Biochemical and Biophysical Research Communications 441 (2013) 59–64

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A rapid screening and production method using a novel mammalian cell display to isolate human monoclonal antibodies

Kosuke Tomimatsu [a,b], Shin-ei Matsumoto [c], Hayato Tanaka [a], Makiko Yamashita [a], Hidekazu Nakanishi [d], Kiichiro Teruya [a,d], Saiko Kazuno [e], Tomoya Kinjo [d], Takeki Hamasaki [a], Ken-ichi Kusumoto [f], Shigeru Kabayama [g], Yoshinori Katakura [a,d], Sanetaka Shirahata [a,d,*]

[a] Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[b] Japan Society for the Promotion of Science, 8 Ichibancho, Chiyoda-ku, Tokyo 102-8472, Japan
[c] Department of Diagnosis, Prevention and Treatment of Dementia, Graduate School of Medicine, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
[d] Graduate School of Systems Life Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[e] Biomedical Research Center, Graduate School of Medicine, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
[f] Biotechnology and Food Research Institute Fukuoka Industrial Technology Center, 1465-5 Aikawa-cho, Kurume, Fukuoka 839-0861, Japan
[g] Nihon Trim Co., Ltd., 1-8-34 Oyodonaka, Kita-ku, Osaka 531-0076, Japan

ARTICLE INFO

Article history:
Received 23 September 2013
Available online 16 October 2013

Keywords:
Human monoclonal antibody
Mammalian cell display
In vitro maturation
CHO cells
Cre recombinase
Human high affinity IgE receptor

ABSTRACT

Antibody display methods are increasingly being used to produce human monoclonal antibodies for disease therapy. Rapid screening and isolation of specific human antibody genes are valuable for producing human monoclonal antibodies showing high specificity and affinity. In this report, we describe a novel mammalian cell display method in which whole human IgG is displayed on the cell surface of CHO cells. Cells expressing antigen-specific human monoclonal IgGs with high affinity on the cell surface after normal folding and posttranscriptional modification were screened using a cell sorter. The membrane-type IgG-expressing CHO cells were then converted to IgG-secreting cells by transfection with a plasmid coding Cre recombinase. This mammalian cell display method was applied to *in vitro* affinity maturation of monoclonal C9 IgG specific to the human high-affinity IgE receptor (FcεRIα). The CDR3 of the C9 heavy chain variable region gene was randomly mutated and inserted into pcDNA5FRT/IgG. A C9 IgG (CDRH3r)-expressing CHO cell display library consisting of 1.1×10^6 independent clones was constructed. IgG-displaying cells showing high reactivity to FcεRIα antigen were screened by the cell sorter, resulting in the establishment of a CHO cell line producing with higher reactivity than the parent C9 IgG.

1. Introduction

Expression of recombinant proteins in mammalian cells is attractive means to produce proteins with proper folding and post-transcriptional modifications suitable for analysis of their character or for therapeutic drugs. Monoclonal antibodies are major proteins produced by mammalian expression system. These are important tools in modern biological research and have great clinical potential, particularly in the analysis and treatment of human diseases.

Several display methods, including phage [1–3], bacterial [4], ribosomal and yeast [5], have been developed to screen for antigen-specific human antibody genes, and many researchers have successfully produced human monoclonal antibodies using these methods. However, because the full-length human monoclonal antibody genes were screened using non-mammalian expression systems with antigen-binding fragments of antibodies such as scFv, the original structures of the intact human antibodies are often lost. Also different codon usages and insertion of mutations by frequent PCR decrease antibody acquisition efficiency. These factors does not ensure the screened antibodies to keep the same structures and characteristics as those when screened after these antibodies are produced on mammalian expression system [6]. To overcome these problems a number of groups tried to develop mammalian cell display systems for antibody generation [7–12]. In most of these methods, antibodies were expressed on the membrane surface of mammalian cells, and antibody genes were recovered after selection and used for secretion of antibody for their characterization. It is more desirable to generate antibodies using identical cell lines for selection and secretion to strictly maintain their characteristics.

* Corresponding author at: Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Fax: +81 92 642 3052.
E-mail address: sirahata@grt.kyushu-u.ac.jp (S. Shirahata).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.007

We previously produced human monoclonal antibodies against the human high affinity IgE receptor alpha chain (FcεRIα), an immune receptor and key regulator of allergy [13], by combining phage display with *in vitro* immunization [14]. The resulting antibodies showed specific binding to FcεRIα and sufficient affinity for therapeutic use [15]. In this study, we present a novel method to construct a Chinese hamster ovary (CHO) cell library consisting of independent cell clones expressing a set of human IgG antibody genes to screen clones expressing high-affinity antibody genes. The method was applied to *in vitro* affinity maturation of the human anti-FcεRIα monoclonal antibody. This system enables efficient screening of full-length human IgG antibody genes with high affinity for antigens in a mammalian environment, to rapidly establish IgG-secreting CHO cells.

2. Materials and methods

2.1. Cell lines and cell culture

CHO cells containing the Flp Recombination Target (FRT) site (Flp-In CHO cells) were purchased from Invitrogen (Carlsbad, CA). The Flp-In CHO cell line contains a single integrated FRT site and stably expresses the lacZ-Zeocin fusion gene. Flp-In CHO cells were cultured in Ham's F12 medium (Nissui, Tokyo, Japan) containing 100 U/ml of penicillin (Meiji Seika, Tokyo, Japan), 100 μg/ml of streptomycin (Meiji Seika), 100 μg/ml of Zeocin (Invitrogen) and 10% fetal bovine serum (FBS, company) at 37 °C in a humidified atmosphere consisting of 5% CO_2 in air.

