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A therapeutic human antibody against the domain 4 of the *Bacillus anthracis* protective antigen shows protective efficacy in a mouse model

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A B S T R A C T

Since *Bacillus anthracis* is a high-risk pathogen and a potential tool for bioterrorism, numerous therapeutic methods including passive immunization have been actively developed. Using a human monoclonal antibody phage display library, we screened new therapeutic antibodies for anthrax infection against protective antigen (PA) of *B. anthracis*. Among 5 selected clones of antibodies based on enzyme-linked immunosorbent assay (ELISA) results, 7B1 showed neutralizing activity to anthrax lethal toxin (LT) by inhibiting binding of the domain 4 of PA (PD4) to its cellular receptors. Through light chain shuffling process, we improved the productivity of 7B1 up to 25 folds. The light chain shuffled 7B1 antibody showed protective activity against LT both *in vitro* and *in vivo*. Furthermore, the antibody also conferred protection of mice from $3 \times LD_{50}$ challenges of fully virulent anthrax spores. Our result expands the possibility of developing a new therapeutic antibody for anthrax cure.

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1. Introduction

Bacillus anthracis, a Gram-positive, rod-shaped, encapsulated and spore-forming bacterium is the causative agent of anthrax. Due to the ability of spores to persist in the harsh environment and be aerosolized, *B. anthracis* has been designated as one of the most critical bioterrorism agents [1,2] and classified as one of the Tier 1 Select Agents and Toxins by the Centers for Disease Control and Prevention (CDC). Antibiotics and vaccines have been suggested as countermeasures for anthrax, but both have limitations. Antibiotics have no effect on clearing anthrax toxin secreted by vegetative cells into the bloodstream, and vaccination requires time and booster doses to become effective and maintain immunity [3]. To this end, passive immunization of neutralizing antibodies was suggested as an alternative treatment, and therapeutic antibody research has been actively carried out for the last decade [3,4]. Raxibacumab (Abthrax) is the first therapeutic human monoclonal antibody approved by Food and Drug Administration (FDA) for anthrax treatment [5]. This therapeutic antibody was derived from a phage display library against protective antigen (PA) of *B. anthracis* [6]. Obiltoxaximab (Anthim), which also got approved by FDA recently, is a humanized mouse monoclonal antibody against PA [7,8].

An anthrax toxin consists of 3 proteins; PA, lethal factor (LF) and edema factor (EF). PA is a central component of anthrax toxin and its binding to cellular receptor allows entrance of LF and/or EF into the host cell [9]. LF is a zinc dependent metalloprotease and inhibits the mitogen-activated protein kinase signaling by cleavage of components of the signal pathway, which results in excessive release of cytokine and apoptosis [10]. EF is a highly active adenylyl cyclase. High concentration of c-AMP increase water efflux from cells and causes massive edema in cutaneous anthrax [11]. LF and EF competitively bind to PA forming lethal toxin (LT) and edema toxin (ET), respectively [12].

A crystal structure study reported that PA consists of 4 domains [13]. Domain 1 (PD1) contains protease cleavage site for LF or EF binding [13]. Domain 2 (PD2) and domain 3 (PD3) are known to be involved in oligomerization of PA [13,14]. Domain 4 (PD4) is the host cell receptor binding site [13], which is the most popular target

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for neutralizing antibodies by inhibiting the interaction between toxins and receptors [15–17].

Here, we report that a human monoclonal antibody (hMAb) 7B1 selected from the phage display library against *B. anthracis* PA binds the PD4 and inhibits PA binding to its cellular receptors. The productivity of the hMAb 7B1 was improved by light chain shuffling method and the resulting hMAb 7B1_3B4 showed protection against LT and spore challenges in mice. Our results expand potential candidates of therapeutic hMAbs for anthrax patients.

2. Materials and methods

2.1. Bacterial strains, culture conditions and reagents

B. anthracis Sterne strain was grown in a brain-heart infusion (BHI) medium (Difco) [18]. PA protein purified as previously described [19] was provided from Green Cross Co. (Korea). *B. anthracis* LF was purchased from List Biological Laboratories.

2.2. Cell lines and culture conditions

J774A.1, a mouse macrophage-like cell line, was purchased from American Type Culture Collection (ATCC, USA). The composition of media and the culture conditions of the cell were determined as previously described [20].

2.3. Screening, light-chain shuffling, and binding of antibodies

The Ymax-ABL Library (Y-Biologics, Korea) was used for phage-scFv (single-chain variable fragment) biopanning against PA (Green Cross Co, Korea). The library was subjected to 3 rounds of panning process, and binding specificities were identified by enzyme-linked immunosorbent assay (ELISA) for each round of selection as previously described [21]. The selected monoclonal phage-scFv clones were converted to human IgG genes using N293F vector (Y-Biologics, Korea), and expressed in HEK293F cells to generate full-length human IgG. Recombinant antibodies were purified by protein A affinity chromatography. Concentrations of purified antibodies were measured by absorbance at 280 nm (NanoDrop, Thermo Fisher Scientific).

