acquired peptide-binding ability indeed is the new kid in an extended family of MHC-I molecules proficient in antigen presentation. It opens exciting new avenues for HLA-F with involvement in immune surveillance and the potential existence of peptide-sequence specific HLA-F restricted receptors. And the search is on…

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The TORC that Gets the GC Cycling

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The signaling pathways regulating positive selection in germinal centers (GCs) are incompletely understood. Ersching et al. (2017) identify a critical but temporal role for the action of the kinase mechanistic target of rapamycin complex (mTORC1), which promotes key changes in GC B cells and thereby facilitates affinity maturation.

Naive B cells differentiate into memory B cells and plasma cells in response to T-dependent antigens within germinal centers (GCs), transient structures located in the follicles of secondary lymphoid tissues. GCs are the site of proliferation, somatic hypermutation, and selection of high-affinity Ag-specific B cells. However, these processes take place in defined regions of the GC; proliferation and somatic hypermutation dominate the dark zone (DZ), and affinity-based selection occurrs in the light zone (LZ) (Mesin et al., 2016). A GC reaction is not a one-way street—appropriate GC responses involve the cyclic re-entry of B cells between the LZ and the DZ (Figure 1). Indeed, positive selection of high-affinity B cells occurs in the LZ as a result of their ability to outcompete low-affinity B cells for antigens and help provided by specialized $CD4^+$ T cells termed T follicular helper (Tfh) cells, which provide the signals that initiate proliferation and migration back into the DZ, where the cycle begins again (Mesin et al., 2016).

A GC reaction is a classic scenario of survival of the fittest. Consequently, to survive a GC reaction, activated B cells must alter their physiology and metabolism. Thus, defining exact signals controlling B cell selection, survival, expansion, and trafficking is key to understanding the regulation of GC responses and how this can go awry, whereupon it leads to dysregulated B cell responses that potentially manifest as production of autoantibodies or B cell malignancy. Previous work identified a number of factors critical for these processes; such factors include the cell-cycle regulator and proto-oncogene c-Myc, which initiates the cell cycle in the LZ (Calado et al., 2012; Dominguez-Sola et al., 2012), and the transcription factor AP4, which allows multiple rounds of division in the DZ (Chou et al., 2016)(Figure 1). However, positive selection and expansion of high-affinity B cells within the GC requires a multitude of signals, the complex temporal regulation of which remains incompletely defined.

Several studies have previously utilized a system that artificially promotes positive selection of B cells in the LZ by using an antibody directed against the surface receptor DEC205 linked to the antigen ovalbumin (DEC-OVA). This enables delivery of antigen to the B cells, resulting in presentation by the B cells and promoting interactions between B cells and Tfh cells, which then drive the migration and proliferation of these B cells into the DZ. Using this system, researchers have shown that the extent of proliferation in the DZ was proportional to the amount of help provided by Tfh cells (reviewed in [Mesin et al., 2016]).

In this issue of *Immunity*, Ersching et al. have used this system to identify additional signaling pathways that might underpin the complex processes of a GC reaction (Ersching et al., 2017). Genes

