Activation of caspase-1 is mediated by stimulation of interferon genes and NLR family pyrin domain containing 3 in monocytes of active systemic lupus erythematosus

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Abstract

Objective

Emerging evidence has shown the importance of inflammasome activation in the progression of autoimmune diseases. In this study, we aimed to identify the main cell types activating inflammasome in autoimmune diseases and to clarify the intracellular pathway of inflammasome activation in systemic lupus erythematosus (SLE).

Methods

Active caspase-1 in each subset of human peripheral blood cells from healthy controls (n=18), SLE (n=51), and other rheumatic diseases (n=36) were fluorescently probed with FLICA™-caspase-1 followed by flow cytometric analysis. The correlation of caspase-1 activation in monocytes and clinical parameters in SLE patients were evaluated. In-vitro experiments were performed to identify the pathway involved in caspase-1 activation induced by SLE serum in monocytes.

Results

Active caspase-1 in monocytes was upregulated in SLE patients. Cluster of differentiation 14 (CD14)-positive and CD16-positive monocytes showed considerable activation of caspase-1 compared with the other subsets of monocytes. Serum titres of anti-double stranded DNA antibodies were positively correlated with active caspase-1 in monocytes, and serum complement component 3 and platelet count were negatively correlated with active caspase-1 in monocytes. The SLE serum-induced activation of caspase-1 and IL-1β secretion were down-regulated by inhibition of NLR family pyrin domain containing 3 (NLRP3), cyclic GMP-AMP synthase (cGAS), or stimulator of interferon genes (STING).

Conclusion

These findings suggest that targeting inflammasome by regulating cGAS/STING and NLRP3 are potential therapeutic strategies for SLE.

Key words

systemic lupus erythematosus, NLRP3, monocyte, cyclic GMP-AMP synthase, stimulator of interferon genes (STING), DNA, inflammasome, caspase-1

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Introduction

Inflammasomes are large protein complexes and play critical roles in the innate immune system. Inflammasomes are comprised of an adaptor protein apoptosis-associated speck-like protein containing CARD (ASC), procaspase-1 and sensor proteins such as absent in melanoma (AIM2), nucleotide-binding domain and leucine-rich-repeat-containing (NLR) family members, and pyrin. Myeloid cells such as macrophages and monocytes are the major cell types equipped with inflammasome components. A sensor protein of inflammasome recognises pathogen-associated molecular patterns (PAMPs) or endogenous danger-associated molecular patterns (DAMPs) followed by a conformational change that allows interaction among inflammasome components in the cytosol. As a result, the inflammasome protein multi-complex is formed leading to the cleavage of pro-caspase-1 into the active form, then substrates of caspase-1 such as pro-interleukin (IL)-1β, pro-IL-18, and gasdermin D are processed. The processed N-terminal domains of gasdermin D execute the cell death called pyroptosis by forming membrane pore, and the secretion of DAMPs and pro-inflammatory cytokines such as active IL-1β and IL-18, which contribute to further activation of the innate and adaptive immune responses (1).

Systemic lupus erythematosus (SLE) has been characterised with the delayed clearance and the accumulation of dead cells and with enhanced type I interferon (IFN) signature resulting in autoimmune responses, originated from both genetic and environmental factors (2). The accumulation of dead cells promotes the production of autoantibodies leading to the formation of immune complexes, which bind to Fc gamma receptor IIA on the cell surface of plasmacytoid dendritic cells (pDCs) and stimulate the secretion of a large amount of type I IFN (3).

While the upregulated type I IFN signature is a considerable hallmark of SLE, non-negligible roles of inflammasomes have been recently indicated (4). Multiple works have shown that the indispensable roles of NLRP3-inflammasome (5-8) and AIM2 inflammasome in murine models of lupus (9). In addition, immune-complexes containing nucleic acids activate NLRP3-inflammasome in human monocytes (10, 11), and IL-18 is elevated in serum from SLE with active lupus nephritis (12-15) or with macrophage activation syndrome (MAS) (16). Furthermore, hyper-activated NLRP3 and AIM2 inflammasomes in macrophages derived from SLE patients were documented (17). Neutrophil extracellular traps (NETs), which are abundantly generated from SLE neutrophils, also activate NLRP3-inflammasome and promote the secretion of IL-1β in human monocytes (18-20). These data implicate considerable roles of the inflammasome in SLE pathogenesis.

Although the activation of caspase-1 in peripheral monocytes from SLE was documented in a previous report (21), detailed cell subsets undergoing inflammasome activation have not been well evaluated. In addition, the intracellular activation pathway of the inflammasome in monocytes has not been precisely clarified yet. Here, we investigated inflammasome activation in each subset of innate immune cells in peripheral blood from SLE patients by detecting active caspase-1 at a single-cell level, and the mechanism of activation of caspase-1 was assessed by *in-vitro* experiments.

