



Engineering Antibodies for the Treatment of Infectious Diseases

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Abstract

Historically, serum therapy was previously used to combat infectious pathogens. However, serum sickness and anaphylaxis limited its broad application. The advancement of antibody engineering technologies has made it feasible to generate monoclonal antibodies. There are divergent methods for antibody engineering and optimization. In this chapter, we summarized the latest developments in engineering antibodies for infectious diseases.

Keywords

Engineering antibodies · Pathogens · Treatment

10.1 Introduction

The discovery of antibiotics has saved countless patients from pathogens. Thus, widespread availability, high efficacy and low costs make antibiotics a cornerstone of modern medicine. However, after many decades of empiric broad-spectrum antibiotics use and misuse, multiple antibiotic-resistant pathogens are emerging. These pathogens include, but not limited to, methicillin-resistant *Staphylococcus aureus*, extreme drug-resistant *Mycobacterium tuberculosis* and *Plasmodium falciparum* [17]. Antibiotic-resistant pathogens pose a threat to global health. Additionally, broad-spectrum antibiotics bring the risk of perturbing normal and beneficial microbiome *in vivo* [36]. The perturbations or dysbioses of normal or beneficial microbiome has been linked to a range of diseases including *Clostridium difficile* associated diarrhea, diabetes, obesity and immune defects [10]. These threats on public health worldwide are detrimental.

Clearly, there is a need to explore new weapons to combat infectious diseases. Among many alternatives, antibody-based therapy represents a major interest with the success in other diseases such as tumors (Opdivo and Keytruda), autoimmune diseases and inflammatory conditions (Remicade, Humira) [58]. With the advancement in antibody engineering, production and optimization, antibodies may hold great promise in combating infectious diseases. If successful, antibody-based therapy will provide precision weap-

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ons to destroy pathogens without comprising normal or beneficial microbiome.

Antibody-based therapy for infectious diseases is not new. As far as in the 1890s, serum immunoglobulin was used to neutralize bacterial virulence [7]. Later, serum therapy was applied to a wide range of other infectious diseases such as *meningococcal meningitis*, *pneumococcal pneumonia*, *streptococcal* scarlet fever, varicella, measles, the pandemic *Spanish influenza* [15] and Ebola virus disease as well as cytomegalovirus [14, 31]. However, adverse reactions such as serum sickness and anaphylaxis harmed the efficacy of serum therapy [9]. With the discovery of antibiotics, serum therapy in infectious pathogens quickly declined. Antibiotics therapy soon became a mainstay of bacterial infections treatment. However, the success did not last long with the emergence of antibiotic-resistant pathogens. The failure to develop new antibiotics with different mechanisms of actions again trapped patients by the super drug-resistant bacteria. Besides, for individuals with compromised immunity, antimicrobial chemotherapy provides a less effective treatment [28]. Obviously, antibiotics are ineffective at eliminating viruses. The emergence of antibiotics resistance calls for renewed efforts. Among the many options, monoclonal antibody (mAb) may be an alternative, reminiscent of the successful application of anti-toxin in fighting against *meningococcal meningitis* in pre-antibiotic era. Indeed, Nebacumab, the first human therapeutic mAb reviewed by a regulatory agency, was engineered for the treatment of sepsis and gram-negative bacteremia [61]. However, the CHES (Centocor: HA-1A Efficacy in Septic Shock) trial conducted in the USA which found non-statistically significant increase in mortality between the monoclonal anti-endotoxin antibody Nebacumab and placebo groups in patients with gram-negative bacteremia lead to the withdrawal of Nebacumab [20]. Currently, only two humanized antibodies Palivizumab and Raxibacumab are approved for infectious diseases. Many other engineering antibodies against infectious pathogens are under development [2, 5, 16, 45, 67]. In this chapter, we summarize existing technologies for antibody engineering and optimization.

