American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Mini Review

How the Signaling Crosstalk of B Cell Receptor (BCR) and Co-Receptors Regulates Antibody Class Switch Recombination: A New Perspective of Checkpoints of BCR Signaling

Chen Z* and Wang JH*

Department of Immunology and Microbiology, University of Colorado, USA

***Corresponding author:** Chen Z & Wang JH, Department of Immunology and Microbiology, University of Colorado, Anschutz Medical Campus, Aurora, CO 80045, USA.

To Cite This Article: *Chen Z, Wang JH, How the Signaling Crosstalk of B Cell Receptor (BCR) and Co-Receptors Regulates Antibody Class Switch Recombination: A New Perspective of Checkpoints of BCR Signaling. 2020 - 10(4). AJBSR.MS.ID.001531.* DOI: 10.34297/AJBSR.2020.10.001531.

Received: July 24, 2020; **Published:** October 07, 2020

Abstract

Mature B cells express B cell antigen receptor (BCR), toll-like receptors (TLR) and TNF family receptors including CD40 and B-cell activating factor receptor (BAFFR). These receptors transduce cellular signals to govern the physiological and pathological processes in B cells including B cell development and differentiation, survival, proliferation, and antibody-mediated immune responses as well as autoimmune diseases and B cell lymphomagenesis. Effective antibody-mediated immune responses require class switch recombination (CSR), a somatic DNA recombination event occurring at the immunoglobulin heavy chain (Igh) gene locus. Mature B cells initially express IgM as their BCR, and CSR enables the B cells to switch from expressing IgM to expressing different classes of antibodies including IgG, IgA or IgE that exhibit distinct effector functions. Here, we briefly review recent findings about how the signaling crosstalk of the BCR with TLRs, CD40 and BAFFR regulate CSR, antibody-mediate immune responses, and B cell anergy.

Why is Antibody Class Switch Recombination (CSR) Important?

Antibody class switch recombination (CSR) is essential for effective humoral immune responses. Mature naïve B cells express IgM as surface B cell antigen receptor (BCR) or secrete IgM antibodies; however, effector functions of IgM are rather limited [1-3]. CSR enables B cells to produce isotype-switched antibodies, such as IgG and IgA, that can combat infectious pathogens or neutralize toxins more effectively than IgM. Consequently, more than 90% of current vaccines deliver protective effects via eliciting isotype-switched antibodies [4]. On the other hand, defects in CSR lead to primary immunodeficiency diseases (PID) such as Hyper-IgM syndrome. PID patients suffer from recurrent infections with a shorter life expectancy [5-7].

Antibody molecules are composed of variable (V) and constant (C) regions. V regions recognize antigens, while C regions mediate effector functions of antibodies. CSR is a DNA rearrangement process that occurs at the C regions of the immunoglobulin heavy chain (Igh) gene locus. The detailed molecular mechanisms of CSR have been extensively reviewed elsewhere [1,8-10]. Briefly, to initiate CSR, B cells need to express a specific enzyme, activation-induced cytidine deaminase (AID) [11,12]. AID introduces DNA lesions to the evolutionarily conserved switch (S) regions preceding each of C regions; subsequently, AID-induced DNA lesions are converted into DNA breaks at the upstream donor S region and one of the downstream acceptor S regions [13]. DNA breaks at S regions are joined by non-homologous end-joining pathway [14-17] that eventually leads to the switching of the C regions of antibody molecules. Of note, AID can potentially target all transcriptionally active genes and induces genome-wide instability that contributes to B cell lymphomagenesis [18,19]. Thus, AID poses a threat to the B cell genome and its expression has to be tightly regulated. Consequently, AID expression is only induced in activated B cells via integrated signals from the BCR and other co-receptors [1].

Can the BCR Induce CSR in the Absence of Co-Stimulation?

