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HIF-1α regulated GLUT1-mediated glycolysis enhances *Treponema pallidum*-induced cytokine responses

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Abstract

Syphilis, caused by *Treponema pallidum* (*Tp*), represents a significant public health challenge. The clinical manifestations of syphilis are attributed to local inflammatory responses induced by *Tp*, notably monocyte infiltration into local lesions and the secretion of inflammatory cytokines. However, the mechanisms driving cytokine production in response to *Tp* infection remain largely unknown. Given that increased glycolysis is associated with inflammatory responses, we aimed to investigate the role of glycolysis in *Tp*-induced secretion of inflammatory cytokines. In this study, we found that *Tp* promotes the secretion of inflammatory cytokines IL-6, IL-8, and CCL2 from monocytes while enhancing glycolysis through increased GLUT1 plasma membrane expression and glucose uptake. Importantly, inhibiting glycolysis and GLUT1 reduced the *Tp*-induced secretion of monocyte inflammatory cytokines. Additionally, *Tp* significantly increased HIF-1α expression and induced its nuclear translocation, thereby promoting glycolysis by upregulating the expression of GLUT1 and LDHA glycolytic enzymes. Knockdown of HIF-1α inhibits *Tp*-induced monocyte cytokine secretion, highlighting the crucial role of HIF-1α-mediated glycolysis in the cytokine response to *Tp*. Also, expression of HIF-1α and an increase in glycolysis were confirmed in patients with syphilis. In conclusion, we demonstrated that HIF-1α-regulated GLUT1-mediated glycolysis enhances inflammatory cytokine secretion following *Tp* infection. Our findings not only elucidate the mechanism of glycolysis in *Tp*-induced inflammatory responses in monocytes but also contribute to the development of a potential biomarker in syphilis diagnosis and treatment.

Highlights

- *Tp* enhances glycolysis in monocytes, leading to increased production of inflammatory cytokines.
- Glucose transporter 1 (GLUT1) drives *Tp*-induced glycolysis and inflammatory cytokines secretion.
- Hypoxia-inducible factor-1α (HIF-1α) regulates GLUT1-mediated glycolysis and inflammatory cytokines secretion.
- Patients with syphilis show increased glycolysis and HIF-1α expression.

Keywords *Treponema pallidum*, Monocyte, Inflammatory, Glycolysis, HIF-1α

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Teaser

The study found that *Tp* enhances GLUT1 expression and glucose uptake via HIF-1α activation, thereby increasing glycolysis and promoting the secretion of inflammation cytokines IL-6, IL-8, and CCL2.

Introduction

Syphilis is a chronic systemic sexually transmitted disease caused by *Treponema pallidum* (*Tp*) infection with a resurgence in recent years, which is a serious threat to human health [1]. Elucidating the pathogenic mechanisms of *Tp* infection is crucial for developing effective treatments and prevention strategies for syphilis. The clinical manifestations of syphilis mainly result from the inflammatory response induced by *Tp* replication in tissues and the body's immune reaction to the pathogen [2, 3]. In secondary syphilis, mucosal and skin lesions show monocyte infiltration in peripheral blood, while in tertiary syphilis, monocyte infiltration and tissue damage are key clinical manifestations of gummas [4, 5]. Monocytes are crucial to the innate immune response, acting as the frontline defense against *Tp* by releasing inflammatory cytokines and chemokines to enhance the local immune response [6, 7]. However, the overall pattern of cytokine responses and the underlying mechanisms in monocytes following *Tp* infection remain unclear.

