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Estrogen receptor alpha promotes lupus in (NZB × NZW)F1 mice in a B cell intrinsic manner

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ABSTRACT

Lupus is a systemic autoimmune disease characterized by the production of autoreactive antibodies against nuclear antigens. Women are disproportionately affected by lupus, and this sex bias is thought to be due, in large part, to the ability of estrogens to promote lupus pathogenesis. Previously, we have shown that global deletion of estrogen receptor alpha (*ERα*) significantly attenuated loss of tolerance, immune cell activation, autoantibody production, and the development of lupus nephritis. Here we show that targeted deletion of *ERα* specifically in B cells retards production of pathogenic autoantibodies and the development of nephritis in lupus-prone (NZB × NZW)F1 mice. Furthermore, we observed that *ERα* deletion in B cells was associated with decreased B cell activation in young, pre-autoimmune (NZB × NZW)F1 females. Altogether, these data suggest that ERα acts in a B cell-intrinsic manner to control B cell activation, autoantibody production, and lupus nephritis.

1. Introduction

Lupus is a systemic autoimmune disease in which patients lose immunologic tolerance and produce B and T cells reactive to nuclear antigens including double stranded DNA (dsDNA). Autoreactive B cells produce autoantibodies, and a subset of these autoantibodies, particularly the anti-dsDNA autoantibodies of the IgG isotype, have the potential to form immune complexes. Immunoglobulin-containing immune complexes that are deposited in tissues cause an inflammatory immune response through activation of complement and Fcγ receptors, which results in tissue damage [1]. Although lupus patients display abnormalities in multiple immune cell lineages, including B cells, T cells, dendritic cells and macrophages [2–9], lupus is considered to be a primarily B cell driven disease. Consistent with this premise, when lupus-prone mice are rendered unable to produce mature B cells, lupus is completely ameliorated [10]. However, when lupus-prone mice are genetically engineered to produce mature B cells that are unable secrete antibody, but are otherwise completely functional, lupus still develops, albeit in an attenuated manner [11]. Altogether, these data suggest that B cells contribute to lupus pathogenesis through both antibody-dependent and antibody-independent mechanisms.

Like most autoimmune diseases, lupus shows a significant sex bias; approximately 90% of lupus patients are female. Furthermore, lupus is primarily a disease that affects young and middle aged women. Female sex hormones, particularly estrogens, have long been thought to promote lupus and to be responsible for the profound sex bias in this disease. Indeed, exposure to both endogenous and exogenous estrogens is a risk factor for developing lupus [12,13]. Cells of the immune system, including B cells, express the cellular receptors for estrogens, estrogen receptor α (*ERα*) and estrogen receptor β [14,15].

Previously, we have shown that targeted disruption of the *ERα* gene attenuates the development of autoantibodies and lupus pathogenesis in female lupus-prone mice, whereas more modest effects are seen in male mice [16]. These studies were conducted using the (NZB × NZW)F1 mouse model of lupus, in which the development of pathogenic dsDNA IgG autoantibodies and fatal glomerulonephritis shows a strong female sex bias. These data indicate that although estrogen signaling through ERα promotes lupus in both sexes, the ability of ERα signaling to enhance autoantibody production and lupus is more pronounced in females than in males. We have also observed that estrogens and ERα signaling are responsible for the sex bias that is seen in mice carrying the lupus susceptibility locus *Sle1*, which controls loss of tolerance to nuclear antigens and immune cell activation. Targeted disruption of *ERα* attenuates the ability of *Sle1* to promote loss of tolerance, autoantibody production and B cell activation preferentially in females [17]. However, in these studies, the cell type or types responsible for these effects could not be identified because *ERα* was knocked out in all cells. Although it is hypothesized that estrogens influence lupus via actions within the immune lineage, there is little concrete evidence to support this hypothesis.

Abbreviations: dsDNA, double stranded DNA; ERα, estrogen receptor alpha; E2, estradiol; BCR, B cell receptor; SSLP, simple sequence length polymorphism; IC, immune complex; ELISA, enzyme-linked immunosorbent assay.

