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"Control of autoreactive B cells by IgM and IgD B cell receptors: maintaining a fine balance"

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Abstract

A substantial fraction of mature naïve B cells recognize endogenous antigens, and this autoreactivity must be controlled to prevent autoantibody secretion. Selective downregulation of the IgM BCR on autoreactive B cells has long been appreciated, and recent findings illustrate how this might impose tolerance. The BCR isotype maintained on autoreactive B cells, IgD, is less sensitive to endogenous antigens than IgM. This reduced sensitivity may be conferred by structural properties of IgD and/or differential association with activating and inhibitory co-receptors. Once activated, autoreactive B cells are normally excluded from rapid plasma cell responses, but they can enter the germinal center and lose their autoreactivity through a mutation-selection process termed clonal redemption.

Introduction

Control of autoreactivity within the B cell repertoire is essential to prevent autoantibody secretion and development of autoimmune disease. Early in development, B cells rearrange their immunoglobulin heavy and light chain genes through V(D)J recombination and express the resulting B cell receptor (BCR) on their surface. A substantial fraction of newly rearranged BCRs are reactive towards self-antigens, and many of these BCRs are removed from the repertoire at early stages of development [1]. This process, termed "central tolerance," (well covered in a recent review [2]) proceeds predominantly via rearrangement of new light chains ("receptor editing") until BCR self-reactivity is censored. If that process fails to eliminate self-reactivity, B cells undergo apoptosis ("deletion"). However, central tolerance is incomplete, and about one fifth of mature B cells in healthy humans harbor reactivity towards nuclear antigens [1]. Moreover, expression of a reporter of BCR signaling, Nur77-eGFP, suggests that the majority of mature B cells in mice recognize endogenous antigens to varying degrees [3]. This review explores recent insights into how mature B cells

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are maintained in a quiescent state in the face of chronic autoantigen recognition, and how their autoreactivity is managed during responses to foreign antigens (Figure 1).

IgM downregulation is a common feature of autoreactive B cells

BCR transgenic (Tg) mouse models have been indispensable in uncovering mechanisms of peripheral B cell tolerance. The most well-studied of these models utilizes a BCR Tg (IgHEL) that binds with high affinity ($K_a = 2 \times 10^9\ M^{-1}$) to its cognate ligand, hen egg lysozyme (HEL) [4,5]. When IgHEL B cells develop in the presence of abundant soluble HEL, they adopt an “anergic” phenotype characterized by functional unresponsiveness and massive surface IgM BCR downregulation, but they express normal levels of IgD. Analogous IgM downregulation is observed in multiple autoreactive BCR Tg models spanning a range of antigen affinities [6]; Ars/A1 B cells are Ars-hapten specific but downregulate surface IgM and adopt an anergic phenotype due to crossreactivity with an unknown endogenous antigen (possibly ssDNA) at low affinity ($K_a < 2.5 \times 10^5\ M^{-1}$) [7]. The large antigen affinity difference between these models suggests that their shared features, such as selective IgM downregulation, are physiologically relevant mechanisms of peripheral B cell tolerance. By contrast, the specific molecular mechanisms that impose anergy, and the intensity with which they do so, differ markedly; while anergic IgHEL and Ars/A1 B cells both display dampened signaling independent of IgM downregulation, the anergic phenotype is rapidly reversed in Ars/A1 cells by treatment with monovalent Ars/Tyr, but anergy persists in IgHEL B cells days after removal of antigen [8,9].

