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Regulation of Autoreactive B Cell Responses to Endogenous TLR Ligands

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Abstract

Immune complexes (ICs) containing DNA and RNA are responsible for disease manifestations found in patients with Systemic Lupus Erythematosus (SLE). B cells contribute to SLE pathology through BCR recognition of endogenous DNA- and RNA-associated autoantigens and delivery of these self-constituents to endosomal TLR9 and TLR7, respectively. B cell activation by these pathways leads to production of class-switched DNA- and RNA reactive autoantibodies, contributing to an inflammatory amplification loop characteristic of disease. Intriguingly, self-DNA and RNA are typically non-stimulatory for TLR9/7 due to absence of stimulatory sequences or presence of molecular modifications. Recent evidence from our lab and others suggests that B cell activation by BCR/TLR pathways is tightly regulated by surface-expressed receptors on B cells, and the outcome of activation depends on the balance of stimulatory and inhibitory signals. Either IFN α engagement of the type I IFN receptor, or loss of IgG ligation of the inhibitory Fc γ RIIB receptor promotes B cell activation by weakly-stimulatory DNA and RNA TLR ligands. In this context, autoreactive B cells can respond robustly to common autoantigens. These findings have important implications for the role of B cells *in vivo* in the pathology of SLE.

Keywords

Systemic Lupus Erythematosus; AM14 B cells; TLR9; TLR7; Fc γ RIIB; Type I IFN

The AM14 Transgenic B Cell System: A Useful System to Study B Cell Responses to BCR/TLR Mechanisms

Systemic autoimmune diseases are characterized by a relatively limited autoantibody repertoire despite the multitude of self-constituents that may potentially serve as target autoantigens. Autoantibodies against DNA, chromatin-associated proteins, RNA, and ribonucleoproteins are particularly common in patients suffering from Systemic Lupus Erythematosus (SLE) and also in animal models of this disease. Although the immune system is typically tolerant to self, under certain circumstances these intracellular autoantigens become visible to the immune system and trigger the activation and differentiation of B cells into antibody-secreting plasma blasts and long-lived plasma cells.

In an effort to understand the mechanisms that lead to loss of tolerance, our laboratory has used genetically modified mice that express a B cell receptor reactive with autologous IgG2a. This AM14 allotype-restricted “rheumatoid factor” receptor is representative of a specificity commonly found in the autoantibody repertoire of autoimmune-prone mice [1]

and binds monomeric IgG2a with relatively low affinity. As a result, AM14 B cells are not tolerized during development and in non-autoimmune-prone mice they differentiate to become conventional mature naive B cells. However in autoimmune-prone mice of the appropriate allotype, a significant proportion of these cells convert to antibody-secreting plasma blasts [2,3].

This activation process can be modeled *in vitro* by stimulating AM14 B cells with immune complexes (ICs) that incorporate IgG2a. Our initial studies demonstrated that IgG2a mAbs reactive with DNA or DNA-associated proteins could very effectively induce AM14 B cells to proliferate, while IgG2a mAbs reactive with haptens or proteins such as BSA, could not [4,5]. Activation depended on the ability of these antibodies to bind to DNA-associated cell debris, such as apoptotic bodies, to form spontaneous “chromatin ICs”. Subsequent studies demonstrated that mAbs reactive with RNA or RNA-associated proteins could also activate AM14 B cells, especially if the B cells had been exposed to type I IFNs [6]. Importantly, the AM14 B cell response to nucleic acid-containing ICs relied on coengagement of endosomal members of the TLR family, TLR9 and TLR7 [4,6], pointing to a critical role for innate immune receptors in the activation of autoreactive B cells. TLR9 recognizes CpG-containing DNA [7], while TLR7 recognizes single stranded RNA [8–10]. Protein ICs fail to trigger AM14 B cell proliferation because engagement of the low affinity AM14 BCR alone cannot maintain sufficient BCR signal strength for a sustained response [5]. Nevertheless, the BCR is required for the delivery of the relevant autoantigen to a cytoplasmic compartment that contains TLR9 and/or TLR7 and is also likely to modulate the functional outcome of TLR9/7 engagement [4,6,11] (Figure 1).

