Transcriptional regulation of memory B cell differentiation

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Abstract | Memory B cells (MBCs) are critical for the rapid development of protective immunity following re-infection. MBCs capable of neutralizing distinct subclasses of pathogens, such as influenza and HIV, have been identified in humans. However, efforts to develop vaccines that induce broadly protective MBCs to rapidly mutating pathogens have not yet been successful. Better understanding of the signals regulating MBC development and function are essential to overcome current challenges hindering successful vaccine development. Here, we discuss recent advancements regarding the signals and transcription factors regulating germinal centre-derived MBC development and function.

Germinal centre
A structure that forms in the B cell follicle of secondary lymphoid organs during an immune response in which B cells engage with antigen and compete for signals necessary for their survival, proliferation and differentiation into plasma cells or memory B cells.

Somatic hypermutation
A cellular process in which proliferating B cells accumulate mutations in antibody complementarity-determining regions, potentially impacting their ability to recognize antigen.

During an immune response, B cells that encounter their cognate antigen become activated and differentiate to form short-lived antibody-secreting cells or germinal centre (GC)-independent memory B cells (MBCs). Within the GC, B cells engage with antigen and compete for limiting amounts of T cell help, which is necessary for B cell survival, proliferation and eventual differentiation into plasma cells or GC-derived MBCs. The GC is also the primary site in which B cells undergo somatic hypermutation, with B cells that accrue productive mutations preferentially receiving T cell help. The GC response is required for the development of affinity-matured plasma cells and MBCs (Box 1).

MBCs are an important component of protective immunity. MBCs are distinguished by their capacity to survive long term and to rapidly differentiate into antibody-secreting cells upon antigen re-encounter. MBCs can also re-enter the GC during recall responses, where they undergo further somatic hypermutation2–5. MBCs tend to emerge from the GC during the early phases of the GC response and typically display reduced levels of somatic hypermutation and affinity maturation relative to plasma cells4–9. In the context of viral infections, the reduced mutational load of MBCs allows these cells to maintain enhanced flexibility in their responsiveness to different viral subtypes compared with plasma cells, which tend to be specific for a particular subtype. Indeed, the MBC population contains an elevated fraction of broadly reactive clones relative to the plasma cell pool for numerous pathogens in both mice and humans8–12. The MBC response comprises multiple subsets, identified based on their expression of CD80 and PD1L2, among other markers9 (Box 2). These MBC subsets emerge from the GC at different times and vary in their capacity to re-enter the GC or differentiate into antibody-secreting cells upon antigen re-encounter9.

Iterative exposure to cross-reactive viral antigens is an emerging vaccination strategy designed to elicit broadly reactive MBCs capable of mediating heterosubtypic immunity against pathogens such as influenza. The potential efficacy of an iterative vaccination strategy is limited by the relative inefficiency with which most MBC clones re-enter the GC response13–15. The secondary GC response tends to consist largely of recently activated naive B cells, with only certain MBC subsets possessing the capacity to efficiently re-enter the GC15,16. Currently, it is unclear why the majority of MBCs fail to participate in secondary GC responses. One possibility is that antigen-specific antibodies limit the ability of MBC clones of the same specificity to access antigen and participate in the GC response4,15.

MBCs can arise through both GC-dependent and GC-independent pathways15–20. GC-independent MBCs largely develop during the early stages of the immune response and contribute to protective immunity against numerous pathogens including Ehrlichia muris and malaria14,21. GC-independent MBCs can be somatically hypermutated but are not thought to undergo affinity maturation, a process that is largely limited to GC-dependent MBCs14,15,20. GC-independent MBCs are also largely not class-switched16,20. By contrast, GC-derived MBCs can undergo affinity maturation and are often class-switched, allowing these cells to have an enhanced ability to mediate clearance of some pathogens. Here, we focus on GC-dependent MBCs.

Considerable progress has been made in recent years in elucidating the interactions and signalling pathways that regulate the GC B cell response. However, a better understanding of the mechanisms that govern MBC development and function is needed for the design of vaccines capable of eliciting broadly reactive MBCs that robustly participate in recall responses. In this Review,
Within the germinal centre (GC), B cells compete for antigen and limiting amounts of T cell help (delivered via CD40 ligand and cytokines). Higher-affinity B cells tend to capture more antigen, receive more T cell help and, subsequently, migrate from the light zone, where T cells reside, to the dark zone. Within the dark zone, B cells undergo rapid proliferation and somatic hypermutation, with B cells that accrue damaging mutations or that are not selected by T cells undergo apoptosis, leading to a progressive increase in B cell affinity over the course of the GC response. The enzyme activation-induced cytidine deaminase (AID) mediates somatic hypermutation and class-switch recombination. AID expression is promoted by the transcription factors basic leucine zipper transcription factor (BATF), PAX5, transcription factor 3 (TCF3) and interferon regulatory factor 8 (IRF8) and is inhibited by the transcriptional inhibitors inhibitor of DNA binding 2 (ID2) and ID3.

