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Epigenetics of the antibody and autoantibody response

Justin B. Moroney, **Daniel P. Chupp**, **Zhenming Xu**, **Hong Zan**, **Paolo Casali**

Department of Microbiology, Immunology & Molecular Genetics, University of Texas Long School of Medicine, UT Health Science Center, San Antonio, TX 78229, USA

Abstract

B cell differentiation driven by microbial antigens leads to production of anti-microbial antibodies, such as those neutralizing viruses, bacteria or bacterial toxin, that are class-switched (IgG and IgA) and somatically hypermutated (maturation of the antibody response) as well as secreted in large volume by plasma cells. Similar features characterize pathogenic antibodies to self-antigens in autoimmunity, reflecting the critical role of class switch DNA recombination (CSR), somatic hypermutation (SHM) and plasma cell differentiation in the generation of antibodies to not only foreign antigens but also self-antigens (autoantibodies). Central to CSR/SHM and plasma cell differentiation are AID, a potent DNA cytidine deaminase encoded by *Aicda*, and Blimp-1, a transcription factor encoded by *Prdm1*. B cell-intrinsic expression of *Aicda* and *Prdm1* is regulated by epigenetic elements and processes, including DNA methylation, histone posttranslational modifications and non-coding RNAs, particularly miRNAs. Here, we will discuss: B cell-intrinsic epigenetic processes that regulate antibody and autoantibody responses; how epigenetic dysregulation alter CSR/SHM and plasma cell differentiation, thereby leading to autoantibody responses, as in systemic lupus; and, how these can be modulated by nutrients, metabolites and hormones through changes in B cell-intrinsic epigenetic mechanisms, which can provide to be therapeutic targets in autoimmunity.

Introduction: Maturation of the antibody and autoantibody response

The generation of pathogenic autoantibodies in organ-specific and systemic autoimmunity is governed by mechanisms similar to those informing the generation of neutralizing antibodies against viruses, bacteria or microbial toxins [1]. Like these antibodies to foreign antigens, those to self-antigens (autoantibodies) are class-switched and somatically mutated, the products of a “maturation” of the antibody response. This starts once naive B cells encounter their cognate antigen and are activated via BCR, CD40 or TLR and cytokine receptors. Activated B cells proliferate and differentiate, generally in germinal centers (GCs) or outside GCs (extrafollicular sites), to undergo CSR and SHM, thereby giving rise to class-switched and hypermutated B cells, plasma cells and memory B cells [2,3]. Extrafollicular

Corresponding author: pcasali@uthscsa.edu.

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GC-like B cell reactions occur in both T-dependent and T-independent responses, and exhibit high levels of CSR and SHM [4,5]. By changing the IgH constant region (Cμ to Cγ, Cα or Cε), CSR endows an antibody with different biological effector functions, e.g., IgMs are limited to the intravascular torrent, IgGs can extravasate and reach virtually all body districts and IgAs dominate the response at barrier tissues. By inserting mainly point-mutations, SHM provides antibodies with the structural correlates for selection of higher-affinity mutants by antigen [2]. Class-switched and somatically hypermutated antibodies are secreted at high rates by B cell-derived plasmablasts and plasma cells. As the primary mechanisms for the maturation of autoantibody responses, CSR and SHM underpin both organ-specific, e.g., Hashimoto's thyroiditis or myasthenia gravis, as well as systemic autoimmunity, e.g., systemic lupus erythematosus (SLE) [6], in which autoantibodies are primarily IgG, somatically hypermutated, high affinity and secreted in large amounts by an expanded plasma cell pool [6,7].

By introducing double-stranded breaks and single-stranded nicks in *Ig* locus DNA, AID, encoded by *AICDA/Aicda*, critically initiates the first step in the cascade of events that lead to CSR/SHM [8–11]. As a potent mutator, it is largely restricted to targeting the *Ig* locus – AID off-targeting (outside the *Ig* locus) can cause genome instability, often promoting neoplastic transformation [12]. AID expression is B cell-restricted and B cell differentiation stage-specific [11]. It is undetectable in resting B cells, is induced at high levels in activated B cells in antibody responses and subsides to undetectable levels in resting memory B cells and plasma cells. AID is also greatly upregulated in activated autoreactive B cells that make class-switched and somatically hypermutated autoantibodies, such as those in systemic lupus [13]. As terminally differentiated elements, plasma cells do not proliferate but secrete large amounts of antibodies or autoantibodies. For their differentiation and maintenance, they are dependent on the master transcription factor (TF) Blimp-1, encoded by *PRDM1/Prdm1*, as well as Irf4 and Xbp1 TFs [14]. Blimp-1 expression level is low or nil in B cells and increases in plasmablasts to plasma cells. These home to unique niches in bone marrow, where they can survive for months to years. Marrow long-lived plasma cells can maintain a durable layer of antibodies against previously experienced microbial pathogens or self-antigens in the case of autoantibodies [14,15]. Additional long-term persistence of recallable high levels of antibodies or autoantibodies is provided by memory B cells, which are reactivated and expanded upon re-exposure to cognate foreign or self-antigens [5,16].

Epigenetic mechanisms, such as DNA methylation, histone post-translational modifications and non-coding RNAs (ncRNA), regulate gene expression, thereby modulating cell functions without changes in DNA sequence [17]. In differentiating B cells, they enable and modulate genetic programs to regulate expression and activity of AID and Blimp-1, thereby modulating CSR/SHM and plasma cell differentiation to inform the antibody and autoantibody response [18–26]. In autoimmune responses, epigenetic mechanisms modulate T cell differentiation and functions, particularly of regulatory T cells, as well as macrophages and dendritic cells [27–32]. The epigenetics of these cells are beyond the scope of this review, which we will focus on the epigenetics of B cells. Here, we will attempt to provide a framework of how B cell-intrinsic epigenetic factors and processes modulate CSR/SHM and plasma cell differentiation, with a focus on regulation of AID and Blimp-1 by DNA methyltransferases (Dnmts) and Tet (ten-eleven translocation) methylcytosine

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dioxygenases, histone deacetylases (HDACs), HDAC inhibitors (HDIs) and non-coding (nc)RNAs, particularly miRNAs. We will conclude by discussing recent findings on how these epigenetic elements and processes can be potential targets of new therapeutic approaches to alleviate antibody-dependent autoimmunity.

