Deregulated NLRP3 and NLRP1 Inflammasomes and Their Correlations with Disease Activity in Systemic Lupus Erythematosus

Qingrui Yang, Chengcheng Yu, Zhaowen Yang, Qing Wei, Kun Mu, Ying Zhang, Wei Zhao, Xiaofeng Wang, Wanwan Huai, and Lihui Han

ABSTRACT. ***Objective.*** NOD-like receptor family, pyrin domain containing 3 and 1 (NLRP3 and NLRP1) inflammasomes are molecular platforms that sense the damage or danger signals of cells. We investigated whether NLRP3/NLRP1 inflammasomes are involved in the pathogenesis and progression of systemic lupus erythematosus (SLE).

Methods. Expressions of inflammasome components at the mRNA and protein levels in the peripheral blood mononuclear cells (PBMC) from patients with SLE and healthy controls were investigated by quantitative real-time transcription PCR and Western blot, respectively. Correlations between NLRP3/NLRP1 inflammasome components' expression and clinical disease progression were investigated. Expressions of NLRP3/NLRP1 inflammasomes before and after treatment in the patients with SLE were also analyzed and compared.

Results. Our data showed that expressions of NLRP3/NLRP1 inflammasomes were significantly downregulated in PBMC from patients with SLE compared with PBMC from healthy controls. Further, expressions of NLRP3/NLRP1 inflammasomes were negatively correlated with the SLE Disease Activity Index, and regular glucocorticoid treatment significantly corrected this deregulation of these inflammasomes. Further analysis showed that type I interferon (IFN) level was significantly negatively correlated with expression of NLRP3/NLRP1 inflammasomes, which indicated that enhanced IFN-I level in patients with SLE was responsible, at least to a great degree, for the deregulation of inflammasomes.

Conclusion. These results indicated deregulation of NLRP3/NLRP1 inflammasomes in patients with SLE, and suggested an important role for inflammasomes in the pathogenesis and progression of SLE. (J Rheumatol First Release Dec 15 2013; doi:10.3899/jrheum.130310)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
DISEASE ACTIVITY
AUTOIMMUNE DISEASE
INFLAMMATION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with multiorgan inflammation, characterized by a myriad of immune aberrations resulting from disturbances of the immune response. Innate and adaptive immunity are the 2 arms of immune response; both are actively involved in all the autoimmune diseases. Disturbance of adaptive immunity in SLE has been extensively addressed; however, the role of innate immunity in the pathogenesis of SLE remains largely unknown. In contrast to adaptive immunity, which uses specific receptors for recognizing antigen, the innate immune system uses pattern recognition receptors (PRR) to recognize molecular patterns derived from pathogens and damaged cells. The NOD-like receptor (NLR) is a recently identified PRR family member that exists in the cell and senses intracellular danger signals. Our understanding of NLR in human diseases has expanded exponentially in recent years, though their roles in autoimmune disease are still elusive.

NLR family, pyrin domain containing 3 and 1 (NLRP3 and NLRP1) are 2 important members of the NLR family. They can recruit the adaptor apoptosis-associated speck protein with a caspase activation and recruitment domain

From the Department of Immunology, Shandong University School of Medicine; the Provincial Hospital Affiliated to Shandong University; and the Department of Pathology, Shandong University School of Medicine, Jinan, China.

Supported by the National Nature Science Foundation of China (No. 81172861 No. 81172352), Science and Technology Development Project of Shandong Province (No. 2011GGE27020, No. 2011GGH21829).

Q. Yang, MD, PhD, Provincial Hospital Affiliated to Shandong University; C. Yu, MD, Department of Immunology, Shandong University School of Medicine; Z. Yang, MD, Provincial Hospital Affiliated to Shandong University; Q. Wei, MS, Department of Immunology, Shandong University School of Medicine; K. Mu, MD, PhD, Department of Pathology, Shandong University School of Medicine; Y. Zhang, BS; W. Zhao, MD, PhD, Department of Immunology, Shandong University School of Medicine; X. Wang, MD, Provincial Hospital Affiliated to Shandong University; W. Huai, BS; L. Han, MD, PhD, Department of Immunology, Shandong University School of Medicine.

Dr. Qingrui Yang and Dr. Yu contributed equally to this work.

Address correspondence to Dr. L. Han, Department of Immunology, Shandong University School of Medicine, 44 Wenhua Xi Road, Jinan 250012, China. E-mail: hanlihui@sdu.edu.cn

Accepted for publication October 22, 2013.

(ASC), and mediate caspase-1 activation and mature interleukin 1β (IL-1β) production through assembly of the inflammasome platform in response to various pathogen-derived factors as well as danger-associated molecules. However, despite the potent role of NLRP3/NLRP1 inflammasomes in triggering innate immunity, their roles in autoimmune diseases have not been studied extensively.

NLRP3 and NLRP1 are reported to be expressed on a wide range of cell types, including macrophages, dendritic cells, lymphocytes, granulocytes, and peripheral blood mononuclear cells (PBMC)[1,2,3]. Whether NLRP3/NLRP1 inflammasomes are involved in the pathogenesis of SLE is not known. In our study, we compared the expression of NLRP3, NLRP1, and inflammasome components in the PBMC from patients with SLE and healthy controls. Surprisingly, our data showed a significant downregulation of NLRP3/NLRP1 inflammasomes in the patients with SLE, and a significant negative correlation between the inflammasome expression and disease progression, which indicated a possible involvement of deregulation of these inflammasome platforms in the pathogenesis of SLE.

