Cellular Immunology 346 (2019) 103996

Contents lists available at ScienceDirect

Cellular Immunology

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

Matters of life and death: How estrogen and estrogen receptor binding to the immunoglobulin heavy chain locus may influence outcomes of infection, allergy, and autoimmune disease

Bart G. Jones[a], Rhiannon R. Penkert[a], Sherri L. Surman[a], Robert E. Sealy[a], Stephane Pelletier[b], Beisi Xu[c], Geoff Neale[d], Robert W. Maul[e], Patricia J. Gearhart[e], J.L. Hurwitz[a,f,*]

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

ABSTRACT

Sex hormones are best known for their influences on reproduction, but they also have profound influences on the immune response. Examples of sex-specific differences include: (i) the relatively poor control of influenza virus infections in males compared to females, (ii) allergic asthma, an IgE-associated hypersensitivity reaction that is exacerbated in adolescent females compared to males, and (iii) systemic lupus erythematosus, a life-threatening autoimmune disease with a 9:1 female:male bias. Here we consider how estrogen and estrogen receptor α (ERα) may influence the immune response by modifying class switch recombination (CSR) and immunoglobulin expression patterns. We focus on ERα binding to enhancers (Eμ and the 3′ regulatory region) and switch sites (Sμ and Sε) in the immunoglobulin heavy chain locus. Our preliminary data from ChIP-seq analyses of purified, activated B cells show estrogen-mediated changes in the positioning of ERα binding within and near Sμ and Sε. In the presence of estrogen, ERα is bound not only to estrogen response elements (ERE), but also to adenosine-cytidine (AC)-repeats and poly adenosine (poly A) sequences, in some cases within constant region gene introns. We propose that by binding these sites, estrogen and ERα directly participate in the DNA loop formation required for CSR. We further suggest that estrogen regulates immunoglobulin expression patterns and can thereby influence life-and-death outcomes of infection, hypersensitivity, and autoimmune disease.

1. Estrogen, a nuclear hormone

Estrogen is a nuclear hormone that is best known for its influence on reproductive organs, but is also critical for the development and function of cardiovascular, skeletal, nervous, and immune systems [1–8]. Estrogen signals at both intra- and extra-nuclear sites [9], but we will focus here on its intra-nuclear functions.

Estrogen is a ligand for estrogen receptors (ER) α and β, which are type I nuclear hormone receptors with DNA binding sites throughout the mammalian genome [9–11]. The receptors are frequently associated with promoters, enhancers, DNA loops, and complex protein-DNA interactions [11–13].

Nuclear hormone receptors have multiple, structurally distinct domains including an N-terminal domain with activation function (AF-1), a central DNA-binding domain (DBD), and a carboxyl-terminal ligand binding domain (LBD) with ligand-dependent function (AF-2). ER binds DNA best as a dimer, but monomer binding is also observed [14,15]. Receptor functions are regulated by ligand binding (e.g. with estradiol or tamoxifen), DNA targets, and interactions with other transcription factors [11].

Typically, two ER monomers bind cooperatively with a consensus ERE (GGTCANNNTGACC), but binding is promiscuous and ER can also bind non-consensus sites, in some cases tethered by other proteins. In fact, a large fraction of ER binding sites (and other nuclear hormone

Abbreviations: ER, estrogen receptor; AID, activation induced deaminase, AC-repeat, cytidine-adenosine repeat; S, switch site; RNA Pol II, RNA polymerase II; CSR, class switch recombination; LPS, lipopolysaccharide; LPS + E, culture condition with estrogen added to LPS; V, variable; D, diversity; J, joining; C, constant; SERM, Selective estrogen receptor modulator; hnRNP, heterogeneous nuclear ribonucleoprotein; HS, (DNase I) hypersensitive site; SLE or 'lupus', systemic lupus erythematosus; IGV, Integrative Genomics Viewer; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein-9 nuclease; ChIP, chromatin immunoprecipitation

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

https://doi.org/10.1016/j.cellimm.2019.103996
Received 16 September 2019; Received in revised form 20 October 2019; Accepted 24 October 2019
Available online 25 October 2019
0008-8749/ © 2019 Elsevier Inc. All rights reserved.

binding sites) in the mammalian genome do not conform to the consensus motif [11,16,17]. Ligand binding directly influences transcriptional regulation by altering ER-DNA conformations and associations with other transcription factors. For example, research using an estrogen-responsive pS2 promoter showed that unliganded ER (apo ER) could bind and repress transcription, and that the addition of estrogen resulted in conformational changes that supported RNA Polymerase II (RNA Pol II) recruitment and improved gene expression [18–21]. Outcomes of interactions between ER and other transcription factors may be enhancing or antagonistic, as molecules may synergize or compete for ligands and DNA binding sites [22].

2. Benefits and risks of high estrogen levels

In mice and humans, immune responses of males and females differ. For example, studies of rodents and humans show sex-specific differences in antibody isotype expression patterns in sera. Overall, females tend to express higher levels of immunoglobulins compared to males (often including IgM, IgG1, IgG2, and/or IgE [23,24]). In small animal studies, the treatment of males with estrogen was shown to up-regulate immunoglobulin levels and antigen-specific antibody-producing cells [25,26]. In humans, estrogen levels correlated with IgG responses toward an influenza virus vaccine [27]. In *ex vivo* cultures of mouse splenocytes, estrogen increased levels of IgM and IgE [28,29].

Differences in immunoglobulin levels between the sexes help explain, at least in part, why adult females gain better control of certain infectious diseases compared to males [30,31]. This benefit for females is not without risk; females suffer more than males from hyper-immune responses toward typically harmless substances or self-antigens [32–36]. Consequences can include life-threatening episodes of allergic asthma or systemic lupus erythematosus (SLE, lupus), particularly among pregnant women [37–43] in whom estrogen levels can soar to > 6000 pg/ml (as compared to the ≤ 100 pg/ml level typical of an adult male) [44,45]. In a mouse model, supplemental estrogen was shown to increase antibody levels against self-antigens including cardiolipin [5,6]. In a separate model, an estrogen inhibitor was shown to reduce levels of allergen-specific IgG1, IgG2a and IgE, as well as clinical disease symptoms [46].