Selective downregulation of IgM, but not IgD, is a feature of autoreactive B cells in humans and mice with diverse, unmanipulated B cell repertoires [3,10–13]. In naive mice harboring a fluorescent reporter of BCR signaling, Nur77-eGFP, B cells exhibit a broad range of reporter expression that correlates with selective IgM downregulation [3]. We showed that endogenous antigen recognition is required for GFP expression, and GFP^{hi} B cells are enriched for nuclearreactive specificities [3]. Although autoreactive GFP^{hi} B cells exhibit dampened signaling in response to IgM ligation, this is attributable entirely to IgM downregulation, and signal transduction downstream of IgD is unperturbed. So, rewiring of BCR signaling does not appear to be a prominent feature of unmanipulated B cell repertoires (at least in mice), perhaps because highly autoreactive clones compete poorly for a limiting supply of the survival factor BAFF and are eliminated from the mature repertoire [14–16]. Therefore, dampened BCR signaling in naturally-occurring, mildly autoreactive B cells may be achieved primarily through IgM downregulation.

IgD senses endogenous antigens more weakly than IgM

IgM and IgD are splice isoforms of the BCR heavy chain that are co-expressed on mature naïve B cells and differ only in their Fc regions. Whether or how IgM downregulation on autoreactive B cells enforces peripheral tolerance has been unclear because such cells retain high expression of the functional IgD BCR. These two BCR isotypes have long been considered redundant because B cell development and immune responses are largely intact in IgM- and IgD-deficient mice [17–19]. Recent work suggests that IgM is uniquely capable of responding to soluble monovalent antigens [20], and IgD is better able to bind to low-density surface antigens than IgM [21]. However, these studies did not directly examine

sensitivity of IgM and IgD to bona fide endogenous antigens, which is particularly relevant given their differential expression on autoreactive B cells.

We hypothesized that IgD is less sensitive to endogenous antigens than IgM and took several orthogonal approaches to test this [22]. Mature B cells expressing IgD alone are less efficient, on a per-receptor basis, at inducing Nur77-eGFP in response to endogenous antigens than cells expressing IgM alone, and this defect is especially profound in the marginal zone (MZ) and B1a compartments. This defect occurs despite robust signaling through IgD following anti-light chain cross-linking *in vitro*. Development of MZ and B1a populations is highly sensitive to BCR signal strength; weak BCR signals are permissive for and favor MZ development, while B1a development requires stronger BCR signals [23,24]. In competition with IgM-expressing B cells, IgD-only B cells have a selective advantage in the MZ compartment, but are strongly disfavored in the B1a compartment [22]. These observations suggest that IgD is less efficient than IgM at sensing bona fide endogenous antigens *in vivo,* but do not establish the properties of IgD accounting for this. Recent studies point towards two potentially overlapping mechanisms (Figure 2): physical properties of BCR-antigen interactions and selective association of IgM and IgD with co-receptors.

Physical properties of BCR-antigen interactions shape signaling by IgM and IgD

In contrast to T cells, which only recognize processed peptides presented on MHC, B cells sense antigens in their native state. Antigens can vary not only in affinity but also in valency and context (soluble or membrane-bound), and requirements for productive signaling differ between antigen forms. Notably, BCR signaling in response to all types of antigens requires Syk kinase, but responses to monvalent antigens uniquely require Src family kinases (SFKs) [25–27]. IgD has a longer and more flexible hinge than IgM [28], and this renders IgD unresponsive to monovalent model antigens while maintaining responsiveness towards multivalent antigens [20]. However, the degree of this unresponsiveness is contested; a subsequent study showed that IgD can respond to soluble monovalent antigen *in vitro* and *in vivo* [29]. It is possible that the phenomenology lies between these two extremes or exists on a continuum between high valency and low valency antigens.

Bona fide endogenous antigens are not restricted to soluble monomers and also encompass cell-surface antigens. 5–10% of naïve B cells in healthy patients express the VH4–34 heavy chain and recognize I/i blood group antigens on erythrocytes as well as B220 on B cells [30,31]. The interaction between the IgM and IgD isotypes and surface bound antigens is complex, with IgM displaying stronger functional avidity for surface-bound antigens at high epitope density and IgD binding more strongly at low epitope density [21]. Thus, structural properties of the BCR could explain reduced endogenous antigen sensing by IgD if relevant antigens are soluble and monomeric, or surface-bound at high density. Since it is thought that conformational changes are important for the initiation of BCR signaling, the relative abilities of different types of antigens to induce such conformational changes in IgM and IgD warrant further investigation [32].

