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Construction of a recombinant full-length membrane associated IgG library

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ABSTRACT

HIV/AIDS has become a global pandemic. Development of an effective HIV-1 vaccine eliciting broadly neutralizing monoclonal antibodies (bnmAbs) remains a big challenge. Before an effective vaccine comes out, passive treatment for prevention and protection of HIV-1 infection may alleviate the burden caused by the pandemic. Numerous bnmAbs have been isolated against different epitopes in HIV-1 envelope glycoprotein via phage/yeast display, EBV-immortalization, single cell sorting and micro neutralization. Recombinant antibody library with extended diversity and enlarged size of units are applicable by phage/yeast display and mammalian cell display for monoclonal antibody isolation. Here, we constructed an immune recombinant membrane associated full length IgGs library based on mammalian cell display system. This library can be used for monoclonal antibody screening/isolation by target cell sorting. A full length antibody mz2F11 was screened with potent neutralizing activities against a panel of viruses tested. Our study provides a novel way of antibody library construction and monoclonal antibody screening. Antibodies screened via this method can help broaden the knowledge in passive treatment and prevention against HIV-1 infection.

1. Introduction

In recent decades, numerous monoclonal antibodies have been isolated and 7studied against HIV-1 by display technique, single cell sorting/cloning, and micro-neutralization (Chuang et al., 2013; Doria-Rose et al., 2017; Liao et al., 2013; Mascola, 2009; Rudicell et al., 2014). Single cell sorting and micro-neutralization are usually labor cost. In comparison, display technique has simple screening/panning procedure, extended size/diversity of antibody repertoires in B cells, and allows display more flexible forms of antibodies including single chain variable fragments (ScFv), antigen-binding fragments (Fab), full length IgGs, and single domain antibodies including nanobodies (Li et al., 2017; Li et al., 2016; Zhu et al., 2011; Zhou et al., 2007).

One most important procedure for the application of display technique is library construction. The quality and format of antibody library affect the results of panning and screening. Thus a library with good quality and stable format will increase the screening efficiency and minimize the work load (Koefoed et al., 2005; Graus et al., 1998; Parren and Burton, 1997; Hexham et al., 1994). In this manuscript, we constructed an immune recombinant membrane-associated full length IgGs library from a patient's memory B cells based on mammalian cell display system. First, according to previous experience, library derived from memory B cells rather than PBMC can better mimic the serum, which means that the efficiency of library based on memory B cells is higher (Sun et al., 2015). Second, antibody of IgG format is more stable than other formats (Mersich et al., 2007; Chukwuocha et al., 1999). Third, mammalian cell display system offers the environment that all of the cellular components will be involved in process of antibody synthesis including the post-modification and folding (Forsyth et al., 2013; Bowers et al., 2011; Zhou et al., 2010; Beerli et al., 2008). Forth, membrane associate antibodies can be located on the surface of mammalian cells, which will facilitate the screening and enrichment of functional antibody repertoires by cell sorting against different fishing immunogens (Wu et al., 2011; Wu et al., 2010; Sun et al., 2017).

In our study, we constructed an immune library with the size of 10^6 individuals. This library allows sorting of antibody repertoires based on antibody neutralization activity by displaying immunized antibody libraries on target cell surface followed by sorting the cells based on the

Abbreviations: PBMCs, peripheral blood mononuclear cells; bnAbs, broadly neutralizing antibodies; bnmAbs, broadly neutralizing HIV-1 human monoclonal antibodies; FBS, fetal bovine serum

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antibody neutralization activity. After several rounds of sorting, a full length IgG mz2F11 was isolated with potency in neutralizing a panel of pseudo-viruses tested in this manuscript. Our study provides a new idea for antibody library construction and isolation of HIV-1 monoclonal antibodies. The monoclonal antibodies isolated have potential applications in HIV-1 therapeutics.

