Emerging Strategies for Therapeutic Antibody Discovery from Human B Cells

18

Saravanan Rajan and William F. Dall'Acqua

Abstract

Monoclonal antibodies from human sources are being increasingly recognized as valuable options in many therapeutic areas. These antibodies can show exquisite specificity and high potency while maintaining a desirable safety profile, having been matured and tolerized within human patients. However, the discovery of these antibodies presents important challenges, since the B cells encoding therapeutic antibodies can be rare in a typical blood draw and are short-lived ex vivo. Furthermore, the unique pairing of V_H and V_L domains in each B cell contributes to specificity and function; therefore, maintaining antibody chain pairing presents a throughput limitation. This work will review the various approaches aimed at addressing these challenges with an eye to next-generation methods for high-throughput discovery from the human B-cell repertoire.

Keywords

Antibody therapeutics · Next-generation sequencing · Microfluidics · Phage display · Hybridoma · High-throughput screening

18.1 Introduction

In the past few years, the use of monoclonal antibodies has seen an explosion as therapeutics, diagnostics, and tools in biomedical research. This is because antibodies carry exquisite specificity to their respective target, persist in the body for many weeks (particularly if endowed with half-life extension technology) and can elicit responses through multiple mechanisms of action, particularly as relates to interactions with their Fc domains. With several improvements in R&D for antibody discovery and development, the number of antibody therapeutics has dramatically increased, with over 570 molecules in clinical development and 12 new molecules approved in 2018 alone [1]. The therapeutic use of monoclonal antibodies spans the breath of therapeutic areas, including infectious disease, cancer, and autoimmune disorders and increasingly ingenious delivery methods, from inhaled to gene therapy, are constantly improving the convenience of administering these therapeutics such that we expect their use to continue increasing with time.

Although monoclonal antibody research began with mouse hybridoma technology [2] and many approved antibodies are mouse-derived, the immunogenicity issues that ensued have led to an interest in antibody discovery from other sources, particularly human. This review will therefore focus on technologies that derive therapeutic antibodies from human sources, though it is acknowledged that many of the next-generation

S. Rajan (✉) · W. F. Dall'Acqua
Antibody Discovery and Protein Engineering, AstraZeneca R&D, Gaithersburg, MD, USA
e-mail: sarav.rajan@astrazeneca.com

B. Yu et al. (eds.), *Single-cell Sequencing and Methylation*, Advances in Experimental Medicine and Biology 1255, https://doi.org/10.1007/978-981-15-4494-1_18

B cell approaches could be applied to mouse or any species for which we have antibody sequence information.

18.2 Antibody Discovery from Synthetic Libraries

Antibody specificity and activity are the result of sequence evolution for a starting set of germline immunoglobulin sequences. One approach to artificially simulate this evolution is through random mutation of a human germline sequence using error-prone PCR [3, 4] or specific mutation of the complementarity determining regions (CDR) using randomized primers [5, 6] and selecting variants with desirable characteristics through one of the display-based methods such as phage display [7]. These approaches can screen through libraries of massive diversity for binders, though the size of most naïve libraries is often dwarfed by the theoretical diversity of the introduced mutations, making full coverage of the sequence space impossible. The resulting antibody candidates therefore tend to be partially evolved sequences with mid-level affinities and modest therapeutic efficacy. They can however serve as a template for secondary evolution through more targeted affinity maturation processes before final therapeutic leads can be chosen.

18.3 Antibody Discovery from Natural Repertoires

Another approach is to use the extraordinary power of natural systems to evolve antibodies, through immunizations of mice or other species, including humans. Here, sequence evolution and selection take place in germinal centers where the antibody expressing B cells compete for limited antigen binding and growth factors. Once isolated, these antibodies can display very high-affinity (often picomolar K_D or lower) for their cognate antigens and target a variety of epitopes, including functional ones. Moreover, antibodies evolved within humans may be better tolerated as therapeutics, having edited out immunogenic sequence variants during evolution. The vast majority of antibodies on the market and in development have been isolated from natural repertoires, most notably in the infectious disease areas, with many prominent reviews on the discovery of neutralizing antibodies against HIV [8], Influenza [9], Ebola [10], Zika [11], and many more. While not as extensive, studying B cells from cancer patients is a burgeoning field and recent analyses of the B cell repertoires from non-progressing cancer patients have led to the identification of specific tumor-inhibiting antibodies that have therapeutic potential [12–14]. Similarly, antibody-mediated autoimmune diseases are benefiting from the analysis and screening of the patient B cell repertoire, including in myasthenia gravis [15, 16], Celiac disease [17, 18], multiple sclerosis [19] and rheumatoid arthritis [20]. While the identification of pathogenic antibodies in these diseases does not directly represent a therapeutic option, the targets they bind can point to potential avenues for valuable therapies that may emerge in the future.

