Redox Biology 28 (2020) 101363

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Impaired inflammasome activation and bacterial clearance in G6PD deficiency due to defective NOX/p38 MAPK/AP-1 redox signaling

Wei-Chen Yen[a,b,1], Yi-Hsuan Wu[c,1], Chih-Ching Wu[b,d,e,1], Hsin-Ru Lin[b], Arnold Stern[f], Shih-Hsiang Chen[g], Jwu-Ching Shu[b], Daniel Tsun-Yee Chiu[b,c,g,*]

[a] Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[b] Department of Medical Biotechnology and Laboratory Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[c] Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan, Taiwan
[d] Molecular Medicine Research Center, Chang Gung University, Tao-Yuan, Taiwan
[e] Department of Otolaryngology - Head & Neck Surgery, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan
[f] New York University School of Medicine, New York, NY, USA
[g] Department of Pediatric Hematology/Oncology, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan

ARTICLE INFO

Keywords:
G6PD
Inflammasome
NADPH oxidase
IL-1β
Bactericidal response
Redox homeostasis

ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the pentose phosphate pathway that modulates cellular redox homeostasis via the regeneration of NADPH. G6PD-deficient cells have a reduced ability to induce the innate immune response, thus increasing host susceptibility to pathogen infections. An important part of the immune response is the activation of the inflammasome. G6PD-deficient peripheral blood mononuclear cells (PBMCs) from patients and human monocytic (THP-1) cells were used as models to investigate whether G6PD modulates inflammasome activation. A decreased expression of IL-1β was observed in both G6PD-deficient PBMCs and PMA-primed *G6PD*-knockdown (*G6PD*-kd) THP-1 cells upon lipopolysaccharide (LPS)/adenosine triphosphate (ATP) or LPS/nigericin stimulation. The pro-IL-1β expression of THP-1 cells was decreased by *G6PD* knockdown at the transcriptional and translational levels in an investigation of the expression of the inflammasome subunits. The phosphorylation of p38 MAPK and downstream c-Fos expression were decreased upon *G6PD* knockdown, accompanied by decreased AP-1 translocation into the nucleus. Impaired inflammasome activation in *G6PD*-kd THP-1 cells was mediated by a decrease in the production of reactive oxygen species (ROS) by NOX signaling, while treatment with hydrogen peroxide (H_2O_2) enhanced inflammasome activation in *G6PD*-kd THP-1 cells. *G6PD* knockdown decreased *Staphylococcus aureus* and *Escherichia coli* clearance in *G6PD*-kd THP-1 cells and G6PD-deficient PBMCs following inflammasome activation. These findings support the notion that enhanced pathogen susceptibility in G6PD deficiency is, in part, due to an altered redox signaling, which adversely affects inflammasome activation and the bactericidal response.

1. Introduction

Although glucose-6-phosphate dehydrogenase (G6PD) deficiency is perhaps the most common sex-linked enzymopathy on earth [1], the biochemical and physiologic roles of this housekeeping enzyme have not been fully explored [2]. Biochemically, G6PD is well known as the rate-limiting enzyme of the pentose phosphate pathway for regenerating nicotinamide adenine dinucleotide phosphate (NADPH) [3–6]. NADPH, an essential cofactor in the redox system, maintains a proper level of reducing equivalence such as reduced glutathione (GSH) and acts as a substrate for NADPH oxidase (NOX) and nitric oxide synthase (NOS), which generate reactive oxygen species (ROS) and

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NOX, NADPH oxidase; NO, nitric oxide; NOS, nitric oxide synthase; UP-LPS, ultrapure lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; ATP, adenosine triphosphate; MAPK, mitogen-activated protein kinases; AP-1, activator protein 1; ROS, reactive oxygen species; PBMC, peripheral blood mononuclear cells; H_2O_2, hydrogen peroxide; THP-1, human monocytic cells; *G6PD*-kd, *G6PD* knockdown

* Corresponding author. Department of Medical Biotechnology and Laboratory Science; Chang Gung University, 259 Wen-Hwa 1st Road, Guishan Dist, Tao-Yuan, Taiwan.
E-mail address: dtychiu@mail.cgu.edu.tw (D. Tsun-Yee Chiu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.redox.2019.101363
Received 23 August 2019; Received in revised form 18 October 2019; Accepted 25 October 2019
Available online 02 November 2019

nitric oxide (NO), respectively, for a subsequent role in signal transduction [1,7,8]. Physiologically, evidence has been emerging to indicate that G6PD deficiency affects glucose metabolism [9], cell growth, embryonic development, lethality [10,11] and susceptibility to infections by modulating redox homeostasis [12,13].

How G6PD deficiency can disrupt immune responses has not been clearly delineated. Since G6PD plays a vital role in cellular redox homeostasis [14], this enzyme can influence the redox microenvironment in cells leading to modulation of physiological functions [15]. NOXs are a major source of ROS [16–18] and are involved in the initiation of cell signaling to modulate inflammatory response and the antimicrobial defense in phagocytes [14,19]. Some transcription factors, such as NF-κB and AP-1, and certain signal transduction pathway proteins, such as MAPKs, are activated by intracellular ROS to induce inflammatory signaling [20–22]. Patients with G6PD deficiency or *G6PD* knockdown cells are more susceptible to pathogen infections [13,23,24], indicating that the immune response is affected by G6PD status.

A key physiological function of the innate immune response is the activation of the inflammasome [25,26]. This mainly leads to the production of pro-inflammatory cytokines, especially interleukin-1β (IL-1β) and IL-18, in response to invading pathogens [27]. The most common inflammasomes include NLRP1, AIM2, NLRP3, and NLRC4, and are classified by their oligomer composition and different stimuli [25]. Among the inflammasomes, NLRP3 is stimulated by environmental- and pathogen/host-derived factors. The processes mediated by inflammasomes are critical during microbial infections, including the regulation of metabolic processes and mucosal immune responses [28]. The activation of the inflammasome requires strict regulation; otherwise, it leads to many diseases [29–32]. How G6PD is involved in the activation of the inflammasome has not been clearly defined.

The activation of the NLRP3 inflammasome is ROS dependent [33,34] and is mediated by the NOX pathway [35]. Decreased ROS production is observed in G6PD-deficient granulocytes upon lipopolysaccharide (LPS) or phorbol 12-myristate 13-acetate (PMA) stimulation and such abnormality has been attributed to impaired NOX signaling [14,36,37]. Increased susceptibility to pathogen infections in G6PD-deficient cells is due to an insufficient ROS-triggered inflammatory response [13]. These findings provide support for the notion that G6PD deficiency impairs ROS production via the NOX signaling pathway. The effect of G6PD on NLRP3 inflammasome activation deserves further attention. In the current study, a decrease in IL-1β was observed in the PBMCs of patients with G6PD deficiency and in *G6PD*-kd THP-1 cells. This led to an investigation of the role of G6PD in inflammasome activation and its association with the bactericidal effect in phagocytes. Mechanistically, G6PD deficiency provides less NADPH as a substrate for NOX, causing less ROS generation to activate the inflammasome.

2. Materials and methods

2.1. Reagents

Ficoll-Paque was purchased from GE Healthcare (Little Halfont, Buckinghamshire, UK). Ultrapure LPS was purchased from InvivoGen (California, USA). Anti-IL-1β, anti-p65, anti-phospho-p65, anti-p38, anti-phospho-p38, anti-ERK, anti-phospho-ERK, anti-JNK, anti-phospho-JNK, anti-c-Jun, anti-phospho-c-Jun, anti-phospho-c-Fos and anti-c-Fos were all purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-G6PD was purchased from Genesis Biotech (Taiwan). ATP, nigericin, PMA, hydrogen peroxide, DPI, NADPH, and anti-β-Actin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-NLRP3 was purchased from AdipoGen Life Sciences (San Diego, CA, USA), and anti-ASC was purchased from Medical & Biological Laboratories (Japan). The anti-caspase-1, anti-pro-IL-1β, anti-mouse and anti-rabbit antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Anti-α-tubulin was purchased from Merck Millipore (Burlington, USA). Anti-lamin B1 was purchased from Proteintech (Illinois, USA). Polyvinylidene difluoride (PVDF) membranes and Immobilon Western Chemiluminescent HRP Substrate (ECL) were purchased from Millipore Corporation (Billerica, MA, USA).

Table 1
Partial characteristics of patients with G6PD deficiency and normal subjects.

No.[a]	G6PD status	Sex (Male/Female)	Age	Point mutation site of *G6PD* gene
1	Normal	Male	22	–
2	Normal	Female	24	–
3	Normal	Female	21	–
4	Normal	Male	22	–
5	Normal	Female	24	–
6	Normal	Male	22	–
7	Normal	Male	21	–
8	Normal	Female	40	–
9	Normal	Male	21	–
10	Normal	Male	21	–
11	Deficiency	Male	22	1376
12	Deficiency	Female	21	1376
13	Deficiency	Female	21	1388
14	Deficiency	Male	22	1376
15	Deficiency	Female	21	1376
16	Deficiency	Male	20	1376
17	Deficiency	Female	21	1376
18	Deficiency	Male	21	1376
19	Deficiency	Male	21	1376
20	Deficiency	Male	70	1376

[a] All volunteers are non-smokers and have no medical background.

2.2. PBMCs isolation and cell culture

This study was approved by the Institutional Review Board at Chang Gung Memorial Hospital, and written informed consent was acquired from every volunteer. Human whole blood was taken from healthy volunteers and G6PD-deficient patients, which are non-smokers and have no medical background. PBMCs were isolated from whole blood using Ficoll-Paque density gradient centrifugation.

THP-1 was a kind gift from Dr. Hsing-I Huang (Chang Gung University) and was cultured in RPMI 1640 (Gibco, USA) supplemented with 10% FBS (Biological Industries, USA), 100 units/mL penicillin and 100 μg/ml streptomycin (Gibco, USA) at 37 °C in a humidified atmosphere of 5% CO_2.

