Estrogen Receptor α Regulates Tripartite Motif–Containing Protein 21 Expression, Contributing to Dysregulated Cytokine Production in Systemic Lupus Erythematosus

Siobhán Smith,1 Joan Ní Gabhann,1 Eoghan McCarthy,1 Barbara Coffey,1 Rebecca Mahony,1 Jennifer C. Byrne,1 Kevin Stacey,1 Elizabeth Ball,2 Aubrey Bell,2 Gaye Cunnane,3 Michele F. Doran,3 Eamonn S. Molloy,4 Ruth Z. Lee,5 Brian Harvey,6 Grainne Kearns,5 and Caroline A. Jefferies1

Objective. To examine the role of 17β-estradiol in the regulation of the autoantigen tripartite motif–containing protein 21 (TRIM-21) in patients with systemic lupus erythematosus (SLE).

Methods. Monocytes isolated from healthy control subjects and patients with SLE were stimulated with 17β-estradiol and/or the estrogen receptor α (ERα) antagonist methyl-piperidino-pyrazole dihydrochloride. TRIM-21, ERα, and CREMα expression was determined by real-time polymerase chain reaction (PCR) analysis. MatInspector software was used to identify putative binding sites within the TRIM-21 promoter. ERα binding to the TRIM-21 gene promoter region in monocytes was analyzed by chromatin immunoprecipitation (ChIP) assay. TRIM-21 and interferon regulatory factor 3 protein levels were analyzed by Western blotting.

Results. Real-time PCR analysis demonstrated a role of estrogen in the regulation of TRIM-21 expression in monocytes, which correlated positively with ERα gene expression in patients with SLE. Investigations into the human TRIM-21 promoter revealed the presence of an estrogen response element, with ChIP assays confirming ERα binding to this site. Studies into estrogen-induced TRIM-21 expression revealed a hyperresponsiveness of SLE patients to 17β-estradiol, which led to the enhanced levels of TRIM-21 observed in these individuals.

Conclusion. Our results demonstrate a role of estrogen in the regulation of TRIM-21 expression through an ERα-dependent mechanism, a pathway that we observed to be overactive in SLE patients. Treatment of monocytes with an ERα antagonist abrogated estrogen-induced TRIM-21 expression and, as a consequence, decreased the expression of interleukin-23. These findings identify TRIM-21 as a novel ERα-regulated gene and provide novel insights into the link between estrogen and the molecular pathogenesis of SLE.

Systemic lupus erythematosus (SLE) is characterized by a wide variety of immunologic abnormalities, including abnormal T and B lymphocyte signaling (1), the production of autoantibodies to self antigens (2), and the defective clearance of immune complexes by macrophages (3). In addition, a strong genetic predisposition is associated with this condition, with a 9:1 ratio of affected females to affected men (4). This sex imbalance has suggested a role of sex hormones, particularly estrogen, as important players in the development of SLE.
Although increased levels of estrogen in SLE patients have not been demonstrated (5), elevated levels of estrogen during pregnancy have been associated with disease exacerbation and an increased incidence of flares (6). Studies in murine lupus models have shown accelerated glomerulonephritis, autoantibody formation, and overall mortality following the administration of estrogen, supporting a role of this hormone in driving disease pathogenesis (7).

Studies investigating the use of hormone-based contraceptives or hormone replacement therapy in menopausal patients, however, have described conflicting findings (8–10). For example, two large trials published concurrently in 2005 demonstrated no link between administration of exogenous hormones and the development of SLE (8,9), whereas an earlier study showed that the use of oral contraceptives by SLE patients induced flares (5). Despite these findings, links between the estrogen system and the development of disease or the occurrence of flares in SLE remains strong. More recently, the estrogen receptor (ER), a nuclear hormone receptor, has come to the fore as the potential link between estrogen and disease severity (11).

The primary effects of estrogen are mediated via activation of the intracellular receptors ERα and ERβ. These receptors exert their regulatory effect on gene expression via 2 classic mechanisms: by directly binding to estrogen response elements (EREs) located within the promoter of target genes or by indirectly interacting with other transcription factors, such as activator protein 1 (AP-1) (12) or NF-κB (13), resulting in modulation of their binding to defined consensus sequences within the promoter regions. Interestingly, autoantibodies against ERs have been described in patients with SLE and were reported to interfere with T cell homeostasis and to correlate with disease activity (14). Although the reports vary, it also appears that ERα and ERβ levels are altered between different immune cells and between SLE patients and healthy controls, with enhanced levels of ERα expression observed in peripheral blood mononuclear cells (PBMCs) from SLE patients (15).

Further underlining the importance of ERα in SLE is a study showing that lupus-prone NZM2410 mice with a deletion of ERα have a better prognosis than their ERα+/+ counterparts, with prolonged survival, less severe renal involvement, and lower proteinuria levels (11). Importantly, immune complex deposition and autoantibody production were not altered in these mice, suggesting that ERα plays a role in the earlier innate response. Interestingly, a number of ERα-regulated genes that are associated with SLE have since been identified, most noteworthy being interferon regulatory factor 5 (IRF-5), a transcription factor that is genetically and functionally associated with SLE (16), BAFF, a B cell growth–promoting cytokine (17), and IFI202, a murine lupus susceptibility gene (18). Proteins known to play a role in T cell function in SLE are also ERα dependent, for example, calreticulin and CREMα (19,20). Estrogen has also been shown to enhance Toll-like receptor 9 (TLR-9)–driven cytokine production and cell activation from B cells and dendritic cells, potentially implicating ERα as a key regulator of gene expression in innate immunity (21,22).