2.2. Recombinant FcεRIα

Recombinant FcεRIα protein was prepared as our previous work [14].

2.3. Plasmid construction

pcDNA5/FRT (Invitrogen) was used to construct the expression system. The coding regions of the leader peptide, variable region (VH, VL) and constant region (CH, Cκ) were derived from C9 human anti-FcεRIα antibodies (C9) in our previous study [14] by PCR with specific primer pairs (Table 1). Internal ribosome entry site (IRES) was amplified from pIRESbleo (Takara bio, Shiga, Japan) by PCR with specific primer pairs (Table 1). The DNA fragment for the transmembrane domain region was synthesized by TaKaRa bio. The fragment consisted of the transmembrane domain between the loxP sequences hidden in the intron and two restriction digestion sites (*BamH* I and *BstX* I), as shown in Supporting Fig. S1. The prepared genes were digested with the relevant restriction enzymes and the DNA fragments were ligated to pcDNA5/FRT yielding the vector pcDNA5/FRT/IgG.

Table 1
Oligonucleotide sequence

Name	Sequence
Not I-Ld	5′-ATAAGAATGCGGCCGCATGGAGACAGACACACTCCT-3′
CL-Apa I	5′-AAAGGGCCCCCTCTAAGACTCTCCCCTGTTG-3′
Nhe I-Ld	5′-AAAGCTAGCATGGAGACAGACACACTCCT-3′
IgGc-BamH I	5′-AAAGGATCCCGGAGACAGGGAGAGGCTCT-3′
BstX I-IRES	5′-CTGCAGAACCAGTGTGGTGGAATTAATTCGCTGTCTGCGA-3′
IRES-NotI	5′-ATAAGAATGCGGCCGCGGTGATCAGATCTGCAGGGC-3′
IgGc-m	5′-GTGGAGTGGGAGAGCAATGGG-3′
IRES-m	5′-CCTCCTGGTTTTTGGAAACTGAC-3′
C9VHCDR3	5′-TCCACCTGAGGAGACGGTGACCAGGGTTCCCAGGCCCCA NNSNNSNNSNNSNNSNNSNNSNNSTCTCGCACAGTAATACAGC-3′
T7-2	5′-AATACGACTCACTATAGGG-3′

2.4. Transfection of Flp-In-CHO cells

Transfection was performed by the lipofection method with cationic amphiphiles [16]. To construct the mammalian cell display library, Flp-In CHO cells were transfected with pcDNA5/FRT-IgG(D) and pOG44. After 48 h of transfection, the culture medium was replaced with Ham's F12 medium containing 10% FBS and hygromycine B for antibiotic selection. Stable clones were collected and used to produce the human monoclonal antibodies. IgG-displaying CHO cells were transfected with pCEP4/Cre coding the nuclear localizing signal and Cre recombinase to remove the transmembrane domain gene by Cre-mediated recombination.

2.5. Flow cytometry

CHO cells (2×10^5 cells) were harvested and incubated with 2 μl of phycoerythrin (PE)-conjugated mouse anti-human IgG (H) antibody (Beckman Coulter, Fullerton, CA) or 2 μl of PE-conjugated mouse anti-human Igκ antibody (Beckman Coulter) in 50 μl of PBS containing 0.5% BSA and 1 mM EDTA (PBE). After incubation for 15 min at 4 °C, the cells were washed with PBE and resuspended in 500 μl of PBE. The fluorescence of living cells was measured using a FACSAria™ Cell Sorter (BD Biosciences, Franklin Lakes, NJ).

2.6. Microscopic observation

CHO cells (2×10^5 cells) were harvested and incubated for 15 min at 4 °C with 2 μl of rabbit anti-human IgG Fc• fragment and 2 μl of mouse anti-human Ig• light chain antibodies in 100 μl of PBE. After incubation, cells were washed and resuspended in 100 μl of PBE with 2 μl of Qdot 525 goat anti-rabbit IgG and 2 μl of Qdot 605 goat anti-mouse IgG. After incubation for 15 min at 4 °C, cells were washed and resuspended in 1 ml of PBE. The co-localization of the heavy and light chain at the plasma membrane of the cell was observed by conforcal laser scanning microscopy LSM 780 (Carl Zeiss, Jena, Germany).

2.7. Confirmation of site-specific recombination

Cre/loxP recombination was confirmed by PCR. Total RNA was isolated from Flp-In-CHO cells by the Fast Pure RNA kit (Takara). cDNA was synthesized using a ReverTra Ace qPCR RT kit (TOYOBO, Osaka, Japan). PCR was performed using the synthesized cDNA or genomic DNA as templates and the specific primer pairs IgGc-m and IRES-m shown in Table 1.

2.8. Measurement of antibody production

The amount of IgG expressed in cells or secreted into the culture supernatant was determined by sandwich enzyme-linked immunosorbent assays (ELISA), as previously described [17].

2.9. ELISA to assess the characteristics of the produced antibodies

The microtiter plates were coated with 1 mg of antigen per well and incubated overnight at 4 °C. After blocking non-specific binding with 1% fish gelatin and washing with PBST, diluted human monoclonal antibodies were added to each well and incubated. After washing with PBST, HRP-conjugated anti-human IgG was added and the plate incubated. The captured IgGs were then incubated with ABTS substrate solution and absorbance was measured at 405 nm using a microplate reader.