The human embryonic kidney cell line (293T) was purchased from the American Type Culture Collection (Rockville, MD, USA) and was cultured in Dulbecco's Modified Eagle's Medium (Gibco, USA) supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. Transfection of siRNAs

Specific sets of human siRNA for *G6PD* and a universal negative control were obtained from Dharmacon RNA Technologies (Lafayette, CO, USA). Transfection of the target siRNA (50 nM per 10^6 cells) was performed by using Lipofectamine 3000 reagent (Invitrogen, CA, USA) based on the manufacturer's instructions. On the next day, the cells were treated with stimuli as described below.

2.4. Cell stimulation

PBMCs isolated from whole blood were incubated with LPS (1 μg/mL) for 3 h and ATP (5 mM) for another 3 h. The supernatants were collected for IL-1β determination by an ELISA kit.

THP-1 cells were cultured in the presence of 150 ng/ml PMA to allow for differentiation into macrophage-like cells. After incubation for 24 h, the cells were incubated with fresh RPMI 1640 for another 24 h, followed by 0.1 μg/ml LPS treatment and 20 μM nigericin treatment.

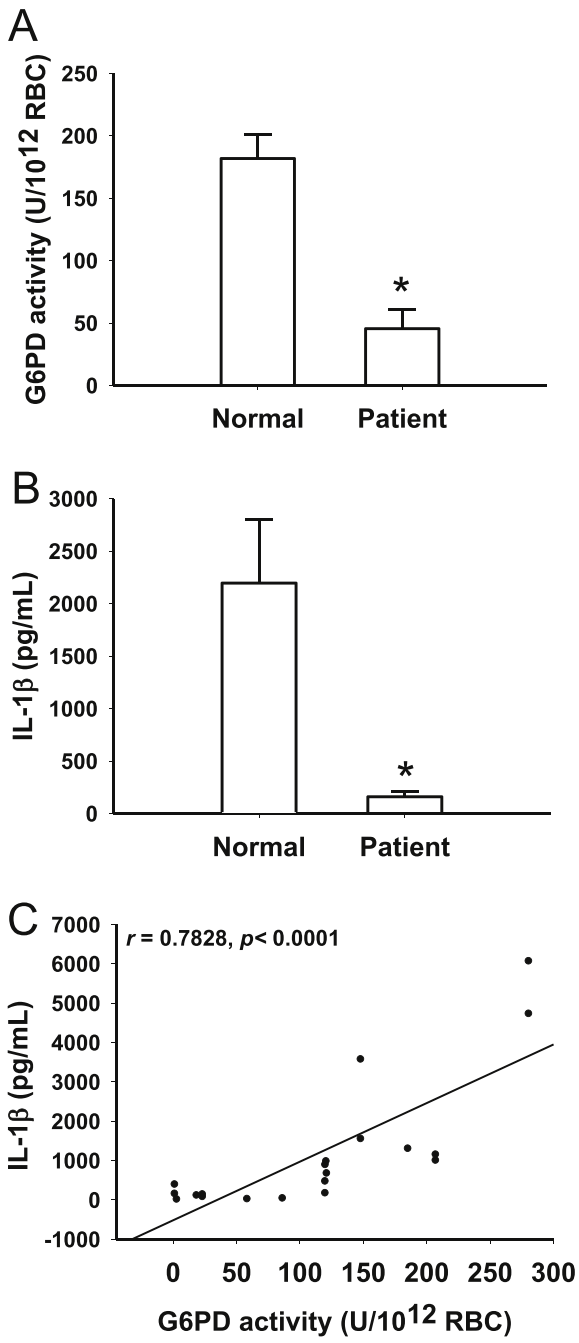

Fig. 1. IL-1β secretion in G6PD-deficient PBMCs. (A) G6PD activity of whole blood from patients with G6PD deficiency and normal subjects. (B) IL-1β concentration in the supernatant determined by ELISA (n = 10 and 10 for normal and G6PD deficiency subjects, respectively, $*p < 0.05$). (C) The correlation of G6PD activity and IL-1β secretion (n = 20, $p < 0.0001$).

The cell lysate and supernatant were collected for the indicated experiments.

2.5. ELISA

Cell supernatants were harvested for human IL-1β quantification using an ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

2.6. G6PD activity

The G6PD activity of whole blood was measured using a G6PD quantitative kit (Trinity Biotech) according to the manufacturer's instructions. In brief, 500 µl assay reagent and 5 µl whole blood were added to the cuvette and incubated for 5 min at room temperature. Then, 1 ml of the substrate solution was added to the cuvette and incubated for 2 min at 37 °C. The reduction of $NADP^+$ was measured spectrophotometrically at 340 nm. The activity of G6PD is expressed as $U/10^{12}$ erythrocytes (RBCs).

G6PD activity was measured spectrophotometrically at 340 nm by the reduction of $NADP^+$ in the presence of glucose-6-phosphate as previously described [38]. In brief, the cells were lysed in lysis buffer (50 mM Tris-HCl, 150 mM $NaCl_2$, 1 mM EDTA, 0.05% SDS, 1 mM NaF, 1% Triton-X 100, pH 7.5). After centrifugation, the cell lysate was reacted with G6PD assay buffer (50 mM Tris-HCl (pH 8), 50 mM $MgCl_2$, 4 mM $NADP^+$, and 4 mM glucose 6-phosphate). The G6PD activity was analyzed at 340 nm by spectrophotometry (Beckman Coulter, USA).

2.7. Western blot analysis

The total protein in lysates and supernatants was analyzed by a Western blot. The cells were collected using lysis buffer (50 mM Tris-HCl, 150 mM $NaCl_2$, 1 mM EDTA, 0.05% SDS, 1 mM NaF, 1% Triton-X 100, pH 7.5), and the protein concentration was determined by the Bradford assay. The protein in supernatants was concentrated by trichloroacetic acid (TCA) precipitation [39]. Samples were denatured, electrophoresed on SDS-polyacrylamide gel, and transferred onto PVDF membranes. The membranes were incubated overnight at 4 °C with an appropriate dilution of a primary antibody (1:1000). The membranes were then incubated with an appropriate dilution of an HRP-conjugated secondary antibody for 1.5 h. The immunoreactive bands were visualized by ECL reagents. ImageJ software was used to analyze the intensity. β-Actin was used as a loading control.

2.8. qRT-PCR

The total RNA was extracted using TRIzol Reagent (Life Technologies, CA, USA). RNA was reverse transcribed into cDNA by oligo-dT (Bioman Scientific, Taipei, Taiwan) as the primer in the presence of reverse transcriptase (Superscript III, Invitrogen). qRT-PCR was conducted using a SsoFast™ EvaGreen® Supermix reagent (Bio-Rad, CA, USA) with an iQ5 real-time thermal cycler (Bio-Rad, CA, USA). The expression levels were normalized to those of endogenous *ACTB*, and the data were analyzed using the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers used in qRT-PCR are as follows: *IL1B*: 5′-TGTCTGGTCCATATGAACTG-3′ and 5′-GCTGTAGAGTGGGCTTATC-3′; *ACTB*: 5′-TCCACCTTCCAGCAGATG-3′ and 5′-GTGTAACGCAACTAAGTCATAG-3’.

2.9. Dual-luciferase promoter assay

The human IL-1β promoter plasmid was a kind gift from Dr. Ben-Kuen Chen (National Cheng Kung University) [40]. The deletion sequence of the AP-1 binding site bearing the luciferase plasmid was constructed using site-directed mutagenesis. The 293FT cells cultured in 24-well plates were transfected with 600 ng of pLKO-scramble or pLKO-*G6PD* [38] together with 300 ng of the IL-1β promoter plasmid (wild-type or deletion) and 100 ng of pRL-TK vector by Lipofectamine® 2000 (Thermo Fisher Scientific, MA, USA). Forty-eight hours after transfection, the luciferase activity was measured by using the Dual-Luciferase Assay (Promega, MA, USA) with a GLOMAX luminometer (Promega, MA, USA). The cellular extracts were assayed for luciferase activity and normalized to the Renilla luciferase levels. The data are presented relative to the pLKO-scramble levels (relative light unit; RLU).

