, with efficien-

cies varying from less than 1% to 20% (15–17). Adenoviral-mediated transfection, on the other hand, is successful but requires a fairly high multiplicity of infection (100–1000) (18,19), and the utility of this approach is limited by the finding that cellular immune responses are elicited against viral proteins as well (20,21), resulting in mild activation of the phenotype of dendritic cells (22–24). Here we describe an improved transfection protocol, using the nonviral nonliposomal lipid polymer, TransIT-TKO[®] transfection reagent (Mirus, Madison, WI, USA), for transfecting murine dendritic cells with the gene that encodes *Coccidioides immitis* antigen 2 (Ag2). Experimental studies in a murine model have established that *C. immitis* Ag2 DNA, a cell wall glycoprotein, induces protective immunity against lethal challenge (25).

MATERIALS AND METHODS

In the present study, murine dendritic cells (JAWS II) (ATCC, Manassas, VA, USA) were transfected with pHYG-enhanced green fluorescent protein (EGFP) (BD Biosciences Clontech, Palo Alto, CA, USA) and *C. immitis* Ag2 cDNA ligated to pVR1012 (Vical, San Diego, CA, USA). The JAWS II dendritic cells were derived from the bone marrow of C57/BL6 mice and express an immature phenotype. The plasmid DNA was prepared using an EndoFree[®] Plasmid Maxi kit (Qiagen,

Figure 1. Fluorescent micrographs of (A) nontransfected and (B) pHYG-enhanced green fluorescent protein (EGFP) and pVR1012-*Coccidioides immitis* antigen 2 (Ag2) plasmid DNA-cotransfected JAWS II cells. The green fluorescence shows expression of GFP. These results are representative of three different experiments. These photographs were taken after a 48-h transfection.

Valencia, CA, USA). The JAWS II cells were maintained in complete α -minimum essential medium containing ribonucleosides and deoxyribonucleosides (Sigma, St. Louis, MO, USA), 20% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 4 mM L-glutamine, 5 ng/mL recombinant mouse granulocyte macrophage colony-stimulating factor (GM-CSF) (Peprotech, Rocky Hill, NJ, USA), penicillin-streptomycin (100 μ g/mL penicillin and 100 U/mL streptomycin; Invitrogen), and 50 μ g/mL gentamycin (Invitrogen). The cells were allowed to grow overnight to 50%–60% monolayer confluency in 24-well plates. The cells were washed with serum-free Dulbecco's minimum essential medium (DMEM; Invitrogen) before transfection. The transfection reagent/DNA complex (4 μ L:2 μ g each plasmid DNA) was prepared in serum-free DMEM by adding plasmid DNAs (2 μ g pHYG-EGFP and 2 μ g pVR1012-*C. immitis* Ag2 plasmid

DNA) to the *TransIT*-TKO reagent (in 150 μ L DMEM). The mixture was kept at room temperature for at least 5 min before adding it to the cells. The cells were incubated at 37°C in a 5% CO₂ incubator. After a 4-h incubation, an additional 250 μ L of complete medium were added. The percent transfection efficiency was evaluated by visual enumeration of the green fluorescence-transfected cells versus the total number of cells using a fluorescent microscope with the appropriate filter (Olympus Optical Co. Ltd., Tokyo, Japan). After a 24-h transfection, both the transfected and nontransfected JAWS II cells were stained with propidium iodide to confirm the viability.

The expression of the *C. immitis* Ag2 protein was detected by dot-blot immunoblotting. Briefly, the cells were washed once with Dulbecco's phosphate-buffered saline (Invitrogen), and the cell lysates were prepared in homogenization buffer containing 1% Igepal CA-630, 0.1% sodium dodecyl sulfate, protease inhibitors (1.1 μ M leupeptin, 1 μ M pepstatin, and 0.2 mM phenylmethylsulfonyl fluoride), and 1 mM EDTA (all from Sigma). Various amounts of cell lysate protein were loaded on prewetted nitrocellulose paper (Schleicher & Schuell, Keene, NH, USA) in Tris-buffered saline (TBS; 20 mM Tris-HCl, pH 7.5, 150 mM NaCl). The nonspecific sites were blocked by incubating with 5% bovine serum albumin in TBST (TBS containing 0.05% Tween® 20). The nitrocellulose sheet was then incubated with goat anti-Ag2 antibody (1:1000) followed by incubation with secondary alkaline phosphatase-labeled rabbit anti-goat immunoglobulin G (IgG) antibody (1:10,000; Sigma). The immunocomplex was detected by an AP Conjugate Substrate kit (Bio-Rad Laboratories, Hercules, CA, USA).

