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'Persistent germinal center responses: slow-growing trees bear the best fruits'

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Abstract

Germinal centers (GCs) are key microanatomical sites in lymphoid organs where responding B cells mature and undergo affinity-based selection. The duration of the GC reaction has long been assumed to be relatively brief, but recent studies in humans, nonhuman primates, and mice indicate that GCs can last for weeks to months after initial antigen exposure. This review examines recent studies investigating the factors that influence GC duration, including antigen persistence, T-follicular helper cells, and mode of immunization. Potential mechanisms for how persistent GCs influence the B-cell repertoire are considered. Overall, these studies provide a blueprint for how to design better vaccines that elicit persistent GC responses.

Introduction

Protection against many pathogens relies on developing an effective humoral immune response. One characteristic feature of this response is the continued increase in antibody affinity for an antigen over time [1]. This affinity maturation occurs in germinal centers (GCs), which are distinct microanatomical structures that form primarily in the follicles of secondary lymphoid organs (SLOs) [2]. Activated B cells that enter the GC undergo iterative rounds of somatic hypermutation (SHM) and proliferation while affinity-maturing their B-cell receptor (BCR) against the inciting antigen [2,3]. Graduates of the GC differentiate into long-lived bone marrow plasma cells (BMPCs) and circulating memory B cells (MBCs) [reviewed in 4]. Additionally, long-lived plasma cells can be retained in the tissue of origin (e.g. spleen) 5] or gut-associated lymphoid tissue [6,7], and tissue-resident MBCs serve

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important defense roles at sites such as surface barriers (reviewed in [8]). Forming these effector B-cell types is critical for mediating long-term protection against a pathogen and is a major goal of vaccine development.

GC formation begins when antigen-experienced cognate B and T cells interact at the interface between the T-cell zone and the border of the follicle [9,10]. Activated B cells that receive survival and co-stimulatory signals from cognate T cells can migrate to the center of the follicle to seed the GC, which is divided into two distinct compartments — the dark zone (DZ) and the light zone (LZ) [11,12]. CXC motif chemokine ligand 12 (CXCL12)-producing stromal cells in the DZ attract CXC motif chemokine receptor 4 (CXCR4)-expressing GC B cells, which proliferate and undergo SHM [11]. After they have undergone SHM, these GC B cells downregulate CXCR4 and migrate into the LZ through CXC motif chemokine receptor 5 (CXCR5) sensing of its ligand CXC motif chemokine ligand 13 (CXCL13), which is produced by follicular dendritic cells (FDCs) in the LZ [11,13]. FDCs are critical sites of antigen deposition in the follicle, retaining and presenting antigen to GC B cells to regulate their affinity maturation [14,15]. FDCs also work with T-follicular helper (Tfh) cells in the LZ to send necessary survival signals to GC B cells [16-18]. Positively selected GC B cells can return to the DZ to undergo additional SHM and proliferation or exit the GC as BMPCs or MBCs [19].

The generation of long-lived effector cells is critical for lasting protection against pathogens. While MBCs and, less frequently, BMPCs, can be produced in a GC-independent manner, those arising from the GC typically have a higher affinity for antigen [20-23]. GC-dependent MBCs are poised to rapidly differentiate into short-lived, extrafollicular plasmablasts (PBs) in the event of pathogen re-exposure, producing high-affinity antibodies that can work in concert with matured antibodies from BMPCs to help clear pathogens quickly [22,24]. Some evidence in mice and humans suggests that upon restimulation with antigen, MBCs can differentiate to PCs that potentially contribute to the BMPC pool [25,26], although direct differentiation to long-lived PCs remains undetermined. MBCs can also re-enter the GC and undergo additional rounds of SHM and affinity maturation, thus enhancing subsequent responses [27].

