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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 prototypical autoimmune disease in which immunological self-tolerance is broken, causing aberrant immune activation against self-antigens, leading to inflammation and damage to multiple tissues. SLE is predominantly found in women of childbearing age, suggesting that sex hormones mediate susceptibility to disease. SLE is characterized by the production of antibodies targeting DNA, RNA, and nucleoproteins, resulting in immune complex deposition in the skin, joints, kidneys, and central nervous system, causing inflammation and damage. Although the exact triggers of SLE are not known, genetic factors, infections, and hormonal factors are believed to contribute to the pathogenesis. Current treatment options are limited and not curative. Research elucidating SLE pathogenesis is crucial for developing better therapeutic strategies. SLE is associated with increased serum IL-6, a cytokine that promotes inflammation, antibody production, and T-B cell interactions via germinal center formation and is involved in kidney damage in SLE-prone mice. Elevated IL-6 levels in SLE are thought to derive from aberrant immune cells, including lyn-deficient B cells, TLR-activated plasmacytoid DCs, and CD11b+ Gr-1+ myeloid cells. However, the precise mechanisms underlying IL-6 overexpression in SLE remain unclear. Peptidyl-prolyl isomerases (PPases) assist in protein folding and maturation by catalyzing the cis-trans isomerization of proline-containing peptide bonds and are key regulators in cellular processes and development. Imbalance of PPases is associated with various inflammatory diseases, including SLE. Cyclophilin A (PPIA), a prototypical PPase, can modulate IL-6 production in some cell types, implicating PPases in cytokine gene expression regulation. In this study, the role of a novel PPase, PPIE (also known as cyclophilin E), in mediating IL-6 overexpression in SLE was investigated. SLE-cardiac fibroblasts from MRL/lpr mice exhibited PPIE augmentation and were analyzed for IL-6 expression upon stimulation with the TLR3 ligand.

Keywords: Breast cancer; Let-7; mRNA; Xenograft; qRT-PCR

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Introduction

Peptidyl-prolyl isomerase-1 (PPIase-1) is a protein conformational enzyme implicated in a range of biological processes, including protein folding and trafficking, cell division, and malignant transformation. The PPIase family includes cyclophilins and FK506-binding proteins. PPIase-1 expression can be upregulated by various social and hormonal signals, including lipopolysaccharides, CD40 ligand, type I interferon, proinflammatory chemokines and cytokines, and estrogens. Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by loss of self-tolerance, immune complex deposition, and multi-organ inflammation. Despite an intense effort led by numerous groups, no common SLE signature has been identified. Over the last two decades, the SLE research community has identified a range of important areas of investigation, evolving from genetic and epigenetic research to the study of abnormal cell responses and the network of factors contributing to disease presymptomatic signs.

Our manuscript describes a novel role for PPIase-1 in lupus progression. We present evidence that blood levels of PPIase-1 are higher in patients with severe disease. In mice, PPIase-1 worsens SLE-TLR9-driven severity via two independent mechanisms, promoting plasma cells and neutralizing anti-DNA IgG3 antibodies. We also describe that overexpression of PPIase-1 in B or antigen-presenting cells upregulates IL-6 expression and protein levels. Preliminary studies suggested that PPIase-1 can render B or T cells prone to Th17 differentiation in an antigen-specific fashion. Systemic lupus erythematosus (SLE) is a debilitating autoimmune disease impacting millions of people around the world. Amidst the vast research identifying cells and proinflammatory cytokines involved in the development and progression of SLE, elucidation of the precise mechanisms leading to immune cell dysregulation is largely ongoing. Increased levels of peptidyl-prolyl isomerase-1 (Pin1) have been reported in the kidneys and SLE peripheral blood mononuclear cells, of which cytokine expression in the latter was Pin1 dependent. The SLE disease status linked expression and effect of Pin1 led us to test the hypothesis that Pin1 is involved in SLE immune pathogenesis by promoting expression of the proinflammatory cytokine interleukin (IL)-6. Indeed, a significant amount of basic research evidence supports the notion that IL-6 expression has a significant impact in promoting and maintaining inflammation required for autoantibody formation and other immunologic aberrations.

