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Binding of estrogen receptors to switch sites and regulatory elements in the immunoglobulin heavy chain locus of activated B cells suggests a direct influence of estrogen on antibody expression

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Abstract

Females and males differ in antibody isotype expression patterns and in immune responses to foreign- and self-antigens. For example, systemic lupus erythematosus is a condition that associates with the production of isotype-skewed anti-self antibodies, and exhibits a 9:1 female:male disease ratio. To explain differences between B cell responses in males and females, we sought to identify direct interactions of the estrogen receptor (ER) with the immunoglobulin heavy chain locus. This effort was encouraged by our previous identification of ER response elements (ERE) in heavy chain switch (S) regions. We conducted a full-genome chromatin immunoprecipitation analysis (ChIP-seq) using DNA from LPS-activated B cells and an ERα antibody. Results revealed ER binding to a wide region of DNA, spanning sequences from the J_H cluster to Cδ, with peaks in Eμ and Sμ sites. Additional peaks of ERα binding were coincident with hs1,2 and hs4 sites in the 3′ regulatory region (3′RR) of the heavy chain locus. This first demonstration of direct binding of ER to key regulatory elements in the immunoglobulin locus supports our hypothesis that estrogen and other nuclear hormone receptors and ligands may

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directly influence antibody expression and CSR. Our hypothesis encourages the conduct of new experiments to evaluate the consequences of ER binding. A better understanding of ER:DNA interactions in the immunoglobulin heavy chain locus, and respective mechanisms, may ultimately translate to better control of antibody expression, better protection against pathogens, and prevention of pathologies caused by auto-immune disease.

Keywords

estrogen response elements; switch site; enhancers; immunoglobulin heavy chain locus; class switch recombination

1. INTRODUCTION

Past literature has revealed differences between the sexes with respect to B cell responses and antibody isotype expression patterns [1]. For example, in some populations, males exhibit higher IgA levels than females, and females exhibit higher IgM levels than males. In other studies, it has been shown that auto-immune diseases like systemic lupus erythematosus are sex-biased and associate with anti-self antibodies of particular isotypes. Estrogen (17-beta-estradiol) is implicated as a mediator of differences between B cells in males and females, because when estrogen supplements are tested *in vivo* or *in vitro*, changes in cytokine levels, B cell growth, and isotype expression patterns are readily apparent [2–17]

The isotype profile of a B cell population is the outcome of sophisticated mechanisms of B cell activation by foreign- or self-antigens. Depending on antigen triggers and the environment of activation, B cells will experience proliferation, cell maturation, somatic hyper-mutation (SHM), and/or class switch recombination (CSR)[18]. In a naïve B cell, the V-D-J gene sequence of an expressed antibody heavy chain is positioned upstream of Cμ, and Cδ, and both IgM and IgD isotypes can be transcribed/translated as a consequence of differential RNA splicing. When CSR is triggered, double strand DNA breaks occur in S regions upstream of donor and acceptor constant (C) region genes. Excision of a large DNA loop and non-homologous end joining between S region segments re-positions V-D-J genes upstream of a new C region gene to support IgG, IgE, or IgA production [19–21]. Enhancer regions upstream of Cμ (the intronic enhancer Eμ) and downstream of Cα (the 3′regulatory region [3′RR]) each influence the quality and magnitude of antibody expression [22,23].

How might estrogen alter the expression of antibody isotypes? Estrogen is classically known for its binding to estrogen receptor (ER), a type I member of the nuclear hormone receptor superfamily. ER modifies DNA accessibility to transcription machinery [24–26], a function that is altered by ligand binding [24–26]. Importantly, estrogen and ER regulate the expression of cytokines, the anti-apoptotic Bcl-2 protein, and AID, an enzyme required for CSR [27–29]. Although these influences may be sufficient to explain estrogen's impact on antibody isotype expression patterns, we asked if ER might also bind the immunoglobulin heavy chain locus directly. We performed chromatin immunoprecipitation (ChIP) analyses with DNA from activated B cells and showed that ER was bound to several DNA elements pertinent to CSR. Based on these data, we present our hypothesis that estrogen and ER

instruct the magnitude and quality of CSR and isotype expression in B cells by binding the immunoglobulin heavy chain locus. Such events may, in turn, define B cell responses to foreign- and self-antigens, explaining, at least in part, differences in disease outcomes between males and females.