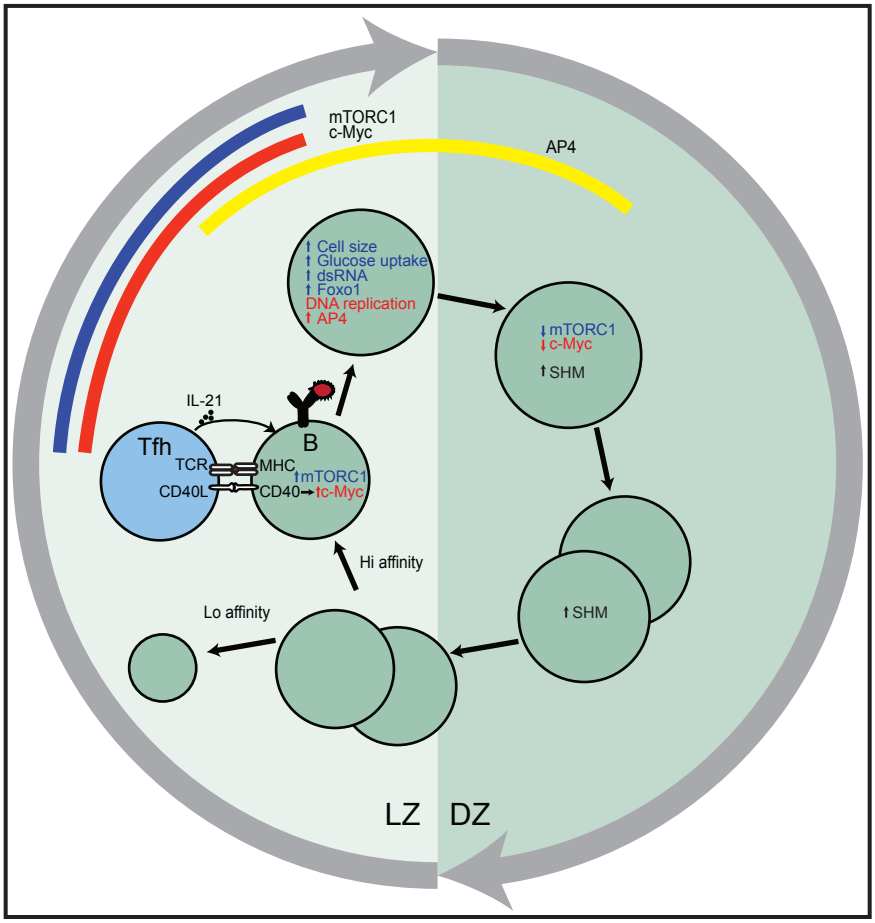

Figure 1. The Role of mTORC1 in GC Cycling

B cells that acquire antigen then present it to Tfh cells in the LZ. The Tfh cells provide B cell help via CD40L stimulation and IL-21. These signals induce upregulation of c-Myc and mTORC1 activity within the LZ. This early activation of mTORC1 is required for inducing an increase in cell size, glucose uptake, and RNA synthesis but does not seem to be essential for entry into cell division, which instead is dependent on c-Myc. Further, mTORC1 activity is not required at later times; instead, other factors, e.g., AP4 (which induced in a c-Myc-dependent manner) are subsequently upregulated and control expansion in the DZ. Upon returning to the LZ, B cells expressing BCR with increased affinity for antigen undergo selection and re-initiate the process of LZ-DZ trafficking.

upregulated early in the LZ after the delivery of help to the B cells were initially identified. Consistent with previous studies, Ersching et al. showed a strong c-Myc-dependent signature. However, they also identified a molecular signature associated with activation of mTORC1 (Ersching et al., 2017). mTORC1 is important for controlling cell growth and metabolism, and its activation is regulated by both nutrient availability and mitogen stimulation (Boothby and Rickert, 2017; Lucas et al., 2016). The significance of the induction of both cMyc- and mTORC1-induced gene signatures was underscored by the finding that cMyc-expressing LZ B cells expressed the highest levels of phospho-S6, a substrate of mTOR, indicating co-activation of these pathways in the same subset of GC B cells undergoing positive selection in the LZ.