Light chain (LC) shuffling was performed to improve the affinity and productivity of 7B1. DNA fragments from another phage-scFv library generated against a human cytosolic protein (Y-Biologics, Korea) were cleaved with BstXI and ligated into the 7B1 LC DNA backbone which was cleaved with the same restriction enzyme to generate LC shuffling library of 7B1. Monoclonal phage-scFv clones of high affinity for PA were selected through 3-rounds of biopanning process, and converted into human IgG form as described above.

The binding affinity of antibodies to PA were analyzed by Bio-Layer interferometry (Octet QK[e], ForteBio Inc.).

2.4. Determination of antibody specificity on PA domains

PD1 was purchased from List Biological Laboratories. PD3 and PD4 were separately expressed and purified as previously described [22]. Specificity of antibodies to each PA domain were determined by ELISA.

2.5. Toxin neutralization assay (TNA)

To evaluate protective effect of PA-specific hMAbs against LT *in vitro*, LT-mediated cytotoxicity experiments with J774A.1 cells were performed with the addition of hMAbs. Cell viability was assessed as previously described [23]. J774A.1 cells (1.5×10^5 cells per well) in 96-well flat-bottom tissue culture plates (SPL Plastic Labware, Korea) were incubated for 2 h at 37 °C in Dulbecco's modified Eagle's medium (DMEM, GIBCO Life Technologies, Germany) containing 10% fetal bovine serum (FBS, GIBCO Life Technologies, Germany). LT (0.8 μg/ml of PA and 0.1 μg/ml of LF) was pre-incubated with 2-fold serially diluted hMAb for 1 h at 37 °C. The LT-hMAb mixture was added to the cell culture medium and incubated for additional 4 h at 37 °C under 5% CO_2. After incubation, 50 μl of 3-[4,5-dimethylthylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT, Sigma-Aldrich) was added to each well in a final concentration of 0.5 mg/ml. After 1 h at 37 °C, the cells were lysed in an extraction buffer as previously described [19]. Absorbance of each well was measured at 570–690 nm. The neutralizing effective doses for 50% of the population (ED_{50}) were calculated by 4-parameter logistic equation regression using SoftMax Pro 5.3 (Molecular Device, USA).

2.6. Inhibition of PA binding to cells by anti-PA hMAbs

Anti-PA hMAbs (5 μg/ml) were incubated for 1 h at 37 °C with 1 μg/ml of fluorescein isothiocyanate (FITC)-conjugated PA_{63} (C-terminal fragment of PA, 63 kDa, List Biological Laboratories, CA, USA) in serum-free DMEM medium. After incubation, this mixture was added to J774A.1 cells (1×10^6 cells/ml/well) in 12-well plates and incubated following 30 min at 37 °C. Cells were washed with chilled PBS containing 2% FBS to remove unbound PA_{63}-FITC or hMAbs. Detached cells were resuspended in PBS containing 2% FBS. The fluorescence level of PA_{63}-FITC binding cells were determined using FACSverse flow cytometer (BD Biosciences, San Diego, CA,

Table 1
Characteristics of hMAbs.

mAb	IgG subclass	V_H[a]	D_H[b]	J_H[c]	V_L[d]	J_L[e]	CDR3[f]		Productivity (mg/L)[g]	Domain specificity[h]
							V_H	V_L		
7A3	IgG1	VH3-9	D3-10	JH6	L5	JK4	ARYFWLGDRAMDV	QQTDSFPLT	89.0	Whole PA/D3
7B1	IgG1	VH3-73	D6-19	JH3	V1-4	JL1	ARYGGLYDRAFDV	SSYSSSTFYV	1.8	Whole PA/D4
15A10	IgG1	VH3-20	D3-10	JH3	A27	JK4	AREIGGAFDI	QHYGGPLRVT	93.25	Whole PA/D4
15B3	IgG1	VH3-11	D3-3	JH3	L5	JK3	ARLTLGRGLV	EQAHSFPLT	104.47	Whole PA/D4
15D8	IgG1	VH3-73	D3-10	JH4	V1-4	JL1	ARGQSTIMEY	SSYSSSTFYV	168.25	Whole PA/D4

[a] H-chain V gene usage.
[b] H-chain D gene usage.
[c] H-chain J gene usage.
[d] L-chain V gene usage.
[e] L-chain J gene usage.
[f] Amino acid sequence of the H-chain and L-chain third complementarity-determining region.
[g] Final amount of recombinant antibodies per cell culture medium. Protein concentration was measured by absorbance at 280 nm.
[h] PA domain specificity of hMAbs based on subunit ELISA.