10.2 Serum Therapy in the Pre-antibiotics Era

Before the discovery of antibiotics, convalescent serum was the first effective strategy to combat infectious pathogens such as *diphtheria*, *tetanus*, *hepatitis B*, *varicella*, and *cytomegalovirus* et al. [13, 14]. It was later discovered that the mechanisms behind it were predominantly due to neutralizing activity and effector functions of immunoglobulins in serum. Antibodies derived from immune sera are polyclonal in nature. However, problems such as lot-to-lot variation and the immune response against animal-derived antibodies contributed to the reduced efficacy of serum therapy. Besides, the preparation of these serum products were expensive and labor-intensive. These complications together with the discovery of antibiotics limited the application of serum therapy in infectious pathogens after 1935. Nevertheless, this early attempt opened up the possibility of antibody-based therapies in infectious diseases.

10.3 Hybridoma Technology

The year 1975 witnessed the development of monoclonal antibody by mouse hybridoma technology [40]. This innovation makes it possible to produce large quantities of antibodies with a defined specificity *in vitro*, paving the way to use therapeutic monoclonal antibodies to treat different diseases. For example, E5 (XOMA Corp, Berkeley, Calif), an IgM antibody produced by hybridoma technology, was designed to neutralize endotoxin, the lipopolysaccharide component of the outer membrane of the J5 mutant of *Echerichia coli* [29]. The inconclusive results generated by clinical trials of E5 led to the withdrawal of this anti-endotoxin mAbs [29, p. 5].

Mouse monoclonal antibodies are limited by short-half life in circulation, inability to trigger human effector functions and the generation of human anti-mouse antibodies (the HAMA response). In an attempt to reduce the immunogenicity of therapeutic antibodies, efforts were directed to produce antibodies by using antibody engineering technology. Teng et al. generated a

human monoclonal IgM antibody HA-1A by fusing human immunized B lymphocytes with heteromyeloma cells [61]. HA-1A was designed to fight against gram-negative bacteremia and shock by binding specifically to the lipid A domain of endotoxin. Clinical trials showed no overall benefit of HA-1A, but significant improvement in the survival rate in a subgroup of patients [76]. The common adverse events (hypersensitivity or allergic reactions) in children with *meningococcal* septic shock who were treated by HA-1A occurred at low rates (<2%) [26].

10.4 Approaches to Generate Humanized Antibodies

Traditional hybridoma technology generates antibodies with high immunogenicity. Such therapeutic antibodies *in vivo* invoke HAMA response. To attenuate the immunogenicity, several approaches are present to reduce non-human fragments in antibodies (Fig. 10.1).

10.4.1 “CDR Grafting” Method

Many other strategies such as recombinant DNA methods are used to attenuate the human immune response, by reducing levels of non-human fragments in mAbs. Chimeric antibodies are produced by replacing mouse constant region with the human constant region. The generated anti-

bodies with mouse variable regions and human constant regions were perceived as less immunogenic than mouse monoclonal antibodies. Nonetheless, human anti-mouse response still exists with the use of chimeric antibodies.

To further minimize the mouse component in the antibodies, murine-derived CDR loops responsible for antigen binding were grafted into the human variable-domain framework. Caution should be noted that such replacements often change the structural relationship between CDRs and framework regions (FRs). Additionally, the change of CDR loops may influence binding properties of humanized Abs [3]. Thus, additional molecule engineering is needed to restore the binding capacity of humanized antibody [53].

“CDR grafting” method are successfully used to generate “humanized” antibodies which are able to evoke immune effector functions such as ADCC and phagocytosis more effectively. However, the murine-derived CDR loops can still evoke the immune response in patients. To minimize the anti-idiotypic response *in vivo*, several strategies were exploited.