MATERIALS AND METHODS

Human subjects. Thirty-nine patients with SLE treated in the outpatient clinics of Rheumatology of the Provincial Hospital Affiliated to Shandong University from May to December 2012 were included in our study. None of these patients had received systemic treatment, especially corticosteroids drugs or other immunosuppressants, as ongoing treatment for at least half a year before enrollment. Thirty-one sex-matched and age-matched subjects with no chronic rheumatic disease were included as healthy controls. SLE activities were assessed by the SLE Disease Activity Index (SLEDAI) scores[4]. All of the enrolled patients meet the criteria of the American College of Rheumatology. At the time of sampling, another 4 patients with SLE were investigated for changes of inflammasome expression before and after the glucocorticoid (GC) treatment. This study was performed with the approval of the Human Ethics Committee of Shandong University, and written informed consent was obtained from all of the enrolled subjects.

Laboratory measurement. For all of the patients with SLE, serum levels of Igs, C3, C4, and CRP were detected by using nephelometry methods, according to the manufacturer's protocol (Dade Behring, BN ProSpec System). ELISA was used for detecting autoantibodies, such as antinuclear antibody, anti-dsDNA, and antinucleosome antibody (AnuA). Erythrocyte sedimentation rates (ESR) were detected according to the manufacturer's instruction (TEST1, ALIFAX). Some other laboratory indices, such as IgG, IgA, IgM, and 24-h urine protein, were also included.

PBMC isolation. All blood samples from patients and healthy controls were deposited in tubes with sodium citrate. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples by Ficoll gradient centrifugation (Lymphoprep, Nycomed Pharma AS). Cells from the interphase were collected, washed twice with HBSS (HyClone) and stored in 1 ml of Trizol (Invitrogen) at –80°C until total RNA extraction.

RNA extraction and quantitative real-time PCR. Total RNA from PBMC was extracted with Trizol, following the manufacturer's instructions. One microgram of RNA was reversely transcribed using a reverse transcription kit (Toyobo) to a total volume of 20 μl. Expressions of objective genes were detected by quantitative real-time PCR (qRT-PCR) using QuantiFast SYBR Green PCR Kit (Qiagen) following the manufacturer's instructions. Primers for the investigated genes were NLRP3 forward 5'-GCA GCA AAC TGG AAA GGA AG-3', reverse 5'-CTT CTC TGA TGA GGC CCA AG-3'; NLRP1 forward 5'-CCA CAA CCC TCT GTC TAC ATT AC-3', reverse 5'-GCC CCA TCT AAC CCA TGC TTC-3'; ASC forward 5'-GCA CTT TAT AGA CCA GCA CCG-3', reverse 5'-GGC TGG TGT GAA ACT GAA GA-3', caspase-1 forward 5'-CCG AAG GTG ATC ATC ATCC A-3', reverse 5'-ATA GCA TCA TCC TCA AAC TCT TCT G-3'; IL-1β forward 5'-TTA CAG TGG CAA TGA GGA TGA C-3', reverse 5'-GTC GGA GAT TCG TAG CTG GAT-3', MX1 forward 5'- TGC TTA TCC GTT AGC CGT GG-3', reverse 5'- CGC CAG CTC ATG TGC ATCT-3'. Reactions of qRT-PCR were carried out using LightCycler CFX96 (Bio-Rad) according to the manufacturer's instructions. Each sample was examined in triplicate and β-actin was used as the internal control. PCR products were loaded in agarose gel, and were in all cases confined to a single band of the expected size. A melting-curve analysis was performed to ensure specificity of the products. The relative mRNA expression was determined using the comparative ($2^{-\Delta\Delta}$CT) method.

Western blot. Total proteins were extracted from PBMC of patients with SLE and from healthy controls and lysed in radioimmunoprecipitation buffer. Sixty micrograms of cell lysate was fractionated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to PVDF membrane by semidry electroblotting. The latter were then probed with specific antibodies against NLRP3 and ASC (#ALX-804-818-C100 and #ADI-905-173, respectively, Enzo Life Sciences); and antibodies against NLRP1, caspase-1, and IL-1β (#4990S, #3866S, and #2022S, respectively, Cell Signaling Technology). Expression of β-actin was assessed as an internal control by using anti-β-actin antibody (#ab6276, Abcam). Immunoreactive bands were detected by radiographic film using the SuperSignal West Pico Substrate Kit (PIERCE) according to the manufacturer's instructions. Band intensity analysis was done by Image J software normalized to β-actin.

ELISA. Serum IL-1β level in patients with SLE and healthy controls were detected by Human IL-1β ELISA Ready-SET-Go! kit from eBioscience according to the manufacturer's protocol. A450 value was used to measure the IL-1β level and draw the standard curve. Serum IL-1β level in the patients and healthy controls were calculated according to the curve.

Statistical analysis. The statistical analyses were carried out using GraphPad Prism software (version 5.0), with statistical significance set at $p < 0.05$. Mann–Whitney U tests were used for comparing NLRP3/NLRP1 inflammasomes expression and serum IL-1β level in patients with SLE and healthy controls. Paired t test was used to analyze the expression level before and after the GC treatment. Correlation analysis was analyzed by Spearman correlation analysis.

RESULTS

Laboratory measurements of the patients with SLE. Thirty-nine patients (30 women and 9 men) were enrolled. The mean age was 37.6 years (range, 17–62 yrs). SLE activities were assessed by SLEDAI scores at the flare time of SLE. SLEDAI scores ranged from 2 to 26, with a mean of 15. Lupus nephritis was one of the most common and serious clinical manifestations, and was observed in 29 of 39 patients. Arthritis is a common symptom in patients with SLE, and was found in 7 of 39 patients. Other clinical symptoms, such as serositis, leukopenia, thrombocytopenia, skin involvement, central nervous system disease, and fever, were found in some of the patients. A summary of clinical manifestations and laboratory measurements of the studied subjects is presented in Table 1.

