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A novel role of peptidyl-prolyl isomerase-1 as inducer of IL-6 expression in systemic lupus erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with various clinical manifestations affecting different tissues. Pro-inflammatory cytokines, such as interleukin 1β, IL-6 and IFN-g are associated with the SLE progression; however, the precise molecular mechanisms that in occurs improper cytokines production in SLE remain unknown. Autoantibody production and renal disease were evaluated in NZB/W F1 mice treated with a specific Pin1 inhibitor, Juglone. Inhibition of Pin1 activity significantly suppressed the IL-6 expression in NZB/W F1 mice and developed milder renal lesions than the lesions developing in non Juglone-treated mice. We further found that Pin1 inhibitor treatment suppresses B-cell differentiation and T-cell activation in NZB/W F1 lupus mice. Finally, stat3 phosphorylation was decreased in T cells from Pin1inhibitor-treated mice at 40 weeks of age as compared to that from the saline and isotype control mAb treatment groups. This is the first study to demonstrate that Pin1 plays critical roles in SLE development. Pin1 inhibition to the appropriate level might provide a novel therapeutic strategy target for future SLE therapies.

Keywords: Pin1; SLE; IL-6; Auto-antibody; Cytokine

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Introduction

Systemic lupus erythematosus (SLE) is a debilitating autoimmune disease characterized by chronic inflammation and extensive dysregulation of the immune system and damage to multiple organs in the body. The pathogenesis of SLE has been attributed to many factors–genetic, environmental, hormonal, epigenetic, or immunoregulatory [1].

SLE leads to disorder of the immune system and to generation of autoantibodies, immune complexes, autoreactive or inflammatory T cells, or inflammatory cytokines that could initiate and intensify inflammation and damage to various vital organs, such as the kidney, skin, lung, brain, and heart [2].

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First-line therapies prescribed for SLE patients include nonsteroidal antiinflammatory drugs, antimalarial agents, glucocorticoids, or immunosuppressive drugs including cyclophosphamide, azathioprine, methotrexate, and mycophenolate mofetil [3], all of which may cause significant side effects. Targeted therapies against SLE have been explored, but only 1 new drug (belimumab) with moderate efficacy has been approved in the last 50 years (7–9). Given the limited therapeutic choices for SLE patients and their adverse side effects, there is an urgent need to develop novel targeted therapies for the disease [4-9].

Notably, recent advances in the understanding of SLE immunopathogenesis have suggested an effective anti-SLE approach of targeting Toll-like receptor 7 (TLR-7) and TLR-9 signaling, because recognition of self-nucleic acids by TLR-7 and TLR-9 on B cells and plasmacytoid dendritic cells is an important step in the pathogenesis of SLE [10].

Moreover, TLR-7/TLR-9 signaling has recently been demonstrated to be under tight regulation and controlled by Pin1, a unique prolyl isomerase governing proline-based *cis-trans* conformational change of its substrates [11]. After TLR-7/TLR-9 stimulation, Pin1 is activated and then in turn interacts with interleukin-1 receptor–associated kinase 1 (IRAK-1) and also dissociates IRAK-1 from the receptor complex, resulting in nuclear translocation of interferon regulatory factor 7 (IRF-7) to induce type I interferons (IFNs).

Consequently, Pin1-deficient cells and mice failed to mount TLR-mediated, IFN-dependent immune responses [12]. These intriguing mechanistic links suggested that targeting activation of TLR-7/TLR-9/IRAK-1/IRF-7 signaling by Pin1 inhibition might represent a promising therapeutic approach for SLE, but the role of Pin1 in SLE is unknown [13].

Pin1, a unique and conserved peptidylprolyl-*cis-trans*-isomerase (PPIase), recognizes the motif consisting of phospho-Ser or phospho-Thr residues preceding Pro (pSer/Thr-Pro). Using its WW domain, Pin1 binds to specific pSer/Thr-Pro motif(s), thus targeting Pin1 close to its substrates, where its PPIase domain catalyzes *cis-trans* isomerization of certain pSer/Thr-Pro motifs [14-16] in a subset of proteins pivotal to a variety of physiologic events and diseases.