18.4 Challenges and Opportunities

Despite many successes, recovering antigen-specific antibody sequences from humans is challenging for reasons listed below and in response to these there has been a steady evolution of technologies to further improve and simplify the process (Fig. 18.1). Each of these approaches have successfully led to the identification of valuable antibodies, some seminal to studying of the disease in question.

18.5 Antigen-Specific B Cells Can Be Very Rare

B cells producing antibodies against any particular antigen tend to be rare in the blood of a healthy or convalescent individual, amidst the vast

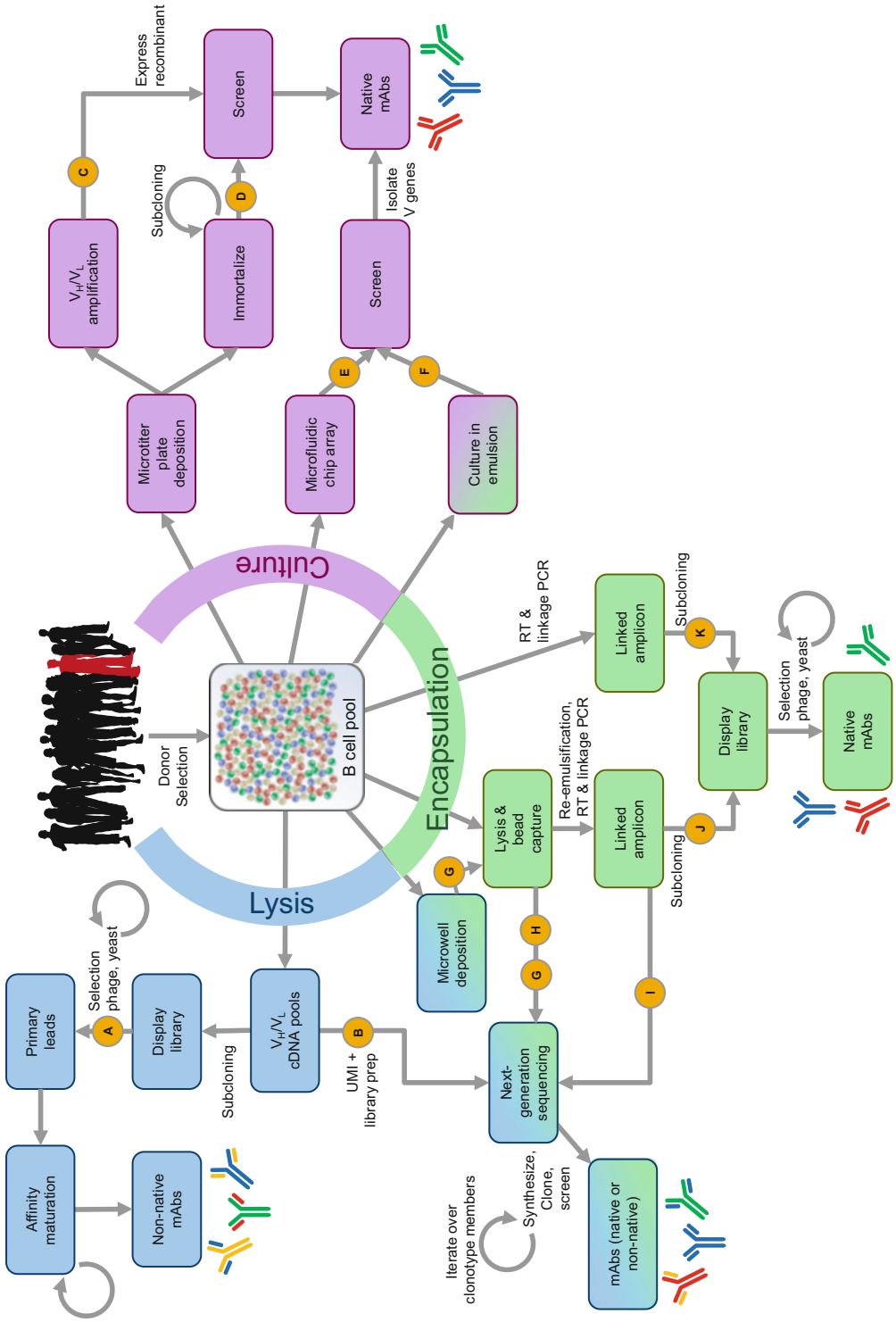

Fig. 18.1 Overview of technologies used to discover antibodies from human B cells. Following selection of reactive donors, polyclonal B cells are isolated from blood or lymphoid tissues and the antibodies which they encode can be screened for antigen specificity through many approaches. Cells can be lysed in bulk and enriched through

number of cells encoding irrelevant antibodies. Finding these cells using the standard practice of depositing single B cells in each well of a microtiter plate therefore becomes very challenging. In some cases, this problem can be circumvented by immunizing the host and collecting blood when antigen-specific B cells reach peak abundance and indeed most discovery campaigns using single B cell cloning have used this approach. Additionally, if therapeutically relevant domains can be purified, these can be used not only for immunization, but also selection of antigen-specific memory B cells through FACS, for instance in the identification of broadly neutralizing anti-HIV antibodies of high therapeutic potential [21]. However, in the absence of a specific domain of interest, immunodominant antibodies elicited through vaccination may not be against epitopes with therapeutic potential, such as sites that mediate neutralization or conserved sites useful for cross-reactivity. Moreover, the availability of a suitable immunogen, adjuvant and relevant host can limit the diseases for which this approach can be used.

18.6 B Cells Are Short-Lived Ex Vivo

Campaigns using primary B cells are significantly time-bound, as the ex-vivo viability of primary B cells is limited to 1–2 weeks, with antibody expression waning prior to that, particularly if grown in isolated cultures. Moreover, the proportion of B cells actively secreting IgG within the blood is very low, so methods need to be in place to differentiate non-secreting cells (i.e., memory B cells) in culture. One method would be to perform RT-PCR on lysed single B cells, followed by amplification of V_H/V_L sequences, and reconstitution of the antibody