Generation of Recombinant Antibodies Against Toxins and Viruses by Phage Display for Diagnostics and Therapy

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Abstract Antibody phage display is an in vitro technology to generate recombinant antibodies. In particular for pathogens like viruses or toxins, antibody phage display is an alternative to hybridoma technology, since it circumvents the limitations of the immune system. Phage display allows the generation of human antibodies from naive antibody gene libraries when either immunized patients are not available or immunization is not ethically feasible. This technology also allows the construction of immune libraries to select in vivo affinity matured antibodies if immunized patients or animals are available.

In this review, we describe the generation of human and human-like antibodies from naive antibody gene libraries and antibodies from immune antibody gene libraries. Furthermore, we give an overview about phage display derived recombinant antibodies against viruses and toxins for diagnostics and therapy.

Keywords scFv • Phage display • Viruses • Toxins • Pathogen • Therapy • Antibody • Antibody engineering

1 Introduction

Antibodies are essential molecules as tools for basic research [24], diagnostics [78] and for therapy [101]. First polyclonal antibodies were produced as serum in horses [126]. A milestone in antibody generation was the development of hybridoma

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technology which allows the production of monoclonal antibodies [62]. But the hybridoma technology has drawbacks like limited number of candidates, possible instability of the aneuploid cell lines [94], inability to provide antibodies against highly conserved antigens and most of all its limited application to generate human antibodies [130]. The hybridoma technology thus essentially allows the isolation of murine antibodies which have a broad detection range and can be applied for diagnostic or research uses. However, their therapeutic applications are limited because repeated administration of murine antibodies can cause human anti-mouse antibody reaction (HAMA), reducing antibody half-life and have severe side effects including anaphylactic shock [26]. A strategy to circumvent these problems is antibody humanization or the use of transgenic animals where the original antibody gene repertoire is replaced with a human gene repertoire [34, 57, 70, 85]. A further strategy is the human hybridoma technology resulting in human antibodies [23, 42], but this technology is subjected – as the murine hybridoma technology – to the limitations of the immune system.

A technology which circumvents the limitation of the immune system is antibodyphage display. This technology is completely independent of any immune system by an in vitro selection process. The display method most commonly used today is based on the work of Georg P. Smith on filamentous phage, which infect *E. coli* [111]. The selection process is called "panning", referring to the gold digger's tool [92].

Phage display technology was further developed 1990/1991 for antibodies in three places in parallel: Heidelberg (Germany), Cambridge (UK) and La Jolla (USA) [12, 14, 22, 76]. Antibody phage display is described in detail by Tomszak et al. "Selection of recombinant human antibodies" in this book or by [35]. In brief, antibody fragments are displayed on the surface of M13 phage and the corresponding antibody gene is packaged in the phage particle, mainly using a phagemid. The most common antibody formats used for antibody phage display are the single chain fragment variable (scFv) [52, 107, 124] or fragment antigen binding (Fabs) [29, 45]. Other antibody formats used for phage display are single chain Fabs (scFab), human VH domains (dAbs), the variable domains of camel heavy chains (VHHs) and immunoglobulins of sharks (IgNARs) [46, 51, 83, 84, 88, 89]. Specific antibody phage can be selected from antibody gene libraries by "panning" on the desired target. Finally, monoclonal antibody phage or monoclonal soluble antibodies can be identified e.g. by ELISA. The antibody fragment genes can be recloned in any other antibody format, e.g. scFv-Fc or IgG [30, 35, 45, 52, 56].

The phage display libraries are built from immunized or non-immunized lymphocytes donors or from synthetic repertoires. Libraries using the naive IgM repertoire of a donor, which corresponds to the primary immune response, or synthetic antibody sequences are summarized as "single-pot" or universal libraries. These universal libraries are designed to isolate antibody fragments against every possible antigen, at least in theory [30, 129]. This library type was used to generate antibodies against viruses [61] or toxins [103]. Immune libraries are constructed from patients or immunized humans or animals. Also from these immune libraries, human antibodies were selected against viruses [105] or toxins [96]. Further details

about antibody gene libraries are given in the chapter 3 "Selection of Recombinant Human Antibodies" by Tomszak et al. or by [35].

