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Mapping and modelling human B cell maturation in the germinal centre

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The maturation of B cells within the germinal centre (GC) is necessary for antigen-specific immune responses and memory. Dysfunction in the GC can lead to immunodeficiencies, autoimmune diseases, or lymphomas. Here we describe how recent advances in single-cell and spatial genomics have enabled new discoveries about the diversity of human GC B cell states. However, with the advent of these hypothesis-generating technologies, the field should now transition towards testing bioinformatic predictions using experimental models of the human GC. We review available experimental culture systems for modelling human B cell responses and discuss the potential limitations of different methods in capturing *bona fide* GC B cell states. Together, the combination of cell atlas–based mapping with experimental modelling of lymphoid tissues holds great promise to better understand the maturation of human B cells in the GC response and generate new insights into human immune health and disease.

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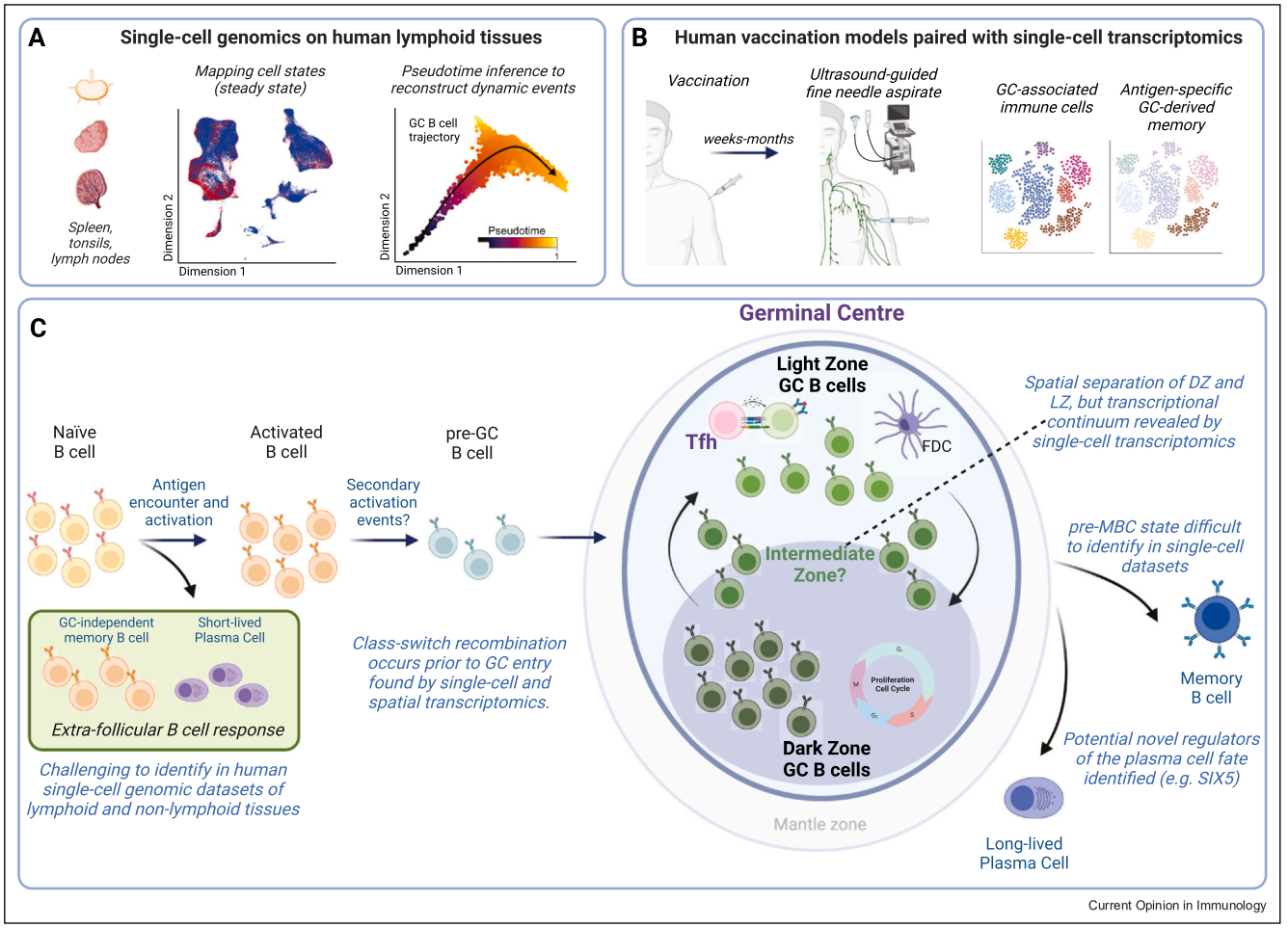
The germinal centre reaction and human health