in recombinant format for screening. The approach works well to find antibodies from immunized donors where the proportion of antigen-specific B cells is high but can be a laborious process if most B cells express irrelevant antibodies [22–24]. Several approaches have focused on immortalizing B cells using viral infection [25–27] or hybridoma generation [28], coupled with cytokine stimulation for cells to secrete antibody for screening. However, each of these steps (immortalization, stimulation, fusion, single-cell cloning) carry inherent inefficiencies and biases that when put together may limit the number of single B cells that can be screened. Successful campaigns would also need large B cell populations to be kept in culture for extended periods with considerable manipulations.

18.7 Antibody Chain Pairing Is Often Important for Function

Antibodies are heterodimeric proteins encoded by uniquely mutated heavy and light chain transcripts whose pairing is often necessary for specificity and activity. Ideally this information

Fig. 18.1 (continued) combinatorial libraries using display technologies (A) or barcoded using unique molecular identifiers (UMI) and sequenced using next-generation sequencing (NGS—B). Various analysis methods can be used to identify dominant clonotypes which need to be paired and synthesized for screening. Individual members of each clonotype can then be subsequently screened for improved function. To preserve the native V_H/V_L pairing, B cells are deposited in microtiter plates through FACS cloning and isolation of individual V_H/V_L sequences (C) by reverse-transcription (RT) and polymerase chain reaction (PCR), followed by recombinant expression and screening. B cells can also be immortalized, stimulated and cultured to allow conditioned media to be screened (D). This can be miniaturized using commercial platforms, either using nanoliter-sized chambers (e.g., AbCellera, Berkeley Lights—E) or picoliter-sized water-in-oil emulsions (e.g., HiFiBiO—F) whereby single B cells can be screened without immortalization, recovered and sequenced. If B cells are colocated with poly-dT beads, they can be lysed and cognate V_H/V_L mRNA species paired for NGS. This can be done using microwells on chips (G) or using emulsions (10x Genomics—H). The beads can also be re-emulsified to generate a linked product suitable for NGS (I). Finally, this linked amplicon can be generated in-frame using beads (J) or directly in droplets (K) to create a natively paired library which can be enriched for antigen specificity using display approaches, thereby combining the advantages of most of the above-mentioned approaches. Donor image designed by Kjpargeter/Freepik (www.freepik.com)

