GPR30 negatively regulates inflammation in gout by inhibiting IL-1β production

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Abstract

Background: Gout is the most common inflammatory arthritis induced by monosodium urate crystal (MSU) precipitation.The incidence of gout attack was significantly higher in men than women, indicating the important role of oestrogen system in pathogenesis of gout, but the specific mechanisms underlying remained to be explored. GPR30, the newly defined estrogen receptor, had been proved participated in regulating inflammation in some diseases. As different estrogen receptors paly different role in inflammation regulation, how GPR30 was involved in the inflammation induced by MSU in gout was still unknown.

Methods: In this work, we investigated the function of GPR30 in inflammation induced by MSU in cultured macrophages and in mouse model by using G-1(the agonist of GPR30). We further tested the expression of the TLR and NOD like receptor protein3 (NLRP3), which are the key receptor involved in pathogenesis of gout. ROS and cleaved-caspase-1 expression were also detected to confirm the regulation role in NLRP3 pathway. Seahorse analysis was used to detect the metabolism profile in macrophages by G-1 to investigate the mechanism of GPR30 in inflammation. The relative expression of GPR30 were detected according to the inflammation state to confirm the role of GPR30 in gout.

Results: Negative regulation role of G-1 in IL-1β expression and NLRP3 expression were found both in vitro and in vivo. Moreover, the negative regulation of ROS production and NLRP3 as well as cleaved-caspase-1 expression were also found in G-1 stimulated macrophages. Our data also showed that G-1 inhibited aerobic glycolysis in LPS activated macrophages, which might be responsible for IL-1β and NLRP3 expression. Higher expression levels of GPR30 were found in patients with remitted gout inflammation.

Conclusion: Together, our data suggested that GPR30 was involved in the negative inflammation regulation induced by MSU and high expression of GPR30 might contribute to part of the mechanism of inflammation remission of gout.

Background

Gout is the most common inflammatory arthritis characterized by abrupt self-limiting attacks of inflammation caused by precipitation of monosodium urate crystal (MSU) in the joint[1]. Although gout is a self-limit inflammatory arthritis within several days or 1–2 weeks, chronic stimulation of MSU in joints can result in joint damage and disfiguring subcutaneous tophi[2–4]. The reported prevalence of gout worldwide ranges from 0.1% to approximately 10%, and incidence from 0.3 to 6 cases per 1,000 person-years[3]. Innate immune cells mainly including macrophages and neutrophils are the key immune cells involved in pathogenesis of gout[5]. Activation of NLRP3 inflammasome and the IL-1β secretion is the most important procedure of inflammation in gout[2, 6]. However, the exact mechanisms of the inflammation regulation in gout remains largely unknown. A lot of questions about gout still needed to be

answered. The study of inflammation regulation mechanisms in gout can provide us more insights in understanding of inflammation regulation mechanisms.

One of the most prominent unanswered questions about gout is the mechanisms underlying the gender difference in incidence of gout attacks. The incidence of gout is significantly higher in men than women [7]. Gout is rarely diagnosed in premenopausal women, while postmenopausal women have a higher risk of incident gout, and moreover hormone users in postmenopausal women have a reduced risk of gout [8, 9]. Those studies suggested hormone, especially the estrogen system might participate in the onset and development of gout. However, the mechanism of estrogen in gout is still controversial and unclear [10, 11].

There are three different estrogen receptors that can bind to estrogen and activate cell signaling pathways. Estrogen receptors (ERs) α and β are well-established classic nuclear receptors of estrogen and have different physiological functions besides in immune regulation [11, 12]. In recent years, a seven-transmembrane domain G protein-coupled receptor was identified as a new estrogen receptor. It was named as G protein-coupled estrogen receptor 1 (GPER-1) or GPR30 and was potentially responsible for non-genomic estrogen signaling[13]. Recent years, GPR30 was indicated to be involved in immune cell activation or proliferation and was responsible for some inflammatory diseases, such as multiple sclerosis[14], endothelial inflammation[15] and inflammatory bowel disease[16]. Also, the GPR30 knocked out mice displayed as insulin resistance, dyslipidemia and proinflammatory state[17, 18]. While role of classic ERs in inflammation have been demonstrated by studies, effect of GRP30 in inflammation regulation in gout still unclear.

In this study, we evaluated the function of GPR30 in inflammation regulation of gout, which was mainly induced by MSU. We used G-1, the specific agonist of GPR30, to examine the function of GPR30 in vivo and in vitro and further confirmed the potential role of GPR30 in gout patients. In addition, we investigated suppressive effects of GPR30 on macrophage cellular metabolism programming to explore the mechanisms of GPR30 in inflammation regulation. These findings may provide more evidences for GPR30 to be a candidate target in treating or researching inflammatory diseases.

Material And Methods

Patients

50 patients with gout were recruited from the Department of Rheumatology and clinical Immunology of the First Affiliated Hospital of Xiamen University. 36 sex and age matched healthy controls were recruited as healthy controls (HCs). This study was approved by the Ethics Committee of the First Affiliated Hospital of Xiamen University. Informed consent was obtained from the patients and the healthy controls in this study. Clinical data were obtained from medical record.

