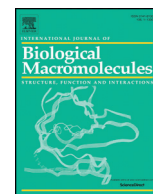




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Review

Infectious disease antibodies for biomedical applications: A mini review of immune antibody phage library repertoire



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ABSTRACT

Antibody phage display is regarded as a critical tool for the development of monoclonal antibodies for infectious diseases. The different classes of antibody libraries are classified based on the source of repertoire used to generate the libraries. Immune antibody libraries are generated from disease infected host or immunization against an infectious agent. Antibodies derived from immune libraries are distinct from those derived from naïve libraries as the host's *in vivo* immune mechanisms shape the antibody repertoire to yield high affinity antibodies. As the immune system is constantly evolving in accordance to the health state of an individual, immune libraries can offer more than just infection-specific antibodies but also antibodies derived from the memory B-cells much like naïve libraries. The combinatorial nature of the gene cloning process would give rise to a combination of natural and un-natural antibody gene pairings in the immune library. These factors have a profound impact on the coverage of immune antibody libraries to target both disease-specific and non-disease specific antigens. This review looks at the diverse nature of antibody responses for immune library generation and discusses the extended potential of a disease-specified immune library in the context of phage display.

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1. Introduction

The threat posed by emerging and re-emerging infectious diseases has highlighted the need to develop novel prophylactic and therapeutic strategies to overcome these infections. Moreover, emergence of

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multidrug-resistant organisms has spurred the need to develop alternate strategies to the standard antibiotics therapy regime. One such strategy is the use of human monoclonal antibodies (mAbs) that can function both in a prophylactic and therapeutic manner [1]. The breadth of mAb application in this area has seen a rapid increase of mAb trials over the past decade for a wide range of pathologies including infectious diseases [2]. The development of antibody display technologies such as phage display [3–5], yeast display [6], mRNA display [7], ribosome display [8], bacterial display [9–11] and mammalian cell surface display [12] have aided the rapid development of new mAbs. The basic principle of these *in vitro* selection technologies stems from the physical link between phenotype (displayed antibody construct) and genotype (antibody genes) tethered to the carrier particle [13]. Even with the availability of different display methods, phage display is widely regarded as the preferred approach for antibody display.

The general requirement for a successful mAb selection process is the existence of a diverse combinatorial repertoire of antibody genes from which to select. This collection of antibody genes is commonly referred to as an antibody library [14]. Antibody libraries can be distinguished by the source of antibody genes used for display. This ranges from naïve (healthy individuals), immune (infected or immunized individuals), synthetic (chemically synthesized) and semi-synthetic (a mixture of natural immune and chemically synthesized genes) libraries [14–16]. The development of human antibody libraries requires B-cells obtained from human donors which sometimes represents a bottleneck due to the specific characteristics of the samples needed such as source of the B-cells, and the strict regulation in terms of human biological samples usage in research. Apart from humans, animals are also a valuable source of B-cells for antibody library generation. In the context of infectious diseases, immune libraries are very attractive options as they are designed to mirror the immune response of an infected individual or immunized animal, reflecting the biased repertoire of antibody genes specific to that infection. This is only true in the case where the B-cells can elicit an immune response to the infection. Therefore, the nature of the immune response to a particular infection is the key to the design of an immune antibody library and shapes the utility, quality, and versatility of the library [17]. However, immune antibody library repertoires constructed *in vitro* by combinatorial mixing of immunoglobulin genes may not entirely reflect the true nature of the natural antibody repertoire as the random pairing of heavy and light chains may result in non-functional antibody clones with incorrect folding [18]. Here we review how the immune response to different infections can influence the identification of anti-infectives from an immune library perspective and refines the considerations of repertoire representation by immune antibody libraries for phage display.