can be captured by sequestering individual B cells in microtiter plates for extraction and cloning of their V genes [23, 24, 29], with the limitation that again only a fraction of the full B cell repertoire of a typical blood draw (one to ten million cells) can be covered. Conversely, it has been a common approach to collect total RNA from a large pool of lysed B cells, separately amplify heavy and light chains, then pair them randomly to form a library of exponentially larger complexity [30–32]. In theory, the diversity of such immune libraries is lower than that of the synthetic libraries mentioned above and could be harnessed using phage display to identify the originally paired and functional sequence and this has been shown with immunized mice with restricted diversity [33]. However, in more diverse libraries, for instance from healthy donors, recovering the original pair is a challenge. Biases in expression and V_H/V_L pairing preferences [34] can lead to the selection to nonnatural solutions and require secondary optimization screens, for instance using light chain shuffling.

Another approach is to sequence the B cell repertoire and synthesize candidate antibodies for screening, for instance after hierarchical clustering of sequences to identify phylogenetic lineages. The application of next-generation sequencing (NGS) has been well described for the characterization of the antibody repertoire, particularly regarding separately prepared heavy and light chain libraries obtained from mRNA isolated from thousands to millions of B cells [35–37]. A recent NGS analysis of the antibody repertoires from ten individuals revealed that their repertoires were largely unique and that the overall diversity of antibody sequences in the human population is extremely large, on the order of 10^{12} unique paired V_H/V_L sequences [37]. While the amplification of B cell mRNA using 5′ RACE provides an unbiased representation of the expressed repertoire for sequencing, current NGS length limitations make assembling such a fragment from paired-end sequencing a challenge. As a result, libraries are often made using multiplex V-gene specific primers to remove the 5′ untranslated region and leader sequences and reduce amplicon size [38] which can introduce bias. Additionally, given that antibodies undergo somatic hypermutation, a significant challenge in the field was to determine if a given mutation was due to natural antibody diversification or a result of PCR and/or sequencing-related artifacts. This issue has been elegantly solved through the addition of unique molecular identifier (UMI) barcodes, where the initial template cDNA can be ligated to a unique tag that is also sequenced to enable error correction at the analysis stage [39, 40]. Using these methods, a population of B cells can be profiled to identify phylogenetic lineages [41] that indicate the maturation of specific clonotypes as evidence of antigen specificity. However, given that maturation at the heavy and light chains occur independently, it is not possible to accurately predict chain pairing based on NGS data and heuristics need to be used to down-select panels of heavy and light chain sequences to synthesize and combinatorially pair for functional testing. Again, in cases where subjects are immunized and B cells harvested at optimal times, this approach can be quite effective, as the most abundant heavy and light chain clonotypes may represent the original pairs. However, for cases involving the identification of antigen-specific antibodies from healthy donors or patients with chronic diseases such as cancer, the selection of antibody function from sequence information alone is likely to be incomplete.

18.8 Next-Generation Microfluidic Technologies

Though successful in many instances, these two broad approaches (display versus B cell cloning/sequencing) suffer from conflicting issues. On the one hand, display-based systems can screen through vast synthetic or combinatorial libraries to identify antigen-specific antibodies of mid to low quality. Conversely, B cell discovery platforms start from B cell pools encoding potentially high-quality antibodies but lack the screening power to identify antigen-specific antibodies,

especially if the B cells encoding them are rare. Over the past 5 years a range of new technologies have emerged promising to solve this tradeoff by miniaturizing the vessel into which B cells are sequestered. Several growing companies (AbCellera, Berkeley Lights, and HiFiBiO) have been successful in directly screening antibody secreting cells within these vessels for binding or even functional activity then exporting antigen-specific B cells into defined locations for V gene capture. Alternatively, several approaches described below have detailed using droplet microfluidics to capture the natively paired repertoire from B cells into a format suitable for next-generation sequencing technology. Finally, the repertoire can be captured via microfluidics in an expressible format to display and/or screen as recombinant protein, effectively combining the benefits of natural antibody evolution with the screening power of display-based approaches.

