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Role of germinal centers for the induction of broadly-reactive memory B cells

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Abstract

Virus-specific memory B cells (B_{mem}) play a crucial role in protecting against variant viruses. The ability to recognize these variant viruses, defined as antibody breadth, is achieved in B_{mem} populations by two very different pathways, germline-encoded cross-reactivity and affinity-driven, somatic evolution in germinal centers (GCs) for conserved viral epitopes. The latter class of broadly-reactive B_{mem} cells are not cross-reactive *per se*, but bind epitopes crucial for viral fitness. Although these conserved epitopes are often weakly immunogenic, the GC reaction is surprisingly permissive for the continued survival/proliferation of B cells that bind with low affinity or react to cryptic epitopes, increasing their chance of memory recruitment. In this review, we discuss the adaptive strategies of B-cell memory to viral antigenic variations.

Introduction

Memory B (B_{mem}) cells have, for some time, been considered little more than back-ups for long-lived plasma cells that continuously secrete antibody to provide immediate humoral protection to homologous re-infection [1]. B_{mem} cells, however, express BCR repertoires that are more broadly-reactive to viral variants than do populations of long-lived plasma cells, and thereby potentiate broader protection than that afforded by circulating antibody [2–4]. Germinal centers (GCs) provide a microenvironment for an affinity-driven selection of memory precursors based on competition for antigen and T-cell help [5], which acts, on average, to increase the affinity and specificity of “winner” B-cell clones but also narrows clonal diversity by removing “loser” clones [6,7].

The observation of an early, GC-independent pathway for entry to the memory cell pools lead to a model proposing that the recruitment of low-affinity/broad specificity (polyreactive) B cells into a B_{mem} compartment serves to increase the breadth of humoral protection [8–10]. On the other hand, the extensive accumulation of V(D)J mutations in

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later, broadly neutralizing Abs (bNAbs) illustrates the contribution of the GC pathways to protective breadth [11,12]. Here, we discuss recent observations on the nature of clonal selection in GCs elicited by complex viral antigens and propose a comprehensive model that accounts for the development of the early and later forms of broadly-reactive B_{mem} cells, with a specific focus on immune responses to influenza virus and HIV-1.

Viral strategies for evading B_{mem} cell responses

Viral envelope proteins (*e.g.*, influenza hemmagglutinin and HIV-1 Env) are the primary targets of efficacious, neutralizing Abs [13–15], but the rapidity with which mutations in these structural proteins accrue enables viral escape from protective Ab [13,15]. Influenza viruses acquire these escape mutations seasonally, within infected populations, while HIV-1 mutates in chronically infected individuals to escape autologous immunity. This escape mechanism for maintaining viral fitness in the face of the immune response appears to have resulted in the evolution of viral envelope proteins comprising variable regions with lesser impact on viral function and conserved regions necessary for transmission and replication [16]. It is epitopes within these conserved, functional regions that are potential targets for broadly-neutralizing Abs (bNAbs). Often, viruses employ a number of strategies to obscure the conserved domains from Ab surveillance, with the functional result that the conserved domains are weakly immunogenic or immunologically subdominant. In consequence, this adaptation restricts the efficacy of standard immunization protocols to elicit high-affinity Ab to conserved but cryptic domains [17].

Another viral strategy for evading immune surveillance that has become increasingly apparent is structural mimicry of host antigens by epitopes within conserved envelope domains. This mimicry serves to mitigate the generation of bNAbs through the effects of immune tolerance [17,18]. Haynes *et al.* first noted auto- and polyreactivity among bNAbs to HIV-1 [19] and later studies showing impaired B-cell development in knock-in mice expressing HIV-1 bNAb BCR transgenes have demonstrated the efficacy of host mimicry [20]. Although the generality of host mimicry as a mechanism to escape immune control remains to be established, the frequent polyreactivity of influenza bNAbs suggest that neutralizing epitopes that elicit auto- or polyreactive antibody may also act to increase the viral fitness of influenza [21].