In addition to cancer and neurodegenerative diseases, Pin1 plays a pivotal role in the regulation of the immune response and related disease.

For example, it binds to and isomerizes the phosphorylated p65 subunit of NF-*κ*B and prevents NF-*κ*B from binding to and thereby being inhibited by the endogenous inhibitory protein I*κ*B, which leads to increased NF-*κ*B activity.

Moreover, Pin1 has an important role in asthma development and in the response to microbial infection [17]. During asthma, Pin1 is activated by dephosphorylation and functions as an essential component of the RNP complex, which is responsible for granulocyte—macrophage colony-stimulating factor messenger RNA stabilization, cytokine secretion, and eosinophil survival [8]. These results all suggest a potential therapeutic role of Pin1 inhibition in immune diseases.

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Development of Pin1 inhibitors has been challenging due to Pin1's unique property of recognizing substrate containing the pSer/Thr-Pro motif. Most Pin1 inhibitors have been shown to lack potency or specificity or have been unable to penetrate cells [9]; this changed with our discovery that all-*trans*-retinoic acid (ATRA) is a Pin1 inhibitor [5].

Use of ATRA for acute promyelocytic leukemia is considered the first example of modern targeted cancer therapy, but its drug target had remained elusive [18]. Unexpectedly, ATRA was discovered to be a Pin1 inhibitor from a high-throughput screening. ATRA was proved to be a potent submicromolar Pin1 inhibitor that specifically binds to, inhibits, and ultimately degrades active Pin1 selectively without cross-reacting with other isomerase members [5].

In the present study, we investigated the efficacy of Pin1-targeted therapy for treating SLE. Activated Pin1 was prevalent in SLE patients, particularly in monocytes. Genetic ablation using validated short hairpin RNA (shRNA) or gene knockout or pharmacologic inhibition of Pin1 using ATRA effectively turned off TLR-7/TLR-9/IRAK-1/IRF-7 signaling in human samples and cultured cells and drastically reversed or prevented a wide range of expression of SLE phenotypes in several lupus-prone animal strains.

Our study offers a novel avenue for developing Pin1-targeted therapy for SLE.

Many of the symptoms that SLE patients develop are congruent with symptoms of patients suffering from influenza or as a side effect of interferon-alpha (IFN-*α*) therapy. Fever, fatigue, and leukopenia are some examples. SLE patients often show enhanced IFN-*α* serum levels [9], and the IFN levels correlate with anti-dsDNA production and disease activity [10]. Furthermore, IFN-*α* therapy may lead to autoantibody production and an SLE-like syndrome [11, 12]. Genetic association studies of patients with SLE identified several genes, amongst which components of the upstream and downstream pathways of type I interferon are the most frequently found [13] including Signal Transducer and Activator of Transcription 4 (STAT4) and interferon regulatory factor 5 (IRF5) [14–16]. STAT4 interacts with type I interferon receptors and is directly involved in IFN signaling. IRF5 is a transcription factor which induces IFN transcription in response to TLR signaling. In fact, the IRF5 risk haplotype in SLE patients is associated with high serum IFN-*α* activity [17]. These genetic association studies are in accordance with the fundamental observations identified by gene expression profiling of SLE PBMCs in the group of Virginia Pascual. These experiments demonstrate a significant upregulation of interferon-regulated gene transcripts in adult and paediatric SLE PBMCs [18, 19]. This characteristic is referred to as the "interferon signature" and assessed as a new biomarker for disease activity [13].