As stated above, the influences of estrogen on immune responses depend on the environment and cross-regulation by other nuclear hormones [47–57]. For example, in a study of C57BL/6 mice, we found that IgG2b levels were higher in females compared to males, but when mice were deficient in vitamin A (a hormone bound by type II nuclear hormone receptors), IgG2b levels rose in male mice and exceeded the IgG2b levels of females [24].

Because estrogen affects many cell types, there are multiple explanations for sex differences in immunity and disease. For example, estrogen may upregulate interleukins that can stabilize B cells [58]. Estrogen also upregulates the expression of activation induced deaminase (AID) in B cells [59,60]. Below, we focus on ERα binding to the immunoglobulin heavy chain locus to highlight a direct mechanism by which estrogen may influence immunoglobulin production and downstream consequences.

3. ERα and the immunoglobulin heavy chain locus

B cells initially produce IgM by transcribing V-D-J-Cμ heavy chain gene segments. Upon B cell activation, isotypes can switch (e.g. from IgM to IgE) by positioning V-D-J upstream of a different constant region gene (e.g. Cε). This class switch recombination (CSR) event involves double stranded DNA breaks at switch (S) sites (e.g. Sμ and Sε) upstream of target C genes followed by ligation of one S region to the other and deletion of intervening sequences. CSR can occur either directly or in succession (IgM to IgE or IgM to IgG to IgE) [61–66].

The process of CSR commences with the production of sterile transcripts, initiated in or near donor and recipient S sites [67–70], a process that creates R loops and exposes single stranded DNA to additional enzyme activity. After splicing, sterile transcripts retain a short intervening exon and a C gene exon (e.g. Iμ-Cμ and Iε-Cε). RNA Pol II stalls during transcription and recruits AID, which converts dC to dU [65,68–70]. Downstream events can include the removal of dU by uracil DNA glycosylase (UNG), DNA cleavage by abasic endonucleases, and ligation of donor and recipient S regions by non-homologous end-joining machinery.

Enhancers that influence the quality and quantity of CSR and antibody expression include Eμ (located upstream of Iμ) and multiple DNase I hypersensitivity sites (including HS1,2) located in the 3′ regulatory region (3′RR) downstream of Cα [71–73]. During CSR, DNA loops integrate enhancers, promoters and S sites [69,74–76].

We previously questioned whether ERα might directly influence CSR and antibody expression and therefore interrogated the immunoglobulin heavy chain locus for ERE. We then discovered hotspots for ERE in S regions including Sμ and Sε [77]. Using ChIP analyses, we next demonstrated that ERα and RNA Pol II exhibited strikingly similar binding patterns within the locus. Sites of peak binding included enhancer sequences Eμ and HS1,2 of the 3′ RR [67,78–80]. When supplemental estrogen was added to purified, activated B cell cultures, ERα and RNA Pol II shifted DNA binding positions synchronously [24]. We additionally discovered that in the presence of estrogen, ERα binding was focused not just on consensus ERE, but on sites containing AC-repeats [78,80] near enhancer regions. It is likely that ERα binding to AC-repeat sequences assists the DNA loop formation that is essential for integrating enhancers, promoters and S sites during CSR. We additionally found that ERE knock-out, either within Eμ or HS1,2 sequences of a B cell line, was sufficient to significantly reduce CSR [78]. The HS1,2 site is of particular interest to us, because it marks a site of polymorphism associated with an unusually high frequency of lupus in humans [81].

Given the differences noted in IgE production and related allergic reactions between males and females (described above), we next focused on IgM and IgE S sites (Sμ and Sε), as these must be cleaved to support the IgM to IgE switch. As a preliminary analysis, we aligned sequences from the previously described ChIP libraries from activated, purified B cells stimulated with lipopolysaccharide (LPS) or LPS plus estrogen (LPS + E) [24,79]. ERα binding within the Sμ region is shown in Fig. 1. This region is a hotspot for ERE (RRYYRNNNTGANY, mapped using IGV software) [77]). In both LPS and LPS + E cultures, there was ERα binding to Eμ (enhancer/promoter) [67,68], Iμ, and Sμ regions, but ERα appeared to be better focused on the Sμ site in the LPS + E culture. Binding peaks were not always coincident with ERE, but in the LPS + E library, peaks were usually aligned either with an ERE or with an AC-repeat or poly A sequence. In the LPS + E culture, ERα preferentially bound to intron rather than exon regions of Cμ and Cδ. Perhaps ERα influences patterns of mRNA splicing, as DNA conformations are known to instruct RNA splicing and certain heterogeneous nuclear ribonucleoproteins (hnRNPs) are known to bind AC-repeats [82–87].

Next, we examined the Sε region (Fig. 2). Again, in the LPS + E library, ERα bound AC-repeats and poly A sequences downstream of the S site. Based on the Sμ result, one might have expected that ERα would also bind Sε in the LPS + E culture, but this was not the case. There was in fact no signal observed within the Sε site. A unique NFKB binding motif (GGGGTTCC) marked the promoter region for sterile Iε-Cε transcription [61,63,64,88,89], but ERα binding was also absent in this position in the LPS + E library, indicating another estrogen-induced ERα binding shift.

