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U1-Small Nuclear Ribonucleoprotein Activates the NLRP3 Inflammasome in Human Monocytes

Min Sun Shin,* Youna Kang,* Naeun Lee,* Sang Hyun Kim,‡† Ki Soo Kang,‡† Rossitza Lazova,§ and Insoo Kang*

The NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome is a caspase-1–containing cytosolic protein complex that is essential for processing and secretion of IL-1β. The U1-small nuclear ribonucleoprotein (U1-snRNP) that includes U1-small nuclear RNA is a highly conserved intranuclear molecular complex involved in splicing pre-mRNA. Abs against this self nuclear molecule are characteristically found in autoimmune diseases like systemic lupus erythematosus, suggesting a potential role of U1-snRNP in autoimmunity. Although endogenous DNA and microbial nucleic acids are known to activate the inflammasomes, it is unknown whether endogenous RNA-containing U1-snRNP could activate this molecular complex. In this study, we show that U1-snRNP activates the NLRP3 inflammasome in CD14+ human monocytes dependently of anti–U1-snRNP Abs, leading to IL-1β production. Reactive oxygen species and K⁺ efflux were responsible for this activation. Knocking down the NLRP3 or inhibiting caspase-1 or TLR7/8 pathway decreased IL-1β production from monocytes treated with U1-snRNP in the presence of anti–U1-snRNP Abs. Our findings indicate that endogenous RNA-containing U1-snRNP could be a signal that activates the NLRP3 inflammasome in autoimmune diseases like systemic lupus erythematosus where anti–U1-snRNP Abs are present. The Journal of Immunology, 2012, 188: 4769–4775.

The innate immune cells detect molecular signals from the invading microorganisms or host cells in danger through specialized receptors, leading to the development of inflammation and cytokine production (1). The cytosolic protein complex inflammasome activates caspase-1, which is involved in processing and secretion of the proinflammatory cytokine IL-1β (2). The production of IL-1β requires the activation of two pathways (3): 1) the pattern recognition receptors such as TLRs with increased pro–IL-1β expression through NF-κB activation; and 2) inflammasomes that convert pro–IL-1β to IL-1β. An array of inflammasome activators has been identified, linking it to various pathologic conditions (2). These activators include self-derived uric acid, cholesterol crystals, and DNA, as well as ones originating from environments and microorganisms like alum, asbestos, silica, and viral nucleic acids (4–8). The NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome that comprises NLRP3, the adaptor protein ASC, and procaspase-1 is the best characterized one (9). Upon detecting triggering molecules, NLRP3 recruits ASC and procaspase-1, which subsequently cleaves pro–IL-1β to IL-1β. Recent studies support the important role for the NLRP3 inflammasome in host defense against pathogens, as well as in the pathogenesis of inflammatory diseases such as gout and atherosclerosis (2).

The U1-small nuclear ribonucleoprotein (U1-snRNP) is an intranuclear molecular complex involved in splicing pre-mRNA (10). This complex that contains U1-small nuclear RNA (U1-snRNA), Sm, and U1-specific proteins is highly conserved from human to insects and the most abundant snRNP in the cell (10, 11). Abs to U1-snRNP are found in patients with systemic lupus erythematosus (SLE) and mixed connective tissue disease, the autoimmune diseases characterized by the systemic inflammation affecting multiple organs including the skin and dysregulated immune responses to self nuclear Ags (10). Of interest, the translocation of U1-snRNP from the nucleus to the cell membrane was found in apoptotic keratinocytes induced by ultraviolet light, a known trigger for SLE, suggesting a potential role for this molecule in initiating and/or propagating autoimmunity (12, 13). Indeed, plasmacytoid dendritic cells (pDC) and B cells, which are involved in the lupus pathogenesis, were activated by the RNA sequences within the U1-snRNP or immune complexes containing U1-snRNP through TLR7 and TLR8 triggering (14–16).