Materials and methods

Patients and blood collection

Blood samples from healthy controls (HC), SLE patients, and patients with other rheumatic diseases were acquired at Kyushu University Hospital. Approval from the Institutional Review Board of Kyushu University Hospital had been obtained before this study. 10–20ml of whole blood was isolated from each subject via venous puncture using collection tubes containing heparin. The classification of SLE was based on 1997 update of the 1982 American College of Rheumatology revised criteria (22). The patients with rheumatoid arthritis (RA) (23), dermatomyositis (DM) (24, 25), and systemic sclerosis (SSc) (26, 27) were defined using classification criteria from the American College of Rheumatology/

European League Against Rheumatism. The diagnosis of Behçet's disease (BD) was made according to the diagnostic criteria of the Behçet's Disease Research Committee and the Ministry of Health, Labor and Welfare of Japan (28). Five HCs, 51 patients with SLE, 10 with DM, 13 with SSc, 10 with DM, and 4 BD subjects were included in the study. For FLICA™ -caspase-1 staining assays, the collected whole blood was used directly within six hours after blood sampling. Peripheral blood mononuclear cells (PBMCs) were separated using Lymphoprep™ (Abbot Diagnosis Technologies AS). Washed PBMCs were resuspended in CELLBANKER® 2 (Takara Bio) and stored at -80°C for further analysis.

Reagents

The small chemical inhibitors used in the present study were purchased as follows: MCC950 (NLRP3 inhibitor) from Cayman Chemical Company; RU.521 (cyclic GMP-AMP synthase (cGAS) inhibitor) from Invivogen; H-151 (stimulator of interferon genes (STING) inhibitor) from Invivogen. Aliquots of stock solution were prepared using dimethyl sulfoxide according to manufacturer's instruction and stored at -20°C. The inhibitors were diluted 1000 times or more using culture media and used at indicated concentrations in each experiment

FLICA-caspase-1 staining and flow cytometry

All fluorescent-labelled antibodies were purchased from BioLegend. For staining whole blood cells, 100 µl of whole blood was incubated with FLICA™ -caspase-1 for 90 minutes at a concentration indicated in the manufacturer's protocol. For staining cultured monocytes, cells were incubated with FLICA™ -caspase-1 for 30 minutes. Red blood cells in whole blood cells were lysed using RBC Lysis Buffer (BioLegend) according to the manufacturer's protocol. The whole blood cells or cultured monocytes probed with FLICA™ -caspase-1 were washed twice with 0.5% bovine serum albumin in phosphate buffer saline, followed by staining with allophycocyanin-conjugated anti-cluster of differentiation 14 (anti-CD14, M5E2), BV510-conjugated anti-CD16 (3G8), and peridinin chlorophyll protein-cyanine 5.5-conjugated anti-CD56 (HCD56). After washing the cells, dead cells were stained with propidium iodide (BioLegend). Stained cells were measured using FACSAria™ cell sorter (BD Biosciences) and data were analysed with FlowJo software (FlowJo).

Quantitative real-time polymerase chain reaction (qPCR)

Total RNA of PBMCs from SLE patients or HC was extracted using RNeasy Mini Kit (Qiagen) in accordance with the manufacturer's protocol. Complementary DNA was synthesised by iScript reverse transcriptase (Bio-Rad) and qPCR was performed using iTaq™ Universal SYBR Green® Supermix (Bio-Rad). The primers used in this study were applied from a previous study (29) or designed using Primer3 software. The primer sequences are as follows: *18s rRNA*, forward, 5'-GGCCCTGTAATTGGAATGAGTC-3'; *18s rRNA*, reverse, 5'-CAAGATCCAACTACGAGCTT -3'; *Mx1*, forward, 5'-TTCCACCTGAAGAAGGGTTACA-3'; *Mx1*, reverse, 5'-TGATTTTCTAACAGGGGCAGAG-3'.

All reactions were normalised to expressions of *18S rRNA* to evaluate the relative gene expressions.

Separation and stimulation of monocytes

Peripheral blood from HC was collected in BD Vacutainer™ Glass ACD Solution A tube (Becton Dickson). Monocytes were purified using RosetteSep™ Monocyte Enrichment Cocktail (STEMCELL Technologies) in accordance with the manufacturer's instruction. The purity of monocytes was confirmed to be >85% by flow cytometry according to the expression of CD14. Purified monocytes were suspended in RPMI 1640 (Thermo Fisher Scientific) with 10% of heat-inactivated (56°C, 20 minutes) autologous serum, MEM Non-Essential Amino Acids Solution (FUJIFILM Wako Pure Chemical Corporation), 50 nM of 2-Mercaptoethanol (Sigma Aldrich) and Penicillin-Streptomycin Solution (FUJIFILM Wako Pure Chemical Corporation), and were seeded on 96-well flat-bottom plate (Falcon) at a concentration of 2 x 10^5 cells/well. Then, the cells were incubated with 5% serum from a healthy donor or an SLE patient for 16 hours to evaluate FLICA™ -caspase-1, or for 24 hours to analyse with enzyme-linked immunosorbent assay (ELISA). In some experiments, monocytes were pre-treated with small chemical inhibitors for one hour prior to incubation with serum.

Measurement of cytokines

IL-1β and IL-6 in the collected supernatants were measured with Human IL-1β ELISA Set II (BD Biosciences) and Human IL-6 Duoset® ELISA (R&D Systems) as indicated in the manufacturer's protocols, respectively.

Ethics

All research involving human participants were approved by the Institutional Ethics Committees of Kyushu University Hospital. Written informed consent was obtained from all participants, and clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Statistics

The statistical analyses were performed using RStudio software, v. 3.5.2 (www.r-project.org). Differences in the percentage of active caspase-1 among monocyte subsets were analysed using Wilcoxon signed-rank test. Correlation analysis among the percentages of active caspase-1 in monocytes, Mx1 mRNA expression in PBMC, and clinical parameters were performed using Spearman's rank correlation test. *p*-values were calculated and adjusted using Holm method (30) in each analysis for multiple comparisons. Differences or correlations were considered to be significant when the corresponding reported *p*-values were <0.05.