The co-activator OBF1, which forms a complex with the transcription factors POU class 2 homeobox 1 (POU2F1; also known as OCT1) and POU2F2 (also known as OCT2), plays a central role in GC development as deficiency in OBF1 and/or OCT2 results in an inability to form GCs. OCT2 directly regulates the expression of the tyrosine kinase SYK and indirectly regulates the expression of the signalling molecule SLAMF1, which have important roles in BCR signalling and T cell:B cell interactions, respectively. OBF1 and OCT2 induce B cell expression of IL-6, which can promote T FH cell differentiation. The E26 transformation-specific (ETS) transcription factor SPI-B is a direct target of OBF1 and is necessary for GC initiation. SPI-B functions in complex with PU.1 to enable B cells to respond appropriately to environmental cues by regulating the expression of BCR signalling pathway components, the receptor CD40, B cell activating factor receptor (BAFFR) and Toll-like receptors (TLRs).

Myc is required for both the initiation and the maintenance of the GC response. MYC is an important regulator of cell proliferation, with MYC- B cells expressing higher levels of cell cycle-promoting genes such as cyclin D2 (Ccn2) and cyclin D3 (Ccn3). MYC also induces the expression of E2F transcription factor 1 (E2F1). E2F1 promotes B cell differentiation through the induction of the histone methyltransferase enhancer of zeste homologue 2 (Ezh2). Ezh2 is the enzymatic component of the Polycomb repressive complex 2 (PRC2) and is responsible for histone 3 trimethylated at lysine 27 (H3K27me3). Ezh2 promotes B cell proliferation via the repression of genes encoding the cyclin-dependent kinase inhibitors (Cdkn1a, Cdkn2a and Cdkn1b). Ezh2 also phosphorylates retinoblastoma (Rb) protein, which enhances E2F1 release from Rb and promotes further Ezh2 expression. MYC is transiently induced in B cells that interact with antigen or T FH cells. MYC is subsequently downregulated as B cells commit to the GC fate, perhaps via direct transcriptional repression by BCL-6.

The transcriptional activators MEF2C and MEF2B also promote GC development. MEF2C also promote GC development. MEF2C also promote GC development. Which in turn activates the transcription factor nuclear factor-kB (NF-kB). Sustained IRF4 expression is sufficient to directly repress BCL-6 expression and promote plasma cell differentiation, which may reflect the dose-dependent function of IRF4 in B cells. IRF8 regulates GC B cell development through the direct induction of BCL-6 expression and can promote GC B cell survival through the induction of the E3 ubiquitin ligase MDM2. IRF8 forms complexes with transcription factors such as SPI-1 (also known as PU.1), which allow it to efficiently bind to target DNA sequences and regulate gene expression. Whereas ablation of IRF8 alone in B cells is not sufficient to overtly impair GC development, compound loss of both IRF8 and PU.1 results in an almost complete loss of GC development. These data suggest that IRF8/PU.1-mediated induction of BCL-6 early after activation is an important regulator of GC initiation.
and survival, including *Cnd2* and B cell lymphoma-extra large (*Bcl2l1)* [6,7]. *MEF2B* is mutated in cases of diffuse large B cell lymphoma (DLBCL) and follicular lymphoma and directly promotes the expression of BCL-6 [REF. 8]. Loss of *MEF2B* results in partial impairment of GC formation, with *MEF2B* regulating the expression of a diverse network of BCL-6-dependent and BCL-6-independent genes that are important for GC development and function [8]. MEF2C and MEF2B can form dimers, with ablation of both proteins resulting in a marked reduction in GC development, suggesting that they may have partially redundant functions [8].

The basic helix–loop–helix transcription factors 3 (TCF3; also known as E2A) and TCF4 function together to promote GC B cell development [8,9]. TCF3 and TCF4 act primarily as transcriptional activators in B cells and regulate the expression of genes critical for GC development, including *Icosl*, *Mef2b* and *Pou2af1* [REFS 10,11]. TCF3 and TCF4 are expressed in B cells before activation but have limited activity owing to inhibition by inhibitor of DNA binding 3 (ID3). B cell activation promotes the downregulation of ID3, leading to increased TCF3/TCF4 activity owing to reduced suppression by ID3 [REF. 12]. Deficiency in ID3 results in reduced GC B cell development, perhaps owing to aberrant activity of TCF3/TCF4 [REF. 12].

The zinc finger transcription factor Yin and Yang 1 (YY1) is also important for the development and maintenance of GC B cell development [8]. YY1 is important for all stages of B cell differentiation and the precise mechanism by which YY1 promotes the GC response is currently unclear. Similarly, the transcription factor early B cell factor 1 (EBF1) is necessary for the development of mature B cell subsets, with deficiency in EBF1 resulting in a reduction in GC B cell development and maintenance [8,9].

**Box 2 | Memory B cell subsets**

Numerous cell surface markers, including CD80, PDL2, CD44, CD62L and CD73, are differentially expressed on memory B cells (MBCs) [8,9]. Three major MBC subsets have been defined in the mouse: CD80<sup>+</sup> PDL2<sup>+</sup> (double positive), CD80<sup>+</sup> PDL2<sup>−</sup> (single positive) and CD80<sup>−</sup> PDL2<sup>−</sup> (double negative). MBC subsets develop during three overlapping periods, with double-positive MBCs developing last and having undergone the greatest amount of somatic hypermutation and class-switching [8,9]. The extent of CD40 signalling may regulate MBC subset development [8]. The MBC isotype has also been reported to regulate MBC function upon recall, with IgM<sup>+</sup> MBCs preferably developing into germinal centre (GC) B cells and IgG<sup>+</sup> MBCs developing into antibody-secreting cells [8,9]. However, subsequent studies found that subset composition, not isotype, was the determining factor for the MBC fate upon recall, with double-negative MBCs preferentially developing into GC B cells and double-positive MBCs developing into antibody-secreting cells [8]. Single-positive MBCs had an intermediate phenotype and could develop into either GC B cells or antibody-secreting cells [8]. MBC subsets express distinct transcriptional signatures, which likely regulate their function upon recall [8,9]. Similar murine MBC subsets have been identified in numerous immune contexts, including following influenza, lymphocytic choriomeningitis virus and malaria infection and during commensal-driven responses in Peyer’s patches [12,13,14]. The relationship between murine and human MBC subsets remains unclear. Considering that human MBCs express CD80, but not PDL2 or CD73, it appears that human and murine MBC subsets express only partially overlapping markers [12,13,14]. Markers of human MBCs include CD27, CD21, CCR2, CEACAM21, Toll-like receptors (TLRs) and Fc-receptor-like proteins [12,13,14]. An improved understanding of the functional capacities of human MBC subsets will be essential for the design of vaccines that are maximally effective in inducing durable immunity.

