Use of bacteriophage for discovery of therapeutically relevant antibodies against infectious diseases

Martina L Jones

National Biologics Facility
ARC Training Centre for Biopharmaceutical Innovation
Australian Institute for Bioengineering and Nanotechnology
The University of Queensland
St Lucia, Qld 4072, Australia
Tel: +61 7 3346 3178
Email: martina.jones@uq.edu.au

Scientists George P Smith and Gregory Winter were recently awarded half of the 2018 Nobel Prize for Chemistry for developing a technology to display exogenous peptides and proteins on the surface of bacteriophage. 'Phage display' has revolutionised the development of monoclonal antibodies, allowing fully human-derived antibodies to be isolated from large antibody libraries. It has been used for the discovery of many blockbuster drugs, including Humira (adalimumab), the highest selling drug yearly since 2012, with US$18.4b in sales globally in 2017[1]. Phage display can be used to isolate antibodies to almost any antigen for a wide range of applications including clinical use (for cancer, inflammatory conditions and infectious diseases), diagnostic use or as research tools. The technology is accessible to any laboratory equipped for molecular biology and bacteria culture.

Displaying exogenous peptides and proteins on bacteriophage

Phage display technology was first demonstrated by Smith in 1985, who showed that DNA encoding peptides could be inserted into the bacteriophage gene III resulting in the expression and display of the corresponding peptides on the surface of the virion as a fusion to the coat protein pIII[2]. Winter then showed that this technology could be used to display antibody fragments on the surface of bacteriophage[3]. His group also showed that highly specific antibodies could be fished out of large libraries of antibody gene sequences cloned into phage expression vectors[4,5]. This now allowed the isolation of fully human antibodies, from cloned human antibody gene repertoires, reducing the impact of immunogenicity of mouse-derived therapeutic antibodies.

The bacteriophage biology that allows the display of peptides and proteins is well reviewed by Russel *et al.*[6]. The most commonly used phage display system uses phagemid vectors, where the antibody-pIII gene fusion is cloned into a bacterial expression vector containing a periplasmic leader sequence, an ampicillin resistance gene and an f1 viral origin of replication. When the phagemid is transformed into *Escherichia coli*, and grown in the presence of ampicillin and M13-derived filamentous helper phage (usually M13K07), the antibody-pIII fusion protein is expressed and incorporated into the newly synthesised phage particles, and the phagemid is replicated as single-stranded DNA and preferentially packaged into the particle (Figure 1). Phage particles are released into the culture media and are purified by precipitation with high salt and polyethylene glycol.

Phage display libraries and biopanning

Phage antibody libraries can either be 'naïve' or 'immunised'. Naïve libraries are usually human derived, and are created by collecting peripheral blood samples from a large group of healthy donors from a general population, with no bias towards any particular disease or condition. Naïve libraries can be used indefinitely to isolate antibodies to almost any target presented to the library. For this reason, naïve libraries are also termed 'single-pot' libraries since the same library can be used for any antigen[7]. Immunised libraries are focussed on the isolation of particular antibodies, with blood samples collected from individuals with a defined condition or from mice immunised with an antigen-of-interest[8]. Immunised libraries increase the likelihood of obtaining highly specific and high affinity antibodies, but also limits their use towards a single antigen.

The process of isolating specific antibodies from a phage antibody library is termed 'biopanning', and is summarised in Figure 2. Biopanning involves incubating the library of phage particles with immobilised antigen, washing away non-binding phage, and then eluting the bound phage using a buffer that breaks the antibody-antigen interaction. After enriching the library for binding phage, individual clones can be isolated, characterised and further developed as either laboratory tools, or as commercial diagnostic and therapeutic antibodies.