Results

Caspase-1 activation in rheumatic diseases

To evaluate active caspase-1 in blood samples obtained from patients with rheumatic diseases, we performed flow

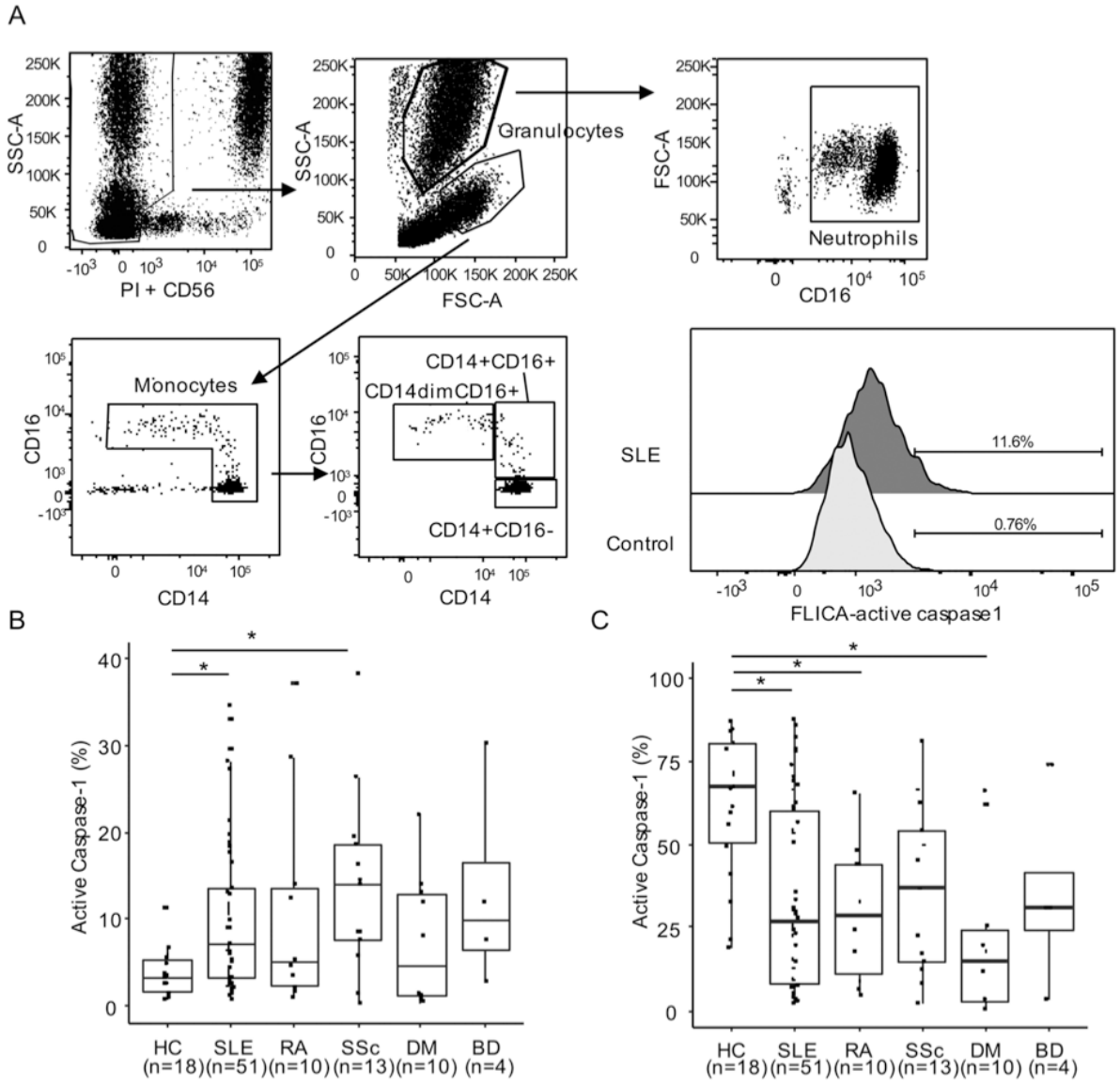

Fig. 1. Active caspase-1 in monocytes and neutrophils from SLE and other rheumatic diseases.
A: Gating strategy for detection of active caspase-1 in human monocytes and neutrophils. The histogram shows active caspase-1 in total monocyte from a healthy donor (control) and a systemic lupus erythematosus (SLE) patient.
B-**C**: Boxplots with active caspase-1 in monocytes (**B**) and neutrophils (**C**) from patients with rheumatic diseases and healthy controls (HC). The top and bottom of the box represent the 25th and 75th percentiles, respectively. The upper whisker represents the smaller of the maximum and upper quartile plus 1.5 x the IQR (interquartile range), and the lower whisker represents the larger of the minimum and lower quartile minus 1.5 x the IQR.
*adjusted *p*-value <0.05, Mann-Whitney U-test. RA: rheumatoid arthritis; SSc: systemic sclerosis; DM: dermatomyositis; BD: Behçet's disease.

cytometry-based detection of active caspase-1 using FLICA™-caspase-1, which has an active caspase-1 targeting sequence sandwiched between green fluorescent labels. Although conventional immune blotting requires a large amount of protein for the detection of active caspase-1, the active caspase-1 detection using FLICA™-caspase-1 allows a single-cell-level detection of active caspase-1 and is suitable for an evaluation of a limited amount of clinical samples.

