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Clonal and cellular dynamics in germinal centers

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Germinal centers (GCs) are the site of antibody affinity maturation, a process that involves complex clonal and cellular dynamics. Selection of B cells bearing higher-affinity immunoglobulins proceeds via a stereotyped pattern whereby B cells migrate cyclically between the GC's two anatomical compartments. This process occurs in a timeframe that is well suited to analysis by intravital microscopy, and much has been learned in recent years by use of these techniques. On a longer time scale, the diversity of B cell clones and variants within individual GCs is also thought to change as affinity maturation progresses; however, our understanding of clonal dynamics in individual GCs is limited. We discuss recent progress in the elucidation of clonal and cellular dynamics patterns.

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Introduction

The high-affinity antibodies typical of a secondary immune response emerge from their lower-affinity precursors through a process of random mutation and targeted selection known as affinity maturation. This process takes place in specialized structures — germinal centers (GCs) — that form within the B cell follicles of secondary lymphoid organs upon infection or immunization [1–5]. While in these structures, B cells undergo somatic hypermutation (SHM) of their immunoglobulin (Ig) genes, triggered by the enzyme activation-induced cytidine deaminase (AID) [6]. A minority of B cells with affinity-enhancing mutations are then selected based on the increased ability of their antigen-binding B cell receptors (BCRs) to retrieve antigen from the surface of follicular dendritic cells (FDCs) and present it to a limiting number of GC-resident T follicular helper (Tfh) cells [4,7••].

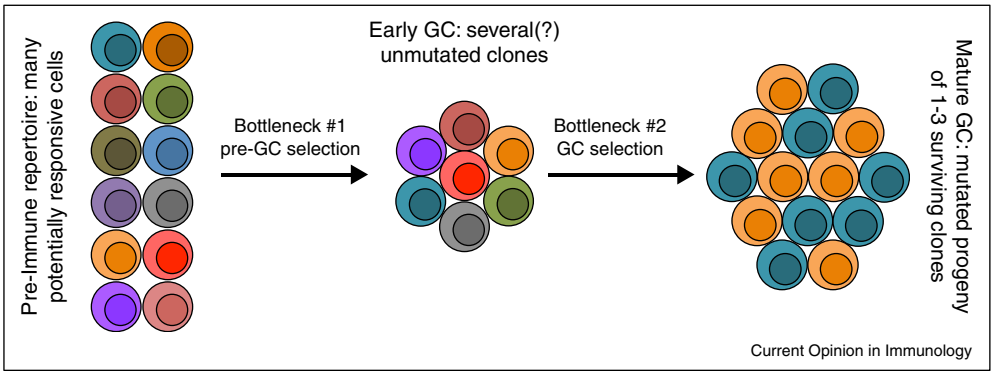
GCs are divided into two anatomically distinct compartments — a dark zone (DZ) and a light zone (LZ). A major feature of the GC reaction is the close association between affinity-based selection and B cell migration between these compartments: upon positive selection in the LZ, GC B cells transit to the DZ, where they proliferate and mutate their Ig genes, subsequently returning to the LZ to test their mutated Igs against antigen retained on FDCs. In recent years, the emergence of multiphoton microscopy has dramatically increased our ability to observe this migratory process in real time, providing invaluable insight into the mechanics of GC selection [7••,8•,9•,10•,11]. These and other studies have been reviewed extensively elsewhere [3,4,12]. In the present review, we discuss specific points regarding the interplay of clonal and cellular dynamics in the GC that in our view remain incompletely understood.

Clonality in the early GC

Before the LZ and DZ form, and thus before *intra*clonal GC selection can begin, GCs must develop by expansion of precursors selected from within a large pool of naïve B cells that compete *inter*clonally (Figure 1). Early studies of GC clonality using allelically marked mixtures of B cells or immunization with two distinct antigens estimated that B cells within mature GCs are the progeny of as few as 1–3 precursor clones [13,14]. Because cells in mature GCs have presumably gone through several cycles of purifying selection, these early studies were in fact reporting on the number of surviving clones rather than of founder clones [15]. Later studies showed that clonal diversity in early GCs can be substantially higher than in mature GCs, suggesting that GCs may initially grow by accretion of many B cell clones that are subsequently filtered by selection to yield the 1–3 clones of mature GCs [16]. Studies in which Ig gene rearrangements were amplified from single cells picked from individual human GCs also support a more complex pattern of GC clonality [15]. Access of B cell clones to the early GC is controlled by a balance between a low B cell-intrinsic activation threshold [17–20] and interclonal competition for T cell signals that regulate B cell entry into the GC [20], possibly by triggering the downregulation of the G-coupled receptor Ebi2 [21,22]. For example, B cells with very low affinity for nitrophenol haptens, which are largely excluded from GCs when transferred into wild-type mice, form normal GCs when in the absence of competition from other B cell clones [18–20]. Interclonal competition is also likely to constrict the breadth of antibody specificities that are allowed entry into the GC. Knowledge of how to manipulate this early selective step may therefore improve our ability to generate antibody responses to non-immunodominant epitopes.