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A growing body of literature suggests that estrogens, acting via ERα, can exert powerful effects on B cells. For example, sustained administration of a high level of the naturally occurring estrogen 17β-estradiol (E2) allows high-affinity dsDNA-reactive B cells to escape mechanisms that maintain tolerance, including clonal deletion at the immature stage and energy induction at the T2 stage [18]. Furthermore, continuous exposure to high levels of E2 leads to upregulation of *CD22* and *SHP-1* in B cells, which likely results in decreased B cell receptor (BCR) signaling, thereby increasing the concentration of antigen required for tolerization of autoreactive B cells and protecting autoreactive B cells from receptor-mediated apoptosis [19,20]. Importantly, the ability of estrogens to induce *CD22* and *SHP-1* expression is dependent upon *ERα*, although it is not known which cell type or types must express *ERα* to elicit this effect. High levels of E2 also cause increased expression of anti-apoptotic *Bcl-2* and the B cell survival factor *BAFF* [19,20]. By decreasing the strength of BCR signaling and increasing the expression of pro-survival molecules, estrogens may enhance the survival of high-affinity dsDNA-reactive B cells. In addition to autoreactive B cells that arise as the result of VDJ recombination, autoreactive B cells can be generated by somatic hypermutation in the periphery. Estrogens promote somatic hypermutation by stimulating the expression of activation-induced deaminase [21,22]. Although these data suggest that estrogens can promote the development of autoreactive B cells at multiple stages of development, it is not known to what extent these various actions are mediated by B cell-intrinsic actions of ERα.

We hypothesize that ERα signaling in B cells promotes lupus. To test this hypothesis, we have generated lupus-prone (NZB × NZW)F1 mice in which *ERα* was deleted specifically in the B cell compartment. To generate (NZB × NZW)F1 mice with B cell specific deletion of *ERα*, we used the CD19-Cre driver strain and a floxed allele of *ERα*. In (NZB × NZW)F1 mice of both sexes, deletion of *ERα* in B cells significantly attenuated autoantibody production and extended survival. However, in female mice, B cell specific deletion of *ERα* also significantly reduced B cell activation, suggesting that *ERα* may enhance lupus in females by acting in a B cell-intrinsic fashion to promote B cell activation and thereby stimulate the production of autoantibodies.

2. Methods

2.1. Production of experimental animals

To produce NZB mice carrying the *CD19-Cre* knockin allele, B6.129P2(C)-*Cd19*$^{tm1(cre)Cgn}$/*J* female mice were purchased (The Jackson Laboratory, Bar Harbor, ME, USA) and crossed with NZB male mice. Genotyping for the *CD19-Cre* knockin allele was performed using primers that amplified the Cre gene (IMR1084 F: 5′-GCGGTCTGGCA GTAAAAACTATC-3′ and IMR1085 R: 5′-GTGAAACAGCATTGCTGTCAC TT-3′). A pair of primers that amplified the IL-2 receptor gene (COO3IC F: 5′-CTAGGCCACAGAATTGAAAGATCT-3′ and COO4IC R 5′-GTAGGTGGAAATTCTAGCATCATCC-3′) were used as an internal positive control. Offspring carrying the *CD19-Cre* knockin allele (*CD19-Cre or CD19*$^{Cre/+}$) were serially backcrossed to NZB mice for 5 generations using simple sequence length polymorphism (SSLP) marker assisted selection as we have described previously [16,23]. Briefly, at each backcross generation, mice were genotyped using 111 polymorphic markers spanning the 19 murine autosomes (detailed in Table 1). PCR genotyping was performed using oligonucleotide primers specific for SSLPs between the B6 and NZB strains. Primer sequences were obtained from the Mouse Genome Informatics database (www.informatics.jax.org). By the N5 generation, an average of only 7% of SSLP markers remained heterozygous in the NZB.*CD19*$^{Cre/+}$ mice whereas 93% were homozygous for NZB allele (data not shown). At the N5 generation, the genetic background of the incipient congenic NZB.*CD19*$^{Cre/+}$ strain was further confirmed at the DartMouse Mouse Speed Congenic Core Facility at Dartmouth Medical School (Supplemental Fig. 1). DartMouse uses the Illumina, Inc. (San Diego, CA, USA) GoldenGate Genotyping Assay to interrogate 1449 possible SNPs spread throughout the genome. The raw SNP data were analyzed using DartMouse's SNaP-Map™ and Map-Synth™ software to determine the SNP genotype, and thus strain of origin of SNP alleles, in each mouse. Analysis of 733 polymorphic SNPs indicated that the incipient congenic NZB.*CD19*$^{Cre/+}$ strain was homozygous for NZB alleles at 96% of markers evaluated (Supplementary Fig. 1). A considerable fraction of markers remaining heterozygous were on distal chromosome 7 and thus linked to the *CD19-Cre* knockin allele. All areas of heterozygosity had been previously identified by our SSLP genotyping, and none of these areas of residual heterozygosity co-localized with known lupus susceptibility loci. Based upon these analyses, the NZB·*CD19-Cre* strain was determined to be extensively backcrossed onto the NZB background.