These observations raised the questions of how the IFN signature in SLE patients develops and how IFNs are involved in pathogenesis of SLE. A hallmark of SLE is the formation of immune complexes (ICs). One cause of immune complex formation is an increased apoptosis and defective clearance of apoptotic material on the one hand and high occurrence of autoantibodies on the other hand [1]. In 1998 Cederblad et al. observed the production of IFN-*α* by PBMCs when serum samples from SLE patients were used as culture supplement [20].

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Further studies showed that immune complexes induce IFN-*α* production by pDCs [21–24]. Immune complexes are internalized after binding Fc gamma RIIa on the surface of pDCs and activate TLR9 and TLR7 in the endosomal compartment, which induces secretion of IFN-*α* [25]. Indeed, pDC are reduced in SLE blood [20], but this reduction might be related to enhanced recruitment to tissues [26, 27].

The overproduction of IFNs in SLE exerts wide effects, which result in the above-mentioned IFN signature. We would like to accent a few of these effects which were intensively observed and papered by Obermoser and Pascual [13].

Methods and Material

Animal studies

Female MRL/MpJ-*Fas*^{lpr}/J (MRL/*lpr*), B6.MRL-*Fas*^{lpr}/J (B6.*lpr*), C57BL/6-Il17atm1Bcgen/J (coex-pressing interleukin-17 [IL-17] and green fluorescent protein [GFP]), and (NZB × NZW)F1/J mouse strains were purchased from The Jackson Laboratory. The Pin1$^{-/-}$-B6.*lpr* genetic background was confirmed by genotyping of Pin1 and *Fas*^{lpr} using poly-merase chain reaction. For the experiment with MRL/*lpr* mice, placebo or 5 mg 21-day ATRA-releasing pellets (Innovative Research of America) was implanted in the backs of these mice. Urea and serum samples were collected weekly for MRL/*lpr* mice and monthly for Pin1$^{-/-}$-B6.lpr and (NZB × NZW)F1/J mice, followed by various examinations. All experiments were performed according to a protocol approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

Cell culture and reagents

THP-1 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and cultured at 37°C in a humidified incubator containing 5% CO_2. R848 and CpG were purchased from Sigma. Antibodies against various proteins were obtained from several sources. Mouse monoclonal antibodies (mAb) included mAb against Pin1 (previously described [36]), against *α*-tubulin and *β*-actin (Sigma), and against cyclin D1 (Santa Cruz Biotechnology). Rabbit antibodies included those against Pin1 phosphorylated at Ser^{16} (Cell Signaling Technology), IRAK-1 (Millipore), and IRF-7 (Abcam). Antibodies against Pin1 phosphorylated at Ser^{71} were previously described (37). A periodic acid—Schiff staining kit was purchased from Sigma. Enzyme-linked immunosorbent assay kits for IgG anti—double-stranded DNA (anti-dsDNA) and IL-17 were from eBioscience. All assays were performed according to the manufacturers' instructions. Samples of human peripheral blood mononuclear cells (PBMCs) were from the Division of Rheumatology at Beth Israel Deaconess Medical Center, and sample collection was approved by the Beth Israel Deaconess Medical Center IRB.

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Pin1 enzymatic assay

Pin1 PPIase enzymatic activity was assayed according to a previously described method (17).

Immunoblotting

PBMCs or THP-1 cells, which received treatments with different doses of ATRA or shRNA knockdown, were lysed for 30 minutes at 4°C in lysis buffer (50 m*M* HEPES, pH 7.4, 150 m*M* NaCl, 1% Triton X-100, and 10% glycerol) with freshly added phosphatase and protease inhibitors consisting of 100 *μM* 4-(2-aminoethyl)benzenesulfonyl fluoride, 80 nM aprotinin, 5 *μM* bestatin, 1.5 *μM* E-64 protease inhibitor, 2 *μM* leupeptin, 1 *μM* pepstatin A, 2 m*M* imidazole, 1 m*M* sodium fluoride, 1 m*M* sodium molybdate, 1 m*M* sodium orthovanadate, and 4 m*M* sodium tartrate dihydrate. After centrifugation at 13,000*g* for 10 minutes, one-tenth of the supernatant was stored as input, and the remainder was incubated for 12 hours with M2 Flag agarose (Sigma). After brief centrifugation, immunoprecipitates were collected, extensively washed twice with the aforementioned lysis buffer, suspended in 2× sodium dodecyl sulfate (SDS) sample buffer (100 m*M* Tris HCl, pH 6.8, 4% SDS, 5% *β*-mercaptoethanol, 20% glycerol, and 0.1% bromphenol blue), boiled for 10 minutes, and subjected to immunoblotting analysis.