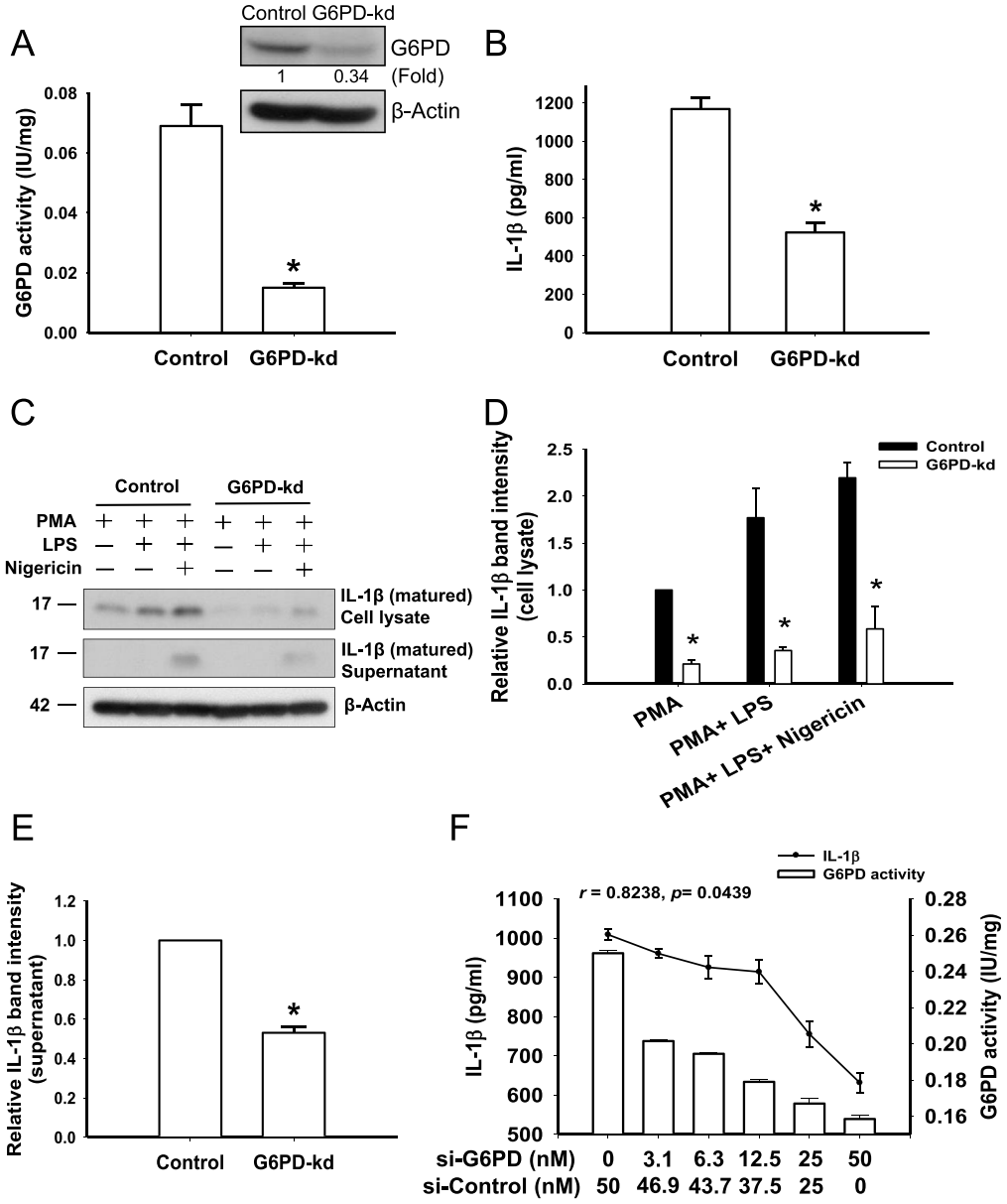

Fig. 2. Inflammasome activation in *G6PD*-kd THP-1 cells. (A) Western blot and spectrophotometry assays of G6PD protein expression and activity in *G6PD*-kd THP-1 cells (n = 3, $^*p < 0.05$). (B) ELISA and (C) Western blot analyses of IL-1β in the supernatant and cell lysate. (D), (E) IL-1β quantitative level in cell lysate and supernatant of (C). β-Actin was used as a normalized loading control of the cell lysate. (F) The correlation of G6PD activity and IL-1β secretion. THP-1 cells were transfected with *G6PD* siRNA (0, 3.1, 6.3, 12.5, 25 or 50 nM). PMA-differentiated THP-1 were treated with LPS and nigericin, cell lysate and supernatant were collected for G6PD activity and IL-1β analysis. These data are representative of three independent experiments (n = 3, $p < 0.05$).

2.10. Detection of ROS production

Cells were incubated for 30 min with 10 μM CM-H_2DCFDA (Thermo Fisher Scientific, MA, USA) at 37 °C. The cells were detached from the wells by a 5 min incubation with PBS (containing 50 mM EDTA) and a 5 min incubation with Trypsin-EDTA at 37 °C. The cells were washed twice and re-suspended in PBS (containing 1% FBS) before flow cytometry (FCM) analysis. FCM data are presented as the mean fluorescence intensity (MFI) for all cells.

2.11. Superoxide measurement by cytochrome c reduction

Superoxide production was measured using cytochrome c reduction according to the previous study [41]. Since cytochrome c cannot enter into cells, this assay exclusively determine superoxide produced from NOX in extracellular medium. In brief, 200 μl PMA-treated cells (10^6/ml) were seeded in 96 well plate to allow for differentiation into macrophage-like cells. After incubation for 24 h, the cells were incubated with fresh RPMI 1640 for another 24 h. Cells were incubated in pre-warmed HBSS containing 100 μM cytochrome c and stimulated 0.1 μg/ml LPS. Absorbance at 550 nm was recorded for 20 min with 1 measurement per 5 min at 37 °C with gentle shaking. The production of superoxide was calculated using an absorption coefficient of 21 $mM^{-1}cm^{-1}$ for cytochrome c.

2.12. Gentamicin protection assay

Staphylococcus aureus and *Escherichia coli* OP50 were incubated in Luria-Bertani (LB) media at 37 °C with shaking one day before the experiment. The density of bacteria was measured at 670 nm in a DU 800 spectrophotometer after washing three times with PBS. LPS-primed THP-1 cells and PBMCs from subjects were infected with bacteria at an MOI (multiplicity of infection) of 10 and were incubated for 2 h at 37 °C in a humidified atmosphere of 5% CO_2. After being infected, the cells were washed three times with HBSS and were incubated with gentamicin (200 μg/ml) containing RPMI 1640 for 30 min to kill extracellular bacteria. After replacing the medium with fresh medium, the cells were incubated with 20 μM nigericin for 30 min to induce inflammasome activation. The cells were washed once by HBSS, followed by lysis with 1% Triton X-100. The cell lysate was serially diluted and spread onto

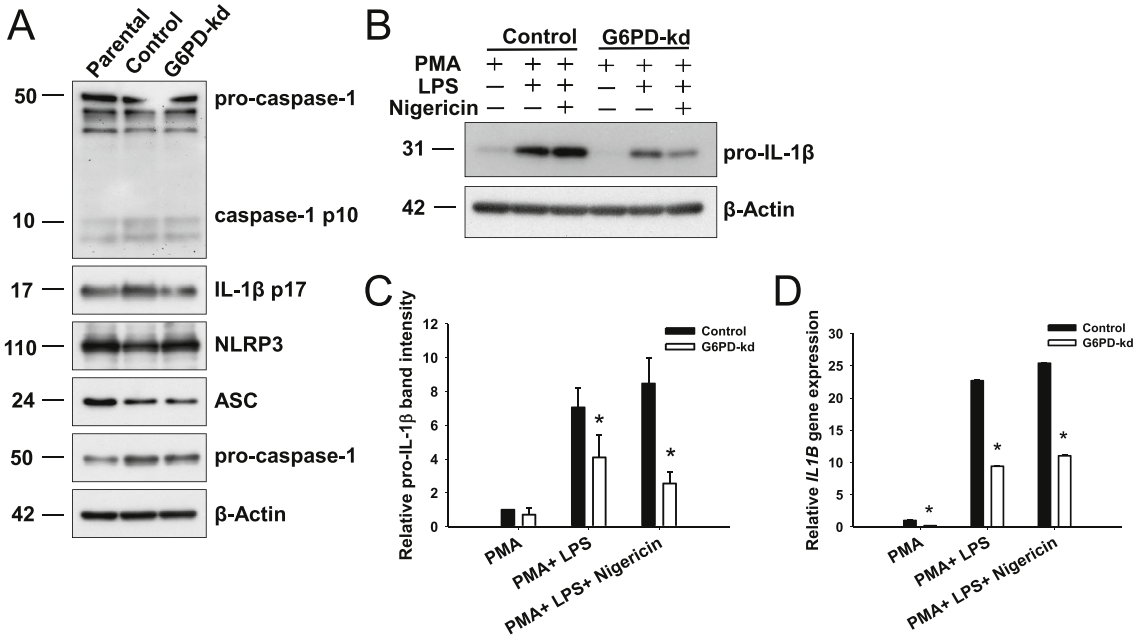

Fig. 3. NLRP3 inflammasome activation in *G6PD*-kd THP-1 cells. (A) Cells were treated with LPS (0.1 μg/mL) for 180 min and with nigericin (20 μM) for 30 min. Western blot of cell lysate and supernatant protein expression of the inflammasome complex. (B) Cells were treated with LPS (0.1 μg/mL) for 180 min with or without nigericin (20 μM) for 30 min. Western blot of the protein expression of pro-IL-1β. (C) pro-IL-1β quantitative level of (B). β-Actin was used as a normalized loading control of the cell lysate in (A) and (B). (D) Gene expression of *IL1B* analyzed by qRT-PCR, where *ACTB* was used as the normalized control. These data are representative of three independent experiments (n = 3, $^*p < 0.05$).

agar plates. The next day, the efficiency of bacterial clearance was determined by counting the colony forming units (CFUs) on agar plates [42].

2.13. Statistical analysis

The significance of the G6PD activity, the IL-1β concentration and the activity of intracellular bacterial clearance from normal and G6PD-deficient patients was evaluated by the Mann-Whitney test. These data were expressed as the mean ± SEM from replicate determinations. The significance of the correlation of G6PD activity and IL-1β was assessed by the Pearson Correlation. Student's *t*-test was used to compare the mean values from the control and *G6PD*-kd THP-1 cells, and for the comparing of superoxide production (expression as the mean ± SEM from replicate determinations). These data are expressed as the mean ± standard deviation of at least three separate experiments. The difference was regarded as significant when the *p* value was < 0.05.

3. Results

3.1. Reduced IL-1β secretion in G6PD-deficient PBMCs

Ten patients with G6PD deficiency and 10 healthy controls were recruited for this study (Table 1). Of the patients with G6PD deficiency and the normal controls, all subjects were within an age range of 20–70. The point mutation sites of the *G6PD* gene from patients with G6PD deficiency are 1376 and 1388 (Table 1). The G6PD activity of whole blood from patients with G6PD deficiency was much lower than that of the normal controls (Fig. 1A). IL-1β secretion was significantly decreased in G6PD-deficient human PBMCs (Fig. 1B) compared to that in normal control PBMCs, indicating that G6PD deficiency is associated with lower IL-1β production. The activity of G6PD was positively correlated with the secretion of IL-1β (Fig. 1C).