2. MATERIALS AND METHODS

2.1 B cell purification

Single cell suspensions from C57Bl/6J mouse spleens were made by mechanically disrupting the tissues and passing through a 70 μm cell strainer. Lymphocytes were separated from erythrocytes by centrifugation on a cushion of Lymphocyte Separation Medium (MP Biomedicals). B cells were purified from the lymphocytes by negative selection with anti-CD43 and anti-CD11b microbeads (Miltenyi Biotec) and passing through a MACS LD Column (Miltenyi Biotec) followed by the elution of the unbound B cells.

2.2 Culture of B cells for ChIP analyses

Purified B cells were plated in a 96-well flat-bottomed tissue culture plate at a final concentration of 4×10^6 cells/ml in a volume of 200 μl/well in freshly prepared RPMI medium (Life Technologies) containing 10% fetal bovine serum (Atlanta Biologicals), 2mM L-glutamine (Life Technologies), 50 μg/ml gentamicin (Lonza), and 55 μM 2-mercaptoethanol (Life Technologies). LPS (Sigma) was added to a final concentration of 5 μg/ml and cultures were incubated overnight at 37°C in 5% CO_2.

2.3 Chromatin preparation for ChIP library

Cultured B cells were harvested and treated with 2 mM disuccinimidyl glutarate (DSG, ProteoChem) in DPBS (Lonza) with the following proteinase inhibitors (PIs); PMSF (Sigma), Pepstatin A (Sigma), and Leupeptin (Sigma). Cells were incubated at room temperature with rotation for 30 minutes. Cells were washed and fixed in DPBS plus PIs and 1% paraformaldehyde (Sigma) for 5 minutes with rotation at room temperature. The reaction was quenched by adding glycine to achieve a 200 mM final concentration and rotating an additional 5 minutes. The cell pellet was washed with DPBS plus PIs and then lysed in Covaris lysing buffer + PIs on ice for 10 minutes. Nuclei were centrifuged at 1500× g for 5 minutes and subjected to a series of washes in Covaris wash buffer and shearing buffer with PIs. The pellet was resuspended in Covaris shearing buffer plus PIs at a concentration of 1 ml per initial 2 X 10^7 cells and sheared in the Covaris E210 (Covaris) in Covaris MilliTubes under the following conditions, 200 cycles/burst, 20 W for 30 minutes. Sheared chromatin was diluted 1:3 with Covaris ChIP dilution buffer and immunoprecipitated with anti-estrogen receptor α antibody (Abcam #32063), 5 μg/2 X 10^7 cell equivalents, overnight with rotation at 4°C. Clean protein A/G magnetic beads were added at 20 μl/ml and incubated with rotation at 4°C for at least 1 hour. Magnetic beads were pelleted using a magnetic rack, and serially washed with a low-salt buffer, a high-salt buffer, a LiCl buffer, and TE buffer. After washing, the beads were resuspended in 130 μl sterile water and heated to 95°C for 10 minutes. NaCl was added to 80 mM final concentration with 10 μg total proteinase K (Ambion) added per tube. The sample was then incubated at 56°C for at least 1 hour. After incubation, the beads were heated to 95°C for 10 minutes and then allowed to cool to room

temperature. The beads were pelleted with the magnetic rack and supernatant was transferred to a new tube. DNA was purified with a PCR Purification Kit (Qiagen) and eluted in 30 μl RNA/DNAse-free water. Sample was submitted to the Hartwell Center at St. Jude for completion of library preparation and sequence analysis.

Libraries were prepared from approximately 10 ng DNA using the NEBNext ChIP-Seq Library Prep Reagent Set for Illumina with NEBNext Q5 Hot Start HiFi PCR Master Mix according to the manufacturer's instructions (New England Biolabs, Ipswich, MA) with the following modifications: a second 1:1 Ampure cleanup was added after adaptor ligation. The Ampure size selection step prior to PCR was eliminated. Completed libraries were analyzed for insert size distribution on a 2100 BioAnalyzer High Sensitivity kit (Agilent Technologies, Santa Clara, California) or Caliper LabChip GX DNA High Sensitivity Reagent Kit (PerkinElmer, Waltham, MA). Libraries were quantified using the Quant-iT PicoGreen ds DNA assay (Life Technologies), Kapa Library Quantification kit (Kapa Biosystems, Wilmington, MA), or low pass sequencing on a MiSeq nano kit (Illumina, San Diego, CA). Fifty cycle single end sequencing was performed on an Illumlina HiSeq 2000 or 2500.