10.4.2 Specificity-Determining Residue (SDR) Grafting

In most cases, only part of the entire CDR residues are involved in antibody-antigen complexes. These residues located in the region of high variability are designated as specificity-determining residues

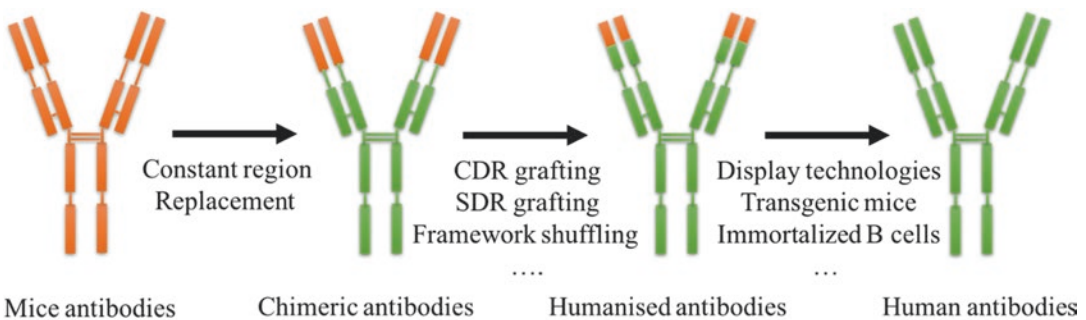


Fig. 10.1 Chimeric antibodies and humanized antibodies
The orange segments originates from murine, the green segments are from human.

(SDRs) [38]. Grafting the murine-derived SDRs onto the frameworks of human antibody generates humanized antibody with minimized immunogenicity. However, this strategy needs the identification of SDRs. Besides, the structure features of the target antibody should be preserved. Residues which are involved in VL-VH contact should also be preserved. Considerations should be taken that SDR grafting strategy usually generates antibodies with a reduced antigen-binding capacity. Thus, additional *in vitro* molecule engineering is needed to enhance antigen-binding affinity [23].

10.4.3 Framework Shuffling

“Framework shuffling” is another humanization approach by synthesizing a combinatorial library comprising CDRs of the non-human antibody fused in frame to pools of synthetic human germline frameworks. The corresponding libraries are then screened for antibodies with specific binding properties. This approach is simple, without requiring prior antibody structural knowledge, CDRs analysis or framework design [21, 22].

10.4.4 Human String Content Optimization (HSC)

Lazar et al. came up with a new paradigm termed “human string content” (HSC) for reducing immunogenic potential [43]. This approach is based on a metric of antibody humanness by comparing the non-human sequence with the human germline gene repertoire. The diversity of substitutions in a given variable heavy or light chain are scored as HSC. The targeted sequence is then humanized by maximizing its HSC.

10.4.5 Superhumanization

Superhumanization can be used to humanize mouse antibody. This method relies on comparison of CDRs. First, hypervariable loops of mice

are compared with those of human. Variable sequences of human which are similar or identical to mice are then selected. Then, within the selected genes, those who share the highest homology to the mice sequence are used to construct a humanized antibody. Using this approach, antibodies with designed antigen-binding specificity and neutralizing activity can be generated [59].

10.5 Engineering Fully Human Monoclonal Antibodies

Although several humanized antibodies are approved for marketing, to some extent, humanized antibody still elicits human anti-human antibodies (HAHAs) response. For example, a patient after receiving adalimumab treatment (an effective treatment for rheumatoid arthritis) over a period of time, lost his initial response to adalimumab due to the formation of HAHAs [4]. Besides, humanization of antibodies can be labor intensive, comprising complex procedures including sequence analysis, developing engineering technology, assessing binding properties, and evaluating HAHAs response. Therefore, developing fully human antibodies is a long pursuit. Several technologies including display technologies, transgenic animals, immortalized B cells and transchromosomal calves are available to generate fully human antibody.

10.5.1 Display Technologies

In vitro display technologies are among of the most commonly used methods for fully human antibody generation. Advantages of *in vitro* display technologies are overcoming immunological tolerance and generating antibodies against defined antigens. Specifically, phage and yeast display systems are best exemplified. Other display systems such as ribosomes, bacteria and virus display are also used to display repertoires of single-chain variable antibody fragments

(scFvs), antigen-binding fragments (Fabs) or domain antibodies (Dabs) on their surface.

These techniques mainly contained three steps:

1. Antibody Library Construction

Amplify human immunoglobulin variable regions derived from human B cells.

2. Surface Display Vector Construction

The antibody library is cloned into the plasmid and transformed (transfected) in the host cell to construct surface display vector.