USA). The flowcytometry data were analyzed using Flow Jo software (Tree Star, San Carlos, CA, USA).

2.7. Mouse challenge

Six-week-old female BALB/c mice (Central Lab. Animal Inc.) were given tail vein injections (intravenous; *i.v.*) of hMAb (40 μg) with LT (40 μg PA and 20 μg LF). Six-week-old female A/J mice (Central Lab. Animal Inc.) with passively transferred hMAbs were challenged with of 3 × LD_{50} of *B. anthracis* Sterne spore by subcutaneous (*s.c.*) injections. Survival rates of mice were traced for 14 days. *B. anthracis* Sterne spores were prepared according to Ivins et al. [24]. The LD_{50}, determined in this study according to Reed-Muench method [25] in A/J mice model by subcutaneous route, was 1795 spores.

Animal study protocol (KCDC-052-17-2A) was approved by the Institutional Animal Care and Use Committee (IACUC) of the Korea Centers for Disease Control and Prevention (KCDC). The procedures involved in housing and care of mice satisfied all guidelines and requirements of the IACUC of the KCDC. Animals were housed in pathogen free facilities and challenged at the BL3 facility of the KCDC.

3. Results and discussion

3.1. Screening of scFv-phage library and characterization of the selected antibodies

Three rounds of panning process were performed against purified PA as an antigen, and 5 clones (7A3, 7B1, 15A10, 15B3, and 15D8) were selected based on binding to PA using ELISA on PA-coated plates. The selected 5 phage clones were converted from scFv to human IgG form (Y-biologic, Korea). Molecular features of the 5 hMAbs are identified in Table 1. Sequences of selected IgG clones were analyzed by IGBLAST (http://www.ncbi.nlm.nih.gov/igblast). All hMAbs belong to IgG1 subclass and ELISA assay showed that all the 5 antibodies specifically recognized PA protein. PA domain specificity was determined by ELISA with recombinant PD1, PD3, and PD4. 7A3 bound specifically for PD3, and all other 4 antibodies (7B1, 15A10, 15B3, and 15D8) bound to PD4.

3.2. In vitro neutralization potency of PA-specific hMAbs against LT

To evaluate the toxin-neutralizing effect of the selected PA-specific hMAbs on LT-mediated cytotoxic activity, LT pre-incubated with hMAbs were treated to J774A.1 cells. Cell viability was assessed using MTT assay after 4-h treatment. Among the 5 clones, 7B1 conferred concentration-dependent toxin neutralizing ability. 7A3 showed 23–25% of toxin neutralizing ability at low concentrations (0.195 and 0.390 μg/ml), while the ability was reduced at high concentrations (Fig. 1A). All the other antibodies did not show significant neutralization efficacy at up to 100 μg/ml (Fig. 1A).

Next, to determine whether the inhibition of LT-mediated cytotoxicity by 7B1 and 7A3 are due to inhibition of PA binding to its cellular receptors, FITC–conjugated C-terminal fragment of PA (PA_{63}-FITC) was pre-incubated with each antibody before determining cell binding by flow cytometry (Fig. 1B). Both 7B1 and 7A3 hMAb decreased the binding activity of PA to J774A.1 cells (Fig. 1C). 7A3 exhibited 60% of inhibitory effect on PA-binding, despite its domain specificity has been addressed to PD3 in ELISA with recombinant proteins. As PD3 is previously known to be involved in oligomerization of PA [13,14], we also tested whether 7A3 inhibits