18.9 Paired Ig Sequencing

In 2013, DeKosky and colleagues devised a method to have B cells deposited within microwells on a microfabricated chip along with magnetic beads conjugated to poly-dT oligonucleotides [42]. The chip could be sealed with lysis reagents such that the cognate heavy and light mRNA strands would be recovered and linked in a format suitable for next-generation sequencing. The repertoire from 68,000 B cells could be captured in a single run, an improvement in throughput of one order of magnitude over traditional 96-well formats. In a follow-up paper, the group expanded the method to have the B cells and magnetic beads encapsulated into water-in-oil droplets, further raising the throughput to one million B cells per run [43]. This falls within the range of B cells obtained from a typical blood draw and enabled the first comprehensive evaluations of the paired antibody repertoire for therapeutic antibodies. As with the single chain NGS studies however, it is a challenge to determine antigen reactivity from antibody sequence alone. An elegant addition to this method therefore has been to overlay paired sequencing data from circulating B cells with proteomic sequence analysis of serum antibodies immunoprecipitated with antigen [44, 45]. Using immunized donors, the authors were able to identify potent neutralizing antibodies targeting influenza and HIV. The advent of 10x Genomics now provides a commercial option for obtaining paired immunoglobulin sequences from primary B cells, albeit from a smaller number of cells (approximately 10,000 cells) and this system has recently been used to sequence B cells from immunized mice to identify antigen-specific antibodies [46].

18.10 Native Library Screening

A natural evolution of these technologies has led to the combination of miniaturizing B cell capture into microfluidic emulsions with paired immunoglobulin capture into a format that can be expressed. Recently, three independent groups have reported in short succession microfluidic methods to capture the repertoire from millions of B cells and rapidly screen them for antigen-specific antibodies.

Adler and colleagues at GigaGen devised an approach to co-encapsulate one to two million B cells in a co-flow setup with poly-dT magnetic beads suspended in lysis/binding buffer [47]. Following bead capture of the mRNA, the emulsions are broken and recovered beads re-emulsified with RT-PCR buffer and a cocktail of primers to generated linked heavy-light amplicons in scFv format. The authors then expressed this library of natively paired scFv amplicons on the surface of yeast and used multiple rounds of fluorescence-activated cell sorting (FACS) to enrich for yeast cells displaying antibodies specific to influenza A and pneumococcal polysaccharide antigens. A subset of recovered scFv-s confirmed to be antigen-specific and functional when expressed in IgG format, and based on NGS analysis, they were estimated to be present at 0.001% of the starting library diversity. The approach was also validated with immunized mice (having a higher

proportion of antigen-specific B cells) for the isolation of antibodies blocking checkpoint inhibitors [48].

Wang and coworkers also reported a similar two-step emulsification strategy to generate a natively paired Fab library, a more aggregation resistant antibody fragment with biophysical properties closer aligned with IgG, that was also displayed the library on the surface of yeast [49]. They used this method to construct libraries from immunized or convalescent patients and panned them over successive rounds to isolate functional antibodies specific to Ebola, HIV, and influenza antigens.

Finally, our group has developed a method of capturing the native repertoire from millions of B cells into natively paired scFv fragments displayed on the surface of phage [50]. Here, B cells are not co-encapsulated with magnetic beads but rather with a highly optimized reaction mix that performs sequential reactions for B cell lysis, amplification of V_H and V_L segments and their pairing by overlap-extension PCR, all within the same droplets. This streamlines the process and obviates the need to handle beads, where captured mRNA species can exchange. The approach was used to rapidly identify very rare and cross-reactive antibodies targeting influenza hemagglutinin.

18.11 Future Directions

Since all three native screening methods use emulsions for amplification of template, this may have an added benefit of normalizing for mRNA expression levels within individual B cells, as the limiting reagents within droplets should saturate with enough cycles of PCR. It would be interesting to see if this bears out in future studies using NGS analysis. On the other hand, since these methods require priming at specific regions to be in-frame (i.e., the start of framework 1 and the end of framework 4), the multiplex primer sets may not be ideally suited to perform as well as other amplification methods (i.e., 5′ RACE) and would benefit from continued development. While not extensively mentioned in this text, these new methods have the potential for screening natively paired repertoires from other species simply by changing primer sets. This has been demonstrated with immunized mice [48], though other species such as rat, rabbit, and even nonhuman primates could provide B cells from which valuable antibodies can be derived.