Pathogen infection or antigen immunization activate multiple receptors on B cells including BCR, CD40, toll-like receptors (TLRs), B-cell activating factor receptor (BAFFR) and cytokine receptors (e.g., IL-4R) depending on different antigen characteristics. The prevailing view of CSR induction is that the BCR cannot induce CSR in the absence of co-stimulation, and the co-stimulatory signals are provided in the form of CD40 ligand (CD40L) expressed by activated T cells for T-cell dependent (TD) antigens, or TLR ligands directly expressed by pathogens or present in the adjuvants for T-cell independent (TI) antigens. Given that it is not practically feasible yet to pinpoint which and how individual receptor(s) induce CSR during *in vivo* immunization, *in vitro* CSR models have been established and widely applied to study underlying mechanisms of CSR [9,20]. It is well-known that engaging CD40 can induce CSR in the presence of proper cytokines such as IL-4. TLRs can also induce a low level of CSR in the presence of cytokines and synergize with the BCR to induce a robust level of CSR [21]. In contrast, engaging the BCR cannot induce CSR in the presence of cytokines [21,22]. Since the BCR recognizes antigen and activates multiple signaling pathways, including nuclear factor kappa B (NF-κB) and phosphatidylinositol 3-kinases (PI3K), to initiate antigen-specific humoral immune response, why the BCR in the presence of cytokines cannot induce CSR [1]?

Our recent study has shed light on this decade long puzzle by showing that there are negative regulatory mechanisms restricting the BCR's ability to induce CSR [23]. Two of such checkpoint molecules are TNF receptor associated factor 2/3 (TRAF2 and TRAF3) (Figure 1). When TRAF2 and/or TRAF3 are deleted in B cells, engaging the BCR can induce CSR in the absence of co-stimulation [23]. These data demonstrate that the BCR has the ability to induce

CSR; however, this ability is normally restrained by TRAF2 and TRAF3. Mechanistically, TRAF3 restricted BCR signaling by preventing the processing of BCR-induced NF- κB2 precursor (p100) into active NF- κB2 (p52) [23]; furthermore, TRAF3 also inhibited BCR proximal signaling. As such, B-cell-intrinsic TRAF3 deletion led to elevated BCR proximal signaling strength, evidenced by increased phosphorylation of bruton tyrosine kinase (BTK) and spleen tyrosine kinase (Syk), and constitutively active NF- κB2 [23]. Intriguingly, NF- κB2 activation is specifically required for the BCR signaling to induce CSR but not CD40 or TLR4 [23,24], suggesting that TRAF3 restricts NF- κB2 activation to specifically limit the BCR's ability to induce CSR. While our recent study addressed how TRAF3 inhibits BCR signaling, it remains unknown how TRAF2 singularly or cooperatively with TRAF3 does so.

How do TRAF2 and TRAF3 Differentially Influence CSR and How Does the BCR Cooperate with Co-receptors to Induce CSR?

Both TRAF2 and TRAF3 are adaptor molecules of TNF receptors (TNFRs) including CD40 and BAFFR [25]. In resting B cells, TRAF2, TRAF3 and cellular inhibitor of apoptosis protein1/2 (cIAP1/2) form a complex to suppress NF- κB inducing kinase (NIK) activity by inducing NIK degradation (Figure 1). In activated B cells, TRAF3 can be degraded [23,26,27], thereby allowing NIK accumulation that eventually activates NF- κB2 (Figure 1). Although TRAF2 and TRAF3 both serve as adaptors of TNFRs, they play differential roles in mediating CSR and *in vivo* antibody responses. We found that TRAF2 is required for CD40-induced AID expression and IgG1 CSR because TRAF2 plays an essential role in CD40-induced NF- κB1 activation [28]. Consistently, B-cell intrinsic deletion of TRAF2 significantly impaired *in vivo* IgG antibody responses against TD antigens [28] that need CD40/CD40L interaction. In contrast.

we found that TRAF2 functions as a checkpoint to prevent the BCR from inducing AID expression and CSR; thus, TRAF2 deletion promotes BCR-induced CSR *in vitro* [23]. In line with these observations, B-cell intrinsic deletion of TRAF2 increased *in vivo* IgG antibody responses against TI antigens [28] that can activate the BCR directly in the absence of T cell help. Contrary to the essential role of TRAF2 in CD40-induced CSR, TRAF3 is completely dispensable for CD40- induced AID expression and CSR [28]. As such, B-cell intrinsic TRAF3 deletion did not affect IgG antibody responses against TD antigens *in vivo* [28,29]. Consistent with TRAF3's role in restricting the BCR's ability to induce CSR, B-cell intrinsic deletion of TRAF3 increased IgG antibody responses against TI antigens [28]. Taken together, these studies highlight the complexity and fine-tuning potential of antibody-mediated immune responses that may have important implications for vaccine design of different types of antigens.