**Regulation of GC B cell maintenance**

BCL-6 expression is essential for maintenance of the GC state through multiple mechanisms, including regulation of the stability of the transcriptional repressor BTB domain and CNC homology 2 (BACH2) [12] (FIG. 2). BCL-6 and BACH2 cooperate to regulate the GC transcription programme and repress expression of plasma cell-defining factors, such as B lymphocyte-induced maturation protein 1 (BLIMP1; also known as PRDM1) [6,7]. BCR and CD40 signalling can transiently disrupt BCL-6 activity through mitogen-activated protein kinase (MAPK) signalling and post-translational modification of the BCL-6 co-repressors SMRT and NCoR [6,7]. Sustained CD40 signalling can promote IRF4 expression, which can directly repress BCL-6 expression, resulting in enhanced expression of BCL-6-repressed BLIMP1 and, ultimately, plasma cell differentiation [7,8]. The T<sup>H</sup><sub>1</sub>-cell-derived cytokines IL-4 and IL-21 can bind to IL-4R and IL-21R on B cells, and downstream signalling from these receptors counteracts BCR signalling-induced degradation of BCL-6 [REFS 29,30]. GC B cells express the E3 ubiquitin-protein ligase CBL, which promotes IRF4 degradation and prevents premature GC exit [11]. CBL ubiquitin ligases also negatively regulate BCR signalling through the degradation of SYK [31,32]. Strong CD40 and BCR signalling can lead to a decrease in expression of CBL and facilitate plasma cell differentiation [11].

There is emerging evidence that the transcription factor requirements for GC responses may differ depending on the type of immune response. For example, T-bet (also known as T-box transcription factor 21 (TBX21)) acts in a B cell-intrinsic manner to both restrict the overall GC response and promote the dark zone GC B cell transcriptional programme following malaria infection [33,34]. T-bet may also be needed for the spontaneous interferon-γ (IFNγ)-driven GC responses that occur in mice prone to autoimmunity [8,9], although this is not universally the case [35]. T-bet<sup>+</sup> MBCs, also known as atypical MBCs, accumulate during autoimmunity, chronic infection and ageing and participate in both protective and pathogenic immune responses (Box 3).

The transcription factor forkhead box O1 (FOXO1) is critical for the development of dark zone GC B cells [36–39]. Ablation of FOXO1 leads to an impairment in the dark zone transcriptional programme and reduced affinity maturation. FOXO1 regulates the expression of numerous genes, including Cxcr4, which encode a G-protein-coupled receptor necessary for migration of B cells to the dark zone [39,40]. Transforming growth factor-β (TGFβ) and IL-10 signalling in GC B cells promote FOXO1 expression [41,42]. The transcriptional programme regulated by FOXO1 largely overlaps with that of BCL-6, suggesting that FOXO1 and BCL-6 cooperate to support the dark zone state [11]. FOXO1 is important for maintenance of the GC proliferative state and is required for the induction of basic leucine zipper transcription factor (BATF) expression in light zone GC cells. FOXO1 does not directly induce BATF but rather is likely necessary to upregulate the expression of CD40 and BCR signalling components that subsequently promote BATF expression [43,44]. BATF is required for GC maintenance
Fig. 1 | Transcriptional regulation of GC B cell commitment. Model for signalling pathways and transcription factors that regulate B cell commitment to the germinal centre (GC) fate. Boxes that indicate signalling molecules are coloured yellow, transcription regulators red, downstream gene targets turquoise and epigenetic modifiers purple. The B cell receptor (BCR), via its signalling subunits Igα and Igβ as well as downstream tyrosine kinases such as SYK and LYN, activates the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT pathways. MAPK signalling induces the expression of the transcriptional activator myocyte enhancer binding factor 2c (MEF2C), which promotes the transcription of B cell lymphoma-extra large (Bcl2l1) and cyclin D2 (Ccdn2). The expression of the transcriptional repressor inhibitor of DNA binding 3 (ID3) is reduced following B cell activation, allowing for transcription factor 3/4 (TCF3/4)-driven induction of Icosl and Mef2b transcription. PI3K/AKT and nuclear factor-κB (NF-κB) signalling also induce the expression of the transcription factor interferon regulatory factor 4 (IRF4). IRF4 and TCF3/4 induce the expression of the co-activator OBF1 (also known as POU class 2 homeobox associating factor 1 (POU2AF1)), which cooperates with OCT2 (also known as POU class 2 homeobox 2 (POU2F2)) to promote transcription of Slamf1, Syk and Il6, OBf1 and OCT2 can also induce the expression of the transcription factor SPI-B, which acts in a redundant fashion with PU.1 to enhance the transcription of genes encoding B cell surface receptors, such as Cd40, B cell activating factor receptor (Baffr), Toll-like receptor 4 (Tlr4) and Tlr9, and many components of the BCR signalling pathway, including Blnk and Btk. Although transiently elevated levels of IRF4 can induce the expression of B cell lymphoma 6 (Bcl2l1) and cyclin D2 (Ccdn2), the expression of IRF4 levels will repress BCL-6 expression. BCL-6 expression is also induced by the transcription factors MEF2B and IRF8 and is also directly regulated by the cytokines IL-4 and IL-21, which bind to their respective receptors (IL-4R and IL-21R) and induce signal transducer and activator of transcription 6 (Stat6) signalling. CD40 and/or TLR-driven NF-κB signalling, alongside PI3K/AKT signalling, will induce the expression of the transcription factor MYC, which promotes cellular proliferation by inducing the expression of Ccnd2/Ccnd3 and the expression of the transcription factor EZF transcription factor 1 (EZF1). EZF1 induces expression of Zeste homologue 2 (Ezh2) that encodes a Polycomb repressive complex 2 (PRC2) enzymatic component. Enhancer of zeste homologue 2 (Ezh2) promotes cell cycle progression by repressing the expression of Cdkn1a, Cdkn2a, and Cdkn2b, which encode cyclin-dependent kinase inhibitors. EZH2 also promotes EZF1 release from the retinoblastoma (Rb) protein via phosphorylation of Rb, thereby enhancing E2F1 activation and further Ezh2 expression. BCL-6 can directly repress MYC expression, thereby limiting the number of cell divisions that GC B cells undergo. BCL-6 promotes GC B cell development through regulation of numerous genes controlling cellular processes including the DNA damage response, B cell migration, apoptosis, BCR and CD40 signalling, plasma cell differentiation and T cell:B cell interactions. Together, these transcriptional regulators allow for the precise control of GC initiation that is necessary to balance the competing needs of the immune system to induce a protective response while limiting immunopathology.