Incipient congenic NZB.*CD19*$^{Cre/+}$ mice were then crossed to congenic NZB.*ERα*$^{+/-}$ mice, which are NZB mice heterozygous for a targeted deletion of exon 2 of *ERα* [16,24], thereby producing NZB mice heterozygous for both *CD19-Cre* and an *ERα* knockout allele. Genotyping for the exon 2 deletion of *ERα* was performed as described (www.jax.org/protocols). The common ERαEx2 F primer: 5′-TACGGC CAGTCGGGCATC-3′ (0.5 μM/rxn) and the ERαEx2wtR: 5′-GTAGAA GGCGGGAGGGCCGGTGTC-3′ (0.06 μM/rxn) or ERαEx2null R: 5′-GCTA CTTCCATTTGTCACGTCC-3′ (2 μM/rxn) primers were used to produce 234 bp (intact exon 2) and ~300 bp (deleted exon 2) products, respectively.

NZW.*ERα*$^{fl/fl}$ mice, which are congenic NZW mice homozygous for an *ERα* allele in which exon 3 is flanked by loxP sites, were previously produced by backcrossing the floxed *ERα* allele from B6.*ERα*$^{fl/fl}$ mice obtained from Ken Korach [25] to the NZW background using marker assisted selection [26]. Genotyping for the *ERα*^{fl} allele was done with the primers N6delcK F: 5′-GACTCGCTACTGTGCCGTGTGC-3′ and N6del3 R 5′-CTTC

Table 1
SSLP markers used for the production of NZB.CD19-Cre strain.

