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Resolve, revise, and relax: The 3 Rs of B cell repertoire adjustment

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Abstract

Competition for limited, cell extrinsic survival factors is a general feature of peripheral selection checkpoints involved in B lymphocyte maturation and activation. Perhaps the best-characterized example involves BLyS (B lymphocyte stimulator), which modulates the size and composition of mature naïve B cell pools, but evidence for analogous competitive checkpoints is emerging for both germinal center B cells and plasma cells. Here we discuss how deliberate alteration of BLyS levels might be used to manipulate B cell repertoire selection in order to restore self-tolerance in autoimmunity, remodel the repertoire to accommodate neo-self antigens introduced through transplantation and gene therapy, or expand repertoire diversity to reveal novel, therapeutically useful specificities.

Keywords

BLyS; BAFF; B cell; selection; repertoire

1. Introduction

The adaptive immune system generates clonally distributed B- and T-cell specificities through random genetic processes. While these afford great receptor diversity, they also impose a need to screen and eliminate cells with self-reactive or ineffective antigen receptors. In general, this is accomplished through negative and positive selection based on antigen receptor signal strength. Among B-lymphocytes, these processes occur during the development of preimmune pools, as well as during the generation of antibody secreting effectors and memory cells. In toto, these selective events shape the composition of naïve and antigen-experienced specificity repertoires.

Recent insights into the molecular processes underlying B cell selection offer the potential to intentionally adjust repertoire selection. Indeed, some settings have long been considered potential targets for such manipulation, such as resolving failed negative selection in humoral autoimmunity. However, the ability to intentionally manipulate specificity-based selection during B cell development and activation raises further groundbreaking possibilities, such as revising specificity repertoires to accommodate neo-self antigens introduced through transplantation or gene therapies, or intentionally relaxing selection to promote the availability of otherwise rare specificities desired for vaccine efficacy. Herein, we consider each of these broad categories of repertoire manipulation in the context of such translational examples.

2.1 Overview of B cell selective checkpoints

Selection based upon BCR specificity occurs at several well-established checkpoints among both developing and activated B cells. These checkpoints vary in terms of the differentiation stages where they act, as well as in the underlying mechanisms that determine cell survival versus elimination. Although some selection occurs at earlier developmental stages based on pre-BCR interactions, the first checkpoint where selection involves a complete BCR occurs during the immature (IMM) stage in the bone marrow (BM). The mechanisms active at this so-called central tolerance checkpoint have been reviewed extensively elsewhere [1–5], and are cell intrinsic, apparently based on BCR signal strength alone. Thus, IMM B cells receiving strong BCR signals are induced to either undergo secondary Ig gene rearrangements or die. This results in the loss of nearly 90% of all IMM B cells prior to their migration to the periphery [6–8].

The remaining selective checkpoints are imposed in peripheral tissues, and act to shape both the pre-immune and antigen-experienced B cell repertoires. The first of these occurs as IMM B cells that have survived central deletion exit the BM and enter the transitional (TR) pools, where they undergo further specificity-based selection [7–10]. In contrast to the exclusively BCR-dependent processes acting among IMM B cells, selection at the TR stages is governed by interplay between BCR signal strength and availability of the cytokine B lymphocyte stimulator (BLyS, also termed BAFF). Survival through this checkpoint allows TR B cells to complete differentiation and organization into the mature, pre-immune follicular (FO) or marginal zone (MZ) compartments.

Although mature B cells in these quiescent, preimmune pools undergo some degree of protracted BCR-mediated selection, the next selective checkpoints critical to effective immunity and tolerance transpire during antigen-driven activation. The best-established post-activation checkpoint is within the germinal center (GC), where B cells undergo somatic hypermutation (SHM) of Ig genes. GC selection involves positive selection for GC B cells with improved antigen affinity, as well as negative selection against autoreactive specificities arising from SHM [11–13]. Selection mechanisms in the GC remain poorly understood, but current models for GC positive selection invoke competition between GC B cells for antigen and T follicular helper interactions that provide co-stimulation and key survival cytokines [14,15]. Two long-lived subsets are derived from GC reactions, memory B cells and long-lived plasma cells (LLPCs). While there is evidence for competitive survival within these populations, the nature and selectivity involved are not yet well characterized.

Because all known peripheral checkpoints involve competition for B cell *extrinsic* signals, their stringency should - at least in theory - be manipulable by either restricting or enhancing the availability of these cell-extrinsic factors. The discovery that BLyS serves as the limiting factor mediating TR B cell selection affords the potential to adjust the selective stringency imposed on the emerging naïve repertoire, thus opening previously unappreciated possibilities for translational applications in autoimmunity, transplantation, gene and cell therapies, and vaccine development. Moreover, this may serve as a conceptual template for intervention strategies in antigen-experienced pools as the selective mechanisms active in these pools are revealed.

2.2 The BLyS family of cytokines and receptors

Over the last decade, members of the BLyS cytokine and receptor family have emerged as key players in the selection and homeostasis of nearly all mature B lineage pools (reviewed in [16–18]). This tumor necrosis factor (TNF) sub-family consists of three receptors and two ligands. The receptors are B cell maturation antigen (BCMA), TACI (transmembrane

activator and calcium-modulator and cyclophilin ligand interactor), and BR3 (BLyS Receptor 3, also termed BAFF-R). The two cytokines in this family are BLyS (also termed BAFF), and APRIL (a proliferation inducing ligand). BLyS can bind to any of the three receptors, with decreasing affinity in the following order: BR3 > TACI > BCMA. In contrast, APRIL binds only BCMA and TACI.

BR3 expression is first observed on IMM B cells in bone marrow, then increases through the TR stages and is highest on FO and MZ B cells ([19] and reviewed in [20]). Once a B cell exits the BM, its persistence in the preimmune pools depends on its ability to compete for BLyS binding and signaling via BR3 [17,21]. Following antigen encounter, the predominance of the BLyS/BR3 axis in the life of a B cell may begin to shift toward different BLyS family members [22,23]. For example, the short-lived plasma cells that develop within a few days of TLR ligation or early in a T-dependent (TD) response upregulate TACI and may require signaling through this receptor for terminal differentiation [24–26], and signaling through TACI or BR3 can induce class switch recombination [20,27–29]. To date, BCMA expression on B cells has been reported only for GC, memory, and LLPC subsets [20,29]; however, BLyS family receptor expression patterns on highly heterogeneous B cell groups, such as those found in the GC or in the memory compartment, are not yet well-characterized. As discussed elsewhere in this review, APRIL signaling through BCMA likely plays a major role in long-term plasma cell retention and survival in the BM [30–32].

2.3 BLyS integrates selection and homeostasis of the preimmune repertoire

Overwhelming evidence indicates that BLyS controls the size and composition of mature preimmune B cell pools because it is the survival factor for which TR, FO, and MZ B cells compete (reviewed in [33]). Thus, eliminating BLyS through either genetic manipulation or exogenous neutralizing agents yields profound decreases in these pools, although developing BM B cell subsets are spared [34,35]. Conversely, BLyS overexpression or exogenous administration yields dose-dependent increases in TR, FO and MZ B cell numbers [36,37]. These effects are mediated primarily through BR3, since deficiencies or mutations in this receptor yield similarly reduced B cell numbers in mice and humans [38,39]. Moreover, TACI-deficient mice have normal or elevated primary B cell numbers, ruling out a central role for this receptor [40].

