Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5690–5692
Bioorganic & Medicinal Chemistry Letters

Selection and characterization of human monoclonal antibodies against Abrin by phage display

Heyue Zhou,[a,†] Bin Zhou,[a,†] Hongzheng Ma,[a] Charlotte Carney[a] and Kim D. Janda[a,b,*]

[a]*Department of Chemistry and Immunology, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA*
[b]*Worm Institute for Research and Medicine (WIRM), The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA*

Received 25 June 2007; revised 12 July 2007; accepted 16 July 2007
Available online 19 August 2007

Abstract—Abrin is a highly potent and lethal type II ribosome inactivating toxin that may be used as a biological warfare agent. To date, no human anti-Abrin antibodies have yet to be reported. Herein, we describe the selection and characterization of two human monoclonal antibodies, termed E12 and RF12, which are capable of binding native Abrin with high affinity and specificity. Through surface plasmon resonance studies, we have determined the association and dissociation rate constants and the cross-reactivity for both antibodies. In our developed Biacore-based Abrin detection system, the limit of detection of antibodies E12 and RF12 is 35 and 75 ng/mL, respectively. These concentrations are about 5×10^4-fold lower than the extrapolated Abrin human LD_{50}. In sum, our data demonstrated the power of human antibody phage display libraries and the promise of these antibodies as detection devices for Abrin.

Abrin is a ribosome inactivating protein (RIP) isolated from the seeds of *Abrus precatorius* (jequirity pea), a vine common to many tropical areas throughout the world. It consists of an enzymatic A-chain and a binding B-chain linked by a disulfide bond. The B-chain of Abrin is a galactose-specific lectin that is responsible for binding the toxin to appropriate galactosides displayed on the target cell surface.[1] The A-chain is an efficient *N*-glycosidase and is transported across the plasma membrane by the B-chain via endocytosis into the cytoplasma of cells. Once internalized, the A-chain removes an adenine from an exposed loop of 28S ribosomal RNA, thus inhibiting protein synthesis and resulting in eventual cell death.[2–5]

Abrin is very similar to Ricin in structure[6] and biochemical mechanism of action, but has been stated to be approximately 75 times more toxic in mice.[7] The estimated human fatal dose is 0.1–1 μg/kg.[8] Although Abrin is not known to have been used as a biological weapon, its extreme toxicity and relatively simple production make it a potential biological warfare agent or terrorist weapon. Both Abrin and Ricin are acknowledged in the latest version of *The Biological and Toxin Weapons Convention (BTWC)* Procedural Report and Rolling Text: Ad Hoc Group 23rd session (23 April–11 May 2001) (http://www.brad.ac.uk/acad/sbtwc/ahg56/ahg56.htm).

The presence of Abrin has been determined most commonly using biosensing systems such as anti-Abrin antibodies, a ribosome-inactivation assay,[9] and most sensitively by direct antigen detection with antiserum against Abrin.[10] Although a rabbit anti-Abrin polyclonal antibody and a mouse monoclonal anti-Abrin antibody have recently become commercially available, there have been to our knowledge no literature reports on anti-Abrin human antibodies. Thus, the development and characterization of Abrin-specific monocolonal antibodies would be useful for rapid detection and possibly protection from Abrin intoxication.

Compared with conventional hybridoma technology, phage display is an efficient, cost-effective alternative for monoclonal antibody generation in vitro. There are two types of antibody libraries which can be used within the phage display format: naïve or immune. With an

Abbreviations: IPTG, Isopropyl-β-D-thiogalactoside; scFv, Single-chain variable fragment; Fab, Antigen binding fragment; mAb, Monoclonal antibody.
Keywords: Phage display; Abrin; Monoclonal antibody.
* Corresponding author. Tel.: +1 858 784 2516; fax: +1 858 784 2595; e-mail: kdjanda@scripps.edu
† These authors equally contributed to this work.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.07.053

immune library there is a strong bias towards the antigen used for immunization, and the antibodies screened from an immune library typically have higher affinities. However, a disadvantage of this type of library is that lymphocytes from infected patients are difficult to obtain, especially when a lethal toxin is involved. Additionally, a new immune library is required for each immunogen to be investigated. Although the affinities of antibodies selected from naïve libraries are usually lower, they can be easily enhanced by affinity maturation.[11] Recently, naïve libraries have been used to select specific human antibodies against different toxins, including Anthrax[12] and Ricin.[13] Here we describe the use of a human naïve scFv phage display library[14] in search for human monoclonal antibodies (mAbs) against Abrin.