Therapeutic antibodies isolated by phage display

As of December, 2018, there were 82 antibodies approved in the US and/or EU for therapeutic use in humans, and 10 of these

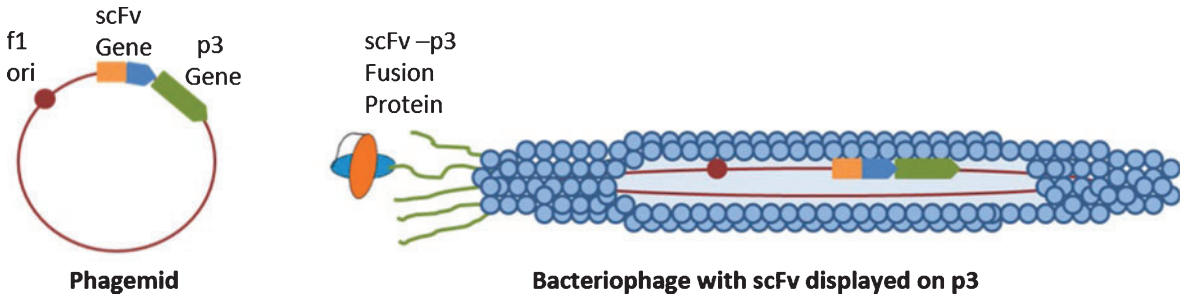

Figure 1. Left: A phagemid cloning vector containing an f1 origin of replication (f1 ori), and antibody variable region genes (Heavy chain (orange) and Light chain (blue)), assembled as a single chain variable fragment (scFv), cloned in frame with the gene for the bacteriophage p3 coat protein (green). Right: A bacteriophage particle containing a phagemid vector inside the particle, and the scFv antibody fragment displayed on its surface as a fusion to the p3 protein.

