ORIGINAL RESEARCH

Role of NINJ1 in Gout Flare and Potential as a Drug Target

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Objective: To determine the role of nerve injury-induced protein 1 (NINJ1) introduced plasma membrane rupture (PMR) and damage-associated molecular patterns (DAMPs) release in the pathogenesis and progression of gout and to explore the potential of NINJ1 as a therapeutic target in gout.

Methods: Both peripheral blood mononuclear cells (PBMCs) and serum sample from gout patients (n = 58) and healthy controls (n = 16) were collected and processed to NINJ1 expression, lactate dehydrogenase (LDH) detection, NINJ1 inhibition, and NINJ1 expression experiments, respectively. NINJ1 knockdown was carried out by lentivirus in a monosodium urate (MSU) induced rat model, and NINJ1 neutralizing antibody was applied in a MSU induced mouse model.

Results: Our results found that NINJ1 was upregulated during a gout flare, and the resulting induction of PMR correlated with gout progression. NINJ1 knockdown significantly reduced the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activation and joint swelling in the rat model, and NINJ1 neutralizing antibody also significantly reduced gout flare in the mouse model and PBMCs. Moreover, NINJ1 expression is under NLRP3 inflammasome produced interleukin (IL)-1β control.

Conclusion: These results support the notion of a pathogenic role of NINJ1 introduced PMR in gout and provide a detailed mechanism for gout pathogenesis involving inflammatory cell death and DAMPs release introduced by IL-1β. In addition, targeting NINJ1 might be a potential therapeutic approach for gout.

Keywords: gout arthritis, NINJ1, NLRP3 inflammasome, plasma membrane rupture, therapeutic target

Introduction

Gout is an inflammatory disease resulting from MSU crystal deposition in joints and other tissues. Patients with gout always suffer from recurrent joints swelling and a great deal of pain.[1] According to the recurrence of a gout flare, gout is subdivided into acute phase and chronic phase, acute gout possesses less recurrence and always self-remission within 7–10 days, chronic gout possesses higher recurrence and lost self-remission.[2] Both genetic and environmental factors affect urate metabolism and can lead to hyperuricemia. This physiological change can result in MSU crystal formation, and activate the NLRP3 inflammasome inside of inflammatory cells synergistically with other stimuli.[3–7] Activated NLRP3 inflammasomes result in caspase-1 activation, which then cleave pro-IL-1β and Gasdermin-D (GSDMD), after which the cleaved GSDMD amino-terminal can insert into the plasma membrane and oligomerize to form a pore, allowing the release of small molecules, such as mature IL-1β to initiate inflammation.[8] IL-1β, in co-ordination with other cytokines such as tumor necrosis factor (TNF)-α and IL-6, promotes the recruitment of neutrophils at the inflamed joint, after which neutrophils were activated by MSU crystals further amplifying the inflammatory process.[9] Once GSDMD was cleaved, inflammatory cells either experience pyroptosis or survive and became super activated with inflammatory cytokines consistently secreted.[10] Pyroptosis occurs with PMR and the release of large molecules including abundant DAMPs, which further activate the NLRP3 inflammasome and other inflammatory pathways promoting inflammation.[11] In addition, the NLRP3 inflammasome can also trigger other types of cell death including necrosis,[12] MSU could also

induce necrosis in a mixed lineage kinase domain-like protein (MLKL) dependent manner.[13,14] Putatively, these inflammatory cell death results in vast PMR and DAMP release, leading to the hyperinflammation responsible for extremely painful gout flare. PMR plays a central role in this inflammation amplification.

NINJ1 was first identified as an adhesion molecule mainly expressed in neurons and Schwann cells and upregulated after nerve injury to induce neurite outgrowth.[15] Later studies found that it was also expressed in other cell types and involved in many diseases including cancer and autoimmune diseases.[16–21] NINJ1 was expressed in myeloid cells and mediates endothelial adhesion in the brains of experimental autoimmune encephalomyelitis (EAE) rats.[21] NINJ1 was upregulated in macrophage to increase cell–cell and cell–matrix adhesion of macrophages in hyaloid vascular system.[20] NINJ1 plays a key role in the transmigration of inflammatory antigen-presenting cells across the blood–brain barrier.[22] NINJ1 expression results in macrophage activation in intestinal inflammatory conditions.[23] Blocked of NINJ1 protected diabetic endothelial cells from high-glucose induced apoptosis in diabetes mellitus.[24] Besides, NINJ1 asp110ala single nucleotide polymorphism is associated with protection in leprosy nerve damage.[19] The role of NINJ1 in inflammation has long owe to its function in inflammatory cell adhesion and migration.[25,26] Until recently, it was reported that NINJ1 is a key regulator of PMR during pyroptosis and other types of cell death, and knockout NINJ1 could prevent PMR and macromolecule release.[27] PMR has long been considered a passive process following plasma membrane permeability change, it is now identified as an automatic process tightly controlled by NINJ1. Specifically, the N-terminal extracellular α-helical of NINJ1 responsible for NINJ1 oligomerizes to form a huge pore on the cell membrane to induce PMR, while factors that induce NINJ1 oligomerization still need further study.[27] At present, clinical treatments for gout are focused on colchicine, glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs),[28,29] which all have side effects and even yield poor outcomes in serious cases.[30] Hence, there is an urgent need for new targets and drugs. A previous study showed that genetic deletion of GSDMD and MLKL did not prevent MSU crystal induced cell death and inflammation,[31] which suggest target GSDMD and MLKL could not inhibit cell death in gout. Whether target NINJ1 could inhibit MSU induced cell death and gout inflammation still need further study.