Equal amounts of protein were resolved on 15% SDS—polyacrylamide gels. After electrophoresis, gel was transferred to nitrocellulose membranes using a semidry transfer cell. The transblotted membrane was washed twice with Tris buffered saline containing 0.1% Tween 20 (TBST). After blocking with TBST containing 5% bovine serum albumin (BSA) for 1 hour, the membrane was incubated overnight at 48° with the appropriate primary antibody (diluted 1:1,000) in TBST containing 2% BSA. After incubation with the primary antibody, the membrane was washed 3 times with TBST for a total of 30 minutes, followed by incubation with horseradish peroxidase—conjugated goat anti-rabbit or anti-mouse IgG (diluted 1:2,500) for 1 hour at room temperature. After extensive washing 3 times with TBST for a total of 30 minutes, the immunoblots were visualized by enhanced chemiluminescence.

Immunostaining

PBMCs, THP-1 cells, or mouse renal tissue was washed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde at room temperature for 20 minutes, followed by permeabilization and blocking with PBS containing 0.1% Triton X-100 and 5% FBS for 1 hour. After another wash with PBS, immunostaining was performed by incubating the cells overnight at 4°C with the appropriate primary antibody (diluted 1:100). Primary antibodies were diluted in PBS containing 0.1% Triton X-100, 0.2% BSA, 0.5 mM phenylmethylsulfonyl fluoride, and 1 m*M* dithiothreitol. After washing with PBS, secondary Alexa Fluor 488—conjugated goat anti-mouse antibodies or Alexa Fluor 564—conjugated goat anti-rabbit antibodies (diluted 1:200;

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Invitrogen) were added for 2 hours at room temperature. Samples were nuclear counterstained with DAPI, mounted, and visualized with an LSM510 confocal imaging system (Zeiss).

Single-cell isolation and T cell culture

Spleens were excised, and single-cell suspensions were obtained. Naive CD4+ T cells were purified using a mouse CD4+CD62L+ T Cell Isolation Kit II according to the instructions of the manufacturer (Miltenyi Biotec). Purified naive T cells were stimulated with plate-bound goat anti-hamster antibodies, soluble anti-CD3 (0.25 μg/ml, 145-2C11; BioLegend), and anti-CD28 (0.5 μg/ml, 37.51; BioLegend). In addition, stimulation with the following was used for each polarized condition: for Th17 cells, IL-6 (20 ng/ml; R&D Systems), transforming growth factor β1 (TGFβ1) (3 ng/ml; R&D Systems), anti—IL-4 (10 μg/ml, C17.8; BioLegend), and anti-IFNγ (10 μg/ml, XMG1.2; BioLegend); for Treg cells, IL-2 (20 ng/ml; R&D Systems), TGFβ1 (3 ng/ml), anti—IL-4 (10 μg/ml), and anti-IFNγ(10 μg/ml).

Flow cytometry

Using a Zombie Aqua Fixable Viability Kit (BioLegend), staining was performed for eliminating dead cells. For Th17 cell detection, surface staining on ice was performed for 20–30 minutes. For Treg cell detection, after surface staining, FoxP3 was stained using a Mouse Regulatory T cell staining kit according to the instructions of the manufacturer (eBioscience). For flow cytometry analysis, the antibodies anti-CD4 (GK1.5) and anti-CD25 (PC61) were purchased from BioLegend, and the antibodies anti-CD3α (17A2) and anti-FoxP3 (FJK-16s) were purchased from eBioscience. All flow cytometry data were acquired on an LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star).