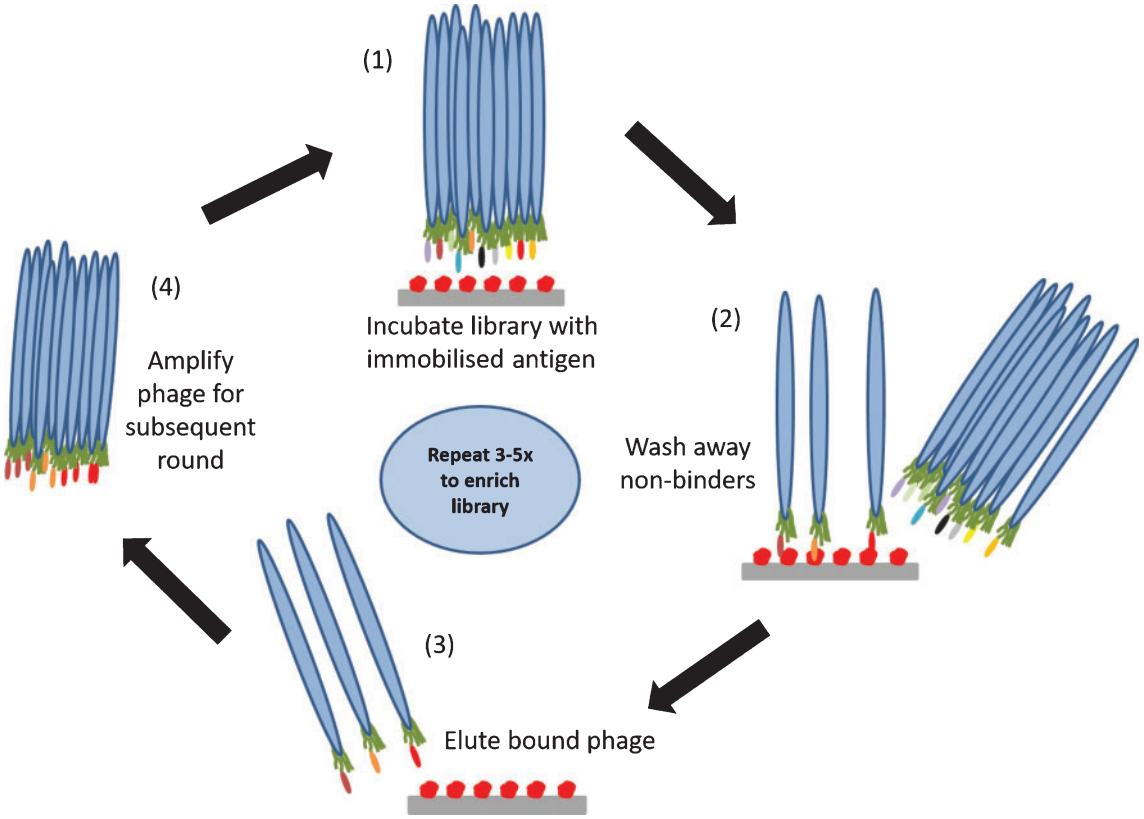

Figure 2. Summary of the biopanning process. The phage particles are depicted in blue with the scFv-p3 fusion protein on their tips. (1) The phage particles displaying a library of scFv is incubated with immobilised antigen (depicted in red), which could be purified proteins, or whole cells or viruses. (2) The surface is washed to remove any non-binding phage. (3) Bound phage are eluted using a low pH, high pH or high salt buffer. (4) The eluted phage are infected into *Escherichia coli* to amplify these phage, enriching the library for specific binders. This process is then repeated with the newly amplified, enriched pool 3–5 times with increasing stringency at step 2 to further enrich the library for strong binders.

were isolated using phage display[9–12] (Table 1). The majority of therapeutic antibodies target endogenous antigens such as proteins involved in the inflammatory response, or cell-surface or circulating proteins overexpressed in cancers.

Phage-derived antibodies against infectious agents

Therapeutic antibodies can also target infectious agents, including bacteria and viruses; examples include bezlotoxumab, which targets the B toxin of *Clostridium difficile*, obiltoxaximab and raxibacumab, which target the anthrax toxin, and palivizumab, which targets the F protein of respiratory syncytial virus. These are currently the only antibodies approved for therapy against infectious agents, and only raxibacumab was isolated using phage display. The others were isolated from mice using traditional hybridoma technology followed by humanisation, or using transgenic mice with humanised immune repertoires.

However, phage display, using immunised human antibody libraries created from individuals who have survived viral infections or from vaccinated individuals, offers a unique advantage for the isolation of neutralising antibodies to infectious agents. Antibodies have been isolated using such techniques from several viruses including Enterovirus 71[14], Ebola virus[15], HIV[16], West Nile Virus[17]

Table 1. FDA approved therapeutic antibodies isolated using phage display technology. Information was obtained from the ImMunoGeneTics antibody database (IMGT/mAb-DB)[11,13], and the numbers following each drug name indicate the IMGT database entry number.

Non-proprietary name	Trade name	Library type	Target	Indication	Year approved (FDA)
Adalimumab (IMGT-165)	Humira	Human naïve	TNF-α	Immune/ inflammatory diseases	2002
Ranibizumab (IMGT-84)	Lucentis	Mutagenic library of bevacizumab	VEGF-A	Immune/ inflammatory diseases	2006
Belimumab (IMGT-266)	Benlysta	Human naïve	B-lymphocyte stimulator	Immune/ inflammatory diseases	2011
Raxibacumab (IMGT-260)	ABthrax	Human naïve	Anthrax protective antigen of *Bacillus anthracis*	Infectious disease	2012
Ramucirumab (IMGT-295)	Cyramza	Human naïve	VEGFR-2	Oncology	2014
Necitumumab (IMGT-294)	Portrazza	Human naïve	EGFR	Oncology	2015
Ixekizumab (IMGT-380)	Taltz	Mouse immunised	IL-17A	Immune/ inflammatory diseases	2016
Atezolizumab (IMGT-526)	Tecentriq	Human naïve	PD-L1	Oncology	2016
Avelumab (IMGT-512)	Bavencio	Human naïve	PD-L1	Oncology	2017
Moxetumomab pasudotox (IMGT-198)	Lumoxiti	Mutagenic library of mouse antibody	CD22	Oncology	2018

and Rabies virus[18]. Neutralising antibodies can also be isolated from naïve human libraries using phage display. m102.4 antibody neutralises Hendra and Nipah viruses, and was isolated by panning a naïve library against the G-protein of Hendra virus[19]. This antibody has recently completed Phase I clinical trials in Australia[20] and has been used as passive immunotherapy in several individuals exposed to Hendra virus[21].

Biopanning strategies for isolation of antibodies to microbial targets requires a source of antigen for incubation with the phage library. The antigen can be a highly purified preparation of the target, for example viral proteins[22–24]or purified bacterial toxins[25,26], or crude preparations such as whole bacterial cells[27,28] or virus particles[29,30].