To date, 44 antibodies and antibody conjugates were approved by EMA and/or FDA (status summer 2015) (http://www.imgt.org/mAb-DB/index) and about 350 antibodies were under development in 2013 [102]. Most approved therapeuticantibodies are for cancer and autoimmune diseases. Mechanisms of therapeutic antibodies are manifold and include neutralization of substances e.g. toxins [97] or cytokines like tumor necrosis factor (TNF) alpha [2], blocking of receptors like epidermal growth factor receptor (EGFR) [95], binding to cells and modulating the host immune system [18], or combinations of these effects [1]. To date, two recombinant antibodies are approved for the treatment of viruses and toxins. Raxibacumab is a human antibody for anthrax treatment derived from a phage display library from Cambridge Antibody Technology (now Medimmune) [75]. The antibody Palivizumab for the treatment of Respiratory syncytial virus (RSV) bronchiolitis is a classical humanised antibody [71].

An overview about recombinant antibodies derived from phage display against viruses and toxins is given in this review.

2 Recombinant Antibodies Against Viruses

Up to now a large panel of antibodies against various viruses has been generated from either naive or immune libraries using phage display technology. Panning against peptides, recombinant viral proteins, or complete virus particles has led to the identification of antibodies directed against human pathogenic viruses such as Sin nombre virus [125], Dengue virus [15, 106], Influenza virus [67, 113], VEEV [61], Norovirus [44], SARS coronavirus [114] or Hepatitis C [112] from naive antibody gene libraries. Other antibodies were selected from immune antibody gene libraries targeting e.g. WEEV [48], HIV [80, 122], SARS [59], Yellow fever virus [27] or Influenza virus [116, 119]. Even semi-synthetic libraries were used to generate antibodies specific for Influenza virus [9].

Libraries originating from different species have been successfully employed to isolate virus specific antibodies in the past. Among others, libraries were constructed from macaque [105], mouse [68], chimpanzee [39], llama [33] and human origin [120].

Most commonly virus specific antibodies have been isolated from libraries in scFv format [41, 131] but Fab libraries [132, 134] and VHH libraries [33] were also successfully used.

In the following paragraphs, we give detailed examples for antibody generation using phage display and antibody engineering against different virus groups.

Vaccinia Virus is the prototype virus in the genus of *orthopoxvirus*. It is a relatively large DNA virus with a genome of about 200 kbp [13]. The genus *orthopoxvirus* includes various species such as monkeypox virus, cowpox virus and especially variola virus which is the causative agent of smallpox in humans. Naturally occur-

ring smallpox has been eradicated in 1977 because of a massive WHO vaccination program that begun in 1967. However, no vaccination of the civilian population is conducted nowadays and potential threat of intentional release has renewed the search for safe and effective smallpox vaccines as case fatality rates of 30 % or more among unvaccinated subjects are reported [43]. Because orthopoxviruses are highly related, it is assumed, that immunity against one poxvirus goes along with immunity against most members of the entire virus family [13, 31]. Using an immune scFvphage display library constructed from vaccinia virus immunized patients, a panel of human vaccinia specific antibodies was selected. Plaque-reduction neutralization tests revealed that seven of these antibodies neutralized vaccinia as well as cowpox virus in vitro. Five of those antibodies additionally neutralized monkeypox virus [120]. Other antibodies were generated from a Fab immune library derived from vaccinia virus immunized chimpanzee. Converted into a chimeric chimpanzee/human IgG format, two antibodies displayed high affinities to vaccinia protein B5 (K_d of 0,2 and 0,7 nM). Antibody 8AH8AL was neutralizing in vitro for vaccinia and smallpox virus and proofed to be protective in mice challenged with vaccinia virus even when administered 2 days after challenge. In this model 8AH8AL proofed to provide significantly greater protection than that of the previously isolated rat anti-B5 antibody 19C2 [20]. Vaccinia had to be used as model, because the final confirmation of protection against smallpox is not possible.