Tripartite motif–containing protein 21 (TRIM-21) was first described as an autoantigen in SLE and has subsequently been shown to regulate antiviral responses and interferon-β (IFNβ) production downstream of antiviral pattern-recognition receptors such as TLRs 3, 7, and 9 (23–25). With regard to its role in IFNβ regulation, TRIM-21 was originally described as a negative regulator of IFN production through its ability to target IRF-3 and IRF-7 for ubiquitination and subsequent degradation downstream of TLR signaling (23). Consistent with this, TRIM-21 knockout mice develop a lupus-like condition following ear-tagging as a result of the overproduction of IFNα and interleukin-23p19 (IL-23p19) downstream of the DNA receptors TLR-3 and TLR-9 (24,25), demonstrating that TRIM-21 plays an important role as a negative regulator of IFN and IL-23 production downstream of the antiviral TLRs.

Given the role of TLR-7 and TLR-9 in driving the production of autoantibodies and, consequently, the deregulated cytokine profile associated with SLE, a role of TRIM-21 in protecting against autoimmunity has been suggested. In addition to its protective role, TRIM-21 has also been shown to stabilize IRF levels in resting cells. Specifically, Kim et al demonstrated that in resting cells, IRF-8 is stabilized by TRIM-21, resulting in enhanced IL-12p40 production, whereas upon stimulation, the p62 sequestosome is recruited to the TRIM-21/IRF-8 complex, leading to the polyubiquitination and destabilization of IRF-8 (26). We have also observed that genetic ablation of TRIM-21 (either via short hairpin RNA [23] or by genetic deletion [24]) results in reduced levels of IRF-3 in resting cells, a phenotype that is reversed upon TLR stimulation of the cells (24). Thus, TRIM-21 plays a dual role in regulating IRF stability, a role that is very much dependent on context or stimulation.

Despite numerous studies into the function of this autoantigen, its regulation at a genetic level is poorly
understood. Interestingly, enhanced levels of TRIM-21 transcripts have been observed in the PBMCs of patients with SLE as compared with healthy controls (27), thus implicating a role for this antigen in the pathogenesis of this condition. The identification of a potential ERE in the human TRIM-21 promoter led us to hypothesize a role for estrogen in the regulation of TRIM-21 expression. Our results demonstrated that the enhanced levels of ERα seen in SLE patients correlate with the increased expression of TRIM-21 observed in these patients. We also demonstrated that 17β-estradiol up-regulates TRIM-21 expression in human monocytes, both at the messenger RNA (mRNA) level and the protein level, through an ERα-dependent mechanism, a pathway that is hyperactive in SLE patients. Importantly, modulation of ERα signaling through the use of the selective ERα antagonist methyl-piperidino-pyrazole dihydrochloride (MPP) abrogated estrogen-induced TRIM-21 expression, leading to the degradation of IRF-3 and thus limiting excessive production of IL-23 and IFNβ. Overall, our results suggest that targeting estrogen and its signaling pathways could hold therapeutic potential in the treatment of SLE.

**MATERIALS AND METHODS**

**Cell culture.** PBMCs were isolated from whole blood of patients with SLE and healthy control subjects with the use of a Ficoll gradient and were cultured in phenol red-free RPMI 1640 medium supplemented with 10% charcoal-stripped fetal calf serum and 100 µg/ml of penicillin/streptomycin. Monocytes were extracted from the PBMCs by positive selection using CD14+ beads (Miltenyi Biotec). Cells were allowed to recover overnight prior to stimulation. Sterile ethanol-soluble 17β-estradiol solution (Sigma) was prepared fresh for each experiment and was used at a final concentration of 10⁻⁷M. Sterile ethanol-soluble MPP (Sigma) was also prepared fresh for each experiment and was used at a final concentration of 10⁻⁶M.

**Study subjects.** All patients with SLE who were included in our study met the American College of Rheumatology classification criteria (28–30) and were recruited from Beaumont Hospital, Dublin, Belfast City Hospital, Belfast, St. James Hospital, Dublin, and St. Vincent’s University Hospital, Dublin. SLE patients included in the study were female and 22–62 years of age. Age- and sex-matched healthy individuals were chosen as controls. The study protocol was approved by the institutional review boards of all involved institutions, and written informed consent was obtained from all participants.

**Real-time quantitative polymerase chain reaction (qPCR).** DNA was extracted from cell cultures using TRIzol reagent (Sigma) and reverse transcribed to complementary DNA using Omniscript reverse transcriptase (Qiagen) according to the manufacturer’s recommendations. Real-time qPCR investigating TRIM-21, CREMα, and ERα gene expression was performed using appropriate primers (Table 1) with SYBR Green Taq ReadyMix (Sigma) according to the manufacturer’s recommendations. Data were analyzed using an ABI Prism 7900 system (Applied Biosystems) and were normalized to an 18S RNA reference. Real-time PCR data were analyzed using the 2⁻ΔΔCt method (31).