3.2. Decreased IL-1β secretion in G6PD-knockdown THP-1 cells

G6PD-kd THP-1 cells expressed approximately 21.7% of the G6PD levels in the control THP-1 cells (Fig. 2A). According to the results of MTT assay and the Cell Counting Kit-8 (CCK8), cell viability was similar between control and *G6PD*-kd THP-1 cells (Fig. S1). Similar to that observed in PBMCs from G6PD-deficient patients (Fig. 1B), *G6PD*-kd THP-1 cells had decreased IL-1β secretion upon inflammasome activation (Fig. 2B), and the pattern of protein expression in the cell lysates and supernatants was the same as that found by ELISA (Fig. 2C). The quantitative intensity of IL-1β in the cell lysate and supernatant is shown in Fig. 2D and E, respectively. The activity of G6PD was positively correlated with the secretion of IL-1β (Fig. 2F).

3.3. Decreased pro-IL-1β expression in G6PD-kd THP-1 cells upon inflammasome activation

G6PD knockdown reduced the expression of mature IL-1β, but not the expression of caspase-1 or the components of the inflammasome (NLRP3, ASC and pro-Caspase-1) (Fig. 3A). A decrease in the translational level of pro-IL-1β upon inflammasome activation was detected by a Western blot (Fig. 3B) in *G6PD*-kd THP-1 cells compared to the level in the controls. The quantitative intensity of pro-IL-1β is shown in Fig. 3C. The mRNA level of IL-1β also decreased in *G6PD*-kd THP-1 cells (Fig. 3D).

3.4. Decreased phosphorylation of p38 MAPK signaling in G6PD-kd THP-1 cells upon inflammasome activation

The phosphorylation of p38 at different time points upon inflammasome activation was decreased in *G6PD*-kd THP-1 cells compared to that in the control cells without a change in the phosphorylation levels of p65, ERK and JNK (Fig. 4A). The quantitative intensity of p-p38 is shown in Fig. 4B. Control and *G6PD*-kd THP-1 cells treated with SB203580 (an inhibitor of p38 MAPK) resulted in p38 inhibition (Fig. 4C). Cells were reacted with LPS, which resulted in impaired pro-

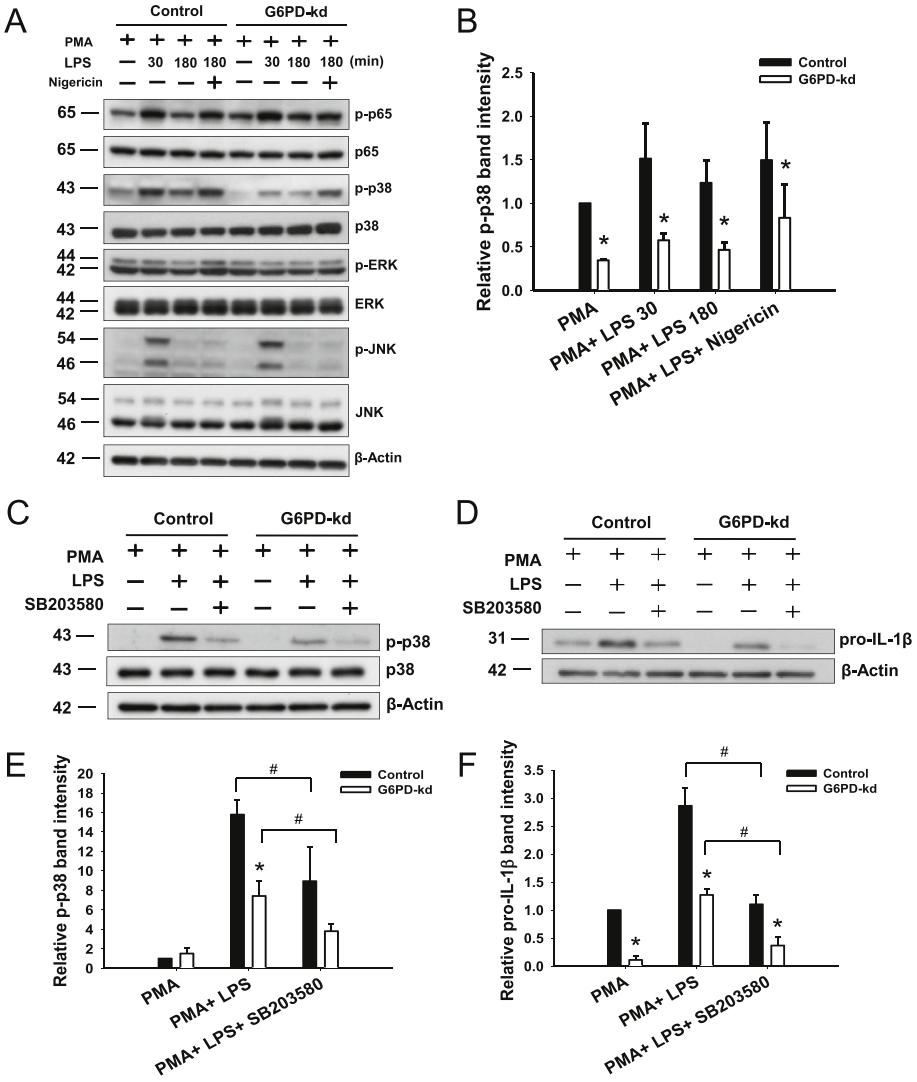

Fig. 4. P38/MAPK pathway in *G6PD*-kd THP-1 cells. (A) Western blot of the expression of p-p65, p65, p-p38, p38, p-ERK, ERK, p-JNK and JNK. PMA differentiated control and *G6PD*-kd THP-1 cells were treated with LPS for 30 or 180 min then with nigericin for 30 min. (B) P-p38 quantitative level of (A). (C), (D) Western blot of the expression of p-p38, p38 and pro-IL-1β. PMA-differentiated control and *G6PD*-kd THP-1 cells were treated with SB203580 for 30 min prior LPS treatment for 30 min for p38 detection or 180 min for pro-IL-1β detection. (E), (F) p-p38, p38 and pro-IL-1β quantitative levels of (C) and (D). β-Actin was used as a normalized loading control. The results are representative of three independent experiments (n = 3, $*p < 0.05$).

IL-1β expression (Fig. 4D). The quantitative intensity of p-p38 and pro-IL-1β is shown in Fig. 4E and F, respectively.

3.5. Inhibition of pro-IL-1β expression through AP-1 signaling in G6PD-kd THP-1 cells

The total and phosphorylated form of c-Fos, but not c-Jun, decreased with or without LPS treatment in *G6PD*-kd THP-1 cells (Fig. 5A). The quantitative intensities of p-c-Fos and c-Fos are shown in Fig. 5B and C, respectively. AP-1 signaling, which is the heterodimer of c-Fos and c-Jun and is downstream of p38, decreased after treatment with SB203580 in both control and *G6PD*-kd THP-1 cells (Fig. 5D). The quantitative intensity of c-Fos is shown in Fig. 5E. LPS treatment promoted the translocation of c-Jun, c-Fos and p65 from the cytoplasm to the nucleus in control and *G6PD*-kd THP-1 cells (Fig. 5F). The translocation of c-Fos was reduced in *G6PD*-kd THP-1 cells, although c-Jun translocation was increased upon *G6PD* knockdown compared with that in the scrambled control cells. No significant difference was observed in the translocation of NF-κB. The quantification of nuclear c-Fos is shown in Fig. 5G. The *IL1B* reporter plasmid construction is shown in Fig. 5H, upper panel. 293FT cells were chosen to measure the binding activity of AP-1 because of the ease of transfection of these cells compared to that of THP-1 cells. The binding activity of AP-1 was lower in 293FT pLKO-*G6PD*-kd cells compared to the binding activity of 293FT pLKO-scrambled cells (Fig. 5H).

3.6. Impaired p38 MAPK/AP-1 signaling by G6PD knockdown in the presence of decreasing ROS production in THP-1 cells

The treatment of differentiated control and *G6PD*-kd THP-1 cells with LPS and nigericin increased ROS production, which was less than that in *G6PD*-kd THP-1 cells (Fig. 6A). The production of superoxide was lower in *G6PD*-kd THP-1 cells than that in control cells upon LPS incubation for 20 min (Fig. 6B). The pretreatment of *G6PD*-kd and control THP-1 cells with DPI, an inhibitor of NADPH oxidase, decreased the expression of p-p38 and pro-IL-1β in both cells (Fig. 6C and E). The quantitative intensity of p-p38 and pro-IL-1β is shown in Fig. 6D and F, respectively. *G6PD*-kd THP-1 cells treated with H_2O_2 (100 μM) and NADPH (100 μM) increased the expression of p-p38 and pro-IL-1β (Fig. 6G and I). The quantitative intensity of p-p38 and pro-IL-1β is shown in Fig. 6H and J, respectively. The expression of IL-1β was improved by overexpress IDH1 in THP-1-LG and *G6PD*-kd cells (Fig. S2). Variable ROS level has different effects on cellular physiology. In Fig. S3, the expression of IL-1β was only induced in high concentrated H_2O_2-treated THP-1 cells. This suggests that G6PD deficiency decreases ROS level by only affecting inflammasome signal 1 pathway but not signal 2.