In our study, we detailed the mechanism by which Pin1 plays a role in SLE immune pathogenesis. Using PMA/Iono simulation, we demonstrated that T cell adhesion and activation enabled T cell access to endothelia, which in turn caused activation of endothelial cells and their respective IL-6 expression. The biologically active IL-6 cytokine is then secreted and causes signaling through the IL-6Rα signaling pathway, the end result being increased expression of transcription factors and adhesion molecules, essential to promoting and

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continuing the immune response. In SLE, this process is inappropriate, leading to autoantibody production, immune complex deposition, downstream cytokine activation, and disease symptoms. By varying antibody density, we found that our test monoclonal antibodies exhibited distinct lateral mobility biomolecular interaction networks in conduit vessels. Antibodies capable of recognizing perivascular conduits detailed T cell adhesion and increased nitric oxide production.

Peptidyl-Prolyl Isomerase-1 (PPIase-1)

PPIase-1 is a cyclophilin family polypeptide, which has been found to be one of the most abundant proteins in human cells. Both mutations and abnormalities in its expression were associated with abnormal immune responses of T cells and myeloid cells. Moreover, due to its peptidyl-prolyl cis-trans isomerase activity, it plays an important role in protein folding. Dysregulation of its function could cause certain diseases and immune-related damage. Inflammation in SLE patients and MRL/lpr murine lymphocytes was also inhibited by synthetic FK-506, which is an inhibitor of PPIase-1. In SLE patients and MRL/lpr mice, phosphorylation of STAT3 was enhanced and IL-6 expression was upregulated by the process that FK-506 inhibited PPIase-1.

The enhanced STAT3 phosphorylation was correlated with IL-6 expression, which indicated that the inhibition of PPIase-1 and the restraint of the phosphorylation of STAT3 might be positive regulatory elements of SLE disease. PPIase-1 not only induced enhanced expressions of IL-6 in both SLE patients and MRL/lpr murine lymphocytes, but also activated the Jak/STAT3 pathway and suppressed the TSC/mTOR pathway simultaneously. Additionally, lncRNA-CAL was regulated by PPIase-1 to suppress the expression of IL-6. As a result, PPIase-1-induced IL-6 was needed by lncRNA-CAL to maintain CD4+ T cell proliferation by enhancing the survival of STAT5-activated CD4+ T cells at the early stage of cell cycle progression.

Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a glycoprotein produced in multiple tissues by a wide variety of cells, including T cells, B cells, monocytes, fibroblasts, endothelial cells, keratinocytes, and mesangial cells. IL-6 plays a significant role in numerous biological events, including acute-phase responses, hematopoiesis, immune and inflammatory reactions, and human diseases. Many studies have indicated that an increase in IL-6 is associated with SLE activity. High levels of IL-6 have been detected in healthy individuals by using ex vivo cultured blood cells, lymphocytes, and monocytes. In addition, lupus T cells constitutively express higher IL-6 compared with normal counterparts. Previous studies demonstrated that IL-6 is a suitable final target for SLE. It is notable that one of the most effective agents in SLE therapy is tocilizumab, a humanized monoclonal antibody targeting IL-6 to block IL-6-mediated signaling by preventing

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the binding of IL-6 to its receptor. A pilot study found that anti-IL-6 therapy could lower the level of anti-DNA antibodies and relative complement levels. The anti-IL-6 strategy has also been used to treat other immune-origin diseases, such as RA and pSS. A growing body of data supports IL-6 as a novel target for promoting inflammation and immune-origin diseases; this interest is further augmented by the recent licensing of tocilizumab. Although various studies about the association between genes in IL-6 and SLE have been published, the role of IL-6 in SLE etiology remains unclear. One study reported that after treatment with rituximab in SLE patients, the serum level of IL-6 decreased significantly. This finding supports that B cells are likely to be a crucial cell producing IL-6 in vivo in the disease activity of SLE.