Real-time PCR analysis

Total RNA of cells was collected by TRIzol (Invitrogen, Carlsbad, CA, USA). Complementary DNAs were synthesized using cDNA synthesis kit (Roche, Indianapolis, IN, USA). RT-PCR was performed with FastStar University SYBR Green master (Roche) according to the manufacturer's instructions. The relative expression of mRNA was normalized to the expression level of β-Actin with the $2^{-\Delta\Delta Ct}$ method. The following primers were designed for testing the human genes: β -Actin: 5'-GTG GGG CGC CCC AGG CAC CA-3' and 3'-CTC CTT AAT GTC ACG CAC GAT TTC-5'; GPER-1: 5'-TTCCGCGAGAAGATGACCATCC -3' and 3'-TAGTACCGCTCGTGCAGGTTGA -5'. And the following primers were used to detect the mice genes *in vivo* and *in vitro*. IL-1β: 5'- TGCCACCTTTTGACAGTGATG-3' and 3'- GAAGGTCCACGGGAAAGACA-5'; IL-6: 5'- GTCCTTCCTACCCCAATTTCCA-3' and 3'- TAACGCACTAGGTTTGCCGA-5'; NLRP3: 5'-CAAGGCTGCTATCTGGAGGAA-3' and 3'-TTCTCGGGCGGGTAATCTTC-5'; TLR4: 5'-CGCTGCCACCAGTTACAGAT-3' and 3'-AGGAACTACCTCTATGCAGGG-5'; MyD88: 5'-CATACCCTTGGTCGCGCTTA-3' and 3'- CCAGGCATCCAACAAACTGC-5'; iNOS: 5'-GAGAACGGAGAACGGAGAACG-3' and 3'-TGAGAACAGCACAAGGGGTTT-5' and GAPDH: 5'-GTCCCTCACCCTCCCAAAG-3' and 3'-GCTGCCTCAACACCTCAACCC-5'

Mouse model of gout

All experimental procedures involving mice were approved by the animal care and use committee of Xiamen University. 6 to 8 weeks age male C57BL/6J mice were used in this study. Uric acid was used to recrystallize MSU crystals as method described previously[6, 19]. Briefly, 3.36 mg uric acid dissolved in 1000ml of 0.01M NaOH (pH 7.1-7.2, 70°C) and then cooled to room temperature to form MSU crystal. After washing with 100% ethanol, MSU crystal were suspended in PBS and used to induced peritonitis. Peritonitis was induced as method previously described[20]. Mice were intraperitoneal injection of 3mg MSU crystals in 0.5 ml PBS. Some mice were received 1μg/ml G-1 one hour before the MSU administration. Mice were scarified after 16 hours of MSU stimulation and the peritoneal cells were collected by lavage with 3 ml PBS. Lavage fluids were separated by centrifugation and supernatants were used to detect the cytokines. The cells were collected to count the total cells and detect the percent of macrophages and neutrophils by flow cytometry. Also, the cells were obtained for detected mRNA expression of some cytokines and receptors by real-time PCR.

Flow cytometry

Cells harvested from the lavage fluids were collected and incubated with Pecy5- anti CD11b, Pecy7- anti Gr-1, PE- anti FC4/80 (eBioscience, San Diego, CA, USA) at room temperature for 20 minutes and then analyzed by flow cytometry (Cytomics FC 500; Beckman Coulter, Fullerton, CA, USA). Neutrophils were identified as CD11b+ Gr-1+ and macrophages were identified as CD11b+ FC4/80+. All the results were analyzed and presented with CXP software (Beckman Coulter).

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of cytokines in lavage fluid of peritonitis the and the supernatants of culture cells induced by MSU *in vitro* were measured using commercially ELISA kits according to the manufacturer's

instructions (R&D, Minneapolis, USA). Absorbance at 450 nm was measured with a microplate reader.

Measurement of ROS levels

reactive oxygen species (ROS) generation was measured using the fluorescent probe DCFH-DA (Merck, New Jersey, USA) as described previously[21]. RAW264.7 were primed with 100ng/ml IFN- γ and activated with 100ng/ml LPS in presence or absence of G-1 for 12h and subsequently collected and incubated in 10μM DCFH-DA for 30 min at 37°C in the dark. After incubated with DCFH-DA, cells were stimulated with MSU and the fluorescence intensity was detected with a multimode microplate reader (Molecular Device, San Jose, USA) with an excitation wavelength at 485 nm and an emission wavelength at 530nm. The fluorescence intensity was assessed every 10 min for continuously 90 min.

Western blot analysis

The western blot was performed as described before. The RAW264.7 were primed with 100ng/ml IFN- γ and activated with 100ng/ml LPS for 12h in presence or absence of G-1 and subsequently stimulated with 200μg/ml MSU for 4 hours. Then, the cells were lysed in RIPA buffer containing protease inhibitor cocktail (Roche, Indianapolis, IN, USA) on ice for 30min. In this study, 30μg protein per lane was loaded with SDS-PAGE and transferred to a polyvinylidene difluoride membranes. Membranes were blocked and then incubated with antibodies specific for NLRP3 (Abcam, Cambridge, UK), cleaved-Caspase-1(Cell signaling technology, Danvers, USA), total caspase-1 and GAPDH. Membranes were washed and then incubated with HRP-conjugated-gout anti-rabbit IgG (Abcam). Protein detection was performed with chemiluminescent horseradish peroxidase substrate system (Bio-Rad, California, USA)