If the BCR has the ability to induce CSR, why do B cells need co-stimulation and what does co-stimulation provide in the context of CSR induction? We propose that CD40 aids BCR- induced CSR *in vivo* by inducing TRAF3 degradation (Figure 1), which is supported by our *in vitro* studies showing that anti-CD40/IL-4 stimulation caused TRAF3 degradation in B cells [23]. However, this hypothesis still needs to be tested in an *in vivo* setting. Regarding BAFFR, our recent studies also suggest that BAFFR's function is to degrade TRAF3, thus permitting the BCR to induce CSR [23] (Figure 1), a point still needs to be confirmed experimentally. TLRs have been shown to synergize with the BCR to induce CSR by enhancing NF-κB2 activation, and such synergistic effects depend on a regulatory subunit of PI3Ks, p85 [21]. However, the catalytic subunits of PI3Ks inhibit AID expression and CSR induced by CD40 [30], TLR4 [22] and BCR (unpublished data). Thus, the precise mechanisms remain

elusive about how TLRs and the BCR synergize to enhance NF- κB2 activation to promote CSR. TLRs can bind TRAF3 via their adaptors myeloid differentiation primary response protein (MYD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF). However, TLRs do not induce TRAF3 degradation like CD40 does, because TRAF3 is required for TLR-induced cytokine production [31]. Hence, we propose that TLRs sequester TRAF3 via adaptors MYD88 and/or TRIF, thereby releasing NIK that eventually activates NF-κB2 (p52) (Figure 1).