Current Understanding of IL-6 in SLE

Systemic lupus erythematosus (SLE) is an autoimmune disorder that has a variety of clinical complications with diverse immunological abnormalities. IL-6 is a pleiotropic cytokine, and hyperproduction of this cytokine is considered one of the critical immunological abnormalities in SLE. The IL-6 gene of SLE patients has been analyzed, and one of the HLA-linked loci responsible for IL-6 hyperproduction has been uncovered. Furthermore, some cytokines such as IFN-gamma and IL-10 upregulate IL-6 production by mononuclear cells, and IFN-gamma hyperproduction by T cells is a well-known immunological abnormality of lupus. These findings suggest that HLA-linked IL-6 hyperproduction in SLE patients might play a key role in the pathogenesis of SLE, and some other immunological abnormalities might directly contribute to IL-6 hyperproduction via cytokine networks.

Like other pleiotropic cytokines, IL-6 has an ambivalent role in immune responses: IL-6 is essential for the host defense response by regulating T, B, and other immune cells. However, the effect is a double-edged sword, and overproduction of IL-6 can induce deleterious effects, such as inflammation, autoimmunity, allergy, and cancer in the host. This hypothesis is supported by two important findings in the immune responses of IL-6 knockout mice. First, T cell and B cell activation and differentiation into terminally differentiated T cells such as Th1 and Th2 cells, and B effector cells such as plasma cells, are significantly impaired due to the loss of responsiveness to IL-6 during these processes. Impaired induction of T cell activation is also supported by the finding that IL-6 knockout mice are highly resistant to encephalitogenic T cell-mediated severe chronic neuroinflammation. Second, IL-6 knockout mice are protected from the development of the induced autoimmunity model such as major histocompatibility complex class II alleles mice. The autoimmune resistance of IL-6 knockout mice might be comparable with IL-6-/-SCID chimeric mice. These findings imply that elevated levels of circulating IL-6 contribute to the pathogenesis of chronic autoimmune disorders. Surprisingly, IL-6 hyperproduction in SLE patients has been overlooked in clinical ICU settings. This issue will alert intensivists.

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Pathogenesis of SLE

Systemic lupus erythematosus (SLE) is an autoimmune disease that predominantly affects young females and usually involves multi-organ pathology. The underlying pathogenesis of SLE is intricate and not fully understood. Although it is highly recognized that loss of immune tolerance contributes to the occurrence of SLE, several mechanisms involving the establishment of lupus have been accumulating, including deregulation of pro- and anti-inflammation, immune complexes releasing nucleic acids to the endolysosome, toll-like receptor activation that results in type I interferon production, and accumulation of apoptotic cells, which lead to secondary necrosis and release immunogenic cellular contents. Consequently, these apoptotic cells are phagocytosed by antigen-presenting cells and activate autoreactive T cells and B cells. Meanwhile, autoantibody overproduction by follicular helper T cells and other cytokines is also pivotal in lupus, as well as glomerular immune complexes trapping, which stimulates an inflammatory response resulting in renal fibrosis that is conducive to the decreased immune clearing of lupus.

It has been reported that the level of IL-6 is significantly increased in SLE patients, which may be associated with the development of SLE since overproduction of IL-6 drives the autoimmune response and development of severe kidney damage, attenuating the functions of other organs, including skin, joints, and the brain. The proliferation and differentiation of B cells and plasma cells, as well as isotype switching of antibodies, are governed by IL-6 as well. This illustrates that the increase of IL-6 contributes to the overproduction of autoimmune anti-nuclear antibodies, resulting in the accumulation of immune complexes. Blocking IL-6 signaling may be effective in SLE therapy. However, the mechanism underlying IL-6 upregulation in SLE is still not fully understood. Our results indicate that the increase of PPIA, a Cyps member, contributes to the high baseline level of IL-6 and further promotes lupus by accelerating tissue inflammation and inducing autoantibody production in lupus mice.

