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Plasma cell and memory B cell differentiation from the germinal center

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Germinal centers (GCs) form in secondary lymphoid tissues in response to antigenic challenge and are the site of somatic hypermutation, generating GC B cells with increasing affinity for the inciting agent that are positively selected over time. However, it is not until GC B cells differentiate into memory B cells and plasma cells and egress from the GC back into the circulation that effective long-lived humoral immunity is conferred upon the host. Here we review what is known about the signals that initiate the transition from a GC B cell into the memory B cell and plasma cell compartments and the downstream transcriptional regulation of these processes.

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Introduction

GCs represent the evolutionary apex of adaptive humoral responses against pathogens, where competitive selection of useful B cell clones occurs resulting in the production of long-lived plasma cells (PCs) and memory B cells (MBCs) [1]. Within the unique microenvironment of the GC, B cells have several potential fates including apoptosis, positive selection for further somatic hypermutation (SHM), or differentiation into MBCs or PCs. Whilst positive selection is more complex to measure and understand because of the iterative nature of B cell recycling within the GC, PC and MBC differentiation offer more linear parameters through which the influence of various GC signals on GC B cell fate may be more readily examined. The process of positive selection is beyond the scope of this review but is examined in depth elsewhere [1,2]. Recent data have shed further light on how to identify MBC and PC precursors within the GC, opening avenues to investigate the cues that direct a GC B cell to initiate differentiation into an MBC or PC, as well as the transcriptional regulation that underpins this critical transition.

Germinal centers: cues in the light zone

The GC is spatially segregated into a light zone (LZ) and dark zone (DZ), permitting compartmentalization of the molecular processes that underpin efficient affinity maturation [1–3]. The DZ is the predominant site of SHM and proliferation. In contrast, the LZ is the site where B cells test their antigen receptor (BCR) against antigen displayed on follicular dendritic cells (FDCs) and compete for limited Tfh-cell help [4]. GC B cells with IgV region mutations rapidly turnover and express their new BCR whilst recent work has also established that GC B cells rapidly renew antigen presentation in the form of MHC:peptide [5], ensuring stringency in positive selection and providing an explanation for how high affinity B cells develop a competitive advantage for LZ survival and selection cues against cells of lower affinity in the same GC.

Thus the model to emerge is one where positive selection for a further iterative round of SHM, or selection into the PC or MBC compartment, is dependent upon signals that reside in the LZ [1–3]. These cues are limited, ensuring a competitive environment for emerging GC B cells. Furthermore, positive selection and MBC/PC differentiation are related but non-synonymous phenomena, and therefore the relative contributions of antigen and Tfh-cell help may differ for each of these processes.

The role of Tfh-cell help in selection into the PC compartment

In a recent series of seminal studies, the Nussenzweig lab has explored the role of Tfh-cell help in positive selection and the generation of PCs with an elegant system that permits the uncoupling of BCR stimulation and Tfh-cell signaling [6–8]. In this system, antigen is delivered to GC B cell via the surface receptor DEC-205, permitting loading of B cells with peptide for presentation to Tfh cells without stimulation of the BCR. In these experiments, peptide-loaded GC B cells exhibited a clear competitive advantage, producing a proliferative burst associated with migration to the dark zone and a wave

of plasmablasts, confirming that strong Tfh-cell help can drive PC production [6].

This work builds on an existing body of data which has included the knockout and overexpression of key signaling molecules between Tfh cells and GC B cells, including CD40/CD40L [9–11], ICOS/ICOSL [12,13], IL-21 [11] and PD1 [14]. Whilst these studies have led to a prevailing view that T-cell signals initiate PC differentiation in the GC, definitive proof of this is still lacking.

The role of BCR signaling in selection into the PC compartment

Using an anti-hen egg lysozyme BCR knock-in system (SW_{HEL} system), our lab previously showed that *Blimp1*$^+$ PCs specifically differentiate from high-affinity but not low-affinity B cell precursors within the GC [15]. Given the relationship between BCR affinity and selection into the PC compartment in T-dependent [15] and T-independent systems [16], BCR signaling may be important in driving PC differentiation *in vivo*. However, experimental systems established to interrogate BCR signaling have yielded contradictory results.