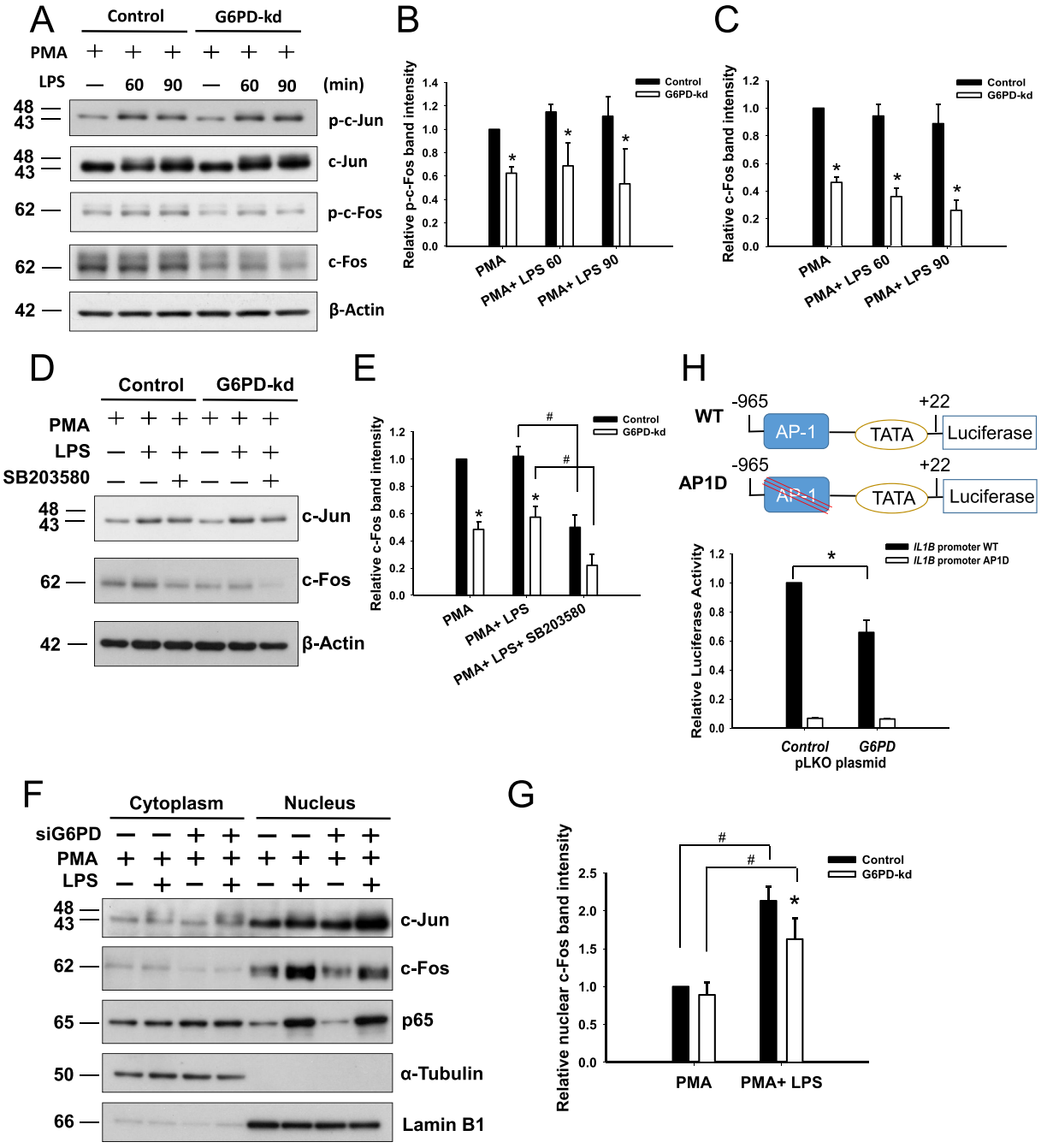

Fig. 5. AP-1 signaling in *G6PD*-kd THP-1 cells. (A) Western blot of the expression of c-Jun and c-Fos. Cells were treated with LPS for 60 or 90 min. (B), (C) p-c-Fos and c-Fos quantitative level of (A). (D) Western blot of the expression of c-Jun and c-Fos. Cells were treated with SB203580 for 30 min prior to LPS treatment for 60 min. (E) c-Fos quantitative level of (D). β-Actin was used as a normalized loading control in (A) and (D). The results are representative of three independent experiments (n = 3, $*p < 0.05$). (F) Western blot of cytoplasmic and nuclear proteins. Cells were treated with LPS for 60 min. α-Tubulin and lamin B1 were used as cytoplasmic and nuclear loading controls. (G) c-Fos quantitative level of (F). The results are representative of three independent experiments (n = 3, $*p < 0.05$). (H) Luciferase activity assay of 293T cells transfected with a firefly luciferase reporter plasmid containing a partial sequence with the putative AP-1 binding site (WT) or the deleted mutation (AP1D). This assay was standardized by Renilla activity after transfection for 48 h ($*p < 0.05$ compared to basal conditions).

3.7. Decreased activity of intracellular bacterial clearance in G6PD-kd THP-1 cells

Gram-positive *S. aureus* (Fig. 7A and B) and gram-negative *E. coli* (Fig. 7C and D) were analyzed for the bacterial clearance activity in *G6PD*-kd THP-1 and PBMCs from patients with G6PD deficiency. The CFU number was higher in *G6PD*-kd THP-1 cells and PBMCs from patients with G6PD deficiency compared to the CFU number in the scrambled control THP-1 cells and PBMCs from normal controls with both pathogens. The impairment of the bactericidal effect due to G6PD deficiency could be improved by adding IL-1β.

4. Discussion

Certain chronic and infectious diseases are associated with G6PD deficiency [13,24,43]. The impact of G6PD status on innate immunity, especially upon inflammasome activation, is unknown. The present study is the first to show that *G6PD*-kd THP-1 cells and PBMCs from patients with G6PD deficiency with different mutations of the enzyme have a decrease in IL-1β expression and NLRP3 inflammasome

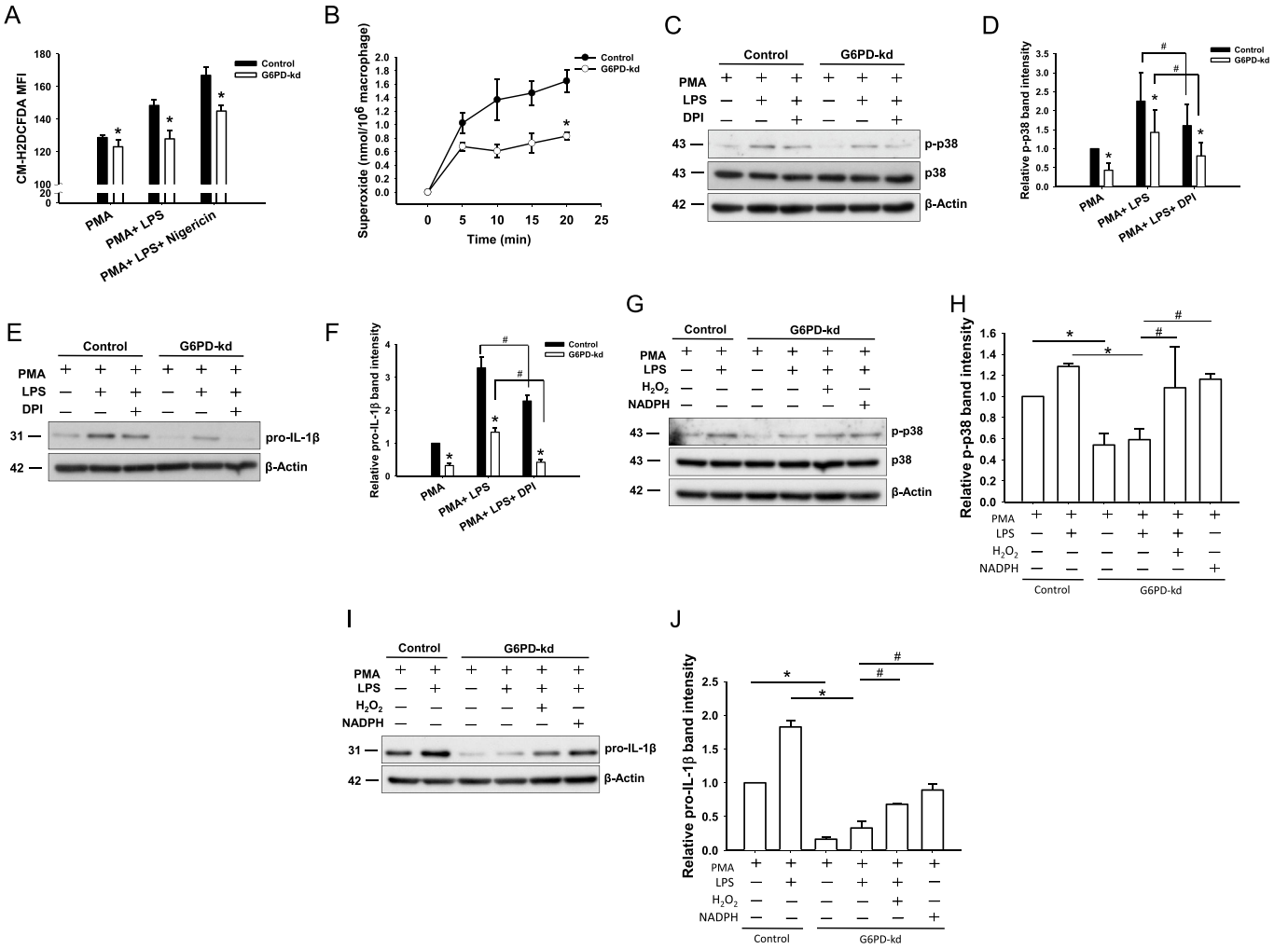

Fig. 6. Signaling of p38 MAPK/AP-1 modulated by the ROS level in *G6PD*-kd THP-1 cells. (A) Flow cytometry for determining the ROS production in PMA-differentiated control THP-1 cells and *G6PD*-kd THP-1 cells. Cells were treated with LPS for 3 h and nigericin for 30 min. Cells were stained with 10 μM CM-H_2DCFDA for 30 min. (B) Superoxide production was measured by cytochrome c reduction. The PMA-differentiated control and *G6PD*-kd THP-1 cells were incubated in cytochrome c containing HBSS with 0.1 μg/ml LPS. The production of superoxide was measured spectrophotometrically at 550 nm. The PMA-differentiated control and *G6PD*-kd THP-1 cells were treated with (C, E) DPI, (G, I) H_2O_2 or NADPH for 60 min prior to LPS treatment for 30 min for p38 detection or 180 min for pro-IL-1β detection. The expression of p-p38, p38 and pro-IL-1β was determined by a Western blot. (D), (F) p-p38 and pro-IL-1β quantitative levels of (C) and (E). (H), (J) p-p38 and pro-IL-1β quantitative levels of (G) and (I). β-Actin was used as a loading control. The results are representative of three independent experiments (n = 3, $^*p < 0.05$).

activation. Such defect in NLRP3 inflammasome activation could be attributed to the inhibition of p38-MAPK and AP-1 signaling upon LPS stimulation. The underlying mechanism is due to decreased superoxide production by NOX, as found in *G6PD*-kd THP-1 cells. Together, these results indicate that G6PD deficiency impairs the cellular innate immune response by a disturbance in redox homeostasis, which can have clinical implications in G6PD-deficient individuals with infectious diseases.