Importantly, the efficiency with which B cells garner and process BLyS-mediated survival signals is tied to BCR signaling. This suggests that BCR and BR3 signaling are integrated through downstream signaling crosstalk, although the molecular mechanisms involved in this signaling are complex and subject to debate [19,21,41–44]. Regardless of the exact basis, this crosstalk allows BLyS to control the stringency of TR B cell selection and thus determine the proportion of cells that complete TR development and join the FO or MZ subsets (reviewed in [45]). Thus, under conditions when BLyS availability is elevated, a higher proportion of B cells survive through TR development, and clonotypes that would normally be deleted instead enter the mature FO or MZ subsets [37,46]. The likelihood of successful TR development is increased when more BLyS is present – indeed, increased BLyS signaling through BR3 results in the “rescue” of autoreactive B cells normally lost at the TR checkpoint [46–48]. Thus, the stringency of this peripheral B cell tolerance checkpoint shows “plasticity” [43]. Taken together, these observations suggest that BLyS levels might be purposefully increased or decreased, in order to either relax or tighten the stringency of TR selection, thereby leading to an expanded or restricted mature B cell repertoire.

2.4 Selection and homeostasis in antigen-experienced B cell subsets

In contrast to naïve B cells, less is known about homeostatic controls and selection checkpoints for antigen-experienced B cell subsets. Nonetheless, there is increasing evidence for a continued dual-control nature following antigen encounter, involving both BCR character and competition for extrinsic factors. It is not yet known if any single factor functions to integrate selection and homeostasis in GC, memory B, or LLPC subsets, as is the case for BLyS and preimmune subsets; however, there are tantalizing clues that suggest similarly elegant -- and potentially manipulable -- control mechanisms.

Long-lived subsets are generally thought to be the “outcome” of GC reactions, wherein antibody genes undergo SHM as well as class switch recombination. Both positive and negative selection of B cells occur during a GC reaction, such that cells with improved antigen affinity are selected for, and those with decreased or self-antigen affinity are eliminated. GC “evolution” culminates with relatively few B cell clones that possess very high affinity for antigen [15]. Selection mechanisms appear to involve competition between GC B cells for limiting amounts of antigen as well as for factors such as CD40L and cytokines produced by T cells (reviewed in [49,50]).

Roles played by BLyS family members in this process are under investigation. Both BLyS and BR3 knockouts, as well as a BR3 signaling mutant mouse, can initiate GCs upon antigen challenge; however, GC kinetics in these animals are altered, GC structure is generally smaller, and the antibody response is reduced (reviewed in [51]). Interestingly, SHM is not affected in BR3 knockouts or BLyS-deficient mice [52,53]. In addition, TD immune responses in knockout mice lacking TACI, BCMA, or APRIL are largely normal, albeit with subtle changes in antibody responses or isotype representation. Work with *ex vivo* human B cells indicates that BLyS enhances differentiation of Ig-secreting cells in TD responses, but attenuates differentiation of such cells in TI responses; moreover, access to BLyS may be limiting in GCs [54].

There is increasing evidence in both mice and humans for checkpoints during the GC reaction, as well as during differentiation and maintenance of memory B cells and plasma cells [12,13,55–59]. Dysregulated GC formation and responses, characterized by positive selection of pathogenic specificities, ultimately result in disease in the *sanroque* mouse model of lupus [60]. In this model, competition among B cells for T cell help may be relaxed as the result of an expansion of T follicular helper cells (TFH). Additional mouse models in which T cell help is aberrant also suggest a competition-based GC checkpoint, where B cells compete for extrinsic factors [61,62].

In healthy humans, cells expressing self-reactive specificities that result from SHM are selected against, and therefore present at very low frequency in the IgM⁺ memory B cell subset; moreover, mutational analyses -- including reversion to germline -- indicate that this selection occurs before SHM, suggesting an early GC checkpoint [63]. Another study of self-reactive IgG⁺ memory B cells in healthy people indicates selection after SHM [64]. Work using a tetrameric ds-DNA “mimeotope” to identify and track ds-DNA-specific B cells in human peripheral blood indicates the existence of an antigen naïve-to-experienced checkpoint that may be defective in SLE patients [65]. Moreover, the exclusion of an autoreactive clonotype from early germinal centers is defective in SLE but not RA patients, indicating a disease-specific tolerance defect [66].

Long-lived subsets include memory B cells and long-lived plasma cells. LLPC are originally generated through the GC reactions of primary immune responses, but also when memory B cells expressing high-affinity antibody undergo a second antigen encounter. LLPC are so named because they may survive for the lifetime of the animal [67]. This subset occupies a

homeostatic niche that is independent of the much larger / more numerous naïve compartment [35,68]. Despite apparent redundancy in LLPC survival cytokines, recent studies indicate a major role for APRIL in LLPC persistence in the bone marrow [69–71]. Chemokine and homing interactions also contribute to LLPC maintenance, both in BM and extramedullary sites [72–75]. Thus, mechanisms affording a degree of plasticity in the size and composition of the LLPC subset seem likely. The current model is based on competition for survival factor(s) between recently-generated and previously-generated plasma cells, as well as selective processes, such that memory for pathogens (antigens) encountered in an animal's distant past is retained / maintained to some degree [72].

Recent work has shown unexpected phenotypic heterogeneity among memory B cells in both mice and humans, with diversity in surface markers as well as BCR/antibody isotype, and including cells that have not undergone somatic hypermutation [76–79]. This suggests different origins, functions, and/or homeostatic niches for various memory B subsets. Little is known about maintenance of the memory B compartment; however, it is clear that memory B cells are long-lived, occupy several anatomical locations, and can differentiate into antibody-secreting plasma cells upon secondary antigen encounter – all suggesting homeostatic mechanisms that are likely integrated for maintenance of memory B and LLPC compartments. Neutralization studies indicate that neither BLyS nor APRIL is required for memory B cell survival or function [68]; however, unswitched memory cells are sensitive to BLyS depletion, while IgG-bearing memory cells are not [35]. Moreover, the proapoptotic BH3-only Bcl-2 family member Puma has recently been shown to regulate memory B cell survival in both mice and humans [80]. These studies raise the intriguing possibility that distinct memory B subsets have distinct survival requisites -- potentially affording targets for repertoire adjustment.

3. Resolve, Revise, and Relax: The 3 Rs of repertoire adjustment

A key feature of all known peripheral checkpoints is that, in addition to depending on BCR signaling and function, *competition* for extrinsic differentiation and survival signals plays an equal role. The plasticity of selection implied by competition affords an opportunity to purposefully manipulate B cell selection, in either direction, to suit a desired outcome [81]. As detailed above, the best understood point of competitive repertoire selection is at the TR checkpoint, where BLyS is the limiting survival factor. Accordingly, this checkpoint may afford a proving ground for repertoire adjustment through the manipulation of selection.

Three general categories of intentional repertoire adjustment can be envisioned and, in fact, are already at varying levels of realization. The notion of reducing available BLyS levels has recently been implemented clinically in SLE (reviewed in [82]), and would be expected to increase the stringency of TR selection. Such an approach might be viewed as “resolving” otherwise aberrant or failed negative selection, with obvious applicability to humoral autoimmunity; however, it is also more broadly applicable to resolving perturbed selection during reconstitution following B ablative therapies in general. The second potential manipulation might be to remodel the repertoire around neo-self antigens. This notion of “revising” the repertoire might be implemented when long term tolerance to recently introduced molecular components would be advantageous, such as during tissue transplantation or gene therapy. Finally, and clearly the least developed concept in terms of implementation, temporarily increasing BLyS levels could broaden the available repertoire by “relaxing” the stringency of TR selection. This may afford an approach for intentionally expanding the B cell repertoire in order to discover or elicit otherwise rare or counterselected specificities.