Highly proliferative GC B cells were recently found to not undergo BCR signaling in response to soluble antigen, with the exception of a period in the G2 phase of the cell cycle [17]. Similarly, a reporter mouse designed to read out antigen receptor signaling (*Nur*77-eGFP [18]) indicated strong signaling in only subset of LZ-enriched GC B cells. On the other hand, *ex vivo* analysis of GC B cells responding to membrane-bound antigen, potentially modeling FDC-displayed antigen, did demonstrate BCR signaling [19••]. Nevertheless, signaling in this case was insufficient to induce NF-κB expression, consistent with a requirement for T-cell help to fully induce activation. Thus, these data argue for a model whereby GC B cells integrate both BCR and Tfh-cell signaling to achieve activation and/or selection. In addition, these data predict the contact time between B cells and antigen-bearing membranes to be in the order of seconds [19••], which may reconcile the previous observations by two-photon microscopy that GC B cells form only brief interactions with FDCs when directly imaged in GCs [20].

In a recent study [21••], we compared the contributions of antigen engagement and Tfh-cell help in the generation of high affinity PCs within the GC. Whilst blocking access to antigen after formation of the GC completely abolished PC production, specific depletion of Tfh cell help did not affect the generation of *Blimp1*$^+$ PC-lineage cells in the GC. However, depletion of CD4 T cells did result in the arrested development of PC-lineage cells in the GC LZ with an immature *Blimp1*^{lo} phenotype. These data indicate that the induction of PC differentiation depends on events directly associated with antigen engagement, with Tfh-cell help providing subsequent signals to progress differentiation and egress of PCs out through the DZ of the GC [22]. Importantly, it remains unclear whether BCR signaling *per se* is the key event associated with antigen engagement or whether co-signals from FDC-expressed or other antigen-associated ligands may play important roles in triggering PC differentiation [23].

Transcriptional regulation of PC differentiation

The molecular re-programming required to undergo PC differentiation has been predominantly studied using *Blimp1*-GFP reporter mice [24], with extensive transcriptional profiling recently undertaken [25•] and recently reviewed in depth by Nutt *et al.* [26]. The up-regulation of *Blimp1* occurs downstream of IRF4 expression, which acts to regulate B-cell differentiation in a temporal and dose-dependent manner [27]. Thus, transient, relatively low-level expression of IRF4 is required for GC B-cell differentiation and expression of *Bcl6* and *Aicda*, whereas sustained and high level IRF4 expression represses the GC B-cell program, resulting in *Blimp1* expression and PC differentiation [27–29]. More recent work confirms a feed-forward loop by which BLIMP1 acts to increase IRF4 expression [30], predicted to stabilize the PC phenotype. BLIMP1 expression induces expression of XBP-1, which is required for PC differentiation [31] and to regulate the intracellular machinery required for PCs to produce large quantities of immunoglobulin [32].

PAX5 is a transcription factor critical to the identity of the B-cell lineage [33,34] whose expression is lost during PC differentiation. This is partly due to repression by BLIMP1 [35] which also suppresses *Aicda* expression [30]. Taken together, the picture of PC differentiation at a molecular level stems from the IRF4-BLIMP1 axis resulting in repression of both the PAX5-dependent mature B cell and the BCL6-dependent GC B cell programs [36,37], upregulation of XBP1 to facilitate high level Ig synthesis, and upregulation of CXCR4 to facilitate migration through the DZ and on to extrafollicular PC niches including the bone marrow [38,39].

Selection for differentiation into the MBC compartment

MBCs arising in an immune response may be GC-derived or GC-independent [40,41]. Therefore, like PCs and early plasmablasts, care must be taken when interpreting experimental results of populations likely to contain both early and GC-derived MBCs. Unlike PCs, where *Blimp1* faithfully reports a PC fate in B cells, the ability to study MBC precursors in the GC has been hampered by a lack of a 'master' transcription factor to identify GC B cells with this fate.