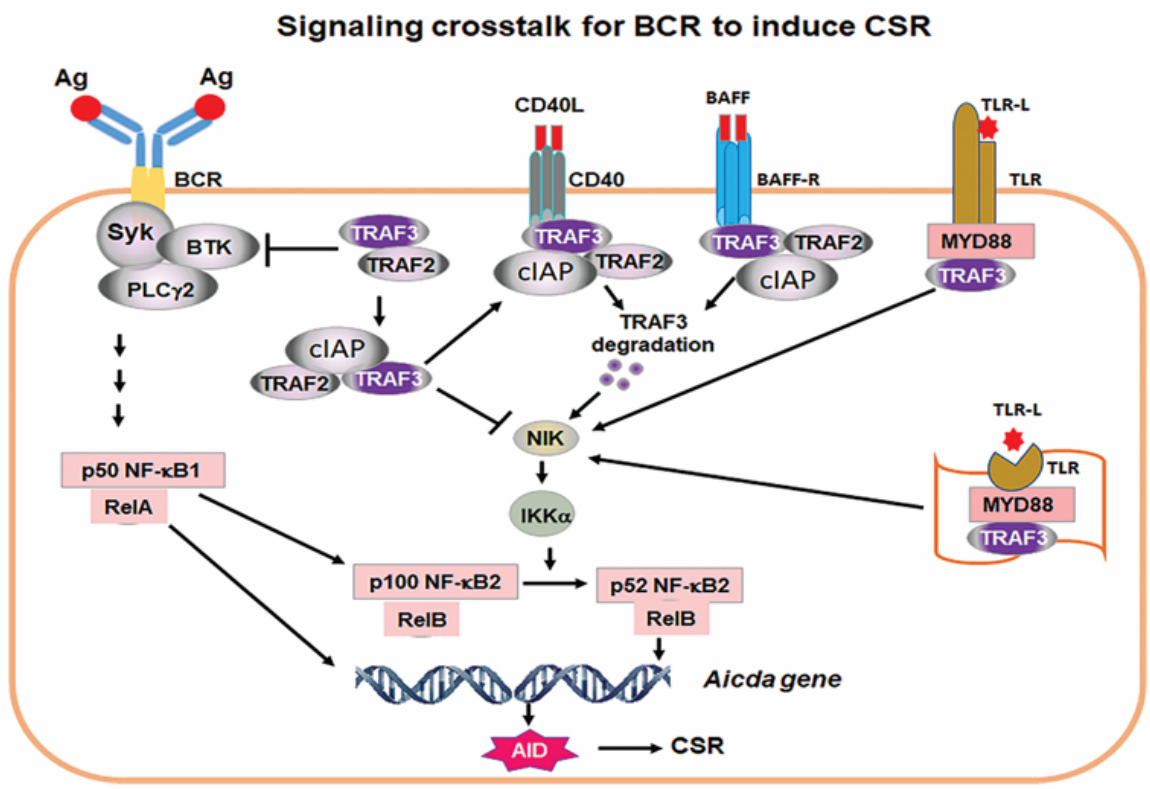

Figure 1: A proposed model of signaling crosstalk for the BCR to induce CSR.

What are the Consequences of BCR Checkpoint Removal?

When TRAF3 is deleted specifically in B cells via CD19Cre (B-TRAF3-KO), mice develop autoimmune manifestations including splenomegaly, lymphocyte infiltration in liver and immune-complex deposition in kidney at the age of 9-12 months [29]. B-TRAF2-KO mice exhibit similar phenotypes to B-TRAF3-KO mice in B cell development and survival as well as lymph organ homeostasis [29,32,33]. However, it remains incompletely understood how TRAF3 deficiency leads to autoimmune manifestations. Our recent studies showed that TRAF3- deficiency-mediated lymphoid organ disorders and autoimmune manifestations were rectified by attenuating BCR proximal signaling strength using a BTK inhibitor, Ibrutinib [23]. Additionally, introducing an antigen-specific BCR recognizing hen egg lysozyme (HEL) into B-TRAF3-KO mice completely rescued autoimmune phenotypes [23]. Taken together, these data suggest that the phenotypes of B-TRAF3-KO mice largely attribute to dysregulated BCR signaling pathway.

Anergy is an important mechanism to maintain B cell tolerance via unresponsiveness of the BCR to antigen stimulation [34]. Anergic autoreactive B cells express a low level of surface IgM that does not induce calcium flux when stimulated with specific antigens or agonist anti-IgM [35,36]. Using a bone marrow chimera system based on HEL, we demonstrated that TRAF3- deficiency breaks autoreactive B-cell anergy [23], possibly via elevating BCR proximal signaling strength. This idea is supported by our findings that TRAF3 deletion enhances BCR-induced phosphorylation of Syk and BTK as well as phospholipase Cγ2-dependent calcium flux [23]; however, it remains to be addressed how TRAF3 restricts BCR proximal signaling strength. We propose that when BCR signaling intensity is increased to a level sufficient to induce AID expression and CSR, it may disrupt autoreactive B cell tolerance and perturb B cell homeostasis. Altogether, our studies present a new concept that may better explain how signaling components of the BCR and co-receptor pathways assure robust humoral immune responses while simultaneously preserve B cell homeostasis and prevent malignancy by fine-tuning the BCR signaling intensity.

Ag stimulation of BCR activates proximal signaling elements, Syk, BTK and PLCγ2, leading to transcription factor NF-κB1 activation. NF-κB1 p50/RelA complex is required for AID transcription. NF-κB1 p50/RelA also induces NF-kB2 p100 transcription. TRAF2/3 restrict BCR proximal signaling strength. TRAF2 and TRAF3 also block NIK activity. Thus, Syk/BTK/PLCγ2 complex cannot signal to generate transcription factor NF-κB2 p52 that is required for AID expression. Removal of TRAF3 and/or TRAF2 leads to NIK accumulation, which activates IKKα pathway, resulting in NF-κB2 p100 being processed into active NF-κB2 p52. NF-κB2 p52/RelB complex and NF-κB1 p50/RelA together with additional factors to initiate AID transcription. AID protein initiates CSR by targeting Igh locus. During humoral immune responses, CD40, BAFF-R as well as cell surface and intracellular TLRs are activated by corresponding ligands, CD40L, BAFF or TLR ligand (TLR-L), respectively. TRAF3/TRAF2 are recruited to cell membrane where TRAF3 is degraded by CD40 and BAFF-R signaling or sequestrated by TLRs. As a consequence, NIK and NF-κB2 complex can be activated. NF-κB2 activation allows the BCR to induce CSR. Thus, the critical function of co-stimulatory signals is to degrade or sequestrate TRAF3 to permit NF-κB2- dependent BCR-induced CSR essential for *in vivo* antibody responses. It is worthy of note that TRAF3 restricts Syk, BTK and PLCγ2 hyper-activation upon Ag stimulation may be especially important for maintaining autoreactive B-cell anergy (Figure 1).