Counteraction by germline-encoded cross-reactivity of B_{mem} cells

After immunization with haptenated protein, B_{mem} cells quickly develop in primary responses, in a week or less, within or outside GCs [8–10]. These early differentiative pathways to humoral memory are relatively less dependent on extensive GC responses, and promote the recruitment of low-affinity/polyreactive B cells, most bearing IgM BCRs, into the memory pool. On exposure to related, variant viruses, these early, cross-reactive B_{mem} cells are postulated to serve as the evolutionary templates for mutation and selection in secondary GCs where they generate high-affinity BCR repertoires to the novel epitopes expressed on variant viruses [9,10,22–24]. The early recruitment of memory repertoires is also supported by the idea that memory precursors are not selected solely on the basis of BCR affinity but by other criteria [25], the details of which are discussed later.

This early type of antibody breadth is the product of virus-specific BCR that, nonetheless, are characterized by binding sites capable of interactions with disparate antigen ligands. In consequence, early B_{mem} cells react with low affinity to structural variants of epitopes that arise in mutant viruses. This model is supported by the findings of McGargill's laboratory showing that rapamycin treatment enhances the breadth of protective Ab responses to influenza viruses in correlation with attenuation of GC responses [26]. This intriguing correlation sets the stage for additional studies to provide a fuller mechanistic explanation of a seemingly counter-intuitive relationship: less selection equals broader protection.

Protection against influenza virus is also mediated by low affinity/polyreactive IgM Abs from B-1 B cells [27], likely reflecting low-affinity interaction between influenza virus and host receptors [28,29]. Whereas the polyreactive IgM Abs provide rapid, albeit low levels of protection, vastly more effective responses to secondary infections are provided by high-affinity, isotype-switched antibodies, a point illustrated by the significantly reduced humoral protection of AID-deficient mice in comparison to normal controls [30].

Cross-reactive B_{mem} cells from the early memory pathways are important, both to provide immediate humoral protection and subsequently to supply evolutionary templates on which later GC mutation and selection – in both primary and secondary responses – act. Essentially, the early B_{mem} pathways illustrate the benefits and limitations of diversity in the primary BCR repertoire. These early BCR are selected for rapid clonal expansion by their frequency and avidity for antigen [31], but are limited by their modest potency in the absence of somatic evolutionary refinement [30].

GC pathways for fine-tuning B_{mem} specificity to conserved, functional epitopes

If the early pathways to B_{mem} cell populations act to provide the templates through a limited selection, Ab fitness, as represented by increasing affinity and specificity to viral conserved domains, is achieved through V(D)J mutation and selection in the later, GC pathways to memory. Indeed, HIV bNAbs are recognized for their extraordinary frequencies of somatic mutations [11]. Influenza bNAbs that target the conserved stem domains are also significantly more mutated than neutralizing Abs to less well conserved HA regions [12]. Although the frequencies and kinetics of V(D)J somatic mutation differ between HIV-1 and influenza bNAbs, both bNAb types are similar in that they expand the breadth of specificity through the accumulation of V(D)J mutations [12,32,33]. In the case of HIV-1, longitudinal analyses have successfully traced bNAb lineages over periods of years and demonstrated the evolution of Ab neutralization breadth during episodes of V(D)J diversification and selection by serial virus escape mutants [32,33]. Intriguingly, during the latent phase when bNAbs emerge, lymph node T cells including follicular helper T (T_{FH}) cells serve as viral reservoirs and continuously release viruses that express mutations that can facilitate escape from humoral immunity [34]. These findings establish a paradigm of virus and B-cell co-evolution that proceeds side-by-side during chronic HIV-1 infection. Significantly, these periodic, co-evolutionary responses focus BCR selection and affinity maturation on those

virus epitopes that remain stable, *i.e.*, on conserved HIV-1 epitopes that cannot readily be altered by mutational change.

