Cytokine 108 (2018) 82–88

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Transfection of bone marrow derived cells with immunoregulatory proteins

Julia N. Khantakova[a], Alexander N. Silkov[a], Valeriy P. Tereshchenko[a], Elena V. Gavrilova[b], Rinat A. Maksyutov[b], Sergey V. Sennikov[a,*]

[a] Federal State Budgetary Scientific Institution "Research Institute of Fundamental and Clinical Immunology", Yadrintsevskaya St. 14, Novosibirsk 630099, Russia
[b] State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk 630559, Russia

ARTICLE INFO

Keywords:
Electroporation
Dendritic cells
Macrophage
Interleukin-10
sTNFRI

ABSTRACT

In vitro electroporation gene transfer was first performed in 1982. Today, this technology has become one of the major vehicles for non-viral transfection of cells. All non-viral transfections, such as calcium phosphate precipitation, lipofection, and magnetic transfection, have been shown to achieve a transfection efficiency of up to 70% in commonly used cell lines, but not in primary cells. Here we describe the use of electroporation to transfect primary mouse bone marrow-derived cells, such as macrophages (Mφ) and dendritic cells (DCs) with high efficiencies (45%–72%) and minimal cell death. The transfection efficiencies and cell death varied depending on the culture duration of the DCs and Mφ. Moreover, the electroporation efficiency was increased when conditioning medium was used for culturing the cells. Furthermore, we demonstrated that measuring the plasmid-encoded secreted proteins is a highly sensitive method for determining the transfection efficiency. In summary, electroporation with plasmid vectors is an efficient method for producing DCs and Mφ with transient expression of immunoregulatory proteins.

1. Introduction

Novel cellular approaches to treating various pathological conditions, such as cancer and autoimmune diseases, and regulating transplantation immunity and tissue repair are being developed worldwide. Dendritic cells (DCs) and macrophages (Mφ) are the most promising immunocompetent cells for clinical applications. DCs are a link between the innate and adaptive immune systems [1] and play a significant role in tolerance induction and the suppression of immune responses [2,3]. Interleukin (IL)-10 secretion is one of the mechanisms used by DCs to maintain a tolerogenic state [4,5]. IL-10-treated DCs acquire the regulatory/tolerogenic phenotype, induce regulatory T cells, and suppress inflammatory responses. Furthermore, IL-10 reduces the production of proinflammatory cytokines by T cells, monocytes, and macrophages [6] and promotes effector T cell anergy [7]. Hence, active IL-10 secretion by DCs promotes the induction of tolerance by immune cells in an antigen-specific manner.

Mφ are involved in the regulation of inflammatory responses at many stages: acute inflammation, removal of damaged cells, recovery of cellular composition and organ repair [8,9]. Tumor necrosis factor (TNF)-α is a key immune regulator derived from Mφ and plays a crucial role in the acute inflammatory phase and maintenance of chronic inflammation. Binding of TNF-α to soluble forms of its receptor regulates its proinflammatory effects, which has been confirmed by numerous experimental and clinical studies [10].

Regulatory proteins often need to be used to modulate the required functional properties of cells: e.g., at stages of *in vitro* cell culture to correct their differentiation or activity, as well as when introducing the generated cells into a mouse or patient to *in situ* modulate production of regulatory proteins. It is desirable to use antigen presenting cells, such as DCs or Mφ, as producers of the required mediators, which can be performed by antigen loading or transfection procedures. The existing approaches are based either on the ability of a cell to capture foreign molecules (phagocytosis or macropinocytosis) or on forced insertion of molecules into the cell using chemical (lipids and polymers), physical (electroporation, sonoporation, a gene gun, or microinjections), or viral exposure. The key drawback of the approaches based on phagocytosis or macropinocytosis is their dependence on the functional status of cells that is often disrupted in pathological conditions, so it is impossible to quantify capture efficiency and the amount of captured material [11]. Application of various chemical, magnetic, and viral transfection methods suggests using artificial or viral particles, which requires additional research into their safety [12]. The use of electroporation; applying a high-voltage discharge to a cell suspension, seems to be the most promising and safe approach for transfecting expression vectors.

The application of electroporation to transfer genes into murine cells was first reported in 1982 [13]. Numerous studies have demonstrated that electroporation can be successfully used to insert not only

* Corresponding author.
E-mail address: sennikovsv@gmail.com (S.V. Sennikov).

https://doi.org/10.1016/j.cyto.2018.03.028
Received 24 January 2018; Received in revised form 16 March 2018; Accepted 19 March 2018

DNA, but other molecules, into various cell types, such as cultured animal cells, protozoa, yeasts, bacteria, and plant protoplasts [14–17]. However, despite a large number of electroporation protocols having been described in publications and on the websites of various equipment manufacturers, in most cases electroporation conditions need to be optimized when performing experimental research to define a protocol that would combine high transfer efficiency and a sufficient level of cell death. The protocol is highly specific for the type and properties of cells being used, the structure of molecules being inserted, the composition of the solutions being used, and parameters of the electric pulse generated by the equipment.

Herein we present the results of optimizing the conditions of electroporation of murine bone marrow-derived DCs and Mφ with DNA plasmids encoding the sequences of immunoregulatory proteins to obtain transient producers of IL-10 and soluble TNF type I receptor (sTNFR1).