2. Immune antibody library repertoires

B-cells, as a major component of the immune system safeguarding our body from harmful antigens, are constantly at work, producing antibodies prior to and following the occurrence of infection. The repertoire of antibodies in a healthy state is diverse enough to generate a response against new infections as well as to remember old encounters. This principal feature of B-cells is possible with the diverse repertoire attained from two primary mechanisms, being V(D)J recombination of the variable (V), diversity (D) and joining (J) gene segments and somatic hypermutation (SHM) [19,20]. This includes to a lesser extent unconventional secondary mechanisms that also increase diversity of the antibody repertoire including non-standard recombination that breaches the 12/23 rule of recombination, SHM-associated genetic insertions and deletions, direct antigen contact by non-complementarity determining regions (non-CDRs) of antibody, post-translational modifications, conformational heterogeneity and employment of non-protein cofactors [20,21]. These mechanisms together contribute to the diverse variations within the antibody CDRs, which form the primary antigen binding site. The events leading up to the generation of a mature

antibody gene are also multifaceted as the recombination of multiple variable genes furnishes a large combinatorial diversity to start with and is further expanded with varying heavy (V_H) and light (V_L) chain combinations (Fig. 1) [21]. Since SHM and related mechanisms are elicited upon encountering an antigen, exposure to an infection would influence the resulting repertoire as the antibody repertoire would be biased and shaped to combat the invading pathogen.

Despite that the repertoire of an immune antibody library offers better prospects in isolating disease-specific binders, the majority of the current phage-displayed antibody approaches focus primarily on the use of naïve repertoires to generate mAbs against infectious diseases, as evident in the majority of the US Food and Drug Administration (FDA) and European Medicines Agency (EMA)-approved phage display-derived mAbs [22]. The reason is mainly attributed to technical and cost implications rather than immunological characteristics. A naïve library, due to the unbiased nature of its repertoire, makes it ideal for the generation of antibodies against virtually any target molecule [15,23,24]. From a utility point of view, naïve library is a preferred single-pot library type for antibody development as it can be 'recycled' for multiple diseases as opposed to a disease-specific immune library which would have to be generated for each new disease. Further, the construction of multiple disease-specific libraries would face the additional challenge of obtaining proper clinical samples for each disease in addition to the constraints on the number of new libraries that can be reasonably generated. The affinity of antibodies obtained from naïve libraries has been reported to be weaker compared to immune libraries due to the lack of *in vivo* affinity maturation [25]. This would mean further downstream *in vitro* affinity maturation processes would have to be carried out. Considering the cost implication, time and effort to generate sub-libraries for a particular clone, having a lower affinity antibody from the naïve library is seen as a small compromise for the broader specificity contained therein.

2.1. Isotype-specific repertoire

The major challenge for library construction is the actual design strategy used for antibody generation. Since the majority of libraries are generated using a primer combination targeting the variable gene segment and the junction gene segment [26,27], no actual discrimination in terms of isotype responses is made unless isotype-specific reverse primers are used for repertoire generation. However, there is a clear benefit to the use of isotype-specific repertoires for immune library generation [28,29]. Analyzing this concept from an immunological standpoint: as B-cells are activated, class-switch recombination (CSR) occurs to allow switching of antibody production in an immature B-cell from IgM or IgD to isotypes IgG, IgA or IgE. IgG is further classified into four subclasses (IgG1, IgG2, IgG3 and IgG4) while IgA is further classified into two subclasses (IgA1 and IgA2) [30]. The CSR event is dependent on the T helper (Th) cell response, which is dependent on the nature of antigen as well as the primary invasion path [31]. To efficiently combat invading antigens, each of the isotypes possess a distinct role and distribution site in the body [32]. IgG is the most abundant isotype in plasma which is responsible for protection against extracellular infection [32]. IgA, the second most abundant immunoglobulin in plasma, also predominant in mucous secretions, may reflect the primary infection at mucosal surfaces [33–35]. IgE is the least abundant immunoglobulin in plasma which bound strongly to mast cells predominate under skin and mucosa layer [32]. Increase in IgE level is associated to parasitic infections such as helminth and protozoan infections [36,37]. The isotypes and subclasses can also reflect the progression and stage of infection, as demonstrated in the changes of the antibody population in early and late infection of measles [38,39] and human herpes virus 6 (HHV-6) [40,41]. Therefore, the choice of immunoglobulin isotype for repertoire generation based on the infection pathway and the infection stage are important aspects for immune library design as isotype-