Statistical analysis

Student's *t*-test was used to analyze data for significant differences. Values of $P < 0.05$ were regarded as significant.

Results

Pin1/IRAK-1/IRF-7 axis suppressed in vitro by treatment with ATRA or knockdown of Pin1

Given the identification of ATRA as a potent inhibitor specifically targeting active Pin1 that is not phosphorylated at Ser^{71} (37), which was prevalent in SLE patients (Figures 1A and B), we examined whether ATRA could inhibit TLR-7/TLR-9/Pin1/IRAK-1/IRF-7 signaling in a monocyte cell line, THP-1. THP-1 cells were treated with various concentrations of ATRA for 72 hours, and cell lysate was collected and subjected to Western blotting for detecting Pin1 expression. As shown in Figure 1A, ATRA at 5 μM effectively induced Pin1 degradation, suggesting that ATRA might inhibit Pin1-dependent IRAK-1/IRF-7 signaling.

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To examine this possibility, we stimulated THP-1 cells with the TLR-7 ligand R848 in the absence or presence of ATRA or in the absence or presence of Pin1 knockdown using a validated Pin1 shRNA (27). Indeed, either ATRA or Pin1 knockdown inhibited the ability of R848 to activate IRAK-1 in a dose-dependent manner (Figure 2B). Moreover, either ATRA or Pin1 knockdown significantly suppressed R848-induced IRF-7 transactivation, as shown by the luciferase reporter assay (Figure 2C), and also blocked R848-induced nuclear translocation of IRF-7, as shown by immunostaining (Figure 2D). Thus, pharmacologic or genetic inhibition of Pin1 potently blocks TLR-7/TLR-9/Pin1/IRAK-1/IRF-7 signaling in vitro.

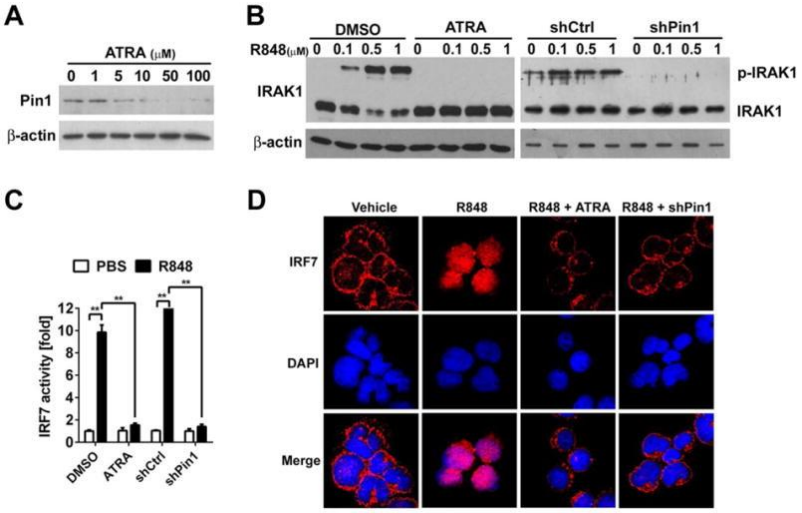

Figure 1.