G6PD is important in maintaining cellular redox homeostasis by regenerating NADPH, the substrate of NOX and NOS. The formation of superoxide by NOX is dependent on the electrons are transferred from cytoplasmic NADPH to extracellular oxygen. Superoxide can be reduced by extracellular superoxide dismutase (SOD1) to produce H_2O_2, which transfer into cells through aquaporin channels, or influx into cytoplasm through the chloride channel-3 to initiate intracellular signaling [44]. Since the activity of NOX is modulated by G6PD status, the pathway-mediated inflammatory response is impaired by G6PD deficiency [12]. G6PD deficiency increases the cellular susceptibility to a variety of pathogen-induced viral and bacterial infections [13,23,24]. Compared to the levels in normal phagocytes, G6PD-deficient phagocytes have lower levels of superoxide and other ROS needed for effective microbial killing [36,45]. Such susceptibility to microbial infections in patients with G6PD deficiency may be due to an impaired response, the absence of NETosis [46], and a still unknown defense mechanism. The present study shows that the inflammasome response is modulated by G6PD status and provides an additional mechanism for why G6PD deficiency increases susceptibility to infections as proposed in Fig. 8.

The level of ROS can have significant consequence on various cellular function [8,12–14,36,38,47–49]. Cytotoxic level of ROS, such as oxidative burst in phagocytic cells, participates in host defense by direct killing of foreign pathogen [48]. On the other hand, cytoregulatory level of ROS mediates intracellular signaling that maintain proper redox environment and regulate many cellular signaling pathways [14,49,50]. In the present study, we provide evidence to support the notion that cytoregulatory level of ROS influenced p38/MAPK/AP-1 pathway and bactericidal effect in G6PD deficient cells. The pathway of inflammasome activation involves two signals, including priming (signal 1) and activation (signal 2) [25]. Our results indicate that G6PD deficiency can influence p38/MAPK/AP-1 pathway (signal 1), but not the expression of inflammasome subunit influenced by signal 2

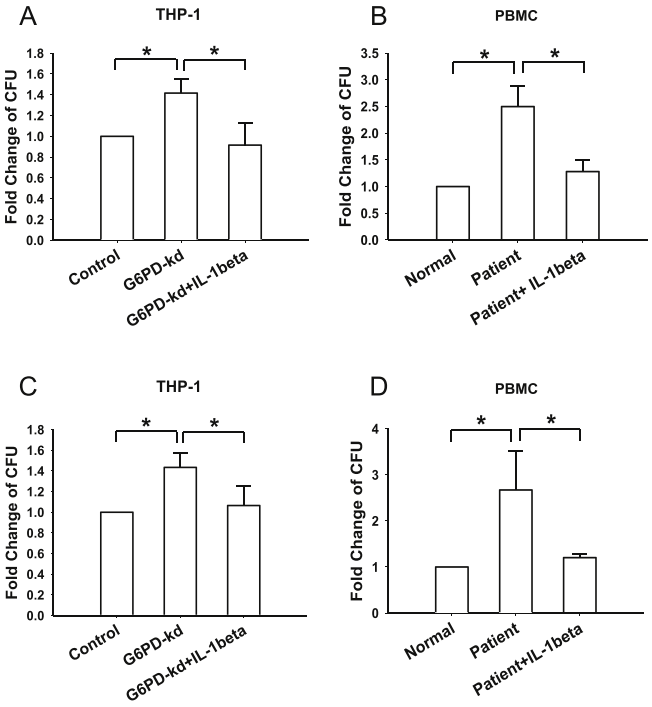

Fig. 7. Bacterial clearance activity in *G6PD*-kd THP-1 cells and PBMCs from patients with G6PD deficiency. LPS-primed THP-1 cells and PBMCs were infected by a bacterial MOI of 10: (A, B) *S. aureus* and (C, D) *E. coli*. The results are representative of three independent experiments (n = 4, $*p < 0.05$).

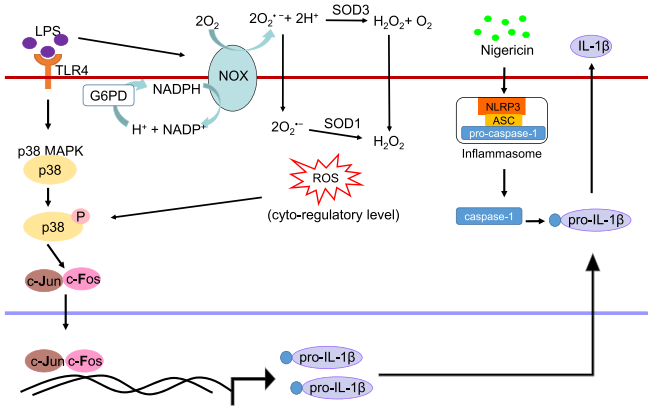

Fig. 8. Proposed schematic representation of the signaling pathways involved in the redox regulation of inflammasome activation in G6PD-deficient cells. *G6PD* knockdown results in the reduced generation of pro-IL-1β and an impairment of the LPS-induced p38/MAPK-AP-1 pathway. IL-1β secretion is also reduced in *G6PD*-knockdown cells.

suggesting that low concentration of ROS in G6PD deficiency can affect signal 1 but not signal 2. Furthermore, the expression of phospho-p38 and pro-IL-1β has been found to be improved in low-dose (0.1 mM) H_2O_2 treated cells (Fig. 6G–J). In contrast, IL-1β secretion has been detected under high level of H_2O_2 stimulation (Fig. S3). High concentration of ROS leads cells undergo apoptosis or pyroptosis, which is the common programmed cell death upon inflammasome activation. In contrast, cyto-regulatory level of ROS is important for cells to regulate cellular signaling transduction [50]. All in all, ROS can affect inflammasome activation via both signals 1 and 2 at high concentration (cytotoxic level), whereas low concentration of ROS is involved in regulating only signal 1 of inflammasome activation.

Cells need to maintain redox homeostasis for regulating proper functioning. Redox pathways have been identified in and between cells for broad functions to relay and generate cellular signals. Since G6PD plays a role in the production of NADPH as the substrate of NOX, it is anticipated that G6PD may be involved in modulating redox-sensitive signaling [49]. When NADPH is decreased, superoxide production is reduced by NOX. This may cause an impaired Nrf2 antioxidant response [51] and a decrease in NF-κB [13,52] and SP-1/HIF-1α signaling [53–55]. AP-1 signaling is a part of the G6PD-regulated redox pathway, thereby contributing to the activity of redox networks. The transcription factor AP-1 is a heterodimer composed of the proteins c-Fos, c-Jun and those from the ATF families [56]. The stability of the Jun-Fos heterodimer allows for higher DNA-binding activity [57]. *G6PD* knockdown decreased p38 phosphorylation and c-Fos expression, which reduced *IL1B* expression. The terminal differentiation of THP-1 cells is associated with the induction of c-Fos, implying that this could be the growth arrest signal in the differentiation of monocytes [58].

A number of redox-related transcription factors are modulated by G6PD status, indicating the importance of G6PD in cellular redox homeostasis. Hemolytic crisis, diabetes [59], hypertension [6], infectious diseases [46,60] and protection against malaria are associated with G6PD deficiency. That patients with G6PD deficiency are susceptible to infection may involve a novel mechanism that includes impaired inflammasome activation. Moreover, NADPH is also the substrate for many protective enzymes. Antioxidant defences may play a role in G6PD-redulated iflammasome activation. NAD(P)H:quinone oxidoreductase-1 (NQO-1) is a major quinone reductases, which requires NADPH as electron donor to catalyze quinones to hydroquinone. The enzyme also plays an antioxidant enzyme in cellular redox homeostasis [61]. The induction of NQO-1 can be mediated by Nrf2-mediated mechanism under a variety of stress responses presumably as a cellular protective system [62,63]. In terms of inflammatory response, NQO-1 as an inhibitor in immune response by promoting IκB-ζ degradation leading to impaired TLR-mediated the production of cytokines [64]. Glutathione is also as antioxidant and plays critical role in many metabolic processes. Our previous metabolomics studies have shown that alteration of G6PD status affects cellular metabolic pathway [9,65]. Abnormal glycerophospholipid metabolism in G6PD deficient embryos leads to defective embryonic development [65]. G6PD deficient cells are unable to regenerate enough NADPH under a stressful situation then rapid switch to GSH biosynthetic supply, which causes energy crisis and ineffective AMPK activation [9]. However, whether G6PD deficiency affected the inflammasome activation via the change of antioxidants or metabolites needs to be further investigated. Since infectious and metabolic diseases in patients with G6PD deficiency may be more severe than in patients with a normal level of G6PD, we concur with the notion that an escalating diagnostic algorithm should include a determination of G6PD activity in these patients [46].