Acknowledgments

We thank Rachel A Woolaver for proofreading the manuscript. We apologize to those whose work was not cited due to length restrictions. This work was supported by University of Colorado School of Medicine and Cancer Center startup funds to J.H.W., Cancer League of Colorado, R21-CA184707, R21-AI110777, R01-CA166325, R21-AI133110, R01-CA229174 and R01-CA249940 to J.H.W., and a fund from American Cancer Society (ACS IRG #16-184-56) to Z.C. The sponsors or funders have no role in the preparation, review, or approval of the manuscript.

References

1. Chen Z, Wang JH (2019) Signaling control of antibody isotype switching. Adv Immunol 141: 105-164.
2. Matter MS, Ochsenbein AF (2008) Natural antibodies target virus-antibody complexes to organized lymphoid tissue. Autoimmun Rev 7(6): 480-486.
3. Pone EJ, Xu Z, White CA, Zan H, Casali P (2012) B cell TLRs and induction of immunoglobulin class-switch DNA recombination. Front Biosci (Landmark Ed) 17: 2594-2615.
4. Plotkin SA, (2010) Correlates of protection induced by vaccination. Clin Vaccine Immunol 17(7): 1055-1065.
5. Parvaneh N, Casanova JL, Notarangelo LD, Conley ME (2013) Primary immunodeficiencies: a rapidly evolving story. J Allergy Clin Immunol 131(2): 314-323.
6. Platt C, Geha RS, Chou J (2014) Gene hunting in the genomic era: approaches to diagnostic dilemmas in patients with primary immunodeficiencies. J Allergy Clin Immunol 134(2): 262-268.
7. Hanna IJ, Wentink M, Zessen DV, Driessen GJ, Dalm VASH, et al. (2015) Strategies for B-cell receptor repertoire analysis in primary immunodeficiencies: from severe combined immunodeficiency to common variable immunodeficiency. Front Immunol 6: 157.
8. Stavnezer J, Guikema JE, Schrader CE (2008) Mechanism and regulation of class switch recombination. Annu Rev Immunol 26: 261-292.
9. Stavnezer J, Schrader CE (2014) IgH chain class switch recombination: mechanism and regulation. J Immunol 193(11): 5370-5378.
10. Vaidyanathan B, Yen WF, Pucella JN, Chaudhuri J (2014) AIDing Chromatin and Transcription-Coupled Orchestration of Immunoglobulin Class-Switch Recombination. Front Immunol 5: 120.
11. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, et al. (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 102(5): 553-563.
12. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, et al. (1999) Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J Biol Chem 274(26): 18470-18476.
13. Chaudhuri J, Basu U, Zarrin A, Yan C, Franco S, et al. (2007) Evolution of the immunoglobulin heavy chain class switch recombination mechanism. Adv Immunol 94: 157-214.
14. Yan CT, Boboila C, Souza EK, Franco S, Hickernell TR, et al. (2007) IgH class switching and translocations use a robust non-classical end-joining pathway. Nature, 449(7161): 478-482.
15. Boboila C, Yan C, Wesemann DR, Jankovic M, Jing H. Wang JH et al. (2010) Alternative end-joining catalyzes class switch recombination in the absence of both Ku70 and DNA ligase 4. J Exp Med 207(2): 417-427.
16. Boboila C, Alt FW, Schwer B (2012) Classical and alternative end-joining pathways for repair of lymphocyte-specific and general DNA double-strand breaks. Adv Immunol 116: 1-49.
17. Boboila C, Oksenych V, Gostissa M, Wang JH, Zha S, et al. (2012) Robust chromosomal DNA repair via alternative end-joining in the absence of X- ray repair cross-complementing protein 1 (XRCC1). Proc Natl Acad Sci USA 109(7): 2473- 2478.
18. Alt FW, Zhang Y, Meng FL, Guo CH, Schwer B (2013) Mechanisms of programmed DNA lesions and genomic instability in the immune system. Cell 152(3): 417-429.
19. Chen Z, Wang JH (2014) Generation and repair of AID-initiated DNA lesions in B lymphocytes. Front Med 8(2): 201-216.
20. Xu Z, Zan H, Pone EJ, Mai T, Casali P (2012) Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. Nat Rev Immunol 12(7): 517-531.
21. Pone EJ, Zhang J, Mai T, White CA, Li G, et al. (2012) BCR-signalling synergizes with TLR-signalling for induction of AID and immunoglobulin class-switching through the non-canonical NF-κB pathway. Nat Commun 3: 767.
22. Heltemes Harris LM, Gearhart PJ, Ghosh P, Longo DL (2008) Activation-induced deaminase-mediated class switch recombination is blocked by anti-IgM signaling in a phosphatidylinositol 3-kinase-dependent fashion. Mol Immunol 45(6): 1799-1806.
23. Chen Z, Krinsky A, Woolaver RA, Wang X, Chen SMY, et al. (2020) TRAF3 Acts as a Checkpoint of B Cell Receptor Signaling to Control Antibody Class Switch Recombination and Anergy. J Immunol 205(3): 830-841.