To answer this question, we first tested the expression and activation of NINJ1 in different gout groups. Second, we investigated whether NINJ1 induced PMR and DAMPs release mediate gout inflammation by using NINJ1 knockdown lentivirus and NINJ1 block antibody. Finally, we found that in gout patients, IL-1β induces the expression of NINJ1 and initiates inflammation. We also identified a critical role of NINJ1 mediated inflammatory cell death in the pathogenesis and progression of gout.

Materials and Methods

Participants

The study compiled with the declaration of the Helsinki and was approved by the Ethics Committee of The First Affiliated Hospital of University of Science and Technology of China. All subjects gave written informed consent. Gout patients, meeting ACR/EULAR criteria,[32] and healthy controls were recruited from the Department of Rheumatology and Immunology at The First Affiliated Hospital of University of Science and Technology of China between January 2018 and April 2021. Gout patients (n = 58) were recruited first and subdivided into three groups according to the recurrence and joints flare, followed by individually matching 16 healthy controls, without past gout. The characteristics of gout patients (n = 58) and health controls (n = 16) can be found in Supplementary Materials Table 1. Acute gout group: gouty patients with gout flare and the recurrence is less than six times within half a year and self-remission. Chronic gout group: gouty patients with gout flare and the recurrence is more than six times within half a year and without self-remission. Gout in diapause: gouty patients without gout flare.[33]

Animals

All animal experiments were approved by the Animal Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China and conducted in conformity with the Guiding Principles for Research Involving Animals and Human Beings. Male C57/BL6 mice weighing between 25 and 30 g and Male Sprague-Dawley rats weighing between 150 and 200 g were purchased from Hangzhou Ziyuan Experimental Animal Technology Co., Ltd.

All animals were kept in pathogen-free animal facilities and were housed six per cage under standard conditions (12 h/ 12 h light/dark cycle and $[22 \pm 2]$ °C with a relative humidity of $[55\% \pm 5\%]$) with free access to feed and water throughout the experiment. All animals used in the experiment were at 8 to 12 weeks of age.

Peripheral Blood Mononuclear Cell Culture

Human PBMCs were freshly isolated from Natrium Citrate-treated blood, and were then isolated by Ficoll-Hypaque (TBD science, cat#LDS1075) density-gradient centrifugation. Cells were cultivated in RPMI 1640 medium (Hyclone, cat# SH30809101) supplemented with 2 mM L-glutamine, penicillin/streptomycin (Solarbio, cat# P1400), and 10% fetal bovine serum (FBS, Hyclone, cat# SH30809.01) in Teflon bags and allowed to rest for 24 h prior to stimulation. All incubations were performed at 37°C in humidified air with 5% CO_2. PBMCs were stimulated with MSU (100ng/mL) and TLR1/2 stimulator Pam3Cys (10 μg/mL) with or without MCC950 (Topscience, cat#T3701, 5 μM) for 6 h, supernatant and cells were collected for ELISA and RT-PCR. PBMC were cultured with or without recombinant human IL-1β (Novoprotein, cat# 0331537, 5 ng/mL), cells were collected at different time points for RT-PCR.

MSU Crystals-Induced Gout Arthritis

MSU crystals were prepared under pyrogen-free conditions by dissolving 10 mg/mL uric acid (Sigma-U2875-5G) in 0.01 M NaOH (pH 7.1) solution. Filter the supersaturated uric acid solution (0.45 μm) and keep it at room temperature for 48 h. The crystals were washed with 100% ethanol and sonicated to reduce their size. The amount of endotoxin present in the injected MSU crystals was <5 pg, which determined by chromogenic Limulus amebocyte lysate assay (Endosafe).

Mice were put under anesthesia, 100 μg MSU crystals (2 mg/mL) were injected into the tibiofemoral knee joints, and the inflammatory parameters were evaluated at different time points (0, 2, 4, 6 and 8 h) after the injection of the MSU crystals by collecting blood samples. Mice were then put to death, both blood samples and joints tissues were collected for further experiments.

