INSIGHTS

“B” aware: Memory lane access is restricted

Ruth Kennedy and Ulf Klein

Understanding the molecular mechanisms that govern the differentiation of high-affinity germinal center (GC) B cells into memory B cells versus plasma cells is a major quest of adaptive immunity. In this issue, Toboso-Navasa et al. (https://doi.org/10.1084/jem.20191933) provide evidence that the MYC–MIZ1 transcriptional repressor complex restricts the differentiation of GC B cells into MBCs.

The germinal center (GC) is the workhorse of antibody-dependent adaptive immunity that manufactures the mediators of the humoral immune response: high-affinity plasma cells (PCs) that eliminate the invading pathogen and memory B cells (MBCs) that, upon reinfection, rapidly mount a pathogen-specific antibody response. Within the GC microenvironment, B cells recirculate between two histologically and functionally defined zones: the dark zone (DZ) and the light zone (LZ). DZ B cells diversify their immunoglobulin variable region genes by somatic hypermutation (SHM) and migrate to the LZ, where they differentiate into LZ B cells, which are selected for improved antigen binding under the guidance of T follicular helper cells (Victora and Nussenzweig, 2012).

In essence, three cellular fates await a positively selected LZ B cell: recirculation to the DZ to undergo iterative rounds of proliferation and SHM, or exiting the GC as an MBC or PC. All three transitions are associated with a drastic change in cell phenotype. A major question of GC biology is, therefore, how are these decision points molecularly controlled? What mechanisms instruct an LZ B cell to differentiate into a DZ B cell, MBC, or PC? Or, conversely, are there molecular “brakes” that prevent certain developmental transitions? Toboso-Navasa et al. have now provided compelling evidence that the MYC and MIZ1 (MYC-interacting zinc-finger protein 1, ZBTB17) transcription factors jointly regulate the fate of positively selected GC B cells, in that they restrict MBC differentiation while promoting effective GC expansion and PC differentiation (Toboso-Navasa et al., 2020).

The congregation of three lines of reasoning drove Toboso-Navasa et al. to investigate the role of MIZ1 in MBC differentiation. First, the up-regulation of MYC expression in positively selected LZ B cells is required both for licensing cell cycle progression before LZ–DZ reentry and for PC differentiation (Domínguez-Sola et al., 2012; Calado et al., 2012; Ise et al., 2018). Second, a phenotypically distinct, MYC-negative LZ population that consists of quiescent LZ B cells, many of which express the transcription factor BACH2 that favors MBC differentiation (Shinnakasu et al., 2012), is enriched for MBC precursors suggested to Toboso-Navasa et al. (2020) hypothesized that MYC–MIZ1 activity in positively selected LZ B cells may positively regulate proliferation and restrict MBC differentiation.

Their hypothesis passed the essential first hurdle: immunofluorescence analysis of spleen sections from mice immunized with a T cell-dependent antigen revealed coexpression of MIZ1 and MYC in positively selected LZ B cells. This finding provided the rationale for elucidating the in vivo function of the MYC–MIZ1 complex in GC B cell development. Central to the study was a transgenic allele harboring an endogenous MYC mutant (valine 394 is replaced with aspartic acid, V→D; Myc<sub>VD</sub> mice; Saba et al., 2011) that abrogates the association of MYC with MIZ1 (top panel of image) while retaining binding to MYC’s obligatory “partner” MAX and the ability to transcriptionally activate. Transcriptional analysis of flow-cytometrically isolated LZ B cells of Myc<sub>VD</sub> mice revealed vastly different gene expression profiles compared with Myc<sub>WT</sub> control mice. By integrating RNA-seq data...
PCs in the Toboso-Navasa et al. (2020) detected fewer dispensable during GC formation. Similarly, a complex is required for GC expansion but type mice, indicating that the MYC was equivocal between mutant and wild-type mice. Nevertheless, the number of GC foci and number of GC B cells in Myc were significantly reduced compared with their wild-type counterparts. V(D)J mice relative to wild-type mice, as was the size of individual GCs. Furthermore, the number of GC foci was equivocal between mutant and wild-type mice, indicating that the MYC–MIZ1 complex is required for GC expansion but dispensable during GC formation. Similarly, Toboso-Navasa et al. (2020) detected fewer PCs in the MycVd mice relative to wild-type mice, which is a relevant finding since positively selected (MYC+) LZ B cells also contain PC precursors (Ise et al., 2018).

Regarding functional consequences, Toboso-Navasa et al. (2020) did not detect changes in GC B cell survival in the absence of MYC–MIZ1 complexes. A first clue for a potential role of the complex in cell cycle regulation came from a gene set enrichment analysis, which revealed that LZ B cells from wild-type animals showed enrichment of gene sets for “GI-S phase transition”; conversely, the transcriptional data from the mutants were enriched for gene sets of the category “cell cycle arrest.” Indeed, pulse experiments where the nucleoside analogue EdU was incorporated into the DNA of actively proliferating cells revealed impaired cell cycle engagement in MycVd mice compared with their wild-type counterparts. So, MYC–MIZ1 complexes facilitate LZ–DZ recycling of positively selected LZ B cells by regulating the expression of cell cycle–associated genes.

A series of well-conceived experiments then provided compelling evidence for the role of the MYC–MIZ1 complex in restricting MBC differentiation. Comparative gene expression analysis identified an altered transcriptional program in LZ GC B cells lacking the MYC–MIZ1 complex that in part is characterized by the up-regulation of MBC-associated genes. In accordance, tracing of MBCs (IgG1*CD273*) in immunized MycVd versus MycWT mice using two different experimental systems revealed increased fractions and numbers of MBCs in the mutant mice (bottom panel of image). The majority of these MBCs originated in the GC, as demonstrated by EdU pulse experiments showing that increased MBC differentiation was linked to GC expansion.