Seahorse analysis

The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of the RAW 264.7 cells were detected using a Seahorse XF96 flux analyser (Agilent, California, USA) according to the method previously described[22, 23]. 1×10^4 cells/well were seeded in XF 96 cell culture plate two days before the experiment. Cells were rinsed with XF assay medium one hour before the experiment and then incubated at 37 for 1 hour without CO2. For OCR analysis, 1.5μM Oligomycin, 1μM FCCP and 0.5μM Rotenone/antimycin A were injected into each sequence to evaluate the maximal and nonmitochondrial oxygen consumption after detecting the basal respiration. For ECAR analysis, 10mM glucose, 1μM Oligomycin and 50mM 2DG were injected successively to detect the glycolytic flux including glycolytic reserve, glycolytic capacity. The OCR and ECAR values were calculated by Seahorse XF-96 software.

Statistical analysis

Three independent experiments were performed. Data were analyzed using SPSS 17.0 software. Data were presented as mean Student's t-test was applied to the data sets, and P values <0.05 were considered to be statistically significant (*P<0.05; **P<0.01; ***P<0.001).

Results

GPR30 significantly inhibited the inflammation induced by MSU in vivo

In order to figure out whether GPR30 participated in inflammation induced by MSU, we firstly induced the animal model of gout by inducing peritonitis with MSU, then treated with GPR30 agonist G-1. The results showed that, intraperitoneal injection of MSU can induce large amount of inflammatory cells infiltration into peritoneal lavage fluids, especially lots of neutrophils. G-1 can significantly decrease the numbers of cells infiltrating in abdominal cavity, and the numbers of neutrophils in cavity were significantly lower when the mice were treated with G-1 (Figure1, C). Besides immune cells, MSU also induced high level of IL-1β and IL-6 secretion, and G-1 can significantly decrease those inflammatory cytokines induced by MSU (Figure1, E-G).

GPR30 negatively regulated IL-1β production induced by MSU *in vitro*

IL-1β has been proved to be one of the most important inflammatory cytokines in pathogenesis of gout, our findings described above suggested a negative regulatory role of GPR30 in IL-1β secretion induced by MSU. To confirm the role of GPR30 in IL-1β production, we then investigated role of G-1 on the inflammatory cytokines produced by macrophage after MSU stimulation in vitro. After primed by IFN-γ and stimulated with LPS in presence or absence of G-1, the macrophages were then stimulated with 200 g/ml MSU to induce inflammatory cytokines secretion. The results demonstrated that, MSU induced large amount of IL-1β and slight increase of IL-6 and TNF-α in macrophage. G-1 significantly decreased the IL-1β secretion in macrophages (figure 2). Those data in vitro further confirmed the protective role of GPR30 in inflammation induced by MSU.

GPR30 inhibited NLRP3 expression and ROS production induced by MSU in macrophages

The results *in vivo* and *in vitro* proved that GPR30 can significant decrease the IL-1β secretion induced by MSU. To further explore the mechanism underlying how GPR30 inhibit IL-1β production, we first investigated effect of G-1 on IL-1β mRNA expression. The results showed, pro-IL-1β as well as TLRs and MyD88 mRNA expression were not significantly changed when treated with G-1. However, the relative expression of NLRP3 induced by MSU were significantly decreased in presence of G-1. IL-1β is produced as an inactive pro-molecule when MSU activated TLRs and MyD88. The pro-IL-1β was then cleaved by cleaved-caspase-1 which were cleaved from caspase-1 with NLRP3 inflammasome. To further described the potential mechanism of GPER-1 in inflammation, we also detected the relative expression of NLRP3, TLR in macrophages induced by MSU. The results showed G-1 also significantly decreased the NLRP3 expression in macrophage stimulated by MSU, suggested the negative regulatory role of GPER-1 in NLRP3 expression. Western blot was also proved the results of G-1 in reducing of NLRP3 expression as well as the cleaved-caspase-1 expression. It suggested the regulation role of G-1 in IL-1β secretion induced by MSU were major through regulating NLRP3 inflammasome. As described in previously studies, activated macrophages produced high level ROS and the ROS expression in macrophages promoted the NLRP3 expression. In order to confirm the regulation role of G-1 in NLRP3 expression, we

detected the ROS expression in macrophage. The results displayed G-1 significantly decreased the ROS expression stimulated by MSU in macrophages and subsequent effected the NLRP3 as well as the cleaved-caspase-1 expression.