Although the induction of the NLRP3 inflammasome contributes to host defenses against infections, dysregulated inflammasomes are associated with many human diseases, such as cancer, autoimmune diseases, and inflammatory disorders [29–32,66]. Our findings that G6PD inhibition can attenuate inflammasome activation suggest a new approach for therapeutic intervention of inflammasome-associated diseases [67,68] with inflammasome as a target. Hence, a G6PD inhibitor such as Polydatin, might be considered as a therapeutic agent to attenuate inflammasome-associated diseases [67,68].

In conclusion, impaired inflammasome activation by G6PD deficiency can present a phenomenon of increased susceptibility to bacterial infections. Mechanistically, defective bacterial clearance found in *G6PD*-kd THP-1 cells is due to impaired NOX/p38 MAPK/AP-1 signaling and may appear in patients with G6PD deficiency. The change in G6PD status contributes to an imbalance in cellular redox homeostasis that affects inflammatory-associated signal transduction to influence cellular infectivity. The care of patients with G6PD deficiency to prevent infection might include appropriate antibiotic prophylaxis and the modulation of inflammasome activity.

Author contributions

All authors co-designed the experiments. The experiments were performed by Wei-Chen Yen, Yi-Hsuan Wu and Hsin-Ru Lin. All authors analyzed the results, wrote the manuscript, contributed substantially to the present work, and then read and approved the final manuscript.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work is made possible by grants from the Ministry of Science and Technology of Taiwan (MOST105-2320-B-182-031-MY2, MOST107-2320-B-182-013 and MOST108-2320-B-182-001 to DTYC, MOST108-2320-B-182-030-MY3 to CCW), from the Ministry of Education of Taiwan (EMRPD1G0181 to CGU), from Chang Gung Memorial Hospital (BMRP098 and CMRPD1I0121 to DTYC, CLRPD190018 and BMRPC77 to CCW), and from Featured Areas Research Center Program within the Framework of Higher Education Sprout Project by Ministry of Education and Ministry of Science and Technology, Taiwan (MOST 107-3017-F-182-001). This manuscript was edited by American Journal Experts.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2019.101363.

References

[1] G. Manganelli, U. Masullo, S. Passarelli, S. Filosa, Glucose-6-phosphate dehydrogenase deficiency: disadvantages and possible benefits, Cardiovasc. Haematol. Disord. - Drug Targets 13 (1) (2013) 73–82.
[2] H.C. Yang, Y.H. Wu, W.C. Yen, H.Y. Liu, T.L. Hwang, A. Stern, D.T. Chiu, The redox role of G6PD in cell growth, cell death, and cancer, Cells 8 (9) (2019).
[3] M.D. Cappellini, G. Fiorelli, Glucose-6-phosphate dehydrogenase deficiency, Lancet 371 (9606) (2008) 64–74.
[4] L. Luzzatto, E. Seneca, G6PD deficiency: a classic example of pharmacogenetics with on-going clinical implications, Br. J. Haematol. 164 (4) (2014) 469–480.
[5] G.H. Wan, S.C. Tsai, D.T. Chiu, Decreased blood activity of glucose-6-phosphate dehydrogenase associates with increased risk for diabetes mellitus, Endocrine 19 (2) (2002) 191–195.
[6] R.S. Gaskin, D. Estwick, R. Peddi, G6PD deficiency: its role in the high prevalence of hypertension and diabetes mellitus, Ethn. Dis. 11 (4) (2001) 749–754.
[7] R.C. Stanton, Glucose-6-phosphate dehydrogenase, NADPH, and cell survival, IUBMB Life 64 (5) (2012) 362–369.
[8] H.Y. Ho, M.L. Cheng, D.T. Chiu, Glucose-6-phosphate dehydrogenase–beyond the realm of red cell biology, Free Radic. Res. 48 (9) (2014) 1028–1048.
[9] H.Y. Tang, H.Y. Ho, P.R. Wu, S.H. Chen, F.A. Kuypers, M.L. Cheng, D.T. Chiu, Inability to maintain GSH pool in G6PD-deficient red cells causes futile AMPK activation and irreversible metabolic disturbance, Antioxidants Redox Signal. 22 (9) (2015) 744–759.
[10] L. Longo, O.C. Vanegas, M. Patel, V. Rosti, H. Li, J. Waka, T. Merghoub, P.P. Pandolfi, R. Notaro, K. Manova, L. Luzzatto, Maternally transmitted severe glucose 6-phosphate dehydrogenase deficiency is an embryonic lethal, EMBO J. 21 (16) (2002) 4229–4239.
[11] H.C. Yang, T.L. Chen, Y.H. Wu, K.P. Cheng, Y.H. Lin, M.L. Cheng, H.Y. Ho, S.J. Lo, D.T. Chiu, Glucose 6-phosphate dehydrogenase deficiency enhances germ cell apoptosis and causes defective embryogenesis in Caenorhabditis elegans, Cell Death Dis. 4 (2013) e616.
[12] H.R. Lin, Y.H. Wu, W.C. Yen, C.M. Yang, D.T. Chiu, Diminished COX-2/PGE2-mediated antiviral response due to impaired NOX/MAPK signaling in G6PD-knockdown lung epithelial cells, PLoS One 11 (4) (2016) e0153462.
[13] Y.H. Wu, D.T. Chiu, H.R. Lin, H.Y. Tang, M.L. Cheng, H.Y. Ho, Glucose-6-Phosphate dehydrogenase enhances antiviral response through downregulation of NADPH sensor HSCARG and upregulation of NF-kappaB signaling, Viruses 7 (12) (2015) 6689–6706.
[14] H.C. Yang, M.L. Cheng, H.Y. Ho, D.T. Chiu, The microbicidal and cytoregulatory roles of NADPH oxidases, Microb. Infect. 13 (2) (2011) 109–120.
[15] K.M. Holmstrom, T. Finkel, Cellular mechanisms and physiological consequences of redox-dependent signalling, Nat. Rev. Mol. Cell Biol. 15 (6) (2014) 411–421.
[16] H.Y. Ho, M.L. Cheng, D.T. Chiu, Glucose-6-phosphate dehydrogenase–from oxidative stress to cellular functions and degenerative diseases, Redox Rep. 12 (3) (2007) 109–118.
[17] T.L. Leto, S. Morand, D. Hurt, T. Ueyama, Targeting and regulation of reactive oxygen species generation by Nox family NADPH oxidases, Antioxidants Redox Signal. 11 (10) (2009) 2607–2619.
[18] B.M. Babior, NADPH oxidase: an update, Blood 93 (5) (1999) 1464–1476.
[19] K.L. Singel, B.H. Segal, NOX2-dependent regulation of inflammation, Clin. Sci. (Lond.) 130 (7) (2016) 479–490.
[20] J.A. McCubrey, M.M. Lahair, R.A. Franklin, Reactive oxygen species-induced activation of the MAP kinase signaling pathways, Antioxidants Redox Signal. 8 (9–10) (2006) 1775–1789.
[21] Y. Son, Y.K. Cheong, N.H. Kim, H.T. Chung, D.G. Kang, H.O. Pae, Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? J. Signal Transduct. 2011 (2011) 792639.
[22] Y. Liang, Y. Zhou, P. Shen, NF-kappaB and its regulation on the immune system, Cell. Mol. Immunol. 1 (5) (2004) 343–350.
[23] H.Y. Ho, M.L. Cheng, S.F. Weng, L. Chang, T.T. Yeh, S.R. Shih, D.T. Chiu, Glucose-6-phosphate dehydrogenase deficiency enhances enterovirus 71 infection, J. Gen. Virol. 89 (Pt 9) (2008) 2080–2089.
[24] Y.H. Wu, C.P. Tseng, M.L. Cheng, H.Y. Ho, S.R. Shih, D.T. Chiu, Glucose-6-phosphate dehydrogenase deficiency enhances human coronavirus 229E infection, J. Infect. Dis. 197 (6) (2008) 812–816.
[25] V.A. Rathinam, S.K. Vanaja, K.A. Fitzgerald, Regulation of inflammasome signaling, Nat. Immunol. 13 (4) (2012) 333–342.
[26] A. Abderrazak, T. Syrovets, D. Couchie, K. El Hadri, B. Friguet, T. Simmet, M. Rouis, NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases, Redox Biol. 4 (2015) 296–307.
[27] T. Yamazaki, T. Ichinohe, Inflammasomes in antiviral immunity: clues for influenza vaccine development, Clin. Exp. Vaccine Res. 3 (1) (2014) 5–11.
[28] K.W. Chen, C.J. Gross, F.V. Sotomayor, K.J. Stacey, J. Tschopp, M.J. Sweet, K. Schroder, The neutrophil NLRC4 inflammasome selectively promotes IL-1beta maturation without pyroptosis during acute Salmonella challenge, Cell Rep. 8 (2) (2014) 570–582.
[29] M. Lamkanfi, L. Vande Walle, T.D. Kanneganti, Deregulated inflammasome signaling in disease, Immunol. Rev. 243 (1) (2011) 163–173.
[30] X. He, Z. Wei, J. Wang, J. Kou, W. Liu, Y. Fu, Z. Yang, Alpinetin attenuates inflammatory responses by suppressing TLR4 and NLRP3 signaling pathways in DSS-induced acute colitis, Sci. Rep. 6 (2016) 28370.
[31] T. Gicquel, S. Robert, T. Victoni, V. Lagente, [The NLRP3 inflammasome: physiopathology and therapeutic application], Presse Med. 45 (4 Pt 1) (2016) 438–446.
[32] H.M. Lee, J.J. Kim, H.J. Kim, M. Shong, B.J. Ku, E.K. Jo, Upregulated NLRP3 inflammasome activation in patients with type 2 diabetes, Diabetes 62 (1) (2013) 194–204.
[33] O. Gross, C.J. Thomas, G. Guarda, J. Tschopp, The inflammasome: an integrated view, Immunol. Rev. 243 (1) (2011) 136–151.
[34] J. Lugrin, N. Rosenblatt-Velin, R. Parapanov, L. Liaudet, The role of oxidative stress during inflammatory processes, Biol. Chem. 395 (2) (2014) 203–230.
[35] A. Sokolovska, C.E. Becker, W.K. Ip, V.A. Rathinam, M. Brudner, N. Paquette, A. Tanne, S.K. Vanaja, K.J. Moore, K.A. Fitzgerald, A. Lacy-Hulbert, L.M. Stuart, Activation of caspase-1 by the NLRP3 inflammasome regulates the NADPH oxidase NOX2 to control phagosome function, Nat. Immunol. 14 (6) (2013) 543–553.
[36] K.J. Tsai, I.J. Hung, C.K. Chow, A. Stern, S.S. Chao, D.T. Chiu, Impaired production of nitric oxide, superoxide, and hydrogen peroxide in glucose 6-phosphate-dehydrogenase-deficient granulocytes, FEBS Lett. 436 (3) (1998) 411–414.
[37] S. Brechard, S. Plancon, E.J. Tschirhart, New insights into the regulation of neutrophil NADPH oxidase activity in the phagosome: a focus on the role of lipid and Ca(2+) signaling, Antioxidants Redox Signal. 18 (6) (2013) 661–676.
[38] Y.H. Wu, Y.H. Lee, H.Y. Shih, S.H. Chen, Y.C. Cheng, D. Tsun-Yee Chiu, Glucose-6-phosphate dehydrogenase is indispensable in embryonic development by modulation of epithelial-mesenchymal transition via the NOX/Smad3/miR-200b axis, Cell Death Dis. 9 (1) (2018) 10.
[39] L. Koontz, TCA precipitation, Methods Enzymol. 541 (2014) 3–10.
[40] S.C. Ko, C.R. Huang, J.M. Shieh, J.H. Yang, W.C. Chang, B.K. Chen, Epidermal growth factor protects squamous cell carcinoma against cisplatin-induced cytotoxicity through increased interleukin-1beta expression, PLoS One 8 (2) (2013) e55795.
[41] Z. Khan, X.Z. Shen, E.A. Bernstein, J.F. Giani, M. Eriguchi, T.V. Zhao, R.A. Gonzalez-Villalobos, S. Fuchs, G.Y. Liu, K.E. Bernstein, Angiotensin-converting enzyme enhances the oxidative response and bactericidal activity of neutrophils, Blood 130 (3) (2017) 328–339.
[42] C.Y. Chen, C.H. Yang, Y.F. Tsai, C.C. Liaw, W.Y. Chang, T.L. Hwang, Ugonin U stimulates NLRP3 inflammasome activation and enhances inflammasome-mediated pathogen clearance, Redox Biol. 11 (2017) 263–274.
[43] Y.T. Hsieh, M.H. Lin, H.Y. Ho, L.C. Chen, C.C. Chen, J.C. Shu, Glucose-6-phosphate dehydrogenase (G6PD)-deficient epithelial cells are less tolerant to infection by Staphylococcus aureus, PLoS One 8 (11) (2013) e79566.
[44] A.B. Fisher, Redox signaling across cell membranes, Antioxidants Redox Signal. 11 (6) (2009) 1349–1356.
[45] B.K. Rada, M. Geiszt, K. Kaldi, C. Timar, E. Ligeti, Dual role of phagocytic NADPH oxidase in bacterial killing, Blood 104 (9) (2004) 2947–2953.
[46] U. Siler, S. Romao, E. Tejera, O. Pastukhov, E. Kuzmenko, R.G. Valencia, V. Meda Spaccamela, B.H. Belohradsky, O. Speer, M. Schmugge, E. Kohne, M. Hoenig, J. Freihorst, A.S. Schulz, J. Reichenbach, Severe glucose-6-phosphate dehydrogenase deficiency leads to susceptibility to infection and absent NETosis, J. Allergy Clin. Immunol. 139 (1) (2017) 212–219 e3.
[47] M. Schieber, N.S. Chandel, ROS function in redox signaling and oxidative stress, Curr. Biol. 24 (10) (2014) R453–R462.
[48] R. Spooner, O. Yilmaz, The role of reactive-oxygen-species in microbial persistence