Studies have shown that the secretion of inflammatory cytokines is regulated by intracellular metabolism [8, 9]. Activated immune cells undergo extensive metabolic reprogramming to meet the dramatic increase in energy requirements, thereby promoting cytokine production and cell proliferation [10, 11]. The Warburg effect, initially described in cancer cells, is characterized by increased glucose uptake and aerobic glycolysis, reduced mitochondrial respiration (oxidative phosphorylation (OXPHOS)), and inhibition of the tricarboxylic acid (TCA) cycle [12, 13]. Subsequent studies have demonstrated that pathogen infection induces a similar metabolic reprogramming phenomenon, playing a key role in pathogen defense and inflammatory response. Mycobacterium tuberculosis causes a shift in human alveolar macrophages from oxidative phosphorylation to aerobic glycolysis, which is essential for controlling intracellular bacillary replication [14]. After infection with *Borrelia burgdorferi*, monocytes induce a shift in cellular metabolism toward glycolysis, which is essential for Borrelia-induced production of IL-22, IL-1β, TNF-α, and IL-6 [15]. Glycolysis is known to convert glucose into lactate through a biocatalytic process involving many important enzymes. Hexokinase (HK) catalyzes the initial rate-limiting step of glycolysis; pyruvate kinase (PK) catalyzes the final rate-limiting step of glycolysis; and L-lactate dehydrogenase (LDH) can promote the production of lactate from pyruvate [16]. Three molecules in the glycolytic pathway described above are directly linked to bacterial infection-induced IL-1β production and inflammation [17, 18]. However, whether glycolysis is involved in the secretion of inflammatory cytokines in response to *Tp* infection is unknown. In addition, previous studies have shown that pro-inflammatory immune cells are powered by glycolysis, which relies on high levels of glucose uptake [19]. Most cells take up glucose by promoting membrane diffusion, a process mediated by the glucose transporters (GLUTs) family of membrane transporters [20, 21]. The GLUT family includes 14 transporters in mammals (GLUT1–14) [20]. However, the range of GLUT transporters utilized by monocytes has not yet been defined, and the mechanisms that regulate GLUT expression and glycolysis in monocytes during *Tp* infection are still poorly understood.

Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric transcription factor composed of two distinct subunits: HIF-1α and HIF-1β [22]. The activated HIF-1α subunit translocates to the nucleus and plays a crucial role in activating glycolysis and inflammation [23, 24]. HIF-1α regulates glycolytic enzymes such as HK, LDH, and GLUT-1, and it is also involved in the transcriptional regulation of cytokines such as IL-1β, IL-6, and TNF-α in the inflammatory response [15, 25]. However, it remains unclear whether HIF-1α is induced in monocytes during *Tp* infection and contributes to the *Tp*-induced inflammatory response.

As key cells of the innate immune system, monocytes initiate and sustain the inflammatory response by releasing cytokines and chemokines. To our knowledge, little is known about whether glycolysis affects the cytokine secretion of monocytes in syphilis, and the related mechanism is unclear. Previous studies have demonstrated that recombinant protein Tp47 of *Tp* induces PKM2-dependent glycolysis, leading to the activation of the NLRP3 inflammasome and subsequently promoting macrophage phagocytosis [26]. However, the single membrane protein Tp47 from *Tp* cannot completely mimic the role of *Tp* in the disease progression of syphilis. Therefore, the link between glycolysis and an uncontrolled inflammation response prompted us to investigate the mechanism by which monocytes respond to *Tp* infection. In this study, we aimed to investigate the interaction between *Tp* and monocytes, specifically the role of HIF-1α in regulating monocyte glycolysis and *Tp*-induced monocyte inflammatory cytokine production.

Materials and methods

Ethics statement

All the experiments involving animals were approved by the Ethics Committee of the University of South China. Experiments were conducted according to the principles expressed in the Declaration of Helsinki.

Preparation of virulent *Tp*

The *Treponema pallidum* Nichols strain was generously provided by Yang Tianci, PhD (University of Xiamen, Xiamen, China), and cultured for virulence in New Zealand rabbits following established protocols [27]. After the virulent live *Tp* was obtained, the isolated live *Tp* was thermally inactivated at 56 ℃ for 30 min to obtain dead *Tp* (DTp). DTp can serve as a negative control to ensure that the observed biological effects are specifically attributable to live *Tp* infection rather than to non-specific factors. In addition, the testicles of uninfected rabbits were removed aseptically, similar to the extraction of viable *Tp*. The PBS solution obtained in this manner does not contain *Tp*, and the PBS solution was used as a control to eliminate the influence of residual rabbit tissue on the experimental results.

Cell culture and treatment

THP-1 cells (human monocytic cell line) were obtained from the American Type Culture Collection (ATCC, USA). Briefly, the cells underwent cultivation in a RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin. Subsequently, the cells were incubated under specific conditions in a humidified incubator at a temperature of 37 °C, while maintaining a 5% CO2 atmosphere.