**Chromatin immunoprecipitation (ChIP) analysis.** ChIP assays were performed using a commercial ChIP assay kit (Active Motif), according to the manufacturer’s instructions. Briefly, cells (1 × 10⁷ in total) were crosslinked for 10 minutes at room temperature with 1% formaldehyde. Nuclei were isolated into 1% sodium dodecyl sulfate (SDS) buffer containing protease inhibitors and subjected to sonication to yield 200–500-bp DNA fragments. Sonicated chromatin was immunoprecipitated overnight at 4°C with 1 µg of anti-ERα (Santa Cruz Biotechnology) or control rabbit IgG (Santa Cruz Biotechnology). Samples were washed, and then bound chromatin was eluted and incubated overnight at 65°C for reversal of crosslinking. After proteinase K digestion for 1 hour, DNA was extracted using a DNeasy kit (Qiagen). To determine the identity of the ERα target genes, ChIP DNA was further analyzed by PCR using primers encompassing the regions of interest on the TRIM-21 promoter. Input DNA was used as a positive control. PCR products were analyzed on 1.5% agarose gels by electrophoresis. The band intensities of products were analyzed using GeneTools (Syngene). IgG control values were subtracted, and the sample values were normalized to those of

**Table 1.** Primers used for reverse transcription–polymerase chain reaction and chromatin immunoprecipitation analyses

| Gene                | Primer sequence                                          |
|---------------------|----------------------------------------------------------|
| Human TRIM-21       | Forward: 5’-GAACGTCCAGGAGGTGATAAA-3’                    |
|                     | Reverse: 5’-AGTTGTCAGAGGTTAATATCCAGGTC-3’               |
| Human ERα           | Forward: 5’-CTGCAAGGAGACTTGCTA-3’                       |
|                     | Reverse: 5’-TGCCGAAAAGTGCCAGT-3’                        |
| Human IRF-3         | Forward: 5’-CTGTTCTGTGCGGGAGTCA-3’                      |
|                     | Reverse: 5’-GCGTGCAGGGTAATGTGCA-3’                      |
| Human αCREM         | Forward: 5’-GAAACAGTCTGACAGTGGGAAGT-3’                  |
|                     | Reverse: 5’-TGCCCCCGTCTCAGTGCATATATG-3’                 |
| TRIM-21 promoter    | Forward: 5’-TGTAGGCATCCAGGGAATC-3’                      |
|                     | Reverse: 5’-CCCATGCTATGTCTGCATATG-3’                    |

* TRIM-21 = tripartite motif-containing protein 21; ERα = estrogen receptor α; IRF-3 = interferon regulatory factor 3.

...
the input DNA. Results are presented as the percentage of input DNA (10× dilution).

**Western blotting.** To prepare whole cell lysates, cells were lysed in 1× loading buffer containing 63 mM Tris HCl (pH 6.8), 10% glycerol, 2% SDS, and 0.0025% bromphenol blue. Equal quantities of whole cell lysates were resolved by electrophoresis on a denaturing SDS–polyacrylamide gel and transferred to a nitrocellulose membrane. Following immunoblotting, the membrane was developed using an enhanced chemiluminescent horseradish peroxidase (HRP) substrate (Millipore). The antibodies used for immunoblotting included anti–α-actinin (Santa Cruz Biotechnology), anti–TRIM-21 (Santa Cruz Biotechnology), anti–β-actin (Santa Cruz Biotechnology), and anti–IRF-3 (Active Motif).

**Statistical analysis.** Statistical analyses were performed using GraphPad Prism version 4.03 software. All data are presented as the mean ± SD. Spearman’s test was used for correlation analyses. P values less than or equal to 0.05 were considered significant.

**RESULTS**

**Estrogen regulation of TRIM-21 expression in human monocytes via an ERE in the TRIM-21 promoter.** A link between estrogen and increased levels of circulating autoantibodies against TRIM-21 has been demonstrated in human keratinocytes, indicating a possible role of this hormone in the regulation of TRIM-21 expression (32). To test this hypothesis, human monocytes were stimulated with 17β-estradiol (10^{-7}M) or ethanol control for 6 hours and 24 hours, following which TRIM-21 expression was analyzed by real-time PCR and Western blotting. We observed significantly increased levels of TRIM-21 expression by human monocytes at both the mRNA (Figure 1A) and protein (Figure 1B) levels following 17β-estradiol treatment, indicating that estrogen and, potentially, the estrogen receptor regulate TRIM-21 expression.