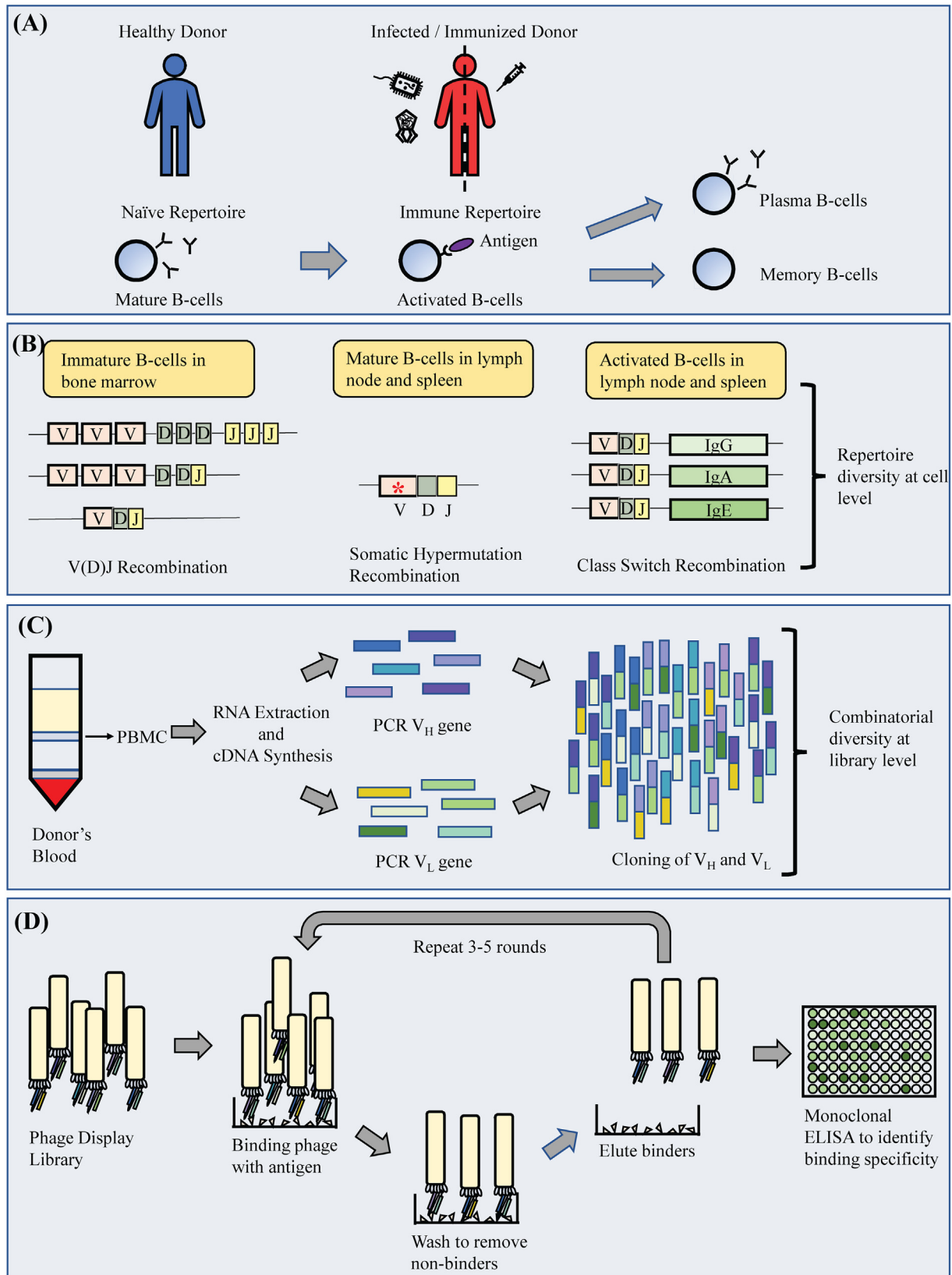


Fig. 1. Schematic diagram of monoclonal antibody generation from sampling to isolation of antibodies. (A) The antibody from mature B-cells of a healthy donor is referred for a naïve repertoire. Upon infection or immunization, the mature B-cells is activated to produce plasma B-cells and memory B-cells. This antibody pool is referred to as immune repertoire. (B) The natural antibody repertoire in a healthy individual is diversified via V(D)J recombination, and this represents a naïve antibody library repertoire. Upon infection or immunization, the natural antibody repertoire undergoes somatic hypermutation (SHM), class switch recombination (CSR) and a series of secondary mechanisms to form an immune antibody repertoire. (C) While the recombination of immunoglobulin genes in the variable region are primarily responsible for the diversity of the antibody repertoire, random combination of the heavy (V_H) and light (V_L) chain during library construction further enlarges the diversity of a library repertoire. (D) The diverse V_H/V_L gene pairs are then displayed on phages to form an antibody library. The phages are exposed to antigen immobilized on various surfaces for selection, usually subjected to 3–5 rounds of selection process. The final pool of antibodies is analyzed through monoclonal ELISA to isolate out monoclonal antibodies with high specificity.

specific gene sampling enables the collection of the most effective antibody repertoire.