Active caspase-1 in monocytes and neutrophils were evaluated as shown in Fig. 1A. Active caspase-1 in monocytes was variably increased among rheumatic diseases and significant upregulation was observed in patients with SLE and systemic sclerosis (SSc) compared with healthy controls (HC) (Fig. 1B). In contrast, active caspase-1 in neutrophils were largely downregulated in rheumatic diseases. Part of SLE patients showed prominent upregulation of caspase-1 in monocytes, we therefore decided to focus on active caspase-1 in monocytes from SLE.

Activation of caspase-1 in CD14-positive and CD16-positive monocytes

Monocytes are divided into functional subsets based on surface expression levels of CD14 and CD16. CD14-positive CD16-positive ($CD14^+CD16^+$) monocytes and $CD14dimCD16^+$ monocytes have more pro-inflammatory potential than $CD14^+CD16^-$ monocyte subsets (31). Among each monocyte subset divided based on the expressions of CD14 and CD16, the proportion of $CD14^+$ $CD16^+$ monocytes tended to

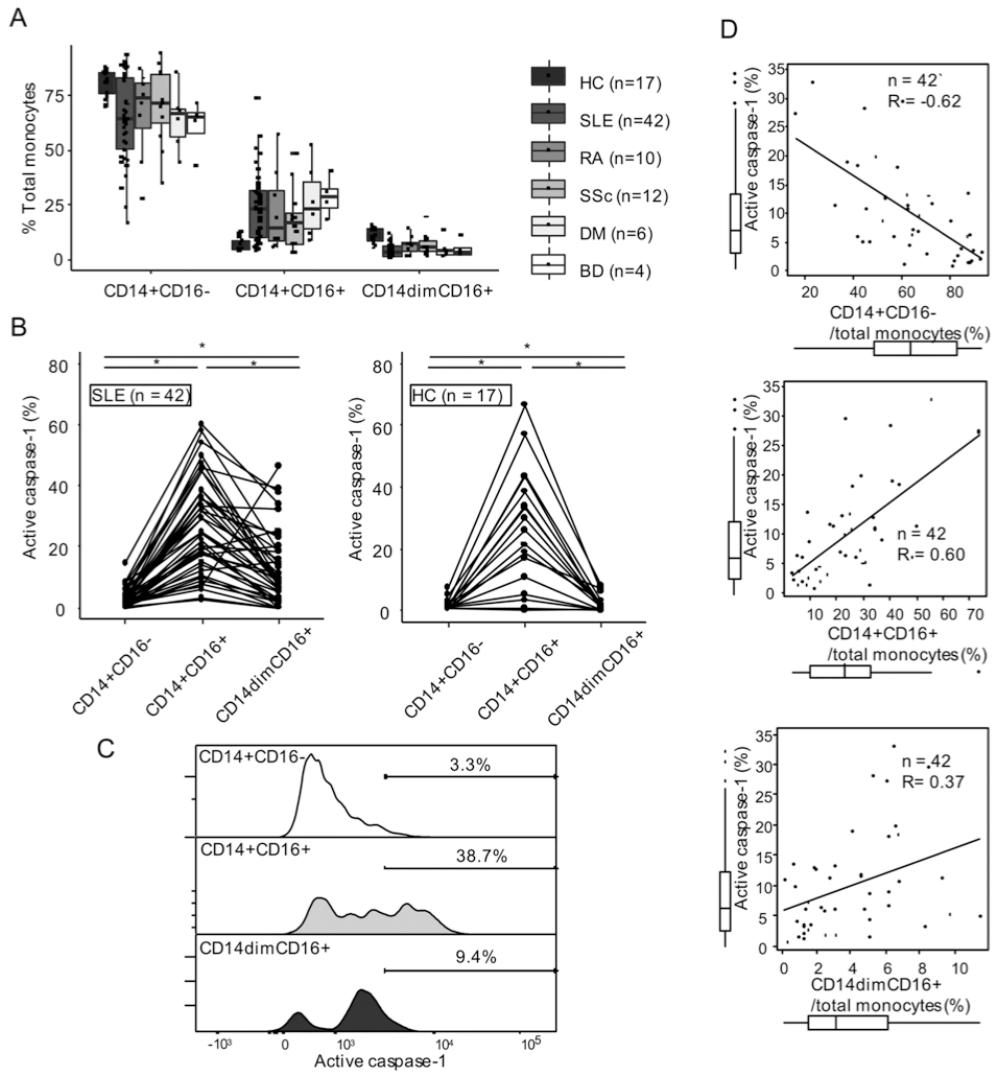

Fig. 2. Most prominent activity of caspase-1 in $CD14^+CD16^+$ monocytes.
A: The proportion of each subset of monocytes in total monocytes from patients with rheumatic diseases and HC. The upper whisker represents the smaller of the maximum and upper quartile plus 1.5 x the IQR (interquartile range), and the lower whisker represents the larger of the minimum and lower quartile minus 1.5 x the IQR.
B: The positive rate of active caspase1 in each subset of monocytes from SLE (left) and HC (right). Lines connect plots of each subsets of monocytes in the same individual.
C: The representative histogram showing positive rate of active caspase-1 in each subset of monocytes from SLE patients.
D: Correlation between the positive rate of active caspase-1 and the proportion of each subset of monocytes in total monocytes. Correlation coefficients (R) were calculated by Spearman's rank correlation test.
*p<0.05, Wilcoxon signed-rank test.