Treatment with ATRA or Pin1 knockdown suppresses the Pin1/IRAK-1/IRF-7 axis in vitro. A, ATRA reduces Pin1 protein levels in a dose-dependent manner. Human monocytic THP-1 cells were treated with different concentrations of ATRA, followed by immunoblotting to detect Pin1 levels. B, ATRA or Pin1 short hairpin RNA (shRNA) suppresses R848-stimulated IRAK-1 activation. THP-1 cells were treated with different concentrations of R848 in the absence or presence of ATRA or Pin1 shRNA (shPin1), followed by detection of IRAK-1 using immunoblotting. IRAK-1 phosphorylation indicates IRAK-1 activation. shCtrl = control shRNA. C and D, ATRA or Pin1 shRNA represses R848-stimulated IRF-7 transactivation. THP-1 cells were treated with R848 in the absence or presence of ATRA or Pin1 shRNA, followed by detection of IRF-7 promoter luciferase activity with *Renilla* luciferase activity as an internal control (n = 3 independent runs of IRF-7 promoter luciferase assay) (C) or followed by detection of IRF-7 subcellular localization using immunostaining (D). Original magnification × 400. In C, values are the mean ± SD. **= $P < 0.01$ by Student's *t*-test.

Expression of SLE phenotypes in lupus-prone mice effectively suppressed by treatment with ATRA

Given that pharmacologic or genetic inhibition of Pin1 effectively blocked activation of the TLR-7/TLR-9 pathway in samples from SLE patients or in a monocyte cell line, we next tested whether inhibition of Pin1 could prevent TLR ligand—induced inflammation in mice. We applied imiquimod, a specific TLR-7 ligand known to induce inflammation of the skin (39), to the skin of wild-type ($Pin1^{+/+}$) mice or Pin1-knockout ($Pin1^{-/-}$) mice for 3 days and examined the skin tissue. Interestingly, imiquimod-induced skin inflammation was significantly attenuated in $Pin1^{-/-}$ mice

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but not in Pin1$^{+/+}$ mice, suggesting that genetic Pin1 depletion might serve as an approach to attenuate a TLR-stimulated immune response such as SLE in mice.

To further confirm this idea, we examined whether ATRA, a small chemical inhibitor [28], could affect Th17 or Treg cell populations, because both of them are essential SLE phenotypes. Naive T cells isolated from the splenocytes of C57BL/6-Il17atm1Bcgen/J (IL-17/GFP–coexpressing) mice were Th17 cell— or Treg cell—polarized in the presence or absence of ATRA.

ATRA significantly reduced differentiation of IL-17—producing Th17 cells but had only a marginal effect on Treg cell differentiation. These results show that pharmacologic depletion of Pin1 by ATRA attenuates the SLE phenotype.

These results encouraged us to examine the effects of ATRA on an SLE-prone mouse strain, (NZB × NZW)F1/J, which spontaneously produces high levels of antinuclear antibodies and proteinuria as well as progressive immune complex glomerulonephritis.

Eight-week-old female mice were implanted with 5 mg 21-day ATRA-releasing pellets or placebo, and urea samples were collected monthly. The pharmacologic effects of ATRA on the mice were validated by the detection of degradation of Pin1 and its substrate, cyclin D1 Treatment with ATRA led to a trend toward reductions in IgG renal deposition, IgG anti-dsDNA (The lifespan of (NZB × NZW)F1/J mice was also moderately extended by ATRA from 8 months to 11 months. Thus, ATRA not only inhibits Pin1 and its downstream target, but also attenuates expression of SLE phenotypes in lupus-prone (NZB × NZW)F1/J mice.

To confirm the above findings, we implanted slow-releasing ATRA pellets in mice of another SLE-prone strain, MRL/*lpr* (43,44). The MRL/*lpr* strain is FasL deficient and presents SLE-like manifestations including skin lesions, lymphadenopathy, splenomegaly, glomerulonephritis, and proteinuria as well as overproduction of anti-dsDNA antibodies, CD4–CD8— T cells, and many cyto-kines, and these mice have an average survival of 120 days [29]. Eight-week-old female MRL/*lpr* mice were implanted with 5 mg 21-day ATRA-releasing pellets or placebo, and urea, blood samples, and survival were assayed or recorded weekly. As expected, compared with treatment with placebo, treatment with ATRA reduced levels of total Pin1 and activated IRAK-1 in MRL/*lpr* mice (Figure 2D). Phosphorylation of Pin1 at Ser^{71} and Ser^{16} also decreased with ATRA treatment, probably due to reduction of total Pin1 (Figure 2D). More importantly, MRL/*lpr* mice that received placebo rapidly developed apparent SLE manifestations at 14 weeks (Figure 2A), including malar rash, skin lesions, and lymphadenopathy. However, these SLE characteristics were thoroughly suppressed in mice implanted with ATRA pellets (Figure 2A).

