Molecular Immunology 77 (2016) 97–102

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

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

Bart G. Jones[a], Rhiannon R. Penkert[a], Beisi Xu[b], Yiping Fan[b], Geoff Neale[c], Patricia J. Gearhart[d], Julia L. Hurwitz (PhD)[a,e,*]

[a] Department of Infectious Diseases, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA
[b] Department of Computational Biology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA
[c] Hartwell Center, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA
[d] Laboratory of Molecular Biology and Immunology, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA
[e] Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN 38163, USA

ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form 17 July 2016
Accepted 21 July 2016
Available online 3 August 2016

Keywords:
Estrogen response elements
Switch site
Enhancers
Immunoglobulin heavy chain locus
Class switch recombination

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 estrogen 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-specific 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 directly influence antibody expression and class switch recombination (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.

1. Introduction

Past literature has revealed differences between the sexes with respect to B cell responses and antibody isotype expression patterns (Reardon, 2016). 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 autoimmune 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 (Dema et al., 2014; Bynoe et al., 2000; Dostal-Johnson et al., 1990; Fan et al., 2014; Kanda et al., 1999; Villalta et al., 2013; Modica et al., 1989; Grimaldi et al., 2002; Quinn and Cidlowski, 2016; Markle and Fish, 2014; Tuero et al., 2015; Klein et al., 2010; Whitacre, 2001; Fish, 2008; Klein and Poland, 2013; Ghazeeri et al., 2011).

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

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 response element; hs, hypersensitive site.

* Corresponding author at: Department of Infectious Diseases, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA.
E-mail address: julia.hurwitz@stjude.org (J.L. Hurwitz).

http://dx.doi.org/10.1016/j.molimm.2016.07.015
0161-5890/

activation, B cells will experience proliferation, cell maturation, somatic hyper-mutation (SHM), and/or class switch recombination (CSR) (Stavnezer and Schrader, 2014). 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 donor and acceptor S regions upstream of 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 (Kumari and Sen, 2015; Guo et al., 2011; Chaudhuri and Alt, 2004). 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 (Ariizumi et al., 1993; Birshtein, 2014).

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, a function that is altered by ligand binding (Mangelsdorf et al., 1995; Klinge, 2001; Evans and Mangelsdorf, 2014). Importantly, estrogen and ER regulate the expression of cytokines, the anti-apoptotic Bcl-2 protein, and AID, an enzyme required for CSR (Pauklin et al., 2009; Pauklin and Petersen-Mahrt, 2009; Maul and Gearhart, 2009). 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), 2 mM 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 min. Cells were washed and fixed in DPBS plus PIs and 1% paraformaldehyde (Sigma) for 5 min with rotation at room temperature. The reaction was quenched by adding glycine to achieve a 200 mM final concentration and rotating an additional 5 min. The cell pellet was washed with DPBS plus PIs and then lysed in Covaris lysing buffer + PIs on ice for 10 min. Nuclei were centrifuged at $1500 \times g$ for 5 min 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 2×10^7 cells and sheared in the Covaris E210 (Covaris) in Covaris MilliTubes under the following conditions, 200 cycles/burst, 20 W for 30 min. Sheared chromatin was diluted 1:3 with Covaris ChIP dilution buffer and immunoprecipitated with anti-estrogen receptor α antibody (Abcam #32063), 5 μg/2×10^7 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 h. 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 min. 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 h. After incubation, the beads were heated to 95 °C for 10 min 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) 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) or Caliper LabChip GX DNA High Sensitivity Reagent Kit (PerkinElmer). Libraries were quantified using the Quant-iT PicoGreen ds DNA assay (Life Technologies), Kapa Library Quantification kit (Kapa Biosystems), or low pass sequencing on a MiSeq nano kit (Illumina). 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 criteria. 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.