Virus and BCR co-evolution also contributes to the generation of broadly reactive B_{mem} cells that recognize invariant regions of influenza virus escape mutants [35]. In contrast to HIV-1 chronicity, influenza virus replications are transient, nonetheless signs of persistent viral replication in the lungs of infected mice can be detected for at least 1 month following pulmonary infection [35,36]. This extended period of viral replication and antigen exposure is reflected in the delayed contraction of local GCs ectopically formed in lungs at sites of viral replication [35]. Importantly, comparing the frequencies of strain-specific and broadly-reactive GC B cells during this persistent GC phase, broadly-reactive B cells were found to be significantly enriched in lung GCs relative to splenic GCs in infected mice [35]. The role for ectopic GCs in generating broadly reactive B_{mem} populations nicely fits with previous observations in mice and humans that respiratory influenza infections increase significantly the breadth of serum IgG responses compared to homologous vaccination by other parenteral routes [37,38].

Permissive selection for maintaining GC clonal diversity

How GCs select BCR repertoires over time is fundamental to understand affinity maturation and specificity breadth achieved in bNAb responses. A major obstacle to the selective expansion of high affinity, broadly-reactive B cells specific for the conserved neutralizing regions of viruses, is the weak immunogenicity or immunological subdominance of the associated viral epitopes [17]. As we discussed earlier, conserved neutralization domains that are crucial to virus survival, replication, and/or transmission are often concealed from immune responses and this concealment is likely responsible for the poor immunogenicity of these epitopes [17].

Recent advances in determining BCR affinity and clonal diversity in GCs elicited by complex, polyepitopic antigens has revealed that BCR selection in GCs is permissive for a diverse spectrum of affinity and clonality. In contrast to responses to genetically restricted hapten responses, B cells activated by complex antigens establish GCs that not only begin with substantial clonal diversity but may actually grow more clonally diverse with time, as B cell clones that were rare initially become numerous [31,39]. A recent and surprising finding by Kuraoka et al.[31] was that half of all GC B cells elicited by complex protein antigens do not bind the native form of the eliciting antigen, even though the non-binding B cells acquire V(D)J mutations and expand clonally at rates and extents similar to that of antigen-binding GC B cells [31]. The proliferation and V(D)J mutations that accrue to GC B cells responding to this unknown agent, called “dark antigen” by Kuraoka [31], most likely represent responses to neoepitopes generated by physiological processes that modify antigen *in vivo*. Even highly stable antigen immunogens carefully adjuvanted to preserve structural stability elicit robust “dark antigen” responses (M. Kuraoka and G. Kelsoe, *unpublished*). We think it likely that humoral responses to dark antigen neoepitopes are common, but overlooked by standard assay methods utilizing native antigen as an assay ligand.

These recent observations of interclonal GC selection make some useful points. First, even in the presence of significant affinity maturation, unrecognized modes of GC selection allow B cells that are initially rare and/or bear low affinity BCR to remain viable and continue within GCs for much longer periods than previously thought [31]. This permissive environment provides an opportunity for less fit, lower affinity and/or polyreactive B cells to enter memory B-cell pools where they may be useful templates for later evolutionary refinement. Second, the observation of GC B-cell responses to dark antigen suggests that complex protein antigens are subjected to structural modifications that may change the balance of epitopic antigenicity *in vivo* ([31]; M. Kuraoka and G. Kelsoe, *unpublished*).

Of note, viral envelop proteins undergo substantial conformational and/or structural modifications during viral replication [13,15]. If these modifications promote the exposure, generation, and recognition of epitopes that are normally cryptic, in some instances including conserved epitopes, to GC B cells, viral replication *per se* may limit the structural constraints that normally minimize the activation and selection of B cells capable of generating bNAbs that recognize stable and conserved virus epitopes. Moreover, the infection-associated, immunological milieu may also attenuate any immunological tolerance that mitigates antigen-driven selection of GC B cells expressing auto- or polyreactive BCR, as B-cell intrinsic signaling through IFN- γ , the cytokine upregulated after virus infection, are suggested to regulate GC B cell tolerance to autoantigens [40,41]. Together, it is tempting to speculate that these viral and immunological factors account for the enhanced elicitation of broadly-reactive antibodies after infection [37,38] and elevated GC selection for broad reactivity at the sites of virus replication [35].