Since the mutational load of GC B cells increases over time, and high levels of mutation enhance antibody affinity, extending the duration of GCs can enhance the production of high-affinity antibodies [21,28]. In this review, we will summarize what is currently known about GC duration, as well as explore multiple factors that may influence GC persistence, including immune complex formation, antigen persistence in the follicle, the ability of GC B cells to acquire T-cell help, and the proliferative capacity of GC B cells. Additionally, we will highlight how the method and context of antigen exposure alter the duration of the GC and how vaccination strategies may be optimized to enhance GC persistence.

How long do germinal centers last?

The ultimate duration of the GC reaction varies depending on the model system studied. Most studies assessing GC kinetics have been done in murine models of vaccination in which an antigen and adjuvant are given as a bolus. These models frequently use a

hapten conjugated to a carrier protein, adjuvanted with alum, and track the hapten-specific GC response in the SLOs. With this immunization method, the antigen-specific mouse GC declines between 2 and 5 weeks after immunization. However, small numbers of antigen-specific GC B cells have been detected in the spleen as late as 21 weeks post immunization [21,29,30]. Using alternate adjuvants may extend GC magnitude and duration, but immunization with other proteins in alum leads to a similar GC kinetics [31-34]. The GC response after injection of sheep red blood cells has also been extensively studied and frequently declines between 2 and 4 weeks post injection in mice [35-37]. Alternate vaccine design and delivery can increase the magnitude and duration of the mouse GC response. Antigens packaged in nanoparticles, particularly those including innate immune system agonists, result in GCs persisting up to 8 weeks post immunization in mice and frequently induce a larger GC compared with a bolus immunization [38,39].

In contrast to vaccination, infection consistently produces more persistent GCs in mice, especially in the SLOs draining the affected tissues. In mice infected with influenza virus, splenic GCs persist until 6–9 weeks post infection, whereas GCs in the lung-draining mediastinal lymph node last between 18 and 24 weeks post infection [40-43]. Notably, later GCs had higher levels of SHM than early GCs and continuously graduated MBCs, highlighting the importance of GC persistence in increasing the affinity of the subsequent antibody response [40,42]. This phenomenon is also observed when comparing GCs generated from acute or chronic infection using the lymphocytic choriomeningitis virus (LCMV) model. Chronically infected mice exhibit more robust GCs at later time points [44,45]. These GCs facilitate higher levels of SHM that lead to an increase in neutralizing antibodies in chronically infected animals compared with acute infection [44].

GC persistence has also been studied in nonhuman primate (NHP) models. Different methods of antigen packaging, adjuvanting, and dosing have also been compared in NHPs. An alum-adjuvanted bolus induces a GC that persists up to 10 weeks post immunization [46]. In contrast, using a saponin-like adjuvant extends GC persistence to 14 weeks post immunization in rhesus monkeys [47]. Escalating the immunization dose over several weeks or delivering antigen through a slow-release osmotic pump also increases the magnitude of the NHP GC response [46,47].

In humans, indirect evidence that GC responses can persist for extended periods is demonstrated by peripheral B-cell SHM rates in antigen-specific BCRs. In subjects vaccinated with a replication-competent adenovirus type-4 recombinant virus expressing influenza H5 hemagglutinin, increases in H5-specific antibody SHM were detected up to 12 months after vaccination [48]. This ongoing SHM persisted beyond the period of active viral replication (2–4 weeks after vaccination) [48]. Similarly, in a longitudinal study, two flavivirus-naive donors vaccinated with the yellow fever 17D vaccine exhibited increased antibody SHM and affinity maturation for 6–9 months post immunization [49]. Ongoing SHM in peripheral B cells has also been observed in humans after influenza H7N9 vaccination [50] and Ebola virus infection [51].

In a study of survivors of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease-2019 (COVID-19) pandemic,

increases in SHM and antibody affinity were observed over several months [52]. This was corroborated by other studies of SARS-CoV-2 infection based on peripheral blood samples [53,54]. However, because postmortem samples of spleen and lymph nodes from severely infected COVID-19 patients showed disrupted GCs, these studies presented no direct evidence of persisting GCs [52].