ER has been shown to enhance gene expression either via binding directly to a putative ERE site or by interacting with transcription factors already bound to a specific promoter (such as AP-1), recruiting the coactivator CREB binding protein and driving transcription. Using a bioinformatic approach with the MatInspector Search program, we analyzed the human TRIM-21 gene promoter for transcription factor–binding sites. Sequence analysis revealed that in addition to a variety of putative transcription factor–binding sites, such as IRF-3 and NF-κB, there was a potential ERE site in the

![Figure 1](image.png)

**Figure 1.** Presence of an estrogen response element (ERE) in tripartite motif–containing protein 21 (TRIM-21) and positive regulation of TRIM-21 expression in monocytes. Monocytes isolated from healthy control subjects were treated with 17β-estradiol or 100% ethanol (vehicle control) for the indicated times. A, TRIM-21 mRNA expression, as determined by real-time polymerase chain reaction (PCR) analysis. Values are the mean ± SD of 3 samples. **= P ≤ 0.01. B, TRIM-21 protein levels, as determined by Western blotting. Results are representative of 3 samples, with α-actinin used as a loading control. C, Identification of potential sites of interest in the TRIM-21 promoter, as determined with the transcription factor prediction program MatInspector. Potential binding sites for an ERE, NF-κB, interferon regulatory factor 3 (IRF-3), and interferon-stimulated response element (ISRE) in the 2-kb region upstream of the transcription start site (TSS) are indicated. D, Binding of estrogen receptor α (ERα) to the human TRIM-21 promoter, as determined by chromatin immunoprecipitation. PCR was carried out using primers encompassing the TRIM-21 promoter, and the products were subjected to electrophoresis on 1.5% agarose gels. IgG was used as a negative control, and input DNA as a positive control. Results are representative of 3 samples.
TRIM-21 promoter lying 2,000 bp upstream of the transcription start site (Figure 1C).

Having identified this potential binding site in the promoter region of the TRIM-21 gene, we hypothesized that estrogen may drive TRIM-21 expression through ERα promoter binding. To test this, we used ChIP assays to investigate whether the ERE identified in the promoter region of human TRIM-21 was functional. Monocytes were stimulated with 17β-estradiol (10⁻⁷ M) or ethanol control for 1 hour and 3 hours. ChIP analysis was performed using an ERα-specific antibody, and PCR was carried out on the resulting immunoprecipitates using primers spanning the potential ERE site within the TRIM-21 promoter region. As shown in Figure 1D, ERα was not bound endogenously to the TRIM-21 promoter; however, following 3 hours of treatment with 17β-estradiol, ERα binding to the promoter was observed. This suggests a classic mode of estrogen-regulated gene expression through induction of the direct binding of ERα to an ERE site located within the TRIM-21 gene promoter.

Correlation of enhanced TRIM-21 expression with ERα expression in SLE patients. We next assessed whether TRIM-21 expression was altered in SLE immune cells and whether any relationship existed between TRIM-21 and ERα expression. Consistent with previously published reports, enhanced expression of TRIM-21 mRNA was observed in SLE PBMCs (Figure 2A) as compared with those from healthy controls, with a concomitant increase in TRIM-21 protein levels, as expected (Figure 2B). Given the role of TRIM-21 in regulating IRF-3 levels, we also observed a similar increase in IRF-3 protein in PBMCs from SLE patients as compared with those from healthy controls (Figure 2B), indicating a potential relationship between TRIM-21 and IRF-3–regulated pathways. Supporting this, we have previously reported increased expression of IL-23p19 in resting monocytes from SLE patients as compared with healthy control subjects (25).

Interestingly, ERα expression was also enhanced in SLE patients as compared with healthy controls (Figure 3A). We observed a strong correlation between TRIM-21 and ERα gene expression levels ($r^2 = 0.7703$, $P = 0.0001$) (Figure 3B), suggesting a direct link between ERα levels and enhanced TRIM-21 expression in SLE patients. Although estrogen levels reportedly do not vary significantly between SLE patients and healthy controls (33), we hypothesized that the increased ERα levels we observed may render PBMCs from SLE patients hyperresponsive to the effects of estrogen, potentially contributing to the enhanced TRIM-21 expression observed. To test this hypothesis, PBMCs from SLE patients and healthy control subjects were treated with 17β-estradiol (10⁻⁷ M) or vehicle control (100% ethanol).
and TRIM-21 expression was analyzed by real-time PCR. As expected, SLE patient PBMCs responded to estrogen treatment to a much higher extent than did their healthy counterparts, thus contributing to the enhanced TRIM-21 expression observed in these patients (Figure 3C).

We also examined the effects of estrogen stimulation on another ERα-regulated gene, CREMα, which in addition to its role as a potent negative regulator of T cell responses, has also been shown to regulate antigen-presentation functions as well as the costimulatory activities of antigen-presenting cells (34). Consistent with the results observed for TRIM-21 expression, SLE patients demonstrated heightened estrogen responses, resulting in enhanced CREMα expression as compared with healthy controls (Figure 3D), further indicating that monocytes isolated from SLE patients exhibit hyperresponsiveness to estrogen.

Abrogated estrogen-induced TRIM-21 expression following ERα inhibition. Given the central role of TRIM-21 in regulating IRF-3 levels and, hence, IFNβ and IL-23 expression, we next tested the effect of estrogen stimulation and ERα inhibition on these pathways. Monocytes from healthy control subjects were left untreated or were pretreated with MPP, a specific pharmacologic antagonist to ERα, followed by stimulation with 17β-estradiol (10^{-7}M). Blocking ERα using this selective antagonist abrogated 17β-estradiol-mediated TRIM-21 mRNA expression, indicating that 17β-estradiol up-regulates TRIM-21 expression in an ERα-dependent manner.