Although the majority of our studies have been carried out with AM14 B cells, we believe that the BCR/TLR paradigm established by these experiments has broad applicability to the activation of autoreactive B cells with other specificities. Any B cell that can directly recognize a DNA/RNA-associated autoantigen is likely to utilize a similar BCR/TLR interaction to achieve a signaling threshold. Moreover, although outside the scope of the current review, the activation of various kinds of antigen presenting cells may also depend on TLR7, TLR9, or other pattern recognition receptors, to attain full activation. In many of these cases, an FcγR may be involved in the delivery of the ligand to the appropriate endosomal compartment [12,13]. Interestingly, recent results from our laboratory suggest that additional surface receptors may communicate with BCR and endosomal TLRs [14] and (Avalos *et al*, submitted) and thereby either enhance or repress the autoantigen response.

AM14 B Cells Stimulated with Chromatin IC Activate BCR and TLR9 Signaling Pathways

The signals triggered upon BCR engagement depend on tyrosine (Tyr) phosphorylation of the ITAMs found in the Igα and Igβ chains associated with the BCR. Upon phosphorylation of ITAMs, phosphotyrosine-binding Src homology 2 domain (SH2) containing Src-protein kinases (Src-PK) Syk and Lyn are recruited to the plasma membrane. BCR aggregation induces activation of phosphatidylinositol-3-kinase (PI3K), which mediates phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP₂), yielding phosphatidylinositol -3,4,5-trisphosphate (PIP₃). Pleckstrin homology (PH) domain-containing signaling molecules, such as the Src PK Btk are thus recruited to the plasma membrane. Btk leads to recruitment of PH-containing PLCγ2, which through breakdown of PIP₂ induces de release of inositol-3-phosphate (IP₃) and diacylglycerol (DAG). The release of these two messengers induces calcium mobilization and MAPK phosphorylation, respectively. These events ultimately induce gene transcription activation and B cell proliferation [15].

In the case of chromatin ICs, engagement of the BCR by these complexes is followed by internalization of the receptor and its cargo (chromatin and associated DNA). Then, TLR9 recognizes the DNA present in ICs and B cells undergo activation [4]. In this context, full activation of the BCR signaling cascade is not required [16]. The BCR and TLR9 share two common signaling pathways, namely activation of MAPKs and NF- κ B [17,18]. p38 and JNK are both activated by BCR and TLR9, whereas ERK is only phosphorylated after BCR engagement [18]. To dissect the contribution of the BCR to IC-activation of AM14 B cells, we have used cells that fail to express the TLR7/9 adapter protein, MyD88. Chromatin IC stimulation of AM14 MyD88-deficient B cells leads to an increase in calcium flux and activation of all three members of the MAP kinase family, thereby implicating the BCR in AM14 activation. However, these BCR-triggered events per se are not sufficient for AM14 B cell proliferation [16]. These data suggest that signals from the both receptors are required for a full response of the autoreactive B cells. The BCR may also contribute to the IC response through a phospholipase D-dependent pathway by promoting the redistribution of cytoplasmic TLRs independently of TLR signaling events per se [11]. As a result, TLRs may traffic to optimally engage the BCR-transported ligand. In anergic B cells, the ability of the BCR to promote TLR9 relocalization may be compromised, thereby preventing optimal BCR/TLR9 colocalization. Reversal of the anergic state restored normal trafficking patterns and allowed these autoreactive cells to respond fully to the autoantigen [19]. Thus, B cell responses to dual BCR and TLR9 engagement depend on proper localization and activation of BCR and TLR9 signals, which appear to be governed by early BCR signaling events.

Identification of the Mammalian Ligands for TLR9 Through the Use of Defined dsDNA ICs

TLR9 was originally identified as a receptor for bacterial DNA, that preferentially bound the hypomethylated CpG motifs found in prokaryotic but not eukaryotic DNA [7]. However, as described above, AM14 B cells responses to spontaneous chromatin ICs (IgG2a-bound cell debris) depend on TLR9 detection of mammalian DNA. The undefined nature of the ligand bound by TLR9 in this experimental setting made it difficult to dissect the identity of the mammalian DNA component, and raised the possibility that any DNA would effectively engage TLR9 as long as it reached the correct compartment [20]. Recent studies have even suggested that TLR9 binds the DNA backbone and thus cannot be sequence specific [21]. To better understand the sequence requirements for TLR9-dependent B cell activation by mammalian DNA, we constructed ICs composed of biotinylated dsDNA fragments of defined sequence combined with an IgG2a anti-biotin mAb, 1D4. As anticipated, (in contrast to an anti-DNA mAb), the 1D4 mAb by itself completely failed to activate AM14 B cells. This mAb could therefore be used in combination with a variety of dsDNA fragments to provide a sensitive method for determining whether TLR9 detection of mammalian DNA is sequence specific.