and contributes to the GC proliferative state. BATF also directly regulates the expression and targeting of activation-induced cytidine deaminase (AID). Phosphoinositide 3-kinase (PI3K) signalling actively restricts FOXO1 expression in light zone GC B cells, with only a small fraction of light zone GC B cells expressing FOXO1. FOXO1-expressing light zone GC B cells also express MYC, suggesting that they are actively receiving T cell help. MYC expression is induced in amounts proportional to the strength of T cell
help and determines the number of cell divisions that occur in the dark zone\(^6\). MYC regulates multiple aspects of cell proliferation including metabolism and the expression of proteins that are necessary to sustain cell division, such as activating enhancer binding protein 4 (AP4; also known as TFAP4) and ubiquitin-like, containing PHD and RING finger domains, 1 (UHRF1)\(^{9,90}\). MYC interacts with the transcriptional activator MYC-interacting zinc finger protein 1 (MIZ1) to form a transcriptional repressor complex that represses MIZ1 target genes\(^\)\(^9\). The MYC–MIZ1 complex promotes cell cycle entry of GC B cells actively receiving T cell help\(^9\). Whereas MYC is rapidly downregulated in GC B cells that enter the dark zone, AP4 expression is maintained and is necessary for dark zone B cells to undergo continued cell division\(^9\).

Fig. 2 | Transcriptional regulation of GC B cell maintenance. Model for signalling pathways and transcription factors that regulate germinal centre (GC) B cell maintenance. Boxes that indicate signalling molecules are coloured yellow, transcription factors red, downstream gene targets turquoise, cytokines blue (drawn inside cell for simplicity) and epigenetic modifiers purple. The B cell receptor (BCR), via its signalling subunits Ig\(\alpha\) and Ig\(\beta\), engages the tyrosine kinases SYK and LYN and the adapter protein BLNK, leading to activation of phosphoinositide 3-kinase (PI3K) and subsequent phosphorylation of AKT at S473. In GC B cells, AKT is already phosphorylated at T308. PI3K/AKT signalling represses forkhead box O1 (FOXO1) expression and attenuates BCR signalling through phosphorylation of the signalling molecules SHP1, CSK and HPK1, which negatively regulate the activity of SYK, LYN and BLNK, respectively. GC B cells also express high levels of the phosphatase and tensin homologue (PTEN), which restrains PI3K-mediated phosphorylation of AKT at S473. PI3K/AKT signalling, along with CD40-driven nuclear factor-xB (NF-xB) activity, can induce mechanistic target of rapamycin complex 1 (mTORC1) signalling and lead to the phosphorylation of the ribosomal protein S6 (pS6) and the adoption of an anabolic metabolic state through the increased accumulation of biomass. mTORC1 can also promote the expression of B cell lymphoma 6 (BCL-6) and repress BTB domain and CNC homology 2 (BACH2) expression. pS6, alongside IL-10 and transforming growth factor-\(\beta\) (TGF\(\beta\), transforms GC B cell migration to the dark zone. FOXO1 also promotes CD40/BCR signalling, indirectly resulting in expression of the transcription factor basic leucine zipper transcription factor (BATF). BATF directly promotes the transcription of Aicda and cell cycle progression, likely through interaction with other transcription factors of the JUN or interferon regulatory factor (IRF) families. BCR signalling also engages the G\(\text{TPase KRAS, which activates the kinase ERK, resulting in repressed BCL-6 expression. CD40 signalling leads to the recruitment of the kinase MEK1 to the adapter molecule TRAF2 and results in activation of the mitogen-activated protein kinases (MAPKs) JNK and p38. MAPK signalling induces the expression of the transcription factors JUN and JUNB, as well as the transcription of Ccnd2. CD40 signalling also signals through TRAF proteins to induce NF-xB. NF-xB signals through both the canonical (REL) and non-canonical (RELB, NF-xB) pathways to regulate GC maintenance. RELB and NF-xB2 induce Icosl expression and promote the induction of the cell cycle. The precise mechanisms by which RELB and NF-xB2 regulate the cell cycle is unclear but may relate to induction of ICOSL expression. REL and PI3K/AKT signalling induce the expression of IRF4 and MYC. MYC acts in a complex with MYC-interacting zinc finger protein 1 (MIZ1) to induce the expression of activating enhancer binding protein 4 (AP4) and the epigenetic regulator ubiquitin-like, containing PHD and RING finger domains, 1 (UHRF1), both of which promote the cell cycle. Strong canonical NF-xB signalling also represses expression of the ubiquitin ligase CBL (not shown). CBL inhibits plasma cell differentiation through repression of IRF4 expression (not shown) and BCR signalling via degradation of SYK. IRF4 and BCL-6 are mutually antagonistic transcription factors. BCL-6 induces BACH2 expression, which represses B lymphocyte-induced maturation protein 1 (BLIMP1) expression. In turn, BLIMP1 can repress BCL-6 expression and induce XBP1 expression, resulting in plasma cell differentiation.
Box 3 | Atypical memory B cells