Epigenetics processes and peripheral B cell differentiation

AID expression and targeting, and, therefore, CSR/SHM are regulated by B cell-intrinsic epigenetic processes, such as DNA methylation and histone post-translational modifications, and mediators, such as miRNAs. Similar B cell-intrinsic epigenetic processes and mediators regulate Blimp-1 expression as well as other TFs that drive plasma cell differentiation, such as Irf4 and Xbp1.

DNA methylation and demethylation critically regulate cell-type specific and context-dependent gene expression [33,34]. Throughout bone marrow B cell development and peripheral differentiation, the B cell genome undergoes progressive demethylation [35–37]. Methylation of cytidine to 5-methylcytosine (5mC) in (mainly) CpG islands of gene promoters and enhancer regions is catalyzed by DNA methyltransferases (Dnmts) and acts to silence gene transcription [23,33]. 5mC is converted to unmethylated state through passive or active demethylation. While passive demethylation occurs through replication dilution, active DNA demethylation, i.e., direct removal of the methyl group, is mediated by Tet (Tet1, Tet2, Tet3) proteins, which oxidize 5mC to 5-hydroxymethylcytosine (5hmC) [33]. 5hmC is enriched at active enhancers and promoters of highly transcribed genes, correlating with chromatin accessibility, yet depleted from the transcription start site (TSS) [33,38]. Tet2 and Tet3 are expressed in B lineage cells [26,37,39], with Tet2 being predominant and correlating with AID and Blimp-1 expression in B cells undergoing CSR/SHM and plasma cell differentiation (our unpublished data) (Figure 1). This together with Dnmt1 requirement for GC formation indicates the importance of maintaining an underlying methylome in such B cells [40,41]. The methylation status of the five regulatory elements located between −29-Kb to +5-Kb of *Aicda* TSS (collectively referred to as the *Aicda* super-enhancer) mediates *Aicda* transcriptional regulation upon B cell activation [26]. The *Aicda* super-enhancer includes Tet-responsive *TetE1* and *TetE2 cis*-elements, which recruit Tet2 as facilitated by Batf, an AP-1 TF, to support active (5hmC) DNA demethylation, sustain super-enhancer accessibility and promote *Aicda* induction [26]. Impairment of Tet2 function in GC B cells correlates with decreased AID levels and reduced CSR [42].

Large-scale DNA demethylation, up to 10% of their DNA methylome, occurs during B cell differentiation to plasma cells, with DNA hypomethylation being prevalent in *Prdm1*, which is enriched in binding motifs for B cell-expressed TFs, including Oct2, NF-κB, IRF4 and AP-1 [22,23]. In bone marrow long-lived plasma cells, epigenetic programming involving extensive Dnmt3A-mediated *de novo* DNA methylation at enhancer elements to suppress expression of over 1,000 gene loci, including *Pax5*, *Bcl6* and *Aicda* [23]. Thus, epigenetic DNA hypomethylation and hypermethylation promotes and restricts plasma cell differentiation.

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Histone post-translational modifications can exert diverse effects on gene expression by altering chromatin structure for selective recruitment of TFs and targeting of other elements. They include phosphorylation of serine or threonine, methylation of lysine or arginine, as well as acetylation, ubiquitylation and sumoylation of lysine. Resting (naïve) B cells display inactive chromatin structure and transcriptional repression, with genome-wide histone H3 methylation, particularly H3K9(me3) and H3K27(me3). In such B cells, the *Aicda* locus is fully quiescent and histone H3 acetylation, a well-defined marker of permissive chromatin, particularly at H3K14ac and H3K27ac, occurs at low levels [25,43,44]. Engagement of BCR and CD40 and/or TLR activates naïve B cells by inducing them to acquire permissive histone marks H3K4me3 and H3K9ac/K14ac, with significant increased H3 acetylation of the *Aicda* enhancer and promoter regions [25,43,44]. This is concomitant with appearance of combinatorial histone mark H3K4acS10ph, which is specifically read by 14-3-3 adaptors to target AID to Ig locus switch region AGCT motifs [19,45]. Histone acetylation is a dynamic process mediated by HATs and HDACs. HATs play an important role in early B cell development and peripheral differentiation, with monocytic leukemia zinc finger (MOZ) protein implicated in GC dark zone B cell expansion and GCN5 promoting expression of membrane IgM and Irf4 [46,47]. HDACs comprise “classical” Class I, II, and IV HDACs and “non-classical” Class III HDACs or Sirtuins (Sirt). Classical HDACs depend on Zn^{2+} as a cofactor, Sirtuins on NAD^{+} [48]. HDAC Class III Sirt1 actively deacetylates acetyl-lysine in multiple histones, including H3K4ac, H3K9Ac, H3K14ac and H3K36ac, to facilitate chromatin compaction and silence gene transcription [49]. Sirt1, the most abundantly expressed Sirtuin in B cells, regulates AID in a B cell differentiation stage-specific fashion. In resting B cells, high levels of Sirt1 mediate extensive histone H3 hypoacetylation across the *Aicda* locus, thereby keeping AID expression in check [25]. B cell activation induces profound downregulation of Sirt1 with concomitant AID upregulation resulting from decreased Sirt1 recruitment to *Aicda* promoter and increased H3K9Ac and H3K14Ac acetylation (Figure 1) [25].