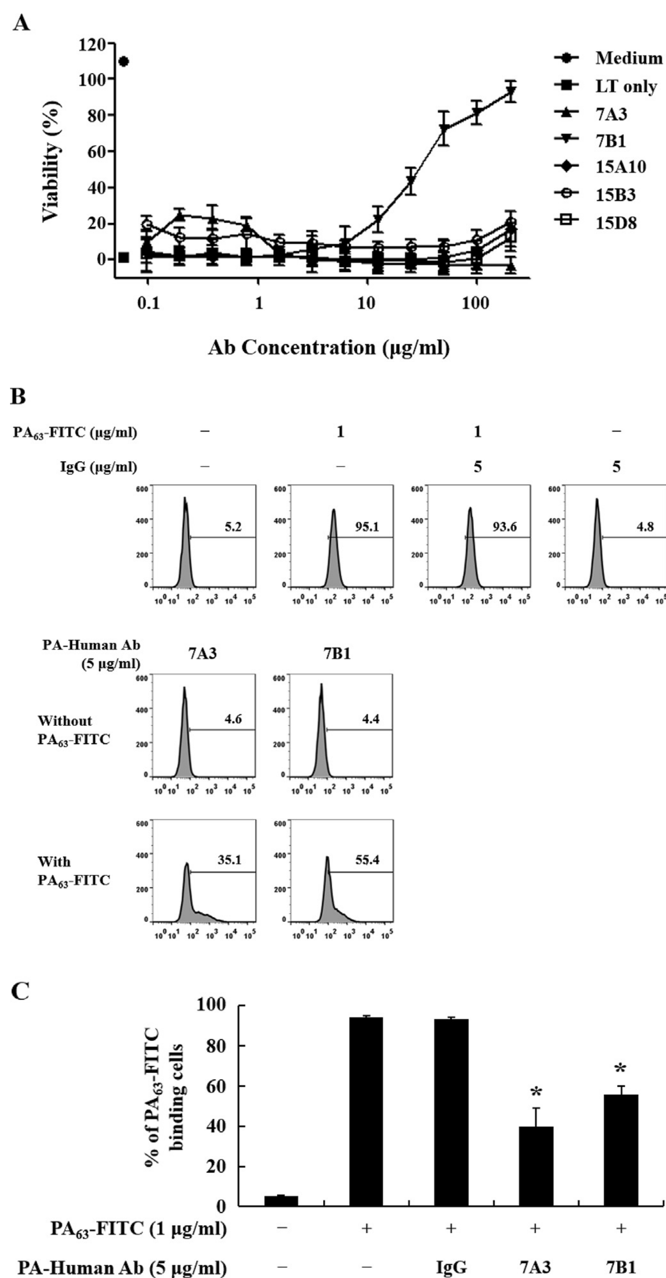

Fig. 1. *In vitro* neutralization of LT-mediated cytotoxicity and inhibition of PA binding to J774A.1 cells by 7B1 and 7A3 hMAbs. (A) hMAbs were serially diluted in 2 folds and mixed with LT before treated to J774A.1 cells. The viability of cells was determined by MTT assay. The means and standard deviations are calculated from three independent experiments. Inhibition of PA_{63}-FITC binding to J774A.1 cell by hMAbs was determined using flow cytometry analysis (B) and the means and standard deviations are calculated from three independent experiments (C). Asterisk (*) indicates statistical significance at $p < 0.05$ compared with the negative control.

oligomerization of PA using a previously described method [17]. It appeared that 7A3 did not affect PA oligomerization (data not shown). Further analysis will be required to reveal the exact binding site and PA-receptor binding inhibition mechanism of this antibody. 7B1, the most effective antibody in toxin neutralization assay, showed 44% of inhibition of PA binding to J774A.1 cell. These results indicate that 4 hMAbs except 15B3 mediate LT neutralizing activity through inhibition of PA binding to cells by binding PD4.

Table 2
Characteristics of the 7B1 light chain shuffled hMAbs.

mAb	IgG subclass	V_H[a]	D_H[b]	J_H[c]	V_L[d]	J_L[e]	CDR3[f]		*In vitro* ED_{50} (μg/ml)[g]	K_D value[h]	Productivity (mg/L)[i]
							V_H	V_L			
7B1	IgG1	VH3-73	D6-19	JH3	V1-4	JL1	ARYGGLYDRAFDV	SSYSSSTFYV	23.4	8.4×10^{-9}	1.8
7B1_2D10	IgG1	VH3-73	D6-19	JH3	V1-4	JL1	ARYGGLYDRAFDV	SSYSSSTFYV	16.3	4.6×10^{-9}	12.9
7B1_2G4	IgG1	VH3-73	D6-19	JH3	V1-4	JL1	ARYGGLYDRAFDV	SSYSSSTFYV	20.4	5.0×10^{-9}	22.3
7B1_3B4	IgG1	VH3-73	D6-19	JH3	V1-4	JL1	ARYGGLYDRAFDV	SSYSSSTFYV	16.4	5.2×10^{-9}	46.3

[a] H-chain V gene usage.
[b] H-chain D gene usage.
[c] H-chain J gene usage.
[d] L-chain V gene usage.
[e] L-chain J gene usage.
[f] Amino acid sequence of the H-chain and L-chain third complementarity-determining region.
[g] Effective dose for 50% of the population determined by cell viability assays.
[h] Dissociation constant determined by biolayer interferometry.
[i] Final amount of recombinant antibodies per cell culture medium. Protein concentration was measured by absorbance at 280 nm.