Ebola Virus and Marburg Virus, two filoviruses, cause severe hemorrhagic fever and possess high mortality of up to 90 % in humans. In addition to public health concerns associated with natural outbreaks, Ebola virus might be a potential agent of biological warfare and bio-terrorism [40]. Human antibodies directed against Ebola Virus were selected from a library originated from patients that recovered from infection in the 1995 Ebola virus outbreak in Kikwit, Democratic Republic of Congo [74]. Several antibodies against various viral proteins such as nucleoprotein, envelope glycoprotein and secreted envelope glycoprotein have been isolated in this study. One antibody (specific for envelope glycoprotein), KZ52 was neutralizing in vitro as Fab (50 % neutralization at 0.4 μg/ml) and as full IgG (90 % neutralization at 2.6 μg/ml) [73]. Follow-up studies were showing effective protection in vivo in a Guinea pig Ebola challenge model when the antibody was administered up to 1 h post viral challenge [93]. Interestingly KZ52 was not protective in macaques challenged with Ebola even if the antibody was given as a two-dose treatment with the first dose 1 day prior viral challenge and the second dose 4 days post challenge [91]. A murine scFv and two shark IgNAR V immune libraries were generated against inactivated Zaire Ebolavirus to yield various antibodies specific for the viral matrix protein VP40 and the viral nucleoprotein [40]. Interestingly, this work represents the first example of a successful targeted IgNAR V isolation from a shark immune response library.

Dengue virus (DENV), a member of the *Flaviviridae* family, is responsible for at least 100 million symptomatic infections each year and has developed into a major health and economic burden in over 50 countries worldwide [28, 81, 82]. It is a positive strand RNA virus with a ~11 kb genome, that compromises a single open reading frame. The four circulating serotypes of dengue virus show approximately 70 %

sequence homology [81, 134]. Fab monoclonal antibodies to dengue type 4 virus were isolated from a chimpanzee immune library. Two Fabs, namely 5H2 and 5D9 neutralized DENV-4 efficiently with a titer of 0.24–0.58 μg/ml by plague reduction neutralization test [77]. Another study selected human scFvantibodies specific to dengue virus envelope protein by panning against recombinant full length envelope protein and its domain III [106]. Because DENV envelope protein is an essential molecule for virion assembly and virus entry, scFvs selected in this study were shown to exhibit inhibitory effects on DENV infection in vitro [106]. Dengue non-structural protein 5 (NS5) is essential for viral replication and host immune response modulation, which makes it an excellent target for dengue-inhibiting antibodies. A naive human Fab-phage library was screened for NS5 specific antibody fragments using various NS5 variants from Dengue Virus serotypes 1–4 as antigens for panning and characterization [134]. Using NS5 from alternating dengue serotypes for each round of panning, this strategy resulted in the identification of two clones that are cross-reactive against all four dengue serotypes. Another study selected antibodies using phage display by panning with Dengue virus particles directly captured from supernatant of infected Vero cells. Here, highly serotype specific antibodies could be generated. From a total of nine antibodies, seven were shown to be specific to only one serotype. One Dengue-3 selected clone cross-reacted with Dengue 1, whereas another clone showed cross-reactivity with all serotypes despite being selected solely on Dengue 2 particles. Interestingly, all of the obtained antibodies recognized several strains of distinct genotypes within the corresponding serotype [15]. Panning against dengue envelope protein identified an antibody (C9) from a mouse/human chimeric Fab library that crossreacts with DENV1-3 and neutralizes DENV2 in cell-based assays after conversion into full length IgG [81]. Besides scFv and Fab, also variable domain heavy-chain antibodies (VHH antibodies) were selected using phage display technology. After four rounds of panning on recombinant DENV 2 NS1 protein, 20 positive clones were selected. Affinity measurements with NS1 revealed a K_D value of $2{,}79 \times 10^{-8}$ M for the best VHH antibody P2 [33].