GPR30 inhibited aerobic glycolysis in LPS activated macrophages

Metabolic profiles changes play important part in immune cells polarization and function. Our result in vitro suggested the negative regulation role of GPR30 in ROS-NLRP3 pathway. ROS and NLRP3 were key molecular involved in mitochondrial respiratory as well as glycolytic pathway. To further investigated the mechanism of GPR30 in inflammation, we detected the metabolic profiles in presence of G-1 using the measurements of OCR and ECAR. The metabolic status of macrophages incubated with LPS/IFN-γ in presence or absence of G1 showed no differences in the levels of proton leak ($OCR_{Oligomycin}$-$OCR_{Antimycin}$), as well as maximal respiration capacity (OCR_{FCCP}- $OCR_{Antimycin}$) and mitochondrial respiratory reserve capacity (OCR_{FCCP}- OCR_{Basal}). However, G-1 significantly reduced the basal (OCR_{Basal}-$OCR_{Antimycin}$) and ATP-linked (OCR_{Basal}- $OCR_{Oligomycin}$). Basal use of the glycolytic pathway for energy demand was slight enhanced by LPS/IFN-γ and slight decreased when treated with G-1. The maximal ECAR was increased after the addition of oligomycin. Total levels of glycolytic capacity ($ECAR_{Oligomycin}$-$ECAR_{Basal}$) were increased when treated with LPS/IFN-γ. However, G-1 significantly decreased the total glycolytic capacity in activated macrophages. Altogether, LPS/IFN-γ treatment had no significant changes in mitochondrial metabolism while activation of glycolytic metabolism. G-1 significantly decreased the glycolytic capacity of activated macrophages, which indicated G-1 regulated cell metabolism and signaling in inflammation in macrophages.

GPR30 negatively regulated inflammation in human PBMCs

We further confirmed the role of GPR30 in patients with gout. First, we isolated the PBMCs from healthy controls and activated PBMCs with 100ng/ml LPS and then stimulated with 200μg/ml MSU with G-1 or not. The result showed that, G-1 significantly reduced the IL-1β secretion induced by MSU, which was consistent with the results found in mouse. Then, to figure out whether the relative expression of GPR30 in gout patients was changed or correlated with the inflammation statues in gout, we analyzed the GPR30 expression level in PBMC of patients with gout by Real-Time PCR. The results showed that, the relative expression of GPR30 in gout patients were quite similar to healthy controls. We also compared the estrogen level in serum and the nuclear receptor, ERα and ERβ relative expression in gout patients and healthy controls. No significantly changes were observed (Supplemental data 1). However, we further analyzed the GPR30 expression level with inflammation state in gout, the GPR30 expression level in patients who were at remission (defined as patients with CRP level were back to normal) were significantly increased compared to the patients who were at active gout attack. The result suggested that high level of GPR30 expression might be helpful for the inflammation remission in gout. Those results in patients further confirmed the protective role of GPR30 in inflammation induced by MSU.

Discussion

Since the estrogen system have been well recognized to play critical role in gout, this study further explored the mechanisms underlying. We found that, instead of classic ERs, the newly defined estrogen receptor GPR30 might be one of the key factors in inflammation regulation in gout. GPR30 inhibited IL-1β production in macrophage, and this effect might due to the negative regulation of NLRP3 and ROS. Moreover, we found that, GPR30 inhibited the aerobic glycolysis of macrophage induced by LPS/IFN-γ.

IL-1β was a key regulatory proinflammatory cytokines that involved in the inflammation induced by MSU[24]. It can promote neutrophil influx into the joint and synovium and initiated the acute inflammation attack. The early IL-1β blockade treatment trials in gout patients demonstrated significant efficacy, further proved the critical role of IL-1β in inflammation induced by MSU[25]. IL-1β was firstly produced as inactive pro-IL-1β by immune cells including macrophages, monocytes and dendritic cells. Then, the pro-IL-1β was cleaved into active IL-1β by cleaved-caspase-1. Researches had proved that, NLRP3 inflammasome complex was a key regulatory that activated the caspase-1 and responsible for the IL-1β production. In our study, we found that G-1 significantly reduced the IL-1β secretion both in vivo and in vitro. Furthermore, we found G-1 negative regulated the NLRP3 protein expression and cleaved caspase-1 production, and then inhibited the IL-1β production. Moreover, the results demonstrated that, the ROS induced by MSU were significantly reduced by G-1. ROS generation has been suggested to be critical for inflammasome activation. TLRs, especially TLR4, also been proved playing indirect part in response to MSU deposition by activating the MyD88 signal and then promoting the pro-IL-1β synthesis[26]. Research had demonstrated that, G-1 can significantly decreased the TLR4 expression in macrophages[27]. However, in this study, we did not find the significantly changes in TLR4 or TLR2 expression by G-1. It might because the concentration of G-1 we used in our study was different. The results suggested G-1 might participate in the inflammation induced by MSU mainly through regulating NLRP3 pathway and then regulating the IL-1β production.

Among the immune cells, resident macrophage played most important role in initiation of inflammation induced by MSU[28]. Cell metabolism profile was essential for the inflammatory process as well as the macrophage function. Immune cells activation depend on intracellular glucose metabolism[29]. In our study, we found that, G-1 significantly reduced the ROS and NLRP3 production, which were responsible for the IL-1 β production. ROS and NLRP3 were also involved in the metabolism pathway and participated in the macrophage function. So, we detected the bioenergetic profiles of mitochondrial respiration and aerobic glycolysis in LPS activated macrophages and compared with the cells pretreated with G-1. Our results showed that IFN-γ and LPS markedly altered cellular metabolism via switching OXPHOS to aerobic glycolysis, which was similar to other reports[22, 30]. Meanwhile, we found G-1 significantly inhibited intracellular aerobic glycolysis. Some researches had reported that glycolytic enzymes, such as hexokinase1 (Hk1) and pyruvate kinase (PKM2) were critical for the NLRP3 inflammasome activation and IL-1β secretion[31, 32]. However, the underlying mechanism of how G-1 inhibited the aerobic effect facilitated by LPS needed to be clarified in future studies.