We also confirmed the phenotype and stimulatory capacity of the pVR1012-*C. immitis* Ag2 plasmid DNA-transfected JAWS II cells. The cells were transfected with pVR1012 or pVR1012-*C. immitis* Ag2 plasmid DNA according to the method described here using *TransIT*-TKO reagent. After a 24-h transfection, cells were incubated with *Escherichia coli* O111:B4 lipopolysaccharide (LPS) (final concentration 100

Figure 2. Flow cytometry analysis of (A) propidium iodide-stained nontransfected and (B) pHYG-enhanced green fluorescent protein (EGFP) and pVR1012-*Coccidioides immitis* antigen 2 (Ag2) plasmid DNA-cotransfected JAWS II cells. Numbers in the upper right indicate the percentage of viable cells.

ng/mL; Sigma) for another 24 h. The cells were stained with fluorochrome-labeled anti-mouse CD14, CD11c, CD86, MHC class II (IA/E), CD80, and CD40 antibodies, according to the method described by the manufacturer (BD Pharmingen, San Diego, CA, USA), and fluorescence analysis was performed using a FACSCalibur™ flow cytometer and CellQuest™ V3.1 software (both from BD Biosciences, San Jose, CA, USA). The mean fluorescent index above background was obtained by using the formula [(geometric mean of fluorescence intensity × % gated cells)/100] - background values.

RESULTS

The transfection efficiency ranged from 30% to 50% in three separate experiments using different batch products of *TransIT*-TKO reagent (Figure 1). Approximately 70% of the transfected cells remained viable after a 24-h transfection, as compared to 88% of the nontransfected cells (Figure 2). The pVR1012-*C. immitis* Ag2 plasmid DNA-transfected JAWS II cells expressed the *C. immitis* Ag2 protein (Figure 3).

The JAWS II cells maintained their immature phenotype during transfection, and there was little or no change in ex-

pression of cell surface markers in either the pVR1012 vector or the pVR1012-*C. immitis* Ag2 plasmid DNA-transfected JAWS II cells, as compared to the nontransfected JAWS II cells, except for CD80 and CD86 (Figure 4A). However, after LPS treatment, nontransfected and pVR1012- or pVR1012-*C. immitis* Ag2 plasmid DNA-transfected JAWS II cells showed up-regulation of MHC class II, CD86, CD80, and CD40 (Figure 4B), and cytokines [tumor necrosis factor α (TNF α), interleukin (IL)-1 β , and IL-12; data not shown] secretion.

DISCUSSION

The present results indicate that we can reproducibly obtain improved transfection in dendritic cells using *TransIT*-TKO nonviral transfection reagent. The transfected JAWS II cells remain viable and functionally active and maintain their immature phenotype. While viral-mediated transfection has limitations of being labor-intensive and its utility for gene therapy has immunogenic and safety concerns, the *TransIT*-TKO system requires only a brief mixing of the commercially available transfection reagent and plasmid DNA. Moreover, the flexibility of this agent for expressing multiple antigens (GFP and *C. immitis* Ag2) offers advantages in delivering multiple immunogens. The transfection protocol reported here can provide a convenient and rapid means of screening novel vaccine candidate genes emerging from genomic and serologic studies for those giving optimal immune responses. *C. immitis* [a biological safety level-3 (BSL-3) class pathogen] causes pulmonary and extrapulmonary systemic infection in endemic areas of the Southwestern U.S. and Mexico. Treatment of coccidioidomycosis with antifungal agents is difficult, and patients often fail to respond to therapy. Immunotherapeutic in-

tervention using transfected dendritic cells could provide an alternative or adjunctive means for reducing the mortality associated with this disease.

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Figure 3. *Coccidioides immitis* antigen 2 (Ag2) expression by transiently cotransfected JAWS II cells after a 24-h transfection as observed by dot-blot immunoblotting. Lanes A–F: 0, 1.75, 2.5, 5.0, 10.0, and 20.0 μ g total cellular protein, respectively, from nontransfected JAWS II cells (N) and pHYG-enhanced green fluorescent protein (EGFP) and pVR1012-*C. immitis* Ag2 plasmid DNA-cotransfected JAWS II cells (T). Lanes A–F: 0, 1.0, 2.0, 5.0, 10.0, and 20.0 μ g, respectively, recombinant *C. immitis* Ag2 protein (Ag2). Black dots show the immunocomplex. The results are representative of three different experiments.

Figure 4. Comparison of cell surface marker expression in nontransfected, pVR1012-transfected, and pVR1012-*Coccidioides immitis* antigen 2 (Ag2) plasmid DNA-transfected JAWS II cells. (A) Cell surface expression of CD14, CD11c, major histocompatibility complex (MHC) class II (IA/E), CD86, CD80, and CD40 in nontransfected (N), pVR1012 vector-transfected (V), or pVR1012-*C. immitis* Ag2 plasmid DNA-transfected (T) JAWS II cells. The numbers in the upper right indicate the mean fluorescent index (MFI) above background. (B) Cell surface expression of CD14, CD11c, MHC class II (IA/E), CD86, CD80, and CD40 in nontransfected, pVR1012 vector plasmid-transfected, or pVR1012-*C. immitis* Ag2 plasmid DNA-transfected JAWS II cells in response to *Escherichia coli* lipopolysaccharide (LPS) (100 ng/mL). The MFI values for cell surface markers presented here on the y-axis show the ratio of MFI values in respective (LPS-treated to untreated) cells. The horizontal dashed line at MFI value 1 represents the MFI in untreated cells. The results are from one representative experiment performed in triplicate.

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