Expressions of NLRP3/NLRP1 inflammasome components were significantly downregulated in patients with SLE. We examined NLRP3, NLRP1, ASC, caspase-1, and IL-1β

Table 1. Demographic characteristics, clinical features, and laboratory measurements of the studied subjects at the onset of disease. Values are n (%) unless otherwise indicated.

Characteristics	Patients with SLE, n = 39	Healthy Controls, n = 31
Female	30 (76)	24 (77)
Male	9 (24)	7 (23)
Age, yrs, mean (range)	37.6 (17–62)	30 (23–44)
Ethnicity	Chinese	Chinese
SLEDAI, mean (range)	15 (2–26)	—
Arthritis	7 (17)	—
Renal disease	25 (64)	—
Serositis	7 (17)	—
Hematological disease		
Leukopenia	2 (5)	—
Thrombocytopenia	7 (18)	—
Skin involvement	21 (54)	—
CNS disease	1 (2)	—
Vasculitis	0 (0)	—
Fever	7 (18)	—
Oral ulcer	5 (13)	—
Alopecia	19 (49)	—
Myositis	0 (0)	—
ANA	39 (100)	—
Anti-dsDNA	21 (54)	—
AnuA	22 (56)	—
C3, g/l, mean (range)	0.75 (0.17–1.54)	—
C4, g/l, mean (range)	0.082 (0.05–0.38)	—
IgG, g/l, mean (range)	19.07 (5.41–45.50)	—
IgA, g/l, mean (range)	3.08 (0.76–7.53)	—
IgM, g/l, mean (range)	2.80 (0.67–27.50)	—
24-h urinary protein, g/l, mean (range)	0.73 (0.08–3.92)	—
ESR, mm/h, mean (range)	53.5 (8–120)	—

SLE: systemic lupus erythematosus; SLEDAI: SLE Disease Activity Index; CNS: central nervous system; ANA: antinuclear antibody; AnuA: antinucleosome antibodies; ESR: erythrocyte sedimentation rate.

mRNA expression levels in PBMC from untreated patients with SLE and control subjects by qRT-PCR. Our results showed that NLRP3 and NLRP1 mRNA expression in PBMC from patients with SLE was significantly decreased compared with that of the healthy controls ($p = 0.0009$, Figure 1A; $p = 0.0001$, Figure 1B). Further, relative expression of inflammasome components' caspase-1 and IL-1β mRNA expression levels was also significantly decreased in patients with SLE compared with healthy controls ($p = 0.0300$, Figure 1C; $p = 0.0357$, Figure 1D).

To further verify the downregulation of inflammasomes in patients with SLE at the protein level, total protein was extracted from PBMC from untreated patients and control subjects, and was further analyzed by Western blot. Our data showed that the protein expressions of NLRP3, NLRP1, caspase-1, and IL-1β were significantly decreased in the patients with SLE compared with the healthy controls ($p < 0.05$, Figure 2A-D). Typical Western blot bands from 4 patients and 4 healthy controls are shown in Figure 2E. ELISA analysis showed that serum IL-1β level in patients with SLE was significantly decreased compared with that of the healthy controls ($p < 0.0001$, Figure 2F). All our data showed that the mRNA and protein levels of NLRP3/NLRP1 inflammasome components were significantly decreased in patients with SLE.

Expressions of NLRP3/NLRP1 inflammasome components were positively correlated with one another. Inflammasomes usually form supramolecular platforms[5] to carry out their effects, so we further investigated whether expressions of inflammasome components have any correlations with one another. Our results showed significant positive correlations between NLRP3 and caspase-1 expression (r 0.7405, $p < 0.0001$, Figure 3A), NLRP3 and IL-1β expression (r 0.6085, $p = 0.0053$, Figure 3B), NLRP1 and caspase-1 expression (r 0.8010, $p < 0.0001$, Figure 3C), NLRP1 and IL-1β expression (r 0.4745, $p = 0.0053$, Figure 3D), caspase-1 and IL-1β expression (r 0.5175, $p = 0.0010$, Figure 3E), and NLRP3 and NLRP1 expression (r 0.7055, $p < 0.0001$, Figure 3F). These data indicated that inflammasome components correlated with one another and these molecules worked as molecular platforms to carry out their functions.

Expression of NLRP3/NLRP1 inflammasomes had negative correlations with disease activity. To explore whether this