2. Materials and methods

2.1. Cells and viruses

TZM-bl cell line (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institute of Health) was grown and maintained in DMEM (Dulbecco's Modified Eagle Medium produced by Life Technologies ®) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. FBS was inactivated by heating at 56 °C for an hour before usage. Cells were cultured at 37 °C incubator with 5% CO_2. FreeStyle™ 293F cell line (Invitrogen™) was cultured in 293 Free-style medium for large scale production of antibodies. 293T cell line (ATCC) was maintained in DMEM (Dulbecco's Modified Eagle Medium produced by Life Technologies ®) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. HIV-1 clade B and C isolates were obtained from NIH ARRP, GXC44 from Dr. Gerald Quinian (USUHS, Bethesda, MD). CH set HIV-1 clade B and C isolates were obtained from China Centers for Disease Control and Prevention, Dr. Yiming Shao. Viruses were packaged in P3 lab with backbone and envelope by transient transfection of 293T cells. Pseudotyped viruses were harvested after 48 h post transfection and titrated before use. IgGs and recombinant Envs were produced in the laboratory using 293F transient transfection system (Invitrogen, Carlsbad, CA) and Protein A/G affinity purification. Clade B' chronically infected and treat-naïve patient samples, 1–5, was obtained from National Center for AIDS/STD Control and Prevention, China CDC (Beijing, China). Heparinized whole blood samples were used to isolate human PBMCs by Ficoll density gradient separation and IgGs by Protein A affinity purification. All these experiments were approved by ethical committees of the respective institutes, and conducted according to local guidelines and regulations. BaL gp120 site mutants, D368R, and G459E, were generated in our laboratory by using site-directed mutagenesis kit (Stratagene). RSC3 and ΔRSC3 expressing plasmids were gifts from Peter Kwong and John Mascola at VRC, NIAID, NIH. Recombinant wild type (WT) Envs and Env mutants, as well as HIV-1 mAbs, including CD4 binding site (CD4bs) mAbs IgG1s b12 and VRC01 and CD4 induced (CD4i) mAb IgG1 × 5, were produced in our laboratory using 293F transient transfection system (Invitrogen) and Protein A affinity purification.

2.2. Memory B cells isolation

PBMCs were derived from one top patient neutralizer, patient 1–5 was provided by Chinese Center for Disease Control and Prevention. Memory B cells were sorted from PBMC of Patient 1–5 by using BD FACS Aria III sorter as described previously (Sun et al., 2015). PBMCs were stained with FITC-anti human CD19 antibody and PE-anti human CD27. Compensation was calculated by standard beads before sorting. About 1% memory B cells were sorted from 1×10^7 PBMCs.

2.3. Total RNA isolation and first strand cDNA synthesis

Total RNA and mRNA were prepared using the total RNA and mRNA isolation kits (QIAGEN) from memory B cells sorted. First-strand cDNAs were synthesized using oligo dT (Invitrogen) as the primer according to the instructions from the manufacturer. RNA was eluted by DEPC treated water. Using RNA eluted as template, first strand cDNA was synthesized. In the first step, up to 5ug RNA was mixed with oligo $(dT)_{20}$ and 10 mM dNTPs, and then incubated at 65 °C for 5 min. In the second step, prepare the following cDNA synthesis mix: 10 × RT buffer, 2 μl; 25 mM $MgCl_2$, 4 μl; 0.1 M DTT, 2 ul; R Nase OUT (40 U/μl), 1 μl, and SuperScript III RT (200 U/μl), 1 μl. Add 10 μl of cDNA synthesis mix to each RNA/primer mixture. Gently mixed and collect mixture by brief centrifugation. Incubate at 50 °C for 50 min. If random hexamer primer was used, incubate 10 min at 25 °C first, and then followed by 50 min at 50 °C. After incubation, the reactions were terminated at 85 °C for 5 min. Then samples were chilled on ice. The first strand synthesized cDNA was stored at −80 °C.

Table 1
Primers for library construction.