Rats were put under anesthesia. Choose the right posterior ankle joint in each group as the puncture point, and flex the ankle joint 90 degrees as much as possible. Inject the MSU solution (100 μL 10 mg/mL MSU) from the inside of the tendon 30–40 degrees downwards into the joint cavity. The above process strictly follows the aseptic operation, with the bulging of the joint capsule as the injection standard. Rats were then put to death at 24 hours after injection, both blood samples and joints tissues were collected for further experiments.

Histopathological Observation of Synovial Tissue of Ankle Joint

At the end of the experiment, the ankle joints were fixed in formalin and embedded in paraffin, samples were then cut into 4μm sections and deparaffinized with xylene, washed with running water for 20 min, stained with hematoxylin for 30 min, washed with running water for 20 min, differentiated with hydrochloric acid and alcohol, stained with eosin for 5 min, finally dehydrated with gradient alcohol, and sealed with resin glue after transparent xylene. Histopathological pictures were then captured in electron microscopy imaging system.

Cytokine and LDH Assay

The concentration of IL-1β was quantified by Human IL-1β kit (RD, cat# DLB50), mouse IL-1β kit (RD, cat# MLB00C), and rat IL-1β kit (RD, cat# RLB00), respectively. All samples were added in triplicate, the protein concentration was calculated from the standard curve. The serum and cultured cells LDH was determined by Lactate Dehydrogenase (LDH) Assay kit (Solarbio, cat# BC0685). All operations are performed in strict accordance with the operating instructions.

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from whole blood using a whole Blood RNA isolation kit (Simgen), total RNA was isolated from cultured cells using Hipure total RNA mini kit (Magen). Isolated total RNA was reverse transcribed with the BioRT Master Hisensi cDNA strand synthesis kit (BioFlux, cat# BSB40M1). ABI Prism 7500 Sequence Detection System (Applied Biosystems) was used for RT-PCR. SYBR Green PCR Master Mix (Muma, cat# A4004M) was used to perform PCR of rat ACTB and NINJ1, IL-1β, TNF-α, IL-6, IL-18, NLRP3, caspase-1, and ASC. The relative level of gene

expression is determined by the comparative threshold cycle method described by the manufacturer, in which the data for each sample is normalized to ACTB constituent genes and expressed as a fold change compared to the control. PCR primers can be found in Supplementary Materials Table 2.

Western Blot

Western blot was processed with a general protocol from Abcam (https://www.abcam.com/protocols/general-western-blot-protocol). Membranes were incubated overnight at 4°C with the following antibodies: anti–IL-1β antibody (Abcam, cat# ab9722, 1/1000 dilution), anti–NLRP3 antibody (Cell Signaling Technology, cat# D4D8T, 1/1000 dilution), anti–β-actin antibody (Cell Signaling Technology, cat# D6A8, 1/1000 dilution), anti–Ninjurin antibody (BD Biosciences, cat# 610777, 1/500 dilution).

Statistical Analysis

All results are expressed as the mean ± standard error of the mean. GraphPad Prism version 7.04 software was used to perform a one-way analysis of variance on the data obtained in the in vivo experiment, and then Dunnett's multiple comparison test was performed. $P < 0.05$ was used as a statistically significant threshold.

Results

Elevated NINJ1 Expression and PMR in Gout Patients

In order to have a comprehensive understanding of NINJ1 function in gout, we divided gout patients into chronic, diapause, and acute groups. We first assessed the level of NINJ1 protein in different patient groups. Western Blot results showed that gout patients possess higher levels of NINJ1 protein than that of healthy control (HC), chronic gout (CG) patients possess even higher levels of NINJ1 protein than acute gout (AG) (Figure 1A), suggesting that NINJ1 may affect gout flare. Because NINJ1 induces PMR during inflammatory cell death, we then analyzed LDH levels, which are a key marker for PMR. We found that both AG and CG patients also have higher levels of LDH than HC (Figure 1B), suggesting that gout patients possess higher level PMR and cell death. NINJ1-induced PMR is associated with gout flare. Moreover, gout in diapause (GD) patients possess a higher NINJ1 protein level and LDH production than that of HC (Figure 1A and B), which could be the reason for gout recurrence.