3. Desired Antibody Screening

Desired antibodies are isolated by panning the library against the target antigen/epitope. Antibodies that don't bind to the antigen are washed away and the binders are retained. The corresponding genes of variable regions are cloned into whole human IgG expression vectors and expressed in orthologous mammalian system to generate human mAbs.

Huse et al. first introduced a lambda phage-based system which expressed a collection of functional antibody fragments in *Escherichia coli* [32]. However, it was laborious to distribute the libraries on agar plates. Besides, this method only assessed repertoires of roughly 1×10^7 , less than the natural antibody repertoire of 1×10^8 in each individual [58]. Later, progress was achieved in the area of display technologies by extending the library size and quantity. Theodore et al. produced mAbs against the West Nile virus (WNV) E protein by yeast surface display [47]. *In vitro* studies showed that one mAb could neutralize 10 different strains of WNV [47]. The animal experiment demonstrated the efficacy of the mAb against WNV by administering the mAb in a mouse model of WNV infection [47].

Different display systems may differ in antibody folding efficiency, post-translational modification and epitope accessibility which may

generate antibodies with different binding properties [46]. For example, Bowley et al. compared yeast display and phage display by using the same HIV-1 immune scFv cDNA library and the same selecting antigen (HIV-1 gp120) [11]. Their results showed that yeast display system generated more full scFvs than phage display system [11]. Besides, yeast display system produced many more novel antibodies [11].

There are many display platforms, including phage display, ribosome display, yeast display et al. Among these display systems, phage display-based selections are most often preferred for the generation of fully human antibodies. Other display methods can be used as complementary methods. Display technologies have become a standard method to generate antibodies with high affinity. However, a drawback is that the antigen must be known prior, in order to select for antibodies with high affinity.

10.5.2 Transgenic Mice

Another strategy to generate human mAbs is by using human immunoglobulin transgenic mice [12]. With the development of embryonic stem cell and gene transfer methods, transgenic mice are generated to carry human immunoglobulin gene. These transgenic mice have a normal humoral immune response [34]. B cells that produce human antibodies are harvested from immunized transgenic mice, and then cloned either as hybridomas or *in vitro* combinatorial library. Antibodies obtained via transgenic mice are of good antigen-binding properties with low immunogenicity. There are three main transgenic mice platforms including Xenomouse, HuMab mouse and VelociMouse [8, 24].

However, flaws may exist when using transgenic mice method. Immune tolerance may arise when using immune antigens that are highly homologous to mouse. Thus, more immunizations or antibody screens are needed.

Transgenic mice are used to generate human antibodies against infectious pathogens including

bacteria and virus. For example, Coughlin et al. generated a human monoclonal antibody against severe acute respiratory syndrome coronavirus (SARS-CoV) using XenoMouse [18]. Paul et al. generated a Human mAb (V2L2-MD) by immunizing humanized VelocImmune mice. The generated mAb is specific for *P. aeruginosa* PcrV, and can protect murine infection models from *P. aeruginosa* challenge [66].

10.5.3 Immortalized B Cells

Memory B cells are produced after infection or vaccination, and these antigen specific B cells could persist for a lifetime. Thus, memory B cells can be used as a source of human mAbs. After immortalization by Epstein Barr Virus (EBV), the antigen-specific B cells can be cultured to secrete a large amounts of antibodies. The surface immunoglobulins of EBV-immortalized B cells can be used to select antigen-specific B cells by fluorescence-activated cell sorting (FACS). Traggiai E et al. used this method to isolate 35 human monoclonal antibodies against SARS-CoV, and found one antibody exerted effective protection *in vivo* [64]. Selection of antibodies against H5N1, HCV, HIV and CMV by EBV-immortalized B cells were also reported [55, 57, 62, 68].

Producing human antibodies by EBV-immortalized B cells was limited by the low efficiency of EBV-immortalization. This limitation is overcome with the discovery that a TLR agonist can increase the efficiency of EBV B-cell immortalization and promote cloning of immortalized B cells [64]. Now, EBV-immortalized B cells has become an interesting shortcut to generate human monoclonal antibodies by isolating memory B cells from a donor who is infected or vaccinated by infectious pathogens.