Although endogenous DNA and microbial nucleic acids are known to activate the inflammasomes (2, 6, 17–20), it is unknown whether and how endogenous RNA-containing U1-snRNP could activate this molecular complex. In this study, we investigated this critical question, focusing on human monocytes, the primary cellular source of IL-1β (21). The results of our study show that U1-snRNP can activate the NLRP3 inflammasome in human CD14+ monocytes dependently of anti–U1-snRNP Abs, leading to IL-1β production. This phenomenon is driven by the generation of reactive oxygen species (ROS) and K⁺ efflux, and is dependent on TLR7 and TLR8. Our findings indicate that endogenous U1-snRNP could be a signal that activates the NLRP3 inflammasome in autoimmune diseases like SLE where anti–U1-snRNP Abs are present.
Materials and Methods

Human monocytes and sera

Human peripheral blood was obtained from the New York Blood Center or healthy adult donors with informed consent. Fresh monocytes were purified from peripheral blood using a negative cell purification kit (Stem Cell Technologies, Vancouver, BC, Canada). Anti-nuclear Ab (ANA)-positive sera with or without anti-U1-snRNP Abs were obtained from the L. Di- agnometrica Laboratory and patients with SLF. Anti-U1-snRNP Abs were measured by ELISA (DiaSorin, Stillwater, MN). Healthy control sera were obtained from peripheral blood of healthy donors. This work was approved by the institutional review committee of Yale University.

Cell stimulation

Purified monocytes (5 × 10^6) were resuspended in 100 μl RPMI 1640 media supplemented with 10% FCS, penicillin, and streptomycin. Bovine U1-snRNP was purchased from AroTec Diagnostics Limited (New Zealand). Monocytes were stimulated with or without bovine U1-snRNP (5 μg/ml) in the presence or absence of serum (final concentration of 5%) or total IgG with or without Abs to U1-snRNP. Total IgG purification from sera was done using a NAb spin kit for Ab purification (Thermo Scientific, Rockford, IL) according to the manufacturer’s protocol. Some monocytes were treated with zymosan (10 μg/ml) according to the manufacturer’s instructions. Some monocytes were additionally pretreated with anti-CD32 (FcγRII) Abs (2.5 μg/ml; R&D Systems, Minneapolis, MN), NF-κB inhibitors (Bay11-7082 [5 μM] and Cenostrol [5 μM]; Invivogen, San Diego, CA) as previously described (15). Anti-U1-snRNP Abs were measured by FAM FLICA Caspase-1 assay kit (Immunochemistry Technologies, Vancouver, BC, Canada). Anti-nuclear Ab (ANA)-positive sera without anti–U1-snRNP Abs were analyzed using a NAb spin kit for Ab purification (Thermo Scientific, Rockford, IL) according to the manufacturer’s protocol. Some monocytes were treated with zymosan (10 μg/ml) according to the manufacturer’s instructions. Some monocytes were additionally pretreated with anti-CD32 (FcγRII) Abs (2.5 μg/ml; R&D Systems, Minneapolis, MN), NF-κB inhibitors (Bay11-7082 [5 μM] and Cenostrol [5 μM]; Invivogen, San Diego, CA) as previously described (15). Anti-U1-snRNP Abs were measured by FAM FLICA Caspase-1 assay kit (Immunochemistry Technologies, Vancouver, BC, Canada).

Depletion of anti-U1-snRNP Abs from autoantibody-positive serum

Anti-U1-snRNP Ab-positive serum diluted with RPMI 1640 media (5% final concentration) was incubated overnight at 4˚C in a sterile ELISA plate coated with 20 μg/ml U1-snRNP. Serum was collected and analyzed for anti–U1-snRNP Abs by ELISA to determine levels of depletion.

Measuring the ROS synthesis

Monocytes were stimulated for 1–4 h with or without U1-snRNP in the presence or absence of healthy serum, anti–U1-snRNP Ab-positive serum (5% final concentration), or IgG purified from anti–U1-snRNP Ab-positive serum. ROS detection reagent carboxy-H2DCFDA (C400; Invitrogen) was added at the end of stimulation. Cells were analyzed on a flow cytometer to determine the intracellular levels of ROS.

Measuring NF-κB activation

To determine the NF-κB activation, we stimulated monocytes for 1–4 h with or without U1-snRNP in the presence or absence of healthy serum, anti–U1-snRNP Ab-positive serum (5% final concentration), or IgG purified from the Ab-positive serum. Stimulated cells were fixed, permeabilized, and stained with anti-phosphorylated NF-κB p65 Abs (pS529; BD Bioscience, San Diego, CA). Cells were analyzed on a flow cytometer.

