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PERSPECTIVE

Animal immunization merges with innovative technologies: A new paradigm shift in antibody discovery

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ABSTRACT

Animal-derived antibody sources, particularly, transgenic mice that are engineered with human immunoglobulin loci, along with advanced antibody generation technology platforms have facilitated the discoveries of human antibody therapeutics. For example, isolation of antigen-specific B cells, microfluidics, and next-generation sequencing have emerged as powerful tools for identifying and developing monoclonal antibodies (mAbs). These technologies enable not only antibody drug discovery but also lead to the understanding of B cell biology, immune mechanisms and immunogenetics of antibodies. In this perspective article, we discuss the scientific merits of animal immunization combined with advanced methods for antibody generation as compared to animal-free alternatives through in-vitro-generated antibody libraries. The knowledge gained from animal-derived antibodies concerning the recombinational diversity, somatic hypermutation patterns, and physiochemical properties is found more valuable and prerequisite for developing in vitro libraries, as well as artificial intelligence/machine learning methods to discover safe and effective mAbs.

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Recently, Gray et al.[1] raised scientific and ethical concerns toward animal immunization for antibody generation, and claimed that non-animal derived universal or naive libraries can generate antibodies with greater versatility and reproducibility than immunization-based methods. Scientific concerns were mainly raised on the use of unsequenced animal-derived polyclonals and hybridomas, which are now commonly replaced with monoclonal antibodies (mAbs) and well-characterized hybridomas, respectively, for affinity reagents and therapeutic applications. Further in their correspondence,[1] Gray et al. stated that non-animal-derived universal antibody libraries contain an enormous repertoire of structurally diverse antibody genes that is equal or greater than that of a naive immune system, from which binders against essentially any target can be generated. In our view, however, mAbs generated from animal-derived methods are highly diverse, antigen-specific, developable and unmatched to those that are derived from the in vitro methods. This is because in vivo-generated mAbs evolve through highly orchestrated B cell immune mechanisms, such as clonal selection specific to antigens with diverse lineages and somatic hypermutation in germinal center B cells, particularly, for complex antigens.[2] In addition, other secondary mechanisms of diversification[3] and rare chromosomal integrations into variable regions[4] also contribute to antibody diversification that cannot be recapitulated by in vitro methods. Specifically, hybridoma technology has a unique advantage in retaining their native heavy and light chain paired assembly, and consequently high solubility.[5] Further, technological advances have blurred species boundaries as the hybridoma approach was made widely applicable across phylogenetically distinct species.[6] This may have an important application in the isolation of mAbs against human targets that could be otherwise limited by self-tolerance to mammalian-conserved epitopes.[7]

In-vitro display-derived libraries cannot yet be regarded as universal, but only as complementary to animal-derived methods. For example, Saggy et al.[8] performed a comparative analysis that evaluated hits from the in vitro phage display vs. next-generation sequencing (NGS) methods using antibodies produced by B cells from immunized mice. Remarkably, they found that phage display hits were often low-abundance sequences in the NGS, whereas NGS-derived high-abundance sequences did not express well in the phage, and thus were not recovered. In another study, it was shown that phage display and hybridoma methods yield antibodies with distinct mechanisms and epitopes.[9] Therefore, these studies demonstrated that, while both the in vivo and in vitro strategies could result in antigen-specific mAbs, they were quite complementary in terms of sequences, targeted epitopes, and functions.

Furthermore, among several in vitro phage display-derived human antibodies approved by the US Food and Drug Administration (FDA),[10,11] adalimumab (Humira®) was the first, and it became the best-selling antibody drug on the market. However, importantly, Humira® was discovered by a process known as "guided selection" using a murine mAb as the original template.[12] Most of the mAbs currently approved by the FDA are from hybridoma technology derived either from wild type or more recently using human immunoglobulin (Ig) transgenic mice, and the list also includes the first immunization-derived, humanized nanobody caplacizumab.[10] At one instance, it was reported that phage display-derived therapeutic antibodies are enriched with aliphatic contents

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along antibody loops and exhibit higher aggregation and polyspecificity compared to non-phage display-derived antibodies.[13] The successful development of any antibody therapeutic, whether animal-derived or non-animal-derived, ultimately depends on key properties such as manufacturability and clinical tolerability of the molecules. The larger number of approved animal-derived antibodies are proven to have these properties as compared to in vitro-derived antibodies.[14]

Gray et al.[1] also viewed animal immunization as the tip of an antibody iceberg and in vitro recombinant antibody generation methods as larger submerged fractions. In doing so, they largely undervalued scientific merits and recent technological innovations that have greatly revolutionized immunization-based methods and enabled the exploration of the antibody repertoire space (Figure 1). Mainly, human immunoglobulin transgenic mice and technological developments, including microfluidic chip-based hybridomas,[15] antigen-specific single B cell isolation,[16–18] single-cell droplet microfluidic screening for antigen-specific antibodies,[19,20] natively paired immune libraries,[21] and NGS-based immune repertoire mining,[22] have allowed a more efficient sampling and capturing of the animal-derived antigen-specific antibody repertoire landscape, which has deepened our understanding of antibody biology. Particularly, the large-scale natively paired V_H-V_L antibody discovery technologies[23–25] have the capacity to impact antibody biological developments. These technologies have enabled merging the benefits of animal immunization with the power of display library screening and human antibody repertoire mining. More recently, we established the use of small volumes of blood from immunized mice to isolate antigen-specific antibodies for potential therapeutic use (unpublished data). Such a cutting-edge antibody discovery approach can be useful in generating antibodies to multiple antigens using the same cohort of mice, thereby potentially restricting the number of animals needed. All these indicate that there is a universe of possibilities yet to be explored in animal-derived antibody repertoire using new technologies.

