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Development of the dual-vector system-III (DVS-III), which facilitates affinity maturation of a Fab antibody *via* light chain shuffling

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ABSTRACT

Light (L) chain shuffling is routinely used to analyze optimal L chains that pair with a specific heavy (Fd or H) chain, which ultimately leads to *in vitro* affinity maturation of a particular antibody. One of the major drawbacks to this procedure is that L chain libraries have to be created for each distinct H chain, which involves complicated cloning procedures. Herein, we designed of the dual-vector system-III (DVS-III), which is composed of a set of pLf1T-3 phagemid and pHg3A-3 plasmid, for L chain shuffling of any given human Fab antibody *via* phage display technology. To demonstrate the feasibility of our system, a human naïve L chain sublibrary, HuNL-D3, constructed in pLf1T-3 phagemid, was combined with the Fd of a human anti-IL-15 Fab, 4H10, subcloned in pHg3A-3 plasmid as a model system. After solution-phase sorting and biopanning the library we obtained eight Fab variants (4H10-LP1–7 and 4H10-LS). Among them, 4H10-LP4 exhibited the highest affinity which is about 36-fold higher than that of the parent molecule 4H10 (K_D = 6 nM versus 200 nM). Our results demonstrate that the DVS-III, along with the HuNL-D3 L chain sublibrary, can be served as a convenient approach for affinity maturation of any given human Fab antibody through L chain optimization.

1. Introduction

For developing therapeutic antibodies, the importance of phage display technology is frequently emphasized because of its capability to obtain fully human antibodies [1], which is supported by the fact that more than a dozen human antibodies created by this technology are currently in clinical trials for a wide range of human diseases including cancer, autoimmune disorders, graft rejection, and infectious diseases [2,3]. In particular, utilization of a human naïve immunoglobulin (Ig) gene repertoire would be advantageous for obtaining therapeutic antibody candidates because one may assume that they are less immunogenic than those obtained from synthetic Ig sources. Unfortunately, however, it seems rather difficult to isolate high affinity antibodies at nanomolar ranges from a naïve Ig repertoire [4], and it is generally acknowledged that lengthy antibody gene manipulations, including site-directed (non-stochastic) or random mutations (stochastic) in the original H and/or L chain antibody genes [5,6,7], are usually followed to improve the relative affinities of antibodies of naïve origin [8]. L chain optimization is an attractive and convenient alternative for improving antibody affinities because it has been known that L chain gene arrangement in peripheral B cells contributes the affinity maturation of antibodies [9], and even a single amino acid modification of the L chain can result in a 5-fold increase of binding affinity for the anti-Gal/GalNAc lectin Fab fragment [10].

We had previously reported the dual-vector system-II (DVS-II) composed of a set of the pHf1g3A-2 phagemid and the pLT-2 plasmid that express Fd-pIII fusions and L chains, respectively [11], and demonstrated that a large combinatorial antibody library can be generated with much less effort using the DVS-II [12]. The recombinant phage generated through the DVS-II display functional Fab molecules but carry H chain genotypes, which enables us to capture an H chain variable repertoire specific for target antigens. In this study, we created the dual-vector system-III (DVS-III) to capture specific naïve L chain variable repertoires and examined its feasibility in affinity maturation of a human anti-IL-15 Fab, 4H10, as a model antibody through L chain shuffling.

Abbreviations: DVS, dual-vector system; PBS, phosphate-buffered saline; IL-15, interleukin-15; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; pAb, polyclonal antibody; Fd or H, $V_H + C_{H1}$; L, $V_L + C_{Lk}$; Ig, immunoglobulin; PDC-E2, pyruvate dehydrogenase complex-E2.

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2. Materials and methods

2.1. Molecular cloning procedure

All DNA cloning experiments were carried out according to standard procedures [13]. PCR sequencing-grade oligonucleotides were synthesized from Bioneer Co., South Korea. *Pfu* or Ex-*Taq* DNA polymerase and all enzymes used in the gene cloning procedures were purchased from Takara, Japan.

2.2. Construction of the recombinant vectors for the DVS-III

A pHg3A-3 plasmid was constructed from the pHf1g3A-2 phagemid [11]. The pHf1g3A-2 vector was treated with *Not* I and *Sal* I, and 5.0 kb of the vector moiety was purified from a 1% agarose gel using a Wizard DNA Clean-Up kit (Promega, USA). The resulting DNA was ligated with the adaptor molecule that was created by annealing two oligonucleotides (5′-GGCCGCGCTAGCCCGCGGG-3′ and 5′-CGCGATCGGGCGCCCAGCT-3′) using T4 DNA ligase. Following phenol/chloroform extraction and ethanol precipitation, the ligated DNA product was electroporated into *E. coli* TG1 cells using Gene Pulser (Biorad, USA) at 2.5 kV, 25 μF and 200 Ω. The transformed cells were plated onto 2× YT agar plates containing 50 μg/ml ampicillin (2× YT/A plates) overnight at 37 °C. To generate the pLf1T-3 phagemid, the pHf1g3T-1 phagemid vector [11] was treated with *Pst* I and *Not* I, and 4.0 kb of the vector moiety was purified through 1% agarose gel as described above. The gene segment containing P_{lac} and the L chain gene ($V_L + C_{Lk}$) of the SP112 Fab in the pLT-2 plasmid [8] was obtained by PCR using a set of PCR primers (5′-GGGATCGATTCAATTGTCTGATTCGTTACCAA-3′ and 5′-GGGACTAGTTCAGTGGAACGAAAACTCACG-3′) (94 °C 1 min, 55 °C 1 min, 72 °C 1 min, 35 cycles), followed by digestion with *Pst* I and *Not* I. Two DNA fragments were ligated and electroporated into TG1 electro-competent cells as described above. The resulting transformed cells were selected using 2× YT plates containing 10 μg/ml tetracycline (2× YT/T plates) overnight at 37 °C.