Fifty base pair single-end reads were obtained. We first aligned the reads to mouse genome mm9 (MGSCv37 from Sanger) by BWA (version 0.5.9-r26-dev, default parameter). Then, duplicated reads were marked with Picard, version 1.65 (1160), and only non-duplicated reads were kept by samtools (parameter "-q 1-F 1024" version 0.1.18 (r982:295)). A cross-correlation plot was generated with the non-duplicated version of SPP (version 1.11) for quality control (QC), and the fragment size was estimated with support of R (version 2.14.0) with packages caTools (version 1.17) and bitops (version 1.0–6). All data passed QC following ENCODE criterion. Upon manual inspection of the cross-correlation plot generated by SPP, the best fragment size estimate (the smallest fragment size estimate by SPP in all cases) was used to extend each read to generate a bigwig file to view on IGV (version 2.3.40). We scaled the bigwig height by a factor normalized to 15M non-duplicated reads.

2.4 qPCR

Chromatin was prepared as described above for ChIP-seq. Samples were diluted for immunoprecipitation. The sample was divided into equal parts for Mouse IgG (Active Motif CHIP-IT Control qPCR Kit-Mouse, Cat#10226) and anti-ERα antibody (Abcam Cat #32063) immunoprecipitations. Input chromatin was precipitated with ethanol. DNA was purified as for ChIP analyses. PCR was performed with primer pairs for the Sμ and hs4. These primers were Sμ 6531: Forward = GAACGAGGTCCTAGCCTAACTG and Reverse = AGCAGAGCTGGGATGAACTG, and 3′RR hs4: Forward = CTCACTGATCCAGGGCCAAC and Reverse = GACTGAAACTCGGGGAACCA. The following qPCR conditions were used; 50°C for 2 minutes, 95°C for 10 minutes for 1 cycle, then 95°C for 15 seconds and 60°C for 1 minute for 40 cycles on a 7300 Realtime PCR (Applied Biosystems). Percent input values were calculated for signals from experiments with ERα and control antibodies, and unpaired T tests were used to identify significant differences.

2.5 Sequence scan for ERE

Mouse and human immunoglobulin heavy chain S regions were accessed from the NCBI Nucleotide database [30–33] for ERE searches (and highlighting) for the sequence G/AG/AT/CT/CG/AnnnTGAG/CC.

2.6 Access IDs

Access IDs for sequences and annotations included gene segments from the following: Human IgA1 : L19121.1, Human IgA2 : AF030305.1, Human IgE : X56797.1, Human IgG1 : U39737.1, Human IgG2 : U39934.1, Human IgG3 : U39935.1, Human IgG4 : X56796.1, Human IgM : X54713.1, Mouse IgA : D11468.1 (Sα 629-4806), Mouse IgE : M57385.1, Mouse IgG1 : M12389.2 (Sγ1 1-9235), Mouse IgG2a and IgG2b : D78344 (Sγ2a 44372-47089; Sγ2b 28278-32080), Mouse IgG3 : M12182.1, Mouse IgM: AC073553 (Sμ 136645-139845), 3′RR hs regions: AF450245.1.

3. RESULTS

Hormones and vitamins have long been considered indirect regulators of antibody expression patterns. As an example, they may modulate cytokine levels, which in turn modulate B cell activities. Experiments were designed to determine if the estrogen receptor, a type I nuclear hormone receptor, binds directly to the immunoglobulin heavy chain locus in regions critical to antibody expression and the CSR.

3.1 Hotspots for ERE in the switch regions of the immunoglobulin heavy chain locus

We previously scanned the murine and human immunoglobulin heavy chain switch regions for nuclear hormone receptor response elements, including ERE [34]. Hotspots for ERE were readily identified, particularly in Sμ, Sε, and Sα regions, but were relatively rare in Sγ regions. Patterns were similar for both mouse and human loci. Positions are illustrated in Figure 1 for the murine Sμ region, and in supplementary Figures 1–14 for additional S regions in murine and human loci.