THP-1 cells were treated with *Tp* at different multiplicities of infection (MOI) (MOI of 1:1, 2:1, 4:1) for 24 h at 37 °C in a 5% CO2 incubator. For inhibition assays, THP-1 cells were treated with various chemicals, including glycolysis pathway inhibitor 2-deoxy-D-glucose (2-DG; HY-13966, Med Chem Express) and GLUT1 inhibitor BAY876 (HY-100017, Med Chem Express) for 2 h before *Tp* stimulation. As for HIF-1α inhibition, small interfering RNA (siRNA) targeting HIF-1α was used to transiently knock-down and then co-culture with *Tp* for 24 h. The siRNA sequences are CTACCCACATACATAAAGA.

Immunofluorescence assay

Cells collected after stimulation were washed twice with PBS to completely eliminate the effect of serum. The cells were coated on a slide coated with poly-lysine, fixed with 4% paraformaldehyde at room temperature for 30 min, and washed three times with PBS. 0.4% Triton X-100 was added to permeabilize cells for 15 min and washed twice with PBS. A 2% bovine serum albumin (BSA) sealing solution was used to block all of the cells, placed at 37 °C for 30 min, and incubated overnight at 4 °C with GLUT1 or HIF-1α antibodies (1:50 dilution). The cells were washed 3 times with PBS for 5 min each time, and then at room temperature with Alexa Fluor 488-labelled or Alexa Fluor 594-labelled goat anti-rabbit IgG (1:200 diluted) and incubated for 1 h. The nuclei were stained with DAPI (1:200 diluted) for 5 min. The cells were washed three times with PBS and observed under a fluorescence microscope.

Western blotting (WB) assay

Total protein was extracted using RIPA lysis buffer containing phosphatase and protease inhibitor, and then total protein concentration was determined using the BCA protein assay kit. According to the manufacturer's instructions, nuclear and cytoplasmic extraction reagents (Thermo Fisher, USA) are used to extract nuclear and cytoplasmic proteins from cells. Typically, a 10% SDS-PAGE gel is used to isolate 15–55 μg of total protein and transfer it to a PVDF membrane. The membrane is treated with 5% skim milk sealer for 2 h at room temperature. Then HIF-1α (1:600, Abmart, China), GLUT1 (1:1000, Abmart, China), HK1 (1:1000, Abcam, England), HK2 (1:1000, Abcam, England), LDHA (1:1000, Cell Signaling Technology, USA), LDHB (1:3000, Proteintech, China), LDH (1:1000, Abcam, England), HDAC1 (1:1000, Abcam, England), and β-actin (1:1000, Cell Signaling Technology, USA) specific primary antibodies were incubated overnight at 4 °C. Use TBST to wash three times for 10 min each time. Incubated with enzyme-labeled secondary antibody (1:3000) at room temperature for 2 h. Wash with TBST again three times, for 10 min each time. Finally, the protein bands were visualized using an ECL reagent (CWBIO, China).

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (TIANGEN, China) in accordance with the protocol. The total RNA concentration was determined by an ultraviolet spectrophotometer and reverse transcribed with a synthesis kit (Vazyme Biotech Co., Ltd., China). Quantitative real-time PCR (qRT-PCR) was performed with SYBR Green Premix (Vazyme Biotech Co., Ltd., China) in accordance with the manufacturer's protocol. The relative change in mRNA was normalized to β-actin and calculated using the $2^{-\Delta\Delta CT}$ method. PCR primers were designed based on sequences from corresponding target genes (Table S1).

Cytokine measurements

Supernatants from cultures of THP-1 cells treated with PBS, DTp, and *Tp* for 24 h, respectively, were collected. The simultaneous measurement of 27 cytokines in cell culture supernatant was performed using luminex detection technology. In addition, cell supernatants were collected, and IL-6, IL-8, and CCL2 in the supernatants were measured according to the ELISA kit (Invitrogen, USA) manufacturer's instructions.

Glycolysis assay and extracellular oxygen consumption assay

Monocytes were seeded into 96-well plates with a density of 5×10^5 cells per well and treated with *Tp* according to the instructions of the extracellular oxygen consumption assay kit (Abcam, ab197243, England) and glycolysis assay kit (Abcam, ab197244, England). Extracellular oxygen consumption and glycolysis levels were measured in real time using SpectraMax iD3 multimode microplate readers (Mol.Devices).

Glucose uptake assay

Following stimulation by *Tp* for 24 h, monocytes underwent two washes in pre-heated glucose-free medium. Subsequently, they were incubated in a 5% CO2 incubator at 37 °C for 15 min with pre-heated glucose-free medium, followed by a 15-min incubation with a pre-heated glucose-uptake probe solution. The cells were then washed three times with a pre-cooled WI solution before being analyzed using flow cytometry.