Presently, it is not clear if the negative binding of ERα in Sε indicates the complete absence of ERα at this site (or a change in configuration that artifactually inhibits ERα immunoprecipitation by the ChIP antibody). It also remains to be determined whether estrogen-driven changes in ERα patterns are transient or durable, and how a positive ERα ChIP signal in Sμ and/or Sε might predict frequencies of

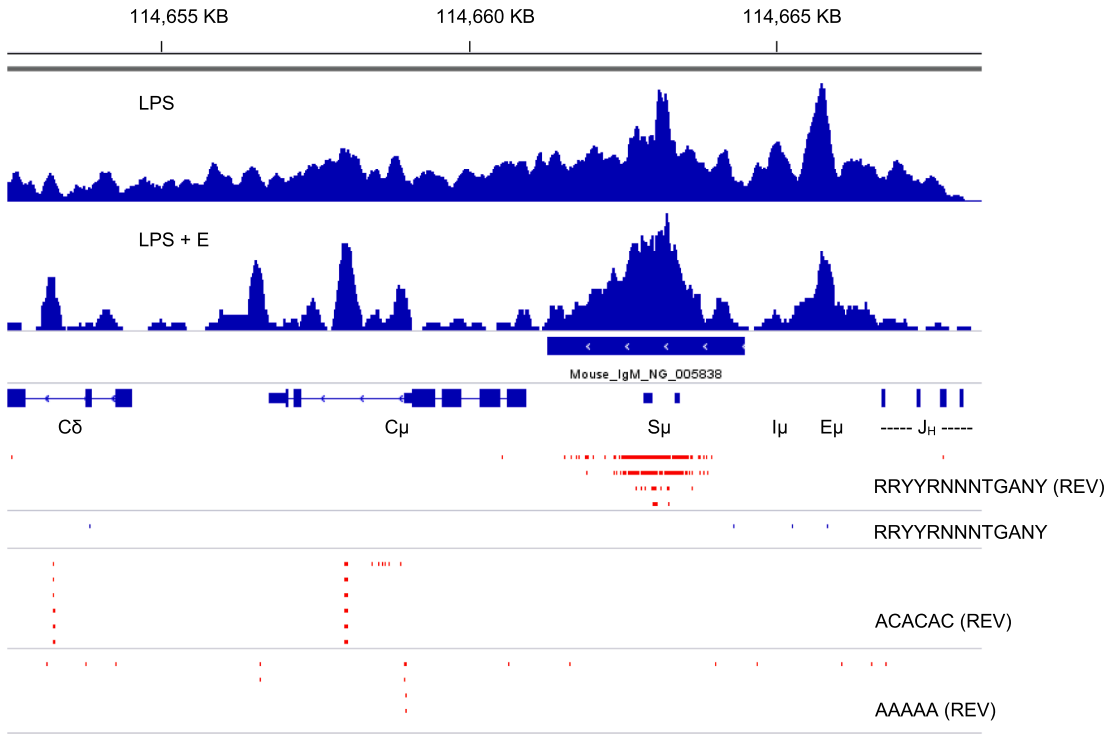

Fig. 1. Estrogen receptorα (ERα) binding in and near Sμ. ChIP-seq libraries were produced from purified B cells one day after stimulation with LPS or LPS + 100 nM estrogen (LPS + E) [24]. Binding patterns were evaluated using IGV software. Positions for J_H, Eμ, Iμ, Sμ, Cμ and a portion of Cδ are shown in murine chromosome 12 (using the mm9 sequence). Estrogen response elements (ERE, RRYYRNNNTGANY), AC-repeats (ACACAC) and poly A (AAAAA) sequences are mapped. Oligonucleotides were mapped in a 5′ – 3′ orientation from left to right, unless labeled 'rev', in which case orientations were from right to left.

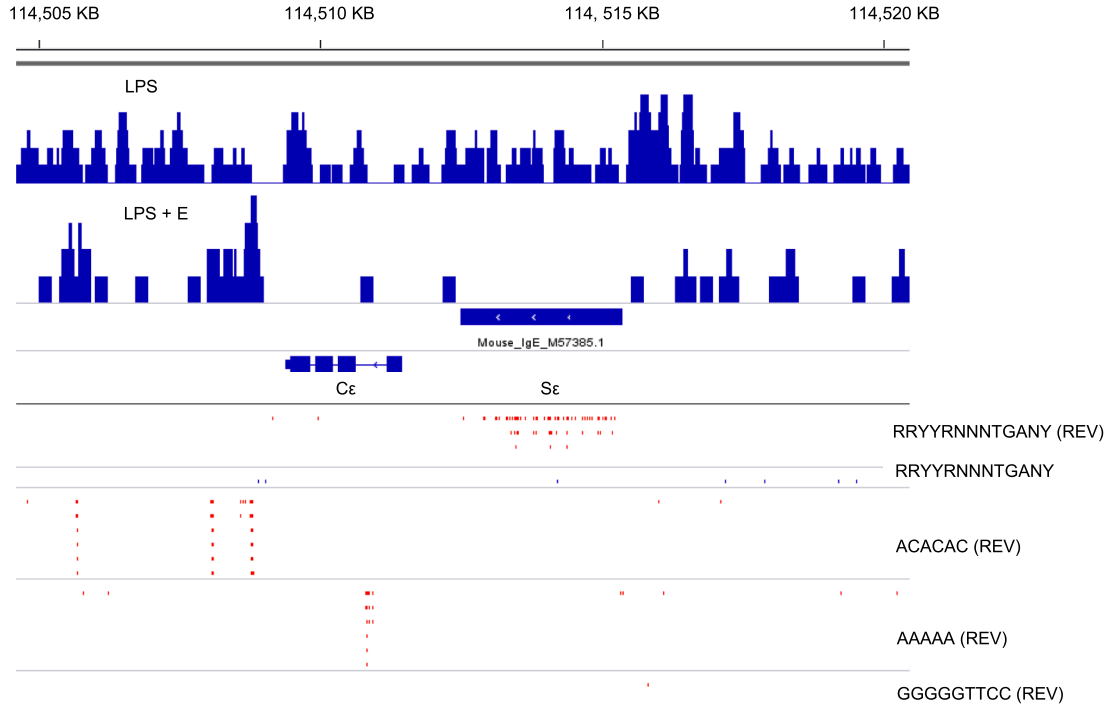

Fig. 2. ERα binding in and near Sε. As described in Fig. 1, ChIP-seq libraries were produced from purified B cells one day after stimulation with LPS or LPS + 100 nM estrogen (LPS + E). Positions for Sε, Cε are shown (mm9). A unique NFKB site (GGGGGTTCC) marks the promoter region for Iε-Cε sterile transcripts. ERE, AC-repeats and poly A sequences are mapped as described in the Fig. 1 legend.