In conclusion, we found that the GPR30 inhibited the aerobic glycolysis induced by LPS and IFN-γ, following by the decreasing of ROS and NLRP3 production which might lead to the negative regulation of

IL-1β production induced by MSU. These findings suggest that the changes of metabolic pathway effect by GPR30 might be a new insight into the inflammation remission from activation of GPR30 including the inflammation induced by MSU. The inflammatory suppression function of GPR30 might be an explanation of why Gout is rarely diagnosed in premenopausal women, and postmenopausal women have a higher risk, because the high-level estrogen in premenopausal women [8]. However, further studies are still required to investigate the downstream signaling molecules and elucidate the mechanism of GPR30 and NLRP3 pathways and glycolysis. These data may help to understand the immune regulation role of GPR30 and provide a new therapeutic target for gout inflammation.

Abbreviations

GPR30: G protein-coupled receptor 30; MSU Monosodium urate crystal; NLRP3: NOD like receptor protein 3; TLR: Toll like receptor; ROS: Reactive oxygen species; ER: Estrogen receptor; HC: Healthy controls; OCR: Oxygen consumption rate; ECAR: Extracellular acidification rate; HK1: Hexokinase 1; PKM2: Pyruvate kinase

Declarations

Ethics approval

Animal studies in this project were approved by the institutional animal care committee of Xiamen University. Informed consents were obtained from patients and healthy controls. This study was approved by the ethics committee of the First Affiliated Hospital of Xiamen University in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Author contributions

Hongyan Qian, Yuan Liu and Guixiu Shi contributed conception and design; Hongyan Qian, Rongjuan Chen performed the experiments and statistical analysis; Shiju Chen and Yan He helped for the data statistical analysis and wrote section of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Figures