Acquisition of permissive histone modifications, including H3K4me1 in active promoters and H3K4me3 in distal enhancers, is associated with changes of methylome and gene expression in differentiating plasma cells [23]. In these cells, loss of repressive histone modifications, such as H3K27me3, occurs at select loci, particularly genes encoding regulators of cell division and metabolism. Such repressive modifications are catalyzed mainly by EZH2 [24], which is differentially recruited by Bcl-6 and Blimp-1 to those gene loci [50,51] as TF dictating B cell fate decisions through recruitment of epigenetic factors. In B cells, inhibition of EZH2 increases expression of genes regulators of cell division and metabolism and premature *Prdm1* transcription and Blimp-1 expression, thereby promoting plasma cell differentiation [52]. *Prdm1* expression is regulated by transcription activators and repressors. It is dependent on acetylation and methylation of H3K9 at a Maf recognition element (MARE) in intron 5 of this gene. GC B cell stage-specific *Prdm1* expression is repressed by Bach2 in cooperation with HDAC3-containing co-repressor complexes through this gene locus reduced histone acetylation [53]. Indeed, TBL1XR1, a core component of these HDAC3-containing SMRT/NCOR1 complexes, plays a pivotal role in GC B cells, preferentially interacting with BCL6, and to a lesser extent Bach2, to repress *Prdm1* gene expression and restrict plasma cell differentiation [54].

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Non-coding RNAs are involved in regulation of B cell differentiation and function. miRNAs, a category of small ncRNAs, regulate gene expression post-transcriptionally by hampering translation through targeting mRNA 3′-untranslated region (3'UTR) for mRNA degradation. Naïve, GC, plasma cells and memory B cells show distinct miRNA expression patterns [55]. In activated B cells, the deletion of Dicer, an RNase III endonuclease crucial for miRNA biogenesis, impairs biogenesis of multiple miRNAs, which directly regulate CSR/SHM and plasma cell differentiation [56]. miR-155, miR-181b, miR-361 and miR-26a bind to evolutionarily conserved *Aicda* mRNA 3′UTR target sites to silence *Aicda* expression [21,57–61]. These miRNAs, particularly miR-155, the most abundant in resting B cells, are downregulated upon B cell activation by CSR-inducing stimuli to allow for AID expression [57,58]. And, ablation of *Aicda* 3′UTR miR-155-target site increases B cell *Aicda* mRNA and AID protein level, thereby upregulating CSR [57,58]. *Aicda* 3′UTR also contains multiple binding sites for miR-181b, which is expressed at high levels in resting B cells and decreases in B cells upregulating AID to undergo CSR [59]. Thus, miR-181b would inhibit premature AID expression and its downregulation would allow for proper AID transcriptional activation at early time points, while miR-155 would restrict AID expression at a later stage of B activation [60].

Prdm1 mRNA contains a long (>2,000 nt) 3'UTR, which is targeted by multiple miRNAs, including miR-9, miR-23b, miR-30, miR-125b, miR-127 and let-7 [20,62–64]. Overexpression of miR-125b inhibits B cell differentiation to plasma cell by impairing Blimp-1 expression through targeting *Prdm1* and *Irf4* 3'UTRs [65]. miR-30c can also downregulate Xbp1 [66], which governs late events in plasma cell differentiation. Thus, dynamic changes in miRNAs control *Prdm1, Irf4* and *Xbp1* genes, which are critical for B cell differentiation to plasma cell. Long non-coding RNAs (lncRNAs) are potentially important regulators of B cell development and peripheral differentiation, but their roles in these cells are still poorly understood [67,68]. Thousands of lncRNAs have been identified across multiple stages of B cell development and activation [67]. Twenty percent of these lncRNAs are promoter-associated RNAs or enhancer-associated RNAs (eRNAs). In activated B cells, expression of many lncRNA closely correlates with the nearest coding gene, thereby pointing to tandem lncRNA/gene locus functional units [67], as epitomized by lncRNAs role in regulating expression of the adjacent *IgH* locus in human memory B cells [69].

Epigenetic and metabolic regulation of the autoantibody response

In addition to providing energy source and biosynthetic building blocks for growth, metabolites instruct cell gene expression, and distinct metabolic pathways regulate immune cell activation, differentiation and survival [70]. Indeed, cross-talks among external environment, B cell signal transduction pathways and TFs rely on metabolites as important mediators [71], thereby pointing to a metabolite-driven epigenetic regulation of B cell differentiation to modulate antibody and autoantibody responses.

Tet2 promotes AID and Blimp-1 expression through active DNA demethylation of *Aicda* and *Prdm1* loci as well as of other elements in GC B cells [26,72,73]. In activated B cells inducted to undergo CSR, *Tet2* transcription remains flat, while Tet2 protein levels increase

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significantly due their stabilization by post-translational phosphorylation, acetylation and glycosylation (our unpublished data). Genome-wide DNA hypomethylation occurs in systemic autoimmune diseases [7,17,74,75], as emphasized by lupus discordance in monozygotic twins, in whom the affected twin displays decreased B cell DNA methylation and increased AID expression [76]. Accordingly, prolonged treatment of hypertensive patients with DNA methylation inhibitors, such as hydralazine, can result in autoantibody production and a lupus-like syndrome. Conversely, B cell-intrinsic deletion of Tet2/Tet3 or Tet2 alone undercuts CSR/SHM and plasma cell differentiation [26,42,72] (our unpublished data). Tet2 would couple B cell metabolic status with effective epigenetic functions, as its transcription level and activity are determined by an array of metabolic factors and changes, such a Vitamin A (retinol), Vitamin C (Ascorbate), $Fe2^{+}$ (co-factor) and α-ketoglutarate (α-KG), a tricarboxylic acid (TCA) cycle metabolite. Mechanistically, retinoic acid enhances 5hmC through activation of TET2 transcription, whereas vitamin C potentiates TET2 activity and 5hmC production through enhanced Fe^{2+} recycling [91]. Indeed, Fe^{2+} withdrawal dampens Tet2-mediated conversion of 5mC to 5hmC [77]. Increased α-KG concentration from excess glucose activates Tet2, thereby enhancing genome-wide 5hmC levels [78]. Conceivably, α-KG, and, likely, vitamin C enhance Tet2-mediated active DNA demethylation in B cells, as α-KG does in stem cells [79]. On the other hand, (pathological) accumulation of 2-hydroxyglutarate (2-HG), succinate and fumarate, metabolites structurally similar to α-KG, leads to a competitive inhibition of Tet2 activity [80], which would result in hypermethylation of *Aicda* as well as other gene loci. Thus, inhibition of Tet2 activity by inhibition or subtraction of its co-factor(s) can conceivably represent an appealing therapeutic approach to autoantibody-mediated systemic autoimmunity involving genomic hypomethylation, such as in lupus.