NLRP3 Inflammasome Sourced IL-1β Regulate NINJ1 Expression

We then download raw data (GSE191054) from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database and reanalyzed with limma R package. Through data processing, we finally obtained phosphate-buffered saline (PBS) treated mouse Bone Marrow-Derived Macrophages (BMDMs) samples (3 cases) as the control group and MSU treated BMDMs samples (3 cases) as the treat group, using |log$_2$ fold change (FC)|>2 and adjusted $p<0.05$ to identify MSU induced differentially expressed gene (MSU-deg). We found that NINJ1 was upregulated in MSU stimulated BMDMs (Figure 1C), suggesting MSU treatment induce NINJ1 expression. We then isolated PBMC from HC and processed it to MSU stimulation with or without sulfonylurea MCC950, a specific NLRP3 inflammasome inhibitor.[34] We found that MSU induced NLRP3 inflammasome activation and NINJ1 upregulation (Figure 1D and E). NLRP3 inflammasome inhibition blocked NINJ1 upregulation (Figure 1D and F), suggesting that NINJ1 expression is NLRP3 inflammasome dependent. Previous results showed that IL-1β, a key indicator of NLRP3 inflammasome activation, induced NINJ1 expression in human endometriotic stromal cells.[35] Our results also showed that NINJ1 is upregulated during IL-1β stimulation in human PBMC (Figure 1G and H). Altogether, support IL-1β as a regulator of NINJ1 expression, MSU activated NLRP3 inflammasome releasing mature IL-1β to upregulate NINJ1 expression, and this upregulated NINJ1 expression correlated with PMR and gout attack. However, this upregulation is time-dependent, since prolonged incubation of IL-1β inhibits NINJ1 expression (Figure 1G and H), this prolonged IL-1β incubation-induced NINJ1 downregulation provides a potential mechanism for gout remission.
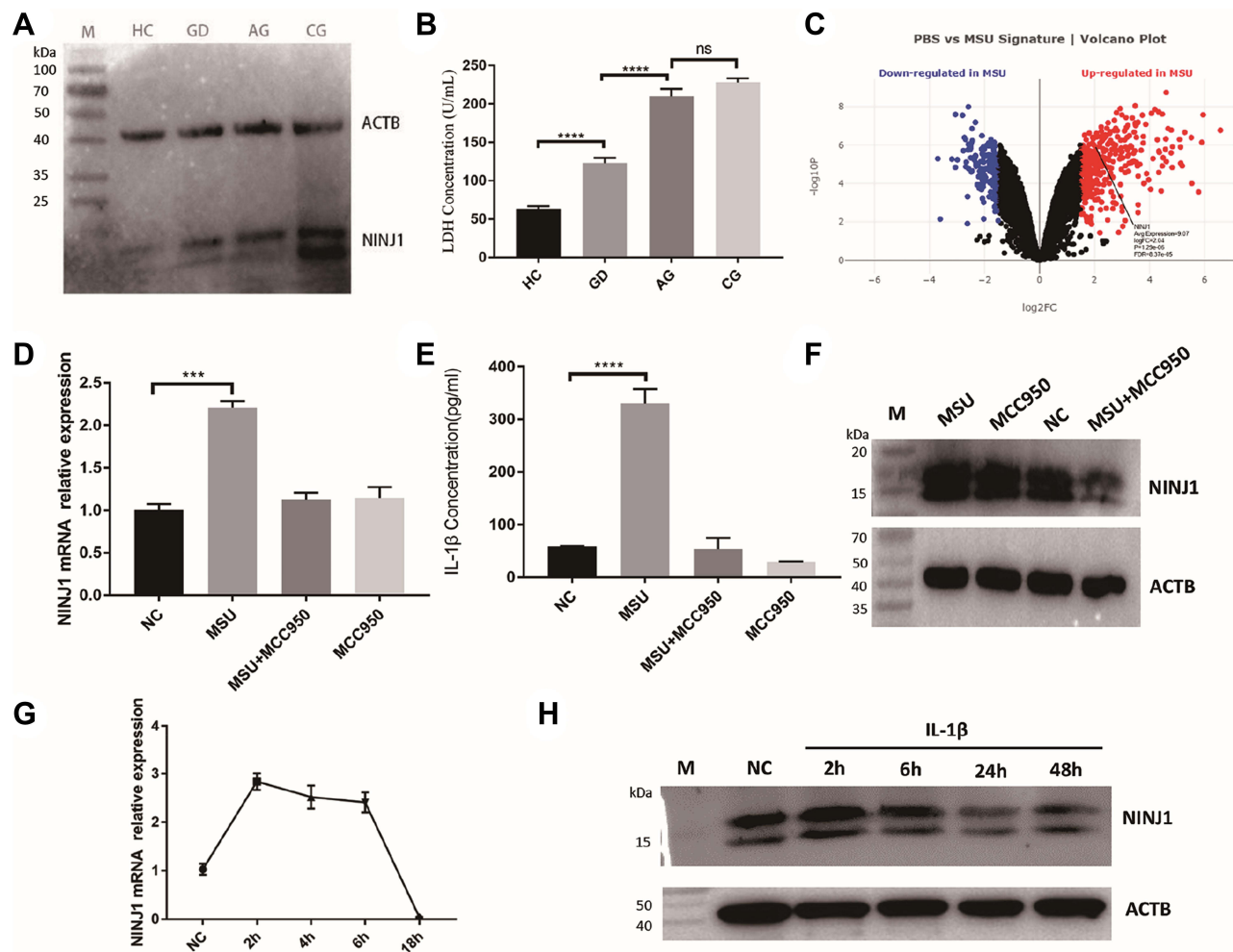
Figure 1 Elevated NINJ1 expression and PMR in gout patients. (**A**) Western blot of NINJ1 protein in PBMCs that isolated from different groups. (**B**) LDH levels in the plasma of different groups. (**C**) DEGs in MSU stimulated BMDMs. (**D**) RT-PCR of NINJ1 expression in PBMCs that stimulated with indicated reagents. (**E**) ELISA test of supernatant IL-1β in PBMCs that stimulated with indicated reagents. (**F**) Western blot of NINJ1 protein in PBMCs that stimulated with indicated reagents. (**G**) RT-PCR of NINJ1 expression in IL-1β treated PBMCs. (**H**) Western blot of NINJ1 protein in IL-1β treated PBMCs. Data are shown as mean± SEM. *** $P<0.001$, **** $P<0.0001$. **Abbreviations**: ns, no significance; HC, healthy control; GD, gout in diapause; AG, acute gout; CG, chronic gout; M, standard protein ladder; FDR, false discovery rate.