increase in SLE and other rheumatic diseases (Fig. 2A). We next evaluated caspase-1 activation in each monocyte subset. $CD14^+CD16^+$ monocytes had the highest amount of active caspase-1 compared with other subsets of monocytes (Fig. 2B, C). $CD14^+CD16^+$ monocytes from HC also showed remarkable upregulation of active caspase-1 compared with $CD14^+CD16^-$ monocyte subsets, which suggests that the $CD14^+CD16^+$ monocytes prone to activate caspase-1 even in a physiological condition (Fig. 2B). Moreover, a significant positive correlation (r=0.60, p=2.5e-4) was observed between the ratio of $CD14^+CD16^+$ monocytes / total monocytes and the positive rate of active caspase-1 in monocytes (Fig. 2D). Therefore, $CD14^+CD16^+$ monocytes presented the most prominent inflammasome activation in general.

Correlation between active caspase-1 in monocytes and disease activity in SLE patients

Next, we evaluated the relationship of active caspase-1 in monocytes and clinical parameters in SLE. The positive rate of active caspase-1 in monocytes moderately correlated with titres of serum anti-double stranded DNA (dsDNA) antibodies (r=0.31). In addition, the positive rate of active caspase-1 in monocytes was negatively correlated with serum levels of complement component 3 (C3) and blood platelet counts (r=-0.29 and r=-0.45, respectively). Interestingly, the positive rate of active caspase-1 in $CD14dimCD16^+$monocytes showed a substantial positive correlation with titres of serum anti-dsDNA antibodies (r=-0.47) and a negative correlation with serum levels of C3 (r=-0.51), C4 (r=-0.29), and total complement activity (CH50, r=-0.47) in comparison with other subsets of monocytes (Fig. 3A, B).

As a previous study has demonstrated increased expression of inflammasome components such as NLRP3 and caspase-1 were associated with elevated type I IFN signature in SLE (32), we therefore examined the correlation between active caspase-1 and Mx1 mRNA in PBMCs, which is one of the canonical interferon-stimulated genes. The expressions of Mx1 mRNA in SLE patients were upregulated compared with HCs and observed the correlation between some clinical parameters such as complement component 4, the number of white blood cells, and the titre of anti-ribonucleoprotein (anti-RNP) antibody (data not shown). Although SLE patients had considerable expres-