Venezuelan equine encephalitis virus (VEEV), an alphavirus of the Togoviridae family, causes equine epidemics but can also cause encephalitis in humans [58, 128]. Because this virus is classified as Category B agent by the Centers for Disease Control and Prevention (CDC), much research has been done to generate neutralizing antibodies against it. Antibodies were generated from an immune library from human donors targeting both VEEV envelope glycoproteins E1 and E2 [49]. The isolated Fabs L1A7 and F5 were neutralizing in vitro, with F5 being 300 times more effective than L1A7. Subsequently, F5 was converted into full IgG format and was employed to generate neutralization-escape variants of VEEV for epitope mapping. Within another study, an immune macaque library was used to generate human-like antibodies [105]. One of these antibodies, scFv-Fc ToR67-3B4, was protective in mice when it was administered 6 h post viral challenge with VEEV Trinidiad strains. Here, 80–100 % of mice survived a lethal viral dose. However, scFv-Fc ToR67-3B4 was not able to neutralize Trinidad strain but other VEEV strains in vitro, showing that neutralization is not mandatory for an in vivo protective antibody [105]. Another study describes the selection of antibodies from a human naive scFv gene library

using complete, active VEEV particles as antigen. Here, specific detection of the VEEV strains TC83, H12/93 and 230 by the selected antibodies was proven. Remarkably, none of the selected scFv phage clones did show any cross-reactivity with Alphavirus species of the Eastern equine encephalitis virus (EEEV) and Western equine encephalitis virus (WEEV) antigenic complex or with Chikungunya virus (CHIKV), making them ideal tools for the immunological detection and diagnosis of Alphavirus species [61]. Two different scFv antibody libraries were constructed from WEEV immunized macaques. Subcloned as scFv-Fc, three antibodies from these libraries specifically bound WEEV in ELISA with little or no cross-reactivity with other alphaviruses and were found to be neutralizing in vitro. In this study, the first antibodies against WEEV, that were shown to be neutralizing in vitro, were developed. About 1 ng/ml of the best antibody (ToR69-3A2) neutralized 50 % of 5×10^4 TCID50/ml WEEV [48].

An overview about recombinant antibodies generated by phage display against viruses is given in Table 1.

3 Antibodies Against Toxins

Several toxins are classified by the Center for Disease Control and Prevention (CDC) as category A or B agents that are relevant for diagnostics and therapeutics. They can easily be disseminated and result in high or moderate mortality rates [36]. Here, the antibodyphage display presents a powerful tool for antibody selection and allows the isolation of neutralizing antibodies against complete active toxins or special domains from different human naive antibody gene libraries with high diversity [5, 54, 86]. For the isolation of high-affinity antibodies against specific targets, animals are immunized with toxoids, non-toxic subunits or selected toxin domains. Alternatively, human material from immunized patients can be used for construction of immune libraries [17, 79, 96, 97].

In the following paragraphs, we give detailed examples for antibody generation using phage display and antibody engineering against different toxins.

So far, antibodyphage display was successfully used for antibody selection against a panel of toxins classified as category A agents, such as from *Clostridium botulinum* (botulism) [16, 25, 53, 79] and *Bacillus anthracis* (anthrax) [97] and also against different category B agents, such as staphylococcal enterotoxin B [66, 110] and ricin toxin from *Ricinus communis* [6, 96]. An example for a high-risk microorganism that produces the most toxic substances known with the highest risk of potential use as bioweapons is the Gram-positive, anaerobic, spore-forming bacterium *Clostridium botulinum* and other *Clostridium* subspecies. They are secreting eight different serotypes (A–H) of botulinum neurotoxin (BoNT). Five serotypes (A, B, E, rarely F and only one case of H) are known to cause human botulism, a disease characterized by flaccid muscle paralysis requiring intensive hospital care and passive immunization [8, 11]. Especially, serotype A is recognized as the most toxic substance known with LD_{50} values (lethal dose) of 1 ng/kg by intravenous and