2. Materials and methods

2.1. Ethical statement

All experimental protocols and methods were approved by the institutional review board of the Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation (Protocol No. 99/2016-02-09). The study followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

2.2. Animals

C57Bl/6 female mice aged 2–6 months were obtained from the breeding facility of the Institute of Cytology and Genetics (Novosibirsk, Russia). The animals were housed in the animal facility at the Research Institute of Fundamental and Clinical Immunology on a standard diet under natural light conditions with unrestricted access to food and water.

2.3. Media and reagents

RPMI-1640 culture media (BioloT), serum-free phenol-red-free OptiMEM (Thermo Fisher Scientific), fetal calf serum (HyClone), 2-mercaptoethanol (Sigma), L-glutamine (BioloT), bovine insulin (Pan-Eco), gentamicin (KRKA), benzylpenicillin (Sintez), HEPES (Sigma), propidium iodide (PI) (Sigma), and Tween 20% (Sigma). Monoclonal antibodies (BioLegend) for flow cytometry phenotyping of DCs: CD11c-FITC, H2b-PE, CD83-PE-Cy7, CD86-APC-Cy7, CD80-Brilliant Violet 421, CD40-PE-Cy5; for phenotyping of Mφ: CD11c-FITC, Ly6c-PE, CD115-APC, and F4/80-PerCP. Recombinant murine cytokines: rmIL-4, rmGM-CSF (Miltenyi Biotec), rmTNF-α (Vector). ELISA kits: mouse IL-10 immunoassay, mouse IFN-γ immunoassay and mouse/rat TNFR1/TNFRSF1A immunoassay (Quantikine ELISA, R&D Systems). Colorimetric assay to assess cell viability MTT (Promega). Culture flasks, plates (TPP, Switzerland), and Petri dishes (Nunclon).

2.4. DNA constructs

The following plasmid DNA constructs were used in this study:

- GFP – control plasmid DNA construct pmax-GFP encoding green fluorescent protein (GFP);
- pIL-10 – plasmid DNA construct pmax-pIL-10 encoding for murine IL-10;
- pTNFR1 – plasmid DNA construct pmax-sTNFR1 encoding murine soluble TNF receptor 1.

2.5. Generation of dendritic cells and macrophages from bone marrow

DCs were generated from the bone marrow monocyte pool [18]. The cell suspension harvested from femoral bones was washed twice with RPMI-1640 medium and incubated for 30 min in Petri dishes to remove the nonadherent cells. The monocyte pool was subsequently cultured in flasks (25 cm^2) at a concentration of 1×10^6 cells per ml in RPMI-1640 supplemented with 10% FCS, 2 mM L-glutamine, 10 mM HEPES, 5×10^{-4} M 2-mercaptoethanol, 80 μg/ml gentamycin, and 100 μg/ml benzylpenicillin with growth factors added (20 ng/ml GM-CSF or 20 ng/mL IL-4); the culture medium was replaced on days 2 and 4.

To generate Mφ, the bone marrow cells were cultured for 7 days in culture medium supplemented with 20 ng/ml recombinant murine M-CSF; the culture medium was replaced and the nonadherent cells were removed on days 2, 4, and 6 (8).

The electroporation of cells was performed at 24 h after the medium replacing.

2.6. Production of plasmid DNA constructs

Artificial genes were designed employing the Gene Designer 2.0 software (DNA2.0, Inc) using the amino acid sequences of the target mouse proteins IL-10 or sTNFR1. Endonuclease restriction recognition sites *Age*I, *Bam*HI, and *Sac*I were deleted to enable subsequent cloning into the pmax vector.

Preparative amounts of each genetic construct with the verified nucleotide sequence were produced and endotoxins were removed using an EndoFree Plasmid Maxi Kit (Qiagen, Germany) in compliance with the manufacturer's recommendations. Concentration of plasmid DNA was measured spectrophotometrically using an Ultrospec 3000 pro spectrophotometer (GE Healthcare Life Sciences, USA). The structures of the purified DNA constructs were confirmed to be correct by restriction analysis with respect to the initial clones and sequencing of both DNA strands.

2.7. Cell electroporation

Mφ were detached using Trypsin/EDTA solution. To detach DCs, phosphate buffered saline (PBS) was added and the cells were carefully resuspended until they were completely detached. The cells were washed twice with PBS and diluted to a concentration of 10–20 $\times 10^6$ per ml with cold OptiMem solution.

To perform the electroporation procedure, a plasmid DNA construct was added to 100 μl of cells at a concentration of 1–100 μg per ml and placed into an electroporation cuvette. The cells were electroporated on a BTX 830 square-wave electroporation system in 2-mm cuvettes (BTX). The electroporation procedure was carried out at different pulse voltage and duration values to determine the optimal conditions. Cells electroporated without adding the DNA construct (CEP) or the cells not exposed to electroporation (Control) were used as an efficiency control and for assessing viability. Immediately after the electroporation, 900 μl of warm working solution was added to the cuvettes containing the cells and the cells were transferred to a 48-well plate for culture. Several working solutions containing the culture medium, rmIL-4 and rmGM-CSF, LPS, M-CSF and the conditioned medium in various combinations, were prepared to determine the optimal conditions for cell culture after the electroporation procedure. The conditioned medium was collected before electroporation from the same cell cultures and centrifuged (10 min 300 g) for use in working solutions.

2.8. Assessment of electroporation efficiency of the cell cultures

The electroporation efficiency and the phenotypic indicators of the DCs and Mφ were assessed on a FACSVerse flow cytometer (Becton Dickinson). Assessment of electroporation efficiency was based on identifying GFP-positive cells. Culture viability was assessed according