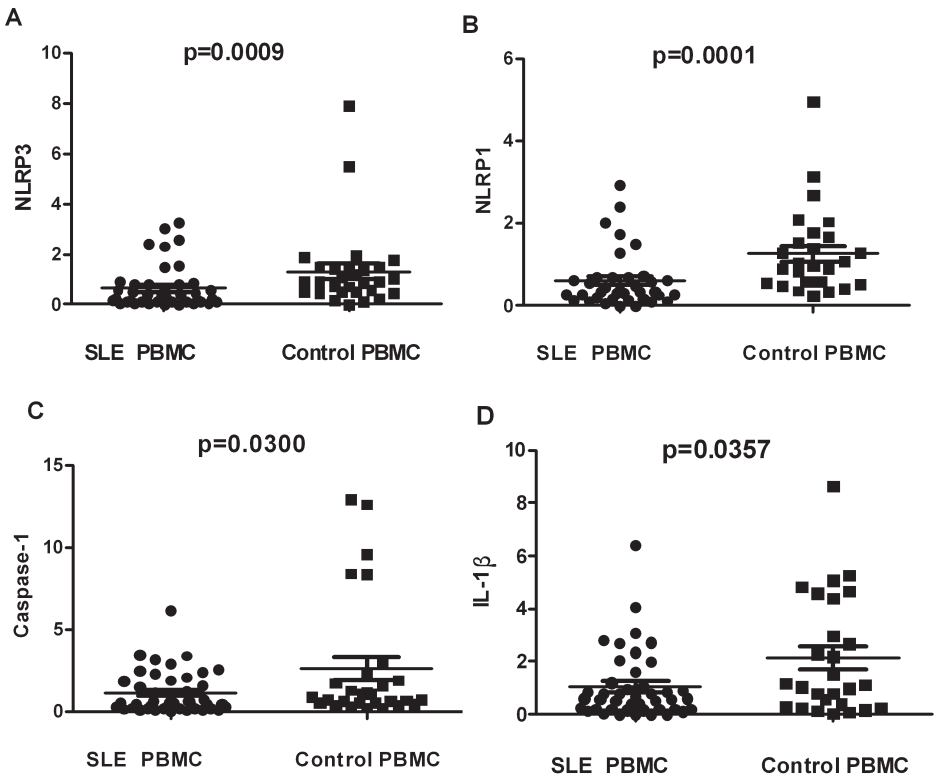

Figure 1. Comparison of NLRP3/NLRP1 inflammasome component mRNA expression from PBMC of patients with SLE and healthy controls. The target gene mRNA expression levels were detected by real-time PCR analysis. The scatter-plot representations showed the quantification levels of normalized mRNA ($2^{-\Delta\Delta CT}$). The p value refers to unpaired nonparametric comparison of the 2 groups (Mann-Whitney U test). SLE: systemic lupus erythematosus; PBMC: peripheral blood mononuclear cell; NLRP3: nucleotide-binding domain, leukine-rich family, pyrin containing 3; NLRP1: NLR, pyrin containing 1.

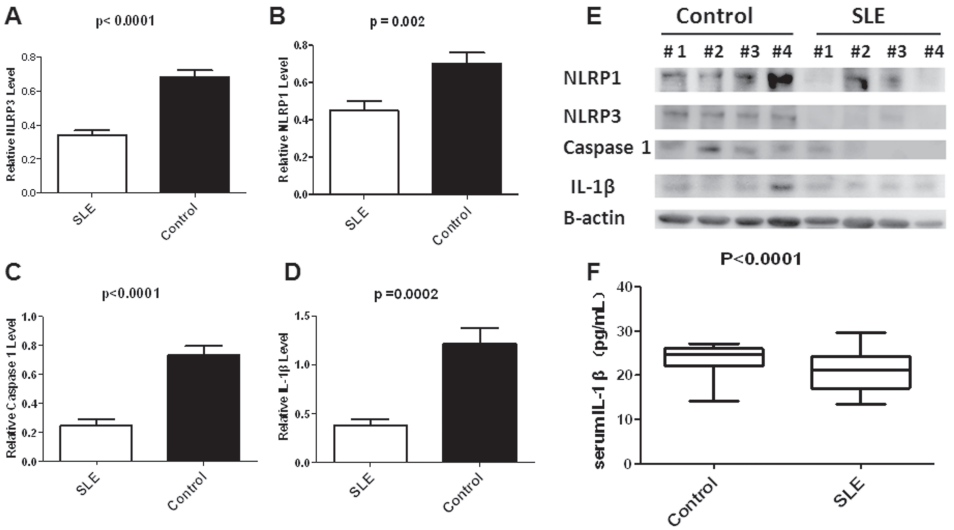

Figure 2. Western blot and ELISA analysis of the NLRP3/NLRP1 inflammasome components expression. Protein levels of NLRP3/NLRP1 inflammasome components expression were detected by Western blot, and the band density analysis of NLRP3 (A), NLRP1 (B), caspase-1 (C), and IL-1β (D) were analyzed by Image J software and normalized to β-actin. Typical Western blot bands from 4 patients with SLE and 4 healthy controls were presented (E). Serum IL-1β level in patients with SLE and healthy controls were analyzed by ELISA (F). SLE: systemic lupus erythematosus; NLRP3: nucleotide-binding domain, leukine rich family, pyrin containing 3; NLRP1: NLR, pyrin containing 1; IL: interleukin.

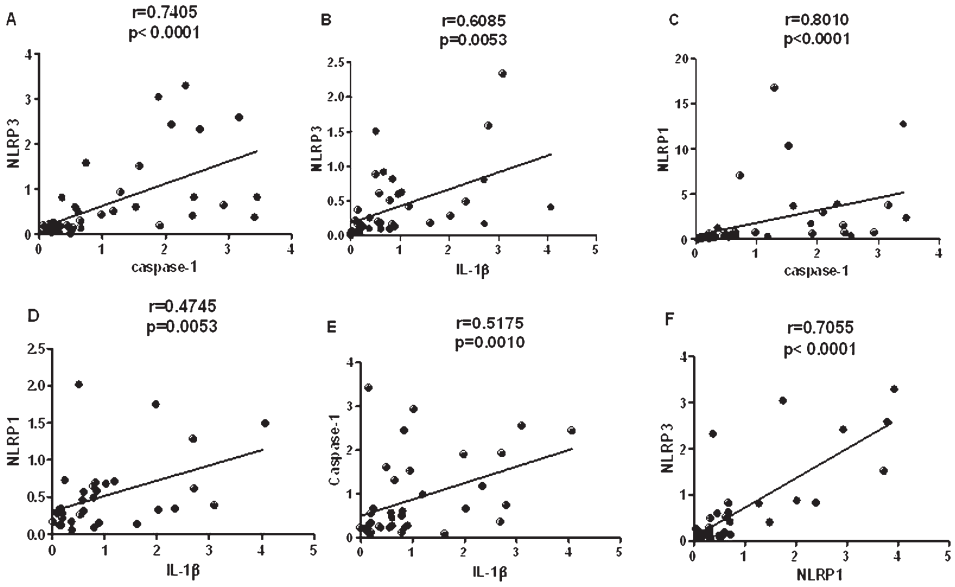

Figure 3. Correlations between expression levels of NLRP3/NLRP1 inflammasome components. Spearman's rank test was used in the correlation analysis. Positive correlations were presented between NLRP3 and caspase-1 (A), NLRP3 and IL-1β (B), NLRP3 and NLRP1 (C), caspase-1 and IL-1β (D), NLRP1 and caspase-1 (E), NLRP1 and IL-1β (F). NLRP3: nucleotide-binding domain, leukine-rich family (NLR), pyrin containing 3; NLRP1: NLR, pyrin containing 1; IL: interleukin.