2.2. Combinatorial repertoire

An immune antibody library can be constructed either in a non-combinatorial or combinatorial manner. A non-combinatorial library uses the original V_H/V_L pairing in a single B-cell whereas a combinatorial library relies heavily on random combinatorial mixing of the heavy and light chain repertoire [18]. The antibody gene repertoire available in an immune setting would provide a cocktail of sequences present during pre- and post-infection with a higher degree of gene complexity post-infection due to affinity maturation [42]. The repertoire from a post-infection environment would result in a repertoire that is biased against the infection. However, the combinatorial strategy will add complexity to the actual enriched gene representation by random pairing of heavy and light chain proteins to yield a final immune library repertoire [43]. Such an approach would lead to the generation of natural as well as unnatural V_H/V_L combinations which could result in functional or non-functional folding of clones within the library. In the context of antibody discovery, having both V_H/V_L gene pairings that are natural and unnatural in combination may expand the functional diversity of the library. Alternately, unnatural V_H/V_L pairing and CDR packing can also result in misfolding that can lead to aggregation of the antibody, rendering them ineffective [15].

2.3. Single variable domain antibody repertoire

As antibodies from mice and humans consist of two variable domains, the same combinatorial diversity is not represented in immune libraries generated from camelid and sharks as the antibodies are in single variable domain antibody (sdAb) format, i.e. VHH for camelid [44] and VNAR for shark [45]. These sdAbs also termed as nanobodies, contains only the V_H domain and exhibits some advantages in terms of solubility, stability, and target accessibility due mainly to their small size [46–48]. However, the absence of the V_L domain has limited repertoire diversification in comparison to humans as combinatorial pairing of V_H and V_L is not achievable. Instead, single variable domain immune library repertoire is depended solely on the inherent germline diversification mechanism upon immunization [49]. Even so, the available repertoire would generally consist of a hyperimmune repertoire with a combination of existing natural repertoires of the immunized host animal. Therefore, the diversity available although seen smaller in comparison to mammals is still adequate to yield high affinity antibodies. The single domain nature of sdAb would also mean that only a relatively smaller sized library is sufficient to adequately represent the immune repertoire making it less cumbersome to generate [50]. Nonetheless, to compensate for the shortcoming of the limited domain repertoire, efficient methods to construct high quality immune libraries are important as the host animal would also yield a mixed repertoire specific to a target as well as other exposed antigens. A classical restriction in antibody library generation processes is the cloning procedure. The quality of a sdAb immune library can be improved through alternative cloning strategies such as Golden Gate Cloning and negative selection using lethal *ccdB* gene [51]. In fact, these strategies could also be applicable to other libraries construction to maximize the library repertoire.

As the immune system is constantly surveilling and producing antibodies, this would mean that an infection-biased repertoire of an individual or host animal would also harbor remnants of antibodies against previously encountered antigens through memory B-cells [52]. Thus, immune repertoires arising from the memory of previous infections co-exist with repertoires from newly encountered infections. The extracted antibody repertoire would then reflect, at any given time, multiple infections derived from both plasma and memory B-cells collectively. In mammals, the bigger diversity caused by the random combination of antibody genes will lead to the generation of new V_H/V_L gene

combinations. This could stretch the value of the constructed repertoire in an immune antibody library for lead candidate discovery. However, the breadth of the antibody repertoire generated by a combinatorial approach can be further augmented by sampling immunoglobulin genes from multiple individuals or host animals to yield a repertoire of higher diversity. This will result in a diverse repertoire that could rival the naïve repertoire, allowing immune libraries to extend its application beyond a specific disease.

3. Application of immune antibody libraries for infectious diseases

The antibodies derived from immune antibody libraries have the potential to be applied as diagnostic reagents or therapeutic agents. Although immune libraries created from recovered patients are ideal for therapeutic mAbs, immune libraries can also be created from immunized animals such as mice, chicken, llama, alpaca, camel, sheep, shark and non-human primates (detailed in Table 1) for other applications. In Table 1 we listed some of the immune libraries developed for infectious diseases, with the details of the format and donor of the library.