3.3. Generation of light chain shuffled antibodies from 7B1

Although 7B1 showed the best neutralizing activity on LT, its productivity was extremely low (1.8 mg/L, Table 1). In order to improve binding affinity and productivity of 7B1, we introduced the light chain shuffling approach. The DNA sequence of 7B1 variable region of light chain was replaced by variable regions from another library generated for antibodies of a human cytosol protein, and expressed for PA-biopanning. Three antibodies were selected from PA-biopanning and converted to IgG form for further analysis (Table 2). IGBLAST identified all derivatives share the same V and J regions of original antibody. Although the full sequences of shuffled antibodies were different from the original one, the sequence of the third complementarity-determining region (CDR3) was revealed to be exactly the same. Compared to original 7B1, the K_D value did not significantly decrease in light chain shuffled antibodies. However, the productivity of 7B1_3B4 increased up to 25 folds compared to the original one. The light chain shuffled antibodies were still effective in neutralizing LT on J774A.1 cells and all 3 antibodies showed 13–30% decreased ED_{50} values against LT-mediated cytotoxicity compared to the original 7B1 (Table 2 and Fig. 2A). We also measured the inhibition ability of the selected three antibodies on PA binding to host cells. PA_{63}-FITC was pre-incubated individually with selected antibodies, and its binding activity to J774A.1 cell was determined by flow cytometry (Fig. 2B). The inhibitory efficacy of PA binding to host cell receptor of 7B1 derivatives, 2D10, 2G4, and 3B4 were 49%, 43%, and 29%, respectively (Fig. 2C). ELISA assay also confirmed that all three derivative antibodies specifically bound to recombinant PD4 as their original one did (data not shown).

3.4. In vivo protective efficacy of 7B1-Light chain shuffled antibody 7B1_3B4

Although the light chain shuffling did not remarkably increased PA binding affinity and *in vitro* toxin-neutralization ability of the antibody, the productivity of the original antibody was highly improved (Table 2). Among the light chain shuffled antibodies, we selected the hMAb 7B1_3B4 based on the highest productivity for *in vivo* test. The protective efficacy against LT was tested in animal model using female BALB/c mice (6 weeks old; n = 10). We also tested the *in vivo* toxin neutralizing ability of 7A3. Even though the *in vitro* neutralization efficacy of this antibody was insignificant at high concentration, it showed 20% of protective effect at low concentrations (Fig. 1A), and more efficiently inhibited PA binding to cell receptors than 7B1 (Fig. 1C).

A Mixture of antibody (40 μg) and LT (40 μg PA and 20 μg LF) was given to the mice via tail vein injection, and the survival rate was traced for 2-weeks. All mice in groups of LT treatment without 7A3 or 7B1_3B4 died within 3–4 days after injection, while passively immunized groups with antibodies showed 80% (7A3) and 90% (7B1_3B4) survival rates (Fig. 3A). This result indicates that the selected candidate 7B1_3B4 has toxin neutralizing ability *in vivo*. It is noteworthy that 7A3 showed notable effect on neutralizing toxin *in vivo*.

Next, to evaluate the protective efficacy of hMAb 7B1_3B4 against spores, female A/J mice (6 weeks old; n = 6) were passively immunized with 40 μg of each antibody through a tail vein injection (intravenous; *i.v.*), and then subcutaneously infected with purified *B. anthracis* spores of $3LD_{50}$. While all mice were dead within 4 days in the PBS and human IgG control groups, the survival rate of immunized mice with 7A3, the domain 3 specific antibody was 16.7%, and 7B1_3B4, the domain 4 specific antibody was 33.3%, respectively (Fig. 3B). The mice group passively immunized with combined antibodies of 7A3 and 7B1_3B4 exhibited enhanced survival rate over 60% (data not shown). Recently, it has been suggested in many infectious diseases including anthrax, that a cocktail of monoclonal antibodies can provide better efficacy than individual single antibodies [3]. This result is in accordance with previous reports that a mixture of monoclonal antibodies which recognize different epitopes can increase the therapeutic efficacy for some infectious diseases, such as botulinum, tetanus, and rabies [26–28].

As a countermeasure against anthrax, considerable kinds of recombinant therapeutic antibodies have been developed [3]. Currently, six monoclonal antibodies against PA are actively investigated, including the mechanism of action and animal testing, and two of them (Raxibacumab and Obiltoxaximab) have been approved by FDA, as previously mentioned [3,5,8]. The binding epitopes of four antibodies among the six are located on domain 4, demonstrating PD4 is the most attractive target for anthrax therapeutics. Similar to previous antibodies, 7B1_3B4 also showed binding affinity to PD4.

In conclusion, we screened a candidate for novel therapeutic antibodies against anthrax toxin using a human antibody phage display library and improved its productivity by light chain shuffling. We confirmed the protective efficacy of the selected antibody 7B1_3B4 both *in vitro* by toxin neutralization assay and *in vivo* by challenge test using mouse model. These results added a potential candidate for therapeutic hMAbs against anthrax.

