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Current Opinion in Immunology

Germinal centers: programmed for affinity maturation and antibody diversification

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The seminal discovery by Eisen that antibodies undergo improvements in antigen-binding affinity over the course of an immune response led to a long running search for the underlying mechanism. Germinal centers in lymphoid organs are now recognized to be critically involved in this phenomenon, known as antibody affinity maturation. As well as improving in affinity for specific epitopes, some antibody responses maintain or even increase their breadth of antigen-recognition over time. This has led to another intense line of research aimed at understanding how broadly neutralizing anti-pathogen responses are generated. Recent work indicates that germinal centers also play an important role in the diversification process. We discuss current understanding of how germinal centers are programmed to support both affinity maturation and antibody diversification.

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Current Opinion in Immunology 2017, **45**:21–30

This review comes from a themed issue on **Lymphocyte development and activation**

Edited by **David Tarlinton** and **Gabriel D Victora**

For a complete overview see the Issue and the Editorial

Available online 12th January 2017

http://dx.doi.org/10.1016/j.coi.2016.12.004

Introduction

Germinal centers (GCs) are sites where B cells undergo antigen-driven somatic hypermutation (SHM) of their immunoglobulin (Ig) variable (V) regions and selection for improved antigen binding [1,2,3•,4]. GCs form over a period of several days following antigen exposure, and individual lymphoid organs can contain a few or dozens of these isolated B cell clusters. GCs become organized into two main zones, a T zone proximal dark zone (DZ) and a distal light zone (LZ). The DZ is the most active site of cell division. DZ GC B cells (also known as centroblasts) express high amounts of activation-induced cytidine deaminase (AID), driving SHM. The LZ contains LZ GC B cells (also called centrocytes) that have mostly left the cell cycle, as well as T follicular helper (Tfh) cells and a dense network of follicular dendritic cells (FDCs) that display surface bound antigens. The LZ is the principle site of antigen-mediated and T cell-mediated selection of GC B cells. During the process of SHM and selection, many B cells undergo cell death within the GC and their corpses are cleared by tingible body macrophages. GC B cells move continually within the GC, traveling to the DZ in response to the CXCR4 ligand, CXCL12, that is made by DZ stromal cells and being directed to the LZ by the CXCR5 ligand, CXCL13 [5,6]. GC B cells undergo repeated rounds of mutation and selection, through a process of cyclic reentry from the LZ to the cycling DZ state [3•]. The output of the GC includes memory B cells and long-lived antibody producing plasma cells (PCs). Here we review the growing evidence that GC responses against complex antigens are not geared solely to generating the most highly focused, highest affinity responses, but that they encompass a permissiveness that allows the generation of diverse responses with a range of antigen binding affinities.

Experimental versus real world antibody affinity maturation

Subsequent to the classical work of Eisen and Siskind that was performed in rabbits [7], the study of antibody affinity maturation has predominantly been performed in mice and has relied largely on two experimental systems; the tracking of endogenous B cells following hapten–protein conjugate immunizations (most commonly NP), and the adoptive transfers of B cells from immunoglobulin (Ig) heavy chain knockin mice such as those specific for hen egg lysozyme (HEL). These approaches have shown that affinity maturation can occur via a process of stringent competition that leads to the best variants rapidly replacing their neighbors. However, in both experimental systems, all cells recognize the same epitope on the antigen (intraclonal competition) and relatively large affinity increases (1–2 log) are conferred via just single amino acid substitutions within the variable region. While this may provide a reasonable model for how 'easy' affinity maturation events occur, the extent to which these responses reflect what is going on during affinity maturation against more complex antigens that have multiple epitopes, and against which many clones may compete, appears more limited.

