Biomaterials 35 (2014) 792–802

Contents lists available at ScienceDirect

Biomaterials

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

Genetic engineering of cell lines using lentiviral vectors to achieve antibody secretion following encapsulated implantation

Aurélien Lathuilière [a], Bernd Bohrmann [b], Erhard Kopetzki [b], Christoph Schweitzer [b], Helmut Jacobsen [b], Marc Moniatte [c], Patrick Aebischer [a], Bernard L. Schneider [a,*]

[a] Neurodegenerative Studies Laboratory, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland
[b] F. Hoffmann-La-Roche Ltd, Pharmaceutical Research Neuroscience, 4070 Basel, Switzerland
[c] EPFL Proteomics Core Facility, EPFL, 1015 Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 21 August 2013
Accepted 5 October 2013
Available online 23 October 2013

Keywords:
Monoclonal antibody
Genetic engineering
Lentiviral vector
Cell encapsulation technology
Macroencapsulation
Passive immunization

ABSTRACT

The controlled delivery of antibodies by immunoisolated bioimplants containing genetically engineered cells is an attractive and safe approach for chronic treatments. To reach therapeutic antibody levels there is a need to generate renewable cell lines, which can long-term survive in macroencapsulation devices while maintaining high antibody specific productivity. Here we have developed a dual lentiviral vector strategy for the genetic engineering of cell lines compatible with macroencapsulation, using separate vectors encoding IgG light and heavy chains. We show that IgG expression level can be maximized as a function of vector dose and transgene ratio. This approach allows for the generation of stable populations of IgG-expressing C2C12 mouse myoblasts, and for the subsequent isolation of clones stably secreting high IgG levels. Moreover, we demonstrate that cell transduction using this lentiviral system leads to the production of a functional glycosylated antibody by myogenic cells. Subsequent implantation of antibody-secreting cells in a high-capacity macroencapsulation device enables continuous delivery of recombinant antibodies in the mouse subcutaneous tissue, leading to substantial levels of therapeutic IgG detectable in the plasma.

1. Introduction

During the last two decades, a rapidly increasing number of monoclonal antibodies (mAbs) have been developed to selectively interfere with various therapeutic targets. Huge progress has been made to engineer more effective mAbs by improving their selectivity and stability, as well as lowering immunogenicity [1]. Monoclonal Abs are now considered as a very successful class of biological drugs, with over 30 molecules currently approved and many more under clinical investigation. Applications include autoimmune and inflammatory diseases, cancer and, more recently, neurodegenerative disorders. In the context of Alzheimer's disease (AD), monoclonal antibodies have been developed against proteotoxic entities such as the amyloid beta (Aβ) peptide and the tau protein (reviewed in Ref. [2]). For Parkinson's disease, antibodies against α-synuclein are considered as a possible therapeutic strategy (reviewed in Ref. [3]). Clinical trials are currently being conducted to test antibody candidates for Alzheimer's and Parkinson's diseases.

However, manufacturing recombinant therapeutic mAbs is an expensive process, and chronic mAb treatments necessitate repetitive high-dose bolus injections. This mode of administration is considered as a limiting factor, in particular for the long-term treatment of chronic diseases such as those affecting the central nervous system (CNS). Hence, there is a need for alternative systems for mAb delivery.

A promising approach is to secrete mAbs *in situ* via implantation of genetically engineered cells. In order to control the amount of grafted cells and halt treatment if needed, the cells can be confined inside a retrievable macroencapsulation device composed of a permeable polymer membrane. In encapsulated cell technology (ECT), the porous membrane allows for both inflow of nutrients and oxygen, as well as outflow of the therapeutic mAb and metabolic by-products. Crucially, the grafted cells are shielded from any direct contact with host immune cells. Hence, the device can support the survival of allogeneic cells by preventing immune rejection.

This approach has been extensively studied as a delivery system for bioactive molecules including erythropoietin [4,5] and neurotrophic factors [6], and more recently, GM-CSF to induce anti-tumor

* Corresponding author. Brain Mind Institute, EPFL, EPFL-SV-BMI-LEN, Station 19, 1015 Lausanne, Switzerland. Tel.: +41 216939519; fax: +41 216939520.
E-mail address: bernard.schneider@epfl.ch (B.L. Schneider).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.10.026

immunotherapy [7]. In addition, it offers the opportunity to chronically deliver biologics to poorly accessible organs, such as the eye or the CNS [8,9].