It has been proposed that MBC differentiation from GC precursors is stochastic [42] in part due to the failure to

identify a master transcription factor associated with MBC identity. In addition, this view has been supported by experiments involving overexpression of anti-apoptotic factors such as BCL2, which result in a marked increase in the number of MBCs without impairing the selection of high-affinity PCs [43].

One consistent characteristic of MBCs emerging in immune responses is their lower affinity and lower mutational load compared to PCs [44]. Shinnakasu *et al.* [45••] recently reported that MBC precursors emerge from the low affinity compartment of the LZ, and that T-cell help signatures were inversely correlated with *Bach2* expression (see next section), suggesting that an affinity-dependent 'threshold' for T-cell help may exist, below which MBC differentiation is favored. This observation is consistent with another recent study, which reported that GC B cells that lacked CXCR4 (and therefore failed to access the DZ) were more likely to enter the MBC compartment, which the authors speculated may be due to T-cell help in the LZ that was sufficient to promote survival and MBC differentiation without driving cyclic-reentry for further SHM or PC differentiation [46].

We recently identified CCR6 as distinguishing GC B cells fated to undergo differentiation into MBCs, the first such marker for this population (manuscript submitted). A selective proclivity for MBCs to emerge from the low affinity compartment of the LZ was demonstrated, consistent with specific upregulation of *Ccr6* mRNA in this GC B cell subpopulation [21••] and the distribution of *Ccr6* mRNA expression in other studies [6,45••,47]. The differences in affinity between MBCs and PCs emerging from GCs are consistent with the recent observation that MBCs arise earlier and long-live PCs emerge later in an immune response, permitting sufficient time for the accumulation of high-affinity mutations in the latter [48].

Transcriptional regulation of MBC differentiation

Globally, the transcriptional program of MBCs share many similarities with naive B cells [49–51]. Of note, *Pax5* is maintained from the naïve to GC to MBC transition, cementing its role in establishing B cell identity across these stages of development [50].

Nevertheless, there are several differences between the gene expression signatures of naïve B cells and MBCs. MBCs express higher mRNA levels encoding the costimulatory receptors *Cd80* and *Cd86* as well as the anti-apoptotic genes *Bcl2* and *Birc6* [50]. Recent data have revealed an important role for *Bach2* in MBC differentiation within the GC response, with haploinsufficiency of *Bach2* resulting in impaired class-switched MBC generation independent of the expression of *Blimp1* [45••].

Finally, molecular control of the fate decision between MBC and PC differentiation was reported to be dependent on *ABF-1* (Activated B cell Factor 1) expression in both humans and mice [52]. *ABF-1* and *Blimp1* mutually repress each other, with ABF-1 expression promoting MBC differentiation. *ABF-1* expression was induced by anti-CD40 and IL-21 signals, further suggesting a role for Tfh-cell help in MBC differentiation.