Detection of lactate production and ATP production

Monocytes were seeded onto 6-well plates and stimulated by *Tp* cultured in 5% CO2 at 37 °C for 24 h. Then, lactate concentrations in the culture supernatant and intracellular were determined using a L-Lactate Assay Kit (Cayman Chemical, 700,510, USA). ATP production was measured by the luciferin-uciferase method with an ATP detection kit (Beyotime Biotechnology, Shanghai, China). Finally, the absorbance was read on a microplate reader (Biotek Instruments, Inc., USA).

Cell isolation and culture in syphilis patients

This study protocol was approved by the Ethics Committee at the First Affiliated Hospital, Hengyang Medical School, University of South China. Written, informed consent was obtained from all participants. Incident syphilis was defined as an RPR titer that was ≥1:8, or a fourfold increase from the prior RPR titer, and a positive TPPA test. Participants were included in the study if they met these criteria for incident syphilis. Patients were excluded from the study if they had infections with other pathogens or were diagnosed with HIV, Chlamydia, hepatitis B, hepatitis C, *Mycoplasma genitalium*, or any malignancies. The anticoagulant blood was collected from healthy participants and syphilis patients, and human peripheral blood mononuclear cells were isolated according to the instructions of human peripheral blood lymphocyte isolation fluid (TBD, LTS10771, China). The cells were cultured using RPMI-1640 medium in a 5% CO2 incubator at 37 °C and were further stimulated with *Tp* (MOI of 2:1) for 24 h.

Statistical analysis

The data are expressed as the mean ± standard deviation (SD) of three independent experiments. Statistical analysis was conducted using GraphPad Prism 8.0 software (San Diego, California, USA), and the Student´s unpaired t test was used for comparison between two groups. A one-way analysis of variance (ANOVA) was used for comparisons among multiple sets of quantitative data. Differences were considered significant when $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Results

Tp promotes inflammatory cytokine secretion in monocytes

To investigate whether *Tp* regulates the inflammatory response of monocytes by inducing specific cytokine secretion, THP-1 cells were stimulated with *Tp* for 24 h. The luminex detection technology was used to detect secretion levels of 23 cytokines related to immune regulation, inflammation and tissue repair. The results showed that *Tp* stimulation significantly increased the secretion of chemokines and pro-inflammatory cytokines, including CCL2, MIP-1β, IL-8, and IL-6 (Fig. 1A). To further confirm the reliability of the luminex results, qRT-PCR and ELISA were used to verify the changes in IL-6, IL-8, and CCL2 expression and secretion (Fig. 1B-G). The results of luminex, qRT-PCR, and ELISA consistently showed that *Tp* stimulation significantly increased the expression and secretion levels of IL-6, IL-8, and CCL2 in monocytes. The increased levels of these pro-inflammatory cytokines may explain the inflammatory response observed in syphilis, which is of great significance for understanding the disease progression and identifying potential therapeutic targets.

Tp enhances glycolysis in monocytes

Metabolic reprogramming plays a critical role in pro-inflammatory activity in monocytes [28]. To investigate whether *Tp* alters the pro-inflammatory response of monocytes by inducing metabolic reprogramming, particularly glycolysis activation, we performed targeted metabolomics after cells were stimulated with *Tp* or DTp for 24 h. Upon *Tp* treatment, we observed