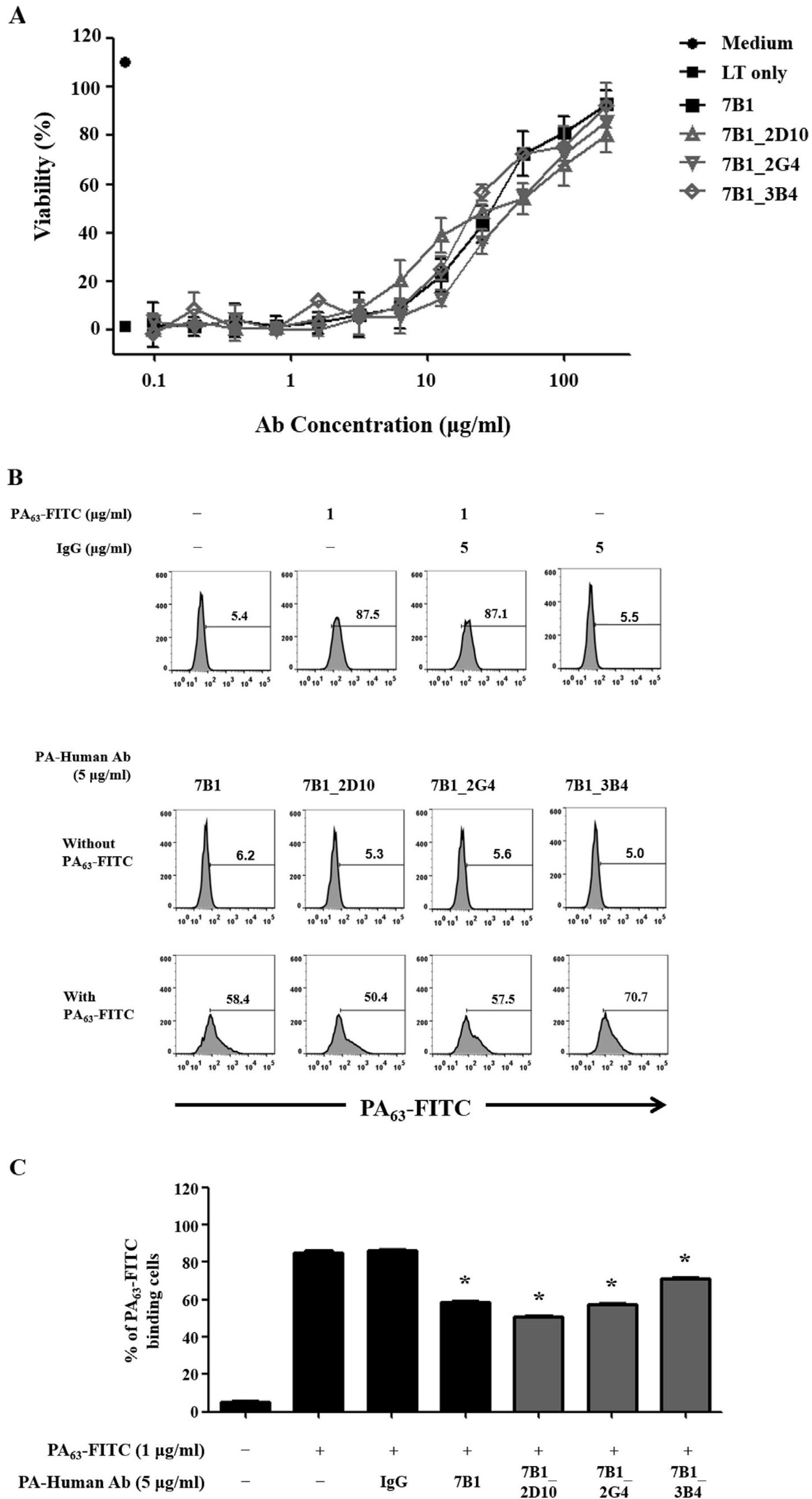

Fig. 2. *In vitro* neutralization of LT-mediated cytotoxicity and inhibition of PA binding to J774A.1 cells by light chain shuffled hMAbs of 7B1. (A) hMAbs were serially diluted in 2 folds and mixed with LT before treated to J774A.1 cells. The viability of cells was determined by MTT assay. The means and standard deviations are calculated from three independent experiments. Inhibition of PA_{63}-FITC binding to J774A.1 cell by hMAbs was determined using flow cytometriy analysis (B) and the means and standard deviations are calculated from three independent experiments (C). Asterisk (*) indicates statistical significance at $p < 0.05$ compared with the negative control.