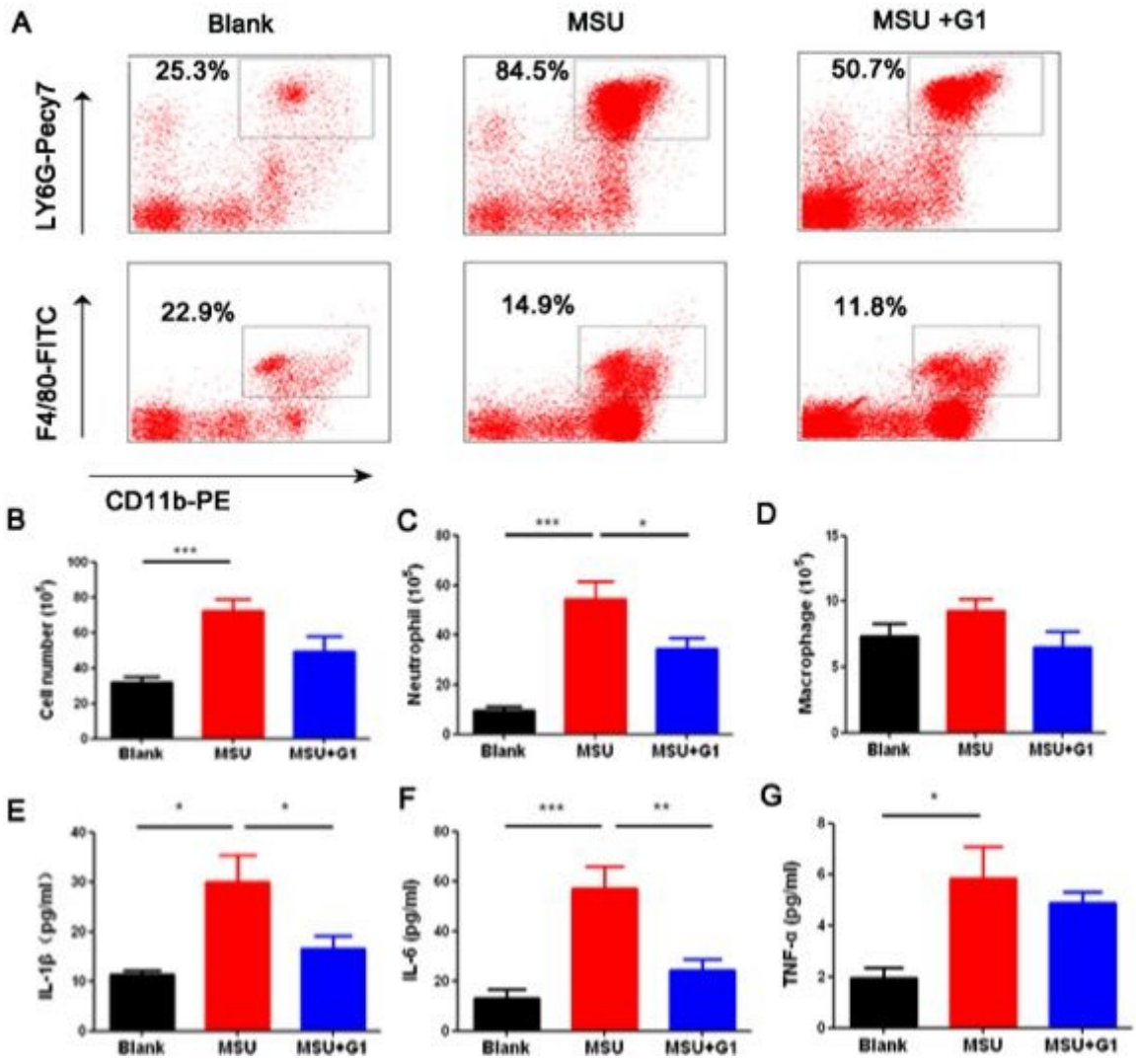

Figure 1

G-1, the agonist of GPR30 reduced the cellular infiltration and cytokines production induced by MSU in vivo. Mice were treated with MSU crystals intraperitoneally (3 mg in 0.5 ml PBS) for 16h to induce the inflammation. The peritoneum cells were harvested by lavage with 3ml PBS and the cell types were identified by flow cytometry. Neutrophils were identified as CD11b+ LY6G+ and monocytes were identified as CD11b+ FC4/80+ (A). Total cell numbers and the numbers of monocytes and neutrophils infiltrating in the peritoneum induced by MSU were decreased when treated with G-1. Supernatants from the peritoneum lavage fluid were analyzed for IL-1β, IL-6 and TNF-a by ELISA. G-1 reduced the expression of inflammation cytokines which induced by MSU (E-G). (n= 6 mice per group)

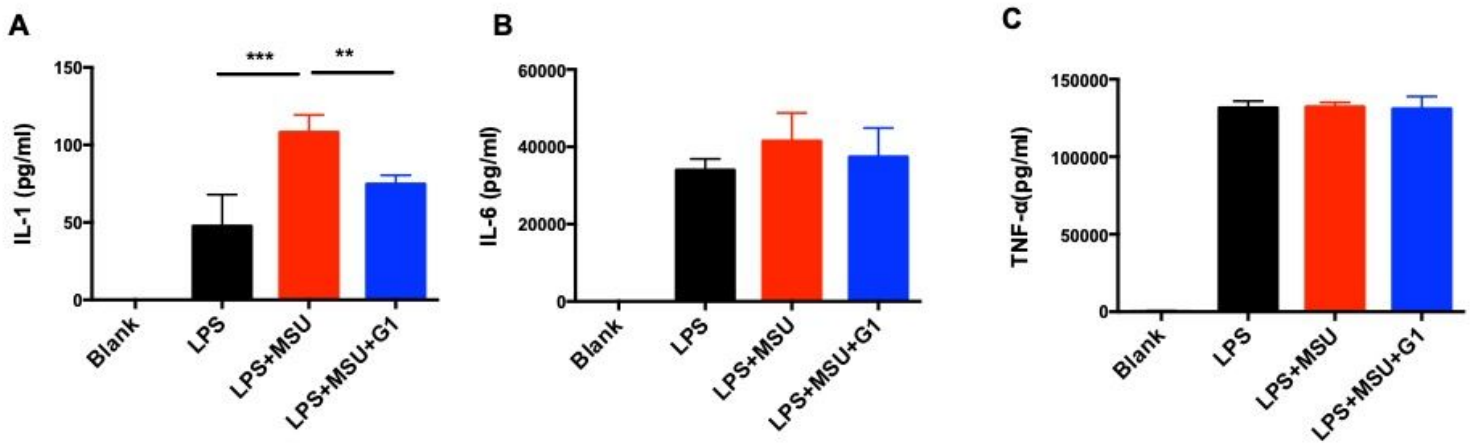

Figure 2

G-1significantly decreased the IL-1β production induced by MSU in activated macrophage. RAW264.7 were primed with 100ng/ml IFN- γ and activated with 100ng/ml LPS and then treated with 200ug/ml MSU in presence or absence of G-1 for 4hours. Activated macrophage produced high level of cytokines, especially IL-6 and TNF-a. MSU induced activated macrophages produced and secreted IL-1β, while G-1 significantly reduced the IL-1β production induced by MSU. Values are the mean and SD. *, p<0.05, **, p<0.01.