Marker (location in cM, Mb)		
D1Mit316 (2.46, 10.3)	*D6Mit123* (27.76, 56.9)	*D12Mit158* (38.14, 83.7)
D1Mit169 (9.9, 24.1)	*D6Mit209* (32.56, 75.5)	*D12Mit7* (ND, 103)
D1Mit123 (17.67, 39.2)	*D6Mit328* (52.62, 112.7)	*D12Nds2* (62.22, 115.1)
D1Mit132 (39.51, 77.1)	*D6Mit14* (77.64, 145.6)	*D13Mit16* (7.26, 20.4)
D1Mit440 (44.98, 90.7)	*D7Mit21* (1.91, 3.3)	*D13Mit275* (14.5, 37.4)
D1Mit495 (55.79, 129.5)	*D7Mit267* (17.09, 30.3)	*D13Mit13* (30.06, 56.6)
D1Mit159 (69.03, 161.6)	*D7Mit82* (32.76, 58.8)	*D13Mit126* (45.05, 85.5)
D1Mit111 (76.73, 170.9)	*D7Mit248* (ND, 63.8)	*D13Mit74* (56.92, 106.7)
D1Mit426 (84.32, 182.3)	*D7Mit323* (54.45, 108)	*D13Mit151* (64.72, 116.3)
D1Mit209 (96.35, 193.3)	*D7Mit98* (60.49, 122.1)	*D14Mit126* (11.94, 22.7)
D2Mit1 (2.33, 3.8)	*D7Mit358* (67.27, 129.9)	*D14Mit60* (24.6, 47.7)
D2Mit83 (19.38, 28.8)	*D7Mit101* (69.01, 132.8)	*D14Mit5* (31.49, 60.3)
D2Mit156 (31.66, 56.9)	*D7Mit332* (77.87, 141.2)	*D14Mit68* (37.61, 72.9)
D2Mit327 (40.88, 69.3)	*D8Mit155* (2.14, 5)	*D14Mit106* (50.9, 100.6)
D2Mit94 (47.93, 80)	*D8Mit289* (16.47, 29.9)	*D14Mit177* (60.21, 116.8)
D2Mit395 (59.97, 119.4)	*D8Mit69* (29.7, 59.2)	*D15Mit252* (8.54, 22.6)
D2Mit411 (80.04, 159.4)	*D8Mit178* (34.43, 73.6)	*D15Mit143* (19.62, 52)
D2Mit145 (86.75, 166.2)	*D8Mit211* (52, 105.2)	*D15Mit67* (32.17, 70)
D3Mit203 (10.82, 26.8)	*D8Mit49* (72.38, 126.6)	*D15Mit107* (39.79, 84.2)
D3Mit51 (ND, 77.0)	*D9Mit90* (17.8, 32.3)	*D15Mit161* (52.78, 96.8)
D3Mit26 (34.97, 79.5)	*D9Mit129* (24.45, 43.7)	*D16Mit131* (3.41, 7.3)
D3Mit311 (40.14, 92.8)	*D9Mit123* (40.88, 73.4)	*D16Mit60* (23.27, 32.7)
D3Mit320 (66.75, 143.2)	*D9Mit355* (51.41, 98.7)	*D16Mit139* (37.28, 65.7)
D3Mit19 (ND, 164.4)	*D9Mit55* (65.28, 114.7)	*D16Mit52* (53.73, 92.7)
D4Mit193 (13.99, 32.3)	*D10Mit213* (9.75, 20.1)	*D17Mit164* (2.11, 3.9)
D4Mit17 (33.96, 63)	*D10Mit20* (34.83, 66.5)	*D17Mit51* (19.74, 43.6)
D4Mit9 (43.34, 94.7)	*D10Mit230* (45.28, 89.7)	*D17Mit10* (ND, 51.0)
D4Mit308 (57.66, 123.8)	*D10Mit233* (61.58, 113.8)	*D17Mit93* (45.2, 74.2)
D4Mit42 (82.64, 150.9)	*D10Mit297* (72.31, 124.5)	*D17Mit122* (52.25, 83.5)
D5Mit348 (11.97, 24.4)	*D11Mit71* (4.7, 6.8)	*D18Mit222* (8.08, 14.7)
D5Mit352 (18.4, 36)	*D11Mit189* (27.39, 45.3)	*D18Mit177* (21.39, 41.1)
D5Mit201 (39.55, 75.6)	*D11Mit5* (40.59, 67)	*D18Mit186* (45.63, 72.2)
D5Mit314 (53.25, 110.1)	*D11Mit285* (54.64, 89.8)	*D18Mit144* (57.79, 85.7)
D5Mit97 (76.1, 137.5)	*D11Mit333* (71.83, 108.6)	*D19Mit96* (15.54, 21.9)
D5Mit143 (89.8, 151.8)	*D12Mit182* (5.52, 10.9)	*D19Mit88* (32.23, 37.3)
D6Mit138 (1.81, 4.5)	*D12Mit60* (15.54, 35.5)	*D19Mit90* (35.97, 42.3)
D6Mit116 (11.5, 21.1)	*D12Mit91* (30.06, 72.8)	*D19Mit103* (48.46, 53.8)