NINJ1 Knockdown Prevents Gout Flare by Inhibiting NLRP3 Inflammasome Activation in a Rat Model

To understand the function of NINJ1 in gout arthritis, we utilized an MSU crystal-induced gout model by direct injection of MSU crystal suspensions into the rat articular cavity.[36] Our previous results showed that rat joint inflammation peaks at 24 h after MSU crystal injection;[37] hence, we selected this time point to estimate rat joint inflammatory levels. Our results showed that MSU crystal injection-induced strong inflammation in rat joints compared with the NC group injected with the same volume of saline. Both joint swelling and inflammatory cell infiltration were dramatically increased (Figure 2A).

According to the manufacturer's protocol, lentiviral vectors for NINJ1 knockdown were injected one week prior to disease induction, and RT-PCR and WB confirmed the knockdown efficacy in rat joints and PBMCs, respectively (Figure 2B and C). Intriguingly, NINJ1 knockdown significantly inhibited rat joint swelling and inflammatory cell infiltration relative to the empty vector control (Figure 2A). Production of inflammatory cytokines such as TNF-α, IFN-γ, and IL-6 was inhibited in NINJ1 knockdown animals (Figure 2D), confirming that NINJ1 knockdown inhibits inflammation. Further work revealed that NINJ1 knockdown inhibited NLRP3 inflammasome activation, and that both NLRP3 and pro-IL-1β protein expression was inhibited (Figure 2C). Moreover, mature IL-1β, a product resulting from NLRP3
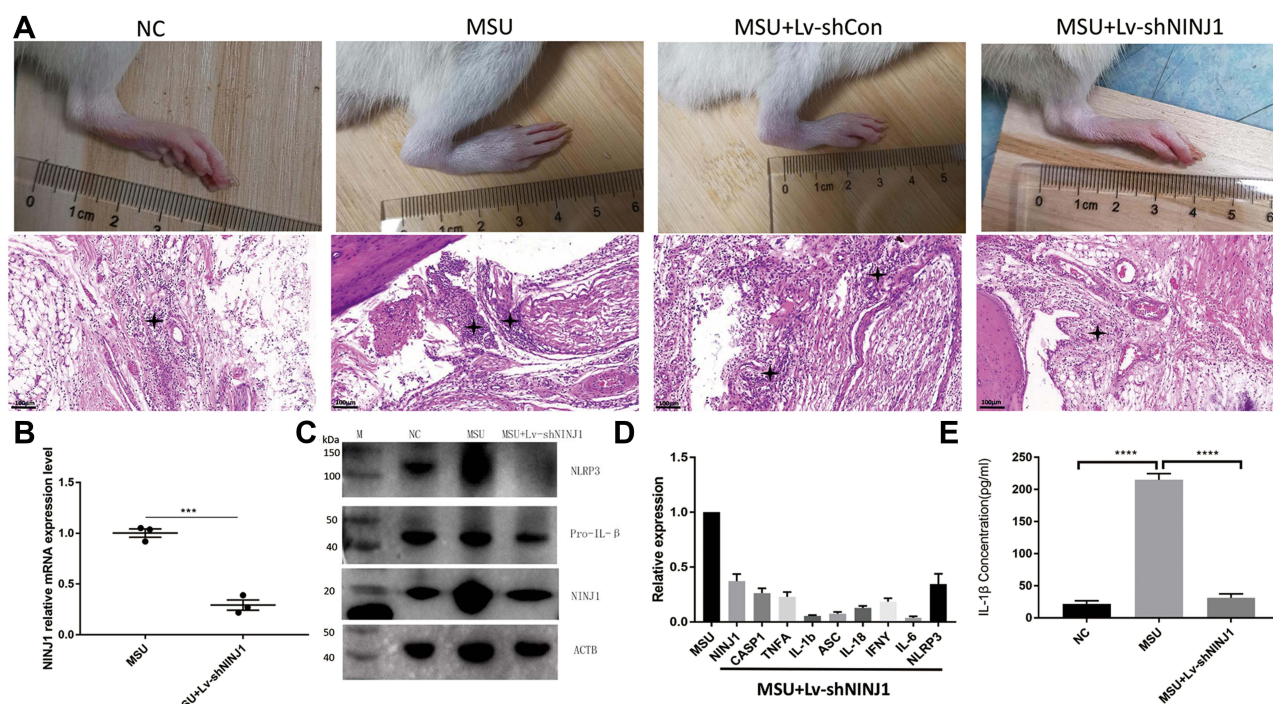
Figure 2 NINJ1 knockdown inhibits rat gout flare. (**A**) Images of rat joint swelling and redness, and hematoxylin and eosin staining of rat joint inflammation. Asterisks indicate the infiltrating leukocytes. (**B**) RT-PCR of NINJ1 expression in rat joints. (**C**) Western blot of NLRP3, pro-IL-1β, and NINJ1 expression in PBMCs of different groups. (**D**) Compare of individual inflammatory genes expression between MUS treated WT and NINJ1 knockdown animals in PBMCs. (**E**) Serum mature IL-1β level quantified by ELISA. ***$P<0.001$, and ****$P<0.0001$. Three rats were included in each group, and the experiment was repeated for four times. (M) standard protein ladder.
Abbreviations: NC, saline injection; MSU, MSU crystal injection; MSU+Lv-shNINJ1, NINJ1 knockdown lentivirus injection.