Table 1 Recombinant antibodies derived by phage display against viruses

Virus	Antigen	Library type	Antibody format	Antibody origin	Application	Reference
Dengue	Dengue virus envelope protein E	Naive	scFv	Human	ELISA, immunofluorescent assay, in vitro neutralization	[106]
Dengue	Dengue NS5 protein	Naive	Fab	Human	ELISA, immunoblot, dot blot,	[134]
Dengue	Dengue virus envelope protein	Naive	Fab, IgG	Human, mouse (panel of hybridoma clones)	ELISA, immunoblot, IHC	[81]
Dengue	Dengue NS3	Naive	Fab	Human	ELISA, in vitro neutralization	[82]
Dengue	NS1 protein	Naive (non-immune)	VHH	Llama	ELISA, lateral flow immunochromatograpic assay	[33]
Dengue	N.d.	Immune	Fab, IgG	Chimpanzee	ELISA, immunoprezipitation, in vitro neutralization	[77]
Ebola	Nucleoprotein, envelope glycoprotein, secreted envelope glycoprotein	Immune	Fab, IgG	Human	ELISA, immunostaining, immunoprezipitation, in vitro neutralization	[73]
Ebola	Nucleoprotein	Synthetic	scFv, IgG	Human	ELISA, immunoblot	[109]
Ebola (Zaire)	Viral matrix protein VP40, nucleoprotein	Immune	scFv, IgNAR V	Mouse, Shark	ELISA, immunoblot	[40]
Hantavirus	Nucleoprotein	Immune	VHH	Llama	ELISA, immunoblot	[98]
Hendra and Nipah virus	Attachment envelope glycoprotein G	Naive	Fab, IgG	Human	ELISA, immunoprezipitation, Immunoblot, in vitro neutralization	[135]
Hepatitis A	Hepatitis A capsid	Immune	Fab, IgG	Chimpanzee	ELISA, in vitro neutralization	[108]

(continued)

Table 1 (continued)

Virus	Antigen	Library type	Antibody format	Antibody origin	Application	Reference
HIV Type 1	gp140 protein	Immune	scFv, scFv-Fc	Human	ELISA, immunoblot, immunoprecipitation, in vitro neutralization	[122]
HIV Type 1	p24	Immune	scFv	Mouse	ELISA	[80]
Infectious haematopoietic necrosis virus	N.d.	Naive (non-immune)	scFv	Mouse	ELISA, immunoblot, IHC	[68]
Influenza A	Hemagglutinin (stem region)	Semi- synthetic	scFv	Human (IGHV1-69)	ELISA, in vitro neutralization	[9]
Influenza A	H5 hemagglutinin ectodomain	Naive	scFv	Human	ELISA, flow cytometry, immunoprecipitation, in vitro neutralization, in vivo protection	[113]
Influenza A	Hemagglutinin (stem region)	Presumably immune	Fab	Human	ELISA, in vitro neutralization	[132]
Influenza A (H5N1)	Hemagglutinin	Synthetic	scFv	Human	ELISA	[131]
Influenza A (H5N1)	Hemagglutinin	Naive	scFv	Human	ELISA, in vitro neutralization, in vivo protection	[72]
Japanese encephalitis virus	Domains I, II, III of envelope protein	Immune	Fab, IgG	Chimpanzee	ELISA, immunoprezipitation, in vitro neutralization, in vivo protection	[39]
Japanese encephalitis virus	Envelope protein	Immune	Fab	Human	ELISA, immunoprezipitation, in vitro neutralization	[7]
Poliovirus	Capsid proteins VP1 and VP3	Immune	Fab, IgG	Chimpanzee	ELISA, in vitro neutralization, in vivo protection	[19]
Rabies virus	Glycoprotein	Synthetic	scFv, scFv-Fc	Human	ELISA, immunostaining, in vitro neutralization	[100]