How broadly-reactive B cells are recruited and maintained in the memory pool?

After bNAbs B cells are selected, they are often recruited into the memory compartment rather than the long-lived plasmacyte compartment [2–4]. Shinnakasu *et al.* [42] have shown the strength of T-cell helper activity provided by T_{FH} cells is among the key determinants for fate decision into the memory compartment; weak signals instruct GC B cells into the memory pools by increasing expression of the Bach2 transcription factor (reviewed in page xx – xx). Broadly-reactive B cells may be guided into the memory compartment by a similar mechanism, as the subdominant nature of the conserved domains lowers the accessibility of BCR ([21,43], Y. Adachi and Y. Takahashi, *unpublished*), thereby limiting the amount of antigens presented to T_{FH} cells.

The maintenance of broadly-reactive B_{mem} cells is crucial to sustain the capacity for broad protection to variant viruses. BCR polyreactivity has negative effects on the maintenance of IgG⁺ memory B cells, and may reduce the life span of broadly-reactive memory B cells [44]. Memory B cells are also maintained in the peripheral tissues where B_{mem} cells with unique phenotypes localize as a tissue-resident memory compartment [35,45,46]. Tissue residency shortens the time for Ab production on secondary infection and substantially improves protective efficacy [47]. Intriguingly, broadly-reactive B_{mem} cells are enriched in tissue-resident memory pools, where they may potentiate broad protection at infection sites [35].

Where and how tissue-resident B_{mem} cells are maintained remains important questions to be addressed.

Concluding remarks

We discussed multiple pathways for memory B cell development, and have highlighted a possible functional partition between the early GC-independent and late GC-dependent pathways. We propose that permissive GC selection based on conformationally modified antigens may be the basis for selecting BCR repertoires targeting conserved viral epitopes, the sites of vulnerability. Whereas antibody secreted by long-lived plasma cells is exactly directed towards past infections and antigen exposures, these non-dividing cells are eventually lost in the absence of additional recruitment by homologous challenge. B_{mem} cells, on the other hand, can persist for extended periods through their capacity for self-renewal even when they carry BCR that are cross-reactive for variant viruses. In this way, the breadth of B_{mem} cells is a key feature of long-lasting memory for future virus infection that have altered their antigenic profiles through mutation. We now know B_{mem} cells are not simply a back-up for long-lived plasma cells but a cell compartment that helps to anticipate virus cells should evolution. A deeper understanding of the biology of broadly-reactive B_{mem} be an important goal for both basic and translational immunology.

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Highlights

- Viral conserved domains are often concealed from the humoral responses.
- Memory B cells counteract with viral mutations by germline-encoded cross-reactivity.
- GC reactions fine-tune the specificity of memory B cells toward the conserved domains.
- Permissive GC selection allows the fine-tuning of memory specificity.
- Broadly-reactive B cells may be recruited into the memory pool with an attenuated T-cell help.

Figure. Takahashi and Kelsoe

Proposed model for GC selection and broad-reactivity of B_{mem} cells after three types of antigen priming. (a) Monoepitopic antigens recruit B cells with better accessibility to antigens into GCs where antigens and T_{FH} cells select somatic variants with high affinity/specificity, resulting in increased affinity and reduced clonality. (b) Polyepitopic antigens elicit GCs where conformational modification of selecting antigens increases the survival and proliferation of B cells that bind to cryptic/conserved epitopes. (c) Viral replication induces substantial conformational modification of antigens that exposes the cryptic/conserved epitopes and promotes the selection of broadly-reactive B cells. (d) GC-independent pathway elicits low-affinity/specificity B_{mem} cells which save germline-encoded cross-reactivity for the later GC responses.