Sirt1 controls, through deacetylation, the intracellular localization, stability and activity of histone and non-histone proteins, making this Class III HDAC an important mediator of multiple epigenetic functions [81]. Under physiological conditions, Sirt1 is highly expressed in resting naïve B cells, profoundly downregulated in activated B cells induced to undergo CSR/SHM, and reset at high levels in memory B cells and plasma cells. Sirt1 critically contributes to quiescent B cell homeostasis by keeping *Aicda* transcription in check through a three-pronged mechanism [25] (Figure 2): (i) deacetylation of *Aicda* promoter histones, which prevents chromatin decondensation, thereby making promoters inaccessible to TFs; (ii) deacetylation of Dnmt1, which activates this DNA demethylase to methylate the *Aicda* promoter; and (iii) deacetylation of the p65 component of NF-κB, inactivating this B cell transcription factor, which together with HoxC4 is critical for *Aicda* transcription [82]. B cells induced to undergo CSR/SHM downregulate Sirt1, leading to upregulation of AID expression [25]. Similarly, B cells in lupus mice and patients show highly reduced intrinsic *Sirt1/SIRT1* expression concomitant with enhanced *Aicda/AICDA* transcription and heightened AID levels [25]. Accordingly, intrinsic B cell deletion leads to production of class-switched ANA autoantibodies and lupus-like symptoms in C57BL/6 mice with a non-autoimmune background [25]. Upon activation, Sirt1 can integrate multiple epigenetic mechanisms to dampen AID expression [25]. Boosting Sirt1 activity by SRT1720 dampens AID expression and CSR/SHM in T-dependent and T-independent antibody responses. Further, in lupus-prone MRL/$Fas^{lpr/lpr}$ mice, Sirt1 activation inhibits anti-dsDNA and anti-

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histone IgG1 and IgG2a autoantibody responses, thereby reducing IgG1/IgG2a kidney deposition and glomerular damage [25]. Finally, as shown in myelodysplastic syndrome stem and progenitor cells, Sirt1 can deacetylate Tet2 protein and restore Tet2 activity [83], thereby further contributing to Sirt1-mediated modulation of the antibody and autoantibody response.

As intracellular levels of free NAD^+ are readily influenced by the balance of nutritional availabilities and metabolic cell state, NAD^+ dependence places Sirt1 at a central interface of metabolism and epigenetic regulation. Consistently, lupus patients with severe clinical outcomes exhibit expanded populations of $CD38^{high}$ T cells and decreased NAD^+ levels. This is in agreement with the nucleosidase activity of CD38, which is also highly expressed on activated human B cells as well as plasma cells [92]. Reduced cellular NAD^+ concentration, resulting from glycolytic conversion of NAD^+ to NADH, leads to decreased Sirt1 activity, increased acetylation of *Aicda* promoter histones, Dnmt1 and NF-κB p65, thereby enhancing *Aicda* expression and CSR [25]. By contrast, increased NAD^+ concentration, as provided by a nicotinamide rich diet [84], would lead to Sirt1 activation and abortive maturation of antibody and autoantibody responses. Thus, Sirt1 induction by a metabolic cofactor or boost by an activator, e.g., SRT1720, would be a promising therapeutic approach to autoantibody-mediated systemic autoimmunity and other states of B cell hyperreactivity. In this vein, it would be important to determine whether Sirt1 activation is effective in dampening activation and/or differentiation of “double negative” $CD27^-$ IgD^- T-bet^+ ($CD11c^{hi}$) ABC B cells. These B cells are expanded in lupus, multiple sclerosis and rheumatoid arthritis patients as well as in autoimmune mice, correlate with disease severity, and would give rise to self-antigen reactive plasma cells upon co-stimulation with TLR ligands and IL-21 [85,86].

HDIs (valproic acid, VPA; Panobinostat, Farydak; vorinostat, SAHA, Zolinza; Trichostatin-A, TSA or romidepsin, Istodax) treatment of lupus-prone MRL/$Fas^{lpr/lpr}$ mice reduces autoantibody levels, autoreactive plasma cell numbers, nephritis and dampen autoimmunity [20,87]. HDIs exert direct and B cell-intrinsic epigenetic effects even at moderate concentrations. In normal C57BL/6 mice, VPA, a well-characterized HDI, effectively downregulates AID and Blimp-1 by upregulating the level of miR-155, miR-181b and miR-361 which target *AICDA/Aicda* as well as miR-23b, miR-30a and miR-125b which target *PRDM1/Prdm1*, by boosting histone acetylation of respective host genes [20,63]. This miRNA downregulation of AID and Blimp-1 is dose-dependent and leads to profound inhibition of class-switched and somatically mutated T-dependent (NP-CGG) and T-independent (NP-LPS) antibody responses [20,63]. Through a similar mechanism and in a B cell-intrinsic fashion, VPA impairs the autoantibody response in lupus-prone MRL/$Fas^{lpr/lpr}$ mice, by reducing ANA IgG levels, autoreactive plasma cell numbers and nephritis [20,87]. As inhibitor of B cell intrinsic AID and Blimp-1 (and Xbp1) expression and restrictor of generation of class-switched high-affinity autoantibodies, HDIs would provide to be potentially effective therapeutics in lupus patients.

Epigenetic HDAC inhibition by dietary catabolites, namely SCFAs, plays an important role in homeostasis and physiological regulation of antibody and autoantibody responses (Figure 3). Butyrate, propionate and acetate are the main SCFAs produced by microbiota processing

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of dietary “resistant” fibers (which escaped digestion by host enzymes in the upper gut) in the cecum and proximal colon [88]. While acetate is mostly consumed by colonocytes, butyrate and propionate are reabsorbed in the colon and routed into the circulatory torrent, making it possible for these SCFAs to mediate their epigenetic effects not only on intestinal B cells (Peyer’s patches, lamina propria), but also in other districts of the body, thereby conditioning antibody production systemically.