Advantages of phage display

Phage display offers several advantages over mouse immunisation strategies for antibody discovery, especially for targets that are either toxic or non-immunogenic in a mouse host, or where precision over epitope targeting is required[31]. Guidance towards particular epitopes can be incorporated into the biopanning strategy, by competing with a ligand, or alternating between mouse and human equivalent antigens, or depleting the library to binders that are cross-reactive to similar antigens. For example, antibodies specific for each of the four serotypes of Dengue virus (DENV) NS1 were isolated from a human naïve phage library[32]. Serotype specificity was achieved by first exposing the library to the other three DENV NS1 serotypes to deplete cross-reactive binders. Such antibodies may be useful in serotyping assays.

Phage display is a simple but powerful tool for antibody discovery, either for therapeutic use or for research tools. It is accessible to any laboratory equipped for standard culturing and molecular biology. Libraries can be created in-house, obtained commercially (Source Bioscience, Creative Biolabs) or shared from other researchers through material transfer agreements. Within Australia, the National Biologics Facility (NBF) at the University of Queensland offers phage display services and access to their naïve human library, and

has experience in isolating antibodies against infectious targets including Dengue virus[32] and the malaria parasite[33]. Isolation of viral neutralising antibodies using phage display of libraries generated from immunised or recovered patients is an emerging field in infectious disease therapy.

Conflicts of interest

Martina Jones is Operations Manager of the Queensland node of the National Biologics Facility, which offers phage display services to industry and academic groups.

Acknowledgements

This research did not receive any specific funding.