In order to initiate productive signaling and discriminate between high and low affinity surface-bound antigens, B cells spread and then contract to bring BCR-antigen complexes

into a central cluster [33]. If an insufficient number of BCRs are occupied, signaling is aborted [33]. B cell spreading requires PKCβ-mediated activation of FAK, and loss of FAK renders B cells unable to discriminate between stiff and soft antigen-containing surfaces [34]. As PKCβ is activated via the canonical BCR signaling cascade [35], productive signaling may require a feed-forward loop where the BCR encounters a surface-bound antigen, initiates a low level of signaling, which enables spreading and additional BCR occupancy. Whether the flexible hinge of IgD disfavors this initial round of signaling is an intriguing possibility warranting further investigation.

BCR isotype clustering and receptor co-localization

The clustering of BCRs and co-receptors on the surface of resting B cells has fostered an intense debate about how these clusters are organized, whether antigen binding alters cluster size, and how this affects BCR signaling [36–40]. IgD clusters are denser and/or larger than IgM clusters [38,40], which may make them more difficult to dissociate or bring in close proximity to co-receptors upon antigen stimulation. Mobile BCR clusters move closer to immobilized CD19 upon antigen encounter [38], and CD19 is more closely associated with IgM on activated B cells, despite being more closely associated with IgD on resting cells [39]. Ligation of the CD19 co-receptor triggers activation of the PI3K pathway, which plays a central role in the survival function of the BCR [41,42]. Preferential association of IgD with CD19 on resting B cells may partially account for the poor survival of IgD-deficient B cells [17,18,29]. By contrast, preferential association of CD19 with IgM on activated B cells might enhance the ability of IgM to sense endogenous antigens.

ITIM-dependent inhibitory signaling, mediated by the SFK Lyn, is required to restrain inappropriate activation of autoreactive B cells in both BCR transgenic models [43–45] and in mice with polyclonal BCR repertoires [46,47]. Inducible deletion of either of the effector phosphatases (SHP-1 and SHIP1) downstream of ITIM-containing receptors triggers activation, proliferation, and differentiation of mature autoreactive B cells into plasma cells [45]. This demonstrates that restraint of autoreactive B cells in the face of chronic autoantigen stimulation requires continuous inhibitory tone. Multiple ITIM-containing receptors maintain inhibitory tone on B cells *in vivo*, but each may do so in response to distinct types of antigens, such as sialic acids (CD22) [48] immune complexes (FcγRIIB) [49], and Sm/RNP (CD72) [50].

It is tempting to speculate that inhibitory tone is differentially applied to the IgM and IgD BCRs, either to explain the reduced responsiveness of IgD to endogenous antigens or because the structure of IgM necessitates additional inhibitory tone. Indeed, we recently showed that IgD-only, but not IgM-only, B cells were protected from differentiating into short-lived plasma cells and autoantibody-secreting plasma cells in the absence of Lyn [22]. As CD22 must diffuse rapidly to surveil abundant BCR molecules on the B cell surface, it is possible that CD22 can more easily access IgM clusters, which are less dense than IgD clusters [38,51]. The relative ability of other co-receptors to restrain and enhance IgM and IgD signaling, and the functional consequences thereof, is of great interest and remains largely unexplored. It was recently reported that IgD is absolutely required for signaling through the chemokine receptor CXCR4, though this finding may be most relevant in

immature and activated populations that lack IgD expression, as opposed to mature B cells with high levels of IgD [52].