Maxisorp® immunotubes (Nunc) were coated with Abrin at 4 °C overnight, the immunotubes were then washed with PBS and blocked (Blocker™ Casein in PBS (Pierce)) for 1 h. Approximately 10^{12} cfu of the phage library was diluted in 4 mL Blocker™ Casein in PBS and added to the Abrin-coated immunotubes. After incubation with gentle shaking for 1 h at room temperature, the tubes were washed with PBS to remove unbound phage. The bound phage were eluted with 0.1 M Glycine–HCl (pH 2.7) and immediately neutralized with 1 M Tris–base (pH 8.5). Titration of eluted phage, their rescue and preparation were performed as previously described.[15]

After the fourth round of panning, 96 single colonies were randomly picked for phage culture and ELISA. Abrin was diluted to 1 μg/mL in PBS and immobilized on Costar ELISA plates (Corning) at 4 °C overnight. After blocking for 1 h at room temperature, the plates were incubated with diluted phage, which were 1:1 in the Blocker™ Casein. Upon washing with PBS, the plates were incubated with 1:1000 diluted horseradish peroxidase (HRP)-conjugated anti-M13 (Amersham) and detected with TMB substrate (Pierce). The reaction was quenched with an equal volume of 2 M H_2SO_4 and the optical density was determined using a UV-plate reader at 450 nm. Sixteen positive clones were obtained, of which five clones were unique in sequence. Ricin was used to detect binding specificity; using this format scFvs B8 and H6 showed cross-reactivity, whereas E12, RF12 and D6 showed good specificity for Abrin over Ricin (Fig. 1).

Because scFv molecules are prone to denaturation and aggregation, we converted the selected scFvs into a more stable Fab format for further assay analysis. Based on the identified scFv sequences, the VH and VL genes of the identified clones were amplified by PCR using their corresponding primers and overlapped with C_{H1} region and C_λ/C_κ region, respectively. A His-tag was appended to the C-terminus of C_λ/C_κ followed by a stop codon; pelB and ompA leader sequences were fused at the N-termini of VH and VL, respectively. The pelB-VH-C_{H1}- STOP and ompA-VL- C_λ/C_κ- His6-STOP fragments were assembled via overlapping PCR and subcloned into pETHis expression vector via flanked

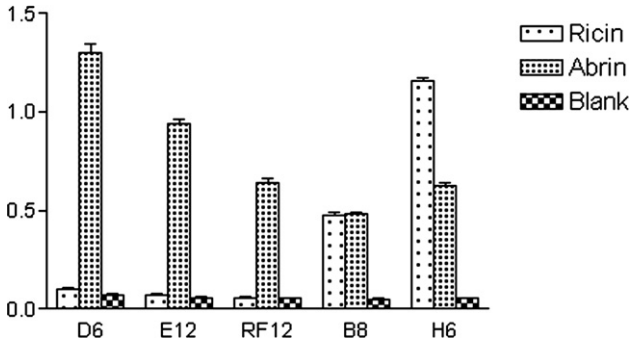

Figure 1. Phage ELISA of selected scFvs. Freshly prepared phage for each clone were added to an ELISA plate coated with Abrin, Ricin, or a blank control. Data shown are from one representative experiment repeated a total of three times.

Sfi I restriction endonuclease sites. The vector containing Fabs RF12, E12 and D6 genes were transformed into Rosetta2 (DE3) *Escherichia coli* (Novagen). Their expression was induced by growth in superbroth containing 0.1 mM IPTG overnight at 25 °C. The Fabs were purified using immobilized metal affinity chromatography (Ni–NTA, Qiagen) and ion exchange chromatography (HiTrap SP, GE HealthCare). All purified Fabs retained their binding activity and specificity when checked by ELISA (Fig. 2).