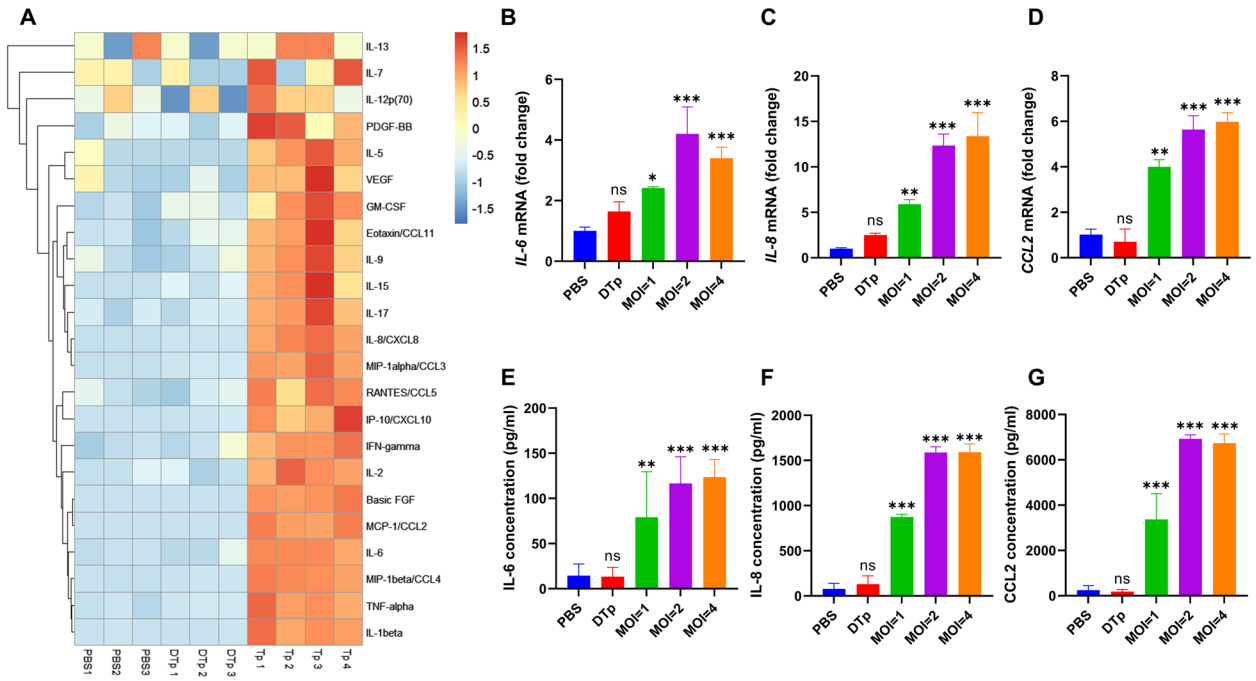

Fig. 1 *Tp* induces cytokine production from monocytes. **A** THP-1 cells were treated with PBS, DTp, or *Tp* (MOI of 2:1) for 24 h. The quantitative detection of 27 cytokines in the cell culture supernatant was conducted using luminex technology. Heat map depicting hierarchical clustering of cytokine concentrations, where red denotes higher concentrations and blue denotes lower concentrations. Three cytokines were randomly selected. **B**-**D** qRT-PCR-based analysis of cytokines mRNA expression in THP-1 cells treated with PBS, DTp, and *Tp* (MOI of 1:1, 2:1, 4:1). **E**–**G** The concentrations of cytokines in the cell culture supernatants of THP-1 cells, treated with PBS, DTp, and *Tp* (MOI of 1:1, 2:1, and 4:1), were confirmed using ELISA. The data are shown as the mean ± SD. Statistical significance tested by one-way ANOVA test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: no significant, *n* = 3

modifications in the abundance of multiple metabolites, and these metabolites were mainly involved in the glycolysis pathway, the pentose phosphate pathway, the tricarboxylic acid cycle, and other pathways (Fig. 2A). Notably, the glycolytic end-products pyruvate and lactic acid were significantly elevated, whereas TCA cycle–related metabolites such as citric acid and succinic acid were markedly decreased (Fig. 2B). Meanwhile, DTp treatment did not induce notable changes of metabolites. Furthermore, additional validation revealed that both intracellular (Fig. 2C) and extracellular (Fig. 2D) lactate levels were increased following *Tp* treatment, while ATP production (Fig. 2E) was significantly reduced. Altogether, these results suggest

(See figure on next page.)