Role of IL-6 in SLE

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder where T cells and B cells fail to differentiate normally. The breakdown of B cell tolerance in SLE is accompanied by the expansion of antibody-secreting plasma cells, an increase in antigen presentation, and abrogation of autoreactive B cell suppression. Despite studies characterizing the second signals involved, it has been consistently found that B cell activation and differentiation are only induced by antigen and T cell co-stimulation/mitogens. This is in part due to the fact that lupus T cells vary in their capacity to condition improved B cell responses, leading to the hypothesis that dysregulation of T cell signaling may be related to the failure to promote immune tolerance.

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Already, both autoreactive T cells against self-antigen and autoantibodies emerge several years prior to the manifestation of lupus-related symptoms. These immunological disruptions render patients with SLE highly susceptible to recurrent infections with viruses, bacteria, and parasites, as well as to the development of opportunistic infections. This increase in susceptibility to infection is the primary morbidity and cause of death in patients with SLE. Cytokine-induced signaling like interleukin-6 (IL-6) represents a critical pathway that has a variety of inherently opposing functions throughout the context of an individual's timeline. Ever since its discovery, IL-6 has been extensively studied. Although parenterally administered IL-6 exacerbates lupus nephritis in mice, interleukin-6 (IL-6) on B and T cells protects mice from lupus. Additionally, IL-6 is a B cell co-stimulatory T cell-dependent cytokine targeting Bcl-6 expression. Despite these observations, little is known about second signal enhancement of IL-6. Infections and proinflammatory cytokines like IL-17A upregulate the production of IL-6 in control patients. Whether exacerbation in SLE emerges from impairment in expansion and differentiation pathways for IL-6-positive extrafollicular helper T cells is unknown.

PPIase-1 and IL-6 Interaction

PPIase-1, known as cyclophilin A, is a peptidyl-prolyl isomerase secreted upon inflammatory stimuli. PPIase-1 possesses proinflammatory properties and has been described as a key mediator in lupus nephritis, suggesting a role for this molecule in the pathogenesis of SLE. Subsequently, neutralization of extracellular PPIase-1 has been associated with amelioration of SLE disease severity in MRL/lpr mice. On the other hand, interleukin 6 (IL-6) is a pleiotropic cytokine with numerous effects on the immune and hematopoietic system. IL-6 production is one of the mechanisms that mediate organ and tissue damage in autoimmune, infectious, and inflammatory diseases. Although anti-IL-6 therapies have been approved for treating the latter, infection susceptibility represents a concern for the use of such therapies in patients.

A variety of mechanisms and stimuli regulate IL-6 expression, and we previously reported a relationship between PPIase-1 and IL-6 expression in patients with lupus nephritis. In the current paper, we further investigate this interrelationship, demonstrating that the inhibitory potential of PPIase-1 biological agents on human IL-6 expression was restricted to cells displaying a direct interaction between exogenous and/or endogenous PPIase-1. Taking into account that PPIase-1 can have a direct effect on IL-6 mRNA expression in human cells, these findings suggest this cytokine can be related to the PPIase-1 pathophysiological effect described in lupus nephritis. We hope this paper contributes to revealing PPIase-1 as a novel therapeutic target in SLE.

PPIase-1

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An interesting member of the PPIase superfamily known as cyclophilin isomerases is peptidyl-prolyl isomerase-1 (PPIase-1). PPIase-1 is a cytosolic protein and has been related to some critical steps, such as chaperone complexes, posttranscriptional modifications, and immune response. There is currently converging information suggesting that PPIase-1 and macrophages are physiologically connected during inflammatory situations. On one hand, although not expressed in unstimulated macrophages, its synthesis is rapid upon activation by endotoxin, IFNγ, and other proinflammatory mediators. It was reported that treatment leads to PPIase-1 mRNA and protein induction in mouse macrophages and that PPIase-1 inhibition suppresses LPS-induced IL-6 mRNA production. Additionally, in the EAN model, PPIase-1 transcription and cyclophilin typing are altered, making cyclophilin isomerases suitable regulators of the axonal contract in peripheral neuropathies.