The success of antibody therapeutics has led to increasing numbers of molecules in clinical trials and as approved medicines and this trend is expected to continue. Fueling this growth is a continued evolution of methods for mining new therapeutic antibodies, both through synthetic and natural repertoires. We can expect that the technologies of the future will continue to harness the natural antibody repertoire with increasing throughput, breadth, speed, and fidelity to reliably generate therapeutic candidates against a continually expanding list of targets.

References

1. Kaplon H, Reichert JM (2018) Antibodies to watch in 2019. mAbs 11(2):219–238
2. Köhler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256(5517):495–497
3. Liu J-L, Hu Z-Q, Xing S, Xue S, Li H-P, Zhang J-B et al (2012) Attainment of 15-fold higher affinity of a Fusarium-specific single-chain antibody by directed molecular evolution coupled to phage display. Mol Biotechnol 52(2):111–122
4. Unkauf T, Hust M, Frenzel A (1701) Antibody affinity and stability maturation by error-prone PCR. Methods Mol Biol 2018:393–407
5. Fellouse FA, Li B, Compaan DM, Peden AA, Hymowitz SG, Sidhu SS (2005) Molecular recognition by a binary code. J Mol Biol 348(5):1153–1162
6. Fellouse FA, Barthelemy PA, Kelley RF, Sidhu SS (2006) Tyrosine plays a dominant functional role in the paratope of a synthetic antibody derived from a four amino acid code. J Mol Biol 357(1):100–114
7. Rajan S, Sidhu SS (2012) Simplified synthetic antibody libraries. Methods Enzymol 502:3–23
8. Sok D, Burton DR (2018) Recent progress in broadly neutralizing antibodies to HIV. Nat Immunol 19 (11):1179–1188
9. Krammer F (2019) The human antibody response to influenza A virus infection and vaccination. Nat Rev Immunol 19(6):383–397
10. King LB, Milligan JC, West BR, Schendel SL, Ollmann Saphire E (2019) Achieving cross-reactivity