In order to fully appreciate the effects of ERα inhibition on TRIM-21 expression at the protein level, monocytes from SLE patients were left untreated or were pretreated with MPP, followed by stimulation with 17β-estradiol (10^{-7}M). Consistent with the results observed in the healthy controls, 17β-estradiol upregulated TRIM-21 protein expression (Figure 4B), the levels of which were then subsequently reduced with MPP treatment. Significantly, levels of IRF-3 protein accumulated in untreated cells, while cells pretreated with MPP demonstrated reduced levels of IRF-3 protein, which corresponded to a decrease in the levels of TRIM-21 protein, confirming a role of TRIM-21 in stabilizing this transcription factor in non–TLR-stimulated cells (Figure 4B).

Given the role of TRIM-21 and IRF-3 in regulating both IL-23 and IFNβ production, we observed that estrogen stimulation drove the expression of both cytokines and that MPP pretreatment resulted in a loss
of expression of both IL-23 and IFNβ, an effect mirroring that observed for TRIM-21 (Figures 4C and D, respectively). Our study strongly suggests that in resting cells, the role of TRIM-21 in regulating IRF-3 levels has a direct impact on the levels of cytokines being produced. In cases where TRIM-21 levels are elevated, the resulting increase in basal IRF-3 results in a corresponding increase in both IFNβ and IL-23. Given the central role of type I IFNs and IL-23 in the pathology of SLE and the published role of TRIM-21 in regulating these cytokines, our study has important implications concerning the role of estrogen in both the development and severity of this disease.

A number of SLE-associated genes have recently been shown to be estrogen dependent, including IRF-5, BAFF, and CREMα (16,17,20). Our results identify TRIM-21 as a novel estrogen-regulated gene, with ERα demonstrated to bind directly to the TRIM-21 promoter and drive gene expression. Importantly, an ERα-selective antagonist MPP was shown to reverse the effect of estrogen on TRIM-21 expression, with a concomitant decrease in inflammatory cytokine and type I IFN levels in SLE patient cells. In addition, a strong correlation was observed between TRIM-21 and ERα mRNA levels in SLE patients, thus underlining the importance of estrogen in regulating TRIM-21 expression, particularly in the context of SLE, in order to maintain the appropriate cytokine balance in the immune system.

The antiviral pattern-recognition receptors TLR-7 and TLR-9 have a well-established role in the development of SLE via their ability to recognize self

**Figure 4.** Estrogen receptor α (ERα) inhibition–modulated tripartite motif–containing protein 21 (TRIM-21) expression and reduced activation of the interferon regulatory factor 3 (IRF-3)/interferon-β (IFNβ)/interleukin-23 (IL-23) axis. Monocytes isolated from systemic lupus erythematosus (SLE) patients and healthy control subjects were treated with ethanol control or 17β-estradiol (E2; 10−7 M) in the presence or absence of methyl-piperidino-pyrazole dihydrochloride (MPP; 10−6 M) for the indicated times. A, TRIM-21 mRNA expression in healthy control subjects, as determined by real-time polymerase chain reaction analysis. Values are the mean ± SD of 3 samples. **B**, Levels of TRIM-21 and IRF-3 in monocytes from SLE patients, as determined by Western immunoblotting (IB), with β-actin used as a loading control. Results are representative of 3 samples. **C** and **D**, Expression of IL-23p19 (C) and IFNβ (D) in healthy control subjects, as determined by reverse transcription–polymerase chain reaction analysis. Values are the mean ± SD of 3 samples, ***P < 0.01, NS = not significant.
RNA and DNA and thus drive B cell and dendritic cell activation, autoantibody production, and type I IFN production, key cytokines associated with the pathology of SLE (39,40). Estrogen has been shown in a number of studies to enhance TLR-9–driven B cell and dendritic cell activation, including the production of proinflammatory cytokines, which suggests that estrogen positively regulates cytokine production and immune cell activation, potentially via direct transcriptional effects, as has been shown for IL-2 in T cells (20), but also perhaps via its ability to positively regulate TRIM-21 expression, as we have shown in this study.

The link between aberrant TRIM-21 activity and SLE is growing, with initial studies in the TRIM-21 knockout mouse demonstrating that deregulated signaling through the TLR-7 and TLR-9 signaling pathways resulted in the development of lupus-like symptoms in the mice, including glomerular nephritis, autoantibody production, and enhanced production of proinflammatory cytokines and type I IFNs (24). More recently, TRIM-21 has been shown to negatively regulate the intracellular DNA receptor DEAD box polypeptide 41 and, in doing so, limit cytokine production in response to DNA detection (41). In both cases, the role of TRIM-21 in the context of DNA or RNA recognition was examined, where loss of TRIM-21 positively contributes to the autoimmune phenotype via increased stability of transcription factors, such as IRF-3 or IRF-7, and enhanced cytokine production as a result.