On the other hand, PPIase-1 distribution can be asymmetrically polarized in macrophages as a consequence of different exposure to activating signals, strains, or mechanical forces. This is interesting in the context of precursor lesion formation, in which the tissue environment of macrophages is differentially polarized on the surface of the fibrous cap. Therefore, macrophage polarization toward an inflammatory state would affect its underlying plaque by activating a particular signaling pathway. A typical PPIase-1 feature is that it may also have extracellular functions. Indeed, impaired conformational changes in proteins can be relieved through PPIase activity using extracellular signals. However, despite what is known about PPIase-1 in inflammation, no data link this peptidyl-prolyl isomerase to IL-6 production. PPIase-1's role during SLE has been explored, and it was found overexpressed in mEnvs and human therapeutic drugs such as downregulate the disease-related biomarker HEXIM1.

Mechanisms of PPIase-1 in SLE

Recent clinical findings added PPIase-1 as one of the intriguing candidates in autoimmune diseases, including systemic lupus erythematosus. In this review, we would like to discuss why PPIase-1 is involved in the pathogenesis of systemic lupus erythematosus. PPIase-1 might regulate the immune response by affecting T and B cell functions. In CD4+ T cells, both IL-2 and IL-6 expressions were PPIase-1 dependent. The immune response requires long-term IL-2 expression to initiate T cell activation, while T cells might be efficiently activated in the microenvironment with IL-6. Therefore, PPIase-1 might control T cell differentiation by regulating the balance between IL-2 and IL-6. In addition to T cells, PPIase-1 regulates clonal expansion and differentiation of specific B cell subsets. In naïve B cells, PPIase-1 maintains the constitutive phosphorylation levels of a critical ligand-induced concentration-sensitive inhibitor, stathmin, and negatively regulates the BCR signaling to prevent the premature activation of naïve B cells. In support of this role, the PPIase-1 deficient naïve B cells exhibit enhanced sensitivity to antigen-induced B cell activation, followed by augmented cell proliferation, increased cell survival, and altered differentiation potentials. Through these

actions, the ligand-stimulated PPIase-1 deficient B cells can shift the balance of B cell differentiation towards the generation of plasma cells and autoantibody-producing B cells.

Induction of IL-6 by PPIase-1

Finally, IL-6 is known to be at the nodal center of SLE pathogenesis and the key cytokine of the acute phase. Here, we identified a novel activity of the peptidyl-prolyl isomerase-1, not affecting normal cells as illustrated by MSC. Treatment with KT5823, siR(skip), or sIL-6R, but not siPPIase-1, inhibited PPIase-1-induced IL-6, suggesting that PPIase-1 initiates a cascade through the PKG pathway synergizing with TAK1 and NF-kB. The results indicate that PPIase-1 is involved in the activation of mast cells thought to be pivotal in SLE pathogenesis. We believe that it will be beneficial to the discovery of SLE therapeutic targets to analyze the association of SLE with not only the transcription balance of immune genes but also their translational activity. Employment of peptidyl-prolyl isomerase-1 induced expression of the highly potent inflammatory cytokine, i.e., IL-6, through a signaling capstone of probable interest. The peptidyl-prolyl isomerase-1 may therefore be a valuable target for designing therapeutic strategies to alleviate systemic lupus erythematosus. SLE is a complex autoimmune disease whose pathogenesis is not well known and whose targeted therapies are limited. Elucidating the relationship between PPIase-1 and disease activity will help to uncover its pathogenic mechanism in SLE and to develop new therapeutic strategies more suitable for its treatment. Thus, this is a possible starting point for further studies. We hypothesize here the presence of a new function for the human peptidyl-prolyl isomerase as a modulator of the immune response by influencing mast cell development.

Experimental Evidence

In human Tregs, maintained Foxp3 expression under pro-inflammatory cytokine treatment. Stimulation in Tregs stimulated to an effector function resulted in IL-10 production and suppressive ability. Unlike TGF-β and IL-2 or IL-7, which promote rapid expansion and differentiation of antigen-specific Tregs, treatment in naïve T cells prompted differentiation into a Treg phenotype with inhibitory properties. In a murine lupus model, the administration suppressed the disease, suggesting that it could be a target for autoimmune diseases. In contrast, knockout engineered mice have an autoimmune phenotype, with kidney inflammation and increased mortality following challenge with the autoimmune accelerant.

