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# The imperfect control of self-reactive germinal center B cells

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Unlike T cells, B cells diversify their antigen receptor (BCR) binding specificities at two distinct stages of differentiation. Thus, in addition to initial variable region gene rearrangements, B cells recruited into T-dependent immune responses further modify their BCR specificity via iterative rounds of somatic hypermutation (SHM) within germinal centers (GCs). Although critical for providing the high-affinity antibody specificities required for long-term immune protection, SHM can also generate self-reactive B cells capable of differentiating into autoantibody-producing plasma cells. Recent data confirm that self-reactive GC B cells can be effectively removed from the secondary repertoire so as to maintain self-tolerance. However, they can also escape deletion under certain circumstances and so contribute to autoimmune disease via production of somatically mutated, pathogenic autoantibodies.

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## Introduction

The ability to direct destructive immune responses against external and internal threats, such as foreign microbes and cancer cells, is one of the key adaptations to have arisen during human evolution [1]. However, the immune system could only evolve this destructive 'yin' if it also possessed the counterbalancing 'yang' of self-tolerance. We now know self-tolerance to be a complex and overlapping system of controls that act collectively to prevent immune attack of the body's own cells and tissues. Nevertheless, the ~5% incidence of autoimmune diseases within the human population indicates that self-tolerance is not absolute and can be subverted in certain circumstances by genetic and/or environmental factors.

Autoantibodies are a hallmark of many autoimmune diseases and result from the differentiation of self-reactive B cells into plasma cells. Whilst there are a number of explanations for how autoantibodies might be produced under various circumstances, the aetiology of pathogenic antibodies in most autoimmune diseases has been difficult to define. A unique challenge to the maintenance of self-tolerance in the B cell compartment is the 'second wave' of BCR diversification within B cells that are recruited into T-dependent immune responses and ultimately enter the germinal center (GC) reaction. Somatic hypermutation (SHM) of the immunoglobulin variable region genes of GC B cells results in the occasional generation of clones with increased affinity for foreign antigen, these cells being specifically perpetuated and subsequently differentiating into the high affinity plasma cells and memory B cells that provide long-term immunity [2]. However, the largely random nature of the SHM process inevitably leads to the generation of self-reactive B cells in the GC that, unless somehow inactivated, have the potential to initiate autoantibody production. The fact that most pathogenic autoantibodies show the hallmarks of SHM and selection strongly suggests that failure to enforce self-tolerance in GCs may contribute to many autoimmune diseases.

This review provides a brief outline of GC structure and cellular dynamics. The reader is referred to recent and excellent overviews both in this volume [3] and elsewhere [2,4] for more details. The major focus here will be on recent insights into how self-tolerance is enforced in the GC how it may break down to generate somatically mutated, pathogenic autoantibodies.

## Constituents and function of the germinal center

The GC is classically divided into the light (LZ) and dark zones (DZ). The LZ is characterized by the presence of follicular dendritic cells (FDCs), non-hematopoietic cells that derive from perivascular precursors [5], which hold antigen on their cell surface in the form of immune complexes. Antigen-specific B cells, previously expanded by T-dependent proliferation outside the GC [6], interact with FDC-bound antigen in the LZ and receive cognate stimuli from $CD4^+$ T follicular helper (Tfh) cells also located in the LZ. Delivery of Tfh signals to LZ GC B cells triggers a phenotypic and positional shift whereby they increase surface CXCR4 levels and undergo migration to the DZ [7]. This migration is most likely supported by a newly identified population of stromal cells that reside within the DZ and express the CXCR4 ligand, CXCL12 [8]. GC B cells undergo cell replication in the DZ as well as SHM of their Ig variable region genes. DZ B cells subsequently return to the LZ expressing their revised BCR variable regions and compete

more successfully for antigen and Tfh help if they have acquired increased antigen affinity following SHM. High affinity GC B cells not only survive to undergo further rounds of SHM and selection but selectively differentiate into plasma cells [9] thus guaranteeing the most effective, high affinity antibodies are produced. GCs remain static in size for long periods of the immune response, meaning that the high rate of GC B cell proliferation must be counterbalanced extensive cell death, particularly among B cells that do not acquire high antigen affinity. The final major component cells within the GC are the tangible body macrophages (TBMs) which act as the 'cleaners' of the GC, rapidly ingesting and degrading apoptotic B cells via the MFGE8 molecules that are produced by FDCs and bind to the surface of apoptotic B cells [10].