Germinal centres (GCs) are transient immunological structures that form within secondary lymphoid organs and are required for activated B cells to mature into antigen-specific memory B cells and antibody-secreting plasma cells. After antigen encounter and activation, naïve B cells can undergo extrafollicular responses and differentiate into short-lived plasmablasts that secrete low-affinity antibodies [1]. Alternatively, they can migrate to interfollicular areas of secondary lymphoid organs to receive cognate interactions from T follicular helper (Tfh) cells and then enter follicles to participate in the GC reaction [2]. Unique cytokine-dependent signalling such as Tfh-derived CD40-LG, interleukin-4, and IL-21 controls the expression of GC regulatory transcription factors such as BCL6, leading GC B cells to become epigenetically and transcriptionally distinct from other B cell populations [3,4]. This rewiring of their cellular identity is required for GC B cells to undergo affinity maturation and selection for antigen-specific clones [2]. Within the GC, B cells cycle between a dark zone (DZ), where cells proliferate and undergo somatic hypermutation of their antibody genes to alter the affinity towards antigen, and a light zone (LZ) for selection of enhanced affinity towards the cognate antigen presented by follicular dendritic cells and Tfh cells [2,5]. GC B cells may then differentiate into either antibody-secreting plasma cells or long-lived memory B cells (Figure 1). Defects in the GC-dependent maturation of B cells result in reduced efficacy in both humoral-mediated immune responses and long-term immune memory [2,5]. The GC is also a site where self-reactive B cell clones can escape selection leading to autoantibody production [6], and lymphomas such as diffuse large B-cell lymphoma and Burkitt's lymphoma can arise from GC B cell populations [5]. Understanding the regulation and processes involved in human B cell maturation in the GC, therefore, has significant potential to enhance immune responses to vaccination and prevent immune disease.

While first identified in human tissue over one hundred years ago, much of what we know about the sequence of molecular and immunological events during B cell maturation in the GC has been learned from animal models. While these experimental approaches have and will continue to enable significant discoveries about the adaptive immune response, questions about translatability remain [7–9]. Here we discuss recent efforts to bridge this gap by 'mapping' or 'modelling' the human GC response, with a focus on GC-dependent B cell maturation: either through examining the cellular diversity and dynamics of GC-associated cells in human tissues with single-cell technologies or through the

Figure 1



Application of single-cell genomics to study GC-associated B cell maturation. **(a)** Example of how single-cell genomic studies have been used to map steady state GC B cell populations (left) or to reconstruct dynamic cellular transitions for the GC (right) [10,11]. **(b)** Recent efforts have studied vaccination-specific GC responses using ultrasound-guided fine needle aspirates to collect GC-associated lymphocytes, in addition to mapping the antigen specificity of B cells. **(c)** Overview of B cell activation and entry into the GC response. Challenges relating to the difficulty identifying extrafollicular B cell responses in human tissue, and recent discoveries about the entry, experience, and exit of B cells in the GC response are highlighted. Created with BioRender.com.

application of new culture systems to model human GC-associated cellular processes *ex vivo*.

