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Advances in Antibody Design

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Abstract

The use of monoclonal antibodies as therapeutics requires optimizing several of their key attributes. These include binding affinity and specificity, folding stability, solubility, pharmacokinetics, effector functions, and compatibility with the attachment of additional antibody domains (bispecific antibodies) and cytotoxic drugs (antibody–drug conjugates). Addressing these and other challenges requires the use of systematic design methods that complement powerful immunization and in vitro screening methods. We review advances in designing the binding loops, scaffolds, domain interfaces, constant regions, post-translational and chemical modifications, and bispecific architectures of antibodies and fragments thereof to improve their bioactivity. We also highlight unmet challenges in antibody design that must be overcome to generate potent antibody therapeutics.

Keywords

IgG; scFv; V_H; Fab; CDR; complementarity-determining region

1. INTRODUCTION

Antibodies are affinity proteins that play a central role in humoral immunity. Their ability to bind to foreign invaders with high affinity and specificity is central to their function. Equally important is their ability to serve as adaptor molecules and recruit immune cells for various effector functions. There are five main classes of antibodies with diverse functions: immunoglobulin (Ig)A IgD, IgE, IgG, and IgM (1). IgGs are the most abundant class of antibodies, as they constitute approximately 75% of the serum immunoglobulin repertoire. There are four subclasses of IgGs, which vary in their abundance and ability to elicit specific effector functions.

The overall architecture of IgGs is conserved across its four subclasses, and consists of two light chains and two heavy chains (Figure 1). The light chains contain variable (V_L) and constant (C_L) domains, and the heavy chains contain one variable (V_H) and three constant (C_H1, C_H2, and C_H3) domains. One notable difference between IgG subclasses is the location of the disulfide bonds between C_H1 and C_L (which link the heavy and light chains)

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and the number of disulfide bonds in the hinge region (which link the heavy chains). The multidomain nature of IgGs elegantly divides their bioactivity into different subdomains. The antigen-binding fragment (Fab) contains both variable domains, and mediates antigen recognition via six peptide loops known as the complementarity-determining regions (CDRs). In contrast, the crystallizable fragment (Fc) contains the constant domains (C_H2 and C_H3) that mediate effector function by binding to immunological receptor molecules such as complement proteins and Fc receptors.

The multifunctional nature of IgGs is only one of the many reasons for the widespread interest in using monoclonal antibodies (mAbs) as therapeutics. The availability and refinement of robust methods for identifying and generating human mAbs, such as immunization and in vitro screening methods (2), have also contributed greatly to the interest in antibody therapeutics. In addition, mAbs typically display excellent pharmacokinetics (long circulation times), low toxicity and immunogenicity (for human or humanized mAbs), and high stability and solubility. It is also notable that the simplicity of expressing and purifying many different mAbs using similar platform processes is highly attractive from a manufacturing perspective and has enabled the production of a staggering number of different mAbs that are in clinical trials (3).

Nevertheless, there are many challenges in generating mAbs for therapeutic applications. At the discovery stage, immunization affords limited control over antibody affinity and specificity due to the difficulty in controlling antigen presentation to the immune system. In vitro methods, such as phage and yeast surface display, enable improved control over antigen presentation. However, these display methods are limited by their need to screen large libraries, their typical use of antibody fragments instead of full-length antibodies, and their reduced quality-control mechanisms relative to mammalian systems. Moreover, antibodies identified via either immunization or display methods have variable and difficult-to-predict solubilities and viscosities at the high concentrations required for subcutaneous delivery (4, 5). Antibody aggregation is particularly concerning due to the potential immunogenicity of such aggregates (6), and abnormally high viscosity can prevent mAbs from being delivered via the subcutaneous route (7). It is also challenging to optimize bispecific antibodies that typically combine binding domains from different parent antibodies, given the large number of possible molecular architectures as well as the complex effects that these nonstandard antibody formats can have on antibody stability. Moreover, developing effective antibody–drug conjugates is extremely challenging due to the need to optimize the linker and conjugation chemistry as well as the location and number of attached drug molecules. Finally, it is difficult to engineer antibodies with the specific types and levels of effector functions that are optimal for a given therapeutic application.

Although each of these challenges can be addressed through screening a large number of antibody variants, it is impractical to use such screening methods alone to address many of the challenges encountered in developing potent therapeutic antibodies. Attempts to optimize each antibody property sequentially are limited by the fact that improving one antibody attribute (such as binding affinity) can lead to defects in other attributes (such as solubility). Attempts to simultaneously optimize multiple antibody properties using mutagenesis and screening methods require libraries that are prohibitively large.