deregulated expression of NLRP3/NLRP1 inflammasomes was involved in the pathogenesis of SLE, we compared mRNA expression levels of inflammasomes and SLEDAI scores in patients with SLE. Our results revealed that expression of NLRP3 mRNA was significantly negatively correlated with SLEDAI (r –0.3959, p = 0.0205, Figure 4A). Further, other inflammasome components also have significant negative correlations with SLEDAI scores, including NLRP1 (r –0.4113, p = 0.0174, Figure 4B), ASC (r –0.4860, p = 0.0087, Figure 4C), and caspase-1 (r –0.4921, p = 0.0107, Figure 4D). These data indicated possible involvement of NLRP3/NLRP1 inflammasomes in the pathogenesis and progression of SLE.

Our data also showed that significant negative correla-

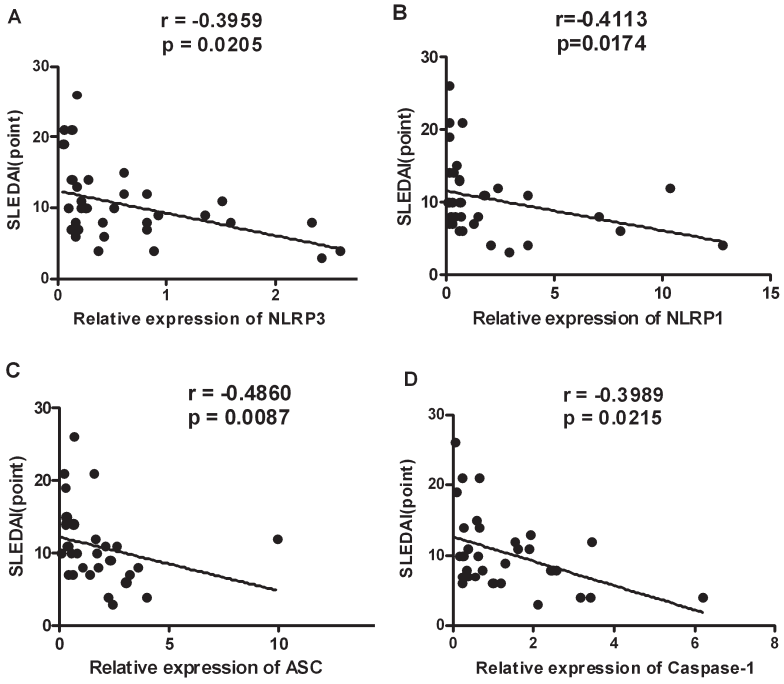

Figure 4. Correlations between NLRP3/NLRP1 inflammasomes mRNA expression levels and SLEDAI scores. Spearman's rank test was used in carrying out correlation analysis between mRNA expression levels and SLEDAI scores. Significant correlation between NLRP3 (A), NLRP1 (B), ASC (C), caspase-1 (D), and SLEDAI scores were presented. NLRP3: nucleotide-binding domain, leukine-rich family (NLR), pyrin containing 3; NLRP1: NLR, pyrin containing 1; ASC: apoptosis-associated speck protein with a caspase activation and recruitment domain; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

tions between the anti-dsDNA antibodies and the expression level of NLRP3 (r –0.5493, p = 0.0011, Figure 5A), NLRP1 (r –0.4077, p = 0.0281, Figure 5B), and caspase-1 (r –0.4002, p = 0.0284, Figure 5C). Analysis of the correlations of inflammasomes expression and AnuA also showed significant negative correlations between AnuA and NLRP1 expression (r –0.3357, p = 0.0486, Figure 5D), AnuA and caspase-1 expression (r –0.3699, p = 0.0372, Figure 5E), AnuA and IL-1β expression (r –0.3544, p = 0.0465, Figure 5F).

Myxovirus resistance 1 (MX1) expression assay and its correlation with the inflammasome components expression. It is well recognized that type I interferon (IFN-I) is involved in the pathogenesis of SLE[6,7,8,9]. However, whether IFN-I is involved in the deregulation of NLRP3/NLRP1 inflammasomes in patients with SLE is not known, and we investigated this topic. Because the IFN-I family includes multiple IFN-α subtypes and IFN-β, it is difficult to measure all the subtypes. It is recognized that measuring the expression of IFN-I-inducible MX1 could represent the total level of type I IFN[10,11,12,13]. Thus we measured the MX1 mRNA expression in PBMC from patients with SLE and healthy controls, and analyzed its correlation with clinical variables. Our data showed that MX1 expression was significantly downregulated in patients with SLE ($p < 0.05$, Figure 6A), and its expression was positively correlated with disease progression ($p < 0.05$, Figure 6B-C), which is consistent with the previous reports[12,13]. Further analysis of its correlation with NLRP3/NLRP1 inflammasome expression showed that the MX1 level was significantly negatively correlated with serum IL-1β level ($p < 0.05$, Figure 6D). Further, MX1 expression was significantly negatively correlated with inflammasome components expression, including NLRP3 (p = 0.0373, r –0.4026, Figure 6E), NLRP1 (p = 0.0147, r –0.05128, Figure 6F), ASC (p = 0.0340, r –0.3882, Figure 6G), and caspase-1 (p = 0.0348, r –0.3932, Figure 6H).