Butyrate and propionate directly inhibit Class I, II and IV HDACs, effectively hyperacetylating histones, enhancing chromatin accessibility and activating gene expression [64]. In human and mouse B cells, these SCFAs act over a broad physiological range and in a dose-dependent fashion to modulate AID and Blimp levels, thereby restricting CSR/SHM and plasma cell differentiation. Like VPA, butyrate and propionate enhance histone acetylation host genes of select miRNAs that target *AICDA/Aicda* and *PRDM1/Prdm1* [64]. In activated B cells, butyrate virtually doubles miR-155 expression, making this *Aicda*-targeting miRNA account for 50% of total miRNA pool [64]. Administration of SCFAs to lupus-prone MRL/$Fas^{lpr/lpr}$ and NZB/WF1 mice effectively decreases IgG autoantibody titers, thereby ameliorating skin lesions and dampening kidney immunopathology [64], thereby suggesting a potential therapeutic use of SCFAs in lupus (Figure 4). Intestinal dysbiosis is a frequent hallmark of lupus patients, exhibiting an altered microbiota composition as compared to healthy subjects, particularly with decreased *Firmicutes* and increased *Bacteroidetes* [94]. Indeed, in healthy subjects, the gut *Firmicutes*/*Bacteroidetes* ratio has been positively correlated with SCFAs concentrations, consistent with *Firmicutes* ability to process dietary resistant fibers into butyrate, propionate and acetate [93]. Thus, intestinal dysbiosis in lupus alters production of SCFAs, thereby contributing to pathological autoantibody responses through dysregulation of AID and Blimp-1 in B cells. The direct activity of SCFAs on B cells would critically contribute to maintain a steady state balance between tolerance to commensal bacteria and immunity to pathogens for gut and systemic immune homeostasis.

Estrogen’s contribution to the immunopathogenesis of lupus is consistent with the 9:1 female-to-male sex ratio of lupus incidence, the typical disease onset during the child-bearing years and the increased risk of disease flares in women administered exogenous estrogens [61]. As a central B cell intrinsic factor, estrogen contributes to the dysregulation of multiple epigenetic mechanisms in lupus B cells, including upregulation of Tet2. Indeed, estrogen boosts Tet2 transcription by binding of estrogen-ER complexes to evolutionarily conserved estrogen response elements (EREs) in the Tet2 promoter (our unpublished data). Estrogen can counteract or even reverse SCFA-mediated modulation of antibody and autoantibody responses [61]. This effect is mediated in great part by estrogen’s downregulation of miR-26a, whose level is boosted by SCFAs to target and downregulate *Aicda* transcripts. Further, miR-26a modulates the expression of Tet2 [89], which regulates AID level by active DNA demethylation of the *Aicda* 5’ super-enhancer [26]. The intertwined activities of estrogen and miR-26a may provide an explanation for the stronger response of women to viral and bacterial vaccines, and the female bias in autoantibody-mediated lupus autoimmunity.

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Conclusions

Epigenetic changes and factors are critical in guiding B cell development and modulating B cell differentiation processes which are central to the maturation of the antibody and autoantibody response. In spite of recent advances, important questions on the nature and role of such epigenetic changes and factors remain to be thoroughly answered. For instance, class-switched high-affinity B cells to foreign antigens or self-antigens are generated through CSR/SHM in GCs and extra-GC sites. After selection for survival and expansion, such B cells undergo differentiation to plasma cells or memory B cells. Much needs to be understood how epigenetic processes determine whether a post-GC or post-extrafollicular B cell differentiates to plasma cell or memory B cell, and what underpins the re-differentiation of a memory B cell to plasma cell [5,16]. Similarly, much light needs to be shed on the nature of resting naïve B cells that seed extrafollicular expansion of pathogenic effector B cells, such as those recently identified in lupus patients. Such B cells are epigenetically poised by a distinct methylation status, which includes enrichment in motifs accessible to AP-1 and EGR TFs synergizing with T-bet [86]. This and other knowledge of epigenetic changes in autoantibody and autoantibody responses has been derived mainly from analysis of disease-associated epigenetic patterns in *ex vivo* human samples, studies of genetically modified mice or autoimmune mouse strains. However, an experimental and fully functioning *in vivo* model of the human immune system is needed to fully understand epigenetic mechanisms impact those underpinning the human antibody and autoantibody response. Although humanized NSG mice have shown some promise as model of systemic autoimmunity [90], they have had fallen short of fully supporting the maturation of human antibody responses to foreign and self-antigens. A novel humanized mouse has become available that upon hormonal conditioning provides a robust *in vivo* platform that allows for generation of mature human T-dependent and T-independent antibody responses and lends itself to *in vivo* modeling of autoimmune disease (our unpublished data). Such new generation of humanized mice can provide accurate and effective models for investigating immunization strategies and candidate vaccines against bacterial and viral pathogens. Also, they are currently used to identify potential therapeutic targets among epigenetic factor coactivators, including metabolites, and inhibitors, such as naturally occurring SCFAs, in systemic autoantibody-mediated autoimmunity.

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Keywords

- B cell-intrinsic epigenetic mechanisms and factors regulate AID and Blimp-1 expression.
- Tet2 actively demethylates *Aicda* super-enhancer elements to derepress *Aicda* transcription.
- Sirt1 uses a three-pronged deacetylation mechanism to downregulate *Aicda* expression.
- Microbiota-derived SCFAs suppress antibody and autoantibody responses through induction of select miRNAs that downregulate *Aicda* and *Prdm1* expression.
- Metabolic factors regulate Sirt1 and Tet2 activity, thereby modulating CSR/SHM and plasma cell differentiation in antibody response to foreign and self-antigens (autoantibodies).