inflammasome activity, decreased in NINJ1 knockdown gouty rats (Figure 2E), suggesting that NINJ1 functions as an NLRP3 inflammasome regulator to control gout disease.

NINJ1 Neutralizing Antibody Has Therapeutic Potential in a Murine Gout Model

We then wondered whether NINJ1 could be a useful therapeutic target in gout arthritis. To test this, we utilized a commercial neutralizing antibody that targets NINJ1.[38] Because this is a mouse antibody, we had to employ a murine model using MSU crystal injection for these experiments. In this case, inflammation and PMR levels peaked 2 h after MSU injection (Figure 3A). The highest IL-1β secretion was observed at 8 hours (Figure 3B), consistent with a pre-inflammatory function of NINJ1 via inducing PMR, responsible for NLRP3 inflammasome over-activation. Therefore, we injected NINJ1 neutralizing antibody before MSU injection and assessed the level of inflammation at 8 h. Mice injected with NINJ1 neutralizing antibody exhibited less joint swelling and redness than controls (Figure 3D). In these mice, joint infiltration by inflammatory cells was also less marked than the IgG control group (Figure 3F). Consistent with this, NINJ1 neutralizing antibody-treated mice had lower IL-1β levels (Figure 3C) and LDH increase (Figure 3E). Altogether, these results support the notion that NINJ1 neutralizing antibody inhibits NLRP3 inflammasome activation and the subsequent inflammatory cascade, thus alleviating gout inflammation.