Challenges and opportunities for the study of the human germinal centre response

Defined model antigens like hen egg lysozyme in murine models have allowed immunologists to temporally track the emergence and evolution of GC-dependent antigen-specific responses. In contrast to environmentally sterile and genetically inbred mouse models, the study of the human GC is made more challenging by genetic diversity between individuals, varied immunological or infection histories, and a highly polyclonal adaptive immune repertoire. These polyclonal responses exist not only within a given lymphoid tissue but even within a single GC, fitting with the idea that GCs are 'open' and can be invaded by other antigens and antigen-specific B cells, further increasing the complexity of the analysis of human GC responses. Another challenge is that GC B cells are absent from peripheral blood and can only be isolated from tissues such as spleen or lymph nodes that typically require invasive surgery to access, usually only performed in the event of major illness like cancer. A common alternative has therefore been the use of palatine tonsils or adenoids (pharyngeal tonsils) routinely removed to treat obstructive sleep apnoea or recurrent tonsillitis. Recent single-cell transcriptomic profiling of different secondary lymphoid organs suggests that many GC-associated cell states are similar between different organs at steady state, and these single-cell maps have been used to reconstruct dynamic cellular transitions with pseudotemporal analyses [10,11] (Figure 1a). An exciting area of development recently has been the use of ultrasound-guided fine needle aspiration to collect lymphocytes from lymph nodes [12,13]. This has allowed the analysis of antigen-specific GC B cells post-vaccination and even to track long-term GC-derived memory through longitudinal sampling of the same patients [14–16] (Figure 1b). One notable study of individuals who received a SARS-CoV-2 mRNA vaccine identified GC-derived memory and plasma cells with affinity to SARS-CoV-2 spike protein as part of persistent GC reactions that lasted over six months [16]. While significant challenges exist in the widespread adoption of fine-needle aspirates, it holds enormous potential to enable the longitudinal analysis of human GC responses, which is valuable for studying immune responses to pathogens for which animal infection models do not exist or do not accurately recapitulate human disease.

New technologies capturing a snapshot of human germinal centre B cell dynamics

Single-cell and spatial genomics now provide increasingly high resolution of the cellular and regulatory dynamics during the human GC response (Figure 1c). The application of single-cell transcriptomics (RNA-seq), epigenomics (e.g. assay for transposase-accessible chromatin with sequencing), antibody gene sequence (e.g. refers to the V, D, J genes of immunoglobulin), antigen specificity (e.g. linking B cell receptor to antigen specificity through sequencing) and cell surface markers (e.g. cellular indexing of transcriptomes and epitopes followed by sequencing, Cytometry by time of flight) to explore GC-associated cell populations in human secondary lymphoid organs has exploded in recent years [10,14–23]. Here we highlight several key studies that have provided new insights into how B cells enter, experience, and exit the GC reaction in human tissue.

Mapping activation dynamics during germinal centre entry

After activation, the dynamic transition from activated B cell to GC B cell has proven challenging to investigate in human tissues. We and others have identified and characterised putative GC precursor cell states with single-cell genomics of human tonsils [10,20,21]. These 'pre-GC' B cells are in a transcriptionally distinct state that appears to be transitioning between classically activated B cell populations and GC cells and likely represent an early GC B cell population. Spatial transcriptomics [11,24] and epigenomics [25] studies support that these cells are found within extrafollicular regions and are absent in existing GCs in human tissue. Similar populations have since been reported in mouse lymphoid tissues [26]. This pre-GC state has elevated expression of genes involved with class switch recombination as well as immunoglobulin germline transcripts, consistent with reports that class switch recombination occurs predominantly outside of the GC that collectively have required a reappraisal of our understanding of how the B cell repertoire is shaped and selected [10,24,27,28]. Spatial analysis of expanded B cell clones in human lymphoid tissue [24] found further evidence for class switching occurring extrafollicularly (where pre-GC B cell state localises), and that while most clonal families are limited to a single GCs, many GCs are polyclonal and contain many different expanded B cell clones. The lack of apparent clonal expansion in the pre-GC state by single-cell VDJ sequencing (in fitting with it being a pre-expansion cell state) has made it difficult to retrospectively reconstruct lineages to support fate decisions of this cell type but will be a key area for future research into the GC response.