10.5.4 Transchromosomal Calves

Transchromosomal calves are recently used to produce large-amounts of human mAbs by transferring mammalian artificial chromosome vectors

carrying human Ig loci to bovine primary fetal fibroblasts to produce cloned transchromosomal (Tc) calves [42]. The cloned Tc calves will contain a high rate of artificial chromosome positive cells. The Ig loci transferred in the cells undergoes rearrangement, diversification and expression. Secreted immunoglobulins in circulation will then be detected [42]. Production of Tc cattle is limited by the mitotic instability of human microchromosomes and the difference in immunophysiology between cattle and humans [42].

10.5.5 Immunospot Array Assay on a Chip (ISAAC)

Immunospot array assay on a chip (ISAAC) is a rapid and efficient method to generate antibodies from single cells [37]. This method uses a microarray chip whose surface is coated with antibodies against immunoglobulins to trap antibody-secreting cells (ACs) [37]. ACs are cultured on a chip for several hours. The antibodies secreted by ACs bind to labeled antigens to form circular spots which are easily identified [37]. Then, the antigen-specific ACs are retrieved to obtain antibody cDNA. The obtained antibody cDNA is inserted into expression vectors, and is transfected into cells for expression. The secreted antibodies can be detected by ELISA assay [37]. Jin et al. applied this system to produce human mAbs against viruses within a week, demonstrating ISAAC to be an alternative strategy for human mAbs generation [37].

10.5.6 New Technologies for Monoclonal Screening and Discovery

1. Next-generation sequencing technologies

Traditional *in vitro* monoclonal antibodies engineering technologies are laborious which depends on high-throughput screening of immortalized B cells. Reddy et al. developed a new method for antibody isolation bypassing antibody screening [50]. They used next generation

sequencing and bioinformatics analysis to analyse the variable-gene repertoires from bone marrow plasma cells. They found that the variable region of immunoglobulin derived from immunized mice became highly polarized. The high-throughput DNA sequencing enabled them to identify several abundant V_L and V_H gene sequences rapidly [50]. V_L and V_H genes with relative frequencies within the repertoire were paired and synthesized by oligonucleotide and PCR assembly. Single-chain variable fragments and full-length IgG were expressed in expression systems. Most of the antibodies produced by this method were antigen specific [50].

Next-generation sequencing is also applied to sequence single antibody-secreting B cells. DeKosky et al. used next-generation sequencing technologies to identify large numbers of V_H and V_L in a single B cell repertoire [25]. They first isolated single B cells from tetanus toxoid immunized human by depositing B cells in a high-density microwell plate. mRNA was then captured and reverse transcribed by RT-PCR. The sequence information of the transcripts was analyzed by next-generation sequencing technology [25].

2. Mass spectrometry

Wine et al. combined next-generation sequencing technology with mass spectrometry to deconvolute the polyclonal serum response after immunization [69]. They sequenced cDNA derived from desired B lymphocyte to generate a database of unique V genes by Roche 454 sequencing. Serum IgGs were pepsin-digested to obtain $F(ab)_2$. Antigen specific $F(ab)_2$ fragments were purified by standard antigen-affinity chromatography and were analyzed by bottom-up, liquid chromatography-high-resolution tandem mass spectrometry. By mapping peptides marking unique V_H CDRH3 sequences, this method can be used to identify a set of V-genes constituting the serum polyclonal responses [69].

3. Fluorescence-activated cell sorting (FACS)

Single B cells from defined subpopulations can be isolated by FACS [63]. The full-length

variable region gene transcripts were obtained and amplified by RT-PCR. *In vitro* antibody were subsequently generated by eukaryotic expression system [63].

10.6 Prolong Half-Life

Once the engineered antibodies enter into the circulation, they would be eliminated by proteolysis, renal elimination and hepatic elimination, as well as neonatal Fc receptor-mediated endocytosis. To prolong serum circulation of engineered antibodies, several strategies are proposed. Extending serum half-life of therapeutic antibodies could reduce of the number of applications and the doses.