For most applications, the main limitation of ECT is to reach a therapeutic dose of the bioactive product. The level of recombinant protein delivered is determined by the cellular capacity of the device and the amount of protein secreted per cell. It is important to use renewable sources of stably modified cells applicable to a significant number of subjects. Thereby, the production of the recombinant protein can be quantitatively controlled prior to implantation in order to predict the efficacy of mAb delivery.

Here, we have devised a genetic engineering approach to generate stable cell lines secreting high levels of a recombinant mAb directed against Aβ to investigate the potential of ECT for the treatment of AD. We use lentiviral (LV) vectors for their ability to stably integrate transgenes in the genome of dividing cells. As a proof-of-principle, this system is applied to adherent cell lines, which display high proliferative capacity in culture and strong contact inhibition, and are therefore compatible with long-term survival in encapsulated devices. This dual LV gene delivery system induces high levels of mAb secretion from C2C12 myoblasts and ARPE19 epithelial cells. This system can also be used for a variety cell lines, progenitor and stem cells, thereby expanding the repertoire of cell sources applicable to ECT for the delivery of biotherapeutics including recombinant mAbs.

In particular, we apply this system to C2C12 myoblasts, and demonstrate that stably infected cells can be combined with high capacity ECD device to generate an effective platform for the production of recombinant mAbs in the subcutaneous tissue.

2. Materials and methods

2.1. Cell culture

Cell lines were obtained from American Type Culture Collection (ATCC) and maintained in regular conditions at 37 °C in 5% CO_2 in medium containing penicillin (100 U/ml) and streptomycin (100 U/ml) (Invitrogen). HeLa cells (ATCC number CCL-2), and C2C12 mouse myoblasts (ATCC number CRL 1772) derived from leg skeletal muscles of an adult C3H (H2k) mouse [10] were both grown in DMEM containing 10% FBS. The human retinal pigment epithelial cell line ARPE-19 (ATCC number CRL 2302) is derived from a 19-year-old male donor [11]. ARPE-19 cells were cultured in a mixture of DMEM and Ham's F12 medium (1:1 mixture) supplemented with 10% FBS. Human bone marrow mesenchymal stem cells (hMSC) were derived from a 34 years-old healthy male. Human MSC were cultured in alpha MEM growth medium (Invitrogen) supplemented with 10% FBS, penicillin and streptomycin and 1 ng/ml FGF-2 (Peprotech).

2.2. MAb-11 IgG antibody

MAb-11 is an anti-amyloid β monoclonal antibody, different by seven amino acids from gantenerumab [12]. It was derived from the MorphoSys HuCAL-Fab1 phage display library [13]. Two cDNAs encoding the heavy and light chains of a human-mouse chimeric version of mAb-11 (human variable domains; mouse IgG2a constant domains) were synthetized for the present study.

2.3. Plasmid construction

To test transduction efficiency, the pRRLSIN.cPPT.PGK-GFP.WPRE vector (Addgene plasmid #12252) encoding GFP under the control of the human PGK promoter was used for the production of LV particles. To generate LV vectors encoding mAb-11 antibody chains, the GFP cDNA was replaced by a fragment containing in 5′- to 3′-order a 5′-UTR from a human antibody heavy chain variable germline gene and a Kozak consensus sequence, a DNA sequence encoding a secretion signal peptide, derived from a mouse antibody heavy chain gene followed by the cDNA coding for either the mAb-11 light or heavy chain, using a flanking BamHI and SalI restriction endonuclease site for insertion in the plasmid. To generate a mAb-11 expressing plasmid for stable transfections, the expression cassettes encoding the heavy and light chains of mAb-11 were ligated into the previously described pPI-ND expression system driven by the mouse PGK-1 promoter [14].

2.4. Recombinant lentiviral vector production and titration

LV particles were produced by transient transfection of HEK293T cells with a packaging construct (pCMV-dR8.2Δvpr), a plasmid encoding for the viral envelope (pMD2.G), a post-transcriptional regulator (pRSV-Rev) and the vector itself [15]. After 48 h, culture medium was concentrated about 1000× and stored at −80 °C until further use. HIV-1 p24 antigen titer was measured using an ELISA kit (Gentaur). Viral titers (infectious particles) were determined by quantifying the number of integrated vector copies following transduction of 1^E5 HeLa cells with serial dilutions of the vector suspension (3.3–10 ng p24) in a 6-well plate. Ten days after transduction, genomic DNA was extracted from infected cells (NucleoSpin Tissue kit; Macherey-Nagel). Real-time PCR was used to determine the number of stably integrated transgene copies per cell, using a TaqMan set of primers for the WPRE and human albumin sequences, as described in Ref. [16].