3.1. Resolving selection

By far the most advanced approaches embracing the idea of modifying B cell repertoire selection involve humoral autoimmunity – spurred in part by the success of B cell depletion therapy (BCDT). Indeed, therapies targeting B cells for ablation in humoral autoimmune syndromes such as RA, SLE, and Sjögren's syndrome have been increasingly adopted over the last decade. For example, Rituximab (anti-CD20), originally developed and approved for the treatment of non-Hodgkin's lymphoma and used to treat other B cell cancers, was subsequently approved for treatment of RA [83,84], and has been used in “off-label” settings for other autoimmune disorders [85–88]. While initially conceived as a straightforward elimination of neoplastic or autoreactive clonotypes, a challenge to the overall success of B cell ablative therapies is to not only obtain a “clean slate,” free of offending neoplastic or autoreactive cells, but to subsequently rebuild a mature B cell pool that has undergone appropriate selection. Indeed, we and others have suggested the temporary but deliberate reduction of BLyS levels as an approach for increasing the stringency of TR selection, thereby “rectifying” inappropriate selection at the TR checkpoint (reviewed in [89]).

Several lines of evidence have suggested that this might be a feasible and efficacious approach. Elevated BLyS levels that correlate with autoantibody production and other aspects of disease activity are observed in SLE and RA (as well as mouse models of autoimmune disease). Moreover, there is evidence that peripheral tolerance is defective in these diseases. For example, frequencies of self-reactive Ab-producing cells are equal between the TR and FO stages in SLE and RA patients, whereas in healthy humans, this frequency decreases by half between the TR and mature stages [90,91]. Furthermore, repertoire analyses indicate that the TR checkpoint is defective in both of these diseases, leading to increased representation of autoreactive specificities in the mature naïve pool [91–94]. Experimental manipulation of BLyS levels in mice shows that elevated BLyS causes increased TR B cell throughput as well as relaxed TR selection, indicated by significantly increased representation of autoreactive or otherwise rare specificities in mature naïve subsets [46,95]. Conversely, long-term BLyS neutralization in NOD mice not only restores negative selection at the TR checkpoint, but also results in reduced insulin autoantibody levels, arrested islet cell destruction, and delayed disease onset [96].

Resolving selection at the TR checkpoint might thus be expected to yield a primary B cell compartment with significantly reduced representation of potentially or frankly pathogenic clonotypes. Clinical trial results with Rituximab and belimumab have been informative in this regard. Rituximab (anti-CD20) treatment ablates mature naïve B cells, and leads to a predominance of TR B cells during a prolonged autoreconstitution period [83,97,98]. However, BLyS levels transiently increase 2- to 3-fold for up to 4 months following rituximab treatment, raising the possibility that selection at the TR checkpoint may be temporarily relaxed [99,100]. Treatment with anti-BLyS might be expected to circumvent this possibility. Belimumab (Benlysta), a human anti-BLyS monoclonal antibody that was recently approved by the FDA for treatment of SLE, ablates mature naïve B cell subsets [82]. Although the mechanism for the therapeutic efficacy of belimumab remains unknown [101], it is tempting to speculate that it acts by reducing serum BLyS levels, thereby ablating primary B cells and simultaneously increasing the stringency of TR selection, thus reducing the likelihood that autoreactive specificities transit to mature naïve subsets during reconstitution [81,102].

Neither rituximab nor belimumab appears to have significant or lasting effects on autoantibodies. Memory B cells may remain depleted for a much longer time than primary B cells following rituximab treatment, and this correlates with clinical efficacy in some cases; nevertheless, autoantibody titers generally show only modest reduction (49, 62–67). Belimumab treatment largely spares some memory B subsets as well as plasma cells, and

also has limited effects on autoantibodies (81). Sequence analysis of a heavy-chain immunoglobulin family (VH4) in patients with active RA showed increased mutation frequency and ratio of amino acid replacement:silent mutations during early autoreconstitution following rituximab [97]. The most highly mutated cells were derived from antigen-experienced, class-switched memory and plasmablast compartments, suggesting that antigen-experienced B cells (re)generated following rituximab treatment may represent a distinct subset resulting from affinity maturation and/or GC selection [97]. Thus, although both rituximab and belimumab may have transient effects on the size and composition of long-lived subsets, pathogenic specificities existing prior to treatment or generated after treatment may be less tractable. It would be of particular interest to determine which of the various memory B subsets is most affected by each agent, since in mice, IgM-bearing memory cells were more sensitive to anti-BLyS treatment compared to IgG-bearing memory [35].

The notion of resolving selection may extend to B ablative approaches in general, rather than only in the context of autoimmunity. By definition, B ablative therapies yield varying levels of B lymphopenia. This paucity of BLyS “consumers” generally results in elevated BLyS levels - as happens in some SLE patients following B cell depletion. Accordingly, as individuals treated with B ablative regimes undergo self-reconstitution, these high BLyS levels may lead to a lack of appropriate selective stringency at the TR checkpoint, increasing the risk of admitting autoreactive or polyreactive clonotypes into the primary pools, or a lack of appropriate germinal center selection upon antigen challenges faced during the reconstitution process [81]. Using titrated doses of anti-BLyS to maintain selective stringency during autoreconstitution of B cells following B cell depletion therapy done for any purpose (e.g., autoimmune therapy, cancer therapy) may prove to be an important tactic in the strategy of repertoire adjustment.

3.2. Revising selection to tolerate neo-self antigens

A growing number of therapeutic interventions introduce structures that act as antigens, either transiently or chronically. Perhaps foremost among these are alloantigens introduced by tissue and organ transplantation. The presence or induction of alloantibodies is increasingly recognized as a major contributing factor to acute and chronic rejection, and clearly play direct roles in sensitization [103,104]. Similarly, gene and cell therapeutics involve long-term introduction of structural gene products, as well as the presence of delivery vectors [105,106] that may elicit alloantibody formation.

While less well developed than the concept of rectifying the B cell repertoire in autoimmunity, the notion of repertoire “revision” around neo-self antigens to afford long-term tolerance has been proposed in recent years [81,107,108]. Lending support to this idea were observations and results implicating B cells as key players in allograft rejection (reviewed in [109–111]). Several strategies have been tested for achieving B cell tolerance in adult transplant patients, and most of these involve B cell depletion following transplantation (reviewed in [108,112,113]). However, despite long-term graft survival and function, many patients ultimately generate donor-specific antibodies or antibody-mediated transplant rejection, indicating a failure of B cell tolerance. Analogous to BCDT in cancer and autoimmunity, while these regimes provide a setting where new antigens might be introduced with impunity, successful subsequent reconstitution will require repertoire selection under conditions sufficiently stringent to prevent the maturation of clones reactive to the neo-self antigens introduced. If this can be accomplished without excess BLyS -- for example using titrated doses of a BLyS neutralizing agent -- then transitional selection stringency can be rigorous as B cells reconstitute, and neo-self specificities should be deleted [108].

Hence the proposed approach: deplete B cells immediately before or at transplantation, then use titrated doses of anti-BLyS to maintain the stringency of TR selection during B cell reconstitution. There is evidence from an alloantigen-specific Ig transgenic mouse model that “permanent” B cell tolerance can be achieved when the primary repertoire is rebuilt in the presence of a tissue graft, with apparently complete clonal deletion of alloreactive specificities from the primary repertoire [114]. However, these results were obtained in a globally immune cell depleted context (*scid* mice). Results currently in press for mice illustrate the potential effectiveness of B cell depletion at transplant followed by periodic, gradually decreasing doses of anti-BLyS [115]. In this work, fully allogeneic transplanted pancreatic islets were maintained for more than 6 months after cessation of anti-BLyS treatment; moreover, subsequent new transplants of the same MHC haplotype were accepted and maintained. Thus, revising the B cell repertoire in the presence of allograft transplants shows promise as an approach for achieving long-term B cell tolerance.