Deregulation of NLRP3/NLRP1 inflammasomes was corrected after regular treatment with GC. To further confirm that deregulation of NLRP3/NLRP1 inflammasome was correlated with disease progression, we investigated the inflammasome expression in 4 patients who responded well to traditional GC therapy (60 mg/day). Therapy effectiveness was evaluated by SLEDAI scores and clinical manifestations. Blood samples of each patient were collected at 2 timepoints, before and 1 month after the treatment. After the GC treatment, clinical symptoms of these enrolled patients declined, and the SLEDAI scores of these patients were significantly improved (19 ± 4.848 vs 4.4 ± 0.894, p = 0.0079), confirming that their diseases were controlled well by therapy. Thus, we compared the NLRP3/NLRP1 inflammasome components expression before and after the treatment. As shown in Table 2, downregulation of NLRP3, NLRP1, caspase-1, and IL-1β expression was significantly corrected by the GC therapy in patients with SLE ($p < 0.05$ for all the comparisons).

DISCUSSION

A wealth of information has emerged in recent years linking deregulated inflammasome signaling to human diseases. SLE is a typical autoimmune disease, driven by immune responses against ubiquitous self-antigens. To date, despite

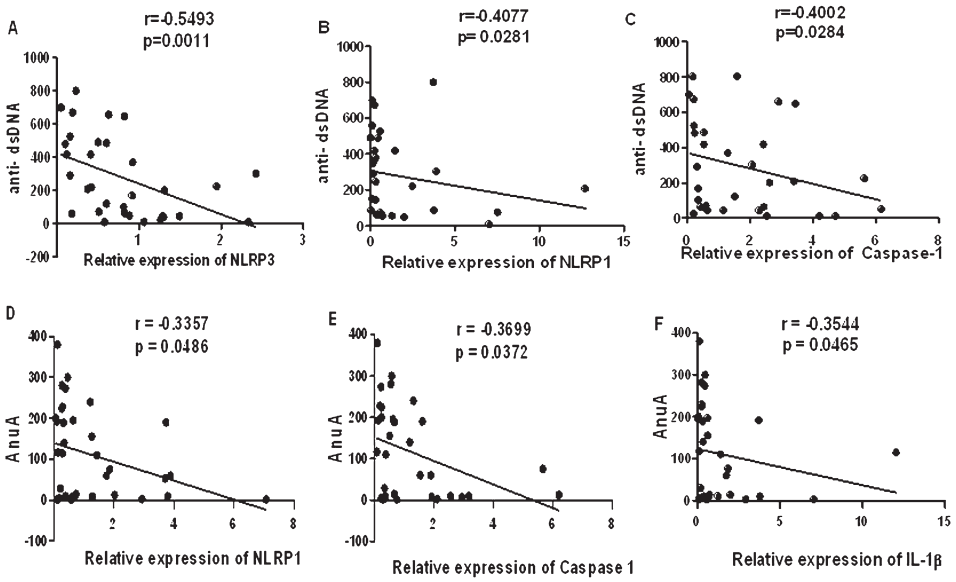

Figure 5. Correlation between NLRP3/NLRP1 inflammasome expression levels and autoantibody levels in patients with SLE. Spearman's rank test was used in the correlation analysis. NLRP3: nucleotide-binding domain, leukine-rich family (NLR), pyrin containing 3; NLRP1: NLR, pyrin containing 1; AnuA: antinucleosome antibodies.

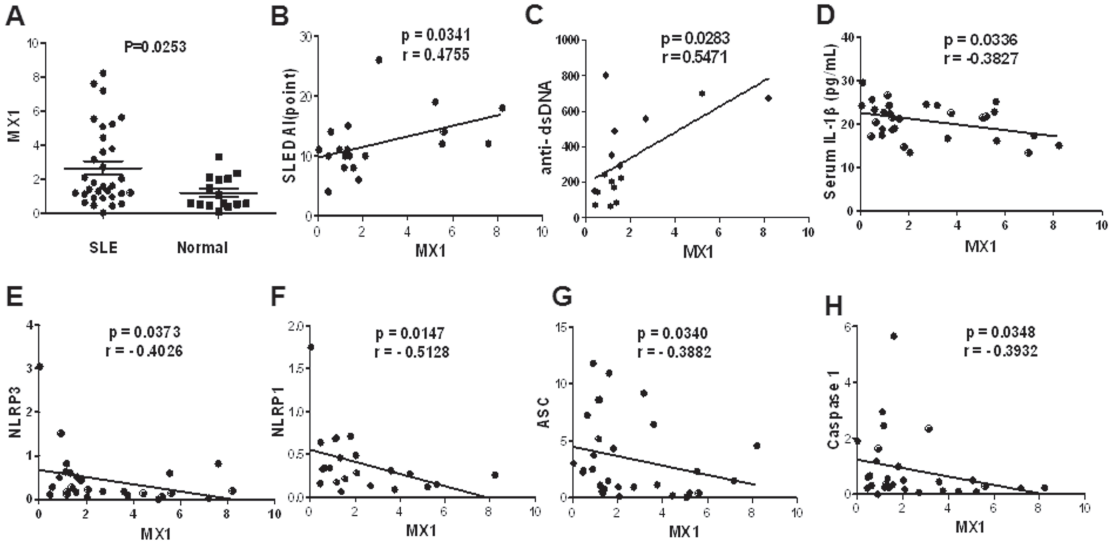

Figure 6. MX1 expression assay and its correlation with the inflammasome components. MX1 mRNA expression in the PBMC from patients with SLE and healthy control was detected by real-time PCR (A). Correlation between MX1 expression and disease activities (B-C), serum IL-1β level (D), NLRP3 mRNA (E), NLRP1 mRNA (F), ASC mRNA (G), and caspase-1 mRNA (H) were presented. NLRP3: nucleotide-binding domain, leukine-rich family (NLR), pyrin containing 3; NLRP1: NLR, pyrin containing 1; ASC: apoptosis-associated speck protein with a caspase activation and recruitment domain; MX1: interferon-I inducible myxovirus resistance 1; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

Table 2. Effect of glucocorticoid treatment on the expression of NLRP3 and NLRP1 inflammasomes.