Measuring caspase-1 activation

Monocytes were stimulated for 3–10 h with or without healthy serum, anti–U1-snRNP Ab-positive serum (5% final concentration), or IgG purified from anti–U1-snRNP Ab-positive serum in the presence or absence of U1-snRNP. The caspase-1 activation was measured by flow cytometry using FAM FLICA Caspase-1 assay kit (Immunochemistry Technologies, Bloomington, MN) according to the manufacturer’s instructions.

Knocking down the NLRP3 gene

Small interfering RNA (siRNA)-human NLRP3 plasmid and scrambled plasmid were purchased from Invivogen. Purified human monocytes were transfected with scrambled siRNA or NLRP3-specific siRNA using the Amaxa transfection system (Lonza, Walkersville, MD). Transfected cells were rested for 6 h followed by incubation for 18 h with or without U1-snRNP and anti–U1-snRNP Ab-positive serum. Knockdown of the NLRP3 gene was confirmed by quantitative PCR (qPCR) with a primer set: forward primer, 5′-CCACAAGATCGTGAGAAAAACC-3′; reverse primer, 5′-CGGTCTCATATGGTCGCT-3′.

ELISA and qPCR

IL-1β in culture supernatants was measured by ELISA (eBioscience, San Diego, CA). Intracellular pro–IL-1β protein was measured using a human pro–IL-1β ELISA kit (R&D Systems). Pro–IL1β gene was determined by qPCR with a primer set: forward primer, 5′-CAGATGCCACCTGATACGACAT-3′; reverse primer, 5′-GGTGGCCATATCCTGTCCT-3′. Total RNA was extracted from cells using RNeasy Plus Midi kit (QIAGEN) and cDNA was synthesized. Each real-time PCR reaction was performed in a mix of 10-μl reaction mixture containing 50 ng cDNA, 2X Brilliant SYBR green master mix (Stratagene), and 3 μM of each primer. The reaction mixture was denatured for 10 min at 94˚C and incubated for 40 cycles (denaturing for 15 s at 95˚C and annealing and extending for 1 min at 60˚C) using Mx3005P QPCR system (Stratagene). ACTINB was used as an endogenous reference. The comparative cycle threshold method (ΔΔCT) was used for quantification of gene expression.

Results

U1-snRNP induces IL-1β production from human monocytes in an anti-U1-snRNP Ab-dependent manner

U1-snRNP induced IL-1β production from human monocytes in the presence of anti–U1-snRNP Ab-positive serum, although U1-snRNP alone or a combination of U1-snRNP and healthy serum could not induce this cytokine production (Fig. 1A, 1B, Supplemental Fig. 1). Monocytes treated with only anti–U1-snRNP Ab-positive serum rarely produced IL-1β. In addition, IL-1β production was not detected from monocytes treated with U1-snRNP along with serum positive for ANA but negative for U1-snRNP Abs (Fig. 1C, Supplemental Fig. 1B).

FIGURE 1. U1-snRNP induces IL-1β production from human monocytes in the presence of U1-snRNP Ab-positive serum. (A–C) IL-1β ELISA at 18 h from cell culture supernatants of human monocytes incubated in the following conditions. (A and B) Monocytes from a single (A) or multiple donors ([B] symbols indicate individual donors) were incubated with or without U1-snRNP (snRNP, 5 μg/ml) in the presence or absence of healthy serum or anti–U1-snRNP Ab-positive [Ab+] serum (5% final concentration) from multiple donors (#S1–#S5). (C) ANA-positive sera without anti–U1-snRNP Abs (donors #A1–#A4) were added to monocytes from a single donor in the presence or absence of U1-snRNP. Representative data from two independent experiments (A, C), *p < 0.05.
To further delineate the specific role of U1-snRNP Abs in producing IL-1β, we incubated monocytes with IgG purified from anti–U1-snRNP Ab-positive serum in the presence or absence of U1-snRNP. Indeed, U1-snRNP induced IL-1β production from monocytes together with IgG purified from anti–U1-snRNP Ab-positive serum (Fig. 2A). Although we could only partially deplete