The development over the last decade of relatively high throughput single B cell antibody cloning technologies has led to an array of insightful studies characterizing the molecular details and maturation pathways of medically and scientifically important antibodies from both humans and mice, with a strong focus towards understanding how antibodies with broadly neutralizing potential function. In line with the mouse studies, affinity maturation of some 'real' antibodies occurs through simple changes in the CDRs of the IgHV region causing cells to rapidly gain neutralizing potential [8•]. Often, however, the developmental pathways of neutralizing antibodies are much more complex, with cells needing to pass through multiple low or moderate affinity intermediate steps en route. One such example is the influenza-specific antibody CR6261, the first identified member of a family of broadly neutralizing human antibodies that utilize IgHV1-69 and recognize a conserved region on the stem of the virus' haemagglutinin glycoprotein [9]. Despite earlier studies indicating that activated B cells compete with one another based upon the affinity of their BCRs even at the time of GC entry [10,11], the unmutated founder of this antibody was determined to have an extremely low affinity (below detection in soluble form with the assay used). To acquire its full activity, CR6261 needed to accumulate a minimum of seven specific mutations in two regions of its IgHV gene. The stem region of the HA glycoprotein that CR6261 recognized is known to be a conformationally challenging site for antibodies to access when in its native form, likely contributing to why multiple mutations are needed to achieve full binding potency [12]. This theme of complex pathways to the generation of high affinity antibodies is reinforced in the large body of work that has been performed to decipher the somatic mutation history of broadly neutralizing HIV-1 gp120 antibodies [13•]. In many cases, these antibodies need to accrue very large numbers of somatic mutations before eventually binding difficult to access conserved regions of this glycoprotein. These findings do not directly contradict simple models of affinity-based discrimination in the GC, however they do infer that GCs should be relatively permissive to low and moderate affinity antibodies so as to not severely restrict the pool of somatic variants from which more complex antibodies can develop. There is also strong evidence that the generation of diversity in GCs contributes to immunity by providing pools of B cells from which recall or maturation towards similar but different antigens can occur following reinfection or in response to viral escape mutants [8•,13•].

Diversity generation and retention in germinal centers

Two recent studies from Tas et al. and Kuraoka et al. have begun to reconcile observations made through human antibody cloning technologies with how affinity maturation might occur *in situ* in GCs by tracking endogenous responses to complex protein antigens [14••,15••]. In both studies, GCs were found to be much more permissive to retaining cells expressing low and moderate affinity receptors than had previously been appreciated. In order to facilitate a large scale analysis of GC derived antibodies, Kuraoka et al. developed a novel feeder cell-based *ex vivo* culture system to expand and differentiate single GC B cells, such that they could screen the antibody products derived from them. When they then compared the relative antigen binding characteristics of the antibodies from 8 days and 16 days post immunization with recombinant proteins derived from influenza A virus and *Bacillus anthracis*, they found clear evidence that the mean antibody affinity improved with time as might be expected. Importantly, however, this did not occur at the expense of retaining low or moderate affinity cells, and it did not cause a narrowing of clonal diversity as defined by usage of different V_H gene segments. Furthermore, order of magnitude affinity differences were observed even when only clonally related cells within the same branch of a phylogenetic tree were considered, indicating significant permissiveness in terms of affinity even when competition was for the same epitope. It will be interesting in the future to determine how long such variants can remain in GCs alongside each other.

Little clonal exchange occurs between GCs during acute responses. This led Tas et al. to interrogate the nature of clonal dynamics and relationships at play within separate GCs from single lymph nodes [14••]. To achieve this, they applied a range of creative 'brainbow', photo-conversion, and micro-dissection techniques that allowed them to both visualize lineage relationships via expression of color combinations and to isolate single cells for antibody cloning-based characterization. This current study revealed that the seeding of primary GCs is a highly oligoclonal affair (50–200 clones per GC) and that the threshold affinity requirement is very low. The visual nature of the analysis in the tamoxifen inducible 'brainbow' system made it possible for the authors to examine multiple GCs from within a single lymph node. Once established, the fate of individual GCs varied greatly, with some foci becoming dominated by single clones while others remained highly diverse throughout the study period. When homogenization did occur, the kinetics could be fast, with some GCs becoming >50% clonally dominated by 9 days post-tamoxifen treatment and >80% by day 11. Remarkably, homogenization occurred even within GCs from Peyer's patches and mesenteric lymph nodes where the responses are to gut-associated organisms. When immunization driven 'clonal bursts' did occur, the expected increases in affinity over the ancestoral seeding cells were observed, suggesting that the outgrowths probably contribute to affinity maturation. However, affinity alone was not sufficient to cause clonal bursts because identical clones that carried the same affinity conferring mutations were also found in non-bursting GCs from the same lymph nodes. Furthermore, selection caused by high affinity antibody-antigen interactions also