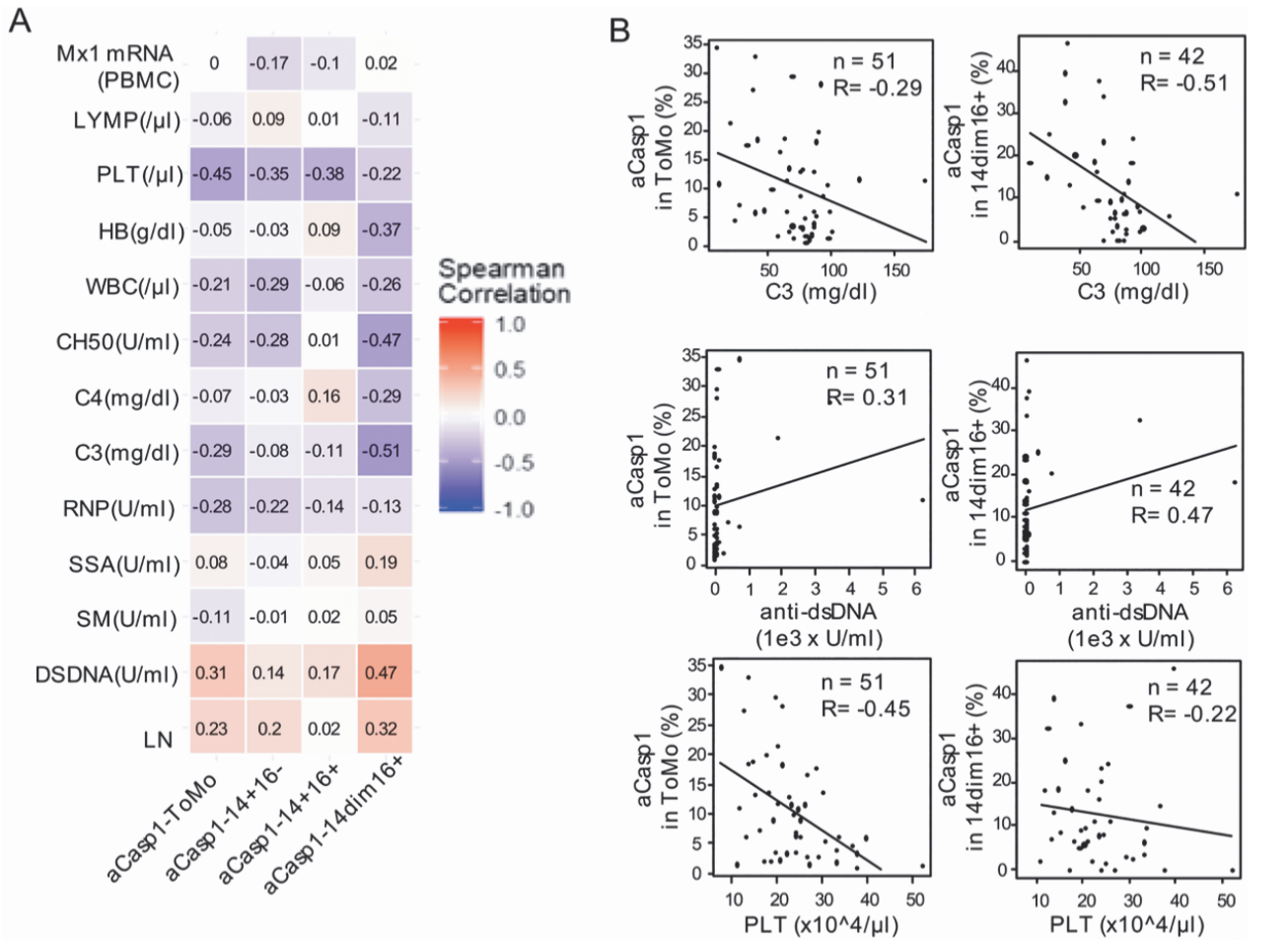

Fig. 3. The associations between active caspase-1 in monocytes and clinical parameters in SLE patients.
A: The heatmap shows correlations between positive rates of active caspase-1 in each subset of monocytes and indicated clinical parameters in SLE patients. The number inside each box denotes Spearman's rank correlation coefficient.
B: Scatterplots showing the correlation between positive rates of active caspase-1 and indicated clinical parameters. Spearman's rank correlation coefficients (R) were indicated.
aCasp1: active caspase-1; ToMo: total monocytes; 14+16-: CD14+CD16- monocytes; 14+16+: CD14+CD16+ monocytes; 14dim16+: CD14dimCD16+ monocytes; LYMP: lymphocytes; WBC: white blood cells; RNP: anti-RNP antibody; SSA: anti-SS-A antibody; SM: anti-Sm antibody; DSDNA: anti-dsDNA antibody; LN: lupus nephritis.

sions of Mx1 mRNA compared with HC, there was no correlation between active caspase-1 in monocytes and Mx1 mRNA in PBMCs (Fig. 3A). Accordingly, inflammasome activation in monocytes may be a critical mediator of exacerbation of SLE, while it is not related to the extent of type I IFN signature of peripheral blood in SLE.

SLE serum activate caspase-1 in monocytes dependently on NLRP3 and cGAS-STING

We speculated that stimulators of monocytes for inflammasome-activation exist in SLE serum. Monocytes from HC were stimulated with serum from HC or active SLE with an elevation of anti-dsDNA antibodies (SLE1-3). SLE-serum induced caspase-1 activation in monocytes compared to HC serum (Fig. 4A). The activation of caspase-1 via SLE serum were suppressed by an NLRP3-specific inhibitor MCC950, indicating that NLRP3 inflammasomes were activated in this system (Fig. 4A). In addition, MCC950 reduced SLE serum-induced IL-1β secretion, while there was no effect in IL-6 secretion (Fig. 4B).

Previous studies showed that microparticles in SLE serum stimulate monocytic leukemia cell line THP-1 cells to promote cyclic GMP-AMP synthase (cGAS)- and stimulator of interferon genes (STING)-dependent type I IFN secretion (33), and that human monocytes activated NLRP3 inflammasome in response to cytosolic DNA through STING-mediated lysosomal rupture (34). These findings compelled us to examine the effect of STING for caspase-1 activation induced by SLE serum. Interestingly, both a cGAS-specific inhibitor RU.521 and a STING-specific inhibitor H-151 inhibited SLE serum-induced caspase-1 activation (Fig. 4C). Secretion of IL-1β as well as IL-6 induced by SLE serum were also suppressed by RU.521 and H-151 (Fig. 4D). On the other hand, LPS-induced IL-1β and IL-6 secretion were not suppressed by RU.521 and H-151 (Fig. 4E). Because STING activation directs nuclear factor-κB (NF-κB)-mediated pro-inflammatory cytokine production such as IL-6 (35, 36) along