Rabies virus	Glycoprotein	Immune	scFv, IgG	Human	ELISA, flow cytometry, in vitro neutralization	[63]
Rabies virus	N.d.	Immune	Fab	Human	ELISA	[47]
Rabies virus	Glycoprotein (antigenic site II)	Immune	Fab, IgG	Human	ELISA, immunostaining, immunoblot, in vitro neutralization, in vivo protection	[115]
SARS-CoV	S1 domain of spike protein	Naive (non-immune)	scFv	Human	ELISA, in vitro neutralization	[114]
Vaccinia, variola virus	Vaccinia B5 envelope protein	Immune	Fab, IgG	Chimpanzee	ELISA, in vitro neutralization, in vivo protection	[20]
VEEV	E1/E2	Naive	scFv, scFv-Fc	Human	ELISA, immunoblot, IHC	[61]
VEEV	E1	Immune	scFv, scFv-Fc	Macaque	ELISA, immunoblot, IHC, in vitro neutralization, in vivo protection	[105]
WEEV	N.d.	Immune	scFv, scFv-Fc	Macaque	ELISA, IHC, in vitro neutralization	[48]
West Nile virus	Domain I and II of WNV envelope protein	Naive (non-immune)	scFv	Human	ELISA, in vitro neutralization, in vivo protection	[41]
Yellow fever virus	Domain II of envelope protein	Immune	scFv	Human	ELISA, immunoblot, immunoprezipitation, in vitro neutralization	[27]

ELISA enzyme linked immunosorbent assays, *IHC* immuno histo chemistry, *VEEV* Venezuelan equine encephalitis virus, *WEEV* Western equine encephalitis virus

subcutaneous routes and 3 ng/kg by the pulmonary route [38]. BoNTs are composed of a disulfide bond-linked 50 kDa light chain and a 100 kDa heavy chain. The heavy chain contains two functional domains (Hc and Hn) that are responsible for toxin uptake into nerve cells by receptor-mediated endocytosis and for the translocation of the light chain across the membrane into the neuronal cytosol. Whereas the catalytic domain of the light chain is responsible for the BoNT toxicity. The current approach for treatment of botulism includes the application of human anti-botulism immunoglobulins, such as Baby botulism immune globulin (BabyBIG), or equine anti-toxin serum. But the human serum stock of BabyBIG is limited and the equine anti-toxin may cause hypersensitivity and serum sickness. Here, antibody phage display provides a technology to generate toxin-neutralizing antibodies against each serotype. For instance, a macaque immune library was used to isolate neutralizing scFv with nm affinities against the light chain of BoNT/A [16, 79], but also antibodies against the heavy chain or other relevant serotypes of BoNT are of therapeutical interest. Phage display technology was also used for isolation of single domain antibodies (VHH) after immunization of a llama with a cocktail of seven BoNT toxoids (A–F) [25]. Another approach was the generation of a human antibody gene library after inducing a BoNT/A-specific immune response by in vitro immunization [53]. Furthermore, antibody phage display was used to generate antibodies against other clostridial toxins such as from *Clostridium tetani* or *Clostridium difficile* [17, 50].

Anthrax, another serious infectious disease is caused by *Bacillus anthracis*, an aerobic, Gram-positive, spore-forming bacterium that is found in soils around the world. *Bacillus anthracis* secrets two toxins, the lethal toxin (LT) and the edema toxin (ET) [69]. Both toxins are composed of two subunits. The LT consists of the lethal factor (LF) and the protective antigen (PA), the ET is formed by the edema factor (EF) and PA. It was demonstrated that only LT has an essential role in the pathogenesis of anthrax [55]. The subunit PA is the basis of current vaccines and induces the generation of neutralizing antibodies. In combination with antibiotics, commercial monoclonal antibodies against PA, such as Raxibacumab, are commonly used for treatment [65]. In 2012, the FDA approved Raxibacumab to treat inhalational anthrax. Due to security issues the use of anti-PA antibodies alone is questionable, since PA could be modified and lose the recognized epitopes while retaining biological activity. An alternative to anti-PA antibodies are antibodies targeting the LF, such as 2LF, which was isolated from an immune library via antibodyphage display technology [97]. A combination of an anti-PA antibody with an anti-LF antibody could lead to a synergistic effect and improve the efficacy of the therapy.