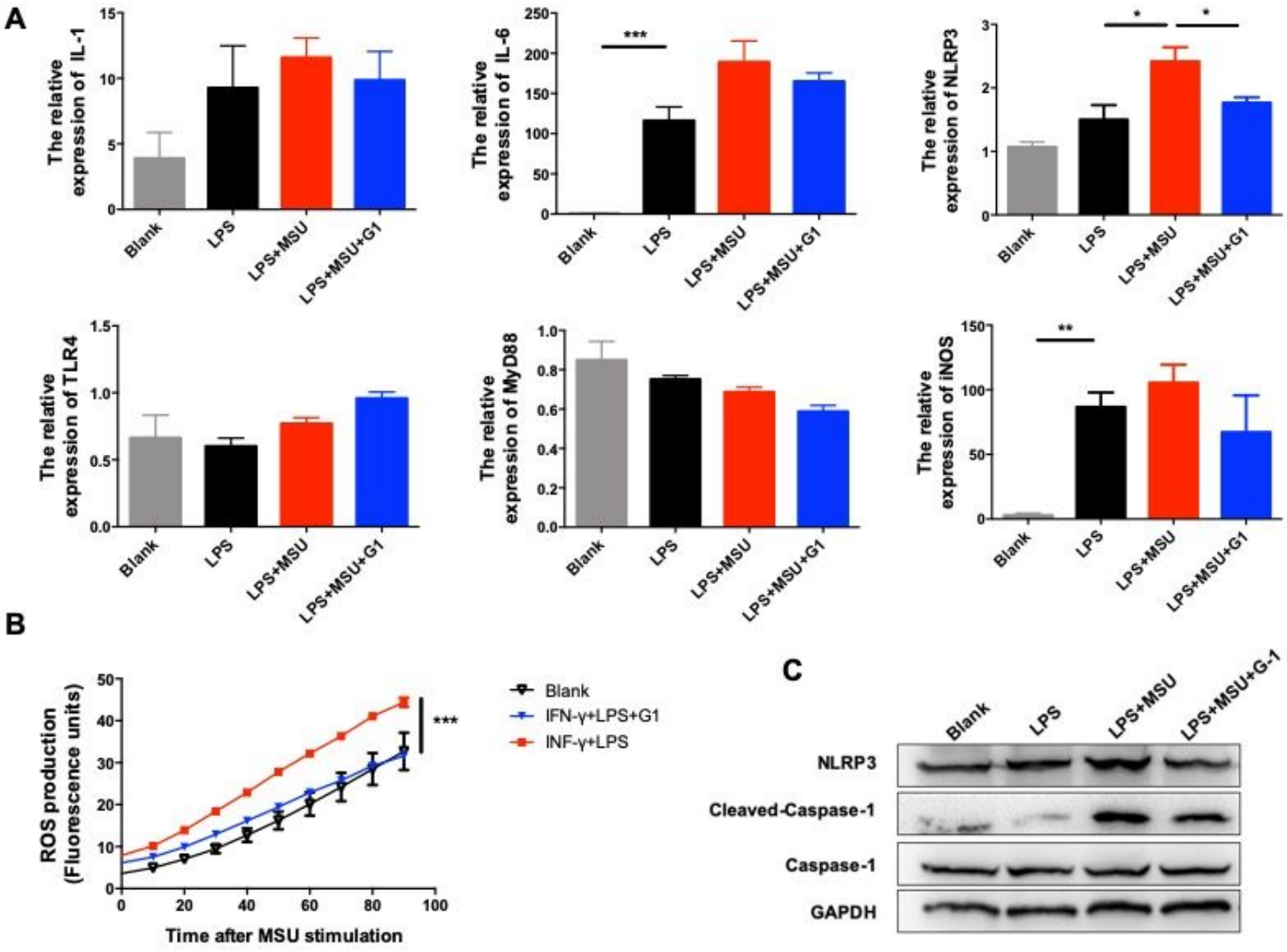

Figure 3

G-1significantly decreased the NLRP3 production induced by MSU in activated macrophage. RAW264.7 were primed with 100ng/ml IFN- γ and activated with 100ng/ml LPS and then treated with 200ug/ml MSU in presence or absence of G-1 for 4 hours. The relative expression of NLRP3 were significantly increased induced by MSU, while G-1 significantly reduced the NLRP3 expression. And the relative expression of TLR4, TLR2 MyD88 and iNOS had no significantly changed induced by MSU and G-1(A-F). ROS production induced by MSU in macrophage significantly reduced in presence of G-1(G). Result of Western blot also confirmed that, MSU induced NLRP3 and cleaved-caspase-1 expression, G-1 significantly decreased the NLRP3 and cleaved-caspase-1 expression induced by MSU(H). *, $p<0.05$,