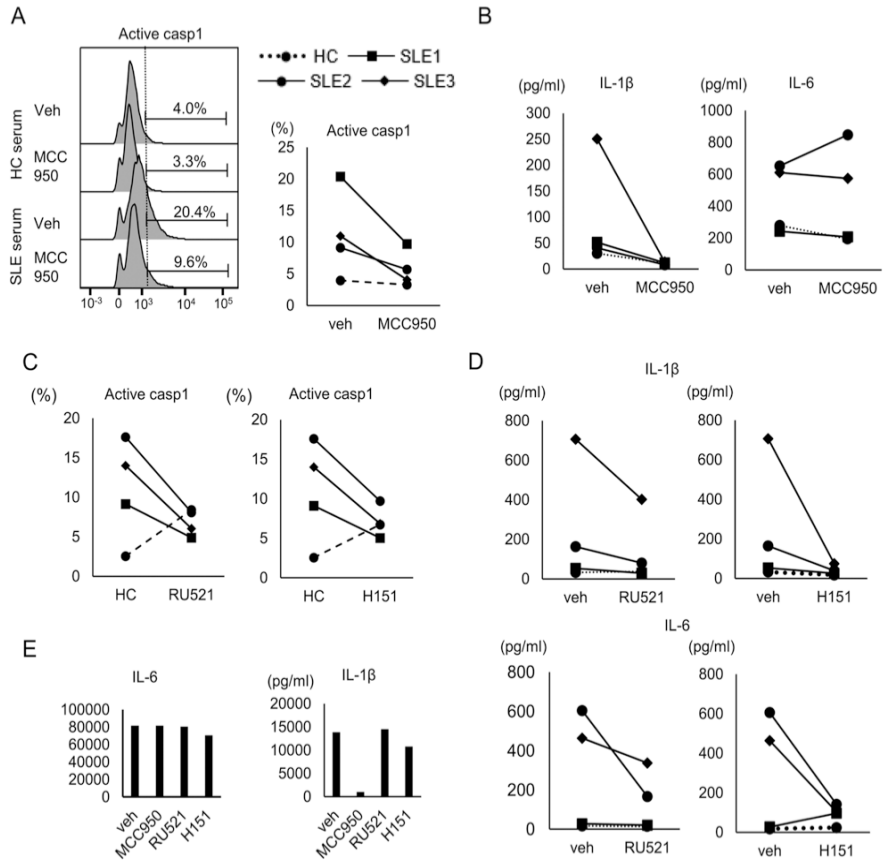

Fig. 4. NLRP3 and cGAS/STING-mediated inflammasome activation by SLE serum.
A: Monocytes from a healthy donor (HC) were pre-incubated with 5 μM of MCC950 (NLRP3 inhibitor) or DMSO (vehicle: veh) for 1 hour, followed by stimulation with 5% serum from SLE or HC. After 16 hours of incubation, active caspase-1 (casp1) was stained with FLICA™-caspase-1 and analysed using flow cytometry. The representative histograms are indicated.
B: Monocytes from a healthy donor are incubated as same as (**A**). The supernatant was collected after 24 hours of incubation and the concentrations of IL-1β and IL-6 in culture supernatants were measured by ELISA.
C: Monocytes from a healthy donor were pre-incubated with 10 μM of RU.521 (cGAS inhibitor), 2 μM of H-151 (STING inhibitor), or DMSO (veh) for 1 hour, followed by stimulation with 5% serum from SLE or HC. Active caspase-1 was evaluated as same as (**A**).
D: Monocytes from healthy donor were stimulated similarly as (**C**). The supernatant was collected after 24 hours of incubation. The concentrations of IL-1β and IL-6 in collected supernatants were measured by ELISA.
E: Monocytes from a healthy donor were pre-incubated with indicated inhibitors. The cells were stimulated with 100 ng/ml of LPS and culture supernatants were collected after 24 hours of incubation for ELISA analysis. Data are representative of two or three independent experiments. The error bars indicate mean±SD. Each analysis was performed in triplicate.

with NLRP3 activation, it explains that STING-inhibition results in suppression of both IL-1β and IL-6. Accordingly, NLRP3 inflammasome may be a dominant inflammasome in SLE pathology and cGAS/STING-induced activation of SLE monocytes can involve in NLRP3 inflammasome activation. These results suggest targeting the cGAS/STING pathway might be a favorable therapeutic strategy for SLE by regulating both NLRP3 inflammasome activation and type I IFN secretion.

Discussion

In the current study, strong caspase-1 activation in $CD14^+CD16^+$ monocytes in SLE patients was identified. These findings may be consistent with the previous report that describe $CD16^+$ monocytes from SLE patients showed an obvious capacity to induce differentiation of T helper (Th) 17 cells (37), whose differentiations are elicited by IL-1β and IL-6. $CD14^+CD16^+$ monocytes can secrete pro-inflammatory cytokines such as IL-1β, IL-6, and tumour necrosis factor-α upon LPS stimulation compared with $CD14^+CD16^-$ and $CD14dimCD16^+$ monocytes (31, 38). Immune complex containing anti-dsDNA antibodies and dsDNA also stimulate $CD14^+CD16^+$ monocytes to produce these pro-inflammatory cytokines (39). As $CD14^+CD16^+$ monocytes in other rheumatic diseases also had greater activation of caspase-1 than other subsets of monocytes in the current study (data not shown), the feature that $CD14^+CD16^+$ monocytes have a high capacity to activate inflammasomes may be a common characteristic among several disorders. On the other hand, some individuals showed equivalent activation of caspase-1 compared with $CD14^+CD16^-$ monocytes and the extent of caspase-1 activation in $CD14^+CD16^+$ were very heterogeneous (Fig. 2B). As previous single cell-based studies documented heterogeneity of monocyte even in $CD14^+CD16^+$ subsets (40, 41), it is conceivable that heterogeneities in $CD14^+CD16^+$ monocytes differ among different individuals, even in healthy ones. Although several markers other than CD14 and CD16 have been proposed to subclassify monocyte subsets, detailed investigations will be required to understand whether these $CD14^+CD16^+$ monocytes undergoing caspase-1 activation can be defined as a distinct monocyte subset (42).