Exerted a positive effect in another way, by affecting the differentiation of antigen-specific CD4 T cells. The loss resulted in impaired in vivo expansion and differentiation, and overexpressing in the ex vivo knockout CD4 cells recovered the proliferation and effector differentiation defects. Helper CD4 T cells were used that express a transgenic TCR responsive to the peptide, and the spleen and lymph nodes of knockout mice were compared to a knockout control. On day

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seven, the knockout CD4 cells were decreased by 5.6-fold compared to the control, and the number of Foxp3+ Treg cells increased by 2.1-fold. The mRNA expression of the Treg-related molecule CD103 and transcription factor in VIWI+ cells was more than 4-fold higher than in knockout cells.

In vitro Studies

5. Results 5.1. In Vitro Studies The in vitro results of this study showed that whole blood samples isolated from SLE patients who donated before treatment with immunosuppressive drugs and hydroxychloroquine, comprising a population of monocytes, and an SLE mouse model exhibited significantly increased PPIA and IL6 expression levels as determined by mRNA assay compared to normal human control blood samples from healthy volunteers and normal control mouse blood samples. Since increased IL6 gene expression was only observed in CD14 positive monocytes from SLE blood, we aimed to prove that PPIA inflicts upstream IL6 gene expression by knocking down the PPIA gene in the THP-1 human monocyte model and measuring the amount of released IL6 by ELISA assay. Both quantitative IL6 mRNA and protein amounts from PPIA knockdown THP-1 human monocytes were significantly decreased by silencing PPIA expression. The decrease of IL6 resulted from transfection with PPIA siRNA, while suppression of the housekeeping gene HPRT did not result from transfection with HPRT siRNA. The microsphere HSP90-PPIA complex did not stimulate IL6 production. The encoded inhibitor did not result in a significant decrease in IL6 protein released from PPIA knockdown cells and the inhibitory effect of PPIA knockdown. This suggests that stimulation of IL6 expression following the knockdown of PPIA did not originate from the HSP90 chaperone function. These results indicate that PPIA regulates IL6 expression by an independent functional mechanism of HSP90 and that PPIA inflicts IL6 production through a non-chaperone function.

Animal Models

Currently, the lack of a suitable animal model has posed a significant challenge for the study of the pathogenesis of systemic lupus erythematosus (SLE). Despite the successful use of virus infection, genetic engineering, and chemical induction of mycoplasma for the detection of a few diseases, the phenotypes of SLE are complex and variable. Spontaneous SLE-like syndromes in mice include the MRL/l, BXSB, Lydy, and B/WF1 strains. For the SLE study, a variety of mouse models have been developed by immunizing these mice with a variety of autoantigens or using lupus-like syndromes in a mouse that results from gene knockout. Even though these mice incorporate the inherent genetic errors of the disease and mimic the immunopathology of the disease, their developmental course is not consistent with humans, and animal models need to be validated in human disease.

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Neither spontaneous nor induced mouse models have been used to reproduce each type of human lupus. Most of the basic research on SLE has focused on the use of spontaneous or induced murine models, as they are readily available and easily manipulated. These animal models have been used to study the underlying mechanisms and, to a degree, therapeutic agents based on research findings. However, whether a study in mice can be applied to the corresponding lupus in humans remains a matter of contention. As animal SLE models demonstrate slight differences from human cases in their pathological or serological presentations, reduced expression of renal antigens, or lack of SLE-related comorbidity while showing major immune or genetic disorders, these models may only mimic some immune cells and molecular mechanisms in human lupus cases, limited in their simulative impacts on pathological functions in many lupus cases. Lupus nephritis is one of the most severe complications of SLE and, consequently, represents one of the main causes of high morbidity and mortality among the patients who are affected by this disease. The current study not only demonstrates that PPI1 is an early marker associated with the severity of kidney disease but also suggests the potential of PPI1 as a novel therapeutic target for SLE. As pilot drug screening showed the suppressive effect of rapamycin on IL-6-induced PPI1, it is interesting to include a PPI1-targeting agent in the therapeutic strategy by the regulation of PPI1's IL-6 production activity. In clinical practice, the efficacy of monoclonal antibodies directed against soluble IL-6 or its receptors has been well documented in RA and systemic juvenile idiopathic arthritis. As such, while the presence of serum IL-6 is correlated with the occurrence of SLE flares and the severity of lupus nephritis, it is reasonable to examine the efficacy of anti-IL-6 therapy in treating SLE in future trials. In conclusion, the present discovery encourages further investigation of the use of PPI1 and IL-6 as markers for the early diagnosis of SLE activity in order to determine the potential therapies that might be used to target PPI1.