3.2 ER binds Eμ, S regions, and the 3′RR in the immunoglobulin heavy chain locus of LPS-activated murine B cells

To determine if ERE hotspots were predictive of ER binding, we conducted ChIP with ERα antibody using DNA from purified, activated, murine B cells. This was accomplished by purifying B cells from C57BL/6 female mice by negative selection with CD11b- and CD43-specific antibodies. Cells were then stimulated overnight with LPS (after which most cells expressed IgM) and harvested. ChIP experiments were performed with ERα-specific antibodies. A ChIP-seq library was then prepared and analyzed as described in the Materials and Methods section. Indeed, as predicted by the identification of ERE hotspots in the Sμ region, we observed ER binding to Sμ. Also as expected, we observed ER binding to non-immunoglobulin loci such as *bcl-2*, consistent with findings from previous literature [3,9,35].

The detailed patterns of ERα binding in the murine heavy chain locus are illustrated in Figure 2. As shown, ER was bound to a region from the J_H locus through to Cμ and Cδ, with

peaks in Eμ (right-most peak) and Sμ. In addition, there was significant binding in the 3′RR downstream of the Cα coding segment. The highest peaks in the 3′RR corresponded to hypersensitive sites, hs1,2 and hs4. Of interest, unlike the the Sμ site, the 3′RR does not contain a large number of ERE, suggesting that in this region, ER may have bound non-canonical RE, or was tethered to other factors that bound DNA [25]. To our knowledge, this is the first demonstration of direct binding of ER to these key regulatory elements in immunoglobulin loci in activated B cells.

The binding observed in the ChIP-seq experiment was confirmed by ChIP-qPCR experiments using primers specific for Sμ and the 3′RR hs4. In each case, we conducted ChIP-qPCR experiments with samples taken from two different LPS-stimulated B cell cultures. For each of the primer sets, we found reproducible and significant increases in PCR signals (% input DNA) when ERα antibodies and IgG control antibodies were compared ($p<.01$ by unpaired T tests). An additional control was with unstimulated B cells, in which case ERα binding to the 3′RR hs4 region, but not the Sμ region, was evident. Results confirmed that ER could bind DNA elements pertinent to antibody expression and the CSR in the immunoglobulin heavy chain locus of activated B cells.

4. DISCUSSION

Results in this report support our hypothesis that ER may be directly involved in the machinery required for antibody expression and the CSR. ER binds to Sμ as predicted by our previous search for ERE, and also binds to Eμ and the 3′RR in activated B cells. In each case DNA-binding by ER might: (i) alter chromatin structure, (ii) alter loop formation, and/or (iii) recruit or inhibit the binding of other enzymes or factors pertinent to antibody expression.

Among the regulatory elements in the heavy chain locus, Eμ was the first enhancer to be discovered near constant region genes [36,37]. Additional enhancers were then identified among multiple DNase I hypersensitive sites in the 3′RR region [23]. These enhancers are characterized by repetitive switch-like regions and are bound by numerous factors including Pax5 and NF B. The 3′RR has been shown to interact in loop formation with Eμ, S regions, and promoters for the germ line transcripts (GLT) that coincide with CSR. When the 3′RR region is absent in mice, V-D-J joining capacity is retained, but mice express low levels of IgM. Mice lacking 3′RR are also deficient in SHM and CSR [23,38–40]. The 3′RR not only influences CSR outcome in normal animals, but a rearrangement involving the 3′RR can serve as a mechanism for B cell suicide [41–44]. Polymorphisms in the hs1,2 sequence associate with particular isotype expression patterns in humans, and risks of the female-predominant systemic lupus erythematosus [45]. Our finding that ER can bind to these key enhancer regions may provide a new explanation for estrogen’s influence on B cell responses, and the sex biases of autoimmunity.

Our results encourage studies to identify potential ER interactions with the plethora of factors known to associate with B cell-development, SHM, and CSR (e.g. AID, RNA polymerase, BATF, cohesin, Med 1, Med 12, histone-modifying enzymes, and the RNA exosome) [18,19,23,31,40,46–59]. ER should perhaps be studied in conjunction with other

members of the nuclear hormone receptor superfamily, as receptors can be promiscuous and cross-regulatory [26,60]. Of note, our previous research identified hotspots for nuclear hormone receptors of both types I and II (e.g. vitamin A-, vitamin D-, and thyroid receptors) in the heavy chain locus [34]. New experiments may help explain differences between male and female immune responses, and differences among individuals with variant levels of hormones and vitamins. Given that hormone and vitamin levels are integral to human health and are often perturbed by drug dosing (e.g. hormone replacement therapy, birth control pills, thyroid hormone supplements, or prednisone), a better understanding of all associated functions, health benefits, and health risks is much desired.