Fates of autoreactive B cells upon stimulation with foreign antigen

If IgM is sensitive to endogenous antigens and its expression is tuned in proportion to autoreactivity, why is IgD expressed at all? After all, it has recently been shown that IgD-deficient human B cells display normal homeostasis and immune responsiveness in a small family of $IgD^{+/-}$ patients (in contrast to impaired survival of IgD-deficient B cells in mice) [17,18,29,53]. One possibility is that IgD provides survival signals essential to retain autoreactive B cell specificities with low surface IgM expression in the mature repertoire. This has been demonstrated for the HEL/sHEL model but has not been extended to the natural B cell repertoire [29]. This further raises the question of why it might be advantageous to the host to retain mildly autoreactive B cells in the mature B cell repertoire instead of deleting them.

Mounting circumstantial evidence suggests that autoreactive B cells may provide a reservoir of protective specificities to defend against pathogens. Indeed, a well-characterized subset of broadly-neutralizing anti-HIV antibodies (bNAbs) harbor self-reactive specificities [54], and transgenic B cells expressing both mutated and germline versions of bNAb antibodies trigger central tolerance mechanisms early in development [55,56]. bNAb Tg B cells that make it into the periphery are controlled by peripheral tolerance mechanisms, and exhibit anergic signaling as well as downregulation of IgM or both IgM and IgD [56–59]. It has been speculated that some pathogens, including HIV, evade immune detection by exploiting “holes” in the B cell repertoire that are created by B cell tolerance mechanisms. Indeed, breaching peripheral B cell tolerance may actually facilitate generation of bNAbs [60]. The autoreactive VH4–34 BCR is expanded in the memory B cell compartment of malaria patients, and VH4–34-derived antibodies rise during acute malarial infection (though the extent of their protective capacity is unclear) [61]. It has recently been shown that VH4–34-derived antibodies can bind commensal bacteria while retaining reactivity to erythrocytes [62]. These data collectively suggest that self-reactivity may be protective, but it remains to be determined whether the autoreactive IgD^{hi} IgM^{lo} BCR repertoire is preferentially enriched in the memory B cell or plasma cell compartments in humans or in mice after various infectious challenges. It is also not known whether mice or humans lacking IgD entirely would express a less autoreactive B cell repertoire and exhibit enhanced susceptibility to infection.

Once self-reactive B cells are recruited into humoral responses, how is frank autoimmunity restrained? We recently demonstrated that cells activated through IgD are normally excluded from rapid plasma cell responses, likely disfavoring direct autoantibody generation by IgD^{hi} IgM^{lo} B cells [22]. Circulating short-lived plasmablast responses in flaring lupus patients derive, in part, directly from naïve self-reactive B cells, suggesting that dysregulation of this ‘checkpoint’ may play an important role in systemic autoimmunity [63]. By contrast, IgD is sufficient to mediate germinal center responses, and may facilitate entry of anergic B cells into the GC in response to immunization [19,22,29]. How are self-reactive BCRs managed in the GC? B cells can be deleted if exposed to abundant soluble antigens in the germinal

center [64,65], and acquisition of novel autoreactivity in the GC is strongly selected against [66]. Furthermore, anergic IgHEL B cells are subjected to strong selection pressure to lose self-reactivity in the GC and can do so via a mutation-dependent process termed “clonal redemption” [67,68].

Unexpectedly, this early pressure to lose self-reactivity may, in specific contexts, set B cells on a faster “trajectory” to enhanced foreign antigen binding than in the absence of this pressure [67]. Indeed, many VH4–34-derived antibodies in humans have acquired mutations that abolish their germline-encoded self-reactivity [68,69]. It is unknown whether or how often this phenomenon occurs in other antigen systems, but clonal redemption may represent an important mechanism by which self-reactive B cells can confer protection against foreign pathogens while minimizing the risk of autoimmunity.