with pan-ebolavirus antibodies. Curr Opin Virol 34:140–148
11. Campos JLS, Mongkolsapaya J, Screaton GR (2018) The immune response against flaviviruses. Nat Immunol 19(11):1189–1198
12. Bushey RT, Moody MA, Nicely NL, Haynes BF, Alam SM, Keir ST et al (2016) A therapeutic antibody for cancer, derived from single human B cells. Cell Rep 15(7):1505–1513
13. DeFalco J, Harbell M, Manning-Bog A, Baia G, Scholz A, Millare B et al (2018) Non-progressing cancer patients have persistent B cell responses expressing shared antibody paratopes that target public tumor antigens. Clin Immunol 187:37–45
14. McDaniel JR, Pero SC, Voss WN, Shukla GS, Sun Y, Schaetzle S et al (2018) High-throughput discovery of antitumor antibodies mined from sentinel lymph node B cells and circulating IgG of breast cancer patients. Cancer Immunol Immunother 67(5):729
15. Makino T, Nakamura R, Terakawa M, Muneoka S, Nagahira K, Nagane Y et al (2017) Analysis of peripheral B cells and autoantibodies against the anti-nicotinic acetylcholine receptor derived from patients with myasthenia gravis using single-cell manipulation tools. PLoS One 12(10):e0185976. [cited 2019 Sep 19]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5645109/
16. Stathopoulos P, Kumar A, Heiden JAV, Pascual-Goñi E, Nowak RJ, O'Connor KC (2018) Mechanisms underlying B cell immune dysregulation and autoantibody production in MuSK MG. Ann N Y Acad Sci 1412(1):154
17. Di Niro R, Mesin L, Zheng N-Y, Stamnaes J, Morrissey M, Lee J-H et al (2012) High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. Nat Med 18(3):441–445
18. Roy B, Neumann RS, Snir O, Iversen R, Sandve GK, Lundin KEA et al (2017) High-throughput single-cell analysis of B cell receptor usage among autoantigen-specific plasma cells in celiac disease. J Immunol 199 (2):782–791
19. Lomakin YA, Zakharova MY, Stepanov AV, Dronina MA, Smirnov IV, Bobik TV et al (2014) Heavy-light chain interrelations of MS-associated immunoglobulins probed by deep sequencing and rational variation. Mol Immunol 62(2):305–314
20. Ge C, Holmdahl R (2019) The structure, specificity and function of anti-citrullinated protein antibodies. Nat Rev Rheumatol 15(8):503–508
21. Krebs SJ, Kwon YD, Schramm CA, Law WH, Donofrio G, Zhou KH et al (2019) Longitudinal analysis reveals early development of three MPER-directed neutralizing antibody lineages from an HIV-1-infected individual. Immunity 50(3):677–691. e13
22. Meijer P-J, Andersen PS, Haahr Hansen M, Steinaa L, Jensen A, Lantto J et al (2006) Isolation of human antibody repertoires with preservation of the natural heavy and light chain pairing. J Mol Biol 358 (3):764–772
23. Tiller T, Meffre E, Yurasov S, Tsuiji M, Nussenzweig MC, Wardemann H (2008) Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. J Immunol Methods 329(1–2):112–124
24. Busse CE, Czogiel I, Braun P, Arndt PF, Wardemann H (2014) Single-cell based high-throughput sequencing of full-length immunoglobulin heavy and light chain genes. Eur J Immunol 44(2):597–603
25. Bernasconi NL, Traggiai E, Lanzavecchia A (2002) Maintenance of serological memory by polyclonal activation of human memory B cells. Science 298 (5601):2199–2202
26. Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR et al (2004) An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. Nat Med 10(8):871–875
27. Kwakkenbos MJ, Diehl SA, Yasuda E, Bakker AQ, van Geelen CMM, Lukens MV et al (2010) Generation of stable monoclonal antibody-producing B cell receptor-positive human memory B cells by genetic programming. Nat Med 16(1):123–128
28. Yu X, McGraw PA, House FS, Crowe JE (2008) An optimized electrofusion-based protocol for generating virus-specific human monoclonal antibodies. J Immunol Methods 336(2):142–151
29. von Boehmer L, Liu C, Ackerman S, Gitlin AD, Wang Q, Gazumyan A et al (2016) Sequencing and cloning of antigen-specific antibodies from mouse memory B cells. Nat Protoc 11(10):1908–1923
30. McCafferty J, Griffiths AD, Winter G, Chiswell DJ (1990) Phage antibodies: filamentous phage displaying antibody variable domains. Nature 348 (6301):552–554
31. Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G (1991) By-passing immunization. Human antibodies from V-gene libraries displayed on phage. J Mol Biol 222 (3):581–597
32. Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC et al (1996) Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library. Nat Biotechnol 14(3):309–314
33. Wang B, Lee C-H, Johnson EL, Kluwe CA, Cunningham JC, Tanno H et al (2016) Discovery of high affinity anti-ricin antibodies by B cell receptor sequencing and by yeast display of combinatorial VH: VL libraries from immunized animals. mAbs 8 (6):1035–1044
34. Jayaram N, Bhowmick P, Martin ACR (2012) Germline VH/VL pairing in antibodies. Protein Eng Des Sel 25(10):523–529
35. Menzel U, Greiff V, Khan TA, Haessler U, Hellmann I, Friedensohn S et al (2014)