Other studies have utilized ultrasound-guided fine-needle aspiration to detect GC B cells in lymph nodes after vaccination in humans [55,56]. Notably, the use of mRNA vaccines in response to the COVID-19 pandemic has highlighted the importance of vaccine design in influencing GC magnitude and persistence. In humans, two doses of BNT162b2 (Pfizer-BioNTech), an mRNA vaccine encoding the spike protein (S) of SARS-CoV-2, produced an S-specific GC response that lasted up to 6 months post immunization [56-58]. In contrast, immunization with the unadjuvanted quadrivalent inactivated vaccine for influenza virus induces an antigen-specific GC that lasts between 2 and 4 months post vaccination [55; unpublished data]. While exposure history and pre-existing memory may complicate the comparison between these vaccines in humans, the strength of the GC response to mRNA vaccination suggests that it could be a useful strategy for increasing GC persistence after immunization.

These studies in murine and NHP models and humans demonstrate that GCs can persist for extended periods. Classical dogma based on hapten immunizations in mice would suggest that B-cell responses are rapidly generated, affinity-matured, and GCs are only maintained for a few weeks. However, the studies above indicate that GC responses can persist for months following infection or vaccination and that the antigen-specific B-cell repertoire can continue to mutate and affinity-mature over this period. To design vaccines that elicit these persistent GC reactions, multiple factors should be considered that may influence the duration of the GC.

Factors influencing germinal center persistence

Antigen persistence

After immunization, the antigen is rapidly delivered to the draining lymph nodes [59-61], but only retained for days to weeks within the B-cell follicles [62]. Opsonization of antigens by complement or immunoglobulins generates immune complexes, which can be efficiently trafficked into follicles by subcapsular sinus macrophages [63,64]. These immune complexes are then captured and retained on the surface of FDCs, which present membrane-bound antigens to B cells for selection within the GC [15,16]. B-cell clones will test the affinity of their BCRs in a mechanosensory process [65,66], interacting with FDCs [67] to extract antigen from their surface [68]. FDCs are unique antigen-presenting cells in that they recycle and retain immune complexes without degradation on their surface for extended periods [14,69], thus acting as a source of intact antigen to fuel ongoing selection in the GC.

Extended antigen persistence may contribute to the durability of GC responses. *In silico* modeling suggests that while T-cell help is necessary for generating GCs, antigen consumption by B cells governs the duration of the GC [70]. Computational modeling also predicts that immune complex formation and antigen concentration on the surface

of FDCs dictate GC kinetics and that increased antigen availability results in increased numbers of GC B cells in mice [39]. *In vivo*, persistent antigen has been found to influence GC B-cell numbers and Tfh cells. In vesicular stomatitis virus (VSV)-infected mice that exhibited GCs up to 100 days post infection, antigen persisted on FDCs for the duration of the GC [71]. Mice immunized with a continuous supply of antigen exhibit prolonged antigen retention on the surface of FDCs and a greater number of GC B cells compared with the bolus immunization [39]. A constant supply of antigens also positively correlates with the magnitude of the Tfh-cell response and the maintenance of the Tfh phenotype [72]. This finding agrees with the result that viral persistence drives CD4 T cells toward a Tfh phenotype [73], suggesting that persistent antigen influences both GC B- and Tfh-cell levels. Additionally, the lack of antigen presentation by B cells reduces Tfh-cell differentiation [74]; thus, a constant source of antigen from FDCs to B cells is likely necessary to sustain cognate B–Tfh interactions.

Limited evidence in human studies also suggests antigen persistence may contribute to GC duration. GC responses that lasted up to 6 months have been detected in humans that received SARS-CoV-2 mRNA vaccines [56]. Röltgen et al. separately demonstrated that SARS-CoV-2 vaccine S-protein is present in lymph node GCs of vaccinees up to 60 days post second dose [75]. If such mRNA vaccine-derived antigen is indeed driving long-lasting GCs in human vaccine recipients, it would support the hypothesis that GC persistence is dependent on antigen availability.