An example for bacterial toxins classified as category B agent is staphylococcus enterotoxin B from *Staphylococcus aureus*. This bacteria are a potential causative agent for food-borne illness and produces 21 types of staphylococcal enterotoxins that cause symptoms of food poisoning including abdominal cramps, vomiting and diarrhea [90, 118]. The staphylococcal enterotoxin B (SEB), a single polypeptide of 28 kDa, is the most potent toxin secreted by *S. aureus*. As a superantigen, it stimulates T cells and leads to an overproduction of cytokines, causing clinical symptoms

such as fever, hypertension and in some cases death. Phage display was used to generate recombinant antibodies from a murine immune library [110] and to identify the epitope of a SEB specific monoclonal antibody using a peptide phage library [123]. Furthermore, a human monoclonal antibody against SEB was isolated from a synthetic human antibody gene library that inhibited SEB binding to MHCII [66].

The phage display technology was also used to isolate antibodies against ricin. Ricin is a 61 kDa glycoprotein from the castor bean plant (*Ricinus communis*), which consists of two distinct subunits (RTA and RTB). RTB is a galactose- and N-acetylgalactosamine specific lectin which binds to specific sugar residues on the cell surface, allowing internalization of the toxin by endocytosis [87], whereas RTA has an RNA N-glycosidase activity that irreversibly inactivates eukaryotic ribosomes resulting in inhibition of protein synthesis [32]. Ricin is also classified as category B agent by CDC. Human-like antibodies were selected by phage display from a macaque immunized with RTA. One antibody, 43RCA, had a picomolar affinity and neutralized the biological activity of ricin in vitro [96]. Furthermore, neutralizing antibodies with high affinities were selected from a llama immune library [6].

In addition to the different toxins that are classified by the CDC as category A or B agents, the number of relevant toxins is almost endless. In addition, different animals are known to produce high potential toxins containing a complex composition. For example, one of them is *Tityus serrulatus*, known as brazilian yellow scorpion, the most dangerous scorpion in Brazil. The major toxic component in the venom of *T. serrulatus* is the gamma-toxin, a polypeptide of 61 amino acid residues [60, 99]. Here, a neutralizing antibody was isolated from a human library via phage display and was protecting in mice [5]. The same procedure was used for *Bothrops jararacussu*, a venomous pit viper species endemic in south america. By using a human antibody gene library different antibodies were selected that inhibit the phospholipase activity of the venom in vitro and reduce the myotoxicity in vivo [104]. Toxins are also produced by marine organism. An example is the tetrodoxin (TTX) of the toxic puffer fish. Here, scFv were selected from a human naive antibody gene library neutralizing the TTX activity [21].

An overview about recombinant antibodies generated by phage display against toxins is given in Table 2.

4 Conclusion

Antibody phage display allows the generation of (human/camel/macaque/shark…) antibodies from mainly two types of sources: immune and naive libraries. Immune libraries should be preferred when immunized animals or convalescent patients are available, offering the chance to directly isolate neutralizing and/or protective antibodies. If immunization is not possible or ethically not feasible, naive antibody gene libraries are an alternative. In an such approach, the antibody generation process is

Table 2 Recombinant antibodies derived by phage display against toxins

Toxin	Antigen	Species	Library type	Antibody format	Antibody origin	Application	Reference
Anthrax	Lethal factor (LF)	*Bacillus anthracis*	Immune	scFv	Macaque	ELISA, in vitro toxin neutralization, in vivo protection	[97]
Anthrax	Protective antigen (PA)	*Bacillus anthracis*	Naive	scFv	Human	In vitro toxin neutralization, in vivo protection	[75]
Bacillus thuringiensis toxin	Cry1C d-endotoxins	*Bacillus thuringiensis*	Semi-synthetic	scFv	Human	ELISA, in vitro toxin inhibition	[127]
Bee venom	Crude venom	*Apis mellifera*	Semi-synthetic	scFv	Human	ELISA, in vitro toxin inhibition	[37]
Botulinum neurotoxin	Serotype A – light chain	*Clostridium botulinum*	Immune	scFv	Macaque	ELISA, in vitro toxin inhibition	[16]
Botulinum neurotoxin	Serotype A – light chain	*Clostridium botulinum*	Immune	scFv	Macaque	ELISA, immunoblot, in vitro toxin inhibition, ex vivo toxin neutralization	[79]
Botulinum neurotoxin	Serotype A – light chain	*Clostridium botulinum*	Immune	VHH	Camel	ELISA, immunoblot, in vitro neutralization	[117]
Botulinum neurotoxin	Serotype A – heavy chain	*Clostridium botulinum*	Immune	scFv	Murine	ELISA, ex vivo toxin neutralization	[3]
Botulinum neurotoxin	Serotype A – heavy chain	*Clostridium botulinum*	Immune	scFv	Human	ELISA, ex vivo toxin neutralization	[4]