Light zone vs dark zone, or something in between

Once B cells have entered the GC reaction, they iteratively cycle between the LZ and the DZ. Identification of CD83 and CXCR4 as cell surface markers that distinguish between LZ and DZ B cells respectively [29] has reinforced a typically binary view of GC cells into these two categories (often referred to as centrocytes and

centroblasts). However, single-cell genomic studies have demonstrated that rather than following a simple binary classification into DZ or LZ cells, GC B cells exist in a multitude of diverse states with distinct features, including intermediary states on a continuum between LZ and DZ gene expression, in addition to more discrete cell states with unique surface marker expression, different phases of the cell cycle, or expression of differentiation markers [10,20–23,30]. As spatial technologies increase in resolution, it will be exciting to explore whether the intermediary LZ-DZ B cell states identified in single-cell datasets (also termed 'grey' zone cells and identified previously in mouse GC responses [31]) and other rare GC B cell states reflect distinct histological niches in the GC.

Exiting the germinal centre — predicting new regulators of plasma and memory fates

Single-cell studies have also reconstructed transcriptional and epigenetic dynamics during exit from the GC, particularly with respect to plasma cell differentiation [10,19–22]. Even for such a well-studied cell fate trajectory, many new hypotheses have been generated about potential regulators of human B cell differentiation, including the transcription factor SIX5 recently identified to be specifically expressed during the later stages of plasma cell differentiation, along with increased chromatin accessibility and selective expression of its predicted target genes [21]. Whether SIX5 is required for plasma cell fate decisions has yet to be determined experimentally. Reconstructing the trajectory for GC-derived memory B cells has proven more challenging. Prememory CCR6^{+} GC B cells were first reported in 2017 [32–34], and several single-cell studies have identified *CCR6^{high}* GC B cells in human tonsil GCs [21,22]. While the trajectory from GC to plasma lineage can be robustly reconstructed with pseudotemporal methods [10,19–22], to our knowledge, no confident pseudotemporal trajectories for the human GC to memory trajectory have been reported. These observations, or lack thereof, could reflect stochastic or passive entry into the memory fate compared with active and transcription factor–directed plasma cell fate determination. Alternatively, technical challenges around the transcriptional similarity of naïve and memory B cells or the rarity of a memory precursor state and intermediates could explain why it has proven difficult to study this trajectory.

There remain many other exciting areas for exploration into the human GC response with advanced single-cell technologies, especially the dissection of diverse Tfh, T follicular regulatory cells, or stromal cell states required to support B cell maturation [35,36]. Single-cell methodologies also offer opportunities to study the role of GC B cells in disease, including B cell lymphomas [21,23,30] or tertiary lymphoid structures in solid tumours that contain GC-like B cells [37–39]. These GC-like tertiary lymphoid structures, forming in non-lymphoid tissues and driving the immune response at the sites of chronic inflammation, are also of interest in autoimmune conditions such as lupus nephritis, rheumatoid arthritis, multiple sclerosis, and Sjögren's syndrome [40]. As the use of advanced single-cell technologies becomes increasingly commonplace, we look forward to continued exploration of the human GC with enhanced resolution in non-diseased lymphoid tissues, comparison of GC cellular dynamics in individuals living with autoimmune diseases or immunodeficiencies, and examination of different GC responses when challenged with different vaccination strategies (e.g. attenuated virus, peptide-based, or mRNA vaccines).