Fig. 2 Monocytes become overtly glycolytic following *Tp* treatment. **A**, **B** Metabolomics analysis was conducted to detect alterations in metabolite levels following treatment of THP-1 cells for 24 h with PBS or *Tp* (MOI of 2:1). **A** Hierarchical clustered heatmap of differential metabolites identified as potential biomarkers. The metabolites are categorized into three major classes: amino acids, carbohydrates, and organic acids. Each row represents an individual metabolite, and each column corresponds to a sample. **B** Schematic representation illustrating changes in metabolites associated with the glycolysis pathway and the tricarboxylic acid (TCA) cycle. Arrows indicate relative changes in the *Tp* group compared to the PBS control. Red arrows represent metabolites that are upregulated following *Tp* treatment, while green arrows indicate downregulated metabolites. **C** Intracellular and (**D**) extracellular lactate concentrations, along with (**E**) intracellular ATP levels, were quantified after 24 h of treatment with PBS, DTp, or *Tp* (MOI of 2:1). Data are presented as mean ± SD. Statistical significance tested by unpaired, two-tailed Student's test. **F**-**G** THP-1 cells were treated with PBS, DTp, or *Tp* (MOI of 1:1, 2:1) for 24 h. **F** The glycolysis assay detects the time course of real-time changes in extracellular acidification. **G** Time course of real-time changes in the OCR after the extracellular oxygen consumption assay. **H** Glucose uptake by THP-1 cells was measured by flow cytometry after 24 h of treatment by *Tp* (MOI of 1:1, 2:1). Statistical significance tested by one-way ANOVA test. **I** TOP 50 pathway enrichment analysis of differential metabolite sets. Western-blot-based analysis of (**J**) HK1, HK2, and (**K**) LDHA, LDHB, and LDH protein expression in THP-1 cells treated with PBS, DTp, or *Tp* (MOI of 1:1, 2:1, 4:1) for 24 h. The data are presented as the mean ± SD of three independent experiments. **P* < 0.05, ***P* < 0.01, *** *P* < 0.001, ns: no significant