B cells expressing T-bet, CD11c, CD11b and/or FCRL5 develop in many individuals with chronic infection or autoimmunity disease and are often referred to as atypical memory B cells (MBCs). Atypical MBCs are a heterogeneous population of antigen-experienced cells that are dependent on CD4+ T cells and IL-21 for their development45,46. Toll-like receptor 7 (TLR7) ligand and interferon-γ (IFNγ) stimulation also promote atypical MBC development47. Plasmid-specific MBCs develop in the absence of germinal centres (GCs) and mature follicular helper T cells, suggesting that GCs may not be required for atypical MBC development48. Atypical MBCs can persist long term and are associated with both protective and pathogenic immune responses49,50. Expansion of atypical MBCs is also associated with progression of autoimmune diseases51. Atypical MBCs expand following infection or protein immunization and rapidly differentiate into GC B cells or antibody-secreting cells upon recall52,53. Atypical MBCs can be either IgM- or class-switched, with the expression of T-bet important for the development of IgG2a+ cells54. T-bet expression also distinguishes MBC subsets with distinct homing and functional properties55,56. The transcription factor MYB represses T-bet expression in B cells57. Whereas B cell intrinsic expression of T-bet in MBCs is required for plasma cell differentiation and protection following viral challenge, T-bet is not required for the development of atypical MBCs58,59. Future studies investigating the impact of atypical MBC depletion on the development of autoimmune disease will be important in assessing the attractiveness of atypical MBCs as a therapeutic target.

Signals downstream of CD40 and the BCR synergistically induce MYC expression in GC B cells60. CD40 signalling activates the transcription factor NF-kB but does not activate PI3K, which is instead induced by BCR signalling61. CD40 signalling can also induce the expression of MAPKs such as JNK and p38, which promote the GC response by inducing expression of the transcription factors JUN and JUNB62. Canonical NF-kB signalling drives activation of the NF-kB subunits REL and RELA (also known as p65)63. REL is required for GC B cell maintenance, with ablation of REL resulting in GC collapse owing to a failure to sustain the metabolic programme necessary for cell growth64. REL can induce IRF4 and MYC expression in B cells65,66. Non-canonical NF-kB signalling also promotes GC maintenance, and experimental deletion of the alternative NF-kB subunits RELB and NF-kB2 result in a reduced GC response67. Non-canonical NF-kB signalling acts independently of REL to promote cell proliferation and the expression of ICOSL in light zone GC B cells68. BCR signalling is generally attenuated in GC B cells owing to negative feedback resulting from increased expression of phosphatase and tensin homologue (PTEN) and phosphoinositide-dependent kinase 1 (PDK1)69. However, BCR signalling can sufficiently overcome this attenuation to signal through PI3K and restrict FOXO1 activity in a protein kinase B (also known as AKT)-dependent manner70,71.

T cell help activates mechanistic target of rapamycin complex 1 (mTORC1) signalling and triggers GC B cells to undergo the anabolic cell growth necessary to sustain rapid cell division72,73. mTORC1 activity is dependent on the phosphorylation of the ribosomal protein S6 (pS6)72. Both BCR and CD40 signalling are necessary for strong phosphorylation of S6 by mTORC1 (REF74). mTORC1 is also important for the induction of BCL-6 expression and repression of BACH2 expression in B cells75,76. Inhibition of mTOR signalling via rapamycin results in a failure to upregulate FOXO1 expression77. Considering that PI3K signalling both promotes mTORC1 and restricts FOXO1, it is unclear why mTORC1 would be necessary for FOXO1 induction78,79. One possibility is that there is temporal separation between the initial BCR signal that represses FOXO1 and the subsequent CD40 signalling that induces mTORC1, which allows sufficient time for FOXO1 to regain activity before migration to the dark zone. Initial repression of FOXO1 would also allow GC B cells that had received a BCR signal to engage with T cells in the light zone and upregulate MYC before migration to the dark zone.