2.3. Electroporation of E. coli cells with the recombinant vectors of the DVS-III

Fresh, electro-competent TG1 cells were transformed with 100 ng of the pHg3A-3 plasmid and spread onto 2× YT/T plates for antibiotic selection overnight at 37 °C. *E. coli* colonies were isolated from the plates, and the TG1 cells containing the pHg3A-3 were grown in 2× YT/T media supplemented with 2% glucose until $OD_{600\,nm} = 0.5$. The cells were then washed three times with sterile dH_2O containing 10% glycerol to render the cells electro-competent. Thereafter, the TG1 cells were transformed with 100 ng of pLf1T-3 phagemid, followed by antibiotic selection using 2× YT agar containing 100 μg/ml of ampicillin and 10 μg/ml tetracycline (2× YT/AT plates) overnight at 37 °C. Recombinant phage particles were prepared by phage rescue using Ex12 helper phage, and a phage ELISA was carried out as previously described [11].

2.4. Construction of a human naïve kappa L chain sublibrary

For preparation of the V_L repertoire, two discrete gene fragments spanning from the framework 1–3 regions and from framework 3 to C_{Lk} regions were amplified from the 10^6 human naïve kappa L chain backbones (IG Therapy, South Korea) by PCR using specific primers (VLFw1-1–4 reverse and VLFw3 forward, and VLFw3 reverse and C_{Lk} forward primers, respectively (IG Therapy, unpublished), with a PCR program of 95 °C 1 min, 60 °C 1 min, and 72 °C 1 min for 22 cycles. The resulting 300 and 400 bp PCR products were purified using 1% agarose gel electrophoresis. Assembly PCR was then carried out using the VFw1-1–4 reverse and C_{Lk} forward primers to obtain human naïve kappa L chain gene repertoires. For constructing a L chain sublibrary, the 700 bp L chain gene fragments prepared above were digested with *Sac* I and *Not* I and then ligated with pLf1T-3 phagemid vector, followed by transformation into *E. coli* TG1 electro-competent cells. The resulting *E. coli* transformants were plated onto 2× YT/T plates, followed by overnight incubation at 37 °C.

2.5. Creation of a phage display combinatorial Fab antibody library

The pHf1g3A-2 phagemid containing the V_H gene, termed IL-15VH1, of a human anti-IL-15 Fab, termed 4H10, was treated with *Sfi* I, and the resulting 350 bp V_H gene fragment was cloned into a pHg3A-3 plasmid vector using T4 DNA ligase, followed by electroporation into *E. coli* TG1 cells. The electro-competent TG1 cells containing the pHg3A-3 plasmid with IL-15VH1 were prepared and transformed with 10 μg of the pLf1T-3 phagemid containing the human naïve light chain repertoire as described above. Selection was performed by growing transformed cells in 20 ml of 2× YT/ACG medium containing 10 μg/ml tetracycline (2× YT/ACGT) for 8 h at 37 °C. Recombinant phage was then prepared by phage rescue using Ex12 helper phage (IG Therapy) as described [14], except for using 2× YT/AT medium supplemented with 70 μg/ml of kanamycin and 0.001% arabinose (w/v) (2× YT/ATKA) [15]. The recombinant phage particles were precipitated by PEG/NaCl solution and resuspended in 1 ml of sterile phosphate-buffered saline (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4, pH 7.3) (PBS) before use.

2.6. Solution-phase sorting and biopanning procedures

A fusion protein containing recombinant human IL-15 fused with maltose-binding protein (MBP-IL-15) was constructed, purified and biotinylated at IG Therapy Co. (unpublished). A solution-phase sorting method was used for the affinity-based selection as described [15], with slight modifications. The first round of solution sorting employed 1 μM biotinylated MBP-IL-15 that was incubated with ~10^9 phage and added into wells coated with 10 μg/ml of streptavidin (Sigma–Aldrich) for 10 min. After washing and elution, fresh *E. coli* TG1 cells were infected with the eluted phage, plated on 2× YT/T plates and grown overnight at 27 °C. The pLf1T-3 phagemid DNA was then purified from the cells and used to transform *E. coli* TG1 cells containing the recombinant pHg3A-3 plasmid with IL-15VH1. The resulting *E. coli* transformants were plated onto 2× YT/AT plates, followed by overnight incubation at 27 °C, and used for phage rescue or identifying *E. coli* clones producing anti-IL-15 Fabs by ELISA. For the second round of selection, 10 nM biotinylated MBP-IL-15 was mixed with phage followed by incubation with 1 μM non-biotinylated MBP-IL-15 at 37 °C for 30 min. Biopanning was carried out using MBP-IL-15 (10 μg/ml) immobilized on Maxisorp ELISA plates (Nunc); 10^{10} phage were used for phage input, and a more extensive wash was applied to remove negative phage [12]. Panning was repeated twice.

2.7. Enzyme-linked immunosorbent assay (ELISA)

To identify *E. coli* clones producing IL-15-specific Fab-pIII fusions, *E. coli* TG1 colonies obtained after the second round of panning were grown in 100 μl of 2× YT/ACTG medium at 27 °C using sterile 96-well plates (Nunc, Denmark) until an $OD_{600\,nm}$ reading reached approximately 0.5. The bacterial cell culture was then centrifuged at 3300 × *g* for 10 min, and the cell pellet was resuspended with 100 μl of fresh 2× YT/ACT medium supplemented with 0.02% arabinose and 0.1 mM IPTG. After overnight incubation at 27 °C, 50 μl of the culture supernatants was added into each well of MaxiSorb ELISA plates (Nunc) coated with 10 μg/ml of MBP-IL-15 or