What about the quality of the MBCs developing in MycVd mice? To address this question, Toboso-Navasa et al. (2020) employed a transgenic B cell receptor (BCR) that is commonly used to analyze antigen affinity maturation (SWHEL allelic system; Paus et al., 2006) and that allows monitoring of the generation of hen egg lysozyme (HEL)–specific B cells by the occurrence of an amino acid exchange that increases HEL affinity by 100-fold. It emerged that MBCs in MycVd versus MycWT mice had fewer SHMs in their antibody genes and a significant reduction in the fraction of high-affinity BCRs. A conceivable explanation for the observed decrease in HEL affinity in the absence of MYC–MIZ1 complexes might be a transitory delay in affinity maturation. However, this possibility was ruled out since MycVd mice lacking AID (an enzyme critical for SHM) similarly showed an increased fraction of MBCs and a concomitant reduction in recycling GC B cells compared with the wild-type counterparts, indicating that the activity of the MYC–MIZ1 complex is cell intrinsic and follows a timed program.

Clearly, this is an intriguing observation because it may imply that the MYC–MIZ1 complex is an evolutionary addition specifically aimed at restricting the exit of B cells from the GC. As Toboso-Navasa et al. further point out, AID and the MYC–MIZ1 complexes appeared in vertebrate evolution around the same time. One may envisage that the joint activities of MYC–MIZ1 and AID allowed for an effective GC expansion.
through facilitating repeated rounds of SHM and selection, thereby substantially improving the affinity of antigen-specific MBCs and PCs. In this regard, an equivalent molecular mechanism that also keeps B cells in the “GC roundabout” and that acts in the PC differentiation arm of GC development is provided by Casitas B-lineage lymphoma (CBL) ubiquitin ligases (Li et al., 2018).

The latter observation—and the work of others that provided insights into the molecular control of GC B cell development that cannot be discussed due to space limitations—highlights the complexity of this B cell developmental stage and reminds us that we are still lacking a deep understanding of why and how the positively selected LZ B cell follows a particular differentiation route. For example, it is well established that over the time course of a GC reaction (which lasts for several weeks), the major cellular output switches from predominantly MBCs early on to predominantly PCs at late stages (Shinnakasu et al., 2016; Weisel et al., 2016). Thus, the findings of Toboso-Navasa et al. (2020) that the MYC-MIZ1 complex restricts MBC differentiation at early and mid-time points of the GC reaction are seemingly at odds with this observation. However, despite the temporal differences in the cellular output, both MBCs and PCs continuously exit the GC during the immune response (Blink et al., 2005), indicating that the MYC-MIZ1-associated restriction of MBC differentiation is likely the subject of a nuanced fine-tuning by multiple parallel mechanisms within the LZ B cell. It seems probable that the fate decisions within an individual LZ B cell, rather than following a binary “on-off” switch, are contingent on the integration of various inputs that establish a gradient of regulation that ultimately reaches a tipping point.

The ultimate expectation of understanding how the differentiation of a positively selected LZ B cell is controlled at the molecular level is to exploit this knowledge for the manipulation of the humoral immune response, with particular relevance for vaccine development. This is an ambitious undertaking, but the new findings by Toboso-Navasa et al. (2020) may bring us one step closer to this goal; interfering with MYC-MIZ1 complexes during an immune response may yield an increased population size of MBCs with overall lower, but much broader, antigen affinity. A broad-spectrum humoral memory induced by vaccination could be advantageous in the recognition of sub-strains or mutants of the immunizing pathogen (Victora and Wilson, 2015), and further rounds of affinity maturation may provide effective protection. It is therefore not outside the bounds of possibility to envisage a therapeutic manipulation of the MYC-MIZ1 axis that would increase MBC output alongside vaccine delivery.

References
Blink, E.J., et al. 2005. J. Exp. Med. https://doi.org/10.1084/jem.20042060
Calado, D.P., et al. 2012. Nat. Immunol. https://doi.org/10.1038/ni.2418
Domínguez-Sola, D., et al. 2012. Nat. Immunol. https://doi.org/10.1038/ni.2428
Ise, W., et al. 2018. Immunity. https://doi.org/10.1016/j.immuni.2018.03.027
Laidlaw, B.J., et al. 2017. J. Exp. Med. https://doi.org/10.1084/jem.20161461
Li, X., et al. 2018. Immunity. https://doi.org/10.1016/j.immuni.2018.03.006
Paus, D., et al. 2006. J. Exp. Med. https://doi.org/10.1084/jem.20060087
Saba, I., et al. 2011. Blood. https://doi.org/10.1182/blood-2010-09-310680
Shinnakasu, R., et al. 2016. Nat. Immunol. https://doi.org/10.1038/ni.3460
Toboso-Navasa, A., et al. 2020. J. Exp. Med. https://doi.org/10.1084/jem.20191933
Victora, G.D., and M.C. Nussenzweig. 2012. Annu. Rev. Immunol. https://doi.org/10.1146/annurev-immunol-020711-075032
Victora, G.D., and P.C. Wilson. 2015. Cell. https://doi.org/10.1016/j.cell.2015.10.004
Wang, Y., et al. 2017. Nat. Immunol. https://doi.org/10.1038/ni.3788
Weisel, F.J., et al. 2016. Immunity. https://doi.org/10.1016/j.immuni.2015.12.004
Wiese, K.E., et al. 2013. Cold Spring Harb. Perspect. Med. https://doi.org/10.1101/cshperspect.a014290