**FIGURE 2.** The production of IL-1β from human monocytes in response to U1-snRNP requires anti–U1-snRNP Abs. (A–D) IL-1β ELISA at 18 h from cell culture supernatants of human monocytes incubated in the following conditions. (A) Monocytes were incubated with or without U1-snRNP in the presence or absence of total IgG purified from anti–U1-snRNP Ab-positive [Ab(+)] serum or healthy serum. (B) Monocytes were incubated with or without U1-snRNP in the presence or absence of serum depleted or undepleted of anti–U1-snRNP Abs. Bars and error bars indicate mean and SEM, respectively (n = 2). (C) Monocytes were treated with anti-CD32 Abs (FcR blocker) followed by incubation with U1-snRNP and anti–U1-snRNP Ab(+) serum. (D) Monocytes were incubated with anti–U1-snRNP Ab(+) serum or U1-snRNP and anti–U1-snRNP Ab(+) serum in the presence or absence of RNase. Bars and error bars indicate mean and SEM, respectively [n = 5–7 donors for (A), (C), and (D)]. *p < 0.05.

**FIGURE 3.** U1-snRNP induces IL-1β production from human monocytes by activating NF-κB through triggering TLR7 and TLR8 in the presence of anti–U1-snRNP Ab-positive serum [Ab(+)]. (A and B) Pro–IL-1β qPCR (A) and ELISA (B) of human monocytes incubated for 6 (qPCR) or 10 (ELISA) hours with or without U1-snRNP (snRNP; 5 μg/ml) in the presence or absence of healthy or anti–U1-snRNP Ab(+) serum (5% final concentration). Bars and error bars indicate mean and SEM, respectively [n = 2 and 3 donors for (A) and (B)]. (C) Flow cytometric analysis of TLR7 and TLR8 expression in monocytes treated for 3 h with anti–U1-snRNP Ab(+) serum (D) or IgG purified from anti–U1-snRNP Ab(+) serum (E) in the presence or absence of U1-snRNP. (F–H) IL-1β ELISA of culture supernatants from monocytes incubated for 18 h with U1-snRNP and anti–U1-snRNP Ab(+) serum in the presence or absence of the NF-κB inhibitors (F, Bay11-7082 and Cenostrol), chloroquine (G), or inhibitory nucleic acid sequences for TLR7/8 (H, ORN). Representative data from 2–3 independent experiments (C–E). Bars and error bars indicate mean and SEM, respectively [n = 4–8 donors for (F)–(H)]. *p < 0.05. ORN, Oligoribonucleic acids.
anti–U1-snRNP Abs in Ab-positive serum (data not shown), such depletion still decreased IL-1β production from monocytes treated with U1-snRNP (Fig. 2B). A previous study reported the involvement of the IgG receptor CD32 (FcγRII) in stimulating pDCs by DNA-containing lupus immune complexes (22). In fact, blocking the CD32 on monocytes with anti-CD32 Abs reduced IL-1β production in response to a combination of U1-snRNP and anti–U1-snRNP Ab-positive serum (Fig. 2C). These findings further support the necessity of U1-snRNP Abs in producing IL-1β from monocytes in the presence of U1-snRNP. The highly conserved molecule U1-snRNP contains U1-snRNA that can trigger TLR7 and TLR8 (15). Incubating monocytes with RNase-pretreated U1-snRNP and anti–U1-snRNP–positive serum almost abrogated IL-1β production (Fig. 2D), suggesting the role of snRNA in inducing IL-1β production. Overall, our findings indicate that U1-snRNP can induce IL-1β production from human monocytes in an anti–U1-snRNP Ab-dependent manner.

**U1-snRNP induces IL-1β production from human monocytes by activating NF-κB through triggering TLR7 and TLR8 in the presence of anti–U1-snRNP Ab-positive serum**

The production of IL-1β requires the activation of two pathways (3): 1) the pattern recognition receptors like TLRs with increased pro–IL-1β expression through NF-κB activation; and 2) inflammasomes that convert pro–IL-1β to IL-1β. Thus, we determined whether U1-snRNP could increase pro–IL-1β gene and protein synthesis in monocytes in the presence of anti–U1-snRNP Abs.