CSR and IgE expression. Nonetheless, evidence of estrogen-driven changes in Sμ and Sε switchosomes (proteins associated with S sites) lend credence to the hypothesis that estrogen directly modifies CSR and isotype expression. In addition to its partnership with RNA Pol II [12,90,91], ER is also known to recruit topoisomerase IIβ to DNA. This topoisomerase associates with poly (adenosine diphosphate-ribose) polymerase-1 enzymatic activity and generates doubled stranded DNA breaks [92], perhaps supplementing the AID/UNG-mediated cleavage mechanism described above. Further experimentation is encouraged to test hypotheses and provide details of how estrogen and ERα binding to the heavy chain locus influences immunoglobulin output among activated B cells.

4. May clinical manipulations of estrogen and ERα-DNA binding patterns improve disease outcomes?

Attempts have been made to treat certain diseases by modifying estrogen levels. Patients may receive estrogen supplements (hormone replacement therapy) or inhibitors. Difficulties are associated with each of these treatment options, because treatments are not well focused on a particular cell type or function. Tamoxifen (a selective estrogen receptor modulator, SERM), for example, is used widely as an antagonist to treat breast cancers, but can act as an agonist in other target tissues [9]. In post-menopausal women who suffer an increased predisposition to infections and impaired vaccine-induced immune responses, the effect of estrogen replacement therapy is a topic of continued debate [93–98].

Today, gene therapy offers an opportunity to focus treatments. New technologies provide researchers with a variety of methods for blocking protein interactions with DNA or RNA (e.g. using CRISPR-CAS9, antisense oligonucleotides [ASO], and/or DNA decoys) [99–113]. We consider that targeted manipulations of ERα binding within enhanceosomes [13] or switchosomes may (i) improve antibody activities when pathogen-specific responses are weak, or (ii) reduce immune responses in cases of allergic asthma or autoimmunity. Important first targets could include the ERα binding sites in HS1,2 (to modify polymorphisms associated with lupus) and ERα binding sites in Sε (to modify the IgE over-expression associated with allergic asthma).

Acknowledgement

Research was supported in part by NCI P30 CA21765, ALSAC, and the Intramural Research Program of the NIH, National Institute of Aging.