Protein immobilized on an ELISA plate can be easily denatured, thus, to verify that these selected antibodies can still recognize native toxin, a dot blot assay was performed with purified Fab samples. A set of boiled (denatured, 5 min in a water bath) and non-boiled (native) Abrin, Ricin and BSA (at a concentration of 0.1 μg) were added onto nitrocellulose membrane strips. The membrane strips were air-dried and blocked with 5% skimmed milk in PBS for 1 h at room temperature. Anti-Abrin Fabs E12, RF12 and D6 were diluted with 0.05% Tween 20 in PBS (PBS–T) and incubated with the membrane strips for 1 h. After washing with PBS–T, the membrane strips were incubated with 1:1000 HRP-conjugated goat anti-Human Fab antibody (Sigma). The signal was detected using WestDura substrate (Pierce). In this format only Fab E12 and RF12 bound to the native Abrin (Fig. 3), suggesting that they recognize conformational epitopes rather than linear

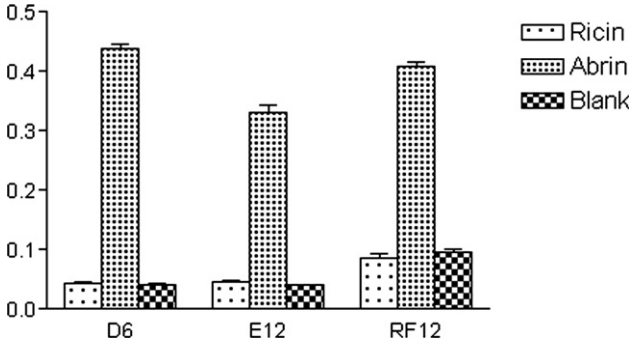

Figure 2. Fab ELISA. Purified Fabs for each clone were added to an ELISA plate coated with Abrin, Ricin, or a blank control. Data shown are from one representative experiment repeated a total of three times.

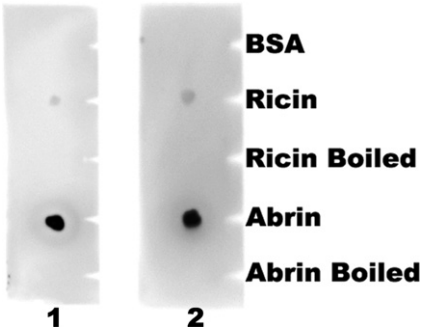

Figure 3. Dot blot assay. (1) Fab E12. (2) Fab RF12. 0.1 μg Abrin and Ricin boiled 5 min or without boiling, together with BSA, was spotted on nitrocellulose membrane strips. Individual Fabs were incubated with the membrane strips and the bound Fabs were detected with an anti-Fab HRP conjugate.

peptide sequences. For Fab D6, no significant signal could be seen even at 1 μg Abrin (data not shown). Interestingly, antibody D6 did not bind denatured Abrin or Ricin, indicating that this antibody may recognize a conformational epitope that is different when presented on ELISA plate in contrast to the nitrocellulose membrane.

Binding kinetics were accomplished using surface plasmon resonance (SPR) on a BIAcore™ 3000 instrument (Biosensor, Piscataway, NJ) at 25 °C. Thus, Fab E12 or RF12 was immobilized on CM5 sensor chip and targeted to 300 RU setting using standard NHS/EDC coupling methodology. All measurements were conducted in HBS-EP buffer with a flow rate of 30 μL/min. Abrin was diluted so as to obtain a series of concentrations. Each was injected into the flow cell for repeated analyses and 4 M $MgCl_2$ was used to regenerate the chip. The association and dissociation rate constants (k_a, $M^{-1}\,s^{-1}$ and k_d, s^{-1}) and affinity (K_D, M) were determined to be $1.37 \times 10^4\ M^{-1}\,s^{-1}$, $7.57 \times 10^{-4}\ s^{-1}$ and 55.2 nM for Fab E12, and $3.58 \times 10^4\ M^{-1}\,s^{-1}$, $3.98 \times 10^{-3}\ s^{-1}$ and 111.0 nM for Fab RF12, respectively, using a model fit to a 1:1 binding assumption. These affinities we view as moderate, however, such binding is reasonable when panning a naïve library, which is constructed from ''hosts'' that were never exposed to a particular antigen.