The dsDNA fragments used for these studies were approximately 600 bp long and corresponded to both experimental and endogenous sequences. One fragment, designated CG50, incorporated 50 canonical CpG motifs that recapitulated the optimal murine TLR9 ligand originally identified through a screen of phosphorothioate-stabilized single-stranded CpG oligodeoxynucleotides [22]. We also tested a series of dsDNA fragments derived from a mammalian CpG library [23]; these fragments were relatively CG-rich but incorporated only minimal numbers of CpG motifs. Other fragments, designated CGneg, Sumo, Senp1 were CG-poor fragments more typical of total genomic DNA. Representative sequences of these fragments are illustrated in Figure 2.

Biotinylated fragments were synthesized by incorporation of biotinylated bases as described [24] and then combined with 1D4 to make defined ICs. We then compared the capacity of

the various ICs to induce AM14 B cell proliferation. CG50 ICs were tested first, to verify the utility of the system, and found to induce a remarkably robust response. By contrast, CG-poor ICs induced only minimal responses [5]. Importantly, ICs that incorporated the CG-rich CpG island derived sequences worked almost as well as the CG50 ICs [14]. These studies clearly demonstrated that TLR9 was sequence specific and could be much more effectively engaged by CG-rich DNA than by CG-poor DNA. Moreover, as we could demonstrate that both the CG-rich and CG-poor complexes induced a comparable level of Ca^{2+} flux and therefore engaged the BCR to a comparable extent, these complexes provide a very useful set of experimental tools for comparing strong and weak endogenous TLR9 ligands.

B Cell Responses to BCR and TLR7 Coengagement

Similarly to the BCR/TLR9 paradigm observed when B cells are stimulated by chromatin ICs, AM14 B cells proliferate in response to RNA ICs through a BCR and TLR7-dependent mechanism [6]. The events that follow BCR recognition and delivery to TLR7 are less well understood. At least in B cells, early studies demonstrated that the response to RNA-associated ligands appeared to depend on the addition of type I IFN [6], and this implied a more complex regulation of BCR/TLR7 responses than BCR/TLR9 responses. As in the case of TLR9-dependent responses, we have studied both spontaneous and defined ICs. For example, the IgG2a RNA-reactive mAb BWR4 binds cell debris and the resulting ICs stimulate AM14 B cells through a TLR7-dependent mechanism. Stimulatory ICs can also be formed by combining an SmD-reactive mAb with purified snRNP [6].

Whether BWR4 recognizes a specific RNA sequence is unknown. There is some data to suggest that TLR7 preferentially recognizes uridine rich-sequences [8,9] or RNA species that lack RNA modifications [25]. Extensive base modification is characteristic of mammalian DNA and especially tRNAs. However, many of the modifications found in tRNA are not found in U1 or other short RNAs associated with known autoantigens.

Regulation of BCR and TLR9/7 Responses by Type I IFN

Both SLE patients and murine models of SLE frequently express an IFN signature [26], and IFN α has been shown to promote plasma cell differentiation [27]. In our initial studies on RNA IC activation of AM14 B cells, we found that IFN α dramatically enhanced the proliferative response elicited by both BWR4 and the snRNP ICs [28]. By contrast, type I IFNs appeared to have only a minimal effect on the response to either spontaneous chromatin ICs or to defined dsDNA ICs. We attributed this difference to the ability of IFN α to significantly upregulate the B cell expression level of TLR7 but not TLR9 [29].

Therefore, we were at first surprised to find that type I IFNs had a quite dramatic effect on the AM14 B cell response to CG-poor defined dsDNA ICs such as those that incorporated CGneg, Sumo, or Senp1. As mentioned above, the CG-poor fragments elicit a minimal response by themselves, but in the presence of type I IFNs the response was markedly enhanced [14]. Because IFN α did not cause any change in the B cell response to a complete dose titration of the TLR9 ligand ODN 1826, we assumed that the response to CG-poor fragments did not reflect any substantial change in the level of expression of any of the major TLR9 signaling cascade components.