Patient	1		2		3		4		
Treatment	BL	AP	BL	AP	BL	AP	BL	AP	p
NLRP3	0.13	5.90	2.75	18.29	0.74	19.44	3.83	13.39	0.0237*
NLRP1	0.53	4.34	5.74	8.56	2.35	5.17	0.14	0.52	0.0436*
Caspase-1	0.47	1.60	0.29	2.20	1.70	5.30	4.34	8.03	0.0252*
IL-1β	1.35	6.62	0.59	5.07	2.01	5.96	0.63	9.52	0.0148*

* $p < 0.05$. BL: baseline; AP: after prednisone; NLRP3: nucleotide-binding domain, leukine-rich family (NLR), pyrin containing 3; NLRP1: NLR, pyrin containing 1; IL: interleukin.

several reports, including the study showing that NLRP1 polymorphisms were associated with SLE (in particular with the development of nephritis, rash and arthritis)[14], the association between NLRP3/NLRP1 inflammasomes and SLE is still far from clear. NLRP3/NLRP1 inflammasomes exert their functions as molecular platforms, including ASC, caspase-1, and effector molecule IL-1β. When expression of inflammasomes is sufficiently high, the inflammasomes could be autoactivated and cleave IL-1β to its active form automatically[5]. Thus, inflammasome component expression reflects their activity and function to a great degree. Our study showed that NLRP3 and NLRP1 inflammasome components were significantly downregulated in the patients with SLE, and their expression levels were significantly negatively correlated with the SLEDAI index. Further, the serum IL-1β level, which actually represented the activation status of inflammasomes, was also significantly downregulated in the patients with SLE. Traditional therapy effectively reversed the deregulation of these inflammasome components, a finding that indicated an active involvement of these molecular platforms in the pathogenesis of SLE.

Inflammasome, as its name indicates, induces inflammation after its activation. One theory suggested that increased inflammation caused by inflammasome formation created a local environment that was favorable for pathogenesis of autoimmune diseases[15]. However, we found a decreased expression of NLRP3 inflammasome in the patients with SLE, indicating a protective role of inflammasome in SLE. Though downregulation and negative correlation of NLRP3/NLRP1 inflammasomes with SLE progression is unexpected, it is reasonable and can be explained by the immune sanctity rationale of inflammasomes. The protective role of inflammasomes in maintaining immune homeostasis, especially in the intestine, has been addressed in several reports[16,17,18,19].

These reports are consistent with our data showing a protective role of inflammasome in autoimmune inflammation-related disease. These may be reflections of the cell sanctity and body guardian role of inflammasomes[20].

One explanation for this inflammasome deregulation in SLE is the direct inhibitory effect of NLRP3/NLRP1 inflammasomes by T lymphocytes. SLE represents as clinical disorders mainly caused by overactivation of adaptive immunity and characterized with overproduction of active T and B lymphocytes. It has been reported that once adaptive immunity was activated, NLRP3/NLRP1 inflammasomes were directly suppressed by T cells[21]. Thus, this significant downregulation of inflammasomes is probably the direct consequence of the inhibitory effect of NLRP3/NLRP1 inflammasomes by overactivation of T cells from adaptive immunity in patients with SLE.

Another explanation for this inflammasome deregulation is attributed to the chaotic activity of type I interferon in patients with SLE. It has been noted for a long time that raised serum level of IFN-I is typical in patients with SLE and these raised levels are correlated with both disease activity and severity[6,7,8,9]. Crosstalk between IFN-I and NLRP3/NLRP1 inflammasomes has been reported, though the molecular mechanism is not clarified. An important consequence of this crosstalk is the suppression of NLRP3/NLRP1 inflammasomes by IFN-I[22,23,24], which is probably one of the mechanisms responsible for downregulation of these inflammasomes in patients with SLE. To investigate whether IFN-I was responsible for this deregulation of NLRP3/NLRP1 inflammasomes, we detected expression of MX1, the common IFN-I inducible gene. MX1 is recognized to reflect the total level of IFN-I[10,11,12,13] and we found that MX1 expression was significantly negatively correlated with inflammasome component expression. Our data indicated that increased expression of IFN-I was responsible, at least to a degree, for this downregulation of NLRP3/NLRP1 inflammasomes in the setting of patients with SLE.

Inflammasomes are pivotal host platforms for sensing danger signals and the subsequent regulation of inflammatory responses. However, tight control is essential because both excessive and inefficient activation of these molecular platforms can cause morbidity[25]. Many of the NLRP3 inflammasome inhibitors not only shut down the NLRP3 itself, but also ASC, caspase-1, and IL-1β, resulting in the dramatically decreased activity of the NLRP3 inflammasome[25]. In the status of patients with SLE, repeated activation of immunity produces huge amounts of inflammatory molecules and cells, many of which act as feedback loops to inhibit NLRP3/NLRP1 inflammasomes. This decreased expression of NLRP3/NLRP1 inflammasomes in patients with SLE may be a consequence of the suppressive effect by these inhibitory molecules produced by overactivation of adaptive immunity. One possible involvement of this decreased level of NLRP3/NLRP1 inflammasomes in patients with SLE can be attributed to their previously defined role associated with programmed cell death[26,27,28]. Appropriated cell death helps to get rid of overactivated lymphocytes and maintain the body's immune homeostasis. Lack of cell death is well recognized to inhibit homeostasis and contribute to autoimmunity in several studies[29,30], which may explain the pathological contribution of deregulated NLRP3/NLRP1 inflammasomes in the pathogenesis and progression in patients with SLE.