3.3. Relaxing selection to capture rare specificities

A final and comparatively unexplored notion is that under some circumstances, temporarily relaxing the stringency of selection might prove informative or beneficial. While a seemingly dangerous assault on the basic notion of *horror autotoxicus* [116,117], the recruitment of polyreactive and self-reactive specificities may prove useful in some scenarios, such as autologous tumor vaccination. Indeed, healthy people may have a range of autoantibodies and self-reactive B cells in both immature and mature pools [90,91,118,119]. Moreover, a weak immune response to tumor-associated antigens -- which may be recognized as self-antigens -- has presented one roadblock in cancer vaccine development [120], and it has been suggested that current data support a beneficial effect of variation in both T and B cell repertoires (via epitope spreading) in cancer vaccines [121].

Similarly, deliberately relaxing the stringency of selection might reveal key specificities for vaccine development that are normally lost during selective processes [81]. A relevant contemporary example of this possibility is the difficulty in generating vaccines capable of eliciting broadly neutralizing antibodies (bNAbs) to human immunodeficiency virus (HIV). bNAbs are extremely rare and may appear only after years of chronic infection [122,123]. Moreover, attempts to elicit bNAbs through conventional approaches have been largely unsuccessful to date [124–126]. However, recent observations suggest that antibodies with the desired characteristics may be those that are more likely to be lost during selective processes. For example, extensive SHM appears to be a hallmark of naturally occurring bNAbs, suggesting a GC origin [127,128] as well as the potential for elimination by negative selection. Further, B cell clonotypes that generate polyspecific bNAbs, including those with known autoreactivity, may be strongly counterselected [129,130]. In transgenic mouse models, such clonotypes are subjected to central and peripheral selection mechanisms, and immature cells can be “rescued” by culture with IL-7 and BLyS [130,131]. In contrast, there is evidence that polyspecific antibodies found in humans are positively selected if they include high-affinity pathogen specificity [132]. During the response to HIV, naïve and memory B cells can produce polyreactive antibodies that recognize two sites on the HIV surface; moreover, mutations that increase affinity also increase both breadth and neutralizing potency [133,134].

Thus, broadly neutralizing clonotypes may be rare because they are under-represented in the mature naïve B cell pool; because they mutate in the “wrong direction” (toward self-reactivity or polyreactivity with decreased affinity for HIV) and are therefore eliminated during GC reactions; because of attrition from long-lived subsets; or for a combination of all of these reasons. Under conditions involving naturally or artificially elevated BLyS levels, TR selection stringency should be relaxed, leading to a generally broadened naïve B cell repertoire. This in turn may result in increased representation of potentially broadly

neutralizing clones in FO and MZ subsets. Manipulation of selection checkpoints in the GC and beyond, with BLYS or APRIL, or other limiting survival factors, should also afford the possibility of enhancing recruitment and retention of broadly neutralizing B cell clonotypes. Recent evidence indeed suggests enhanced neutralizing antibody titers using APRIL containing viral vaccine constructs, as well as in mice treated with BLYS prior to vaccination [135,136].

Whether regimes designed to relax selection temporarily could be implemented safely remains unclear. Nonetheless, this approach might be used immediately in experimental animals to discover and characterize rare, therapeutically useful B cell clonotypes, as well as to reveal the basis for their relative paucity under normal conditions.

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Abbreviations

Ab	antibody
BCMA	B cell maturation antigen
BCDT	B cell depletion therapy
BCR	B cell receptor
BLYS	B lymphocyte stimulator
BM	bone marrow
bNAbs	broadly neutralizing antibody
BR3	BLYS receptor 3
FO	follicular
GC	germinal center
HIV	human immunodeficiency virus
Ig	immunoglobulin
IMM	immature LLPC long-lived plasma cell
MZ	marginal zone
MHC	major histocompatibility complex
RA	rheumatoid arthritis
SHM	somatic hypermutation
SLE	systemic lupus erythematosus
TACI	transmembrane activator and calcium-modulator and cyclophilin ligand interactor
TD	T-dependent
TFH	T follicular helper cell
TI	T-independent

TLR	Toll-like receptor
TNF	tumor necrosis factor
TR	transitional

References

- [1]. Nemazee D, Weigert M. Revising B cell receptors. *J Exp Med*. 2000; 191:1813–1817. [PubMed: 10839798]
- [2]. Goodnow CC, Cyster JG, Hartley SB, Bell SE, Cooke MP, Healy JJ, Akkaraju S, Rathmell JC, Pogue SL, Shokat KP. Self-tolerance checkpoints in B lymphocyte Development. *Advances in Immunology*. 1995; 59:279–368. [PubMed: 7484462]
- [3]. Nossal GJ. Negative selection of lymphocytes. *Cell*. 1994; 76:229–239. [PubMed: 8293461]
- [4]. Goodnow CC, Adelstein S, Basten A. The need for central and peripheral tolerance in the B cell repertoire. *Science*. 1990; 248:1373–1379. [PubMed: 2356469]
- [5]. Nossal GJV. Cellular mechanisms of immunological tolerance. *Ann Rev Immunol*. 1983; 1:33–62. [PubMed: 6399977]
- [6]. Osmond D. Proliferation kinetics and the lifespan of B cells in central and peripheral lymphoid organs. *Curr Opin Immunol*. 1991; 3:179–185. [PubMed: 2069745]
- [7]. Allman DM, Ferguson SE, Lentz VM, Cancro MP. Peripheral B cell maturation. II. Heat-stable antigen(hi) splenic B cells are an immature developmental intermediate in the production of long-lived marrow-derived B cells. *J Immunol*. 1993; 151:4431–4444. [PubMed: 8409411]
- [8]. Rolink AG, Andersson J, Melchers F. Characterization of immature B cells by a novel monoclonal antibody, by turnover and by mitogen reactivity. *Eur J Immunol*. 1998; 28:3738–3748. [PubMed: 9842916]
- [9]. Allman DM, Ferguson SE, Cancro MP. Peripheral B cell maturation. I. Immature peripheral B cells in adults are heat-stable antigenhi and exhibit unique signaling characteristics. *J Immunol*. 1992; 149:2533–2540. [PubMed: 1383316]
- [10]. Carsetti R, Kohler G, Lamers MC. Transitional B cells are the target of negative selection in the B cell compartment. *J Exp Med*. 1995; 181:2129–2140. [PubMed: 7760002]
- [11]. Klinman NR. The “clonal selection hypothesis” and current concepts of B cell tolerance. *Immunity*. 1996; 5:189–195. [PubMed: 8808674]
- [12]. Vuyyuru R, Mohan C, Manser T, Rahman ZS. The lupus susceptibility locus Sle1 breaches peripheral B cell tolerance at the antibody-forming cell and germinal center checkpoints. *J Immunol*. 2009; 183:5716–5727. [PubMed: 19828626]
- [13]. Rahman ZS, Alabyev B, Manser T. FcγRIIB regulates autoreactive primary antibody-forming cell, but not germinal center B cell, activity. *J Immunol*. 2007; 178:897–907. [PubMed: 17202351]
- [14]. Goodnow CC, Vinuesa CG, Randall KL, Mackay F, Brink R. Control systems and decision making for antibody production. *Nat Immunol*. 2010; 11:681–688. [PubMed: 20644574]
- [15]. MacLennan IC. Germinal centers. *Annu Rev Immunol*. 1994; 12:117–139. [PubMed: 8011279]
- [16]. Mackay F, Silveira PA, Brink R. B cells and the BAFF/APRIL axis: fast-forward on autoimmunity and signaling. *Curr Opin Immunol*. 2007; 19:327–336. [PubMed: 17433868]
- [17]. Miller JP, Stadanlick JE, Cancro MP. Space, selection, and surveillance: setting boundaries with BLyS. *J Immunol*. 2006; 176:6405–6410. [PubMed: 16709796]
- [18]. Cancro MP. The BLyS family of ligands and receptors: an archetype for niche-specific homeostatic regulation. *Immunol Rev*. 2004; 202:237–249. [PubMed: 15546397]
- [19]. Stadanlick JE, Kaileh M, Karnell FG, Scholz JL, Miller JP, Quinn WJ 3rd, Brezski RJ, Trembl LS, Jordan KA, Monroe JG, Sen R, Cancro MP. Tonic B cell antigen receptor signals supply an NF-κappaB substrate for prosurvival BLyS signaling. *Nat Immunol*. 2008; 9:1379–1387. [PubMed: 18978795]