10.6.1 PEGylation

Attaching highly flexible hydrophilic molecules such as polyethylene glycol (PEG) could increase the hydrodynamic volume of engineered antibodies [27]. The increased volume thus improves the serum half-life. Careful attention should be paid that the number and size of PEG labeled may decrease the activation or binding affinity of the engineered antibodies [41]. Different coupling methods including random and site-directed approaches exist. Site-directed approach may be a better method to conjugate a single PEG chain to the antibody.

10.6.2 Fusion to Human Serum Albumin

Fusing human serum albumin to engineered antibodies provides an alternative way to extend serum half-life of antibodies. The binding site of albumin on FcRn does not alter the affinity for antigen or the affinity for FcRn. Covalent linkage of albumin can be achieved either by chemical coupling or genetic engineering methods. Albumin and IgG taken up by cells through macropinocytosis will bind to the FcRn of the early endosome. This binding will protect IgG from

degradation in the lysosomal compartment. Antibodies are then redirected to the plasma membrane and released back into the blood.

10.7 Affinity Optimization

Humanization may hamper the affinity of antibodies. Sometimes, the affinity of engineered antibodies may not satisfy actual needs. Affinity optimization is, therefore, desired. Most affinity optimization methods depend on optimizing the CDR residues [71]. Targeted or random mutagenesis is used to generate libraries of variants. Normally, libraries of individual CDRs are cloned and screened for improved binding to antigens. Yang et al. successfully generated high affinity human antibodies against human envelope glycoprotein gp120 of HIV-1 by saturation mutagenesis of CDRs [71]. The Fab fragments were displayed and selected for improved binding to the immobilized gp120 [71]. Among the CDR residues, residues in CDR3 are often concentrated for affinity optimization [52]. It is speculated that key variants in CDR3 regions play a major role in antigen affinity mainly for the reason that CDR3 regions are located in the center of the antibody combining site [52].

10.8 Fc Engineering

The therapeutic efficacy of the antibody is directly influenced by the affinity of antibodies to pathogens. However, effector functions mediated by the Fc domain also modulates the efficacy of these antibodies. Fc domain plays an important role in opsonophagocytic killing activity, toxin neutralization efficiency [1, 72]. Additionally, Fc domain is involved in mediating effector function, namely complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP).

Optimizing the affinity of the Fc domain for Fc receptors is an alternative to increase the efficacy

of therapeutic antibodies [56]. Several approaches including alanine scanning, site-directed mutagenesis, computational structure-based design and selection-based method are available to improve the affinity for Fc receptor [49].

Besides, the N-linked glycans in the Fc region of IgG also influence the interaction of the Fc region with its receptors. IgG1 antibodies that are deficient in fucose attached to the Asn²⁹⁷-linked carbohydrate show improved binding affinities with FcγRIIIA receptor [54]. Several different methods can be used to modulate the fucose content on the IgGs. Different expression platforms may generate antibodies with different fucose content. For example, IgG produced by rat hybridomas technologies often has low fucose content [33]. Yeast-and plant-based systems produce antibodies with quite different glycosylation patterns [19, 44].

Antibodies of IgG isotype have a prolonged circulation through FcRn-mediated recycling. Research showed that some mutations in Fc domain may increase the affinity of Fc to its receptor at acidic pH instead of neutral pH. Incorporating such mutations in the Fc domain of IgG will extend the half-life of antibodies [75].

10.9 Immunoglobulin Isotype Selection

There are five major classes of immunoglobulin: IgG, IgA, IgD, IgE and IgM. Among these, the IgG class is the most preferred to develop therapeutic antibodies.

IgG contains four isotypes: IgG1, IgG2, IgG3 and IgG4. The main differences between IgG isotypes are located in the Fc domain. To date, most approved human mAbs are mainly of IgG1. Palivizumab (Synagis; MedImmune Inc) approved against RSV infections is a humanized IgG1 monoclonal antibody [30]. IgG1 is preferred for the reason that IgG1 can induce robust cell killing activities by activating various effector cells.