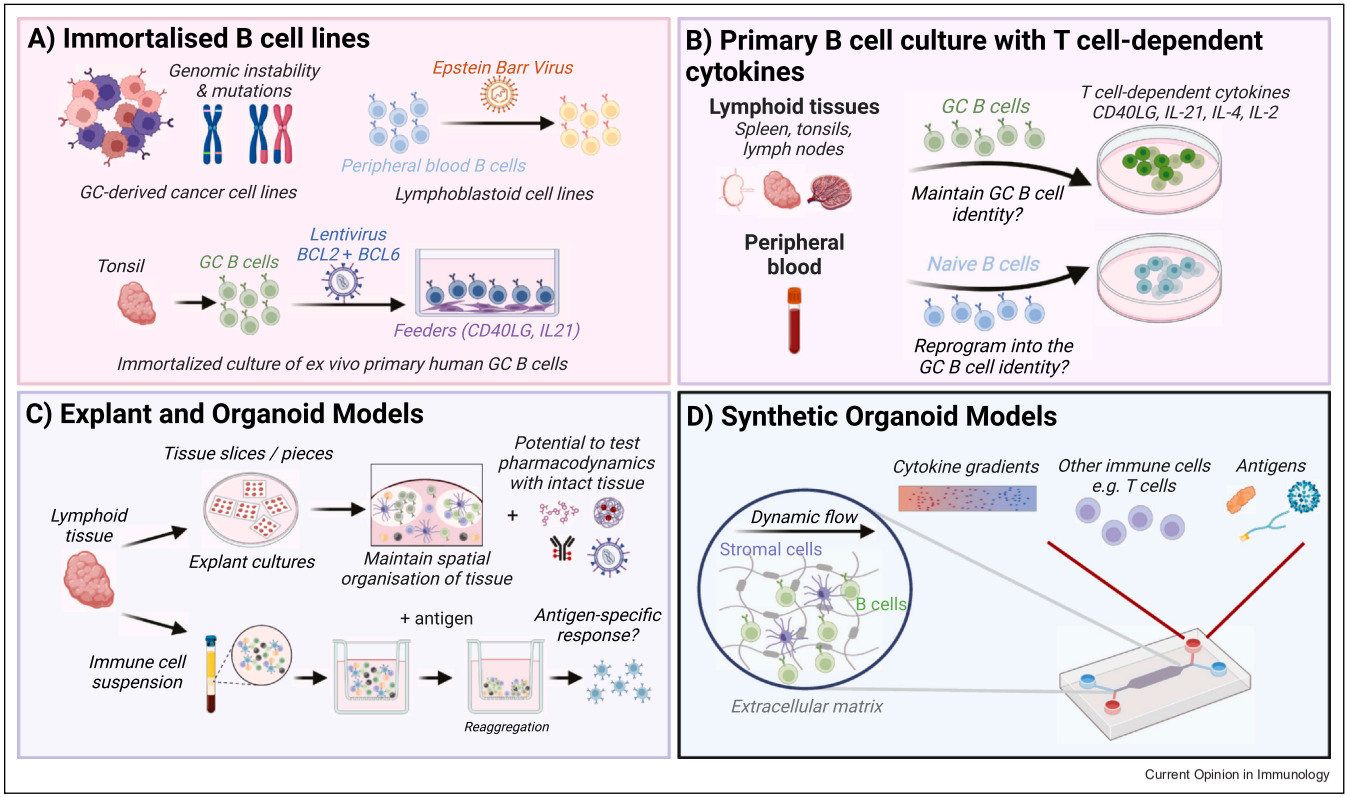
Modelling the human germinal centre response *ex vivo* — from cells to organoids

As discussed above, increasingly high-throughput and 'unbiased' single-cell genomic methods have generated an enormous amount of data about different cell states and potential regulators in the human GC. However, many of these observations remain correlative and yet to be experimentally tested, in part due to limitations of available cellular and genetic tools for studying primary B cells. Here we review some of the methods available to study human GC B cells and how recent advances in tissue explants and organoids now offer increasingly sophisticated models that incorporate the multicellular complexity of the GC (Figure 2). We propose that in addition to measuring functional outputs of GC B cells (e.g. survival, differentiation to antibody-secreting cells, class switch recombination, somatic hypermutation, and antigen specificity), the increasingly comprehensive single-cell maps of the human GC provide an opportunity for a more quantitative assessment of B cell identity (gene expression, chromatin-based regulatory networks, and cell surface marker expression) in *ex vivo* cultures to determine how closely they model human GC B cells.