IFN α treatment has been reported to lead to partial B cell activation. It results in the expression of CD69 [30] but does not change the level of expression of surface IgM [14]. Therefore, the enhanced response to CG-poor dsDNA ICs is most likely due to effects on the BCR signaling cascade that lower the BCR-initiated activation threshold. This is consistent with the observation that pretreatment of B cells with IFN α results in a greater

level of Ca^{2+} flux following suboptimal BCR engagement [14,30]. Just as the response of IFN-primed AM14 B cells to RNA ICs remains TLR7-dependent, the response of AM14 B cells to CG-poor dsDNA ICs remains TLR9-dependent and IFN-primed AM14 B cells do not respond to protein ICs [14].

Regulation of BCR and TLR9/7 Responses by FcγRIIB

FcγRs bind IgGs or IgG ICs and can transduce activating or inhibitory signals [31]. B cells only express the inhibitory receptor, FcγRIIB, which can modulate cell responses emanating from the BCR. FcγRIIB contains an immunoreceptor tyrosine-based inhibitory (ITIM) motif in the cytoplasmic domain. FcγRIIB-BCR co-engagement by ICs results in phosphorylation of the ITIM motif leading to recruitment of the 5'-inositol phosphatase SHIP to the plasma membrane. SHIP in turn affects the phosphorylation status of PIP_3 , and thus the association of PH-containing signaling molecules such as PLCγ2 and Btk. FcγRIIB/BCR coengagement therefore results in inhibition of calcium mobilization, MAPKs activation and ultimately, B cell proliferation [31].

Several lines of evidence support the notion that FcγRIIB deficiencies are associated with predisposition to autoimmune disease in a B cell autonomous manner. In agreement with its suppressive function, mice with a deletion in the FcγRIIB gene develop enhanced humoral and anaphylactic responses to foreign antigens [32]. In addition, deletion of the FcγRIIB gene promotes responses to self-antigens, and on the B6 background FcγRIIB deficiency can lead to overt SLE [33]. Lupus-prone mice such as NZB, BXBS and MRL/lpr contain polymorphisms in the FcγRIIB gene leading to decreased protein expression in germinal center B cells [34]. In addition, SLE patients have been shown to fail to appropriately augment FcγRIIB expression on memory B cells and plasma cells [35–37].

Historically, antigens incorporated into ICs were considered poor B cell ligands because the IC invariable cross linked the BCR and FcγRIIB [38]. However, as discussed above, despite the presence of the inhibitory FcγRIIB, chromatin ICs activate low-affinity AM14 B cells very effectively [5,39], and the dose response curves of AM14 B cells and FcγRIIB^{-/-} AM14 cells to both spontaneous chromatin ICs and CG-rich dsDNA ICs are essentially identical (Avalos *et al.*, submitted). We therefore assumed that the apparent lack of regulation by the FcγRIIB receptor in the context of BCR/TLR9 co-activation simply reflected the inability of FcγRIIB to traffic to the appropriate endosomal compartment where the TLR9 signal ensues, or the fact that FcγRIIB binds IgG2a with relatively low affinity (~20-fold lower than its affinity for IgG2b) and therefore was not effectively activated by our IgG2a ICs [40].

However, just as the effects of IFNα are more apparent when CG-poor defined ICs were used to activate AM14 B cells, we subsequently found that FcγRIIB^{-/-} AM14 B cells, in contrast to FcγRIIB⁺ AM14 B cells, responded quite robustly to ICs containing CG-poor dsDNA fragments. Interestingly, FcγRIIB^{-/-} AM14 B cells also responded to RNA ICs much more vigorously than FcγRIIB⁺ AM14 cells, even in the absence of IFNα priming, indicating that B cell responses to RNA ICs signals are also efficiently regulated by FcγRIIB (Avalos *et al.*, submitted). Thus, in the context of a strong TLR ligand, FcγRIIB was unable to regulate the B cell response, but in the context of a relatively weak TLR ligand, FcγRIIB exhibited profound regulatory activity.

We then went on to re-examine the potential ability of FcγRIIB to regulate responses to CG-rich dsDNA in the context of an IC that incorporated its high avidity ligand, IgG2b. Here we found that even in the presence of a strong TLR9 ligand, FcγRIIB could effectively regulate BCR/TLR9-dependent responses. Thus, whether or not low affinity auto reactive B cells

will respond to their cognate autoantigens depends on the combined strength of signals emanating from BCR, TLR9 (or TLR7), IFNAR and FcγRIIB (Figure 3).