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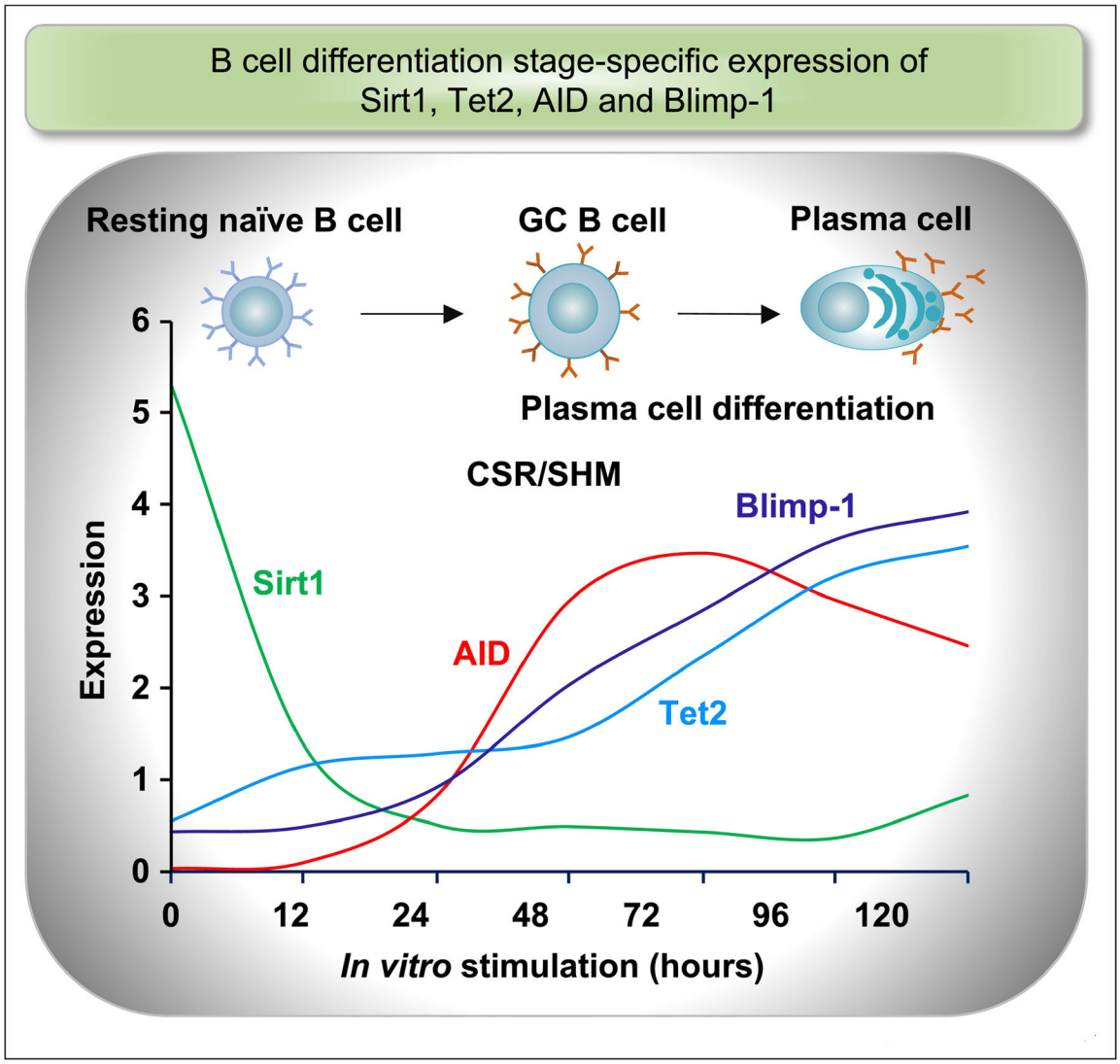

Figure 1. B cell differentiation stage-specific expression of Sirt1, Tet2, AID and Blimp-1.

Bi-phasic B cell differentiation and associated dynamic changes in gene expression. B cell CSR/SHM precedes plasma cell differentiation, concomitant with induction of AID and Blimp-1, respectively. AID upregulation coincides with downregulation of Sirt1 transcript and protein. AID and Blimp-1 induction tracks expression of Tet2 protein. In B cells induced to express AID and Blimp-1, Tet2 protein, but not *Tet2* transcript, is increased, likely through post-translational modifications that enhance the stability of this epigenetic mediator.

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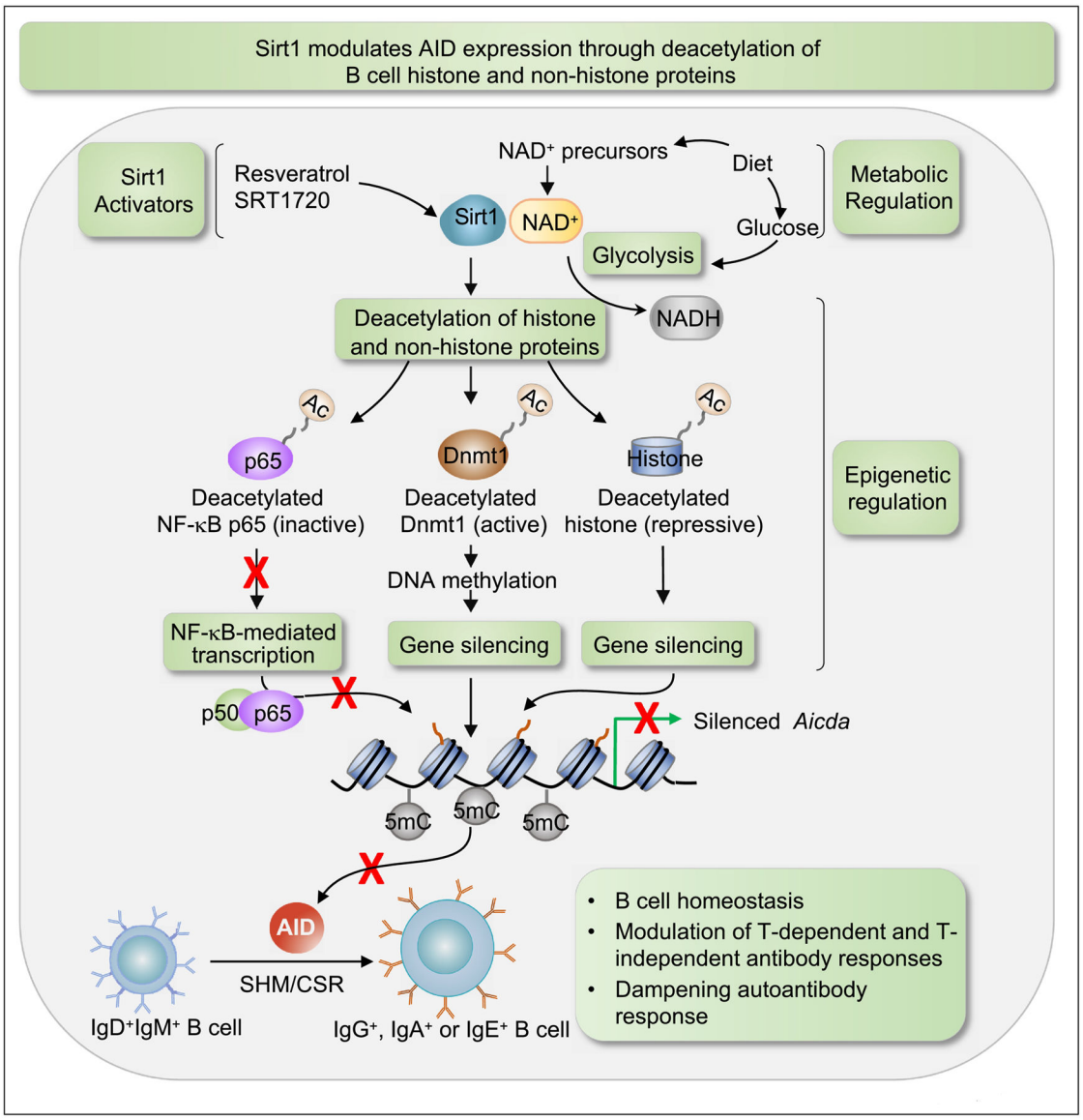