NINJ1 is a Potential Therapeutic Target for Gout Arthritis

Although targeting NINJ1 inhibited gout flare in both animal models, the potential effects of targeting it in human gout remained unknown. Therefore, we further tested the NINJ1 neutralizing antibody on human samples in vitro. PBMCs from either AG or CG patients were isolated and stimulated with MSU in the presence or absence of NINJ1 neutralizing antibody. Our results showed that NINJ1 neutralizing antibody inhibited LDH production and IL-1β secretion (Figure 4A and B), thus preventing PMR production and the following inflammation.
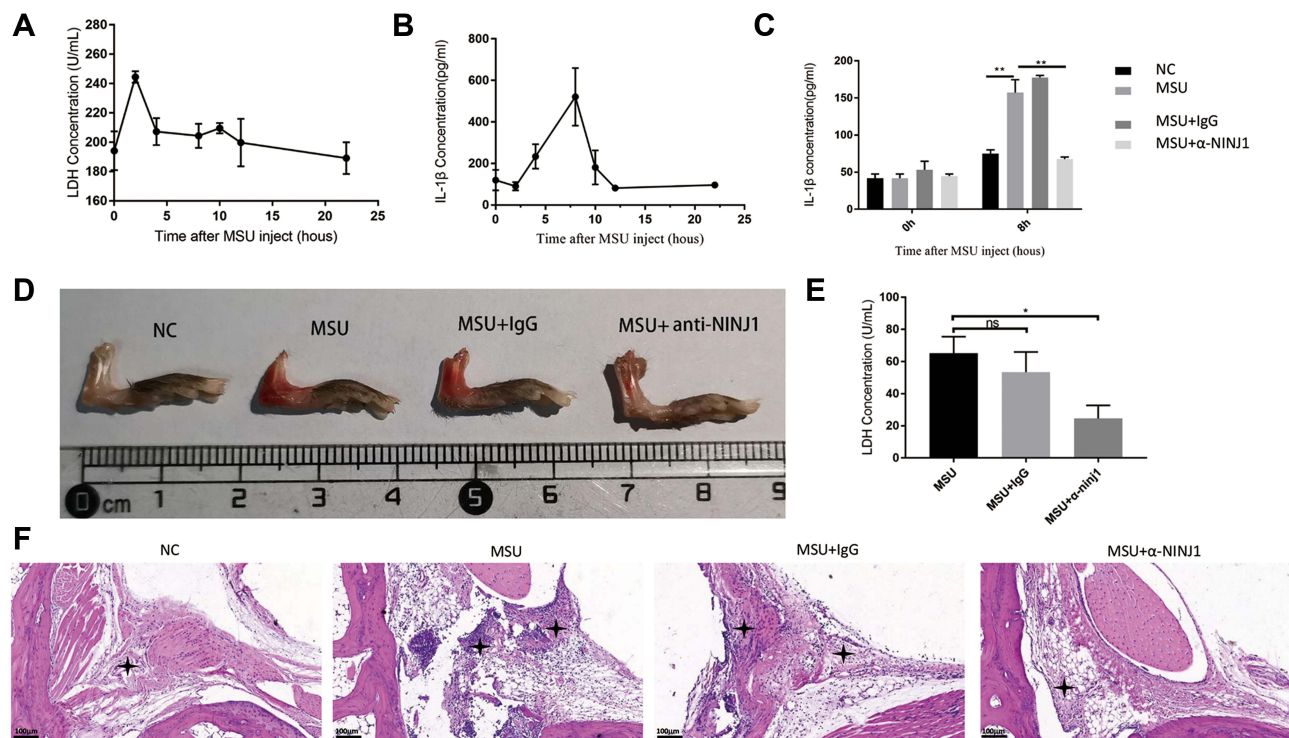
Figure 3 NINJ1 neutralizing antibody prevents murine gout. (**A**) LDH production after MSU injection into mouse joints at different time points. (**B**) IL-1beta production after MSU injection into mouse joints at different time points. (**C**) IL-1β production in different groups. (**D**) Images of mouse joint swelling and redness in different groups. (**E**) Mice LDH increase after MSU injection. (**F**) Histochemical analysis of mouse joint inflammation. Asterisks indicate the infiltrating leukocytes. *$P<0.05$, **$P<0.01$. Three mice were included in each group, and the experiment was repeated for four times.

Abbreviations: ns, no significance; NC, saline injection; MSU, MSU crystal injection; IgG, isotype antibody injection; anti-NINJ1, NINJ1 antibody injection.
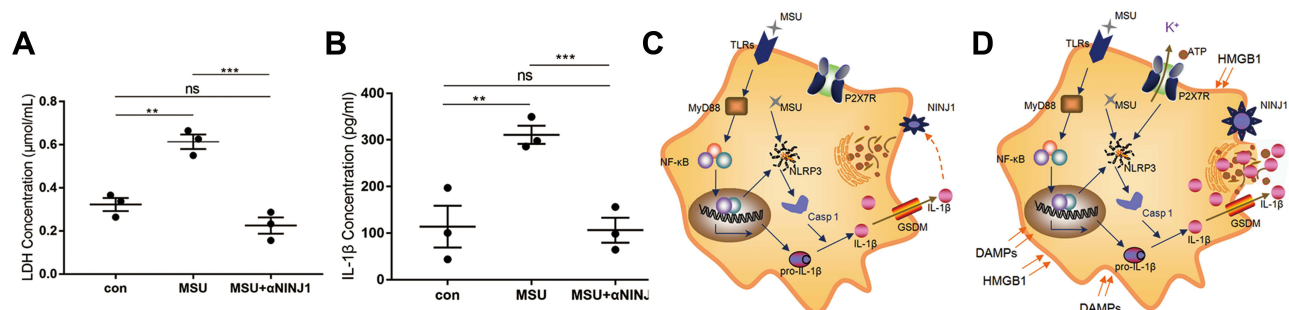

Figure 4 NINJ1 is a potential target for human gout arthritis. (**A**) LDH levels in different groups. (**B**) Production of IL-1β from PBMC. (**C**) MSU activate NLRP3 inflammasome inside of macrophage, which results GSDMD activation and IL-1β secretion. (**D**) Production of IL-1β upregulate NINJ1 expression on macrophage, NINJ1 then facilitate pyroptosis and PMR, releasing DAMPs including ATP, DNA, LDH, HMGB1 into surroundings. Released DAMPs in turn synergistic with MSU to activate NLRP3 inflammasome leading hyperinflammation and gout flare. ** $P<0.01$, *** $P<0.001$.