References

[1] E. Morselli, R.S. Santos, A. Criollo, M.D. Nelson, B.F. Palmer, D.J. Clegg, The effects of oestrogens and their receptors on cardiometabolic health, Nat. Rev. Endocrinol. 13 (2017) 352–364.
[2] M. Kim, M.D. Neinast, A.P. Frank, K. Sun, J. Park, J.A. Zehr, L. Vishvanath, E. Morselli, M. Amelotte, B.F. Palmer, R.K. Gupta, P.E. Scherer, D.J. Clegg, ERalpha upregulates Phd3 to ameliorate HIF-1 induced fibrosis and inflammation in adipose tissue, Mol. Metab. 3 (2014) 642–651.
[3] N. Kanda, K. Tamaki, Estrogen enhances immunoglobulin production by human PBMCs, J. Allergy Clin. Immunol. 103 (1999) 282–288.
[4] D.C. Nguyen, F. Masseoud, X. Lu, F. Scinicariello, S. Sambhara, R. Attanasio, 17beta-Estradiol restores antibody responses to an influenza vaccine in a post-menopausal mouse model, Vaccine 29 (2011) 2515–2518.
[5] S.A. Ahmed, D. Verthelyi, Antibodies to cardiolipin in normal C57BL/6J mice: induction by estrogen but not dihydrotestosterone, J. Autoimmun. 6 (1993) 265–279.
[6] D. Verthelyi, S. Ansar Ahmed, Characterization of estrogen-induced autoantibodies to cardiolipin in non-autoimmune mice, J. Autoimmun. 10 (1997) 115–125.
[7] M. Yilmazer, V. Fenkci, S. Fenkci, O. Aktepe, M. Sonmezer, G. Kurtay, Association of serum complement (C3, C4) and immunoglobulin (IgG, IgM) levels with hormone replacement therapy in healthy post-menopausal women, Hum. Reprod. 18 (2003) 1531–1535.
[8] J. Asaba, M. Bandyopadhyay, M. Kindy, S. Dasgupta, Estrogen receptor signal in regulation of B cell activation during diverse immune responses, Int. J. Biochem. Cell Biol. 68 (2015) 42–47.
[9] P. Yasar, G. Ayaz, S.D. User, G. Gupur, M. Muyan, Molecular mechanism of estrogen-estrogen receptor signaling, Reprod. Med. Biol. 16 (2017) 4–20.
[10] S.C. Hewitt, K.S. Korach, Estrogen receptors: new directions in the new millennium, Endocr. Rev. 39 (2018) 664–675.
[11] R.M. Evans, D.J. Mangelsdorf, Nuclear receptors, RXR, and the big bang, Cell 157 (2014) 255–266.
[12] M.J. Fullwood, M.H. Liu, Y.F. Pan, J. Liu, H. Xu, Y.B. Mohamed, Y.L. Orlov, S. Velkov, A. Ho, P.H. Mei, E.G. Chew, P.Y. Huang, W.J. Welboren, Y. Han, H.S. Ooi, P.N. Ariyaratne, V.B. Vega, Y. Luo, P.Y. Tan, P.Y. Choy, K.D. Wansa, B. Zhao, K.S. Lim, S.C. Leow, J.S. Yow, R. Joseph, H. Li, K.V. Desai, J.S. Thomsen, Y.K. Lee, R.K. Karuturi, T. Herve, G. Bourque, H.G. Stunnenberg, X. Ruan, V. Cacheux-Rataboul, W.K. Sung, E.T. Liu, C.L. Wei, E. Cheung, Y. Ruan, An oestrogen-receptor-alpha-bound human chromatin interactome, Nature 462 (2009) 58–64.
[13] D. Panne, The enhanceosome, Curr. Opin. Struct. Biol. 18 (2008) 236–242.
[14] S. Mader, P. Chambon, J.H. White, Defining a minimal estrogen receptor DNA binding domain, Nucl. Acids Res. 21 (1993) 1125–1132.
[15] M.A. Kuntz, D.J. Shapiro, Dimerizing the estrogen receptor DNA binding domain enhances binding to estrogen response elements, J. Biol. Chem. 272 (1997) 27949–27956.
[16] M.D. Driscoll, G. Sathya, M. Muyan, C.M. Klinge, R. Hilf, R.A. Bambara, Sequence requirements for estrogen receptor binding to estrogen response elements, J. Biol. Chem. 273 (1998) 29321–29330.
[17] F.J. Shu, N. Sidell, D. Yang, C.B. Kallen, The tri-nucleotide spacer sequence between estrogen response element half-sites is conserved and modulates ERalpha-mediated transcriptional responses, J. Steroid Biochem. Mol. Biol. 120 (2010) 172–179.
[18] J.M. Hall, D.P. McDonnell, K.S. Korach, Allosteric regulation of estrogen receptor structure, function, and coactivator recruitment by different estrogen response elements, Mol. Endocrinol. 16 (2002) 469–486.
[19] L.P. Freedman, B.F. Luisi, On the mechanism of DNA binding by nuclear hormone receptors: a structural and functional perspective, J. Cell. Biochem. 51 (1993) 140–150.
[20] R. Metivier, G. Penot, R.P. Carmouche, M.R. Hubner, G. Reid, S. Denger, D. Manu, H. Brand, M. Kos, V. Benes, F. Gannon, Transcriptional complexes engaged by apo-estrogen receptor-alpha isoforms have divergent outcomes, EMBO J. 23 (2004) 3653–3666.
[21] Y. Shang, X. Hu, J. DiRenzo, M.A. Lazar, M. Brown, Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription, Cell 103 (2000) 843–852.
[22] D.K. Biswas, S. Singh, Q. Shi, A.B. Pardee, J.D. Iglehart, Crossroads of estrogen receptor and NF-kappaB signaling, Sci. STKE 2005 (2005) pe27.
[23] G. Pasternak, A. Lewandowicz-Uszynska, K. Pentos, Analysis of differences between total IgG and sum of the IgG subclasses in children with suspected immunodeficiency – indication of determinants, BMC Immunol. 19 (2018) 22.
[24] B.G. Jones, R.E. Sealy, R.R. Penkert, S.L. Surman, R.W. Maul, G. Neale, B. Xu, P.J. Gearhart, J.L. Hurwitz, Complex sex-biased antibody responses: estrogen receptors bind estrogen response elements centered within immunoglobulin heavy chain gene enhancers, Int. Immunol. (2018).
[25] L.M. Charles, T. Nicol, Effect of diethylstilboestrol on the serum gamma-globulin in the guinea pig, Nature 192 (1961) 565–566.
[26] J.F. Kenny, P.C. Pangburn, G. Trail, Effect of estradiol on immune competence: in vivo and in vitro studies, Infect. Immun. 13 (1976) 448–456.
[27] F. Engelmann, A. Rivera, B. Park, M. Messerle-Forbes, J.T. Jensen, I. Messaoudi, Impact of estrogen therapy on lymphocyte homeostasis and the response to seasonal influenza vaccine in post-menopausal women, PLoS ONE 11 (2016) e0149045.
[28] A. Keselman, N. Heller, Estrogen signaling modulates allergic inflammation and contributes to sex differences in asthma, Front. Immunol. 6 (2015) 568.
[29] D. Han, M.S. Denison, H. Tachibana, K. Yamada, Effects of estrogenic compounds on immunoglobulin production by mouse splenocytes, Biol. Pharm. Bull. 25 (2002) 1263–1267.
[30] E.N. Fish, The X-files in immunity: sex-based differences predispose immune responses, Nat. Rev. Immunol. 8 (2008) 737–744.
[31] K. Sue, The science behind “man flu”, BMJ 359 (2017) j5560.
[32] R.H. Straub, The complex role of estrogens in inflammation, Endocr. Rev. 28 (2007) 521–574.
[33] A.C. Neto, R.D. Annes, N.M. Wolff, A.P. Klein, F.C. Dos Santos, J.L. Dullius, M. Gressler, L.S. Muller, C.F. Angonese, S. Menna-Barreto, Prevalence and severity of asthma, rhinitis, and atopic eczema in 13- to 14-year-old schoolchildren from southern Brazil, Allergy Asthma Clin. Immunol. 2 (2006) 3–10.
[34] S. Narita, R.M. Goldblum, C.S. Watson, E.G. Brooks, D.M. Estes, E.M. Curran, T. Midoro-Horiuti, Environmental estrogens induce mast cell degranulation and enhance IgE-mediated release of allergic mediators, Environ. Health Perspect. 115 (2007) 48–52.
[35] V. Hox, A. Desai, G. Bandara, A.M. Gilfillan, D.D. Metcalfe, A. Olivera, Estrogen increases the severity of anaphylaxis in female mice through enhanced endothelial nitric oxide synthase expression and nitric oxide production, J. Allergy Clin. Immunol. 135 (2015) 729–736 e725.
[36] R. Munoz-Cano, M. Pascal, G. Araujo, M.J. Goikoetxea, A.L. Valero, C. Picado, J. Bartra, Mechanisms cofactors, and augmenting factors involved in anaphylaxis, Front. Immunol. 8 (2017) 1193.
[37] T.A. Platts-Mills, The role of immunoglobulin E in allergy and asthma, Am. J. Respir. Crit. Care Med. 164 (2001) S1–5.
[38] M.E. Clowse, Lupus activity in pregnancy, Rheum. Dis. Clin. North Am. 33 (2007) 237–252.

[39] J.F. Cohen-Solal, V. Jeganathan, C.M. Grimaldi, E. Peeva, B. Diamond, Sex hormones and SLE: influencing the fate of autoreactive B cells, Curr. Top. Microbiol. Immunol. 305 (2006) 67–88.

[40] K. Schreiber, K. Stach, S. Sciascia, Lupus nephritis and pregnancy outcome, Autoimmun. Rev. 16 (2017) 433–434.

