# Mapping the secrets of the antibody pool

Jonathan R McDaniel, Gregory C Ippolito & George Georgiou

**The progression of antibody-mediated immunity can now be monitored at high-throughput on a single-cell level.**

The polyclonal antibody response generates proteins that recognize potentially pathogenic molecules and then orchestrate a series of functions in an effort to protect against disease. Interrogating the ensemble of antibody-secreting cells (ASCs) with respect to the quality, quantity, and sequence of antibodies they produce is essential for a systems-level understanding of humoral immunity and its many roles in fighting infection, clearing apoptotic debris, and regulating immunity. In this issue, Eyer *et al.*[1] describe DropMap, a powerful and elegant technology for the phenotypic analysis of ASCs at the single-cell level with unprecedented throughput and resolution. With this approach, both antibody quality (antigen affinity and selectivity) and antibody secretion can be observed simultaneously over several hours for thousands of ASCs.

Following antigen stimulation, activated B lymphocytes experience affinity maturation in germinal centers and then proliferate and differentiate into ASCs. Early in ASC development, B cells differentiate into short-lived plasmablasts that circulate in blood; a fraction of this population may then mature to long-lived plasma cells that reside preferentially in the bone marrow, secrete a copious amount of antibody, and can survive for years if not decades in humans[2]. The totality of plasmablasts and long-lived plasma cells produce the antibody pool, or repertoire, present in blood and secretions.

While methods for separately determining the affinity and the rate of secretion of antibodies from plasmablasts have been reported[3,4], there was no way to measure both at the same time for the same cell. DropMap uses a microfluidic chip to physically isolate individual ASCs into picoliter-sized water-in-oil emulsions. In-droplet miniaturized sandwich immunoassays monitor antibody secretion, measure the affinity of the antibody–antigen interaction, and detect polyreactivity. Each active droplet contains four components: a single ASC, several hundred magnetic nanoparticles, fluorescently labeled antigen (green), and fluorescently labeled anti-Fc $F(ab')_2$ molecules (red) (**Fig. 1**). The magnetic nanoparticles are coated with a κ light-chain-specific nanobody ($V_HH$) that captures antibodies secreted from the encapsulated ASC onto the surface of the magnetic nanoparticles.

One of the many innovations in the DropMap technology is the use of a magnetic field to align the nanoparticles into a beadline. The ratio of the intensity of the beadline to the diffuse free red $F(ab')_2$ fluorescence background in the droplet is used to estimate the antibody concentration, track the rate of antibody secretion from individual cells, and determine whether the antibody is polyreactive. Likewise, the fluorescent ratio in the green channel together with the antibody concentration is used to calculate antigen equilibrium dissociation constants ($K_d$ values). Importantly, the authors demonstrated that antibody affinities and secretion rates can be determined with a dynamic range of more than three orders of magnitude for thousands of ASCs in parallel.

Eyer *et al.*[1] apply DropMap to study antibody responses to immunization in mice. They show that the frequency of individual IgG-ASCs decreases rapidly after reaching a peak in both

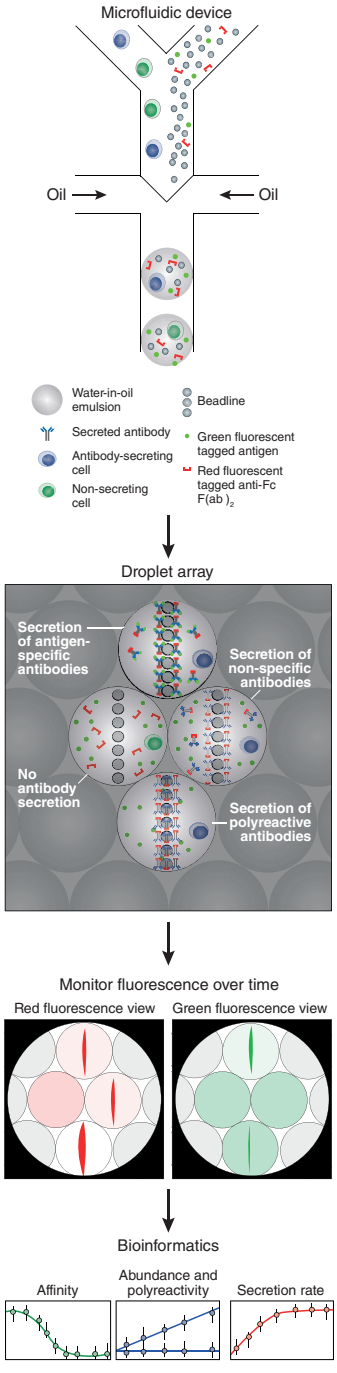


**Figure 1** DropMap workflow. A microfluidic device is used to compartmentalize cells together with magnetic nanoparticles, antigen, and anti-Fc $F(ab')_2$. Application of a magnetic field to the droplet array assembles the nanoparticles into a beadline, and fluorescence measurements over time monitor the relocation of fluorescence intensities from the diffuse background to the beadline. The rate and magnitude of these changes indicate antibody affinity, specificity, and rate of secretion.