- [20]. Trembl JF, Hao Y, Stadanlick JE, Cancro MP. The BLyS family: toward a molecular understanding of B cell homeostasis. *Cell Biochem Biophys*. 2009; 53:1–16. [PubMed: 19034695]
- [21]. Cancro MP. Signalling crosstalk in B cells: managing worth and need. *Nat Rev Immunol*. 2009; 9:657–661. [PubMed: 19704418]
- [22]. Trembl LS, Crowley JE, Cancro MP. BLyS receptor signatures resolve homeostatically independent compartments among naive and antigen-experienced B cells. *Semin Immunol*. 2006; 18:297–304. [PubMed: 16919470]
- [23]. Crowley JE, Trembl LS, Stadanlick JE, Carpenter E, Cancro MP. Homeostatic niche specification among naive and activated B cells: a growing role for the BLyS family of receptors and ligands. *Semin Immunol*. 2005; 17:193–199. [PubMed: 15826824]
- [24]. Ozcan E, Garibyan L, Lee JJ, Bram RJ, Lam KP, Geha RS. Transmembrane activator, calcium modulator, and cyclophilin ligand interactor drives plasma cell differentiation in LPS-activated B cells. *J Allergy Clin Immunol*. 2009; 123:1277–1286. e1275. [PubMed: 19427685]
- [25]. Mantchev GT, Cortesao CS, Rebrovich M, Cascalho M, Bram RJ. TACI is required for efficient plasma cell differentiation in response to T-independent type 2 antigens. *J Immunol*. 2007; 179:2282–2288. [PubMed: 17675489]
- [26]. Trembl LS, Carlesso G, Hoek KL, Stadanlick JE, Kambayashi T, Bram RJ, Cancro MP, Khan WN. TLR stimulation modifies BLyS receptor expression in follicular and marginal zone B cells. *J Immunol*. 2007; 178:7531–7539. [PubMed: 17548587]
- [27]. He B, Santamaria R, Xu W, Cols M, Chen K, Puga I, Shan M, Xiong H, Bussell JB, Chiu A, Puel A, Reichenbach J, Marodi L, Doffinger R, Vasconcelos J, Issekutz A, Krause J, Davies G, Li X, Grimbacher B, Plebani A, Meffre E, Picard C, Cunningham-Rundles C, Casanova JL, Cerutti A. The transmembrane activator TACI triggers immunoglobulin class switching by activating B cells through the adaptor MyD88. *Nat Immunol*. 2010; 11:836–845. [PubMed: 20676093]
- [28]. Castigli E, Wilson SA, Scott S, Dedeoglu F, Xu S, Lam KP, Bram RJ, Jabara H, Geha RS. TACI and BAFF-R mediate isotype switching in B cells. *J Exp Med*. 2005; 201:35–39. [PubMed: 15630136]
- [29]. Darce JR, Arendt BK, Wu X, Jelinek DF. Regulated expression of BAFF-binding receptors during human B cell differentiation. *J Immunol*. 2007; 179:7276–7286. [PubMed: 18025170]
- [30]. Mackay F, Schneider P. TACI, an enigmatic BAFF/APRIL receptor, with new unappreciated biochemical and biological properties. *Cytokine Growth Factor Rev*. 2008; 19:263–276. [PubMed: 18514565]
- [31]. Salzer U, Jennings S, Grimbacher B. To switch or not to switch--the opposing roles of TACI in terminal B cell differentiation. *Eur J Immunol*. 2007; 37:17–20. [PubMed: 17171762]
- [32]. Schneider P. The role of APRIL and BAFF in lymphocyte activation. *Curr Opin Immunol*. 2005; 17:282–289. [PubMed: 15886118]
- [33]. Cancro MP, Smith SH. Peripheral B cell selection and homeostasis. *Immunol Res*. 2003; 27:141–148. [PubMed: 12857963]
- [34]. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, Frew E, Scott ML. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science*. 2001; 293:2111–2114. [PubMed: 11509691]
- [35]. Scholz JL, Crowley JE, Tomayko MM, Steinel N, O'Neill PJ, Quinn WJ 3rd, Goenka R, Miller JP, Cho YH, Long V, Ward C, Migone TS, Shlomchik MJ, Cancro MP. BLyS inhibition eliminates primary B cells but leaves natural and acquired humoral immunity intact. *Proc Natl Acad Sci U S A*. 2008; 105:15517–15522. [PubMed: 18832171]
- [36]. Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J, Browning JL, Mackay F. BAFF mediates survival of peripheral immature B lymphocytes. *J Exp Med*. 2000; 192:1453–1466. [PubMed: 11085747]
- [37]. Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, Brink R. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity*. 2004; 20:785–798. [PubMed: 15189742]

- [38]. Lentz VM, Cancro MP, Nashold FE, Hayes CE. Bclm governs recruitment of new B cells into the stable peripheral B cell pool in the A/WySnJ mouse. *J Immunol.* 1996; 157:598–606. [PubMed: 8752907]
- [39]. Warnatz K, Salzer U, Rizzi M, Fischer B, Gutenberger S, Bohm J, Kienzler AK, Pan-Hammarstrom Q, Hammarstrom L, Rakhmanov M, Schlesier M, Grimbacher B, Peter HH, Eibel H. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proc Natl Acad Sci U S A.* 2009; 106:13945–13950. [PubMed: 19666484]
- [40]. Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T, Tumas D, Grewal IS, Dixit VM. Activation and accumulation of B cells in TACI-deficient mice. *Nat Immunol.* 2001; 2:638–643. [PubMed: 11429549]
- [41]. Sasaki Y, Casola S, Kutok JL, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and -independent roles for BAFF in B cell physiology. *J Immunol.* 2004; 173:2245–2252. [PubMed: 15294936]
- [42]. Otipoby KL, Sasaki Y, Schmidt-Supprian M, Patke A, Gareus R, Pasparakis M, Tarakhovsky A, Rajewsky K. BAFF activates Akt and Erk through BAFF-R in an IKK1-dependent manner in primary mouse B cells. *Proc Natl Acad Sci U S A.* 2008; 105:12435–12438. [PubMed: 18713867]
- [43]. Stadanlick JE, Cancro MP. BAFF and the plasticity of peripheral B cell tolerance. *Curr Opin Immunol.* 2008; 20:158–161. [PubMed: 18456486]
- [44]. Srinivasan L, Sasaki Y, Calado DP, Zhang B, Paik JH, DePinho RA, Kutok JL, Kearney JF, Otipoby KL, Rajewsky K. PI3 kinase signals BCR-dependent mature B cell survival. *Cell.* 2009; 139:573–586. [PubMed: 19879843]
- [45]. Cancro MP. Peripheral B-cell maturation: the intersection of selection and homeostasis. *Immunol Rev.* 2004; 197:89–101. [PubMed: 14962189]
- [46]. Hondowicz BD, Alexander ST, Quinn WJ 3rd, Pagan AJ, Metzgar MH, Cancro MP, Erikson J. The role of BLyS/BLyS receptors in anti-chromatin B cell regulation. *Int Immunol.* 2007; 19:465–475. [PubMed: 17369193]
- [47]. Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu HB, Cyster JG. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. *Immunity.* 2004; 20:441–453. [PubMed: 15084273]
- [48]. Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, Brink R. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone microenvironments. *Immunity.* 2004; 20 in press.
- [49]. Vinuesa CG, Linterman MA, Goodnow CC, Randall KL. T cells and follicular dendritic cells in germinal center B-cell formation and selection. *Immunol Rev.* 2010; 237:72–89. [PubMed: 20727030]
- [50]. Vinuesa CG, Sanz I, Cook MC. Dysregulation of germinal centres in autoimmune disease. *Nat Rev Immunol.* 2009; 9:845–857. [PubMed: 19935804]
- [51]. Kalled SL. Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production. *Semin Immunol.* 2006; 18:290–296. [PubMed: 16931038]
- [52]. Vora KA, Wang LC, Rao SP, Liu ZY, Majeau GR, Cutler AH, Hochman PS, Scott ML, Kalled SL. Cutting edge: germinal centers formed in the absence of B cell-activating factor belonging to the TNF family exhibit impaired maturation and function. *J Immunol.* 2003; 171:547–551. [PubMed: 12847217]
- [53]. Rahman ZS, Rao SP, Kalled SL, Manser T. Normal induction but attenuated progression of germinal center responses in BAFF and BAFF-R signaling-deficient mice. *J Exp Med.* 2003; 198:1157–1169. [PubMed: 14557413]
- [54]. Darce JR, Arendt BK, Chang SK, Jelinek DF. Divergent effects of BAFF on human memory B cell differentiation into Ig-secreting cells. *J Immunol.* 2007; 178:5612–5622. [PubMed: 17442944]
- [55]. Shokat KM, Goodnow CC. Antigen-induced B-cell death and elimination during germinal-centre immune responses. *Nature (Lond).* 1995; 375:334–338. [PubMed: 7753200]