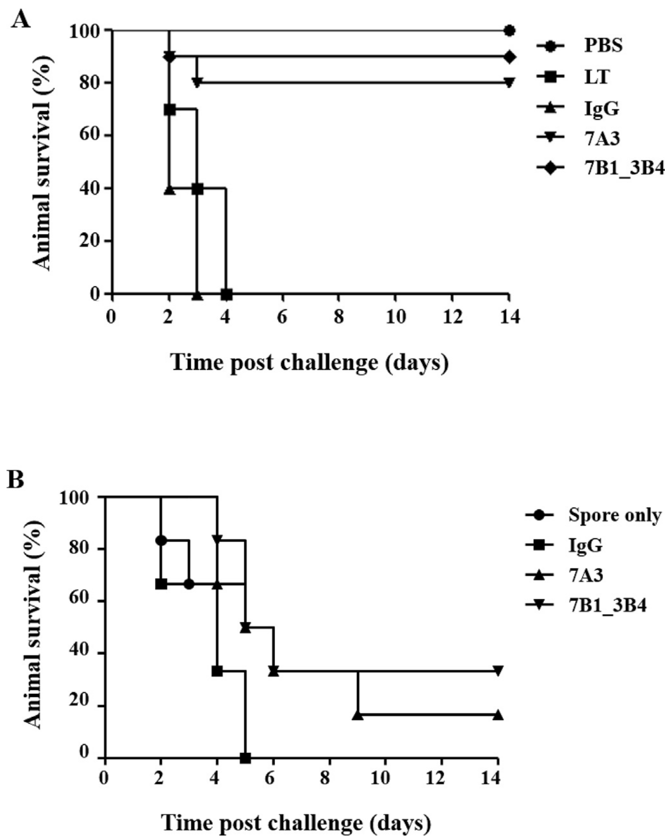

Fig. 3. *In vivo* protective efficacy of 7A3 and 7B1_3B4 against LT (A) or spore (B) challenge. BALB/c mice (n = 10/group) were given tail vein injections (intravenous; *i.v.*) of the hMAb (40 μg) with LT (40 μg PA and 20 μg LF) (A). A/J mice (n = 6/group) with passively transferred hMAbs were challenged with of 3 × LD_{50} of *B. anthracis* Sterne spores by subcutaneous (*s.c.*) injections to determine the *in vivo* protection (B). Survival rates were observed for 14 days.