*Jonathan R. McDaniel and George Georgiou are in the Department of Chemical Engineering at the University of Texas at Austin, Austin, Texas, USA, and Gregory C. Ippolito is in the Department of Molecular Biosciences at the University of Texas at Austin, Austin, Texas, USA.*
*e-mail:* gg@che.utexas.edu

spleen and bone marrow compartments following secondary and boost immunizations with the model antigen tetanus toxoid, even though the serum level of anti-tetanus toxoid IgG antibody persists and remains elevated for a period of time that exceeds the known half-life of mouse IgG. This result raises the provocative possibility that a sizable fraction of circulating antibodies may be produced by a reservoir of ASCs residing in other anatomical compartments.

The authors also find that the secretion rates of antibodies from ostensibly homogenous ASCs can vary by several orders of magnitude. It is unlikely that such vast differences in protein synthesis are due solely to stochastic effects and transcriptional noise[5]. Could it be that unknown developmental mechanisms or external signals program antibody secretion rates from long-lived plasma cells, and if so, what is the function of such a stratification of antibody production? It is noteworthy that secretion rates do not seem to correlate with antibody affinity, suggesting that selection for high affinity and high expression may be uncoupled.

Yet another interesting observation by Eyer *et al.*[1] is that following immunization, a significant percentage of ASCs secrete IgGs that bind antigens other than the evoking tetanus toxoid immunogen, reminiscent of a previously described hypothesis regarding polyclonal bystander activation of human memory B cells. It would be of interest to determine whether the degree of cross-activation reflects an intrinsic difference between "short-term serological memory" and "long-term serological memory"[6].

As with every new technology, DropMap at present relies on custom-made equipment and demands considerable expertise. However, given the great interest in single-cell analysis tools, the system is likely to be commercialized. As it becomes more widely available, DropMap should facilitate the study of a range of questions of fundamental interest for vaccinology and for the understanding of serological memory. The unique insight provided by tracing single-cell trajectories may help parse the underlying causes of ASC heterogeneity. Is the observed variability in antigen specificity and antibody secretion dynamics a result of other immune cell subsets or of gradations in immune cell function at a single-cell level due to factors such as age and senescence?

Collectively, the results of Eyer *et al.*[1] underscore the differential contributions of ASCs to the serological repertoire, with some ASCs contributing little to the IgG pool. The sheer number and tissue distribution of ASCs at hard-to-access anatomical sites, sampling size limitations, the complex kinetics of antibody secretion, and the long circulatory half-life of antibodies make it impossible to infer the polyclonal serum response to an antigen from single-cell measurements, at least for the foreseeable future. Recent advances in liquid chromatography–tandem mass spectrometry proteomics coupled with B-cell-receptor sequencing now enable the quantification and identification of the monoclonal antibodies in the serum antibody repertoire[7,8]. The combination of serum proteomics and DropMap-based phenotypic analysis of ASCs could be a powerful strategy to investigate the key aspects of ASC phenotypic diversity that shape antibody-mediated immunity.

While Eyer *et al.*[1] only report the analysis of ASCs expressing IgG containing a κ light chain (which account for >95% of antibodies in mice), for human studies it will be necessary to analyze both the λ and κ repertoires, which are distinctly encoded and tend toward equivalent expression in humans. Ultimately, it will be important to integrate the phenotypic data generated using DropMap with transcriptional data on single ASCs[9]. Techniques for single-cell transcriptional analysis with droplet microfluidics are advancing rapidly[10], but combining the complex technologies of DropMap and single-cell transcriptomics is still likely to be quite challenging. Nonetheless, the benefits would be truly transformative. Antibody affinity, antigen selectivity, and sequencing from single cells could be analyzed at an unprecedented scale. Transcriptional profiling of ASCs independent of cell-surface phenotype may identify novel ASC subsets based solely on a molecular definition of the genes they transcribe and the antibodies they secrete. And insights from transcriptomic analyses may provide a more complete understanding of the basis of the secretion heterogeneity observed by Eyer *et al.*[1].

**COMPETING FINANCIAL INTERESTS**
The authors declare no competing financial interests.

1. Eyer, K. *et al. Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3964 (2017).
2. Nutt, S.L., Hodgkin, P.D., Tarlinton, D.M. & Corcoran, L.M. *Nat. Rev. Immunol.* **15**, 160–171 (2015).
3. Mazutis, L. *et al. Nat. Protoc.* **8**, 870–891 (2013).
4. Clargo, A.M. *et al. MAbs* **6**, 143–159 (2014).
5. Duffy, K.R. *et al. Science* **335**, 338–341 (2012).
6. Bernasconi, N.L., Traggiai, E. & Lanzavecchia, A. *Science* **298**, 2199–2202 (2002).
7. Lee, J. *et al. Nat. Med.* **22**, 1456–1464 (2016).
8. Williams, L.D. *et al. Sci. Immunol.* **2**, eaal2200 (2017).
9. Shi, W. *et al. Nat. Immunol.* **16**, 663–673 (2015).
10. Macosko, E.Z. *et al. Cell* **161**, 1202–1214 (2015).

Published online 11 September 2017; doi:10.1038/nbt.3972