- [56]. Pulendran B, Kannourakis G, Nouri S, Smith KGC, Nossal GJV. Soluble antigen can cause enhanced apoptosis of germinal-centre B cells. *Nature*. 1995; 375:331–334. [PubMed: 7753199]
- [57]. Alabyev B, Rahman ZS, Manser T. Quantitatively reduced participation of anti-nuclear antigen B cells that down-regulate B cell receptor during primary development in the germinal center/memory B cell response to foreign antigen. *J Immunol*. 2007; 178:5623–5634. [PubMed: 17442945]
- [58]. Fearon DT, Carroll MC. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu Rev Immunol*. 2000; 18:393–422. [PubMed: 10837064]
- [59]. Han S, Zheng B, Dal Porto J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. IV. Affinity-dependent, antigen-driven B cell apoptosis in germinal centers as a mechanism for maintaining self-tolerance. *J Exp Med*. 1995; 182:1635–1644. [PubMed: 7500008]
- [60]. Linterman MA, Rigby RJ, Wong RK, Yu D, Brink R, Cannons JL, Schwartzberg PL, Cook MC, Walters GD, Vinuesa CG. Follicular helper T cells are required for systemic autoimmunity. *J Exp Med*. 2009; 206:561–576. [PubMed: 19221396]
- [61]. Luzina IG, Atamas SP, Storrer CE, daSilva LC, Kelsoe G, Papadimitriou JC, Handwerker BS. Spontaneous formation of germinal centers in autoimmune mice. *J Leukoc Biol*. 2001; 70:578–584. [PubMed: 11590194]
- [62]. Munthe LA, Corthay A, Os A, Zangani M, Bogen B. Systemic autoimmune disease caused by autoreactive B cells that receive chronic help from Ig V region-specific T cells. *J Immunol*. 2005; 175:2391–2400. [PubMed: 16081810]
- [63]. Tsuiji M, Yurasov S, Velinzon K, Thomas S, Nussenzweig MC, Wardemann H. A checkpoint for autoreactivity in human IgM+ memory B cell development. *J Exp Med*. 2006; 203:393–400. [PubMed: 16446381]
- [64]. Tiller T, Tsuiji M, Yurasov S, Velinzon K, Nussenzweig MC, Wardemann H. Autoreactivity in human IgG+ memory B cells. *Immunity*. 2007; 26:205–213. [PubMed: 17306569]
- [65]. Jacobi AM, Zhang J, Mackay M, Aranow C, Diamond B. Phenotypic characterization of autoreactive B cells--checkpoints of B cell tolerance in patients with systemic lupus erythematosus. *PLoS One*. 2009; 4:e5776. [PubMed: 19488401]
- [66]. Cappione A 3rd, Anolik JH, Pugh-Bernard A, Barnard J, Dutcher P, Silverman G, Sanz I. Germinal center exclusion of autoreactive B cells is defective in human systemic lupus erythematosus. *J Clin Invest*. 2005; 115:3205–3216. [PubMed: 16211091]
- [67]. Manz RA, Thiel A, Radbruch A. Lifetime of plasma cells in the bone marrow. *Nature*. 1997; 388:133–134. [PubMed: 9217150]
- [68]. Benson MJ, Dillon SR, Castigli E, Geha RS, Xu S, Lam KP, Noelle RJ. Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. *J Immunol*. 2008; 180:3655–3659. [PubMed: 18322170]
- [69]. Belnoue E, Pihlgren M, McGaha TL, Tougne C, Rochat AF, Bossen C, Schneider P, Huard B, Lambert PH, Siegrist CA. APRIL is critical for plasmablast survival in the bone marrow and poorly expressed by early-life bone marrow stromal cells. *Blood*. 2008; 111:2755–2764. [PubMed: 18180376]
- [70]. Chu VT, Berek C. Immunization induces activation of bone marrow eosinophils required for plasma cell survival. *Eur J Immunol*. 2012; 42:130–137. [PubMed: 22057654]
- [71]. Chu VT, Frohlich A, Steinhäuser G, Scheel T, Roch T, Fillatreau S, Lee JJ, Lohning M, Berek C. Eosinophils are required for the maintenance of plasma cells in the bone marrow. *Nat Immunol*. 2011; 12:151–159. [PubMed: 21217761]
- [72]. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, Hiepe F. Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat Rev Immunol*. 2006; 6:741–750. [PubMed: 16977339]
- [73]. Moser K, Muehlinghaus G, Manz R, Mei H, Voigt C, Yoshida T, Dorner T, Hiepe F, Radbruch A. Long-lived plasma cells in immunity and immunopathology. *Immunol Lett*. 2006; 103:83–85. [PubMed: 16280169]
- [74]. Muehlinghaus G, Cigliano L, Huehn S, Peddinghaus A, Leyendeckers H, Hauser AE, Hiepe F, Radbruch A, Arce S, Manz RA. Regulation of CXCR3 and CXCR4 expression during terminal