Interestingly, TRIM-21 has also been reported to be a high-affinity Fc receptor (42) that can bind antibody–self-antigen complexes, thus facilitating immune signaling and activation (43). Consistent with this, we report that TRIM-21, and as a consequence IRF-3, levels were elevated in resting SLE immune cells, which may suggest that enhanced TRIM-21 expression in SLE patients could enhance the detection of antibody–self-antigen complexes present in these patients, thereby exacerbating inflammation. Critically for this study, TRIM-21 also appeared to stabilize members of this transcription factor family, namely, IRF-3 and IRF-8, in resting immune cells, as evidenced by the decreased levels of IRF-3 and IRF-8 observed in the absence of TRIM-21 (23,24,26).

In terms of immune stimulation, most likely through recruitment of additional factors such as the p62 sequestosome, as has been shown for IRF-8 (26), the role of TRIM-21 is to ubiquitinate and degrade IRF-3, IRF-7, and IRF-8 (23,26,44). Consistent with this, we report that TRIM-21 levels were elevated in resting PBMCs and monocytes from SLE patients and that the increased expression of TRIM-21 observed at both the mRNA and protein levels was accompanied by increased levels of IRF-3.

We observed enhanced levels of TRIM-21 expression as a result of estrogen regulation, which corresponds to IRF-3 levels in monocytes. Although we cannot definitively rule out a direct effect of estrogen on IL-23p19 or IFNβ gene expression, our results suggest that the ability of estrogen to drive TRIM-21 expression and the role of TRIM-21 in regulating IRF-3 stability potentially contribute to the enhanced basal levels of type I IFNs and IL-23 observed in SLE patients. Upon investigation into the mechanism of this estrogen-induced TRIM-21 expression, we identified a potential ERE site in the TRIM-21 promoter. This GGTTANNNTGACC region differs from the ERE consensus sequence GGTCANNNTGACC by 1 nucleotide. Although base mutations from this consensus sequence can decrease the binding affinity between the ERs and their target DNA, previous studies have shown such sites to be functional (12). Similarly, we found the potential ERE site located in the TRIM-21 promoter to be functional, with estrogen inducing ERα binding to this region and, hence, regulating gene expression.

Estrogen antagonists have previously been investigated for their ability to ameliorate SLE symptoms and pathology, both in murine studies and in clinical trials. Modulation of estrogen signaling using the well-characterized ERα antagonist tamoxifen in female NZB/W mice has proved highly successful, with significantly reduced anti-DNA antibody production, improved nephritis, and improved overall survival (7). Similarly, in lupus-prone MRL-1pr/1pr mice, estrogen-driven enhancement of inflammatory cytokines, proteinuria, and autoantibody levels were reversed by coadministration of tamoxifen (45).

Unfortunately, these promising results observed in murine studies have yet to be realized in humans, with a small study in 11 SLE patients demonstrating no beneficial effects of tamoxifen on disease activity or serologic markers (46). However, in a double-blind, placebo-controlled clinical trial of fulvestrant, an estrogen receptor–selective down-regulator, patients receiving fulvestrant for 12 months showed improved scores on the Systemic Lupus Erythematosus Disease Activity Index and a reduction in the level of conventional medication required to manage disease symptoms, thus highlighting the therapeutic potential for estrogen receptor down-regulators (47). Our current findings indicate that TRIM-21 levels are intricately linked with IL-23p19 and IFNβ levels, with increased TRIM-21...
expression as a result of estrogen stimulation, yielding a corresponding increase in these cytokines, whereas co-treatment with the ERα antagonist MPP completely blocked the estrogen-driven responses and, importantly, restored IL-23p19 and IFNβ levels to baseline.

While studies of estradiol levels in healthy females as compared with females with SLE demonstrated no difference in estrogen levels, abnormal levels of estrogen metabolites, such as 2- and 16-hydroxyestrone, were noted in the SLE patients (48). Furthermore, it has been suggested that estrogen sensitivity may be enhanced in SLE patients due to variations in ER expression in these patients. In the present study, we observed significantly enhanced levels of ERα expression in PBMCs from SLE patients that correlated positively with their TRIM-21 levels. We also demonstrated enhanced estrogen-induced TRIM-21 expression in PBMCs from SLE patients as compared with those from healthy controls, thus supporting the idea that an overactive ERα/TRIM-21 pathway may hold potential as a therapeutic target for use in SLE. Overall, our results identify a novel link between estrogen and the deregulated immune system seen in SLE and further support estrogen and ER signaling as important therapeutic targets for the treatment of SLE.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Jefferies had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Smith, McCarthy, Coffee, Mahony, Byrne, Stacey, Ball, Bell, Doran, Molloy, Lee, Harvey, Kearns, Jefferies.

Acquisition of data. Smith, McCarthy, Coffee, Mahony, Byrne, Stacey, Ball, Bell, Doran, Molloy, Lee, Harvey, Kearns, Jefferies.

Analysis and interpretation of data. Smith, Ní Gabhann, McCarthy, Coffee, Mahony, Byrne, Stacey, Bell, Cunnane, Doran, Molloy, Lee, Harvey, Kearns, Jefferies.