2. OVERVIEW OF APPROACHES FOR DESIGNING ANTIBODIES

The complexity of optimizing several different antibody attributes (summarized in Figure 2) using traditional immunization and screening methods has led to intense interest in developing antibody-design methods. The most important antibody attributes are binding affinity and specificity, which involve optimizing the variable domains and the CDRs in particular. Colloidal stability (solubility) and conformational (folding) stability are also critical attributes of antibodies because therapeutic mAbs must be soluble for high-concentration delivery and stable for long-term storage. This typically requires optimizing solvent-exposed residues for solubility and solvent-shielded residues for conformational stability. The effector functions of antibodies are also critical to their bioactivity, and can be tailored by manipulating the hinge and Fc regions.

Another increasingly important antibody attribute—which is uncommon in natural antibodies—is bispecificity for either multiple antigens or multiple epitopes on the same antigen. Achieving bispecificity requires methods for combining multiple antibodies into a single one as well as optimizing the key attributes of conventional antibodies. A second nonconventional attribute of antibodies that continues to grow in importance is their bioactivity when attached to small-molecule drugs. Developing antibody–drug conjugates (ADCs) requires optimizing many aspects of the chemistries and linkers used to derivatize antibodies in addition to the other key attributes of conventional antibodies.

This review highlights progress in designing and optimizing each of these key antibody attributes. Given the large size and complexity of antibodies, most design efforts have focused on redesigning or optimizing existing antibodies rather than on de novo design of new antibodies. These design methods vary greatly, and range from knowledge-based methods based on previous mutagenesis results to advanced computational methods based on first principles. A commonality of these diverse methods is that they attempt to guide the design of antibodies in a systematic manner to reduce the need for screening and immunization methods. We discuss these design methods and their application to improve the properties of antibodies that are critical for their activity and stability.

3. ANTIBODY BINDING AFFINITY AND SPECIFICITY

The most important property of antibodies is their ability to recognize targets with high affinity and specificity. This binding activity is largely mediated by the CDRs. Several innovative approaches have been developed for designing CDRs that range from de novo design methods to those that involve the redesign of existing antibodies. Some of these design methods have used motif-grafting approaches to mimic natural protein interactions, and directed evolution approaches to achieve specificities for difficult-to-target antigens.

3.1. De Novo Design

The holy grail of antibody design is to accurately and reliably predict the sequences of antibodies that will bind with high affinity and specificity based solely on the sequence or composition of the antigen. Toward this ambitious goal, a computational approach named OptCDR (Optimal Complementarity Determining Regions) has been developed for

designing the CDRs of antibodies to recognize specific epitopes on a target antigen (8). This method uses canonical structures to generate CDR backbone conformations that are predicted to interact favorably with the antigen. Amino acids are then chosen for each position in the CDRs using rotamer libraries, and this process is repeated many times to refine the backbone structures and amino acid sequences. This leads to the prediction of several sets of CDR sequences, which can be grafted onto antibody scaffolds for evaluation.

This approach has been tested for developing antibody–antigen complexes involving a hepatitis C virus capsid peptide, fluorescein, and vascular endothelial growth factor (VEGF) (8). The investigators predicted mutations that are expected to increase binding affinity (which were not evaluated experimentally) as well as evaluated mutations that had been reported previously to improve binding affinity for fluorescein antibodies. There is some correlation between predictions by OptCDR and experimental data for fluorescein antibodies. It will be important to further evaluate the ability of OptCDR and closely related methods (9) to make de novo predictions of CDR mutations as well as entire CDR sequences that either generate or improve antibody binding.

3.2. Design by Mimicking Natural Protein Interactions

Another fruitful approach for designing antibodies with specific binding activities has been to mimic natural protein interactions. For example, Williamson and colleagues (10) designed antibodies to recognize misfolded conformers of the prion protein (PrP) by mimicking natural interactions between cellular PrP (PrP^{C}) and its misfolded counterpart (PrP^{Sc}). Previous studies had found that PrP residues 96–104 and 133–158 govern the ability of PrP^{Sc} to catalyze misfolding of soluble PrP^{C} (11, 12). This led to the hypothesis that grafting such PrP peptides into heavy chain CDR3 (HCDR3) of an IgG—which originally lacks PrP-binding activity—would create antibodies that specifically recognized PrP^{Sc} (10). Indeed, they found that antibodies with PrP residues 89–112 or 136–158 in HCDR3 bound to PrP^{Sc} with apparent affinities in the low nanomolar range (2–25 nM), and these same antibodies weakly interacted with PrP^{C}. Follow-up studies also identified a third region near the N terminus of PrP (residues 19–33) that resulted in binding activity for grafted PrP antibodies (13). Interestingly, grafting peptides from other PrP regions (such as those from the C-terminal domain) into the same CDR loop failed to generate binding activity. Moreover, the antibodies grafted with PrP residues 19–33 and 89–112 appear to bind via electrostatic interactions because mutating positively charged residues to alanine in these grafted peptides eliminated binding.