5. CONCLUSION

In conclusion, we discovered that ER binds to the immunoglobulin heavy chain locus in activated B cells at positions critical for antibody expression and CSR. Results support our hypothesis that nuclear hormone receptors directly influence machinery pertinent to antibody expression. ER binding (and the binding of other nuclear hormone receptors) to DNA may alter chromatin architecture, factor recruitment, and/or formation of DNA loops. Endogenous vitamin/hormone levels and drugs (e.g. birth-control pills, hormone replacement therapy, thyroxine, prednisone, vitamin supplements) may thus influence B cell responses to self- and foreign-antigens. Studies are now warranted to evaluate mechanisms by which vitamins/hormones may influence antibody expression machinery. These mechanisms might then be employed to diagnose and correct conditions of B cell malfunction and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CSR	class switch recombination
V	variable region
D	diversity region
J	joining region
S	switch region
C	constant region
AID	activation induced cytidine deaminase

ER	estrogen receptor
ERE	estrogen receptor response element
hs	hypersensitive site

References

1. Reardon S. Infections reveal inequality between the sexes. Nature. 2016; 534:447. [PubMed: 27337319]
2. Dema B, Pellefigues C, Hasni S, Gault N, Jiang C, Ricks TK, et al. Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis. PloS one. 2014; 9:e90424. [PubMed: 24587356]
3. Bynoe MS, Grimaldi CM, Diamond B. Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. Proc Natl Acad Sci U S A. 2000; 97:2703–2708. [PubMed: 10694576]
4. Dostal-Johnson D, Rote NS, Branch DW. IgG1 and IgG2 are the predominant subclasses of antiphospholipid antibody in women with the lupus anticoagulant. Clin Immunol Immunopathol. 1990; 54:309–319. [PubMed: 2104789]
5. Fan H, Dong G, Zhao G, Liu F, Yao G, Zhu Y, et al. Gender differences of B cell signature in healthy subjects underlie disparities in incidence and course of SLE related to estrogen. J Immunol Res. 2014; 2014:814598. [PubMed: 24741625]
6. Kanda N, Tsuchida T, Tamaki K. Estrogen enhancement of anti-double-stranded DNA antibody and immunoglobulin G production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Arthritis Rheum. 1999; 42:328–337. [PubMed: 10025928]
7. Villalta D, Bizzaro N, Bassi N, Zen M, Gatto M, Ghirardello A, et al. Anti-dsDNA antibody isotypes in systemic lupus erythematosus: IgA in addition to IgG anti-dsDNA help to identify glomerulonephritis and active disease. PloS one. 2013; 8:e71458. [PubMed: 23951169]
8. Modica MA, Freddi S, Caruso C. Blood IgA, IgM and IgE levels are influenced by sex and HLA phenotype. Exp Clin Immunogenet. 1989; 6:251–257. [PubMed: 2488685]
9. Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B. Estrogen alters thresholds for B cell apoptosis and activation. The Journal of clinical investigation. 2002; 109:1625–1633. [PubMed: 12070310]
10. Quinn MA, Cidlowski JA. Endogenous hepatic glucocorticoid receptor signaling coordinates sex-biased inflammatory gene expression. FASEB J. 2015
11. Markle JG, Fish EN. SeXX matters in immunity. Trends Immunol. 2014; 35:97–104. [PubMed: 24239225]
12. Tuero I, Mohanram V, Musich T, Miller L, Vargas-Inchaustegui DA, Demberg T, et al. Mucosal B Cells Are Associated with Delayed SIV Acquisition in Vaccinated Female but Not Male Rhesus Macaques Following SIVmac251 Rectal Challenge. PLoS Pathog. 2015; 11:e1005101. [PubMed: 26267144]
13. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis. 2010; 10:338–349. [PubMed: 20417416]
14. Whitacre CC. Sex differences in autoimmune disease. Nature immunology. 2001; 2:777–780. [PubMed: 11526384]
15. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nature reviews Immunology. 2008; 8:737–744.
16. Klein SL, Poland GA. Personalized vaccinology: one size and dose might not fit both sexes. Vaccine. 2013; 31:2599–2600. [PubMed: 23579257]
17. Ghazeeri G, Abdullah L, Abbas O. Immunological differences in women compared with men: overview and contributing factors. Am J Reprod Immunol. 2011; 66:163–169. [PubMed: 21749547]
18. Stavnezer J, Schrader CE. IgH chain class switch recombination: mechanism and regulation. Journal of immunology. 2014; 193:5370–5378.