IgG2 is chosen as a therapeutic backbone when weak effector function is needed. Now, several

therapeutic IgG2 antibodies are either on market or in clinical development [51]. However, IgG2 dimers occur *in vivo* naturally. A more in-depth analysis by cleaving recombinant IgG2 suggested that Cys residues in the hinge are involved in the formation of covalent dimers. Besides, the reduced environments *in vivo* facilitate the formation of IgG2 dimers [74]. The mechanism of dimerization and the exact structure of IgG2 dimers have yet to be investigated. The report of IgG2 disulfide linkages interconverting *in vivo* may shed some light on IgG2 dimer formation [74]. One consequence of IgG2 dimerization is to enhance antibody avidity, which may play a better protective role [51]. IgG2 dimers exist at low levels (<1%) *in vivo*, and their speculative roles should not be magnified [73].

IgG3 is less chosen as a backbone of therapeutic antibodies for several reasons. First of all, IgG3 accounts for a minor component of all IgGs in humans (~10%) [51]. Secondly, IgG3 is susceptible to proteolysis and has a short half-life of ~7 days *in vivo*. Finally, IgG3 displays extensive polymorphism, within the constant domains [35].

IgG4 can't activate the classical complement pathway effectively and has reduced effector function [65]. Besides, IgG4 antibodies exchange half-molecules *in vivo* to form undesired cross-link [70]. To abrogate half-molecule exchange of IgG4 and to eliminate the possible unseen adverse effects, Ser 228 to Pro (S228P) mutation is introduced by antibody engineers to optimize IgG4-based therapeutic antibodies. This mutation not only reduces half-molecule change to a large extent, but also extends serum half-life.

10.10 Bispecific Antibodies

Bispecific antibodies (BsAbs) have two different binding specificities, which enables them to simultaneously recognize two different mediators/pathways that exert important roles in pathogenesis [27]. This dual binding capacity will increase targeting specificity or redirect specific

immune cells to pathogens or infected cells, thus enhancing pathogen elimination. Recently, bispecific antibodies BiS4 α Pa against *Pseudomonas aeruginosa* was generated [39]. BiS4 α Pa was designed to bind to PsI and PcrV. PsI is an extracellular polysaccharide which involves in immune evasion and biofilm formation. PcrV takes part in the secretion of virulence factors. Animal studies showed that binding to PsI and PcrV led to superior protective activity of BiS4 α Pa [39]. Now BiS4 α Pa is in clinical candidate for the treatment of *P. aeruginosa* [39]. Berg et al. constructed a bispecific antibody by linking one heavy/light chain pair from an antibody against CD3 on cytotoxic T cells to a heavy chain whose variable region was replaced by sequence from CD4 [6]. The constructed antibody has dual binding specificity with one arm binding to CD3 on cytotoxic T cells and the other arm which contained sequences from CD4 binding to viral envelope protein gp120 of HIV [6]. Thus, the bispecific antibody redirected cytotoxic T cells to HIV-infected cells whose surface express integral viral proteins to eliminate pathogens. Bispecific antibodies hold great promise in the elimination of HBV, bacteriophages and other pathogens [48, 60].

The formats of bispecific antibodies can be roughly divided into two categories: IgG-like molecules and non-IgG-like molecules (Fig. 10.2). Divergent approaches are used to engineer bispecific antibodies (Table 10.1). For details, please refer to Fan et al. [27].

10.11 Conclusion

In the era of antibiotic resistance, antibody-based therapy holds great promise in treating infectious pathogens. It is a holy grail to produce human antibodies with high therapeutic efficacy. The therapeutic potential of antibodies is derived from less immunogenicity, proper serum circulating time, high antigen-binding affinity, exquisite specificity and robust effector function. Advancement in antibody engineering technolo-