Other computational modeling experiments demonstrate that while increased antigen availability extends the length of the GC reaction, it can also influence the degree of affinity maturation the B-cell repertoire undergoes [76], as lower-affinity B-cell clones can compete for help with increased antigen availability. These results have been confirmed *in vivo*, where sequential immunization with antigen variants has been shown to broaden the reactivity of the B-cell response [77], suggesting that increased antigen persistence may not only lengthen GC responses but also influence repertoire diversity and affinity [78].

T-follicular helper cells

Tfh cells play an important role in the GC reaction by providing ‘help’ to antigen-specific B- cell clones in the form of CD40 ligand (CD40L), interleukin (IL)-21, and IL-4 [79]. B cells that do not receive co-stimulation after binding antigen likely die [80], and Tfh-cell impairment in mice results in reduced or absent GCs [81-83]. While Tfh cells are not only involved in initiating GC responses, they likely contribute to determining GC duration, as GCs stimulated by T-independent antigens such as 4-Hydroxy-3-nitrophenylacetic (NP)-Ficoll do not persist as long as conventional T-dependent GCs [84]. T-cell help is considered a possible limiting factor in GC selection, with GC B cells competing for survival signals by presenting antigen to Tfh cells to enter the DZ and clonally expand [18]. However, some data suggest that T-cell help may not be necessary for LZ–DZ cycling [85]. Instead, increased Tfh-cell help ‘refuels’ GC B cells for improved survival and proliferation in the DZ, promoting selection and polyclonal responses [85]. This model demonstrates how cognate B–T-cell interactions may not be necessary for B-cell GC cycling, but are required

to sustain prolonged GC responses. Without Tfh-cell help, GC B cells cannot receive growth and proliferation signals [85,86] necessary for long-lived GCs.

In addition to controlling selection and proliferative capacity, Tfh cells also influence the terminal differentiation of GC B cells to plasma cells or MBCs, in part by regulating GC persistence. Increased plasma cell differentiation due to constitutive CD40L expression in mouse B cells results in premature termination of GCs [87]. The Tfh-cell repertoire also likely plays a role in determining GC persistence. Experiments suggest low-affinity T-cell antigens result in early GC collapse and reduced memory B-cell output [88]. The length of the GC reaction may also be determined by the differentiation of $Foxp3^+$ Tfh cells, also known as Tfh-regulatory cells [89]. These cells have been found to expand in mice before GC contraction, with late-stage GC B cells displaying prolonged interactions with $Foxp3^+$ Tfh cells [90]. Tfh-regulatory cells have also been shown to suppress Tfh numbers [89]. Disruption of B-cell interactions with Tfh-regulatory cells results in elevated GC responses [91]. Thus, Tfh phenotypic changes likely influence the length of GCs. These studies demonstrate that by influencing GC entry, proliferation of GC B cells, and GC termination, Tfh cells are critical in determining GC length by controlling many of the ‘on’ and ‘off’ switches for sustained B-cell responses.

Proliferative capacity and B-cell receptor signaling

As GCs are derived from the clonal expansion of proliferating B cells, the persistence of GC responses is influenced by the divisional capacity and internal signaling of B cells. The formation and maintenance of GCs are dependent on the expression of the cell cycle regulator c-Myc in B cells [92,93]. Downstream of BCR signaling, c-Myc controls the expression of D-type cyclins necessary for the DZ GC B-cell proliferation [94]. The levels of c-Myc expression proportionally dictate the amount of cellular expansion in the DZ [95], and proliferative capacity is controlled by the Tfh-cell help [85] and BCR/CD40 signaling [96]. Blocking these signals or knocking out cyclin D3 reduces proliferation in the GC [97]. Inhibition of c-Myc also results in reduced GC size [93]. Thus, the degree to which B-cell clones continuously receive proliferative signals likely influences the overall duration of the GC response.