Human (germinal centre) B cell lines

Human B cells isolated from lymphoid tissues or peripheral blood require specific activation and stimulation signals to survive *ex vivo* but even then, they will rapidly die and/or differentiate within 2–14 days. Immortalisation of human B cells using Epstein–Barr virus to make lymphoblastoid cell lines (GM12878) [41], GC-derived lymphoma cell lines (Ramos, Raji, and Daudi), or transformation with pro-survival genes such as *BCL2* and *BCL6* or *MYC* [42,43] offer long-term culture models to investigate some cell-intrinsic features of human GC B cells. However, while these methods provide more tractable experimental models for perturbation studies of gene function in GC B cells, it is not clear how closely these models reflect *bona fide* GC B cell states due to viral-dependent gene expression or

Figure 2



Cell culture and organoid models for human GC B cells. **(a)** Multiple cell line models have been used to study human GC processes in B cells, including lymphoma-derived cell lines, Epstein-Barr virus-transformed lymphoblastoid cell lines from peripheral blood B cells, and immortalisation of tonsillar GC B cells with BCL2 and BCL6/MYC overexpression. **(b)** Culture of either naïve B cells or GC B cells with T cell–dependent cytokines has been widely used to keep human B cells alive *ex vivo* and model differentiation. How closely these methods either reprogram or maintain GC B cell states is unclear. **(c)** Recent advances in lymphoid tissue explants or organoid/aggregate cultures provide new opportunities to investigate the spatial organisation of GC reactions and the interactions between different cell types driving antigen-specific responses *ex vivo*. **(d)** Synthetic organoid models give the possibility for a high degree of control of cellular, cytokine, and other culture system dynamics to create 'GC-on-a-chip' systems to experimentally investigate the human GC response. Created with BioRender.com.

extensive genomic mutations and rearrangements in cancer cell lines.

Modelling T cell–dependent germinal centre B cell cultures with cytokines — too much of a good thing?

In many human B cell cultures, CD40-LG and IL-21 mimic signals from Tfh cells in the GC [3,4], and these two cytokines are sufficient to enable short-term B cell survival and differentiation *ex vivo*. These cytokines can be delivered as soluble recombinant proteins or by feeder cell lines engineered to express human CD40-LG and IL-21 [42]. In addition, a multitude of different combinations of these cytokines with additional stimuli (e.g. IL-2, IL-4, and anti-IgM) exist, of which the full breadth is beyond the scope of this review. Instead of isolating *bona fide* GC B cells from secondary lymphoid organs for cell culture experiments, it is more common to isolate naïve B cells from peripheral blood and to treat them with T cell–dependent cytokines to model the GC B cell response. While this can approximate several features of GC B cells, such as increased expression of activation markers and GC regulators like BCL6 and AICDA, plasma cell differentiation, and, in some cases, class switch recombination, it is not clear how well cells in these culture conditions recapitulate GC B cell states. One recent study used single-cell transcriptomics to explore human naïve B cell cultures after growth with IL-4, IL-21, and CD40-LG and reported B cells with a GC-like phenotype *in vitro* [44]. This was based on the expression of key marker genes and scoring of GC-derived marker gene sets between different cell states in the culture, providing a relative, rather than absolute, quantification of their GC-like state.

Another underexplored question is whether the saturating concentrations of recombinant cytokines in B cell cultures accurately reflect the increasingly 'signal-poor' conditions B cells experience in an ongoing GC response. Competition for signals is likely to be key to prevent self-reactive B cell clones from arising in the GC [45–47] — could the saturating cytokine concentrations in *ex vivo* culture, therefore, be a closer approximation of aberrant signalling that occurs in autoimmunity or other disease? Similarly, most current models fail to account for the hypoxic conditions of lymphoid tissues and the GCs within them, and hypoxic cell culture models have been reported to enhance B cell differentiation *ex vivo* [48]. Finally, a common output from 'T cell–dependent' culture methods is the differentiation of antibody-secreting plasma cells, although it is also possible that this plasma cell differentiation is more akin to extrafollicular B cell responses, especially given the lack of somatic hypermutation and affinity maturation in many such culture systems. To understand the advantages and limitations of different approaches, more unbiased and data-driven comparisons between *ex vivo* cultured B cells and *in vivo* GC B cell states will be important. For example, increasingly comprehensive single-cell maps of GC B cell states from lymphoid tissues can either be directly integrated with culture-derived gene expression datasets (although this faces technical batch effect challenges) or used as a reference data framework to 'project' query *in vitro* datasets on to *in vivo* reference datasets. Both methods, carefully implemented, should provide a more quantitative assessment of the cellular identity of *in vivo* cell states compared with GC B cell populations in tissue.