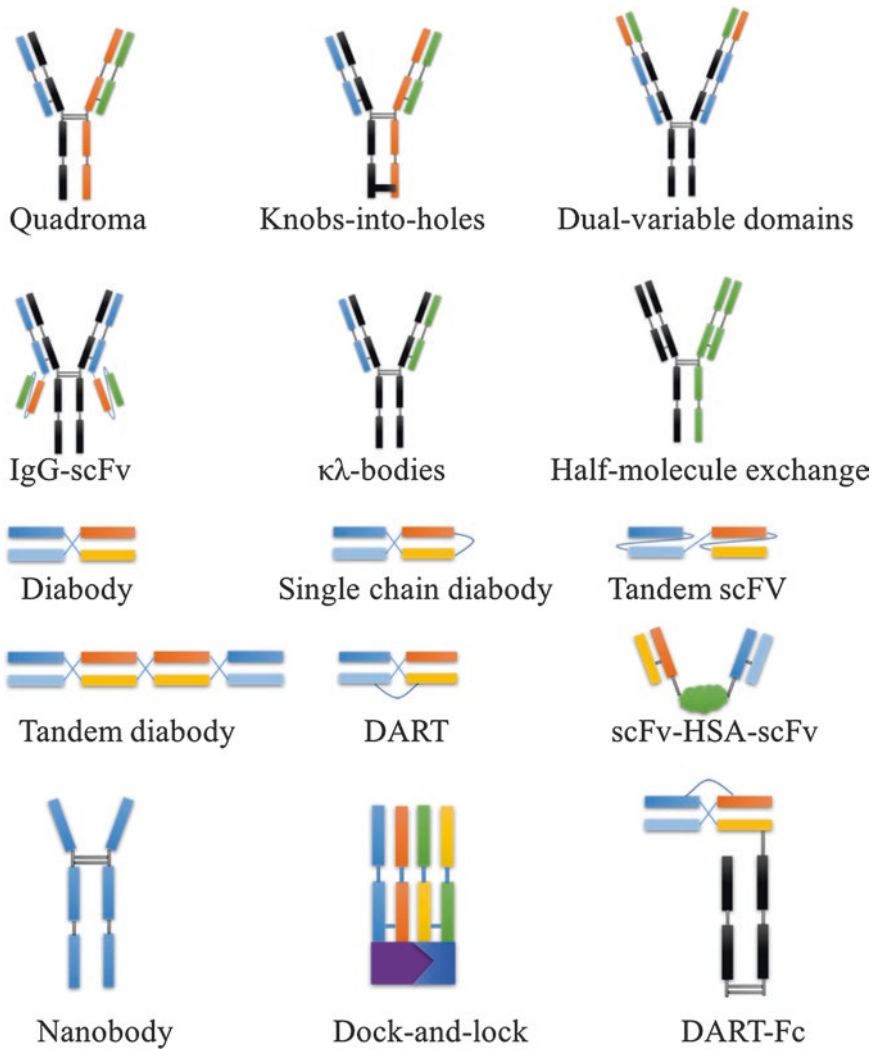


Fig. 10.2 Formats of bispecific antibodies

gies makes it possible to produce antibodies tailored to different infection pathogens. Over the past decades, the “magic bullet” has obtained fruitful success in a wide range of diseases including tumors, autoimmune diseases and inflammatory conditions. However, in the field of infections, there is a gap between preclinical researches and clinical applications. Many engineered antibodies are still making their way

through preclinical/clinical tests, only few are approved for market. Here, we provided a review of technologies for antibody engineering and optimization. We are optimistic about the application of engineered antibodies to treat infectious pathogens. With the emergence of new infectious pathogens and multidrug resistant bacterial, engineered antibodies may offer another choice to combat these threats.

Table 10.1 Methods to engineer bispecific antibodies

Formats	IgG-like molecules	Non-IgG-like molecules
Methods	Quadromas	Tandem scFvs
	Knobs-into-holes	Diabody format
	Dual-variable domains Ig (DVD-Ig)	Single-chain diabodies
	IgG-single-chain Fv (IgG-scFv)	Tandem diabodies (TandAbs)
	Dual action Fab (DAF)	Dual-affinity retargeting molecules (DARTs)
	Half-molecule exchange	Nanobodies
	$\kappa\lambda$ -bodies	Dock-and-lock (DNL)
		scFvs connected to other molecules to form bispecific

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