Recently, in silico rational design of antibodies in a modular-[26] and epitope-specific manner[27] and computational method for immune repertoire mining[28] have emerged as third-generation antibody discovery methods. These newly developed in silico methods have utilized the sequence and structural information from antibodies derived from immunization and in vitro methods. More recently, immune organoids from human tonsils and other lymphoid tissues have been developed with a potential for the discovery of antigen-specific antibodies, mimicking key germinal center features including somatic hypermutation and affinity maturation.[29] We expect that artificial intelligence (AI)- and machine learning (ML)-based methods[30–32] could essentially exploit the best of both worlds of in vivo- and in vitro-generated methods, large-scale naïve and antigen-specific antibody sequence and structure data,[33–35] knowledge of immune repertoire and literacy,[36–38] help design feature-controlled antibody libraries and developable antibodies,[39,40] which, in turn, would ultimately solve scientific and ethical problems in antibody generation.

In conclusion, any advanced biomedical scientific research in general can raise ethical concerns when involving the in vivo use of animals or patients as data subjects. As of now, animal-free antibody universal libraries exist only as utopian alternatives to immunization methods and are neither well-defined nor matured enough for the replacement of animal immunization. In contrast, there has been a substantial and concerted effort in academic and biopharmaceutical research communities to improve immunization strategies. In this regard, DNA-based immunization has evolved as a powerful technology platform for mAb generation using animals.[41] In addition, use of animals has benefited the development and validation of mRNA immunization,[42] which has contributed to the successful development of COVID-19 vaccines. Several anti-SARS-CoV-2 neutralizing human mAbs that are now in clinical studies were identified from immunization strategies, such as VelocImmune® (Regeneron), and through use of convalescent blood samples from patients.[43] Thus, immunization-based methods along with the evolution and advent of new technologies will lead to the rapid identification and

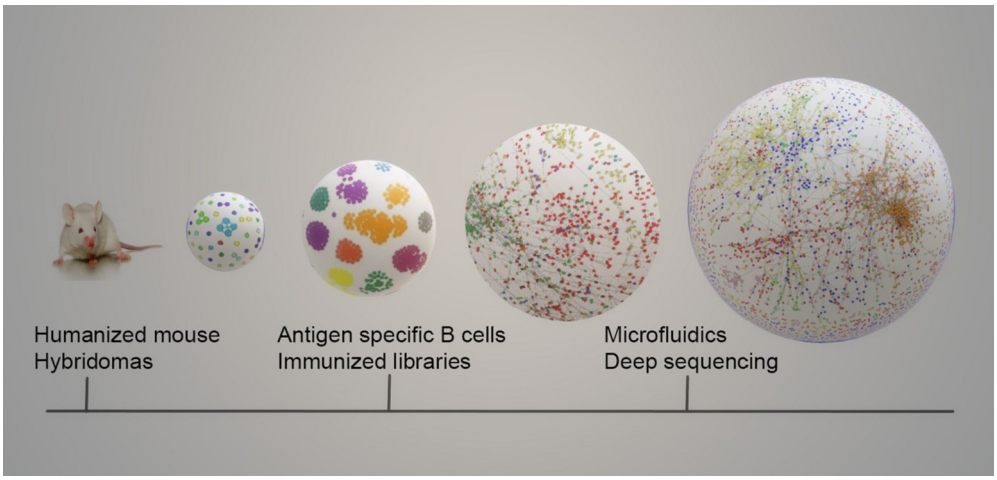

Figure 1. Advanced technologies expose the vastness of animal-derived antibody repertoire. Recent developments using humanized mouse, advanced hybridomas, isolation of antigen specific bulk and single B cells, immunized display libraries, droplet microfluidics technique and immune repertoire data mining through NGS have paved the way for capturing the expanding universe of animal-derived antibodies that are schematically shown as isolated, clustered and networks of dots within the growing spheres.

generation of mAbs in the areas of therapeutics and other applications. Because the knowledge of complete immunogenetic diversity and other characteristics required for developing large, universal in vitro libraries are not yet substantiated and still in its infancy, immunization-dependent antibody generation will continue to be a powerful method that can complement and co-exist with in vitro and in silico methods. We envisage that in the future, advanced experimental and AI/ML-enabled technologies may merge the unique capabilities of in vivo, in vitro and in silico methods for antibody discovery.

Abbreviations

AI, artificial intelligence; FDA, Food and Drug Administration; Ig, immunoglobulin; mAb, monoclonal antibody; ML, machine learning; NGS, next-generation sequencing

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All authors discussed and contributed to the manuscript.

Competing interests

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