Indeed, pro–IL-1β gene and protein were highly expressed in monocytes treated with U1-snRNP along with anti–U1-snRNP Ab-positive serum (Fig. 3A, 3B). Viral RNAs can trigger TLR7 and TLR8, leading to NF-κB activation that induces pro–IL-1β synthesis (20). Also, endogenous RNA could stimulate pDCs and B cells by triggering TLR7 and TLR8 (14, 16, 23, 24). Thus, we explored whether TLR7 and TLR8 with NF-κB activation are involved in producing IL-1β from monocytes in response to a combination of U1-snRNP and its autoantibodies. Human monocytes that expressed these TLRs had activation of NF-κB upon stimulation with U1-snRNP in the presence of anti–U1-snRNP Ab-positive serum or IgG purified from the autoantibody–positive serum (Fig. 3C–E). However, U1-snRNP alone or in combination with healthy serum hardly induced the activation of NF-κB in monocytes (Supplemental Fig. 2). Furthermore, IL-1β production from these cells was blocked by NF-κB inhibitors (Fig. 3F). TLR7 and TLR8 recognize their target molecules in the endosome (1). The endosomal inhibitor chloroquine and inhibitory nucleic acid sequences for TLR7 and TLR8 (14, 16, 23, 24) reduced both pro–IL-1β and IL-1β production by monocytes treated with U1-snRNP and anti–U1-snRNP Ab-positive serum (Fig. 3G, 3H; Supplemental Fig. 3A, 3B). Incubating monocytes with RNase-pretreated U1-snRNP and anti–U1-snRNP–positive serum also reduced pro–IL-1β production (Supplemental Fig. 3C). These findings indicate that NF-κB activation by U1-snRNP through TLR7 and TLR8 triggering in the endosome is essential for IL-1β production from monocytes in the presence of anti–U1-snRNP Abs.

**FIGURE 4.** The production of IL-1β from human monocytes in response to U1-snRNP and anti–U1-snRNP Ab-positive serum requires caspase-1 activation and NLRP3 inflammasome. (A–C) Flow cytometric analysis of active caspase-1 in human monocytes stimulated for 7 (A) or indicated hours (B) with or without U1-snRNP (5 μg/ml) in the presence or absence of anti–U1-snRNP (snRNP) Ab-positive [Ab(+) ] serum (5% final concentration), healthy serum (A, B), or total IgG purified from anti–U1-snRNP Ab(+) serum (C). (D) IL-1β ELISA of culture supernatants from monocytes incubated for 18 h with U1-snRNP and anti–U1-snRNP Ab(+) serum in the presence or absence of caspase-1 inhibitor. (E) IL-1β ELISA of culture supernatants from monocytes transfected with scrambled or NLRP3-specific siRNA followed by the incubation for 18 h with U1-snRNP and anti–U1-snRNP Ab(+) serum. Representative data from three independent experiments (A, C). Numbers in histograms indicate the frequency of cells stained positive. Symbols and error bars indicate mean + SD (n = 2) (B). Bars and error bars indicate mean and SEM, respectively [n = 4–7 donors for (D) and (E)]. *p < 0.05.
The production of IL-1β from human monocytes in response to U1-snRNP and anti–U1-snRNP Ab-positive serum is dependent on caspase-1 activation, ROS synthesis, K⁺ efflux, and NLRP3.

Active caspase-1 cleaves pro–IL-1β into the mature form IL-1β for secretion (3). Thus, we next determined whether U1-snRNP could activate caspase-1 in monocytes. Active caspase-1 was detected at high levels in monocytes treated with U1-snRNP in the presence of anti–U1-snRNP-positive serum (Fig. 4A, 4B). However, U1-snRNP alone or in combination with healthy serum could not induce the activation of caspase-1. A similar finding was found when monocytes were stimulated with IgG purified from the autoantibody-positive serum (Fig. 4C). The caspase-1 inhibitor suppressed IL-1β production from monocytes treated with a combination of U1-snRNP and anti–U1-snRNP Ab-positive serum (Fig. 4D), indicating the essential role of caspase-1 activation. To determine whether this caspase-1 activation was dependent on the NLRP3 inflammasome, we knocked down NLRP3 gene expression in human monocytes and measured IL-1β production from these cells in response to U1-snRNP and anti–U1-snRNP Ab-positive serum. Indeed, reducing NLRP3 gene expression decreased IL-1β production (Fig. 4E).