19. Kumari G, Sen R. Chromatin Interactions in the Control of Immunoglobulin Heavy Chain Gene Assembly. Adv Immunol. 2015; 128:41–92. [PubMed: 26477365]
20. Guo C, Gerasimova T, Hao H, Ivanova I, Chakraborty T, Selimyan R, et al. Two forms of loops generate the chromatin conformation of the immunoglobulin heavy-chain gene locus. Cell. 2011; 147:332–343. [PubMed: 21982154]
21. Chaudhuri J, Alt FW. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. Nature reviews Immunology. 2004; 4:541–552.
22. Ariizumi K, Wang Z, Tucker PW. Immunoglobulin heavy chain enhancer is located near or in an initiation zone of chromosomal DNA replication. Proc Natl Acad Sci U S A. 1993; 90:3695–3699. [PubMed: 8475117]
23. Birshtein BK. Epigenetic Regulation of Individual Modules of the immunoglobulin heavy chain locus 3′ Regulatory Region. Frontiers in immunology. 2014; 5:163. [PubMed: 24795714]
24. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, et al. The nuclear receptor superfamily: the second decade. Cell. 1995; 83:835–839. [PubMed: 8521507]
25. Klinge CM. Estrogen receptor interaction with estrogen response elements. Nucleic Acids Res. 2001; 29:2905–2919. [PubMed: 11452016]
26. Evans RM, Mangelsdorf DJ. Nuclear Receptors, RXR, and the Big Bang. Cell. 2014; 157:255–266. [PubMed: 24679540]
27. Pauklin S, Sernandez IV, Bachmann G, Ramiro AR, Petersen-Mahrt SK. Estrogen directly activates AID transcription and function. The Journal of experimental medicine. 2009; 206:99–111. [PubMed: 19139166]
28. Pauklin S, Petersen-Mahrt SK. Progesterone inhibits activation-induced deaminase by binding to the promoter. Journal of immunology. 2009; 183:1238–1244.
29. Maul RW, Gearhart PJ. Women, autoimmunity, and cancer: a dangerous liaison between estrogen and activation-induced deaminase? The Journal of experimental medicine. 2009; 206:11–13. [PubMed: 19139165]
30. Pan-Hammarstrom Q, Zhao Y, Hammarstrom L. Class switch recombination: a comparison between mouse and human. Adv Immunol. 2007; 93:1–61. [PubMed: 17383538]
31. Wu X, Stavnezer J. DNA polymerase beta is able to repair breaks in switch regions and plays an inhibitory role during immunoglobulin class switch recombination. The Journal of experimental medicine. 2007; 204:1677–1689. [PubMed: 17591858]
32. Mills FC, Brooker JS, Camerini-Otero RD. Sequences of human immunoglobulin switch regions: implications for recombination and transcription. Nucleic Acids Res. 1990; 18:7305–7316. [PubMed: 2124350]
33. Mills FC, Mitchell MP, Harindranath N, Max EE. Human Ig S gamma regions and their participation in sequential switching to IgE. Journal of immunology. 1995; 155:3021–3036.
34. Hurwitz JL, Penkert RR, Xu B, Fan Y, Partridge JF, Maul RW, et al. Hotspots for Vitamin-Steroid-Thyroid Hormone Response Elements Within Switch Regions of Immunoglobulin Heavy Chain Loci Predict a Direct Influence of Vitamins and Hormones on B Cell Class Switch Recombination. Viral Immunol. 2016; 29:132–136. [PubMed: 26741514]
35. Dong L, Wang W, Wang F, Stoner M, Reed JC, Harigai M, et al. Mechanisms of transcriptional activation of bcl-2 gene expression by 17beta-estradiol in breast cancer cells. The Journal of biological chemistry. 1999; 274:32099–32107. [PubMed: 10542244]
36. Sakai E, Bottaro A, Alt FW. The Ig heavy chain intronic enhancer core region is necessary and sufficient to promote efficient class switch recombination. Int Immunol. 1999; 11:1709–1713. [PubMed: 10508189]
37. Lennon GG, Perry RP. C mu-containing transcripts initiate heterogeneously within the IgH enhancer region and contain a novel 5′-nontranslatable exon. Nature. 1985; 318:475–478. [PubMed: 3934561]
38. Zaller DM, Eckhardt LA. Deletion of a B-cell-specific enhancer affects transfected, but not endogenous, immunoglobulin heavy-chain gene expression. Proc Natl Acad Sci U S A. 1985; 82:5088–5092. [PubMed: 3927296]
39. Su LK, Kadesch T. The immunoglobulin heavy-chain enhancer functions as the promoter for I mu sterile transcription. Molecular and cellular biology. 1990; 10:2619–2624. [PubMed: 2111440]