As the GC reaction proceeds, B cell selection shifts from interclonal competition to a system increasingly dominated

Figure 1



Model for clonal dynamics during germinal center formation. GCs are seeded by a small fraction of the large repertoire of naïve B cells potentially responsive to the immunizing antigen by pre-GC competition for T cell help (Bottleneck #1), generating GCs composed of a limited number of clones. These GCs are then further purged from less competitive clones by a second round of competition for T cell help in the mature GC (Bottleneck #2), yielding the 1–3 clones observed in experiment.

by competition among variants of a single clone generated by SHM [16]. This progressive 'monoclonalization' is limited by the segregation of individual GCs from the B cell perspective, which allows several different clonal trees to evolve simultaneously in different GCs. A further contributing factor may be the invasion of ongoing GCs by newly activated B cells with a competitive advantage in antigen binding or access to T cells [9•,23]. The need for competitive advantage limits invasion to special circumstances, such as when T-cell help specific for the incoming B cells is available [23]. Thus, GC invasion and reutilization may be confined to sites prone to constant antigenic stimulation, such as mucosal associated lymphoid tissue. Accordingly, following oral immunization, circulating B cells repeatedly re-enter antigen-experienced GCs, as revealed by the clonal relationship between GC B cells derived from multiple Peyer's Patches [24].

Dynamics of selection in the mature GC

Within mature GCs, selection and cell division are polarized towards LZ and DZ, respectively, although the extent of cell division in the LZ may vary slightly depending on the model employed [7••,8•,25,26•]. Polarization of GCs into these two compartments relies on gradients of the chemokines CXCL13 and CXCL12, whose receptors — CXCR5 and CXCR4 — are upregulated in LZ and DZ B cells, respectively [7••,27••]. Early attempts at mathematical modeling of the GC reaction concluded that efficient affinity maturation could only be attained if cells were allowed to cycle functionally between periods of proliferation and selection, and, by extension, to cycle physically between DZ and LZ [28,29]. These predictions led to a model for affinity maturation known as *cyclic re-entry*, which would be confirmed experimentally only after the introduction of intravital microscopy to the field a decade later [7••,8•,9•,10•].

The close association between interzonal migration and affinity maturation has raised interest in the mechanisms that trigger the transition of GC B cells from one zone to another (Figure 2). Because LZ to DZ migration is equivalent to positive selection [7••], most of the emphasis has been placed on the cellular and molecular mechanisms driving this transition. It was predicted mathematically [30,31] that LZ to DZ migration is restricted by the ability of a GC B cell to recruit help from a limiting number of GC-resident Tfh cells, which is in turn determined by the ability of the B cell to retrieve and present antigen deposited on FDCs. To directly test this hypothesis, we developed a system in which the density of cognate peptide on a small subset of GC B cells expressing the surface lectin DEC205 could be artificially increased by injection of the T cell antigen fused to a monoclonal antibody to this protein. DEC205-expressing B cells thus targeted followed the predicted steps of selection, migrating in mass from LZ to DZ and then proliferating vigorously within the GC or differentiating into plasmablasts [7••]. Importantly, the larger fraction of untargeted ($DEC205^{-/-}$) GC B cells almost entirely disappeared from GCs 48 to 72 h following treatment, leading to the conclusion that Tfh are the limiting factor in GC selection [7••].

In such a model, the primary function of the BCR would be that of an endocytic receptor, and the role of signaling downstream of the BCR would be limited to tasks other than executing the selection of higher affinity clones and variants. Indeed, when cognate peptide levels are equalized among all GC B cells by anti-DEC205 targeting, affinity maturation ceases to operate despite the variation in BCR affinity among GC B cells [7••]. The notion that BCR signaling plays a limited role in selection is further supported by work by Shlomchik and colleagues showing that phosphorylation of proximal components of the BCR