Regulation of MBC differentiation

Low-affinity light zone GC B cells generally undergo apoptosis owing to a failure to receive T cell help104,105 (FIG. 3). GC B cells that receive low levels of T cell help have low activation of mTORC1 and display a reduced accumulation of biomass relative to cells that had received T cell help106. These cells also fail to express MYC, which is required for both the accumulation of metabolites necessary for cell division and for entry into the cell cycle107. MYC functions in complex with MIZ1 to promote plasma cell development and restrict MBC differentiation41. GC B cells that receive low levels of T cell help also maintain elevated BACH2 expression, perhaps owing to reduced repression of BACH2 transcription by mTORC1 (REFS108,109). BACH2 expression predisposes GC B cells to differentiate into MBCs7. The precise mechanisms by which BACH2 functions to promote MBC development remain to be elucidated but might relate to repression of cyclin-dependent kinase inhibitor genes (Cdkn1a, Cdkn2a) and the induction of the anti-apoptotic gene Bcl2110. Expression of prosurvival genes, such as Bcl2, and repression of pro-apoptotic genes facilitate MBC differentiation109,110. Together, these studies suggest a model where low levels of T cell help promote MBC differentiation by limiting mTORC1 and MYC-driven cell growth and cell cycle progression, thereby promoting cell survival.

Precursor memory (PreMem) B cells have been identified in the GCs of both mice and humans110–112. PreMem B cells tend to localize at the edge of the light zone and they transcriptionally and functionally resemble MBCs, despite expressing GC B cell surface markers. PreMem B cells are somatically mutated and have recently undergone cell division, indicating that they are mature GC B cells that are in the process of differentiating into MBCs. It is likely necessary for PreMem B cells to exit the antigen-rich light zone to complete their differentiation into MBCs. Downregulation of BCL-6 is important in allowing PreMem B cells to efficiently exit the GC. BCL-6 induces the expression of the GC-confinement factor S1PR2 and represses the expression of pro-migratory receptors that are likely to be involved in GC exit, such as EBI2 and S1PR1 (REF113). BCL-6 also promotes GC B cell apoptosis through repression of BCL-2 expression114. Loss of the BCL-6 transcriptional programme is one of the main drivers of the PreMem B cell gene signature115.

The finding that BCL-6 represses MBC differentiation is consistent with a model in which a low level of T cell help favours MBC differentiation116. GC B cells that receive low levels of T cell help have less exposure to the
T<sub>FH</sub> cell-derived cytokines IL-4 and IL-21, which would normally serve to counteract BCR signalling-mediated degradation of BCL-6 (REF. 29). Considering that BCL-6 and BACH2 cooperate to regulate the GC B cell transcriptional programme, it will be important to understand the distinct roles of BACH2 and BCL-6 in promoting MBC differentiation<sup>10–112</sup>. Both PreMem B cells and MBCs express lower levels of BACH2 relative to bulk GC B cells, suggesting that BACH2 alone is not sufficient to induce MBC fate commitment<sup>110–112</sup>.

**Fig. 3 | Transcriptional regulation of GC B cell differentiation.** Germinal centre (GC) B cell differentiation is regulated by the extent of signals received by the B cell through the antigen-engaged B cell receptor (BCR) and CD40 (via CD40L-expressing T cells). Boxes indicating signalling molecules are coloured yellow, transcription factors red, cytokines blue and downstream gene targets turquoise. A red cross indicates a pathway that is not functional under the condition illustrated.