Figure 2. Sirt1 modulates modulation AID expression through deacetylation of B cell histone and non-histone proteins.

By deacetylating histone and non-histone proteins, the NAD^+-dependent class III HDAC Sirt1 exerts a three-pronged repression of *Aicda*: (1) by deacetylating histones in the *Aicda* promoter, Sirt1 directly represses *Aicda* expression; (2) by deacetylating p65, Sirt1 reduces the activity of NF-kB, which together with HoxC4 is a key activator of *Aicda* promoter; (3) by deacetylating Dnmt1, Sirt1 enhances the DNA methylation activity of this methyltransferase, leading to an increased DNA methylation of the *Aicda* promoter, thereby further repressing *Aicda* expression. As intracellular levels of free NAD^+ are readily influenced by the balance of nutritional availabilities and the metabolic state of the cell, the NAD^+ dependence places Sirt1 at the interface of metabolism and epigenetic regulation. NAD^+ precursors, available in food, increase cellular NAD^+ level and enhance Sirt1 activity, while glucose, which metabolized through glycolysis converts NAD^+ to NADH, lowers cytosolic NAD^+/NADH ratio and Sirt1 activity. In addition, some naturally occurring and

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synthetic compounds, such as resveratrol and SRT1720 which facilitate NADH oxidation to NAD^+ and increase Sirt1 affinity for NAD^+ and acetylated substrates, function as Sirt1 activators.

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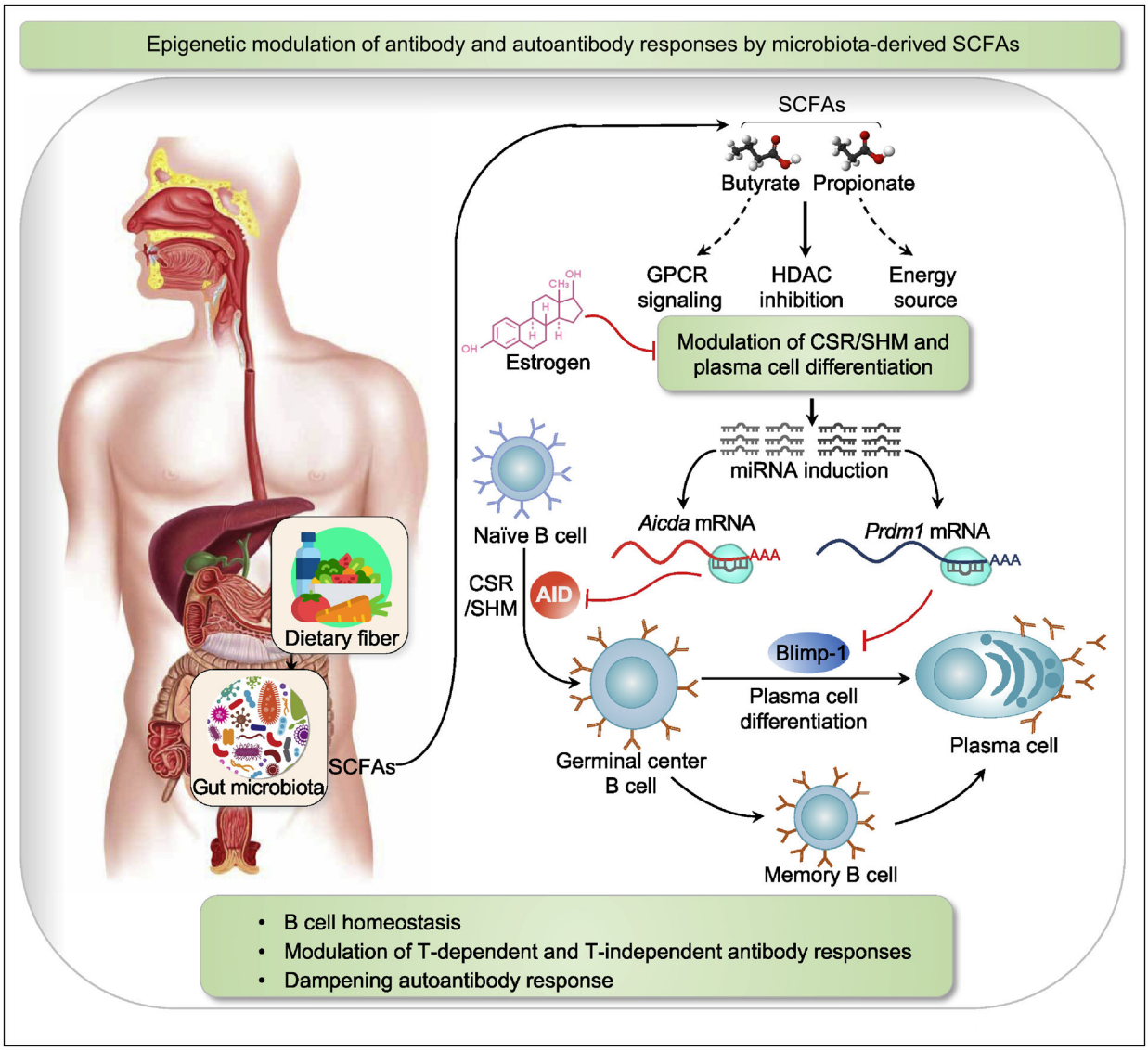