$CD14dimCD16^+$ monocytes is another novel subset of monocytes, which are further divided into several functional subsets according to expressions of 6-sulfo LacNAc (slan) and CD9 (43). These subsets showed DC-like property and greater responsiveness toward toll-like receptor 7 (TLR7) and TLR8 ligands in terms of IL-1β secretion than other subsets of monocytes (31, 44, 45). Considering the pathological roles of these endosomal TLRs for SLE (46), these subsets of monocytes may undergo activation of caspase-1 and secretion of IL-1β in the SLE context. In the current study, caspase-1 activation in $CD14dimCD16^+$ monocytes were robustly correlated with serum complement factors and titres of anti-dsDNA antibodies in comparison with other monocyte subsets, therefore $CD14dimCD16^+$ monocytes might be an intriguing population as the target of SLE treatment.

Essential consequences of inflammasome activation are secretion of a large amount of IL-1β and IL-18, which lead to activation of both innate and adaptive immune system (47). Fever is a common manifestation in SLE (48) and IL-1β is one of fever-associated cytokines in SLE (49). It has been demonstrated that IL-1α/β-double knockout mice were resistant to the development of lupus (50), and elevated plasma concentration of IL-1β was observed in SLE patients (51). Moreover, increased IL-1β protein was detected in kidney tissue of lupus nephritis (52), and expanded Th17, differentiation of which is promoted by IL-1β in human, were observed in renal tissue in lupus nephritis (53). In addition, IL-1 blockade for SLE patients was partially effective for arthritis (54) and MAS (55). These facts suggest IL-1 may be one of the key mediators of SLE pathology. On the other hand, IL-18 is a pleiotropic cytokine belonging to the IL-1 superfamily (56) and has been initially referred to as IFN-γ inducing factor and associated with Th1 and NK cell response in synergy with IL-12 (57-59). This cytokine is known as an essential driver of macrophage activation syndrome through induction of IFN-γ (60-62) and elevated in severe SLE complicated by lupus nephritis (12-15) and MAS (16). It has been also suggested that dysfunctions of endothelial cells (63) and keratinocyte (64) in SLE induced by IL-18. Although, the results of clinical trials for the therapeutic antibodies of these cytokines have not been available, inhibiting IL-1 and/or IL-18 may be effective for SLE patients with inflammasome activation.

Among several autoinflammatory and autoimmune disorders, excessive activations of inflammasomes have been reported. NLRP3-inflammasome is activated by a diverse range of DAMPs and PAMPs such as silica crystal (65), TLR ligands (66), bacterial toxins, ATP (67), and immune complex containing nucleic acid (10, 11). As documented above, several studies and the current study suggest NLRP3 could be a dominant inflammasome in SLE. Multiple factors, such as apoptotic microparticles, immune complexes containing anti-dsDNA antibodies, and NETs, may be associated with the activation of the NLRP3 inflammasome in the SLE context, because of following reasons; 1) NETs induce IL-1β secretion from human monocytes (18-20), 2) immune complex containing anti-dsDNA antibody trigger NLRP3-mediated IL1β secretion (10, 39), and 3) apoptotic microparticles from SLE patients with high titre of anti-dsDNA antibody induce STING-mediated type I IFN production (33), which also results in NLRP3 inflammasome activation (34). Considering NETs can be internalised by monocytes and reach their cytosol (68), NETs and apoptotic microparticles might be a serum factor activating NLRP3 inflammasome. As SLE serum also trigger STING-mediated type I IFN secretion from monocytes (33), it is conceivable that STING-targeting could be efficient for SLE treatment through inhibiting both type I IFN secretion and STING-mediated NLRP3-inflammasome activation.

Meanwhile, our data showed poor correlations between inflammasome activation in monocytes and Mx1 mRNA, which is one of the representative interferon-stimulated genes (69-71). It has been known that type I IFN signature was not necessarily correlated with disease activity of SLE (72, 73) but reflected disease development (74) or future SLE flare (75, 76). On the other hand, one report showed type I IFN signature leads to enhanced expression of inflammasome components like caspase-1 in SLE (32). These facts suggest type I IFN signature may predispose, but not trigger, inflammasome activation by increasing expression of inflammasome components.

The strong correlation between active caspase-1 in monocytes and platelet count is one of the intriguing findings in the current study. There was no correlation between caspase-1 activation in monocytes and anti-phospholipid antibody or lupus anticoagulant, and therefore complicated antiphospholipid syndrome may not be associated with caspase-1 activation. Although it cannot be denied the possibility that the correlation between platelet count and active caspase-1 simply reflect the disease severity of SLE (77), the correlation may mean augmentation of caspase-1 activation induced by activated platelet. Activation of platelets was observed in SLE patients (78), and causes decreased platelet counts (79). Activated platelets mediate immune activations through various mechanisms as follows; activation of the complement system, the release of granules or microparticles, membrane protein such as CD40L and MHC molecules, and secretion of pro-inflammatory factors such as IL-1β and granzyme A (80–82). As the activation status of platelet (*e.g.* surface expression of CD62P and PAC-1 on platelets) was not addressed in the current study, however, further examinations are required to clarify the association between decreased platelet count and activated caspase-1 in monocyte.

To summarise, active caspase-1 in SLE monocytes were identified and $CD14^+CD16^+$ monocytes showed greater activation of caspase-1 than other subsets of monocytes. Because caspase-1 activation was associated with the disease activity of SLE, inflammasome activation may play a pivotal role in SLE pathogenesis. Activation of caspase-1 induced by SLE serum was largely NLRP3-dependent, and the cGAS/STING system contributed to the activation of NLRP3 inflammasome. Therefore, targeting NLRP3 and cGAS-STING system are the potential therapeutic strategies against SLE.

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