- differentiation of memory B cells into plasma cells. *Blood*. 2005; 105:3965–3971. [PubMed: 15687242]
- [75]. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. *Immunity*. 1998; 8:363–372. [PubMed: 9529153]
- [76]. Anderson SM, Tomayko MM, Ahuja A, Haberman AM, Shlomchik MJ. New markers for murine memory B cells that define mutated and unmutated subsets. *J Exp Med*. 2007; 204:2103–2114. [PubMed: 17698588]
- [77]. Tomayko MM, Steinel NC, Anderson SM, Shlomchik MJ. Cutting edge: Hierarchy of maturity of murine memory B cell subsets. *J Immunol*. 2010; 185:7146–7150. [PubMed: 21078902]
- [78]. Berkowska MA, Driessen GJ, Bikos V, Grosserichter-Wagener C, Stamatopoulos K, Cerutti A, He B, Biermann K, Lange JF, van der Burg M, van Dongen JJ, van Zelm MC. Human memory B cells originate from three distinct germinal center-dependent and - independent maturation pathways. *Blood*. 2011; 118:2150–2158. [PubMed: 21690558]
- [79]. Wu YC, Kipling D, Leong HS, Martin V, Ademokun AA, Dunn-Walters DK. High-throughput immunoglobulin repertoire analysis distinguishes between human IgM memory and switched memory B-cell populations. *Blood*. 2010; 116:1070–1078. [PubMed: 20457872]
- [80]. Clybourn C, Fischer S, Auffredou MT, Hugues P, Alexia C, Bouillet P, Raphael M, Leca G, Strasser A, Tarlinton DM, Vazquez A. Regulation of memory B-cell survival by the BH3-only protein Puma. *Blood*. 2011; 118:4120–4128. [PubMed: 21868573]
- [81]. Trembl LS, Quinn WJ 3rd, Trembl JF, Scholz JL, Cancro MP. Manipulating B cell homeostasis: a key component in the advancement of targeted strategies. *Arch Immunol Ther Exp (Warsz)*. 2008; 56:153–164. [PubMed: 18512030]
- [82]. Stohl W, Scholz JL, Cancro MP. Targeting BlyS in rheumatic disease: the sometimes-bumpy road from bench to bedside. *Curr Opin Rheumatol*. 2011; 23:305–310. [PubMed: 21330926]
- [83]. Anolik JH, Friedberg JW, Zheng B, Barnard J, Owen T, Cushing E, Kelly J, Milner EC, Fisher RI, Sanz I. B cell reconstitution after rituximab treatment of lymphoma recapitulates B cell ontogeny. *Clin Immunol*. 2007; 122:139–145. [PubMed: 17008130]
- [84]. Looney RJ, Anolik J, Sanz I. A perspective on B-cell-targeting therapy for SLE. *Mod Rheumatol*. 20:1–10. [PubMed: 19669389]
- [85]. Calero I, Sanz I. Targeting B cells for the treatment of SLE: the beginning of the end or the end of the beginning? *Discov Med*. 2010; 10:416–424. [PubMed: 21122473]
- [86]. Ramos-Casals M, Garcia-Hernandez FJ, de Ramon E, Callejas JL, Martinez-Berriotxo A, Pallares L, Caminal-Montero L, Selva-O'Callaghan A, Oristrell J, Hidalgo C, Perez-Alvarez R, Mico ML, Medrano F, Gomez de la Torre R, Diaz-Lagares C, Camps M, Ortego N, Sanchez-Roman J. Off-label use of rituximab in 196 patients with severe, refractory systemic autoimmune diseases. *Clin Exp Rheumatol*. 2010; 28:468–476. [PubMed: 20525449]
- [87]. Van Allen EM, Miyake T, Gunn N, Behler CM, Kohlwe J. Off-label use of rituximab in a multipayer insurance system. *J Oncol Pract*. 2011; 7:76–79. [PubMed: 21731512]
- [88]. Murray E, Perry M. Off-label use of rituximab in systemic lupus erythematosus: a systematic review. *Clin Rheumatol*. 2010; 29:707–716. [PubMed: 20155295]
- [89]. Liu Z, Davidson A. BAFF inhibition: a new class of drugs for the treatment of autoimmunity. *Exp Cell Res*. 2011; 317:1270–1277. [PubMed: 21333645]
- [90]. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. *Science*. 2003; 301:1374–1377. [PubMed: 12920303]
- [91]. Wardemann H, Nussenzweig MC. B-cell self-tolerance in humans. *Adv Immunol*. 2007; 95:83–110. [PubMed: 17869611]
- [92]. Yurasov S, Wardemann H, Hammersen J, Tsuiji M, Meffre E, Pascual V, Nussenzweig MC. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J Exp Med*. 2005; 201:703–711. [PubMed: 15738055]
- [93]. Samuels J, Ng YS, Coupillaud C, Paget D, Meffre E. Impaired early B cell tolerance in patients with rheumatoid arthritis. *J Exp Med*. 2005; 201:1659–1667. [PubMed: 15897279]

- [94]. Yurasov S, Tiller T, Tsuiji M, Velinzon K, Pascual V, Wardemann H, Nussenzweig MC. Persistent expression of autoantibodies in SLE patients in remission. *J Exp Med.* 2006; 203:2255–2261. [PubMed: 16966430]
- [95]. Quinn WJ 3rd, Noorchashm N, Crowley JE, Reed AJ, Noorchashm H, Naji A, Cancro MP. Cutting edge: impaired transitional B cell production and selection in the nonobese diabetic mouse. *J Immunol.* 2006; 176:7159–7164. [PubMed: 16751358]
- [96]. Zekavat G, Rostami SY, Badkerhanian A, Parsons RF, Koeberlein B, Yu M, Ward CD, Migone TS, Yu L, Eisenbarth GS, Cancro MP, Naji A, Noorchashm H. In vivo BLYS/BAFF neutralization ameliorates islet-directed autoimmunity in nonobese diabetic mice. *J Immunol.* 2008; 181:8133–8144. [PubMed: 19018006]
- [97]. Palanichamy A, Roll P, Theiss R, Dorner T, Tony HP. Modulation of molecular imprints in the antigen-experienced B cell repertoire by rituximab. *Arthritis Rheum.* 2008; 58:3665–3674. [PubMed: 19035487]
- [98]. Roll P, Palanichamy A, Kneitz C, Dorner T, Tony HP. Regeneration of B cell subsets after transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. *Arthritis Rheum.* 2006; 54:2377–2386. [PubMed: 16869000]
- [99]. Cambridge G, Stohl W, Leandro MJ, Migone TS, Hilbert DM, Edwards JC. Circulating levels of B lymphocyte stimulator in patients with rheumatoid arthritis following rituximab treatment: relationships with B cell depletion, circulating antibodies, and clinical relapse. *Arthritis Rheum.* 2006; 54:723–732. [PubMed: 16508933]
- [100]. Cambridge G, Isenberg DA, Edwards JC, Leandro MJ, Migone TS, Teodorescu M, Stohl W. B cell depletion therapy in systemic lupus erythematosus: relationships among serum B lymphocyte stimulator levels, autoantibody profile and clinical response. *Ann Rheum Dis.* 2008; 67:1011–1016. [PubMed: 17962238]
- [101]. Liu Z, Davidson A. BAFF and selection of autoreactive B cells. *Trends Immunol.* 2011; 32:388–394. [PubMed: 21752714]
- [102]. Scholz J, Lunink Prak E, Cancro M. Targeting the BLYS family in autoimmunity: a tale of mouse and man. *Clinical Investigation.* 2011; 1:951–967.
- [103]. Susal C, Morath C. Current approaches to the management of highly sensitized kidney transplant patients. *Tissue Antigens.* 2011; 77:177–186. [PubMed: 21299521]
- [104]. Akalin E, Pascual M. Sensitization after kidney transplantation. *Clin J Am Soc Nephrol.* 2006; 1:433–440. [PubMed: 17699242]
105. Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, Forman SJ. Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biol Blood Marrow Transplant.* 2010; 16:1245–1256. [PubMed: 20304086]
106. Marcus A, Waks T, Eshhar Z. Redirected tumor-specific allogeneic T cells for universal treatment of cancer. *Blood.* 2011; 118:975–983. [PubMed: 21653325]
107. Parsons RF, Redfield RR 3rd, Rodriguez E, Mustafa MM, Vivek K, Murayama M, Naji A, Noorchashm H. Primary B cell repertoire remodeling to achieve humoral transplantation tolerance. *Semin Immunol.* 2011
108. Parsons RF, Vivek K, Redfield RR, Migone TS, Cancro MP, Naji A, Noorchashm H. B-cell tolerance in transplantation: is repertoire remodeling the answer? *Expert Rev Clin Immunol.* 2009; 5:703. [PubMed: 20161663]
109. Vongwiwatana A, Tasanarong A, Hidalgo LG, Halloran PF. The role of B cells and alloantibody in the host response to human organ allografts. *Immunol Rev.* 2003; 196:197–218. [PubMed: 14617206]
110. Colvin RB, Smith RN. Antibody-mediated organ-allograft rejection. *Nat Rev Immunol.* 2005; 5:807–817. [PubMed: 16175181]
- [111]. Pescovitz MD. B cells: a rational target in alloantibody-mediated solid organ transplantation rejection. *Clin Transplant.* 2006; 20:48–54. [PubMed: 16556153]
- [112]. Vivek K, Mustafa MM, Rodriguez E, Redfield RR 3rd, Parsons RF, Rostami S, Migone TS, Cancro MP, Naji A, Noorchashm H. Strategies for B-lymphocyte repertoire remodeling in transplantation tolerance. *Immunol Res.* 2011; 51:1–4. [PubMed: 21948256]