**a Weak BCR signalling + weak T cell help**

| BCR | KRAS  |
|-----|-------|
| CD40 | NF-κB |

BCL-6, BCL-2

Apoptosis

**b Weak T cell help**

| BCR | KRAS  |
|-----|-------|
| CD40 | NF-κB |

BCL-6, BCL-2

IL-4/IL-21

Memory B cell

**c Intermediate T cell help**

| BCR | PI3K/AKT |
|-----|----------|
| CD40 | NF-κB |

pS6, FOXO1

Dark zone state

**d Strong T cell help**

| BCR | PI3K/AKT |
|-----|----------|
| CD40 | NF-κB |

IRF4, BLIMP1

Plasma cell

IRF4, BLIMP1

Plasma cell

Fig. 3 | Transcriptional regulation of GC B cell differentiation. Germinal centre (GC) B cell differentiation is regulated by the extent of signals received by the B cell through the antigen-engaged B cell receptor (BCR) and CD40 (via CD40L-expressing T cells). Boxes indicating signalling molecules are coloured yellow, transcription factors red, cytokines blue and downstream gene targets turquoise. A red cross indicates a pathway that is not functional under the condition illustrated. **a** Weak BCR signalling and weak T cell help results in a failure of GC B cells to receive sufficient signals through the BCR and CD40 to induce KRAS and nuclear factor-xB (NF-xB)-driven repression of B cell lymphoma 6 (BCL-6) expression. Repression of BCL-2 expression by BCL-6 in these cells will result in apoptosis owing to a failure to receive sufficient survival signals. **b** GC B cells that receive weak T cell help (transmitted via CD40) will not receive sufficient CD40-induced NF-xB signals to indirectly repress BCL-6 and cyclin-dependent kinase inhibitor family genes (Cdkn1a, Cdkn2a). BACH2 also represses B lymphocyte-induced maturation protein 1 (BLIMP1) and, accordingly, restricts plasma cell differentiation. The continued survival of B cells that receive weak T cell help is dependent on receiving sufficient BCR-driven KRAS signalling to repress BCL-6 expression. Exposure to T cell-derived IL-4 and IL-21 normally stabilizes BCL-6 expression in GC B cells. However, B cells that receive weak T cell-derived CD40 will also have low exposure to IL-4 and IL-21, resulting in impaired BCL-6 expression and induction of BCL-6-repressed genes such as haematopoietically expressed homeobox (Hhex) and Bcl2. HHEX interacts with the co-repressor transducin-like enhancer 3 (TLE3) to further repress BCL-6 expression and promote memory B cell development through the upregulation of BCL-2 and the transcription factor Sloan-Kettering Institute (SKI). **c** GC B cells that receive intermediate T cell help receive both phosphoinositide 3- kinase (PI3K)/AKT and NF-xB signalling, resulting in phosphorylation of the ribosomal protein S6 (pS6) and expression of MYC. pS6 and MYC subsequently induce expression of forhead box O1 (FOXO1) and activating enhancer binding protein 4 (AP4), respectively, ultimately leading to the adoption of the highly proliferative dark zone state. Intermediate levels of CD40 signalling do not drive sufficient NF-xB signalling to repress expression of the ubiquitin ligase CBL, allowing CBL to continue to repress interferon regulatory factor 4 (IRF4) expression and prevent plasma cell differentiation. **d** GC B cells that receive strong T cell help receive both PI3K/AKT and NF-xB signalling. Strong NF-xB signalling represses CBL expression and allows for upregulation of IRF4. IRF4 represses BCL-6 expression and leads to expression of BLIMP1 and plasma cell differentiation.
The transcription factor haematopoietically expressed homeobox (HHEX) has recently been identified as an important promoter of MBC differentiation. Ablation of Hhex in GC B cells leads to a marked decrease in the development of PreMem cells and MBCs. BCL-6 directly represses the expression of HHEX in GC B cells. Ablation of Hhex leads to increased expression of BCL-6 and reduced expression of the BCL-6 target gene Bcl2. Overexpression of BCL-2 is sufficient to rescue MBC differentiation in Hhex-deficient cells. HHEX also functions to promote MBC differentiation through BCL-2-independent mechanisms. For example, HHEX promotes the expression of the transcriptional cofactor Sloan-Kettering Institute (SKI), with overexpression of SKI sufficient to rescue MBC development in the absence of Hhex. The promotion of MBC development by SKI might relate to the observation that retroviral overexpression of SKI imparts a proliferative advantage to CD40-engaged B cells in vitro. Overexpression of the transcription factor Krüppel-like factor 2 (KLF2) also provides a competitive advantage in vitro and modestly promotes MBC differentiation.

Ablation of transducin-like enhancer 3 (Tle3), which encodes a transcriptional co-repressor, in GC B cells also leads to a marked reduction in MBC differentiation. TLE3 does not directly bind to DNA but rather functions through interaction with DNA-binding transcription factors and subsequent recruitment of histone deacteylases (HDACs), which mediate gene repression. TLE3 can directly interact with HHEX via an EH-1 motif and mutation of this motif is sufficient to prevent HHEX from promoting MBC development. TLE family members interact with numerous transcription factors, including TCF1, lymphoid enhancer-binding factor 1 (LEF1) and runt-related transcription factor 1 (RUNX1) and RUNX3, to regulate cell differentiation. Ablation of Runx3 in GC B cells leads to a slight increase in MBC differentiation, suggesting that competition for TLE3 binding between HHEX and other transcription factors might serve as a mechanism for regulating MBC differentiation.

HHEX is not required for MBC maintenance but can regulate the ability of MBCs to respond upon antigen re-encounter in certain immune settings. IL-9R signalling is also a regulator of MBC recall upon antigen re-encounter. Ablation of IL-9R results in attenuated recall antibody responses to T cell-dependent antigens. IL-9R-deficient MBCs had augmented ability to differentiate into GC B cells upon recall. IL-9R is not required for MBC maintenance, with contrasting data on whether IL-9 acts to promote MBC differentiation. IL-9 is produced by MBCs and a very small proportion of T memory cells and can signal through STAT3 and STAT5. MBCs also express the transcription factor zinc finger and BTB-containing 32 (ZBTB32), which acts to negatively regulate the magnitude and duration of the antibody recall response through regulation of the cell cycle and mitochondrial function. The increased expression of ZBTB32 in CD80/PDL2- MBCs, relative to CD80/PDL2+ MBCs, might help regulate the differential fate of these subsets upon secondary challenge.

Numerous genes besides Zbtb32, including Thr7 and Thr9, are differentially expressed between MBC subsets. How differences in naive B cell and MBC responsiveness to inputs such as TLR, CD40 and BCR signalling determine the relative ability of these cells to proliferate and differentiate upon reactivation remains to be fully understood.