Figure 3. Epigenetic modulation of antibody and autoantibody responses by microbiota-derived SCFAs.

Resistant dietary fibers are fermented by gut commensal bacteria to yield SCFAs, such as butyrate and propionate. Beyond their GPCR-signaling-capacity and use as an energy source, butyrate and propionate display potent HDAC inhibitory functions. In B cells, butyrate and propionate act as HDIs to effect chromatin decondensation and induce the expression of select miRNAs that target the 3' UTR region of *Aicda* and *Prdm1* transcripts. Such miRNAs silence the expression of AID and Blimp-1, and reduce CSR/SHM and plasma cell differentiation, thereby inhibiting antibody and autoantibody responses. The HDI-mediated downregulation of AID expression and its impact on maturation of antibody and autoantibody responses are reversed by estrogen. Estrogen's reversion of HDI-mediated inhibition of AID occurs at least partially through downregulation of B cell miR-26a.

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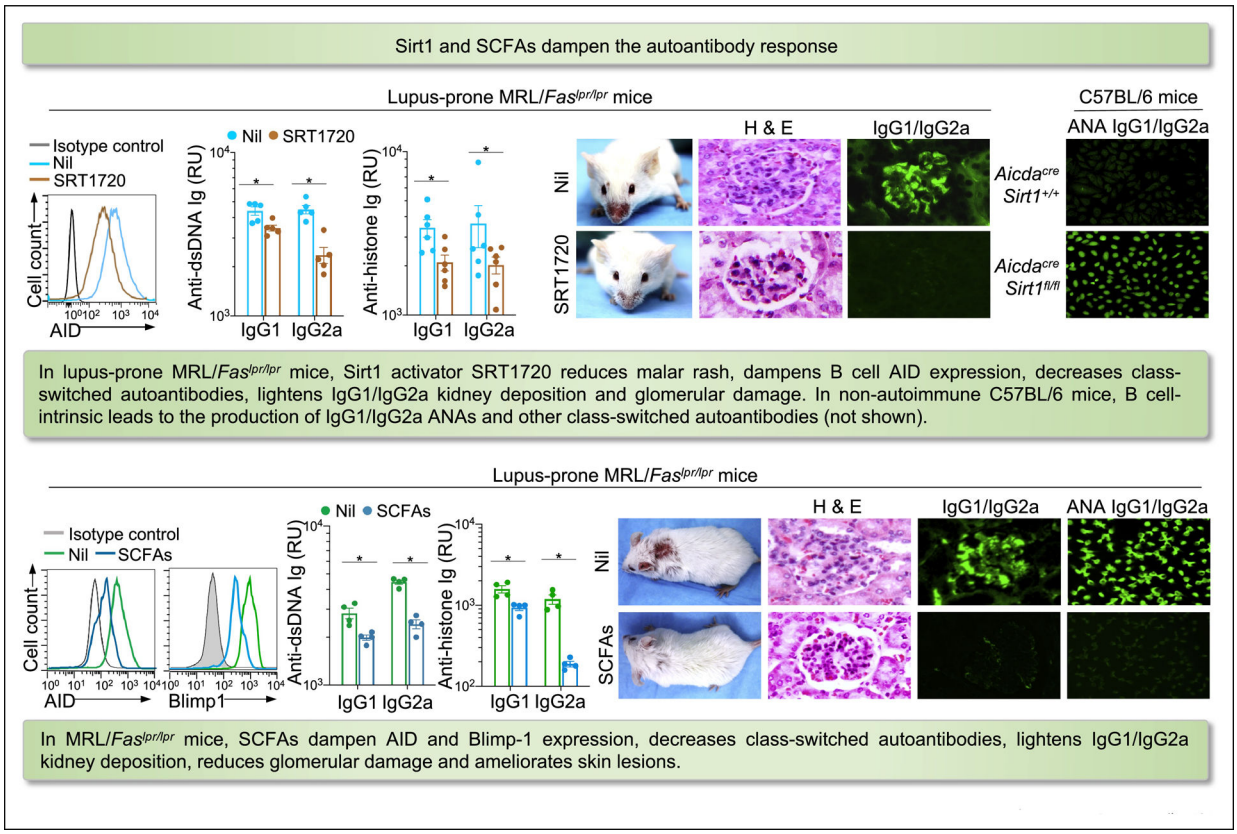

Figure 4. Sirt1 and SCFAs dampen autoantibody response.

In lupus-prone MRL/*Fas*$^{lpr/lpr}$ mice, Sirt1 activator SRT1720 reduces malar rash, dampens B cell AID expression, decreases class-switched autoantibodies, lightens IgG1/IgG2a kidney deposition and glomerular damage. In non-autoimmune C57BL/6 mice, B cell-intrinsic Sirt1 deletion leads to production of IgG1/IgG2a ANAs and other class-switched autoantibodies. In MRL/*Fas*$^{lpr/lpr}$ mice, SCFAs dampen AID and Blimp-1 expression, reduce class-switched autoantibodies, lighten IgG1/IgG2a kidney deposition, and inhibit glomerular damage and skin lesions.