- [113]. Parsons RF, Vivek K, Redfield RR 3rd, Migone TS, Cancro MP, Naji A, Noorchashm H. B-lymphocyte homeostasis and BLyS-directed immunotherapy in transplantation. *Transplant Rev (Orlando)*. 2010; 24:207–221. [PubMed: 20655723]
- [114]. Parsons RF, Vivek K, Rostami SY, Zekavat G, Ziaie SM, Luo Y, Koeberlein B, Redfield RR, Cancro MP, Naji A, Noorchashm H. Acquisition of humoral transplantation tolerance upon de novo emergence of B lymphocytes. *J Immunol*. 2011; 186:614–620. [PubMed: 21084661]
- [115]. Parsons RF, Ming Y, Vivek K, Zekavat G, Rostami S, Ziaie S, Luo Y, Koeberlein B, Redfield RR 3rd, Ward C, Migone T-S, M.P. C, Naji A, Noorchashm H. Murine Islet Allograft Tolerance Upon Blockade of the B-Lymphocyte Stimulator, BLyS/BAFF. *Transplantation*. 2012 in press.
- [116]. Silverstein AM. Autoimmunity versus horror autotoxicus: the struggle for recognition. *Nat Immunol*. 2001; 2:279–281. [PubMed: 11276193]
- [117]. Mackay IR. Travels and travails of autoimmunity: a historical journey from discovery to rediscovery. *Autoimmun Rev*. 2010; 9:A251–258. [PubMed: 19883799]
- [118]. Meffre E, Schaefer A, Wardemann H, Wilson P, Davis E, Nussenzweig MC. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J Exp Med*. 2004; 199:145–150. [PubMed: 14699083]
- [119]. Jennette JC, Falk RJ. The rise and fall of horror autotoxicus and forbidden clones. *Kidney Int*. 2010; 78:533–535. [PubMed: 20805814]
- [120]. Vergati M, Intrivici C, Huen NY, Schlom J, Tsang KY. Strategies for cancer vaccine development. *J Biomed Biotechnol*. 2010; 2010
- [121]. Sioud M. Does our current understanding of immune tolerance, autoimmunity, and immunosuppressive mechanisms facilitate the design of efficient cancer vaccines? *Scand J Immunol*. 2009; 70:516–525. [PubMed: 19906192]
- [122]. Montefiori DC, Mascola JR. Neutralizing antibodies against HIV-1: can we elicit them with vaccines and how much do we need? *Curr Opin HIV AIDS*. 2009; 4:347–351. [PubMed: 20048696]
- [123]. Verkoczy L, Kelsoe G, Moody MA, Haynes BF. Role of immune mechanisms in induction of HIV-1 broadly neutralizing antibodies. *Curr Opin Immunol*. 2011; 23:383–390. [PubMed: 21524897]
- [124]. Hessel AJ, Haigwood NL. Neutralizing Antibodies and Control of HIV: Moves and Countermoves. *Curr HIV/AIDS Rep*. 2011
- [125]. Gamble LJ, Matthews QL. Current progress in the development of a prophylactic vaccine for HIV-1. *Drug Des Devel Ther*. 2010; 5:9–26.
- [126]. Wijesundara DK, Jackson RJ, Ramshaw IA, Ransinghe C. Human immunodeficiency virus-1 vaccine design: where do we go now? *Immunol Cell Biol*. 2011; 89:367–374. [PubMed: 20956986]
- [127]. Scheid JF, Mouquet H, Ueberheide B, Diskin R, Klein F, Oliveira TY, Pietzsch J, Fenyo D, Abadir A, Velinzon K, Hurley A, Myung S, Boulad F, Poignard P, Burton DR, Pereyra F, Ho DD, Walker BD, Seaman MS, Bjorkman PJ, Chait BT, Nussenzweig MC. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science*. 2011; 333:1633–1637. [PubMed: 21764753]
- [128]. Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A, Kwon YD, Scheid JF, Shi W, Xu L, Yang Y, Zhu J, Nussenzweig MC, Sodroski J, Shapiro L, Nabel GJ, Mascola JR, Kwong PD. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. *Science*. 2010; 329:811–817. [PubMed: 20616231]
- [129]. Haynes BF, Moody MA, Verkoczy L, Kelsoe G, Alam SM. Antibody polyspecificity and neutralization of HIV-1: a hypothesis. *Hum Antibodies*. 2005; 14:59–67. [PubMed: 16720975]
- [130]. Verkoczy L, Diaz M, Holl TM, Ouyang YB, Bouton-Verville H, Alam SM, Liao HX, Kelsoe G, Haynes BF. Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. *Proc Natl Acad Sci U S A*. 2010; 107:181–186. [PubMed: 20018688]
- [131]. Verkoczy L, Chen Y, Bouton-Verville H, Zhang J, Diaz M, Hutchinson J, Ouyang YB, Alam SM, Holl TM, Hwang KK, Kelsoe G, Haynes BF. Rescue of HIV-1 broad neutralizing antibody-

- expressing B cells in 2F5 VH × VL knockin mice reveals multiple tolerance controls. *J Immunol.* 2011; 187:3785–3797. [PubMed: 21908739]
- [132]. Mouquet H, Nussenzweig MC. Polyreactive antibodies in adaptive immune responses to viruses. *Cell Mol Life Sci.* 2011
- [133]. Mouquet H, Scheid JF, Zoller MJ, Krogsgaard M, Ott RG, Shukair S, Artyomov MN, Pietzsch J, Connors M, Pereyra F, Walker BD, Ho DD, Wilson PC, Seaman MS, Eisen HN, Chakraborty AK, Hope TJ, Ravetch JV, Wardemann H, Nussenzweig MC. Polyreactivity increases the apparent affinity of anti-HIV antibodies by heterologation. *Nature.* 2010; 467:591–595. [PubMed: 20882016]
- [134]. Mouquet H, Warncke M, Scheid JF, Seaman MS, Nussenzweig MC. Enhanced HIV-1 neutralization by antibody heterologation. *Proc Natl Acad Sci U S A.* 2012
- [135]. Melchers M, Bontjer I, Tong T, Chung NP, Klasse PJ, Eggink D, Montefiori DC, Gentile M, Cerutti A, Olson WC, Berkhout B, Binley JM, Moore JP, Sanders RW. Targeting HIV-1 envelope glycoprotein trimers to B cells using APRIL improves antibody responses. *J Virol.* 2011
- [136]. Kanagavelu SK, Snarsky V, Termini JM, Gupta S, Barzee S, Wright JA, Khan WN, Kornbluth RS, Stone GW. Soluble multi-trimeric TNF superfamily ligand adjuvants enhance immune responses to a HIV-1 Gag DNA vaccine. *Vaccine.* 2011