Botulinum neurotoxin	Serotype A – heavy chain	*Clostridium botulinum*	Naive	scFv	Human	ELISA, ex vivo toxin neutralization	[4]
Botulinum neurotoxin	Serotype A – heavy chain	*Clostridium botulinum*	Immune	scFv	Macaque	ELISA, ex vivo toxin neutralization	Avril et al. (in revision)
Botulinum neurotoxin	Serotype B – light chain/heavy chain	*Clostridium botulinum*	Immune	scFv	Macaque	ELISA, in vitro toxin inhibition, ex vivo toxin neutralization	Rasetti-Escargueil et al. (accepted)
Botulinum neurotoxin	Serotype E – light chain	*Clostridium botulinum*	Immune	scFv	Macaque	ELISA, in vitro toxin inhibition, ex vivo toxin neutralization	Miethe et al. (in revision)
Botulinum neurotoxin	Serotype E – heavy chain	*Clostridium botulinum*	Immune	VHH	Dromedary	ELISA, in vivo protection	[10]
Botulinum neurotoxin	Serotype A/B/C/D/E/F	*Clostridium botulinum*	Immune	VHH	Llama	ELISA, in vitro toxin inhibition	[25]
Brazilian yellow scorpion venom	Gamma-toxin	*Tityus serrulatus*	Naive	scFv	Human	ELISA, in vivo protection	[5]
C. difficile toxin	TcdA	*Clostridium difficile*	Immune	VHH	Llama	ELISA, immunoblot, in vitro neutralization	[50]
C. difficile toxin	TcdA, TcdB	*Clostridium difficile*	Immune	VHH, bispecific VHH	Alpaca	ELISA, in vitro toxin inhibition, in vivo protection	[133]
Jararacussu venom	Phospholipase A2 (PLA_2)	*Bothrops jararacussu*	Naive	scFv	Human	ELISA, in vitro and in vivo protection	[104]
Mexican scorpion venom	Cn2	*Centruroides noxius*	Naive	scFv	Human	ELISA, in vivo protection	[103]

Table 2 (continued)

Toxin	Antigen	Species	Library type	Antibody format	Antibody origin	Application	Reference
Ricin	Chain A	*Ricinus communis*	Immune	scFv	Macaque	ELISA, in vitro toxin neutralization	[96]
Ricin	Chain A	*Ricinus communis*	Immune	VHH	Llama	ELISA, in vitro toxin neutralization	[6]
Shiga toxin	Stx1, Stx2	*E.coli* (*STEC*)	Immune	VHH	Alpaca	ELISA, in vitro toxin inhibition, in vivo protection	[121]
Staphylococcus enterotoxin B	SEB	*Staphylococcus aureus*	Immune	scFv	Murine	ELISA	[110]
Staphylococcus enterotoxin B	SEB	*Staphylococcus aureus*	Synthetic	scFv	Human	ELISA, immunoblot, in vitro toxin inhibition	[66]
Tetanus	Tetantus toxoid	*Clostridium tetani*	Immune	Fab	Macaque	ELISA	[17]
Tetanus	Tetantus toxoid	*Clostridium tetani*	Naive	scFv	Human	ELISA, in vitro toxin inhibition	[54]
Tetrodotoxin (TTX)	$C_{11}H_{17}N_3O_8$	*Lagocephalus lunaris*	Naive	scFv	Human	ELISA, in vitro and in vivo protection (prolonged survival)	[21]
Thai cobra venom	Neurotoxin	*Naja kaouthia*	Naive	scFv	Human	ELISA, immunoblot, in vivo protection	[64]

not limited by the immune system. Antibody phage display has provided a panel of antibodies useful for diagnostics and therapy against toxins and viruses.

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