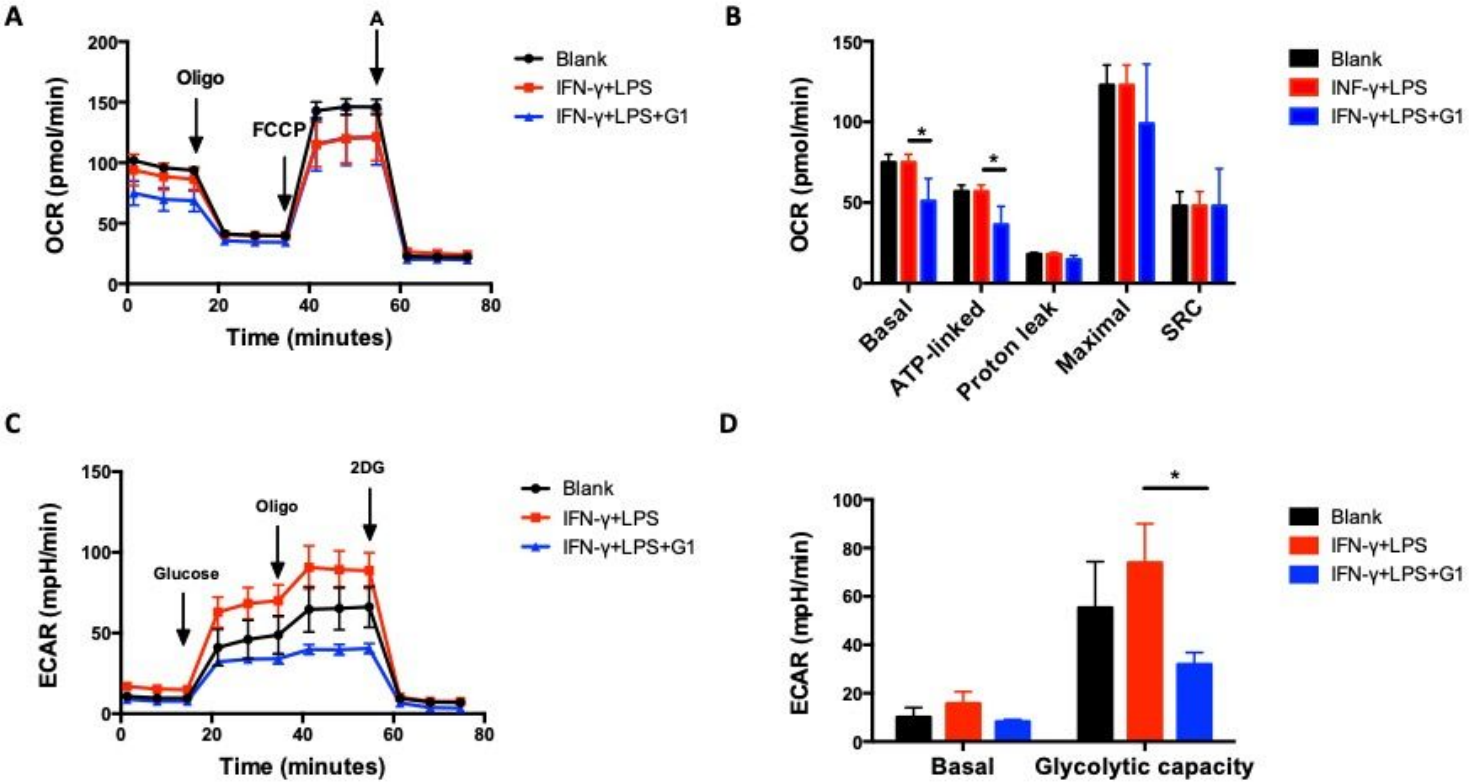

Figure 4

G-1 significantly inhibited the Warburg metabolism (aerobic glycolysis) of activated macrophages. To detect the metabolism status of macrophage with G-1. RAW264.7 were seeded in XF96 plated and incubated with IFN- γ and LPS for 4h and detected the Mitochondrial stress and glycolysis stress. The oxygen consumption rate (OCR= Mitochondrial oxidative metabolism rate) and extracellular acidification rate (ECAR=anaerobic glycolytic metabolism) were evaluated. Bioenergetic profiles obtained by plotting the maximal ECAR and OCR as quantified in (B) and (D). All data are showed as the mean and SD. *, $p<0.05$.

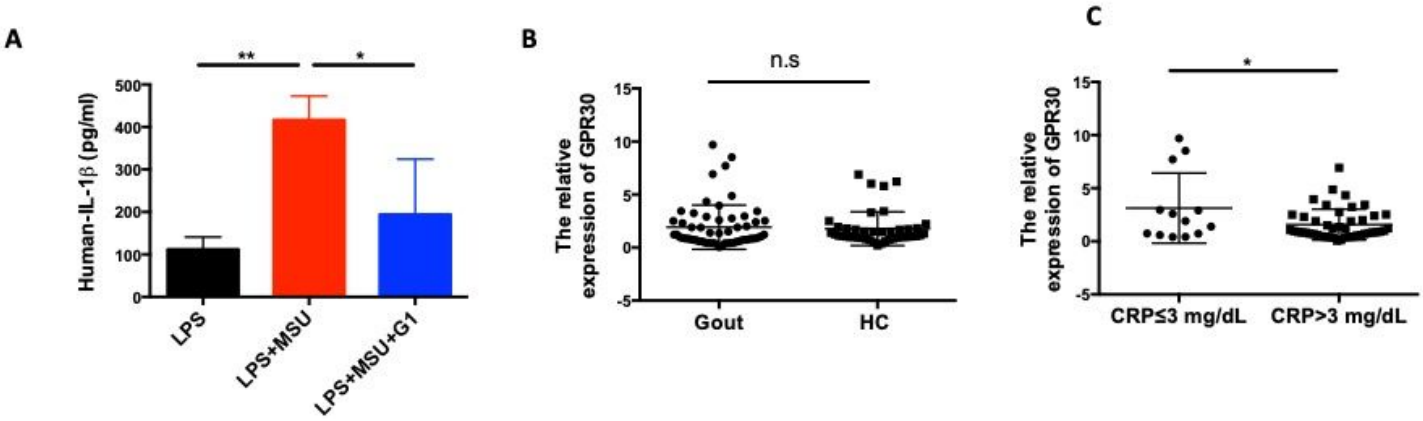

Figure 5

GPR30 negatively regulated the inflammation induced by MSU in human. PBMCs were isolated from healthy controls and activated with 100ng/ml LPS and then stimulated with 200ug/ml MSU. G-1 significantly reduced the IL-1β stimulated by MSU (A). The relative expression of GPR30 were detected in patients with gout and healthy controls. No significantly change was found between gout patients and healthy controls (B). However, the GPR30 expression level in patients who were at remission were significantly increased compared to the patients who were at active gout attack (C). *, $p<0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaldata1.jpg