Conclusions and unanswered questions

Many B cells are chronically stimulated by endogenous antigens and maintain quiescence through a combination of ITIM-dependent inhibitory tone and predominant expression of IgD. Upon recruitment into immune responses, they can be shunted away from rapid plasma cell responses and into the GC, where selection favors loss of their autoreactivity. Exactly how IgM and IgD mediate these processes is unclear. Do structural properties of IgM and IgD influence their ability to sense surface-bound antigens, cluster, or localize with co-receptors? Do qualitative or quantitative differences in BCR signaling influence the propensity of autoreactive B cells to adopt the germinal center fate? Furthermore, what signals in the germinal center mediate the redemption process, and are these distinct from those that govern affinity maturation? The answers to these questions will improve our understanding of peripheral B cell tolerance and may inform both treatment of B cell-mediated autoimmune diseases and optimization of immunization strategies.

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Highlights.

- IgM, but not IgD, downregulation is a shared feature of autoreactive B cells
- IgD is less sensitive to endogenous antigens and monovalent antigens than IgM
- IgM and IgD BCRs may differentially associate with B cell co-receptors
- Autoreactive B cells are preferentially shunted into the germinal center (GC)
- Autoreactive B cells may be “redeemed” by somatic hypermutation in the GC

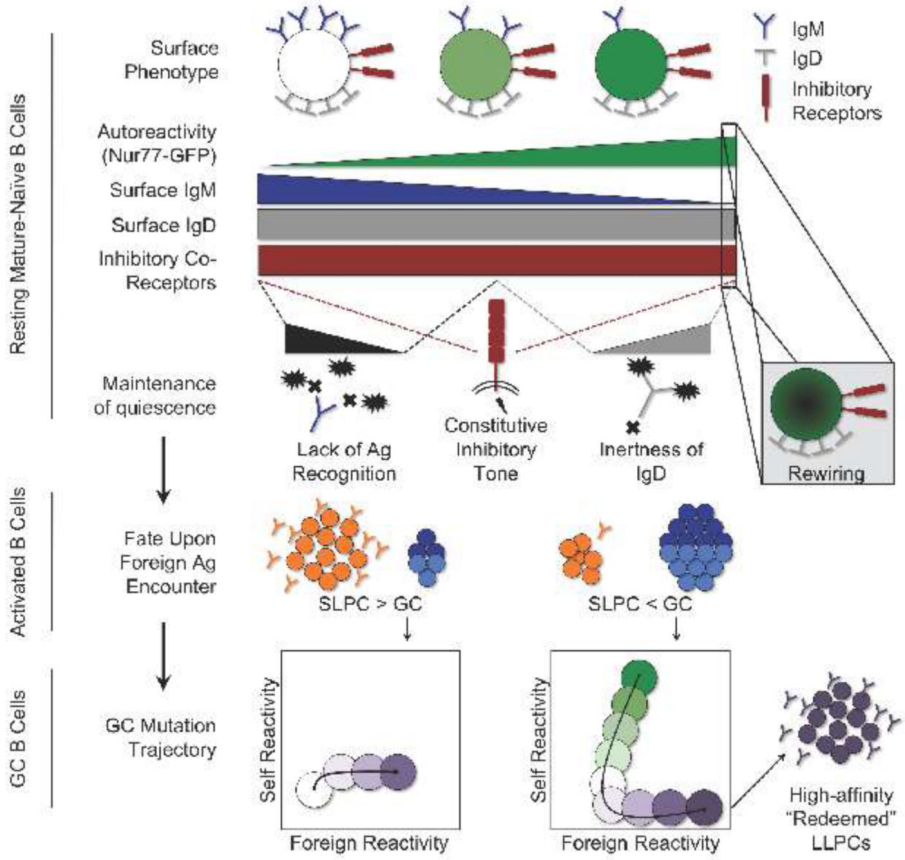

Figure 1. Maintenance of quiescence and fate skewing in polyclonal B cell repertoires

Polyclonal B cell repertoires contain a broad range of autoreactivity, and autoreactivity is associated with selective IgM downregulation. While the most highly autoreactive cells may be tolerized through rewiring of BCR signaling, more mildly autoreactive cells are tolerized by a combination of inhibitory tone and inefficient endogenous antigen sensing by IgD. The least autoreactive cells may be maintained in a quiescent state because they do not recognize endogenous antigens strongly enough to initiate signaling. Activation of cells through IgD disfavors the short-lived plasma cell (SLPC) fate while permitting germinal center (GC) entry. Recent work suggests that autoreactive GC B cells face strong selection pressure to lose autoreactivity before increasing foreign antigen reactivity [67]. This redemption process may allow autoreactive B cells to participate in humoral responses while minimizing autoantibody production.

