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

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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. (First Release Dec 15 2013; J Rheumatol 2014;41:444–52; 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...
(ASC), and mediate caspase-1 activation and mature interleukin 1β (IL-1β) production through assembly of the inflammasome platform in response to various patho-gen-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) scores4. 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 and disease progression, which indicated a possible involvement of deregulation of these inflammasome platforms in the pathogenesis of SLE.
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 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 \(2^{-\Delta\DeltaCT}\). 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, leucine 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, leucine rich family, 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 3](image1.png)

![Figure 4](image2.png)
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. 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. 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. 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...
several reports, including the study showing that NLRP1 polymorphisms were associated with SLE (in particular with the development of nephritis, rash and arthritis)\textsuperscript{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-1\textbeta. When expression of inflammasomes is sufficiently high, the inflammasomes could be autoactivated and cleave IL-1\textbeta to its active form automatically\textsuperscript{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-1\textbeta 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\textsuperscript{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\textsuperscript{16,17,18,19}.

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment</th>
<th>1 BL</th>
<th>1 AP</th>
<th>2 BL</th>
<th>2 AP</th>
<th>3 BL</th>
<th>3 AP</th>
<th>4 BL</th>
<th>4 AP</th>
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<td>8.56</td>
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<td>5.17</td>
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<td>0.52</td>
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<tr>
<td>Caspase-1</td>
<td>BL</td>
<td>0.47</td>
<td>1.60</td>
<td>0.29</td>
<td>2.20</td>
<td>1.70</td>
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<td>4.34</td>
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<tr>
<td>IL-1\textbeta</td>
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<td>6.62</td>
<td>0.59</td>
<td>5.07</td>
<td>2.01</td>
<td>5.96</td>
<td>0.63</td>
<td>9.52</td>
<td>0.0148*</td>
</tr>
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</table>

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

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. 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. Crosstalk between IFN-I and NLRP3/NLRP1 inflammasomes has been reported, though the molecular mechanism is not clarified. A significant consequence of this crosstalk is the suppression of NLRP3/NLRP1 inflammasomes by IFN-I, 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 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. 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. 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. 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, 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**