2.5. Cell transduction

All transduction conditions were tested in duplicates. The day prior to transduction, 5000 cells per well was plated in a 24-well plate. Two extra wells were prepared for cell counting at the time of transduction. Virus was diluted in culture medium with 5 μg/ml protamine sulfate and added to the well to a final volume of 250 μl. One day later, 250 μl of culture medium was added to each well. Cells were passaged and maintained in culture for at least 10 days before any analysis was performed, to make sure that all non-integrated transgene copies were eliminated.

2.6. Clone sorting

Clones were isolated from a pool of cells generated by transduction with 1500 TU/cell with each of the vectors encoding light and heavy IgG chains. Clones were isolated by limiting dilution. Briefly, cells were harvested, diluted in culture medium and plated in 96-well plates at a concentration of 0.4 cells/well. Eight hours after plating, every well was observed under the microscope and wells with two or more cells were discarded. As soon as clonal populations of C2C12 myoblasts had grown to confluence, the cells were passaged and amplified as individual clones in regular culture conditions.

2.7. Flow cytometry

Ten days after transduction, cells were detached using trypsin, harvested and washed in phosphate buffer saline (PBS) (Invitrogen). GFP fluorescence was analyzed using a flow cytometer (Accuri C6; BD bioscience).

2.8. ELISA for antibody quantification

The day prior to antibody quantification, 1^E5 cells were plated in duplicate in a 6-well plate. The day after, cells were washed with HBSS (Invitrogen) before addition of 1 ml of fresh culture medium. One hour later, the supernatant was collected, kept on ice or frozen at −80 °C. Cells were detached with trypsin (Lonza) and counted using an automated cell counter (Countess, Invitrogen). For the ELISA to determine the amount of amyloid β-binding antibodies, 96-well microtiter plates were coated with 7 μg/ml amyloid β in coating buffer (500 mM Tris, 140 mM NaCl, 0.02% Bronidox L, pH 7.4) for three days at 37 °C on an orbital shaker. Plates were then decanted and dried for further use. Serial dilutions of purified mAb-11 in fresh culture medium were used as quantification standards. 100 μl of culture medium samples were loaded in duplicates. Plate was incubated for 1 h at room temperature (RT) on a shaker. After three washes with PBS/0.05% Tween 20, POD-conjugated goat anti-mouse IgG (Jackson Immunoresearch; diluted in Low Cross Buffer, Candor) was applied for 1 h at RT for antibody detection. After three washes, peroxidase substrate (ABTS solution, Roche Applied Science) was applied and colorimetric reaction was quantified by measuring absorbance at 405 nm on a plate reader. A standard curve was generated by non-linear 4-parameter fit. Antibody concentration in the culture supernatant was calculated and cell expression levels were converted into pg/cell/day.

2.9. Western blot analysis

Cells were homogenized in 50 mM HEPES buffer with 150 mM NaCl, 1% NP40 and protease inhibitor cocktail (Roche Applied Science). Western blot detection of the antibody heavy chain was performed on 20 μg of total proteins loaded in a 7.5% polyacrylamide gel under non-reducing conditions, using a Fc fragment specific goat anti-mouse IgG (1:1000; Jackson Immunoresearch). For light chain detection on 12% polyacrylamide gels, we used a light chain specific goat anti-mouse IgG (1:1000; Jackson Immunoresearch). Detection of surplus free light chains was performed on culture supernatants collected under the same conditions as for ELISA analysis.

2.10. DNA and RNA extraction and RT-qPCR

RNA was extracted with an RNAeasy Mini Kit (Qiagen). cDNA was prepared using an Omniscript Reverse Transcription Kit (Qiagen). Briefly, total RNA was reverse transcribed in a final volume of 20 μl with OligodT primers at 37 °C for 1 h according to manufacturer's instructions. The mRNA levels of mAb-11 heavy and light chains were measured by RT–qPCR using Taqman assays. Specific primers sets were designed for the variable regions in the heavy and light antibody chains to avoid cross hybridization. Beta-tubulin primers were used to measure a housekeeping gene reference, as described in Ref. [17].

Each sample was run in duplicate with the Rotor-Gene Probe PCR Kit (Qiagen) on a Rotor-Gene Cycler with the following cycling conditions: 3 min 95 °C, and 40 cycles