[41] M. Cutolo, S. Capellino, A. Sulli, B. Serioli, M.E. Secchi, B. Villaggio, R.H. Straub, Estrogens and autoimmune diseases, Ann. N. Y. Acad. Sci. 1089 (2006) 538–547.

[42] V.E. Murphy, Managing asthma in pregnancy, Breathe (Sheff) 11 (2015) 258–267.

[43] J. Ritchie, A. Smyth, C. Tower, M. Helbert, M. Venning, V. Garovic, Maternal deaths in women with lupus nephritis: a review of published evidence, Lupus 21 (2012) 534–541.

[44] D.J. Handelsman, J.D. Newman, M. Jimenez, R. McLachlan, G. Sartorius, G.R. Jones, Performance of direct estradiol immunoassays with human male serum samples, Clin. Chem. 60 (2014) 510–517.

[45] D. James, P. Steer, C. Weiner, B. Gonik, C. Crowther, S. Robson, M. Ramsay, Pregnancy and laboratory studies: a reference table for clinicians, Obstet. Gynecol. 115 (2010) 868 author reply 868-869.

[46] M. Babina, F. Kirn, D. Hoser, D. Ernst, W. Rohde, T. Zuberbier, M. Worm, Tamoxifen counteracts the allergic immune response and improves allergen-induced dermatitis in mice, Clin. Exp. Allergy 40 (2010) 1256–1265.

[47] L.I. McKay, J.A. Cidlowski, Cross-talk between nuclear factor-kappa B and the steroid hormone receptors: mechanisms of mutual antagonism, Mol. Endocrinol. 12 (1998) 45–56.

[48] D. Kalaitzidis, T.D. Gilmore, Transcription factor cross-talk: the estrogen receptor and NF-kappaB, Trends Endocrinol. Metab. 16 (2005) 46–52.

[49] H. Keller, F. Givel, M. Perroud, W. Wahli, Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements, Mol. Endocrinol. 9 (1995) 794–804.

[50] C. Rousseau, F. Pettersson, M.C. Couture, A. Paquin, J. Galipeau, S. Mader, W.H. Miller Jr., The N-terminal of the estrogen receptor (ERalpha) mediates transcriptional cross-talk with the retinoic acid receptor in human breast cancer cells, J. Steroid Biochem. Mol. Biol. 86 (2003) 1–14.

[51] Y. Wang, C.H. Cheng, ERalpha and STAT5a cross-talk: interaction through C-terminal portions of the proteins decreases STAT5a phosphorylation, nuclear translocation and DNA-binding, FEBS Lett. 572 (2004) 238–244.

[52] C.R. Wira, C.P. Sandoe, Sex steroid hormone regulation of IgA and IgG in rat uterine secretions, Nature 268 (1977) 534–536.

[53] C.R. Wira, C.P. Sandoe, Regulation by sex hormones of immunoglobulins in rat uterine and vaginal secretions, Adv. Exp. Med. Biol. 107 (1978) 531–539.

[54] C.R. Wira, D.A. Sullivan, C.P. Sandoe, Estrogen-mediated control of the secretory immune system in the uterus of the rat, Ann. N. Y. Acad. Sci. 409 (1983) 534–551.

[55] T.A. Paula Couto, N. Falsarella, C. Mattos Cde, L.C. Mattos, Total IgE plasma levels vary according to gender and age in Brazilian patients with allergic rhinitis, Clinics (Sao Paulo) 69 (2014) 740–744.

[56] T. Yamatomo, M. Okano, T. Ono, E. Nakayama, T. Yoshino, A.R. Satoskar, D.A. Harn Jr., K. Nishizaki, Sex-related differences in the initiation of allergic rhinitis in mice, Allergy 56 (2001) 525–531.

[57] M. Becerra-Diaz, A.B. Strickland, A. Keselman, N.M. Heller, Androgen and androgen receptor as enhancers of M2 macrophage polarization in allergic lung inflammation, J. Immunol. 201 (2018) 2923–2933.

[58] D. Verthelyi, D.M. Klinman, Sex hormone levels correlate with the activity of cytokine-secreting cells in vivo, Immunology 100 (2000) 384–390.

[59] T. Mai, H. Zan, J. Zhang, J.S. Hawkins, Z. Xu, P. Casali, Estrogen receptors bind to and activate the HOXC4/HoxC4 promoter to potentiate HoxC4-mediated activation-induced cytosine deaminase induction, immunoglobulin class switch DNA recombination, and somatic hypermutation, J. Biol. Chem. 285 (2010) 37797–37810.

[60] S.R. Park, H. Zan, Z. Pal, J. Zhang, A. Al-Qahtani, E.J. Pone, Z. Xu, T. Mai, P. Casali, HoxC4 binds to the promoter of the cytidine deaminase AID gene to induce AID expression, class-switch DNA recombination and somatic hypermutation, Nat. Immunol. 10 (2009) 540–550.

[61] S. Delphin, J. Stavnezer, Regulation of antibody class switching to IgE: characterization of an IL-4-responsive region in the immunoglobulin heavy-chain germline epsilon promoter, Ann. N. Y. Acad. Sci. 764 (1995) 123–135.

[62] W.A. Dunnick, J. Shi, K.A. Graves, J.T. Collins, The 3' end of the heavy chain constant region locus enhances germline transcription and switch recombination of the four gamma genes, J. Exp. Med. 201 (2005) 1459–1466.

[63] H.J. Gould, R.L. Beavil, D. Vercelli, IgE isotype determination: epsilon-germline gene transcription, DNA recombination and B-cell differentiation, Br. Med. Bull. 56 (2000) 908–924.

[64] C.S. Mao, J. Stavnezer, Differential regulation of mouse germline Ig gamma 1 and epsilon promoters by IL-4 and CD40, J. Immunol. 167 (2001) 1522–1534.

[65] A.J. Matthews, S. Zheng, L.J. DiMenna, J. Chaudhuri, Regulation of immunoglobulin class-switch recombination: choreography of noncoding transcription, targeted DNA deamination, and long-range DNA repair, Adv. Immunol. 122 (2014) 1–57.