and inflammation, Int. J. Mol. Sci. 12 (1) (2011) 334–352.
[49] H.C. Yang, Y.H. Wu, H.Y. Liu, A. Stern, D.T. Chiu, What has passed is prolog: new cellular and physiological roles of G6PD, Free Radic. Res. 50 (10) (2016) 1047–1064.
[50] I. Silva, T. Peccerella, S. Mueller, V. Rausch, IL-1 beta-mediated macrophage-hepatocyte crosstalk upregulates hepcidin under physiological low oxygen levels, Redox Biol. 24 (2019) 101209.
[51] B.M. Hybertson, B. Gao, S.K. Bose, J.M. McCord, Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation, Mol. Asp. Med. 32 (4–6) (2011) 234–246.
[52] M. Ham, J.W. Lee, A.H. Choi, H. Jang, G. Choi, J. Park, C. Kozuka, D.D. Sears, H. Masuzaki, J.B. Kim, Macrophage glucose-6-phosphate dehydrogenase stimulates proinflammatory responses with oxidative stress, Mol. Cell. Biol. 33 (12) (2013) 2425–2435.
[53] M. Philippe, Y. Larondelle, F. Lemaigre, B. Mariame, H. Delhez, P. Mason, L. Luzzatto, G.G. Rousseau, Promoter function of the human glucose-6-phosphate dehydrogenase gene depends on two GC boxes that are cell specifically controlled, Eur. J. Biochem. 226 (2) (1994) 377–384.
[54] A. Franze, M.I. Ferrante, F. Fusco, A. Santoro, E. Sanzari, G. Martini, M.V. Ursini, Molecular anatomy of the human glucose 6-phosphate dehydrogenase core promoter, FEBS Lett. 437 (3) (1998) 313–318.
[55] G.P. Laliotis, I. Bizelis, A. Argyrokastritis, E. Rogdakis, Cloning, characterization and computational analysis of the 5' regulatory region of ovine glucose 6-phosphate dehydrogenase gene, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 147 (4) (2007) 627–634.
[56] M. Karin, Z. Liu, E. Zandi, AP-1 function and regulation, Curr. Opin. Cell Biol. 9 (2) (1997) 240–246.
[57] T. Kouzarides, E. Ziff, The role of the leucine zipper in the fos-jun interaction, Nature 336 (6200) (1988) 646–651.
[58] J. Lee, K. Mehta, M.B. Blick, J.U. Gutterman, G. Lopez-Berestein, Expression of c-fos, c-myb, and c-myc in human monocytes: correlation with monocytic differentiation, Blood 69 (5) (1987) 1542–1545.
[59] N.Y. Spencer, R.C. Stanton, Glucose 6-phosphate dehydrogenase and the kidney, Curr. Opin. Nephrol. Hypertens. 26 (1) (2017) 43–49.
[60] S. Zamani, A.Z. Hoseini, A.M. Namin, Glucose-6-phosphate dehydrogenase (G6PD) activity can modulate macrophage response to Leishmania major infection, Int. Immunopharmacol. 69 (2019) 178–183.
[61] J.M. Park, D.H. Kim, H.K. Na, Y.J. Surh, Methylseleninic acid induces NAD(P)H:quinone oxidoreductase-1 expression through activation of NF-E2-related factor 2 in Chang liver cells, Oncotarget 9 (3) (2018) 3014–3028.
[62] C.H. Song, N. Kim, S.M. Lee, R.H. Nam, S.I. Choi, S.R. Kang, E. Shin, D.H. Lee, H.N. Lee, Y.J. Surh, Effects of 17beta-estradiol on colorectal cancer development after azoxymethane/dextran sulfate sodium treatment of ovariectomized mice, Biochem. Pharmacol. 164 (2019) 139–151.
[63] D. Ross, D. Siegel, Functions of NQO1 in cellular protection and CoQ10 metabolism and its potential role as a redox sensitive molecular switch, Front. Physiol. 8 (2017) 595.
[64] A. Kimura, M. Kitajima, K. Nishida, S. Serada, M. Fujimoto, T. Naka, Y. Fujii-Kuriyama, S. Sakamato, T. Ito, H. Handa, T. Tanaka, A. Yoshimura, H. Suzuki, NQO1 inhibits the TLR-dependent production of selective cytokines by promoting IkappaB-zeta degradation, J. Exp. Med. 215 (8) (2018) 2197–2209.
[65] T.L. Chen, H.C. Yang, C.Y. Hung, M.H. Ou, Y.Y. Pan, M.L. Cheng, A. Stern, S.J. Lo, D.T. Chiu, Impaired embryonic development in glucose-6-phosphate dehydrogenase-deficient Caenorhabditis elegans due to abnormal redox homeostasis induced activation of calcium-independent phospholipase and alteration of glycerophospholipid metabolism, Cell Death Dis. 8 (1) (2017) e2545.
[66] S. Tartey, T.D. Kanneganti, Differential role of the NLRP3 inflammasome in infection and tumorigenesis, Immunology 156 (4) (2019) 329–338.
[67] R. Lv, L. Du, X. Liu, F. Zhou, Z. Zhang, L. Zhang, Polydatin alleviates traumatic spinal cord injury by reducing microglial inflammation via regulation of iNOS and NLRP3 inflammasome pathway, Int. Immunopharmacol. 70 (2019) 28–36.
[68] J. Tang, Y. Li, J. Wang, Q. Wu, H. Yan, Polydatin suppresses the development of lung inflammation and fibrosis by inhibiting activation of the NACHT domain-, leucine-rich repeat-, and pyd-containing protein 3 inflammasome and the nuclear factor-kappaB pathway after Mycoplasma pneumoniae infection, J. Cell. Biochem. 120 (6) (2019) 10137–10144.