3.1. Bacterial infections

Historically, bacterial infections were treated with anti-serum from animals and later with antibiotics. The evolution of bacteria has led to the surge of drug-resistant strains that are dampening the efficacy of antibiotic-based therapeutic strategies. As such, mAbs are touted as potential alternatives for antibiotic resistant bacterial infections. In general, bacterial toxins play a major role in enhancing infection. The tetanus toxoid immunized library is one of the first combinatorial immune antibody libraries constructed from the peripheral B-cell repertoire of an immunized human, from which multiple mAbs were successfully isolated but not evaluated for their neutralizing potential [53]. Since then, several immune libraries have been generated for studies against bacterial infections. An immune library for *Clostridium botulinum* reported antibodies that identified two key epitopes that were shown to prolong the time to neuroparalysis [54]. Another set of immune library-derived antibodies against the botulinum toxins possessed neutralizing activity in a phrenic nerve-hemidiaphragm assay [55]. An immune antibody library for *Pseudomonas aeruginosa* was reported to generate neutralizing antibodies targeting the Psl exopolysaccharide [56]. A recent report highlighted the development of neutralizing diphtheria antibodies from an immune library [57]. Raxibacumab, obiltoxaximab and bezlotixumab are exotoxins neutralizing antibodies approved by FDA for treatment of bacterial infections. However, mAbs currently under clinical trials apply a different approach by targeting cell surface proteins or polysaccharides. The antibody-bacteria complex formed will promote antibody-mediated opsonophagocytosis and antibody-dependent complement activation for bactericidal effect [58,59]. Of the three FDA-approved mAbs only raxibacumab is isolated by phage display platform while obiltoxaximab and bezlotixumab are chimeric- and hybridoma-derived mAbs, respectively.

3.2. Viral infections

Immune antibody libraries have also been utilized extensively to discover neutralizing mAbs against viral infections such as dengue fever [60], Ebola virus disease [61,62], hepatitis B [63–65], human immunodeficiency virus (HIV) infection [66–70], influenza [71], measles [72], rabies [73] and respiratory syncytial virus (RSV) [74]. The common and effective strategy for majority of commercial anti-viral mAb development is focused on inactivating the virus *via* binding of antibodies to the virus surface receptor such as envelope glycoprotein (gp), spike protein and the receptor binding domain (RBD) at the initial stage of infection so that internalization of the virus into host cells can be inhibited [75]. The diversity of the antibody repertoire of an immune antibody

Table 1
Summary of some infectious disease-specific immune libraries from different hosts, constructed in different formats and displayed on phage display platform.