[66] P.J. Gearhart, J.L. Hurwitz, J.J. Cebra, Successive switching of antibody isotypes expressed within the lines of a B-cell clone, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 5424–5428.

[67] G.G. Lennon, R.P. Perry, C mu-containing transcripts initiate heterogeneously within the IgH enhancer region and contain a novel 5'-nontranslatable exon, Nature 318 (1985) 475–478.

[68] L.K. Su, T. Kadesch, The immunoglobulin heavy-chain enhancer functions as the promoter for I mu sterile transcription, Mol. Cell. Biol. 10 (1990) 2619–2624.

[69] J. Stavnezer, C.E. Schrader, IgH chain class switch recombination: mechanism and regulation, J. Immunol. 193 (2014) 5370–5378.

[70] M.Z. Xu, J. Stavnezer, Regulation of transcription of immunoglobulin germ-line gamma 1 RNA: analysis of the promoter/enhancer, EMBO J. 11 (1992) 145–155.

[71] B.K. Birshtein, Epigenetic regulation of individual modules of the immunoglobulin heavy chain locus 3' regulatory region, Front. Immunol. 5 (2014) 163.

[72] S. Chatterjee, Z. Ju, R. Hassan, S.A. Volpi, A.V. Emelyanov, B.K. Birshtein, Dynamic changes in binding of immunoglobulin heavy chain 3' regulatory region to protein factors during class switching, J. Biol. Chem. 286 (2011) 29303–29312.

[73] F.E. Garrett, A.V. Emelyanov, M.A. Sepulveda, P. Flanagan, S. Volpi, F. Li, D. Loukinov, L.A. Eckhardt, V.V. Lobanenkov, B.K. Birshtein, Chromatin architecture near a potential 3' end of the igh locus involves modular regulation of histone modifications during B-Cell development and in vivo occupancy at CTCF sites, Mol. Cell. Biol. 25 (2005) 1511–1525.

[74] B.K. Birshtein, The role of CTCF binding sites in the 3' immunoglobulin heavy chain regulatory region, Front. Genet. 3 (2012) 251.

[75] S. Feldman, I. Achour, R. Wuerffel, S. Kumar, T. Gerasimova, R. Sen, A.L. Kenter, Constraints contributed by chromatin looping limit recombination targeting during Ig class switch recombination, J. Immunol. 194 (2015) 2380–2389.

[76] A.S. Thomas-Claudepierre, I. Robert, P.P. Rocha, R. Raviram, E. Schiavo, V. Heyer, R. Bonneau, V.M. Luo, J.K. Reddy, T. Borggrefe, J.A. Skok, B. Reina-San-Martin, Mediator facilitates transcriptional activation and dynamic long-range contacts at the IgH locus during class switch recombination, J. Exp. Med. 213 (2016) 303–312.

[77] J.L. Hurwitz, R.R. Penkert, B. Xu, Y. Fan, J.F. Partridge, R.W. Maul, P.J. Gearhart, 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. 29 (2016) 132–136.

[78] R.E. Sealy, B.G. Jones, S.L. Surman, R.R. Penkert, S. Pelletier, G. Neale, J.L. Hurwitz, Will attention by vaccine developers to the host's nuclear hormone levels and immunocompetence improve vaccine success? Vaccines (Basel) 7 (2019).

[79] B.G. Jones, R.R. Penkert, B. Xu, Y. Fan, G. Neale, P.J. Gearhart, J.L. Hurwitz, 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, Mol. Immunol. 77 (2016) 97–102.

[80] B.G. Jones, R.E. Sealy, R.R. Penkert, S.L. Surman, B. Birshtein, R.W. Maul, B. Xu, G. Neale, P.J. Gearhart, J.L. Hurwitz, From flu to lupus: synchronous estrogen receptor α and RNA Polymerase II binding within the immunoglobulin heavy chain locus, Viral Immunol. (2019) Submitted.

[81] D. Frezza, B. Tolusso, V. Giambra, E. Gremese, M. Marchini, M. Nowik, E. Serone, P. D'Addabbo, C. Mattioli, S. Canestri, L. Petricca, G. D'Antona, B.K. Birshtein, R. Scorza, G. Ferraccioli, Polymorphisms of the IgH enhancer HS1.2 and risk of systemic lupus erythematosus, Ann. Rheum. Dis. 71 (2012) 1309–1315.

[82] H. Li, D. Chen, J. Zhang, Analysis of intron sequence features associated with transcriptional regulation in human genes, PLoS ONE 7 (2012) e46784.

[83] J. Hui, L.H. Hung, M. Heiner, S. Schreiner, N. Neumuller, G. Reither, S.A. Haas, A. Bindereif, Intronic CA-repeat and CA-rich elements: a new class of regulators of mammalian alternative splicing, EMBO J. 24 (2005) 1988–1998.

[84] M. Levine, C. Cattoglio, R. Tjian, Looping back to leap forward: transcription enters a new era, Cell 157 (2014) 13–25.

[85] J.J. Wong, D. Gao, T.V. Nguyen, C.T. Kwok, M. van Geldermalsen, R. Middleton, N. Pinello, A. Thoeng, R. Nagarajah, J. Holst, W. Ritchie, J.E.J. Rasko, Intron retention is regulated by altered MeCP2-mediated splicing factor recruitment, Nat. Commun. 8 (2017) 15134.

[86] Y.E. Guo, J.C. Manteiga, J.E. Henninger, B.R. Sabari, A. Dall'Agnese, N.M. Hannett, J.H. Spille, L.K. Afeyan, A.V. Zamudio, K. Shrinivas, B.J. Abraham, A. Boija, T.M. Decker, J.K. Rimel, C.B. Fant, T.I. Lee, P.A. Cisse II, D.J. Sharp, R.A. Young Taatjes, Pol II phosphorylation regulates a switch between transcriptional and splicing condensates, Nature 572 (2019) 543–548.

[87] S. Naftelberg, I.E. Schor, G. Ast, A.R. Kornblihtt, Regulation of alternative splicing through coupling with transcription and chromatin structure, Annu. Rev. Biochem. 84 (2015) 165–198.