It was further shown that mTORC1 was activated in B cells after engagement of CD40 by CD40L expressed on Tfh cells (Figure 1). This resulted in an increase in glucose uptake, ribosomal biogenesis, and blastogenesis. Remarkably, these changes in cell physiology peaked 36 hr after antigen uptake and sharply declined over the following 36 hr. These dynamic changes in GC B cell physiology preceded the onset of intense proliferation in the DZ. Thus, it was predicted that this increase in cell size and anabolic activity would prepare B cells for subsequent divisions that occur in the DZ and that the return of these physiological changes to baseline measures would coincide with trafficking of DZ B cells to the LZ. Accordingly, assessment of the in situ movement of selected B cells within the GC zonal compartments revealed that B cells remaining in the DZ were larger than cells that had recently exited the DZ and returned to the LZ. Furthermore, inhibition of mTORC1 with the immunosuppressive drug rapamycin shortly after DEC-OVA treatment, and before cell growth had occurred, led to a reduction in subsequent expansion of the cells. However, if mTORC1 was inhibited after cell growth was initiated, then expansion of the cells proceeded relatively normally. Thus, mTORC1 activation primes antigen-specific GC B cells for expansion; however, this is temporally constrained to the early phase of B cell activation. Remarkably, despite the reduction in expansion as a result of early inhibition of mTORC1, there was no effect on the distribution of cells within the cell cycle, suggesting an effect on survival, rather than the unique halting of proliferation. Inhibition of mTORC1 at different times after receipt of T cell help also disrupted positioning of B cells within the discrete zones of the GC; fewer GC B cells acquired a phenotype of DZ-resident cells, and many more cells retained a LZ phenotype. This was attributable to the finding that rapamycin inhibits upregulation of the transcription factor Foxo1, which is required for GC B cells to acquire a DZ phenotype (Dominguez-Sola et al., 2015; Sander et al., 2015). Assessing the temporal location of antigen-specific B cells in rapamycin-treated mice histologically would unequivocally demonstrate the requirement for early activation of mTORC1 in the cyclic re-entry of B cells within the GC LZ and DZs.

Recent studies of gene-targeted or pharmacologically treated mice established a key role for mTORC during a GC reaction. Thus, deletion of components of the mTOR complex, or inhibition by rapamycin, strongly impedes GC responses and antibody formation in response to T-dependent antigens (reviewed in [Boothby and Rickert, 2017]). Analysis of conditional mTOR deletion from B cells or T cells has also revealed important roles for mTOR signaling in generating functional GC B cells and Tfh cells (Boothby and Rickert, 2017; Zeng et al., 2016). Thus, the finding by Ersching et al. that

rapamycin treatment temporally impacted the expansive burst of GC B cells did not delineate whether this effect was due to a direct consequence of mTOR inhibition in B cells or whether it was secondary to an impact of Tfh cells. To establish a B cell intrinsic requirement for mTORC1, the authors generated an elegant model with which to examine lineage-specific effects of timed delivery of rapamycin. They achieved this by CRISPR/Cas9-induced editing of the *Mtor* gene to introduce a mutation that renders mTORC1 insensitive to the inhibitory effects of rapamycin. By transferring wild-type rapamycin-sensitive B cells into *Mtor* mutant rapamycin-resistant hosts, they established that mTORC1 functioned intrinsically in B cells to promote expansion and trafficking within GCs. Thus, although mTOR is critical for generating Tfh cells (Zeng et al., 2016), regulated inhibition of mTOR in Tfh cells once they had formed had minimal, if any, effects on their ability to promote B cell differentiation.

Lastly, Ersching et al. investigated the effect of dysregulated mTORC1 activation on GC B cell selection by using B cells lacking the mTORC1 inhibitor Tsc1. Although constitutive mTORC1 activity resulted in an exaggerated phospho-S6 response and an enlarged DZ compartment, these B cells were unable to compete with wild-type B cells and failed to undergo affinity maturation. Thus, although mTORC1 has a unique role in priming GC B cells during a defined and narrow window of time to undergo physiological and metabolic changes to induce proliferation and movement within GCs, unbridled mTORC1 activity is deleterious for high-affinity B cell responses. This highlights the need to tightly regulate mTORC1 function and might also explain the paradoxical findings of impaired humoral immune responses in humans and mice with either hyper-active or hypo-active PI3 kinase signaling, which functions upstream of mTORC1 and other signaling modalities (Lucas et al., 2016). It will also be of interest to explore more fully how mTORC1 activation interacts with c-Myc and other key regulators of GC biology. Interestingly, Ersching et al. showed that rapamycin treatment did not completely ablate c-Myc upregulation, nor entry into division. Rather, they observed a modest decrease in c-Myc activation, indicating that these two factors might have overlapping regulation and effects.

Overall, this study elegantly pinpoints the critical role and temporal requirement for mTORC1 in mediating the initial biological changes required for generating high-affinity, antigen-specific effector cells after receipt of cognate T cell help in GCs. Ersching et al. also note that mTORC1 exhibits a "Goldilocks effect" on the GC response—the amount of mTORC1 function needs to be "just right" to elicit optimal humoral immunity.

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