Comprehensive evaluation and optimization of amplicon library preparation methods for high-throughput antibody sequencing. PLoS One 9(5): e96727
36. Turchaninova MA, Davydov A, Britanova OV, Shugay M, Bikos V, Egorov ES et al (2016) High-quality full-length immunoglobulin profiling with unique molecular barcoding. Nat Protoc 11 (9):1599–1616
37. Briney B, Inderbitzin A, Joyce C, Burton DR (2019) Commonality despite exceptional diversity in the baseline human antibody repertoire. Nature 566 (7744):393–397
38. Bernat NV, Corcoran M, Hardt U, Kaduk M, Phad GE, Martin M et al (2019) High-quality library preparation for NGS-based immunoglobulin germline gene inference and repertoire expression analysis. Front Immunol 10:660. [cited 2019 Sep 19]. https://www-ncbi-nlm-nih-gov.iscape.idm.oclc.org/pmc/articles/PMC6459949/
39. Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B (2011) Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci U S A 108(23):9530–9535
40. Kivioja T, Vähärautio A, Karlsson K, Bonke M, Enge M, Linnarsson S et al (2011) Counting absolute numbers of molecules using unique molecular identifiers. Nat Methods 9(1):72–74
41. Briney B, Le K, Zhu J, Burton DR (2016) Clonify: unseeded antibody lineage assignment from next-generation sequencing data. Sci Rep 6:23901. [cited 2019 Sep 19]. https://www-ncbi-nlm-nih-gov.iscape.idm.oclc.org/pmc/articles/PMC4840318/
42. DeKosky BJ, Ippolito GC, Deschner RP, Lavinder JJ, Wine Y, Rawlings BM et al (2013) High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. Nat Biotechnol 31 (2):166–169
43. DeKosky BJ, Kojima T, Rodin A, Charab W, Ippolito GC, Ellington AD et al (2015) In-depth determination and analysis of the human paired heavy- and light-chain antibody repertoire. Nat Med 21(1):86–91
44. Lee J, Boutz DR, Chromikova V, Joyce MG, Vollmers C, Leung K et al (2016) Molecular-level analysis of the serum antibody repertoire in young adults before and after seasonal influenza vaccination. Nat Med 22(12):1456
45. Williams LD, Ofek G, Schätzle S, McDaniel JR, Lu X, Nicely NI et al (2017) Potent and broad HIV-neutralizing antibodies in memory B cells and plasma. Sci Immunol. 2(7). [cited 2019 Sep 18]. https://www-ncbi-nlm-nih-gov.iscape.idm.oclc.org/pmc/articles/PMC5905719/
46. Goldstein LD, Chen Y-JJ, Wu J, Chaudhuri S, Hsiao Y-C, Schneider K et al (2019) Massively parallel single-cell B-cell receptor sequencing enables rapid discovery of diverse antigen-reactive antibodies. Commun Biol 2:304
47. Adler AS, Mizrahi RA, Spindler MJ, Adams MS, Asensio MA, Edgar RC et al (2017) Rare, high-affinity anti-pathogen antibodies from human repertoires, discovered using microfluidics and molecular genomics. mAbs 9(8):1282–1296
48. Adler AS, Mizrahi RA, Spindler MJ, Adams MS, Asensio MA, Edgar RC et al (2017) Rare, high-affinity mouse anti-PD-1 antibodies that function in checkpoint blockade, discovered using microfluidics and molecular genomics. mAbs 9(8):1270–1281
49. Wang B, DeKosky BJ, Timm MR, Lee J, Normandin E, Misasi J et al (2018) Functional interrogation and mining of natively paired human VH:VL antibody repertoires. Nat Biotechnol 36 (2):152–155
50. Rajan S, Kierny MR, Mercer A, Wu J, Tovchigrechko A, Wu H et al (2018) Recombinant human B cell repertoires enable screening for rare, specific, and natively paired antibodies. Commun Biol 1(1):5

Saravanan Rajan is a Senior Scientist in the department of Antibody Discovery and Protein Engineering at AstraZeneca. Dr Rajan obtained his PhD in Human Genetics from McGill University and a postdoctoral fellowship from the University of Toronto, specializing in using high-throughput molecular biology in genomics and the discovery of therapeutic antibodies. Over the past 8 years at AstraZeneca he has contributed to multiple discovery campaigns and earned several distinctions, including major awards for innovation and entrepreneurship and large external grants tied to new technology he developed. He currently manages a team in Gaithersburg, USA that uses a multidisciplinary approach to push the boundaries of antibody discovery.

William F. Dall'Acqua is VP, Research and Development at AstraZeneca, a biopharmaceutical company headquartered in Cambridge, UK. Dr. Dall'Acqua has been studying protein structure, function, and engineering for over 20 years and has coauthored over 70 papers and three book chapters in the field. He has also been named inventor on more than 80 patent applications and on 18 issued patents. He has Bachelor's and Master's Degrees in Biochemistry from University of Paris, and a PhD in Immunology from University of Paris in conjunction with University of Maryland. He also spent 2½ years as a postdoctoral fellow at Genentech in the Molecular Oncology Department before joining AstraZeneca. At AstraZeneca, Dr. Dall'Acqua supervises a group of over 30 scientists in the Department of Antibody Discovery and Protein Engineering, working on discovery of new therapeutic antibody drugs and establishing novel technology platforms.