40. Chatterjee S, Ju Z, Hassan R, Volpi SA, Emelyanov AV, Birshtein BK. Dynamic changes in binding of immunoglobulin heavy chain 3′ regulatory region to protein factors during class switching. The Journal of biological chemistry. 2011; 286:29303–29312. [PubMed: 21685395]
41. Garot A, Marquet M, Saintamand A, Bender S, Le Noir S, Rouaud P, et al. Sequential activation and distinct functions for distal and proximal modules within the IgH 3′ regulatory region. Proc Natl Acad Sci U S A. 2016; 113:1618–1623. [PubMed: 26831080]
42. Peron S, Laffleur B, Denis-Lagache N, Cook-Moreau J, Tinguely A, Delpy L, et al. AID-driven deletion causes immunoglobulin heavy chain locus suicide recombination in B cells. Science. 2012; 336:931–934. [PubMed: 22539552]
43. Morvan CL, Pinaud E, Decourt C, Cuvillier A, Cogne M. The immunoglobulin heavy-chain locus hs3b and hs4 3′ enhancers are dispensable for VDJ assembly and somatic hypermutation. Blood. 2003; 102:1421–1427. [PubMed: 12714490]
44. Pinaud E, Khamlichi AA, Le Morvan C, Drouet M, Nalesso V, Le Bert M, et al. Localization of the 3′ IgH locus elements that effect long-distance regulation of class switch recombination. Immunity. 2001; 15:187–199. [PubMed: 11520455]
45. Frezza D, Tolusso B, Giambra V, Gremese E, Marchini M, Nowik M, et al. Polymorphisms of the IgH enhancer HS1.2 and risk of systemic lupus erythematosus. Ann Rheum Dis. 2012; 71:1309–1315. [PubMed: 22294636]
46. Thomas-Claudepierre AS, Schiavo E, Heyer V, Fournier M, Page A, Robert I, et al. The cohesin complex regulates immunoglobulin class switch recombination. The Journal of experimental medicine. 2013; 210:2495–2502. [PubMed: 24145512]
47. Kenter AL, Kumar S, Wuerffel R, Grigera F. AID hits the jackpot when missing the target. Curr Opin Immunol. 2016; 39:96–102. [PubMed: 26845615]
48. Montefiori L, Wuerffel R, Roqueiro D, Lajoie B, Guo C, Gerasimova T, et al. Extremely Long-Range Chromatin Loops Link Topological Domains to Facilitate a Diverse Antibody Repertoire. Cell Rep. 2016; 14:896–906. [PubMed: 26804913]
49. Kenter AL, Feldman S, Wuerffel R, Achour I, Wang L, Kumar S. Three-dimensional architecture of the IgH locus facilitates class switch recombination. Ann N Y Acad Sci. 2012; 1267:86–94. [PubMed: 22954221]
50. Kenter AL. AID targeting is dependent on RNA polymerase II pausing. Semin Immunol. 2012; 24:281–286. [PubMed: 22784681]
51. Thomas-Claudepierre AS, Robert I, Rocha PP, Raviram R, Schiavo E, Heyer V, et al. Mediator facilitates transcriptional activation and dynamic long-range contacts at the IgH locus during class switch recombination. The Journal of experimental medicine. 2016; 213:303–312. [PubMed: 26903242]
52. Basu U, Meng FL, Keim C, Grinstein V, Pefanis E, Eccleston J, et al. The RNA exosome targets the AID cytidine deaminase to both strands of transcribed duplex DNA substrates. Cell. 2011; 144:353–363. [PubMed: 21255825]
53. Pefanis E, Wang J, Rothschild G, Lim J, Kazadi D, Sun J, et al. RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity. Cell. 2015; 161:774–789. [PubMed: 25957685]
54. Maul RW, Saribasak H, Martomo SA, McClure RL, Yang W, Vaisman A, et al. Uracil residues dependent on the deaminase AID in immunoglobulin gene variable and switch regions. Nature immunology. 2011; 12:70–76. [PubMed: 21151102]
55. Rajagopal D, Maul RW, Ghosh A, Chakraborty T, Khamlichi AA, Sen R, et al. Immunoglobulin switch mu sequence causes RNA polymerase II accumulation and reduces dA hypermutation. The Journal of experimental medicine. 2009; 206:1237–1244. [PubMed: 19433618]
56. Keim C, Kazadi D, Rothschild G, Basu U. Regulation of AID, the B-cell genome mutator. Genes & development. 2013; 27:1–17. [PubMed: 23307864]
57. Bonaud A, Lechouane F, Le Noir S, Monestier O, Cogne M, Sirac C. Efficient AID targeting of switch regions is not sufficient for optimal class switch recombination. Nat Commun. 2015; 6:7613. [PubMed: 26146363]