Interplay of TLRs in Murine Lupus Models

Several lines of evidence support a role for endosomal TLRs in autoreactive B cell activation and in autoimmune disease manifestations in mouse models of SLE. For example, AM14 B cells in autoimmune-prone Fas-deficient mice spontaneously proliferate and differentiate at extrafollicular sites to form antibody-producing cells [41]. At these sites, B cells undergo somatic hyper mutation (SHM) and secrete high titers of antibody. On a non-autoimmune prone background, AM14 B cells can be induced to undergo a similar form of seroconversion by injecting these mice with the mAb PL2–3, which presumably then forms chromatin ICs, and is then bound by the AM14 receptor [42]. Even though SHM is normally a T-cell dependent process, extrafollicular SHM of AM14 B cells is a T cell-independent process. However, extrafollicular expansion of AM14 B cells depend on TLR signaling events, as it is not found in TLR7/TLR9 double-deficient mice [43].

TLRs also appear to play a key role in spontaneous autoimmune prone murine models. As mentioned above, B6 FcγRIIB^{-/-} mice spontaneously develop an SLE-like disease. When these mice are bred to mice expressing the Y-linked autoimmune acceleration mutation, Yaa, disease onset occurs more rapidly and these mice exhibit increased mortality, more extensive renal pathology, and higher autoantibody titers than B6 FcγRIIB^{-/-} littermates. Moreover, the specificity of the autoantibody repertoire found in this mouse model changed from predominantly DNA-reactive antibodies found in FcγRIIB^{-/-} mice to include much higher titers of autoantibodies reactive with RNA-associated autoantigens [44]. It is now known that the Yaa phenotype is due to the duplication and translocation to the Y chromosome of an X-chromosomal interval that includes TLR7 [45,46]. Deletion of the endogenous TLR7 gene significantly decreases autoantibody titers and renal disease in the Yaa mice [47,48] although certain aspects of the Yaa phenotype appear to be TLR7 independent and due to duplication of other genes within the translocated interval [49]. Consistent with a major role for TLR7 in SLE pathogenesis, in Fas-deficient MRL/*lpr* mice, TLR7 deficiency leads to a dramatic decrease in autoantibodies reactive with RNA-associated autoantigens (but not chromatin-associated autoantigens) and reduced clinical disease [50]. Similar effects have been seen in a pristane-induce model of SLE [51,52].

TLR3 is also found in endosomal compartments and is known to be activated by viral dsRNA. Although TLR3-deficiency does not appear to have an effect on immune activation or clinical disease in Fas-deficient mice [53], recent studies suggest that TLR3 can be activated by certain mammalian RNAs that form structures with regions of dsRNA [54].

The contribution of TLR9 to SLE is less clear-cut. Surprisingly, TLR9-deficient mice have decreased titers of autoantibodies reactive with DNA-associated autoantigens, as evidenced by decreased homogeneous ANA staining patterns of HEp2 cells [53]. However TLR9-deficient autoimmune-prone mice develop exacerbated clinical disease [50,55,56]. Exactly how TLR9 might promote the development of autoimmune disease in this context is unclear. Possibilities include a role for TLR9 in the production of antibodies involved in the clearance of apoptotic debris as well as in the activation of pathways that might negatively regulate TLR functional activity.

Importantly, autoimmune-prone mice that do not express functional UNC93B, a chaperone protein regulating translocation of TLR7, TLR9 and TLR3 to endolysosomal compartments, develop much less severe disease than their UNC93B-sufficient counterparts [57]. This recent study further supports a major role for endosomal TLRs in systemic autoimmune

disease and shows the TLR7 (TLR3) deficiency overrides the disease promoting activity of TLR9-deficiency.

In vivo and *in vitro* evidence also indicate that TLRs induce class switch recombination, promoting the production of antibody isotypes that avidly engage activating FcγRs. TLR9-deficient B6 FcγRIIB^{-/-} mice exhibit significant decreases in IgG2a and IgG2b antibody titers [58]. Moreover, immunization of mice using microspheres containing antigen and CpG ODN promoted B cell responses and T cell-independent production of antigen-specific IgG2a and IgG2b isotype antibodies [59]. These isotypes bear high affinity for activating FcγRs [40] and have been shown to promote immune cell activation also through complement deposition, in contrast to less pathogenic IgG1 and IgG3 isotypes [60].