Type of infection or antigen exposure

How long GCs persist is influenced by whether a pathogen generates an acute or chronic infection. Mouse models utilizing LCMV, which can produce acute or chronic infections depending on the strain used and infection dose, demonstrate that chronic viral infections drive more extended GC responses. Mice chronically infected with LCMV had GC B cells that persisted up to 60 days post infection [44]. Chronic LCMV infection generates higher numbers of GC B cells and increased antiviral antibody titers over time compared with acute infection [44,45]. While B cells accumulate mutations in acute and chronic infections, long-term chronic infections generate higher-neutralizing antibody titers dependent on SHM of antibody clones, and sustain clonal diversity throughout the response [44,45]. Additionally, for some pathogens, the generation of a potent neutralizing antibody response is dependent on appreciable levels of affinity maturation, as evidenced by longitudinal analysis of responses to Ebola virus in humans [51]. These results in mice strongly suggest that

persistent GCs are more likely to be established by chronic pathogen infections. However, evidence for persisting GCs in mice in response to influenza [42] or VSV [71] infection indicates that the persistence of antigen may continue to drive GC responses even after viral infection has been resolved.

Infection or vaccination with pathogens (live or inactivated) is more likely to generate long-lived GC responses than vaccination with protein antigens. A longitudinal study in humans found that half-lives for antibody responses to viral antigens were far greater (50–200 years) than for responses to nonreplicating protein antigens (11–19 years) [98]. Mice immunized with protein antigens typically do not generate GCs that persist for more than a few weeks [21,99]. Still, modulation of the delivery method of protein antigen to better stimulate pattern recognition receptors of the innate immune system can increase GC duration to over one year [38]. The method of vaccination used to stimulate GC responses likely greatly influences whether GCs are maintained for long periods.

Method of vaccination

Modulating the vaccine delivery method beyond bolus injection or the adjuvant used has been shown to contribute to the development of prolonged GC responses. An escalating dose regimen of antigen delivery, in which mice were immunized with exponentially increasing doses of a protein antigen, was shown to increase both the magnitude of the antibody response and the numbers of GC B cells [39]. It is hypothesized that this method of antigen delivery improves the GC response by providing a continuous supply of intact antigens rather than degraded epitopes for diverse B-cell selection [100]. Another possibility is that greater antigen supply increases the probability that antigen–antibody immune complexes form due to early PB induction. FDCs can then capture and retain these immune complexes for GC B-cell selection. Increased antigen dosing may also overcome pre-existing high-affinity antibody titers that clear antigen before it can be presented in GCs, thus limiting the GC response.

Multiple studies have validated the continuous supply of antigens as a robust method for optimizing antibody responses. Osmotic pump immunization utilized in NHPs demonstrated that extended immunogen release could improve neutralizing antibody titers to human immunodeficiency virus (HIV) versus bolus injection of antigen [101]. Osmotic pump delivery of antigen [102] and mini collagen pellets [103] have also been shown to modulate mouse antibody responses. Finally, slow antigen release devices have been shown to induce high-titer antibody responses and long duration in sheep [104]. In addition to the method of antigen delivery, modulating the site of booster vaccination may contribute to improving GC persistence and MBC recall. Data in mice indicate that ipsilateral boosting with antigen rather than contralateral boosting more efficiently recalls MBC to secondary GC reactions [105]. This result may be observed because ipsilateral boosting better coordinates cognate antigen delivery with persistent GCs in draining lymph nodes, thus helping to expand the MBC repertoire. In agreement with the studies supporting increased antigen availability contributing to persisting GCs, altering vaccination strategies to supply antigen continuously will likely extend GC response duration.