Models of MBC development

The asymmetric fate model. Multiple models of MBC development have been proposed. The asymmetric fate model suggests that MBCs arise in a stochastic manner from GC B cells. This is supported by data from some animal models showing that a genetically induced decline in GC B cells results in a proportional loss in MBCs. It is thought that GC B cells that interact with Tfh cells can undergo asymmetric cell division, resulting in an unequal distribution of cell fate-determining molecules such as BCL-6, IRF4 and IL-21R among their cell progeny. Daughter cells that inherit IRF4 will preferentially differentiate into plasma cells, whereas cells that inherit BCL-6 and IL-21R will maintain GC B cell identity or differentiate into MBCs. However, in vitro work with activated B cells and mathematical modelling studies suggest that GC B cells generally undergo symmetric cell division, calling into question the functional relevance of asymmetric cell division in the regulation of MBC differentiation.

The instructive fate model. The instructive fate model posits that MBC development is actively regulated by cell-extrinsic signals, such as cytokines and cell-contact-dependent signals. This model is supported by the finding that perturbations in the ability of GC B cells to receive T cell help (for example, an inability to sense IL-21 or migrate to the dark zone) results in an accumulation of MBCs that is disproportionate to GC size. A central mechanism underlying the instructive fate model is antigen affinity, as higher affinity GC B cells will receive stronger T cell help, which promotes the expression of plasma cell-defining transcription factors such as IRF4 and BLIMP1. The instructive fate model is still probabilistic as cells with the same antigen affinity can have different fates, with the affinity influencing the probability of a particular fate. The probabilistic nature of the GC likely facilitates the differentiation of high-affinity GC B cells into MBCs and is reflected in the widely disparate rates at which individual GCs lose clonal diversity. However, the instructive fate model does not directly account for the observation that MBCs tend to emerge from the GC before plasma cells.

The decreasing-potential fate model. The decreasing-potential fate model posits that the cumulative signals received by GC B cells regulate cell fate. During the early GC reaction, B cells will have undergone fewer rounds of cyclic re-entry and will have received less cumulative T cell help. This reduced overall selection signal will predispose early GC B cells to develop into MBCs. As the GC response matures, the cumulative T cell help received by GC B cells will also increase,

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leading to a shift in GC output towards increased plasma cell differentiation. The decreasing-potential fate model is supported by the observation that early and late GC B cells are transcriptionally and functionally distinct. The precise mechanisms underlying this model remain to be elucidated.

The integrative fate model. The instructive fate model and the decreasing-potential fate model are not mutually exclusive. It is possible that GC B cells integrate both the current quality and the cumulative amounts of received signals when making cell fate decisions. The integrative fate model postulates that, although GC B cells are predisposed to certain fates based on their signalling history, the ultimate cell fate is still influenced by the quality of T cell help received before differentiation. Therefore, the shift in GC output that occurs over the course of the GC response may be partially a result of changes in the quality of T cell help. This is supported by the finding that TFH cells change their pattern of cytokine production and surface ligand expression over the course of the GC response. Late TFH cells express elevated levels of IL-4 and CD40L and preferentially induce the development of plasma cells during type 2 immune responses. It remains to be determined whether changes in the number and phenotype of FOXP3+ regulatory T cells present in the GC could also impact GC output.

Concluding remarks and perspective
Significant progress has been made towards understanding the signals and transcription factors regulating GC B cell differentiation. However, there remain many knowledge gaps that limit our ability to harness MBCs therapeutically and that need to be addressed in...
subtypes of follicular lymphoma and DLBCL. The t(14;18) translocation is a hallmark of follicular lymphoma and results in constitutive activation of BCL-2. BCL-2 overexpression predisposes B cells to develop into MBCs. BCL-2 overexpressing MBCs appear to be capable of re-entering the GC multiple times, accruing further mutations and spreading to distant lymphoid organs. These data suggest that MBCs possessing driver mutations might migrate from the primary lymphoma to extra-nodal sites where they accrue further malignant mutations. This process may also be relevant to the MCD/cluster 5 subtype of DLBCL (defined based on co-occurrence of Myd88 and Cd79b mutations), which has extensive extra-nodal involvement and has one of the lowest survival rates of DLBCL subtypes.

MBCs were recently found to promote the progression of MCD/cluster 5 DLBCL. A more precise understanding of the pathways that regulate MBC development and survival could offer new opportunities for the design of therapeutics capable of limiting follicular lymphoma and MCD–DLBCL progression.

MBCs also contribute significantly to disease pathology in individuals with allergy. High-affinity allergen-specific IgG1 MBCs arise from repeated allergen encounters and can sequentially isotype switch and differentiate into long-lived IgE plasma cells capable of producing increased levels of antibodies. IL-13-producing T helper 2 cells appear to have a role in the development of high-affinity IgE-producing cells following allergen exposure but not helminth infection. This raises the intriguing notion that there are also unique pathways regulating the differentiation of allergen-specific MBCs. The identification of such pathways would afford the opportunity to selectively target allergen-specific MBC differentiation.

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A better understanding of the functional heterogeneity of MBCs will also be important moving forwards. The MBC population comprises numerous subsets with unique transcriptional profiles and developmental pathways. For example, MBCs can establish residence in mucosal tissues such as the lungs following viral infection and contribute to protection upon pulmonary challenge. It is likely that the transcriptional circuitry governing MBC development will differ between MBC subsets, with unique transcription factors required for migration to and maintenance within different anatomical sites. Understanding these differences will be critical for the development of therapeutics that can modulate MBC differentiation to overcome the bottleneck in MBC recall responses and to specifically induce the population best equipped to combat a particular pathogen.

Another key emerging area is the study of MBC differentiation in contexts beyond infectious disease. MBCs may contribute to the pathogenesis of certain...
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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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