Type I IFN Pathways and Autoimmunity

FcγRIIB signaling cascades can also interface with type I IFN pathways. For example, FcγRIIB-deficiency in DCs appears to promote the maturation and the expression of IFN-inducible genes [61]. In B cells, in addition to the effects described above, engagement of IFNAR by IFNα promotes B cell activation [14,30] and plasma cell differentiation [27]. Moreover, IFNα also induces maturation of DCs and presentation of apoptotic antigens to T cells [62]. Surprisingly, IFNAR^{-/-} MRL.*lpr* mice display more severe disease than IFNAR + littermates [63]. However, IFNAR deficiencies were protective in a model of pristane-induced lupus [64] and in the B6.Nba2 and (B6.Nba2xNZW) F1 mice [65]. Therefore, whether type I IFNs strongly promote clinical disease or simply serve as a biomarker of inflammation remains to be determined.

Concluding Remarks

Under normal conditions, cells are simultaneously exposed to a variety of stimuli. Crosstalk between diverse receptors normally facilitates a rapid yet regulated response to exogenous and endogenous environmental cues by adding new amplification loops and checkpoints onto existing networks. However, under certain pathological conditions, the regulatory networks involved in distinction of self from non-self can be perturbed. AM14 B cells have proven to be very useful experimental system for the analysis of the signaling pathways involved in B cell activation by physiologically relevant autoantigens. In the case of BCR/TLR activation of autoreactive B cells, it has been clearly demonstrated that a “healthy” equilibrium can be perturbed by the elevated levels of type I IFNs and/or the loss of FcγRIIB inhibitory activity, thereby highlighting the interplay between BCR, TLR9, IFNAR and FcγRIIB (Figure 3). Further studies will be required to elucidate the specific pathways that connect these receptors.

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Figure 1.

Immune complexes (ICs) incorporating a TLR7 or TLR9 ligand induce AM14 B cell proliferation. IgG2a anti-TNP antibodies in complex with TNP-conjugated BSA are recognized by the AM14 BCR (anti-IgG2a) and internalized, but do not promote AM14 B cell proliferation due to failure to engage TLR7 or 9 (left panel). Spontaneous ICs comprised of IgG2a anti-DNA or anti-chromatin in complex with endogenous DNA or CG-rich DNA fragments promote AM14 B cell proliferation through engagement of TLR9 (middle panel). AM14 B cells proliferate in a TLR7-dependent manner when anti-RNA IgG2a antibodies are complexed with endogenous RNA, in the presence of IFN α (right panel).

[illegible]

Figure 2. CpG-motif and CG-rich containing DNA fragments activate TLR9

The canonical CpG motif recognized by TLR9, PuPuCGPyPy is depicted in turquoise. The strong B cell mitogen 1826, a single stranded phosphorothioate (PS-DNA) oligonucleotide (ODN), contains two repeats of the CpG motif. CG50 is an experimentally designed phosphodiester dsDNA fragment that includes 50 repeats of the canonical CpG motif. Clone 11 is a dsDNA fragment corresponding to an endogenous CpG island that contains two CpG motifs (in turquoise) and 42 CG-repeats (in yellow). SenP1 is a DNA fragment representative of genomic DNA.

Figure 3. The AM14 B cell response to ICs reflects the combined signal strength of the BCR, TLR7/9, IFNAR and FcγRIIB

(a) ICs that incorporate CG-poor DNA weakly engage the BCR (thin red arrow) and TLR9 (thin black arrow) and activation is prevented by weak engagement of FcγRIIB by IgG2a (thin blue arrow). This equilibrium can be disrupted by the addition of type I IFNs (green arrow), or loss of FcγRIIB engagement, resulting in B cell proliferation. (b) ICs that incorporate CG-rich DNA weakly engage the BCR and strongly engage TLR9 (thick black arrow) and activation cannot be prevented by weak engagement of FcγRIIB by IgG2a. (c) ICs that incorporate RNA weakly engage the BCR and TLR7 (thin purple arrow), and activation is prevented by weak engagement of FcγRIIB by IgG2a. This equilibrium can be disrupted by the addition of type I IFNs, or loss of FcγRIIB engagement, resulting in B cell proliferation. (d) ICs that incorporate CG-rich DNA weakly engage the BCR and robustly engage TLR9, and activation is suppressed by strong engagement of FcγRIIB by IgG2b (thick blue arrow). This response is amplified by loss of FcγRIIB engagement.