ROS is an essential mediator for the activation of the NLRP3 inflammasome by multiple molecules such as asbestos, silica, and cholesterol crystals (9). ROS could induce the separation of thioredoxin interacting protein from thioredoxin, resulting in the NLRP3 inflammasome activation through binding thioredoxin interacting protein to NLRP3 (25). Thus, we measured ROS in monocytes treated with U1-snRNP in the presence of serum or purified IgG containing Abs to this molecule. The increased generation of ROS was found in such treated monocytes (Fig. 5A–C). However, monocytes treated with U1-snRNP alone or a combination of U1-snRNP and healthy serum could not increase ROS synthesis. Furthermore, blocking the generation of ROS with the NADPH oxidase inhibitor DPI decreased IL-1β production (Fig. 5D). In addition to ROS, K⁺ efflux has been suggested as an activator for the NLRP3 inflammasome (9). Indeed, monocytes stimulated with U1-snRNP and anti–U1-snRNP Ab-positive serum in the presence of exogenous KCl had decreased IL-1β production (Fig. 5E). DPI and exogenous KCl also decreased caspase-1 activation in monocytes treated with U1-snRNP and anti–U1-snRNP Ab-positive serum, although the endosomal inhibitor chloroquine could not suppress caspase-1 activation (Fig. 5F). The latter finding suggests that the activation of TLR7 and TLR8 by U1-snRNP is primarily involved in the generation of pro–IL-1β rather than caspase-1 activation. This point is further supported by the suppression of pro–IL-1β production in monocytes treated with chloroquine or inhibitory nucleic acid sequences for TLR7 and TLR8 (Supplemental Fig. 3A, 3B). Of interest, the levels of active caspase-1 in monocytes treated with DPI or KCl were lower than those in untreated monocytes. This is likely secondary to the presence of constitutively activated caspase-1 at low levels in unstimulated human monocytes as previously reported (21). Overall, our findings indicate the role for ROS and K⁺ efflux in activating the NLRP3 inflammasome and inducing IL-1β production from human monocytes in response to U1-snRNP in the presence of anti–U1-snRNP Abs.

![FIGURE 5](http://www.jimmunol.org/)

The production of IL-1β and activation of caspase-1 by human monocytes in response to U1-snRNP and anti–U1-snRNP Ab-positive serum requires ROS synthesis and K⁺ efflux. (A–C) Flow cytometric analysis of ROS in monocytes stimulated for 3 (A) or indicated hours (B) with or without U1-snRNP (5 μg/ml) in the presence or absence of anti–U1-snRNP (snRNP) Ab-positive [Ab(⁺)] serum (5% final concentration), healthy serum (A, B), or total IgG purified from anti–U1-snRNP Ab(⁺) serum (C). (D and E) IL-1β ELISA of culture supernatants from monocytes incubated for 18 h with U1-snRNP and anti–U1-snRNP Ab(⁺) serum in the presence or absence of the ROS inhibitor DPI (D) or KCl (E). (F) Flow cytometric analysis of active caspase-1 in monocytes treated for 7 h with U1-snRNP and anti–U1-snRNP Ab(⁺) serum in the presence or absence of DPI, KCl, or chloroquine. Representative data from 3–4 independent experiments (A, C, F). Symbols and error bars indicate mean ± SD (n = 2) (B). Bars and error bars indicate mean and SEM, respectively [n = 4–7 donors for (D) and (E)]. *p < 0.05.
Discussion

The NLRP3 inflammasome is a caspase-1–activating cytosolic protein complex that is essential for processing and secretion of IL-1β. U1-snRNP, which contains U1-snRNA, is a highly conserved intranuclear molecular complex. Although endogenous DNA and microbial nucleic acids are known to activate the inflammasomes (2, 6, 17–20), it is unknown whether endogenous RNA-containing U1-snRNP could activate this molecular complex. In this study, we show that U1-snRNP activates the NLRP3 inflammasome in CD14+ human monocytes dependently of anti-U1-snRNP Abs, leading to IL-1β production. This phenomenon is driven by the generation of ROS and K+ efflux, and is dependent on NLRP3, caspase-1, and TLR7 and TLR8 pathways. Our findings indicate that endogenous RNA-containing U1-snRNP could be a signal that activates the NLRP3 inflammasome in autoimmune diseases like SLE where anti–U1-snRNP Abs are present.