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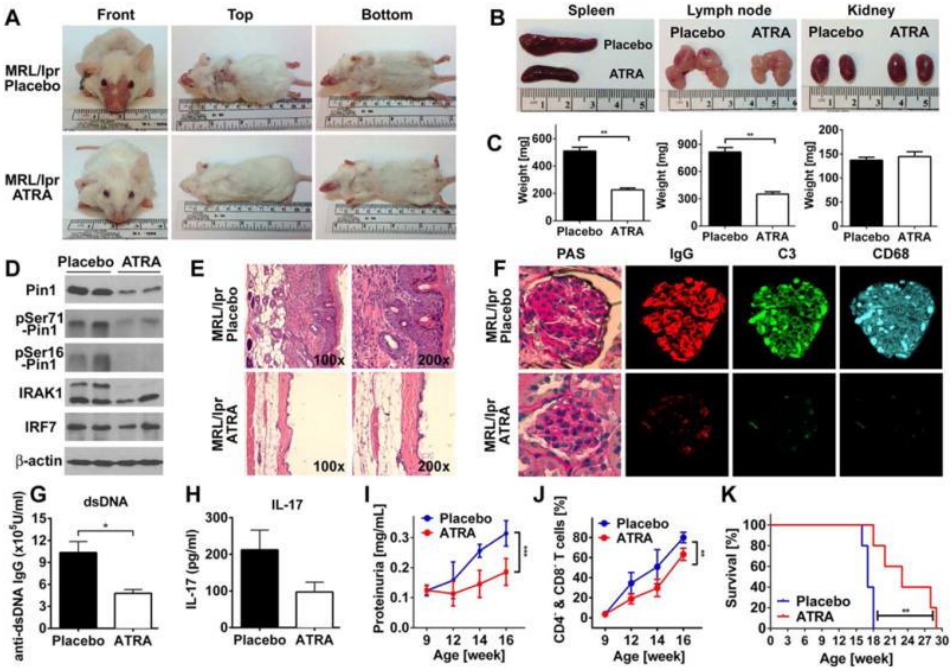

Figure 2.

Treatment with ATRA effectively suppresses expression of SLE phenotypes in SLE-prone MRL/MpJ-*Fas*^{lpr}/J (MRL/*lpr*) mice. Female MRL/*lpr* mice were implanted with slow-releasing ATRA (5 mg over 21 days) or placebo pellets at age 8 weeks, and expression of various SLE phenotypes was examined. A, ATRA treatment inhibits exterior SLE phenotypes in MRL/*lpr* mice, including malar rash (left), skin lesions (middle), and lymphadenopathy (right). B, C, and E, ATRA treatment inhibits internal SLE phenotypes in MRL/*lpr* mice, including splenomegaly (B and C, left), lymphadenopathy (B and C, middle), and keratosis (E), without affecting kidney size (B and C, right). D, ATRA treatment reduces expression of total Pin1, Pin1 phosphorylated at Ser^{71}, Pin1 phosphorylated at Ser^{16}, and activated IRAK-1 in MRL/*lpr* mice, as detected by immunoblotting. Samples were spleens shown in B. F, ATRA treatment inhibits glomerulonephritis in MRL/*lpr* mice, including deposition of IgG, C3, and monocyte/macrophages (CD68) in the renal glomerulus, as shown by immunostaining.