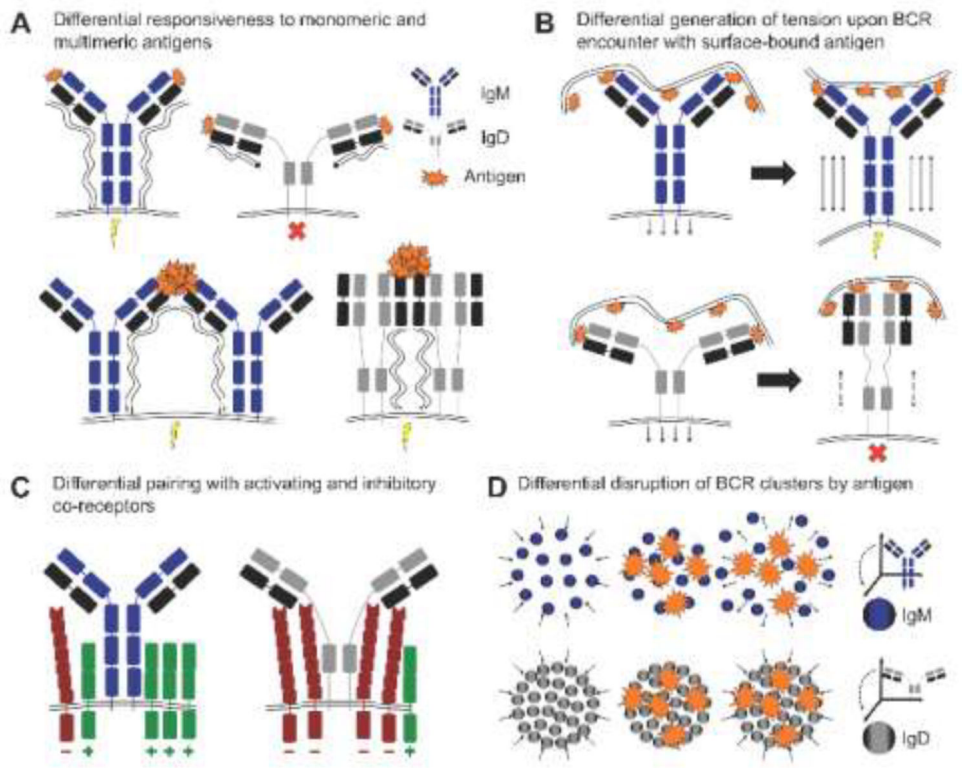

Figure 2. Potential biophysical and biochemical mechanisms for weak *in vivo* antigen sensing by IgD.

(A) The rigid hinge of IgM allows for sensing of monovalent antigens, but IgD may require crosslinking by polyvalent antigens to initiate signaling. (B) The flexible structure of IgD may preclude generation of tension and conformational changes in response to surface-bound antigens. (C) Differential co-localization with activating and inhibitory co-receptors may quantitatively modulate signaling by IgM and IgD. The activating co-receptor CD19 is preferentially associated with IgD on resting cells and with IgM on activated cells [39]. Endogenous antigen encounter may promote re-localization of CD19 away from IgD and towards IgM, or other co-receptors may display differential co-localization on resting cells. (D) BCR molecules exist as clusters on the surface of resting B cells (depicted from above in this diagram), and one model proposes that dissociation of these clusters initiates BCR signaling [37]. Structural properties of IgD or the high density of IgD clusters may make it more difficult for antigens to overcome the forces holding IgD clusters together.