Our data surprisingly presented a significant downregulation of NLRP3/NLRP1 inflammasomes in patients with SLE, and this downregulation was negatively correlated with disease progression. Though it is difficult to discern whether deregulation of NLRP3/NLRP1 inflammasomes is a reason or an outcome of the disease progression, this deregulation is clear, and it must be considered when using the IL-1 blockade with anakinra as therapy for patients with SLE. Altogether, these data indicated a potential involvement of NLRP3/NLRP1 inflammasomes in the pathogenesis and progression of SLE, and highlighted the need for a better understanding of inflammasomes in these conditions.

REFERENCES

1. Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, et al. Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity 2006;24:317-27.
2. Guarda G, Zenger M, Yazdi AS, Schroder K, Ferrero I, Menu P, et al. Differential expression of NLRP3 among hematopoietic cells. J Immunol 2011;186:2529-34.
3. Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, et al. Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. J Histochem Cytochem 2007;55:443-52.
4. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992;35:630-40.
5. Broz P, von Moltke J, Jones JW, Vance RE, Monack DM. Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. Cell Host Microbe 2010;8:471-83.
6. Elkon KB, Wiedeman A. Type I IFN system in the development and manifestations of SLE. Curr Opin Rheumatol 2012;24:499-505.
7. Dall'era MC, Cardarelli PM, Preston BT, Witte A, Davis JC Jr. Type I interferon correlates with serological and clinical manifestations of SLE. Ann Rheum Dis 2005;64:1692-7.
8. Ronnblom L, Alm GV, Eloranta ML. The type I interferon system in the development of lupus. Semin Immunol 2011;23:113-21.
9. Niewold TB. Interferon alpha as a primary pathogenic factor in human lupus. J Interferon Cytokine Res 2011;31:887-92.
10. Lee PY, Li Y, Richards HB, Chan FS, Zhuang H, Narain S, et al. Type I interferon as a novel risk factor for endothelial progenitor cell depletion and endothelial dysfunction in systemic lupus erythematosus. Arthritis Rheum 2007;56:3759-69.
11. Li D, Song L, Fan Y, Li X, Li Y, Chen J, et al. Down-regulation of

TIPE2 mRNA expression in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Clin Immunol 2009;133:422-7.
12. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, et al. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis Rheum 2006;54:2951-62.
13. Hua J, Kirou K, Lee C, Crow MK. Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. Arthritis Rheum 2006;54:1906-16.
14. Pontillo A, Girardelli M, Kamada AJ, Pancotto JA, Donadi EA, Crovella S, et al. Polimorphisms in inflammasome genes are involved in the predisposition to systemic lupus erythematosus. Autoimmunity 2012;45:271-8.
15. Shaw PJ, McDermott MF, Kanneganti TD. Inflammasomes and autoimmunity. Trends Mol Med 2011;17:57-64.
16. Allen IC, TeKippe EM, Woodford RM, Uronis JM, Holl EK, Rogers AB, et al. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. J Exp Med 2010;207:1045-56.
17. Hirota SA, Ng J, Lueng A, Khajah M, Parhar K, Li Y, et al. NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. Inflamm Bowel Dis 2011;17:1359-72.
18. Zaki MH, Boyd KL, Vogel P, Kastan MB, Lamkanfi M, Kanneganti TD. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity 2010;32:379-91.
19. Zaki MH, Lamkanfi M, Kanneganti TD. The Nlrp3 inflammasome: contributions to intestinal homeostasis. Trends Immunol 2011;32:171-9.
20. Lamkanfi M, Dixit VM. Inflammasomes: guardians of cytosolic sanctity. Immunol Rev 2009;227:95-105.
21. Guarda G, Dostert C, Staehli F, Cabalzar K, Castillo R, Tardivel A, et al. T cells dampen innate immune responses through inhibition of NLRP1 and NLRP3 inflammasomes. Nature 2009;460:269-73.
22. Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Forster I, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity 2011;34:213-23.
23. Inoue M, Williams KL, Oliver T, Vandenabeele P, Rajan JV, Miao EA, et al. Interferon-beta therapy against EAE is effective only when development of the disease depends on the NLRP3 inflammasome. Sci Signal 2012;5:ra38.
24. Veeranki S, Duan X, Panchanathan R, Liu H, Choubey D. IFI16 protein mediates the anti-inflammatory actions of the type-I interferons through suppression of activation of caspase-1 by inflammasomes. PLoS One 2011;6:e27040.
25. Rathinam VA, Vanaja SK, Fitzgerald KA. Regulation of inflammasome signaling. Nat Immunol 2012;13:333-2.
26. Schroder K, Tschopp J. The inflammasomes. Cell 2010;140:821-32.
27. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature 2012;481:278-86.
28. Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 2009;10:241-7.
29. Gray DH, Kupresanin F, Berzins SP, Herold MJ, O'Reilly LA, Bouillet P, et al. The BH3-only proteins Bim and Puma cooperate to impose deletional tolerance of organ-specific antigens. Immunity 2012;37:451-62.
30. Zhang J, Xu X, Liu Y. Activation-induced cell death in T cells and autoimmunity. Cell Mol Immunol 2004;1:186-92.