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References

[1] W.L. Nicholson, N. Munakata, G. Horneck, H.J. Melosh, P. Setlow, Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments, Microbiol. Mol. Biol. Rev. 64 (2000) 548–572.
[2] P. Setlow, Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals, J. Appl. Microbiol. 101 (2006) 514–525.
[3] Z. Chen, M. Moayeri, R. Purcell, Monoclonal antibody therapies against anthrax, Toxins 3 (2011) 1004–1019.
[4] J.W. Froude, B. Stiles, T. Pelat, P. Thullier, Antibodies for biodefense, MAbs 3 (2011) 517–527.
[5] C.W. Tsai, S. Morris, Approval of raxibacumab for the treatment of inhalation anthrax under the US Food and Drug administration "animal rule, Front. Microbiol. 6 (2015) 1320.
[6] S. Mazumdar, Raxibacumab, MAbs 1 (2009) 531–538.
[7] N. Mohamed, M. Clagett, J. Li, S. Jones, S. Pincus, G. D'Alia, L. Nardone, M. Babin, G. Spitalny, L. Casey, A high-affinity monoclonal antibody to anthrax protective antigen passively protects rabbits before and after aerosolized Bacillus anthracis spore challenge, Infect. Immun. 73 (2005) 795–802.
[8] S.L. Greig, Obiltoxaximab: first global approval, Drugs 76 (2016) 823–830.
[9] L. Abrami, N. Reig, F.G. van der Goot, Anthrax toxin: the long and winding road that leads to the kill, Trends Microbiol. 13 (2005) 72–78.
[10] N.S. Duesbery, C.P. Webb, S.H. Leppla, V.M. Gordon, K.R. Klimpel, T.D. Copeland, N.G. Ahn, M.K. Oskarsson, K. Fukasawa, K.D. Paull, G.F. Vande Woude, Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor, Science 280 (1998) 734–737.
[11] C.L. Drum, S.Z. Yan, R. Sarac, Y. Mabuchi, K. Beckingham, A. Bohm, Z. Grabarek, W.J. Tang, An extended conformation of calmodulin induces interactions between the structural domains of adenylyl cyclase from Bacillus anthracis to promote catalysis, J. Biol. Chem. 275 (2000) 36334–36340.
[12] J.L. Elliott, J. Mogridge, R.J. Collier, A quantitative study of the interactions of Bacillus anthracis edema factor and lethal factor with activated protective antigen, Biochemistry 39 (2000) 6706–6713.
[13] C. Petosa, R.J. Collier, K.R. Klimpel, S.H. Leppla, R.C. Liddington, Crystal structure of the anthrax toxin protective antigen, Nature 385 (1997) 833–838.
[14] J. Mogridge, M. Mourez, R.J. Collier, Involvement of domain 3 in oligomerization by the protective antigen moiety of anthrax toxin, J. Bacteriol. 183 (2001) 2111–2116.
[15] M.J. Rosovitz, P. Schuck, M. Varughese, A.P. Chopra, V. Mehra, Y. Singh, L.M. McGinnis, S.H. Leppla, Alanine-scanning mutations in domain 4 of anthrax toxin protective antigen reveal residues important for binding to the cellular receptor and to a neutralizing monoclonal antibody, J. Biol. Chem. 278 (2003) 30936–30944.
[16] B. Zhou, C. Carney, K.D. Janda, Selection and characterization of human antibodies neutralizing Bacillus anthracis toxin, Bioorg. Med. Chem. 16 (2008) 1903–1913.
[17] X. Chi, J. Li, W. Liu, X. Wang, K. Yin, J. Liu, X. Zai, L. Li, X. Song, J. Zhang, X. Zhang, Y. Yin, L. Fu, J. Xu, C. Yu, W. Chen, Generation and characterization of human monoclonal antibodies targeting anthrax protective antigen following vaccination with a recombinant protective antigen vaccine, Clin. Vaccine Immunol. 22 (2015) 553–560.
[18] A. Cataldi, E. Labruyere, M. Mock, Construction and characterization of a protective antigen-deficient Bacillus anthracis strain, Mol. Microbiol. 4 (1990) 1111–1117.
[19] G.E. Rhie, Y.M. Park, J.H. Chun, C.K. Yoo, W.K. Seong, H.B. Oh, Expression and secretion of the protective antigen of Bacillus anthracis in Bacillus brevis, FEMS Immunol. Med. Microbiol. 45 (2005) 331–339.
[20] J. Jang, M. Cho, J.H. Chun, M.H. Cho, J. Park, H.B. Oh, C.K. Yoo, G.E. Rhie, The poly-gamma-D-glutamic acid capsule of Bacillus anthracis enhances lethal toxin activity, Infect. Immun. 79 (2011) 3846–3854.
[21] K. Rangnoi, N. Jaruseranee, R. O'Kennedy, P. Pansri, M. Yamabhai, One-step detection of aflatoxin-B(1) using scFv-alkaline phosphatase-fusion selected from human phage display antibody library, Mol. Biotechnol. 49 (2011) 240–249.
[22] D.A. Sarkes, J.M. Kogot, I. Val-Addo, D.N. Stratis-Cullum, P.M. Pellegrino, Cloning and Expressing Recombinant Protective Antigen Domains of B. Anthracis, Army Research Laboratory, 2011.
[23] J.H. Chun, O.J. Choi, M.H. Cho, K.J. Hong, W.K. Seong, H.B. Oh, G.E. Rhie, Serological correlate of protection in Guinea pigs for a recombinant protective antigen anthrax vaccine produced from Bacillus brevis, Osong Public Health Res Perspect 3 (2012) 170–176.
[24] B. Ivins, P. Fellows, L. Pitt, J. Estep, J. Farchaus, A. Friedlander, P. Gibbs, Experimental anthrax vaccines: efficacy of adjuvants combined with protective antigen against an aerosol Bacillus anthracis spore challenge in Guinea pigs, Vaccine 13 (1995) 1779–1784.
[25] L.J. Reed, H. Muench, A Simple method of estimating fifty per cent endpoints12, Am. J. Epidemiol. 27 (1938) 493–497.
[26] A. Nowakowski, C. Wang, D.B. Powers, P. Amersdorfer, T.J. Smith, V.A. Montgomery, R. Sheridan, R. Blake, L.A. Smith, J.D. Marks, Potent neutralization of botulinum neurotoxin by recombinant oligoclonal antibody, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11346–11350.
[27] H.W. Ziegler-Heitbrock, C. Reiter, J. Trenkmann, A. Futterer, G. Riethmuller, Protection of mice against tetanus toxin by combination of two human monoclonal antibodies recognizing distinct epitopes on the toxin molecule, Hybridoma 5 (1986) 21–31.
[28] T.Y. Chao, S. Ren, E. Shen, S. Moore, S.F. Zhang, L. Chen, C.E. Rupprecht, E. Tsao, SYN023, a novel humanized monoclonal antibody cocktail, for post-exposure prophylaxis of rabies, PLoS Neglected Trop. Dis. 11 (2017) e0006133.