Insights from ‘naturally’ persisting germinal centers — gut and tertiary lymphoid structures

In addition to the studies cited above contributing evidence regarding the factors that extend GC duration, some information may be gleaned by studying two scenarios where GCs persist naturally for extended periods. In contrast to the typically assumed transient GCs of SLOs, GCs persist chronically in the Peyer’s patches of the murine gut due to continuous stimulation by commensal bacteria [reviewed in 106,107]. However, there is underdeveloped gut-associated lymphoid tissue and an absence of GCs in the gut of germ-free mice [108,109], suggesting a lack of antigen results in an abrogation of the persistent GC response. Murine gut GCs have been shown to robustly select antigen-specific clones and facilitate SHM and affinity maturation of BCRs [110], despite a lack of immunization or infection. The rate of B-cell selection is tunable based on the presence or absence of microbiota, with faster clonal dominance occurring in germ-free mice [110]. Collectively, data from gut GCs indicate that persistent GC reactions likely require continuous antigen stimulation, but also that long-lived GCs can support complex clonal selection. Thus, stimulating persisting GCs in SLOs will likely support diverse B-cell repertoires.

Further support for the benefit of persisting GCs comes from tertiary lymphoid structures (TLSs). TLSs are ectopic lymphoid structures that develop outside of SLOs at sites of inflammation or chronic antigen stimulation and are associated with both autoimmune diseases and tumors [reviewed in 111,112]. TLSs associated with tumors can generate mature GCs and support antigen-driven B-cell selection [reviewed in 111]. In several forms of cancer, the presence of TLSs with GCs has been associated with improved patient prognosis. Patients with pancreatic ductal adenocarcinoma that develops TLS GCs show increased rates of B-cell SHM and improved survival [113]. Improved prognosis was also associated with TLS GCs in patients with lung squamous cell carcinoma [114] and colorectal cancer [115]. B-cell signatures and GCs in tumors are enriched in patients that respond positively to immune checkpoint blockade therapy [116], and CXCL13-producing Tfh cells have been found to be a positive biomarker for survival in patients with breast cancer (presumably indicative of GC activity) [117]. The development of TLS GCs in the presence of chronic antigen stimulation (tumors) and the improved association of these GCs with patient survival provides another ‘natural’ example of persistent GCs that require a continuous source of antigen and generate enhanced humoral effector output.

Why should we care about persistent germinal centers?

The goal of understanding the factors that influence the GC reaction is to design better vaccines that produce long-lasting humoral immunity. This is contingent on the ability of a vaccine to generate effective MBC repertoires and long-lived BMPCs. Part of the difficulty in generating vaccines against certain infectious agents is the high degree of antigenic variability that allows the pathogen to evade the humoral immune system. This is seen with HIV, malaria, influenza, and, most recently, SARS-CoV-2. Generating more persistent GC responses may facilitate protection against such pathogens by boosting antibody titers and generating greater SHM in MBCs and BMPCs [46,57]. Extended GC responses may contribute to generating a repertoire of responding B cells that more broadly bind pathogen strains [56], potentially generating MBC pools that can anticipate pathogen mutants.

Persistent GCs can support a complex polyclonal selection environment, with ‘invader’ clones continuously entering the ongoing GC reactions [31,41]. This likely contributes to the ‘permissive’ selection of a diversity of clones spanning various affinities [118], and thus is more likely to produce highly mutated B-cell clones while preventing responses from being biased toward a limited repertoire.

One potential mechanism for how persistent GCs benefit the B-cell repertoire could be extended access to novel antigen epitopes (Figure 1). If early PB responses generated by MBCs produce secreted antibodies to conserved epitopes, these antibodies could form immune complexes or bind antigens on the surface of FDCs. These early secreted antibodies would block access by GC B-cell clones to the conserved epitopes unless BCRs were of sufficiently high affinity to outcompete the MBC clonotypes. This would drive B-cell selection toward clonotypes targeting nonconserved or novel epitopes, thus ensuring a more diverse repertoire while simultaneously supporting the development of high-affinity clones. In a scenario where GC duration is relatively short, this process could be ‘shut down’ early by secreted antibodies reducing intact antigen persistence. The result would be limited B-cell selection and maintenance of a MBC pool targeting conserved, previously experienced epitopes (i.e. ‘antigenic imprinting’ phenomenon observed with influenza). However, if GC reactions were extended for long periods, it would allow for B-cell selection against the exposed epitopes and increase the diversity of exported MBC and BMPC clones. Greater SHM loads and increased diversity would facilitate broader binding to pathogen strains. Thus, persistent GCs would yield the best ‘fruit’ for future humoral responses.