To examine the ability of these antibodies to detect native Abrin in solution, Fab E12 or RF12 was immobilized on a CM5 sensor chip targeted to 8000 RU. The injection was conducted at 30 μL/min for 5 min. Thirty cycles of blank samples (buffer only) were injected, and the standard deviation (SD) was calculated for each antibody. We used 10× SD as the limit of detection (LOD), which is the cutoff value for determining a positive sample (Biacore Concentration Analysis Handbook, Version AA). To determine the lowest limit of quantitation (LLOQ) for each antibody, thirty cycles of Abrin samples were injected through the antibody immobilized flow cells; we note that the lowest Abrin concentration within all responses was equal to or greater than the LOD, which was thus defined as the LLOQ. The LLOQ for Fab E12 and RF12 were determined to be 35 ng/mL (or 0.56 nM) and 75 ng/mL (or 1.17 nM), which translates to approximately 5×10^4-fold lower than the extrapolated human LD_{50}. Injection of Ricin and its agglutinin (RCA_{120}) at a concentration of 100-fold greater than the LLOQ under the same assay conditions revealed that the cross-reactivity to these closely related toxins (Ricin and RCA_{120}) was lower than 0.5% for both antibodies.

In conclusion, we have demonstrated that human mAbs that specifically bind to Abrin can be discovered from naïve phage display libraries. The data we have presented suggest that mAb E12 and RF12 may prove to be valuable starting points for the development of biosensors for Abrin detection. Further affinity maturation and IgG production of these two mAbs may provide enhanced sensitivity and stability for future real-time Abrin detection as well as possible therapeutics for Abrin intoxication.

Acknowledgments

This work was supported by The Skaggs Institute for Chemical Biology. We thank Dr. Gao Changshou for preparing the human PIII scFv-phage display library.

References and notes

1. Sandvig, K.; Olsnes, S.; Pihl, A. *J. Biol. Chem.* **1976**, *251*, 3977.
2. Olsnes, S.; Refsnes, K.; Pihl, A. *Nature* **1974**, *249*, 627.
3. Endo, Y.; Mitsui, K.; Motizuki, M.; Tsurugi, K. *J. Biol. Chem.* **1987**, *262*, 5908.
4. Griffiths, G. D.; Leek, M. D.; Gee, D. J. *J. Pathol.* **1987**, *151*, 221.
5. Hughes, J. N.; Lindsay, C. D.; Griffiths, G. D. *Hum. Exp. Toxicol.* **1996**, *15*, 443.
6. Tahirov, T. H.; Lu, T. H.; Liaw, Y. C.; Chen, Y. L.; Lin, J. Y. *J. Mol. Biol.* **1995**, *250*, 354.
7. Olsnes, S.; Sandvig, K.; Eiklid, K.; Pihl, A. *J. Supramol. Struct.* **1978**, *9*, 15.
8. Houston, L. L. *J. Toxicol.* **1982**, *19*, 385.
9. Dickers, K. J.; Bradberry, S. M.; Rice, P.; Griffiths, G. D.; Vale, J. A. *Toxicol. Rev.* **2003**, *22*, 137.
10. Godal, A.; Olsnes, S.; Pihl, A. *J. Toxicol. Environ. Health* **1981**, *8*, 409.
11. Levin, A. M.; Weiss, G. A. *Mol. Biosyst.* **2006**, *2*, 49.
12. Steiniger, S. C.; Altobell, L. J.; Zhou, B.; Janda, K. D. *Mol. Immunol.* **2007**, *44*, 2749.
13. Gao, C.; Mao, S.; Kaufmann, G.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12612.
14. Gao, C.; Brummer, O.; Mao, S.; Janda, K. D. *J. Am. Chem. Soc.* **1999**, *121*, 6517.
15. Mao, S.; Gao, C.; Lo, C. H.; Wirsching, P.; Wong, C. H.; Janda, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6953.