REFERENCES

1. Tenbrock K, Juang YT, Kyttaaris VC, Tsokos GC. Altered signal transduction in SLE T cells. Rheumatology (Oxford) 2007;46:1525–30.
2. Zhang J, Jacobi AM, Wang T, Berlin R, Volpe BT, Diamond B. Polyreactive autoantibodies in systemic lupus erythematosus have pathogenic potential. J Autoimmun 2009;33:270–4.
3. Salmon JE, Kimberly RP, Gibofsky A, Fotino M. Defective mononuclear phagocyte function in systemic lupus erythematosus: dissociation of Fc receptor-ligand binding and internalization. J Immunol 1984;133:2525–31.
4. Cervera R, Khamashia MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. The European Working Party on Systemic Lupus Erythematosus. Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. Medicine (Baltimore) 1993;72:113–24.
5. Junger P, Dougados M, Pelissier C, Kuttten F, Tron F, Lesavre P, et al. Influence of oral contraceptive therapy on the activity of systemic lupus erythematosus. Arthritis Rheum 1982;25:618–23.
6. Doria A, Iaccarino L, Sarzi-Puttini P, Ghirardello A, Zamperi S, Ariensi S, et al. Estrogens in pregnancy and systemic lupus erythematosus. Ann N Y Acad Sci 2006;1069:247–56.
7. Carlsten H, Tarkowski A, Holmdahl R, Nilsson LA. Oestrogen is a potent disease accelerator in SLE-prone MRL lpr/lpr mice. Clin Exp Immun 1990;80:467–73.
8. Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, et al. Combined oral contraceptives in women with systemic lupus erythematosus. N Engl J Med 2005;353:2550–8.
9. Sanchez-Guerrero J, Uribe AG, Jimenez-Santana L, Mestanza-Peralta M, Lara-Reyes P, Seue AH, et al. A trial of contraceptive methods in women with systemic lupus erythematosus. N Engl J Med 2005;353:2539–49.
10. Costenbader KH, Feskanich D, Stampfer MJ, Karlson EW. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. Arthritis Rheum 2007;56:1251–62.
11. Scovenn JL, EuDaly J, Ruiz P, Korach KS, Gilkeson GS. Impact of estrogen receptor deficiency on disease expression in the NZM2410 lupus prone mouse. Clin Immunol 2008;128:259–68.
12. Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson JA, Kushner PJ, et al. Differential ligand activation of estrogen receptors ERα and ERβ at API sites. Science 1997;277:1508–10.
13. McKay LI, Cidlowski JA. Cross-talk between nuclear factor-κB and the steroid hormone receptors: mechanisms of mutual antagonism. Mol Endocrinol 1998;12:45–56.
14. Colasanti T, Maselli A, Conti F, Sanchez M, Alessandi C, Barbati C, et al. Autoantibodies to estrogen receptor α interfere with T lymphocyte homeostasis and are associated with disease activity in systemic lupus erythematosus. Arthritis Rheum 2012;64:778–87.
15. Inui A, Ogasawara H, Naito T, Sekigawa I, Takasaki Y, Hayashida Y, et al. Estrogen receptor expression by peripheral blood mono-nuclear cells of patients with systemic lupus erythematosus. Clin Rheumatol 2007;26:1675–8.
16. Shen H, Panchanathan R, Rajavelu P, Duan X, Gould KA, Chouby D. Gender-dependent expression of murine IRF5 gene: implications for sex bias in autoimmunity. J Mol Cell Biol 2010;2:284–90.
17. Panchanathan R, Chouby D. Murine BAFF expression is upregulated by estrogen and interferons: implications for sex bias in the development of autoimmunity. Mol Immunol 2013;53:15–23.
18. Panchanathan R, Shen H, Bupp MG, Gould KA, Chouby D. Female and male sex hormones differentially regulate expression of Hif2α, an interferon-inducible lupus susceptibility gene within the Nba2 interval. J Immunol 2009;183:7031–8.
19. Ward J, Rider V, Abdou N, Kiemler B. Estradiol differentially regulates calreticulin: a potential link with abnormal T cell function in systemic lupus erythematosus? Lupus 2013;22:583–96.
20. Moulton VR, Holcomb DR, Jazdel MC, Tsokos GC. Estrogen upregulates cyclic AMP response element modulator α expression and downregulates interleukin-2 production by human T lymphocytes. Mol Med 2012;18:370–8.
21. Li X, Xu Y, Ma L, Sun L, Fu G, Hou Y. 17β-estradiol enhances the response of placacytid dendritic cell to Cpg. PLoS One 2009;4:e8412.
22. Siracusa MC, Overstreet MG, Housseau F, Scott AL, Klein SL. 17β-estradiol alters the activity of conventional and IFN-producing killer dendritic cells. J Immunol 2008;180:1423–31.
23. Higgs R, Ní Gabhann J, Larbi NB, Breen EP, Fitzgerald KA, Jefferies CA. The E3 ubiquitin ligase Ro52 negatively regulates IFNβ production post-pathogen recognition by polyubiquitin-mediated degradation of IRF5. J Immunol 2008;181:1780–6.
24. Espinosa A, Dardalhon V, Brauner S, Ambrosi A, Higgs R,
Quintana FJ, et al. Loss of the lupus autoantigen Ro52/Trim21 induces tissue inflammation and systemic autoimmunity by disregulating the IL-23–Th17 pathway. J Exp Med 2009;206:1661–71.