The activators of the NLRP3 inflammasome are heterogeneous, ranging from self-derived ones to molecules from the environments and pathogens (2). It has been a subject of intense research of how molecules with distinct structures and chemical properties can activate the NLRP3 inflammasome. Several models have been suggested including the ones mediated by ROS synthesis and K+ efflux (9). The diverse types of NLRP3 inflammasome activators such as uric acid, asbestos, silica, and extracellular ATP could induce ROS synthesis and/or K+ efflux, leading to the caspase-1 activation and IL-1β secretion (reviewed in Ref. 9). We found that U1-snRNP induced ROS production in monocytes in the presence of anti–U1-snRNP Abs. Furthermore, the NADPH oxidase inhibitor DPI blocked caspase-1 activation and IL-1β production by such treated monocytes, indicating the involvement of NADPH oxidase in inducing this ROS. In fact, NADPH oxidase was implicated in generating ROS by the monocytic THP1 cells in response to asbestos and monosodium urate crystals (7). In addition to ROS, K+ efflux is likely linked to activating the NLRP3 inflammasome in monocytes in response to U1-snRNP and anti–U1-snRNP Abs in that adding KCl to tissue culture media blocked inflammasome in monocytes in response to asbestos and monosodium urate crystals (7). In fact, inhibiting cathepsin B also reduced caspase-1 activation and IL-1β production by these monocytes. A role of cathepsin B in activating NLRP3 inflammasome has been reported (5, 26, 27). In fact, inhibiting cathepsin B also reduced caspase-1 activation and IL-1β production by these monocytes. A role of cathepsin B in activating NLRP3 inflammasome has been reported (5, 26, 27). In fact, inhibiting cathepsin B also reduced caspase-1 activation and IL-1β production by these monocytes. A role of cathepsin B in activating NLRP3 inflammasome has been reported (5, 26, 27). In fact, inhibiting cathepsin B also reduced caspase-1 activation and IL-1β production by these monocytes.

Autoantibodies to U1-snRNP are found in patients with autoimmune diseases like SLE. The immune complex containing this molecule could activate pDC via the triggering of TLR7 and TLR8, leading to increased IFN-α production in lupus patients (14, 15). Increased IL-1β gene or protein expression is found in the PBMCs and skin lesions of lupus patients, as well as in the kidneys of lupus-prone mice (28–32). Furthermore, IL-1β–deficient mice were resistant to induction of lupus (33), and the rL-1R antagonist anakinra improved arthritis in lupus patients (34). Although these findings suggest a pathogenic role of IL-1β in lupus and the NLRP3 inflammasome is essential for IL-1β production (21), little is known about the mechanism for increased IL-1β production in lupus. Indeed, the results of our studies provide a possible mechanistic explanation for increased IL-1β production in lupus. The data from our study demonstrated that the highly conserved U1-snRNP activated the caspase-1 in human monocytes through triggering the NLRP3 inflammasome, leading to IL-1β production. This event that required U1-snRNP and anti–U1-snRNP Abs raises a question of how U1-snRNP located in the nucleus can be recognized by anti–U1-snRNP Abs. Of interest, keratinocytes damaged by the ultraviolet light, which is a well-known precipitating factor for SLE, expressed this molecule on the cell surface in a form of apoptotic bodies, allowing the access of autoantibodies to the U1-snRNP (12, 13). Thus, U1-snRNP could become a danger signal that triggers the activation of the NLRP3 inflammasome in autoimmune diseases like SLE where anti–U1-snRNP Abs are produced. Such activation with IL-1β production would induce and/or aggravate inflammation with tissue damage, although this mechanism may not account for why breaching of self-tolerance occurs at the beginning of autoimmunity.

Taken together, we show that U1-snRNP induces IL-1β production from human monocytes in the presence of serum or purified IgG containing U1-snRNP Abs by activating the NLRP3 inflammasome. This process involves ROS synthesis and K+ efflux, as well as the activation of TLR7, TLR8, and NF-κB. Our findings provide new insights into how the endogenous molecule U1-snRNP could serve as an NLRP3 inflammasome activator in the diseases with aberrant autoimmune responses to U1-snRNP, possibly contributing to the pathogenesis of such diseases.

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Disclosures

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