58. Ise W, Kohyama M, Schraml BU, Zhang T, Schwer B, Basu U, et al. The transcription factor BATF controls the global regulators of class-switch recombination in both B cells and T cells. Nature immunology. 2011; 12:536–543. [PubMed: 21572431]

59. Romanello M, Schiavone D, Frey A, Sale JE. Histone H3.3 promotes IgV gene diversification by enhancing formation of AID-accessible single-stranded DNA. The EMBO journal. 2016; 35:1452–1464. [PubMed: 27220848]

60. Bugge TH, Pohl J, Lonnoy O, Stunnenberg HG. RXR alpha, a promiscuous partner of retinoic acid and thyroid hormone receptors. The EMBO journal. 1992; 11:1409–1418. [PubMed: 1314167]

HIGHLIGHTS

1. Hotspots for estrogen receptor response elements (ERE) exist in Sμ, Sε, and Sα regions within murine and human immunoglobulin heavy chain loci.
2. ERE are rare within all Sγ regions in immunoglobulin heavy chain loci of mice and humans.
3. A chromatin immunoprecipitation (ChIP-seq) experiment using purified female mouse B cells stimulated for 1 day with LPS revealed binding of estrogen receptor α (ERα) to the Sμ region in the immunoglobulin heavy chain locus.
4. Additional peaks of binding were coincident with the intronic enhancer Eμ and the 3′regulatory region (3′RR), particularly hs1,2 and hs4 DNA hypersensitivity sites.
5. The finding that nuclear hormone receptors interact with S and regulatory regions pertinent to immunoglobulin gene expression and class switch recombination (CSR) in activated B cells suggests that there may be a direct influence of estrogen and other hormones or vitamins on antibody expression patterns.

Figure 1. Hotspots for ERE in the Sμ switch site of the immunoglobulin heavy chain locus

The sequence G/AG/AT/CT/CG/AnnnTGAG/CC was highlighted within the Sμ region of the mus musculus genome. Sequences are highlighted in purple.

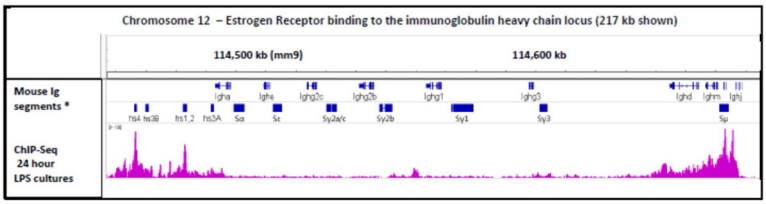

Figure 2. ERα binding in the immunoglobulin heavy chain locus

Results of Chip-Seq with the ERα antibody in the immunoglobulin heavy chain locus are shown.