24. Caamano JH, Rizzo CA, Durham SK, Barton DS, Raventós Suárez C, et al. (1998) Nuclear factor (NF)- κB2 (p100/p52) is required for normal splenic microarchitecture and B cell-mediated immune responses. J Exp Med 187(2): 185-196.

25. Lin WW, Hostager BS, Bishop GA (2015) TRAF3, ubiquitination, and B-lymphocyte regulation. Immunol Rev 266(1): 46-55.

26. Hostager BS, Haxhinasto SA, Rowland SL, Bishop GA (2003) Tumor necrosis factor receptor-associated factor 2 (TRAF2)-deficient B lymphocytes reveal novel roles for TRAF2 in CD40 signaling. J Biol Chem 278(46): 45382- 45390.

27. Liao G, Zhang M, Harhaj EW, Sun SC (2004) Regulation of the NF-κB-inducing kinase by tumor necrosis factor receptor- associated factor 3-induced degradation. J Biol Chem 279(25): 26243-26250.

28. Woolaver RA, Wang X, Dollin Y, Xie P, Wang JH (2018) TRAF2 Deficiency in B Cells Impairs CD40-Induced Isotype Switching That Can Be Rescued by Restoring NF-κB1 Activation. J Immunol 201(11): 3421-3430.

29. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA (2007) Tumor necrosis factor receptor-associated factor 3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. Immunity 27(2): 253-267.

30. Chen Z, Getahun A, Chen X, Dollin Y, Cambier JC, et al. (2015) Imbalanced PTEN and PI3K Signaling Impairs Class Switch Recombination. J Immunol 195(11): 5461-5471.

31. Hoebe K, Beutler B, (2006) TRAF3: a new component of the TLR-signaling apparatus. Trends Mol Med 12(5): 187-189.

32. Gardam S, Sierro F, Basten A, Mackay F, Brink R (2008) TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. Immunity 28(3): 391-401.

33. Grech AP, Amesbury M, Chan T, Gardam S, Antony Basten A (2004) TRAF2 differentially regulates the canonical and noncanonical pathways of NF-κB activation in mature B cells. Immunity 21(5): 629-642.

34. YarkoniY, Getahun A, Cambier JC, (2010) Molecular underpinning of B-cell anergy. Immunol Rev 237(1): 249-263.

35. Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith Gill SJ, et al. (1988) Altered Immunoglobulin Expression and Functional Silencing of Self-Reactive Lymphocytes-B in Transgenic Mice. Nature 334(6184): 676-682.

36. Duty JA, Szodoray P, Zheng NY, Koelsch KA, Zhang Q, et al. (2009) Functional anergy in a subpopulation of naive B cells from healthy humans that express autoreactive immunoglobulin receptors. J Exp Med 206(1): 139-151.