## Some but not all self-reactive B cells are removed from the GC

It has been recognised for over 25 years that pathogenic autoantibodies can be generated by SHM and antigen-driven selection [11], most likely in GCs but also potentially in extrafollicular niches [12]. Whilst the very existence of such autoantibodies indicates that self-tolerance in the GC is not absolute, their absence from most individuals suggests that self-reactive GC B cells are normally kept in check and are only rarely permitted to differentiate into autoantibody-producing plasma cells. Experiments performed nearly 20 years ago in which self-antigen was mimicked by an acutely administered bolus of exogenous (foreign) antigen, suggested that B cells that acquire self-reactivity in the GC are deleted upon contact with self-antigen [13–15]. However, there has been little progress since this time in identifying the fate of self-reactive GC B cells. In particular, the fate of GC B cells that recognise a *bona fide* self-antigen has been difficult to uncover due to the dynamic nature of the GC response and the absence of a suitable model system for identifying and tracking such cells within the GC [16].

A solution to this problem was provided in a recent study by Chan and colleagues [17••] in which B cells expressing a defined BCR against the foreign protein hen egg lysozyme (HEL), obtained from '$SW_{HEL}$' mice [18], could undergo affinity maturation when immunized with a HEL variant ($HEL^{3X}$) [19]. A transgenic mouse line was produced which expressed a related HEL variant ($HEL^{4X}$) as a self-antigen. Importantly, $SW_{HEL}$ B cells did not bind to $HEL^{4X}$ but acquired cross-reactivity to it when they underwent affinity maturation in response to $HEL^{3X}$ immunization [17••]. In mice ubiquitously expressing $HEL^{4X}$, GC B cells that bound $HEL^{4X}$ self-antigen were prevented from developing (Figure 1(b)). Strikingly, self-reactive anti-$HEL^{4X}$ GC B cells and anti-$HEL^{4X}$ autoantibodies did develop following $HEL^{3X}$ immunization of transgenic mice that expressed $HEL^{4X}$ self-antigen in a tissue specific manner (e.g. in the liver or kidney) [17••] (Figure 1(c)). In summary, this study indicated first that self-reactive B cells generated in the GC could indeed be removed from the secondary repertoire, but also showed this is not always the case. In particular, if the self-antigen in question is not expressed at sufficient levels in the GC microenvironment, it appears that self-reactive GC B cells remain 'ignorant' of their self-reactivity and can differentiate unimpeded into autoantibody secreting plasma cells [16] (Figure 1).

## Selection of self-reactive GC B cells by foreign versus self-antigen

In terms of the potential mechanisms for the production of autoantibodies, the system of Chan and colleagues most accurately models the concept of 'molecular mimicry' — that is, the idea that immune responses generated against foreign antigens (typically infectious pathogens) can give rise to cross-reactive antibodies that bind to a specific self-antigen as well as the foreign antigen. Cross-reactive autoantibodies have been characterized in a number of autoimmune diseases that can occur following particular infections, including hepatitis C-related immune thrombocytopenia, pauci-immune focal necrotizing glomerulonephritis, Chagas disease, Guillain-Barré syndrome and rheumatic carditis (for references see [17••]). A key property of this model of autoantibody production is that it does not require T cell self-tolerance to be compromised, since Tfh cells recognising foreign epitopes can in theory drive the selection of the self-reactive GC B cells since the B cells cross-react with foreign antigen. If self-tolerance has been breached at the T cell level, however, high affinity pathogenic autoantibodies may be selected in the GC based purely on their affinity for self-antigen. It has been reasoned that the nature of the antigen responsible for driving autoantibody production might be clearer if the primary BCR specificity (i.e. that expressed on the original, unmutated B cell clone) from which the autoantibody was derived could be identified.

## Deriving the primary specificity of somatically mutated autoantibodies

By the time they are identified, pathogenic autoantibodies are the product of terminally differentiated plasma cells that have long since exited the GC and acquired somatic mutations that obscure the primary specificity encoded in the original naïve B cell clone. A number of recent studies have employed the strategy of 'reverting' the variable region sequences of hypermutated autoantibodies back to putative primary specificity generated by V(D)J recombination.

Autoantibodies associated with the autoimmune skin and mucous membrane disease pehmphigus vulgaris (PV) primarily target the epithelial desmosome protein desmoglein-3 (DSG3). Di Zenzo and colleagues recently reported the results of reverting the variable region sequences of four somatically mutated anti-DSG3 autoantibodies