Type of infection	Species	Targeting site	Antibody format	Donor	Reference	
Bacterial	<i>Bacillus anthracis</i>	Live spore	scFv	Mouse	[89]	
		S-layer protein EA1	VHH	Llama	[90]	
		Lethal factor (LF)	scFv	Macaque	[91]	
		Edema toxin (EF)	Fab	Chimpanzee	[92]	
	<i>Brucella melitensis</i>	Whole cell	scFv	Mouse	[93]	
		<i>Clostridium botulinum</i>	Botulinum neurotoxin serotype A (BoNT/A)	scFv	Human	[54]
	BoNT/A, BoNT/B		scFv	Macaque	[55]	
	<i>Clostridium difficile</i>	Toxin A (TcdA)	VHH	Llama	[94]	
	<i>Clostridium tetani</i>	Tetanus neurotoxin (TeNT)	scFv	Human	[95]	
	<i>Corynebacterium diphtheriae</i>	Diphtheria toxin (DT)	scFv	Human	[57]	
	<i>Escherichia coli</i>	Shiga toxin (Stx)	VHH	Alpaca	[96]	
	<i>Haemophilus influenzae</i>	Capsular polysaccharides	Fab	Human	[97]	
	<i>Helicobacter pylori</i>	Cell lysate, urease	scFv	Human	[98]	
	<i>Mycobacterium avium</i>	Cell lysate	scFv	Sheep	[99]	
	<i>Mycobacterium tuberculosis</i>	α-Crystalline	scFv	Human	[100]	
	<i>Staphylococcus aureus</i>	Staphylococcal enterotoxin B (SEB)	scFv	Mouse	[101]	
	Viral	<i>Dengue virus</i>	–	Fab	Chimpanzee	[60]
		<i>Ebola virus</i>	Envelope glycoprotein	Fab	Human	[61]
			VP40	scFv	Mouse	[62]
			Nucleoprotein	VNAR	Shark	
Foot-and-mouth disease virus		3ABC	scFv	Chicken	[102]	
Hantavirus		Nucleoprotein	VHH	Llama	[103]	
		Envelope G2 protein	Fab	Human	[104]	
Hepatitis A virus		Capsid	Fab	Chimpanzee	[105]	
Hepatitis B virus		Surface antigen	Fab	Human	[63,64]	
		Surface antigen	IgG	Human	[65]	
Hepatitis C virus		Core protein, envelope E2 protein	scFv	Human	[106]	
		core protein	Fab	Human	[107]	
Hepatitis E virus		ORF2	Fab	Chimpanzee	[108]	
Herpes simplex virus		Glycoprotein, virus lysate	Fab	Human	[109,110]	
Human immunodeficiency virus Type 1 (HIV-1)		gp120	Fab	Human	[66]	
		gp120	scFv	Human	[68]	
		gp140	Fab	Human	[69]	
		gp140	scFv	Human	[111]	
		gp140	VHH	Llama	[70]	
		gp125	Fab	Human	[67]	
Human immunodeficiency virus Type 2 (HIV-2)		Hemagglutinin (HA) glycoprotein	scFv	Human	[71]	
		HA	Fab	Human	[112]	
Japanese encephalitis		Envelope protein	Fab	Human	[113]	
Marburg virus		Glycoprotein	scFv	Macaque	[114]	
Measles virus		Measles virus protein	Fab	Human	[72]	
Polio virus		Capsid protein	Fab	Chimpanzee	[115]	
^a Rabies virus		Glycoprotein	scFv	Human	[73]	
Respiratory syncytial virus		F glycoprotein	Fab	Human	[74]	
SARS-CoV		Spike protein	scFv	Chicken	[116]	
	S1 protein	scFv	Human	[117]		
VEEV	S and M protein	Fab	Human	[118]		
	TC83	scFv	Mouse	[119]		
West Nile virus	Envelope protein	scFv	Human	[120]		
	Envelope protein	Fab	Human	[121]		
Parasitic	<i>Brugia malayi</i>	BmR1	scFv	Human	[83]	
	<i>Brugia malayi</i>	BmSXP	scFv	Human	[84]	
	<i>Plasmodium falciparum</i>	Pfs48/45	scFv	Human	[79]	
		MSP-1	scFv	Human	[80]	
	<i>Plasmodium vivax</i>	DBP	scFv	Human	[122]	
	<i>Taenia solium</i>	TS14	VHH	Camel	[81]	
	<i>Toxoplasma gondii</i>	TgMIC2	scFv	Mouse	[82]	

^a mAb against rabies virus isolated from immune library is named as foravirumab (CR4098) and it is currently under clinical review together with rafvirumab (CR57) in the form of a cocktail (CL184) [73,86].

library can also be leveraged for the isolation of broadly neutralizing mAbs. Studies on mAbs from immune antibody repertoires demonstrate cross-reactivity against conserved epitopes on multiple viruses from the same family or subtype, as evident in a study of H5N1 influenza, where more than 300 monoclonal antibodies isolated from a combinatorial immune library created from H5N1 influenza survivors are able to neutralize H1 and H5 subtype of influenza viruses [76]. This illustrates the application of a combinatorial immune library for the selection of antibodies against closely related antigens that carry similar epitopes. Immune libraries are also used extensively in HIV studies, mainly to isolate neutralizing antibodies against the glycoprotein

gp120 and gp41 [66,69,77]. The panel of antibodies generated from these HIV-1 immune libraries demonstrated broadly neutralizing characteristics against different subtypes [70,77]. The use of an immune library for other disease has also been demonstrated. HIV-1 positive patients are often associated with opportunistic infections. Therefore, an immune library constructed from HIV-1 patients was successfully used to isolate neutralizing antibodies against RSV FG glycoprotein [74]. Collectively, these studies illustrate the utility of combinatorial immune antibody library repertoires towards isolating functional antibodies with inter-species and inter-strain specificity beyond the original infection. This is especially true in the case of viral infections as viruses

undergoes rapid mutation, often resulting in multiple subtypes that share a certain degree of conserved regions or mechanism of infection. The identification of broadly neutralizing mAbs from combinatorial immune libraries demonstrates diversity of the antibody repertoire used in the library that is able to provide coverage for closely related antigenic motifs.