Abbreviations: ns, no significance; Con, control treatment; MSU, MSU crystal stimulation; MSU+αNINJ1, MSU crystal stimulation plus NINJ1 antibody.

Discussion

NINJ1 is a cell-surface adhesion molecule that regulates cell migration and attachment. It is widely expressed and upregulated during inflammatory conditions, such as following lipopolysaccharide (LPS), poly(I:C), or TNF-α stimulation.[39] Increased oxidative stress, endoplasmic reticulum (ER) stress (ERS) or NF-κB activation also enhances NINJ1 expression.[40] NINJ1 is involved in many inflammatory diseases, including diabetes mellitus,[24] nervous system inflammatory lesions,[22] intestinal inflammatory conditions,[23] and experimental autoimmune encephalomyelitis.[21] However, these previously established studies on the roles of NINJ1 in inflammation were mainly focused on NINJ1-

mediated leukocyte migration, although NINJ1 can also interact with LPS and regulate Toll-like receptor 4 (TLR4) signaling.[39,41] No data from these studies are related to PMR.

MSU-induced NLRP3 inflammasome activation plays a central role in gout arthritis, but MSU alone is insufficient to trigger gout in humans because only a small fraction of hyperuricemia patients develop overt clinical gout. Our results showed that NINJ1 was only upregulated during the acute inflammatory phase in gouty patients. Both AG and CG, MSU induce NINJ1 upregulation in an NLRP3 inflammasome-dependent manner, suggesting that NINJ1 plays a key role in gout flare. NINJ1-induced PMR promotes NLRP3 inflammasome activation and inflammation amplification, as both NINJ1 knockdown and NINJ1 neutralizing antibody inhibited NLRP3 inflammasome activation and gout flare, supporting the notion that during gout development, elements that induce cell death are also pathological factors for gout, and that cell death is the key step for inflammatory disease. Consistent with this view, our previous results revealed that ATP, a product of cell death, facilitates gout flare through the P2X7 receptor.[4,5] Further work may reveal other key factors involved in cell death and synergistic with MSU to induce human gout.

NLRP3 inflammasome is a multicomponent cellular platform responsible for various PAMPs and DAMPs detection. MSU activated NLRP3 inflammasome releasing mature IL-1β that immediately promote NINJ1 expression, however, this directly release of IL-1β is insufficient to induce gout flare, upregulated NINJ1 then introduce PMR and cell death releasing abundant DAMPs, which in turn activate NLRP3 inflammasome, triggering abundant IL-1β release leading hyper inflammation and gout flare (Figure 4C and D). Once activated, gout patients maintain a relatively higher NINJ1 expression than healthy control (Figure 1A). Although there is no inflammation, the maintained NINJ1 made a lower threshold for gout happening again and possibly explains the recurrence of gout. As inflammation progresses, prolonged IL-1β then downregulates NINJ1 expression and together with other inhibitory mechanisms to control gout flare, leading to self-remission.

The limitations of this study include the fact that although NINJ1 is mainly expressed in inflammatory cells, such as monocytes and neutrophils, it is also expressed in other cell types, including T cells and B cells.[42] NINJ1-induced cell death also regulates the homeostasis of these other cells, and targeting NINJ1 might lead to side effects. Moreover, appropriately-regulated inflammation is essential for adaptive immune responses to clear pathogens and maintain health, so targeting NINJ1 might increase infections. At present, there are mainly three NINJ1 inhibitory strategies, namely, small molecule inhibitors, neutralizing antibodies, and peptide mimics.[26] Recently, antibody engineering has progressed dramatically, and artificial antibody constructs have become mainstays in immune therapy. These approaches may allow the generation of bispecific antibodies that target only NINJ1 and inflammatory cells simultaneously to minimize side effects on other cells.

Altogether, these results strongly suggest that NINJ1 crosstalk with NLRP3 inflammasome and plays a key role in gout inflammation. Thus, NINJ1 may be a potential therapeutic target for human gout arthritis. Both inhibitors and humanized antibodies that target NINJ1 can be used in clinical treatment, and the clinical development of these drugs is awaited. We conclude that broadly targeting NINJ1 to inhibit NLRP3 inflammasome over-activation should control gout flare, but might increase infections and have other unknown risks. More exquisite targeting strategies will be needed to reduce these side effects and bring NINJ1 targeting to gout treatment in the clinic in the future. Moreover, NINJ1 may also be a target of choice in other inflammatory diseases characterized by excessive inflammation.

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Disclosure

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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