Tissue explants and organoids — adaptive immune responses in a dish

For all their advantages, the culture of purified B cells in suspension or on feeder cells lacks the spatial dynamics and cell-to-cell interactions that normally exist within GCs in tissue. Lymphoid explants, either as blocks or thin slices of tissue, retain this 3D and spatial organisation and thus may be a more accurate model for microenvironmental or histological features of the GC response. These methods have been used to explore questions ranging from anti-inflammatory drug effects to viral infection routes in human lymphoid tissue [18,49–51]. The ability to model perfusion of chemical treatments or antigens through solid tissue holds significant promise for pharmacological or other translational studies by offering a more representative and physiologically relevant model of human tissue than cells in suspension [50]. However, their utility may be limited due to tissue deterioration even in short-term cultures (< 3 days) and higher rates of technical variability compared with cellular suspensions from the same tissue.

Lymphoid organoid models that involve the cultured reaggregation of mononuclear cells dissociated from lymphoid tissues have recently been reported [52,53]. Remarkably, these culture systems have been proposed to form a GC-like spatial organisation, and when incubated with varied antigens such as influenza vaccine or SARS-CoV2 peptides, antigen-specific B cells could be detected after several weeks in culture. A recent analysis of B cells isolated from tonsil organoids has a low number of GC-like B cells [53], raising the question of whether these cultures may keep existing GC B cells alive but do not sustain differentiation from naïve to GC B cells. The inclusion of additional recombinant cytokines in these organoid cultures could be trialled to enhance the survival and differentiation of cells. Several years after the publication of this protocol, it remains unclear what potential and promise these immune organoids hold for the field to model antigen-specific GC responses in humans.

Finally, synthetic microenvironmental culture systems are gaining popularity to model the lymphoid organ environment of the GC [54]. Culture of peripheral blood-derived B cells with a synthetic extracellular

matrix, CD40L-expressing fibroblasts and tonsillar stromal cells, allowed class switching and differentiation into antibody-secreting cells [55]. A further step towards modelling the GC is the use of microfluidic chips that can integrate dynamic fluid flow as well as these environmental architectures. A recent GC organ-on-a-chip model reported spontaneous aggregation of B cells into structures resembling lymphoid follicles, wherein cells demonstrated class switch recombination and plasma cell differentiation, as well as even production of antigen-specific IgG when stimulated with an antigen in the presence of follicular dendritic cells [56]. The potential for control over cytokine gradients experienced by B cells could be used to build even better models for the human GC.

Future directions — will better maps mean better experimental models?

The emergence of single-cell and spatial technologies has empowered human immunologists to start exploring the GC response in detail, which was not possible even a decade ago, except in mouse or animal studies. Now a challenge for the field is to transition from 'mapping' and correlative predictions of human GC B cell states to experimental modelling of the GC *ex vivo*. This poses many challenges, not just with respect to the nature or reproducibility of B cells in varied cultured systems but also regarding the application of experimental tools like CRISPR/Cas9 to these cell culture models to perturb gene function. Although many B cell culture systems have been routinely used for decades now, the advent of unbiased single-cell technologies and expansive *in vivo* cell atlases of human B cell states now provides an opportunity for a critical assessment of how well, if at all, these model culture systems reflect the true GC experience for human B cells. Recent efforts in creating a unified data framework for myeloid lineages [57] could be applied to the human GC to provide a data-driven paradigm to better understand whether our *in vitro* models capture *in vivo* cellular states or not. Cellular signalling and transcriptional information from single-cell datasets could then be used to redesign or refine culture models, including integration with disease-specific datasets to model pathological GC reactions, such as in the context of autoimmune disease where it is difficult to study the loss of peripheral tolerance in autoimmunity in humans. Together, different emerging technologies and culture systems mean we can continue tackling bigger questions about human GC biology, although we should be cautious and critical of the models and conclusions that we draw — there may not be one size fits all.

Declaration of Competing Interest

We, the authors, declare no conflict of interest for the manuscript entitled 'Mapping and modelling human B cell maturation in the germinal centre'.

Data Availability

No data were used for the research described in the article.

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- •• of outstanding interest

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