VH forward primers	
scVHlB7AF	5′-CAGGTGTCCACTCCCAGRTGCAGCTGGTGCARTCTGG-3′
scVHlCF	5′-CAGGTGTCCACTCCSAGGTCCAGCTGGTRCAGTCTGG-3′
scVH2bF	5′-CAGGTGTCCACTCCCAGRTCACCTTGAAGGAGTCTGG-3′
scVH3bF	5′-CAGGTGTCCACTCCSAGGTGCAGCTGGTGGAGTCTGG-3′
scVH3cF	5′-CAGGTGTCCACTCCGAGGTGCAGCTGGTGGAGBCYGG-3′
scVH4bF	5′-CAGGTGTCCACTCCCAGGTGCAGCTACAGCAGTGGGG-3′
scVH4CF	5′-CAGGTGTCCACTCCCAGSTGCAGCTGCAGGAGTCSGG-3′
scVH5bF	5′-CAGGTGTCCACTCCGARGTGCAGCTGGTGCAGTCTGG-3′
scVH6aF	5′-CAGGTGTCCACTCCCAGGTACAGCTGCAGCAGTCAGG-3′
VL forward primers	
spVk1bF	5′-CAGATGCCAGATGTGACATCCAGWTGACCCAGTCTCC-3′
spVk2F	5′-CAGATGCCAGATGTGATGTTGTGATGACTCAGTCTCC-3′
spVk3bF	5′-CAGATGCCAGATGTGAAATTGTGWTGACRCAGTCTCC-3′
spVk4bF	5′-CAGATGCCAGATGTGATATTGTGATGACCCACACTCC-3′
spVk5F	5′-CAGATGCCAGATGTGAAACGACACTCACGCAGTCTCC-3′
spVk6F	5′-CAGATGCCAGATGTGAAATTGTGCTGACTCAGTCTCC-3′
spVllAF	5′-CAGATGCCAGATGTCAGTCTGTGCTGACTCAGCCACC-3′
spVl1bF	5′-CAGATGCCAGATGTCAGTCTGTGYTGACGCAGCCGCC-3′
spVllcF	5′-CAGATGCCAGATGTCAGTCTGTCGTGACGCAGCCGCC-3′
spVl2F	5′-CAGATGCCAGATGTCARTCTGCCCTGACTCAGCCT-3′
spVl3aF	5′-CAGATGCCAGATGTTCCTATGWGCTGACTCAGCCACC-3′
spVl3bF	5′-CAGATGCCAGATGTTCTTCTGAGCTGACTCAGGACCC-3′
spVl4F	5′-CAGATGCCAGATGTCACGTTATACTGACTCAACCGCC-3′
spVl5F	5′-CAGATGCCAGATGTCAGGCTGTGCTGACTCAGCCGTC-3′
spVl6F	5′-CAGATGCCAGATGTAATTTTATGCTGACTCAGcccCA-3′
spVl78F	5′-CAGATGCCAGATGTCAGRCTGTGGTGACYCAGGAGCC-3′
spVl9F	5′-CAGATGCCAGATGTCWGCCTGTGCTGACTCAGCCMCC-3′
Reverse primers	
CH3R	5′- GTCGCACTCA TTT ACC CRG AGA CAG GGA gag gct ctt −3′
CkappaFOR	5′- GATCAAT GAATTC TAACACTCTCCCCTGTTGAAG −3′
ClamdaFOR	5′- GATCAAT GAATTC TATGAACATTCTGTAGGGGCCAC −3′

2.4. IgG VL/VH gene repertoires amplification

VH/VL gene repertoires were amplified from synthesized cDNA, a panel of primer pairs were designed according to V-base (human antibody sequence database, http://vbase.mrc-cpe.cam.ac.uk/) and strategies used by Scheid et al. Table 1 shows the synthesized primer pairs designed. In brief, cDNA was synthesized from the total RNA and antibody heavy chain variable regions (VHs) and light chains (LCs) fragments were amplified by PCR using the cDNAs as the template with the following PCR cycles: 94 °C for 5 min, followed by 10 cycles of 95 °C for 15 s, 45 °C for 30 s and 72 °C for 45 s, and 20 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 45 s, and final extension at 72 °C for 10 min. Amplified DNA was loaded on the DNA gel, and was cut for gel purification. The same amount DNA of each sub-family was taken and mixed together for digestion. VHs and LCs were digested with SacI/XbaI and HindIII/EcoRI respectively, and ligated to a mammalian expression vector PTM. Ligation mixtures were desalted before eletroporatation into TG1 electroporation competent cells.

2.5. Recombinant full length antibody library construction

After all gene fragments of VHs and Vls were amplified, a full length IgG expressing vector was undertaken to construct the full length IgG libraries based on PTM vector. Products were purified and transformed