25. Smith S, Ni Gabhann J, Higgs R, Stacey K, Wahren-Herlenius M, Espinosa A, et al. Enhanced interferon regulatory factor 3 binding to the interleukin-23p19 promoter correlates with enhanced interleukin-23 expression in systemic lupus erythematosus. Arthritis Rheum 2012;64:1601–9.

26. Kim JY, Ozato K. The sequestosome 1/p62 attenuates cytokine gene expression in activated macrophages by inhibiting IFN regulatory factor 8 and TNF receptor-associated factor-6/NF-κB activity. J Immunol 2009;182:2131–40.

27. Espinosa A, Zhou W, Ek M, Hedlund M, Brauner S, Popovic K, et al. The Sjögren's syndrome-associated autoantigen Ro52 is an E3 ligase that regulates proliferation and cell death. J Immunol 2006;176:6277–85.

28. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1982;25:1271–7.

29. Hochberg MC, for the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.

30. Heinlen LD, McClain MT, Merrill J, Akbarali YW, Edgerton CC, Harley JB, et al. Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. Arthritis Rheum 2007;56:2344–51.

31. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. Methods 2001;25:402–8.

32. Furukawa F, Lyons MB, Lee LA, Coulter SN, Norris DA. Estriol enhances binding to cultured human keratinocytes of antibodies specific for SS-A/Ro and SS-B/La: another possible mechanism for estradiol influence of lupus erythematosus. J Immunol 1988;141:1480–8.

33. Lahita RG. The role of sex hormones in systemic lupus erythematosus. Curr Opin Rheumatol 1999;11:352–6.

34. Ahlmann M, Varga G, Sturm K, Lippe R, Benedyk K, Viemann D, et al. The cyclic AMP response element modulator a suppresses CD86 expression and APC function. J Immunol 2009;182:4167–74.

35. Bolland S, García-Sastre A. Vicious circle: systemic autoreactivity in Ro52/TRIM21-deficient mice. J Exp Med 2009;206:1647–51.

36. Jefferies C, Wynne C, Higgs R. Antiviral TRIMs: friend or foe in autoimmune and autoinflammatory disease? Nat Rev Immunol 2011;11:617–25.

37. Weckerle C, Niewold T. The unexplained female predominance of systemic lupus erythematosus: clues from genetic and cytokine studies. Clin Rev Allergy Immunol 2011;40:429–32.

38. Mok CC, Lau CS, Ho CT, Wong RW. Do flares of systemic lupus erythematosus decline after menopause? Scand J Rheumatol 1999;28:357–62.

39. Hwang SH, Lee H, Yamamoto M, Jones LA, Dayalan J, Hopkins R, et al. B cell TLR7 expression drives anti-RNA autoantibody production and exacerbates disease in systemic lupus erythematosus–prone mice. J Immunol 2012;189:5786–96.

40. Christensen SR, Kashgarian M, Alexopoulou L, Flavell RA, Akira S, Shlomchik MJ. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. J Exp Med 2005;202:321–31.

41. Zhang Z, Bao M, Lu N, Weng L, Yuan B, Liu YJ. The E3 ubiquitin ligase TRIM21 negatively regulates the innate immune response to intracellular double-stranded DNA. Nat Med 2013;19:172–8.

42. Keeble AH, Khan Z, Forster A, James LC. TRIM21 is an IgG receptor that is structurally, thermodynamically, and kinetically conserved. Proc Natl Acad Sci U S A 2008;105:6045–50.

43. McEwan WA, Tam JC, Watkinson RE, Bidgood SR, Mallery DL, James LC. Intracellular antibody-bound pathogens stimulate immune signaling via the Fc receptor TRIM21. Nat Immunol 2013;14:327–36.

44. Higgs R, Lazzari E, Wynne C, Ni Gabhann J, Espinosa A, Wahren-Herlenius M, et al. Self protection from anti-viral responses—Ro52 promotes degradation of the transcription factor IRF7 downstream of the viral Toll-like receptors. PLoS One;5:e11776.

45. Wu WM, Suen JL, Lin BF, Chiang BL. Tamoxifen alleviates disease severity and decreases double negative T cells in autoimmune MRL-lpr/lpr mice. Immunology 2000;100:110–8.

46. Sturgess AD, Evans DT, Mackay IR, Riglar A. Effects of the oestrogen antagonist tamoxifen on disease indices in systemic lupus erythematosus. J Clin Lab Immunol 1984;13:11–4.

47. Abdou NI, Rider V, Greenwell C, Li X, Kimler BF. Fulvestrant (Faslodex), an estrogen selective receptor downregulator, in therapy of women with systemic lupus erythematosus. Clinical, serologic, bone density, and T cell activation marker studies: a double-blind placebo-controlled trial. J Rheumatol 2008;35:797–803.

48. Lahita RG, Bradlow HL, Kunkel HG, Fishman J. Alterations of estrogen metabolism in systemic lupus erythematosus. Arthritis Rheum 1979;22:1195–8.