References

1. Philippidis, A. (2018) The top 15 best-selling drugs of 2017. https://www.genengnews.com/a-lists/the-top-15-best-selling-drugs-of-2017/ (accessed 31 January 2019).
2. Smith, G.P. (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* **228**, 1315–1317. doi:10.1126/science.4001944
3. McCafferty, J. *et al*. (1990) Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* **348**, 552–554. doi:10.1038/348552a0
4. Clackson, T. *et al*. (1991) Making antibody fragments using phage display libraries. *Nature* **352**, 624–628. doi:10.1038/352624a0
5. Marks, J.D. *et al*. (1991) By-passing immunization. Human antibodies from V-gene libraries displayed on phage. *J. Mol. Biol.* **222**, 581–597. doi:10.1016/0022-2836(91)90498-U
6. Russel, M. *et al*. (2004) Introduction to phage biology and phage display. In *Phage Display* (Clackson, T. and Lowman, H.B. eds). pp. 1–26. Oxford University Press.
7. Nissim, A. *et al*. (1994) Antibody fragments from a 'single pot' phage display library as immunochemical reagents. *EMBO J.* **13**, 692–698. doi:10.1002/j.1460-2075.1994.tb06308.x
8. Ubah, O. and Palliyil, S. (2017) Monoclonal antibodies and antibody like fragments derived from immunised phage display libraries. *Adv. Exp. Med. Biol.* **1053**, 99–117. doi:10.1007/978-3-319-72077-7_6
9. Frenzel, A. *et al*. (2016) Phage display-derived human antibodies in clinical development and therapy. *MAbs* **8**, 1177–1194. doi:10.1080/19420862.2016.1212149
10. Kaplon, H. and Reichert, J.M. (2018) Antibodies to watch in 2019. MAbs.
11. Lefranc, M.P. (2011) IMGT, the International ImMunoGeneTics Information System. *Cold Spring Harb. Protoc.* **2011**, 595–603. doi:10.1101/pdb.top115
12. Reichert, J.M. (2018) Approved antibodies. https://www.antibodysociety.org/news/approved-antibodies/ (accessed 31 January 2019).
13. Lefranc, M.P. IMGT/mAb-DB. http://www.imgt.org/mAb-DB/query (accessed 31 January 2019).
14. Chen, Z. *et al*. (2017) An elaborate landscape of the human antibody repertoire against enterovirus 71 infection is revealed by phage display screening and deep sequencing. *MAbs* **9**, 342–349. doi:10.1080/19420862.2016.1267086
15. Maruyama, T. *et al*. (1999) Ebola virus can be effectively neutralized by antibody produced in natural human infection. *J. Virol.* **73**, 6024–6030.
16. Trott, M. *et al*. (2014) Functional characterization of two scFv-Fc antibodies from an HIV controller selected on soluble HIV-1 Env complexes: a neutralizing V3- and a trimer-specific gp41 antibody. *PLoS One* **9**, e97478. doi:10.1371/journal.pone.0097478
17. Duan, T. *et al*. (2009) Human monoclonal Fab Antibodies against West Nile virus and its neutralizing activity analyzed *in vitro* and *in vivo*. *J. Antivir. Antiretrovir.* **1**, 36–42. doi:10.4172/jaa.1000005
18. Kramer, R.A. *et al*. (2005) The human antibody repertoire specific for rabies virus glycoprotein as selected from immune libraries. *Eur. J. Immunol.* **35**, 2131–2145. doi:10.1002/eji.200526134
19. Zhu, Z. *et al*. (2006) Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J. Virol.* **80**, 891–899. doi:10.1128/JVI.80.2.891-899.2006
20. ANZCTR (2016) ACTRN12615000395538. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=368110 (accessed 6 January 2019).
21. Broder, C.C. *et al*. (2013) A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res.* **100**, 8–13. doi:10.1016/j.antiviral.2013.06.012
22. Zhang, D. *et al*. (2013) Generation and characterization of a novel recombinant antibody against LMP1-TES1 of Epstein–Barr virus isolated by phage display. *Viruses* **5**, 1131–1142. doi:10.3390/v5041131
23. Jo, G. *et al*. (2018) Generation and characterization of a neutralizing human monoclonal antibody to hepatitis B virus PreS1 from a phage-displayed human synthetic Fab library. *J. Microbiol. Biotechnol.* **28**, 1376–1383.
24. Wu, Y. *et al*. (2017) Neutralization of Zika virus by germline-like human monoclonal antibodies targeting cryptic epitopes on envelope domain III. *Emerg. Microbes Infect.* **6**, e89. doi:10.1038/emi.2017.79
25. Rukkawattanakul, T. *et al*. (2017) Human scFvs that counteract bioactivities of *Staphylococcus aureus* TSST-1. *Toxins (Basel)* **9**, 50. doi:10.3390/toxins9020050
26. Wang, D. *et al*. (2017) Preparation and characterization of a human scFv against the *Clostridium perfringens* type A alpha-toxin. *Toxicon* **130**, 79–86. doi:10.1016/j.toxicon.2017.02.021
27. Kuhn, P. *et al*. (2017) Human Anti-Lipopolysaccharid (LPS) antibodies against *Legionella* with high species specificity. *Hum. Antibodies* **26**, 29–38. doi:10.3233/HAB-170318
28. Nian, S. *et al*. (2016) Development and identification of fully human scFv-Fcs against *Staphylococcus aureus*. *BMC Immunol.* **17**, 8. doi:10.1186/s12865-016-0146-z
29. Wu, D. *et al*. (2011) Phage displayed peptides to avian H5N1 virus distinguished the virus from other viruses. *PLoS One* **6**, e23058. doi:10.1371/journal.pone.0023058
30. Liu, H. *et al*. (2014) Selection and characterization of single-chain recombinant antibodies against infectious haematopoietic necrosis virus from mouse phage display library. *J. Virol. Methods* **205**, 61–67. doi:10.1016/j.jviromet.2014.04.008
31. Frenzel, A. *et al*. (2017) Designing human antibodies by phage display. *Transfus. Med. Hemother.* **44**, 312–318. doi:10.1159/000479633
32. Lebani, K. *et al*. (2017) Isolation of serotype-specific antibodies against dengue virus non-structural protein 1 using phage display and application in a multiplexed serotyping assay. *PLoS One* **12**, e0180669. doi:10.1371/journal.pone.0180669
33. Leow, C.H. *et al*. (2014) Production and characterization of specific monoclonal antibodies binding the Plasmodium falciparum diagnostic biomarker, histidine-rich protein 2. *Malar. J.* **13**, 277. doi:10.1186/1475-2875-13-277

Biography

Dr Martina Jones is the Operations Manager of the Queensland node of the National Biologics Facility, located at the Australian Institute for Bioengineering and Nanotechnology (AIBN) at The University of Queensland. She is also Deputy Director of the ARC Training Centre for Biopharmaceutical Innovation, also located at AIBN. Her research interests are in antibody discovery and antibody engineering, and bioprocessing of biopharmaceuticals.