This exciting study raises the question of whether grafting peptides from other aggregation-prone proteins into the CDRs of antibodies would also lead to specific binding activity. Our lab recently tested this question using the Alzheimer's Aβ42 peptide (14). There are two hydrophobic segments within Aβ (residues 17–21 and 30–42) that mediate amyloid formation and are located within the β-sheet core of Aβ fibrils (15). We posited that grafting these peptide segments into CDR3 of a single-domain (V_H) antibody would lead to antibody domains with Aβ-specific binding activity. Indeed, we found that grafted V_H domains displaying the central hydrophobic region of Aβ (residues 17–21) in CDR3 bound to Aβ fibrils with submicromolar affinity (300–400 nM), and they weakly bound to Aβ monomers

or oligomers (14). Interestingly, V_H domains grafted with the hydrophobic C terminus of Aβ (residues 30–42) bound both Aβ fibrils and oligomers with submicromolar affinity (300–700 nM) and weakly recognized Aβ monomers. We refer to these grafted antibodies as gammabodies (Grafted AMyloid-Motif AntiBODIES). We have also verified that this grafting approach can be applied to other amyloid-forming proteins, including α-synuclein (associated with Parkinson's disease) and IAPP (associated with type 2 diabetes) (16). Nevertheless, future work will need to develop more systematic methods for selecting amyloidogenic peptides for grafting because we currently do not understand why some sequences mediate binding and others do not. Moreover, it will be important to evaluate how multiple CDR loops can be engineered to display amyloidogenic peptides on the surface of single- and multidomain antibodies to improve the affinities of these grafted antibodies.

3.3. Semirational Design Combined with Directed Evolution Methods

Despite these key advances in the de novo design of CDRs, it has been extremely challenging to use such rational approaches to generate antibodies with subnanomolar (or lower) dissociation constants. Nevertheless, several innovative approaches have been developed that involve designing some CDR residues while randomizing others, and screening such libraries using in vitro display methods to select variants with high binding affinity and specificity. One of the first examples of this hybrid approach was the design of antibody libraries specific for integrins (17). The RGD sequence (arginine-glycine-aspartate) was inserted in the middle of HCDR3, and three flanking residues were randomized on each side of the RGD sequence. In addition, cysteines were introduced at each edge of HCDR3 to constrain the loop, which was posited to be necessary to generate high affinity for antibody binding that is mediated primarily via a single CDR. The investigators displayed a Fab library with these HCDR3 sequences on the surface of phage and screened for binding to integrins. Impressively, several antibody variants were identified with subnanomolar binding affinities, and these antibodies retained the same binding epitope as natural integrin ligands. This and related work (18, 19) has demonstrated the potential of using natural protein interactions to guide the design of high quality antibody libraries.

Another example of this hybrid approach is a method for generating antibodies that recognize post-translational modifications (20, 21). It is difficult to isolate antibodies that recognize chemical modifications such as phosphorylation, especially for phosphoserine and phosphothreonine, because of the relatively small size of their side chains. Therefore, the investigators sought to introduce a common phosphate-binding motif from proteins such as kinases into the CDRs of antibodies (Figure 3). By identifying an antibody with a CDR loop (HCDR2) that naturally displays a similar anion-binding motif, the investigators first confirmed that this antibody bound weakly to phosphorylated peptides. Next, the affinities and specificities of such antibodies for phosphorylated serine, threonine, and tyrosine were evolved by randomizing sites within the anion-binding motif. After mutants were identified by phage display with selective and improved affinity for each type of modification, CDR residues outside the phospho-binding pocket in HCDR2 as well as in LCDR3 and HCDR3 were randomized, and antibodies were selected for binding to different phosphorylated targets. Impressively, this approach generated many phospho-specific antibodies for a wide range of target peptides with modified serine and threonine in addition to tyrosine. Although

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