Evidence for such a mechanism has been identified by Tas and colleagues in mice. Broadly binding, low-affinity primary antibody responses can enhance recruitment of naive cells to GCs upon secondary challenge, while high-titer, high-affinity primary responses focused on specific epitopes reduce later cognate B-cell recruitment to GCs [119]. This inhibition of naive B-cell recruitment can be overcome with excess antigen doses [119], lending credence to the hypothesis that antigen availability influences GC responses. High-affinity primary responses may result in increased antigen clearance upon subsequent rechallenge, limiting GC duration. These data demonstrate the importance of considering how primary responses influence subsequent GC reactions when designing vaccine regimens, particularly when antigenic variability is a concern.

Conclusion

This review summarizes how GCs, once previously believed to last only a few weeks, are structures that can be persistently maintained in SLOs for months after the pathogen has been cleared. Many factors likely contribute to whether a GC subsides or is continuously renewed, including antigen persistence, ongoing Tfh-cell help, B-cell proliferative capacity, and the mode of infection or immunization. While GCs are perceived as transient in the spleen and lymph nodes, studying persistent GCs under chronic stimulation conditions, such as the gut and TLSs, may provide evidence for how to prime long-term reactions. The development of new strategies for vaccination will permit GC responses that persist long after the initial immunization. Such GCs may better generate MBCs and BMPCs that protect against antigenically variable pathogens.

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Data Availability

No data were used for the research described in the article.

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Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

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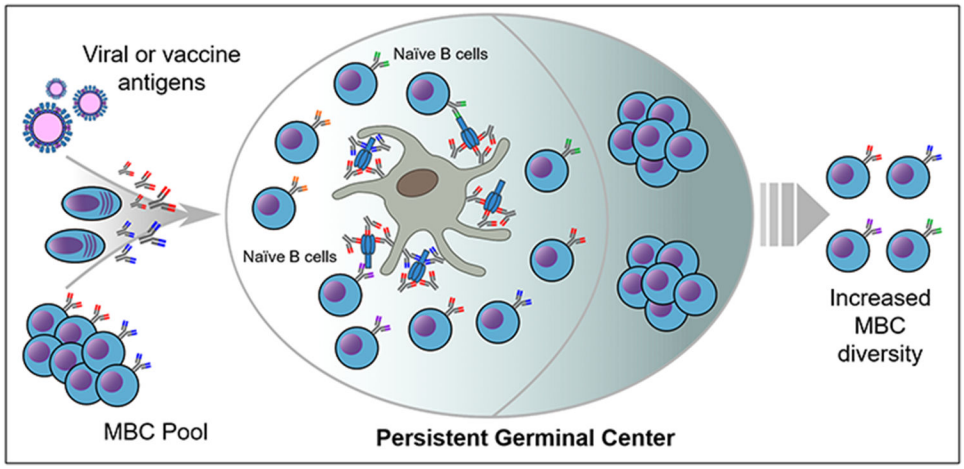

Figure 1.

Potential mechanism for how persistent GCs could increase memory B-cell repertoire diversity. Upon encountering antigens, MBCs rapidly generate PB responses that produce antibodies to previously encountered epitopes. These antibodies bind antigen to form immune complexes, which are then deposited on the surface of FDCs within GCs. These immune complexes may impede repertoire diversification in short-lived GCs by blocking access to epitopes or reducing the duration of antigen presentation. However, persistent GCs could allow for sufficient time for prolonged access of naive B cells to exposed novel epitopes. These clones could be selected and exported as MBCs, expanding the repertoire and driving future responses away from conserved epitopes or antigenic imprinting.