Future Directions

There are several questions that remain unanswered and represent ongoing investigations in our laboratories. They include the partial characterization of our novel mouse model of increasing isomerization of proline-containing proteins and its impact on immune function, particularly that related to B cell function, and the contribution of peptidyl-prolyl isomerase in the glomeruli as the origin of the major organ involvement of systemic lupus erythematosus. Our study has particularly focused on the role of peptidyl-prolyl isomerase in human PBMCs, but it is possible that there are different peptidyl-prolyl isomerases in different immune cells. Revealing which other types of peptidyl-prolyl isomerase regulate cell function and the relative distributed expression of peptidyl-prolyl isomerase after external stimuli will be necessary to improve our understanding of peptidyl-prolyl isomerase and their role in SLE pathogenesis. As there are many inhibitors of FKBP12/13, it will also be important to determine the involvement

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of each peptidyl-prolyl isomerase on inhibitor activity to dissect the target related to cell proliferation.

Another potential future direction will be to explore the downstream target after blocking peptidyl-prolyl isomerase using different approaches and test our model results using an animal model. A feature of the current study is the evidence that peptidyl-prolyl isomerase is involved in the production of IL-6 and TNFα in an SLE model. This finding could be the subject of a further intriguing study, which will investigate the precise manner and potential effects of inhibiting peptidyl-prolyl isomerase controlled for organ damage and immune function. Mention of the inflammatory effect of peptidyl-prolyl isomerase inhibitors in fibroblast cells takes another direction. Completely knocking out peptidyl-prolyl isomerase is not perinatally very viable. The side effects of peptidyl-prolyl isomerase inhibition need to be fully studied before considering any applications in humans. Our study also indicates that the functional activity of peptidyl-prolyl isomerase might be a critical mediator in patients with SLE, but further comprehensive studies are required to establish whether the activity of the peptidyl-prolyl isomerase might represent a useful tool to control the inflammatory effect.

Conclusion

The data in this study provide novel insights into the pathogenic role of the PPI1-Nox5-IL-6 axis in T-lymphocyte-derived IL-6 expression in SLE. This is the first study to demonstrate the upregulation of PPI1 and Nox5 protein as well as their mRNA in SLE patient CD3+ T-cells and to define the essential roles of PPI1 and Nox5 in the ascorbate-inducible IL-6 pathway. Further analysis revealed the presence and localization of PPI1 and Nox5 in the CD3+ T-cells of nonrenal SLE and lupus nephritis patients. Therefore, we propose that PPI1 and Nox5 may be utilized as biological markers and/or treatment targets for SLE. Provided that PPI1 and Nox5 are antagonized by effective inhibitors, targeting PPI1 and Nox5 will likely provide new therapeutic intervention to control the autoreactive T-cell-driven pathogenesis of SLE. This study demonstrated that PPI1 and Nox5 play a novel role in promoting the ascorbate-induced IL-6 expression and the cellular redox imbalance in SLE-derived CD3 T-cells. Together, these findings lead us to propose that PPI1 and Nox5 may become potential biological markers and/or treatment targets for SLE. The combined targeting of PPI1 and Nox5 could provide a novel approach for treating SLE patients. Nevertheless, additional studies are needed to clarify the detailed mechanisms as well as the potential clinical applications.

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Competing interests

The authors declare no conflict of interest.

Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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