Chemokine expression for egress from the GC

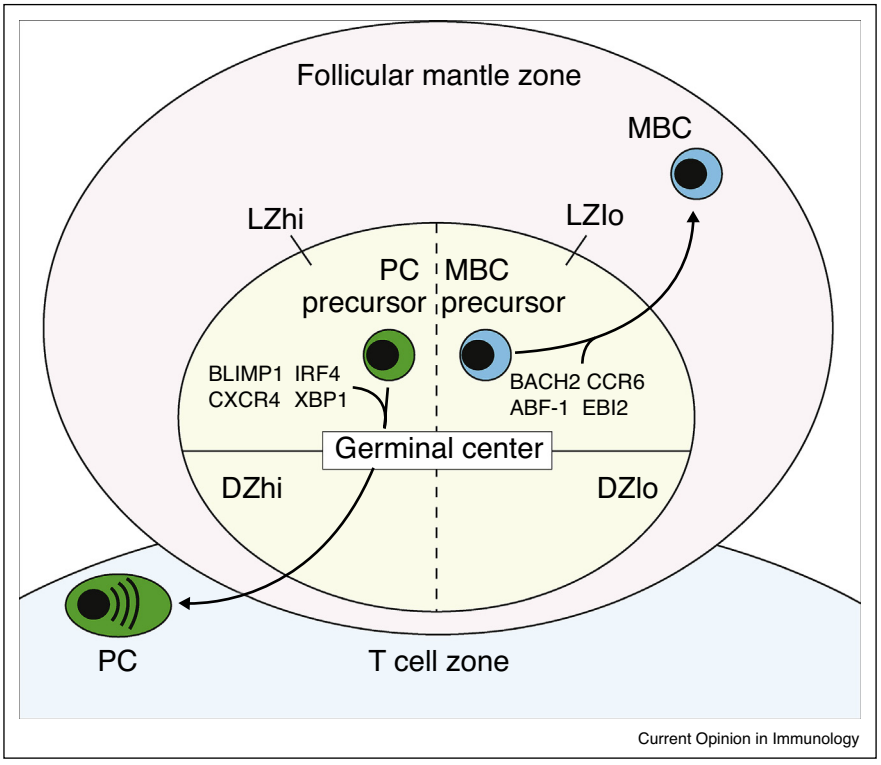
PCs and MBCs generated by GC responses alter their chemokine expression to facilitate egress from the GC and position themselves to assume different roles in immunological memory. PCs upregulate CXCR4 expression and downregulate the B-cell follicle homing chemokine receptor CXCR5 and the T-cell zone homing receptor CCR7 [39]. Plasmablasts do not express, or only express low levels of CCR6 [53] and EBI2 [54], while expression of the receptor S1P1 is critical in the egress from secondary lymphoid organs of PCs destined for the bone marrow [55]. Consistent with these observations regarding chemokine expression, antigen-specific, BrdU-labeled, $CD138^+$ PCs have been visualized exiting the DZ side of the GC [22] and we have also observed a predominant DZ localization [21••].

In contrast, MBCs are known to express a similar chemokine profile to naïve B cells [42], likely reflecting the fate of MBCs to egress to the tissues and recirculate to survey for antigen. Recent work has revealed a crucial role for CCR6 in positioning MBCs in lymphoid tissues for recall responses [56•]. Whilst CCR6 is dispensable to the formation of MBCs in the primary response to antigenic challenge, secondary responses are impaired in CCR6-deficient mice due to the malpositioning of MBCs within the inner follicle and remnant GCs, rather than in the marginal zone and outer follicle where antigen encounter is more likely to occur. MBCs also express higher levels of EBI2 than naïve B cells (unpublished observation), predicted to contribute to the position of MBCs in the outer follicle [54].

Conclusion

There appears to be a fundamental dichotomy between MBCs and PCs in their differentiation and egress. The model to emerge is one of elegant symmetry in which differentiating MBCs and PCs primarily populate opposite anatomical zones and exhibit contrasting antigen affinities (Figure 1). Whilst only high-affinity GC B cells are selected on the basis of antigen engagement in the LZ to initiate the PC differentiation, subsequent Tfh-cell interaction completes their differentiation, up-regulating CXCR4 and driving migration through the DZ. In contrast, MBCs preferentially emerge from the LZ low affinity compartment, where they re-acquire CCR6 to re-position themselves in the marginal zone and perifollicular areas [56•], Thus the differentiation of both PCs

Figure 1



The differentiation of PCs and MBCs from the GC by anatomical zone and affinity. Selection for export occurs in the LZ of the GC. Only high-affinity GC B cells are selected to differentiate into PCs. These PC precursors migrate rapidly to the DZ and exit the GC, returning to the bone marrow to secrete high-affinity antibodies. In contrast, MBC precursors are enriched in the low-affinity compartment of the LZ and emerge from the LZ-side of the GC, returning to the marginal and peri-follicular areas in secondary lymphoid organs, poised to survey for antigen and prepared for secondary responses. LZhi = light zone high affinity, LZlo = light zone low affinity, DZhi = dark zone high affinity, DZlo = dark zone low affinity.

and MBCs from GC precursors is not stochastic but regulated by distinct affinity-dependent mechanisms. This regulatory regime not only provides the effective humoral responses required to deal with active infections but also helps maintain broad antigen specificity in recall responses, which may serve to mitigate original antigenic sin [57].

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