Discussion

It is worth noting that a recent study indicated improvements in some organs but worsening in others in MRL/*lpr* mice [23]. One of the most important differences between our experiments and those reported by other [24] involves the administration of ATRA. In our experiments, we implanted pellets releasing 10 mg ATRA subcutaneously over 21 days, which has been reportedly shown to maintain the serum concentration of ATRA constant at 0.6 *μM* [25]. This is because we have shown that ATRA is a submicromolar Pin1 inhibitor that can inhibit and degrade Pin1 at ~0.5–1.0 *μM*, as reported, while Liao et al administered ATRA orally at 6 mg/kg body weight per day.

It has been well documented that oral ATRA is quickly metabolized in the liver, with a very short half-life both in mice and in humans. For example, as reported [26], following oral dosing of

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mice with 10 mg/kg, tissue ATRA levels reach a maximum within 30–120 minutes, then decline after 3 hours in an exponential manner with half-life values of 25–68 minutes. Similarly, the half-life of ATRA in humans is 45 minutes [27]. With such a short half-life, it is impossible to have ATRA concentrations sufficient to keep Pin1 function disabled at all times, as we have previously shown. However, at these low concentrations, ATRA can still activate its receptors (RARs or retinoid X receptors [RXRs]) because their affinity is low nanomolar [28].

Whether worsening phenotypes in some tissues observed by Liao et al could be due to activation of RARs or RXRs is not known. However, it is also worth noting that there was a significant therapeutic response to ATRA in 2 patients with steroid-resistant SLE. SLE patients received ATRA treatment for 6 months and achieved complete remission, with reduced proteinuria and anti-dsDNA antibody levels [29]. Disease did not recur, and neither patient experienced any adverse effects during > 1 year of follow-up.

Moreover, ATRA was previously used to treat mice of 2 representative SLE-prone strains, MRL/*lpr* (52) and (NZB × NZW)F1/J [30], and it significantly inhibited a number of SLE symptoms, although the mechanisms of inhibition were not determined. ATRA-treated MRL/*lpr* mice had reductions in SLE manifestations, including lymphadenopathy, splenomegaly, proteinuria, glomerulonephritis, and cytokine overproduction [31]. ATRA-treated (NZB × NZW)F1/J mice also had reductions in SLE manifestations, including glomerulonephritis and cytokine overproduction, and had an increased rate of survival [32].

It remains to be determined why the effect of ATRA treatment seems to be weaker in (NZB × NZW)F1/J mice than in MRL/*lpr* mice in our experiments, but it may be because lupus-like pathogenesis differs between these strains [33]. Nevertheless, these studies in humans and mice provide proof of the concept that ATRA is a promising therapeutic tool for SLE, although the molecular targets underlying its therapeutic efficacy were previously unknown [34].

The quest for an ideal cancer therapy began when cancer itself was described as a disease and many promising targets have been investigated in the past with varying results [35]. Since a cancer cell starts as a normal cell that has become deregulated, the ability to selectively target only cancer cells by identification of proteins/processes unique to cancer cells remains elusive for many cancer types and stages.

Such targeting should minimize adverse effects while obtaining an effective treatment [36]. As a further complication, the pathways that lead to cancer are numerous and varied, with confounders like immunoediting, persistence of cancer stem cells, etc. Here we propose a target common to all cells: isomerization-mediated apoptosis, but in such a specifically targeted way that normal cells are spared [37]. The isomerization of ATR by Pin1 is an important biological process that should be studied further since the existing evidence points to exciting possibilities for drug/genetic regulation of this singular process. There would be significant potential translational implications in disease diagnosis and treatment.

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Conclusion

This is the first study to demonstrate that Pin1 plays critical roles in SLE development. Pin1 inhibition to the appropriate level might provide a novel therapeutic strategy target for future SLE therapies.

Competing interests

The authors declare that they have no competing interests.

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