[88] S. Delphin, J. Stavnezer, Characterization of an interleukin 4 (IL-4) responsive region in the immunoglobulin heavy chain germline epsilon promoter: regulation by NF-IL-4, a C/EBP family member and NF-kappa B/p50, J. Exp. Med. 181 (1995) 181–192.

[89] F. Wan, M.J. Lenardo, Specification of DNA binding activity of NF-kappaB proteins, Cold Spring Harb. Perspect. Biol. 1 (2009) a000067.

[90] W.J. Welboren, F.C. Sweep, P.N. Span, H.G. Stunnenberg, Genomic actions of estrogen receptor alpha: what are the targets and how are they regulated? Endocr. Relat. Cancer 16 (2009) 1073–1089.

[91] W.J. Welboren, M.A. van Driel, E.M. Janssen-Megens, S.J. van Heeringen, F.C. Sweep, P.N. Span, H.G. Stunnenberg, ChIP-Seq of ERalpha and RNA polymerase II defines genes differentially responding to ligands, EMBO J. 28 (2009) 1418–1428.

[92] B.G. Ju, V.V. Lunyak, V. Perissi, I. Garcia-Bassets, D.W. Rose, C.K. Glass, M.G. Rosenfeld, A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription, Science 312 (2006) 1798–1802.

[93] B. Weinberger, B. Grubeck-Loebenstein, Vaccines for the elderly, Clin. Microbiol. Infect. 18 (Suppl 5) (2012) 100–108.

[94] R. Solana, R. Tarazona, I. Gayoso, O. Lesur, G. Dupuis, T. Fulop, Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans, Semin. Immunol. 24 (2012) 331–341.

[95] S. Kumru, A. Godekmerdan, B. Yilmaz, Immune effects of surgical menopause and estrogen replacement therapy in peri-menopausal women, J. Reprod. Immunol. 63 (2004) 31–38.

[96] M. Ghosh, M. Rodriguez-Garcia, C.R. Wira, The immune system in menopause: pros and cons of hormone therapy, J. Steroid Biochem. Mol. Biol. 142 (2014) 171–175.
[97] V.R. Porter, G.A. Greendale, M. Schocken, X. Zhu, R.B. Effros, Immune effects of hormone replacement therapy in post-menopausal women, Exp. Gerontol. 36 (2001) 311–326.
[98] A.A. Ewies, F. Alfhaily, Topical vaginal estrogen therapy in managing post-menopausal urinary symptoms: a reality or a gimmick? Climacteric 13 (2010) 405–418.
[99] H. Eimer, S. Sureshkumar, A. Singh Yadav, C. Kraupner-Taylor, C. Bandaranayake, A. Seleznev, T. Thomason, S.J. Fletcher, S.F. Gordon, B.J. Carroll, S. Balasubramanian, RNA-dependent epigenetic silencing directs transcriptional downregulation caused by intronic repeat expansions, Cell 174 (2018) 1095–1105 e1011.
[100] L.H. Wang, X.Y. Yang, X. Zhang, K. Mihalic, W. Xiao, W.L. Farrar, The cis decoy against the estrogen response element suppresses breast cancer cells via target disrupting c-fos not mitogen-activated protein kinase activity, Cancer Res. 63 (2003) 2046–2051.
[101] G. Cabrini, V. Bezzerri, I. Mancini, E. Nicolis, M.C. Dechecchi, A. Tamanini, I. Lampronti, L. Piccagli, N. Bianchi, M. Borgatti, R. Gambari, Targeting transcription factor activity as a strategy to inhibit pro-inflammatory genes involved in cystic fibrosis: decoy oligonucleotides and low-molecular weight compounds, Curr. Med. Chem. 17 (2010) 4392–4404.
[102] R. Gambari, Recent patents on therapeutic applications of the transcription factor decoy approach, Expert Opin. Ther. Pat. 21 (2011) 1755–1771.
[103] M. Hecker, A.H. Wagner, Transcription factor decoy technology: a therapeutic update, Biochem. Pharmacol. 144 (2017) 29–34.
[104] S.M. Rad, L. Langroudi, F. Kouhkan, L. Yazdani, A.N. Koupaee, S. Asgharpour, Z. Shojaei, T. Bamdad, E. Arefian, Transcription factor decoy: a pre-transcriptional approach for gene downregulation purpose in cancer, Tumour Biol. 36 (2015) 4871–4881.
[105] N. Dias, C.A. Stein, Antisense oligonucleotides: basic concepts and mechanisms, Mol. Cancer Ther. 1 (2002) 347–355.
[106] S. Barolo, How to tune an enhancer, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) 6330–6331.
[107] V.K. Rao, D. Kapp, M. Schroth, Gene therapy for spinal muscular atrophy: an emerging treatment option for a devastating disease, J. Manage. Care Spec. Pharm. 24 (2018) S3–S16.
[108] Y. Hua, T.A. Vickers, H.L. Okunola, C.F. Bennett, A.R. Krainer, Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice, Am. J. Hum. Genet. 82 (2008) 834–848.
[109] N.K. Singh, N.N. Singh, E.J. Androphy, R.N. Singh, Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron, Mol. Cell. Biol. 26 (2006) 1333–1346.
[110] C.F. Bennett, B.F. Baker, N. Pham, E. Swayze, R.S. Geary, Pharmacology of antisense drugs, Annu. Rev. Pharmacol. Toxicol. 57 (2017) 81–105.
[111] H.Y. Shin, Targeting super-enhancers for disease treatment and diagnosis, Mol. Cells 41 (2018) 506–514.
[112] E. Callaway, CRISPR cuts turn gels into biological watchdogs, Nature 572 (2019) 574.
[113] S. Aguiar, J. Dias, A.M. Manuel, R. Russo, P.M.P. Gois, F.A. da Silva, J. Goncalves, Chimeric small antibody fragments as strategy to deliver therapeutic payloads, Adv. Protein Chem. Struct. Biol. 112 (2018) 143–182.