3.3. Parasitic infections

Parasitic diseases caused by protozoa, helminths and ectoparasites are also a major healthcare burden. One of the most common examples is the mosquito-borne protozoa, *Plasmodium*, that causes malaria [78]. Immune libraries have been generated to isolate mAbs against *Plasmodium falciparum* Pfs48/45 gamete surface protein [79] and Block 2 region of *P. falciparum* merozoite surface protein-1 [80] as well as other parasites such as *Taenia solium* Ts14 glycoprotein [81] and *Toxoplasma gondii* MIC2 protein [82]. Only the mAbs targeting gametocyte surface protein of *P. falciparum* blocked development of the parasite in mosquito upon bloodmeal ingestion [79]. This showed the potential use of immune library-derived antibodies to block transmission of parasitic disease. The other mAbs developed were only evaluated based on the binding capacity to the target antigen. Another immune antibody library derived from lymphatic filariasis (LF)-infected donors was developed to isolate mAbs against LF-related antigens, BmR1 and BmSXP [83,84]. A parallel experiment using immune and naïve libraries resulted in more unique clones with better binding from the immune library, suggesting the ability of immune libraries to better generate mAbs against targets of a specific infection [83].

Currently, raxibacumab, a naïve antibody library-derived mAb against *Bacillus anthracis* is the only anti-infective approved by the FDA for market [85]. Raxibacumab functions as a neutralizing antibody that prevents binding of the protective antigen (PA) of anthrax toxin to host receptors, curbing the subsequent release of lethal factor (LF) and edema factor (EF) into the cell, thereby halting disease progression [85]. A human immune single-chain variable fragment (scFv) library isolated antibody against rabies virus glycoprotein antigenic site III, named foravirumab (CR4098), is currently under phase II clinical trial in the form of a cocktail with rafivirumab (CR57), a mAb derived from somatic cell hybridization targeting rabies virus glycoprotein antigenic site I [73,86]. Despite that the only anti-infective approved clinically to date was derived from a naïve phage display library, the growing number of neutralizing antibodies described from immune libraries in research laboratories and the clinical trial of foravirumab suggests that immune library derived mAbs will soon be making an impact in the pharmaceutical industry. Nonetheless, the cost of mAbs as passive immunotherapy, mainly incurred by the perishable characteristic and complicated administration, is remained as the chief obstacle for widespread usage of mAbs especially in low income families and countries. With the maturity of mAbs discovery technologies and availability of more mAbs, it would hopefully provide a more cost-effective solution for infectious diseases with no effective drug treatment [87,88].

4. Conclusion

Antibody gene repertoire is the vital parameter for the success of any antibody library generated. The ability to extract the antibody gene repertoire of an individual post recovery and display it for functional binding permits the discovery of target-specific antibodies for therapeutic and diagnostic applications. This disease-constrained repertoire can be an important source of high affinity antibodies for disease-specific antigens. Immune libraries have the added potential of generating cross-reactive antibodies with homologs of protein from the same family and closely related diseases like viral disease that are caused by different strains of the same virus. Therefore, in the context of immune antibody library repertoires, one should understand that it is a collection of preferentially expressed, disease preferring antibodies resulting from gene

segment rearrangements or random combination of heavy and light chain sequences in mammals. Several considerations should be put in place when designing an immune antibody library. This includes the isotype source as a reflection of infection route, agent or stage, and recombination as a potential source of novel reactivity. In conclusion, the potential of immune antibody libraries transcends its ability to just generate high affinity disease specific mAbs but also has the diversity to produce mAbs against other target proteins making it an indispensable alternative to naïve libraries in antibody phage display development laboratories.

Author statement

Jing Yi Lai drafted and edited the manuscript.

Theam Soon Lim conceptualised the article, reviewed and edited the manuscript.

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Author contributions

JYL and TSL wrote the manuscript.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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