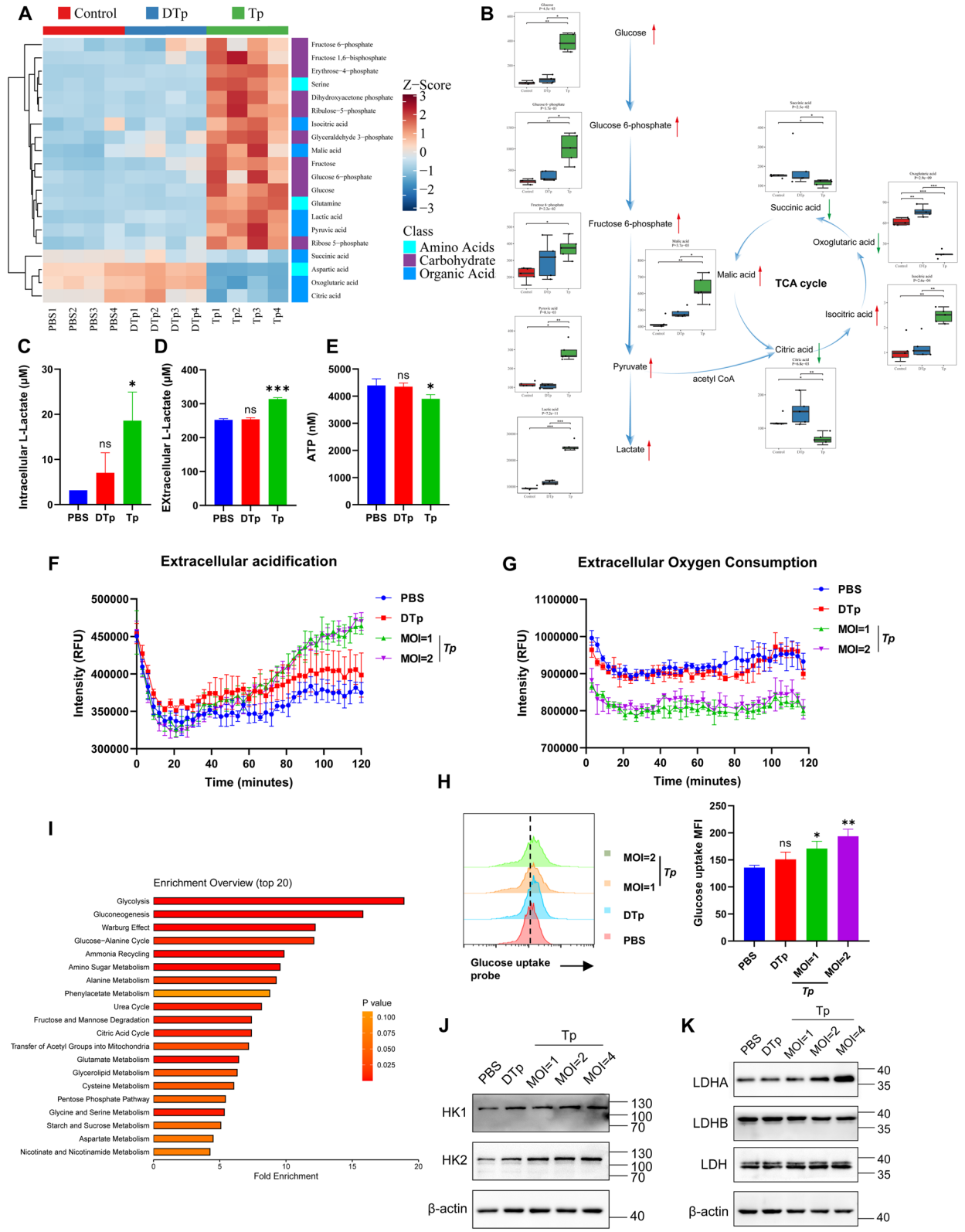

Fig. 2 (See legend on previous page.)

that *Tp* enhances glycolytic activity to meet the energy demands of the induced inflammatory response, likely facilitating the rapid mobilization of pro-inflammatory functions in monocytes.

To further confirm the impact of *Tp* on monocyte glycolytic pathways, we conducted metabolic flux analysis and examined the expression of key enzymes. Extracellular metabolic flux analyses showed that *Tp* treatment significantly reduced extracellular oxygen consumption and, conversely, increased extracellular acidification (Fig. 2F, G). Moreover, the metabolism of monocytes after *Tp* treatment is highly dependent on the glycolytic pathway and increases glycolytic capacity (Supplementary Fig. 1 A, B). Glycolysis depends on high levels of glucose uptake, and we found that *Tp* stimulation significantly increased the glucose uptake capacity of monocytes (Fig. 2H). The combined data indicate that monocytes overtly increase their glycolytic activity after *Tp* treatment. On the other hand, we used the selected pathway-associated metabolite sets (The Small Molecule Pathway Database, SMPDB) library for pathway enrichment analysis of differential metabolites, which showed strong correlation with glycolytic pathways (Fig. 2I). Hexokinase (HK) is the first rate-limiting enzyme of glycolysis, controlling glycolytic flux [29]. HK1 and HK2 increased significantly in both mRNA and protein levels after *Tp* stimulation of monocytes (Figure J; Supplementary Figures S1C, D and S2A, C). To investigate the mechanism of lactate increase, qRT-PCR and Western blot were used to detect the expressions of LDHA, LDHB, and LDH. LDHA mRNA and protein expression were significantly increased (Fig. 2K, and supplementary figure S1E, 2B, 2D), but LDHB mRNA expression was significantly reduced and protein expression remained unchanged (Supplementary Figures S1F, 2B, D), indicating that *Tp* infection promoted lactate production through up-regulation of LDHA expression. Taken together, our results indicate that *Tp* overtly increases monocyte glycolysis.

Tp induces IL-6, IL-8, and CCL2 secretion by enhancing glycolysis

Previous studies have shown that pro-inflammatory immune cells rely on glycolysis for energy [30]. To determine the specific role of glycolysis in the *Tp*-induced inflammatory response, we used 2-DG as a glycolysis inhibitor to block the glycolytic pathway and assess its impact on cytokine secretion. To evaluate the role of glycolysis in *Tp*-induced inflammation, THP-1 cells were pretreated with the glycolysis inhibitor 2-DG for 2 h, followed by stimulation with *Tp* (MOI of 2: 1) for 24 h. Real-time glycolysis analysis showed that 2-DG inhibited *Tp*-induced extracellular acidification of monocytes (Fig. 3A). Additionally, the production of cytokines IL-6, IL-8 and CCL2 was significantly reduced in the *Tp*+ 2-DG group compared to *Tp* treatment alone (Fig. 3B-D). This suggests that glycolysis may rapidly mobilize monocyte functions by providing the energy required for the inflammatory response, thereby promoting the secretion of IL-6, IL-8, and CCL2.

GLUT1 drives *Tp*-induced glycolysis and IL-6, IL-8, and CCL2 secretion

At times of peak inflammation, immune cells preferentially select glycolysis as an energy source, which in turn depends on high levels of glucose uptake [31]. We observed a significant increase in monocyte glucose uptake after *Tp* stimulation (Fig. 2H). Glucose uptake is mediated by glucose transporters, and there are 14 glucose transporters in mammals, of which GLUT5, GLUT6, and GLUT13 are not involved in glucose transport [32]. However, it is unclear which glucose transporters control monocyte glucose uptake during *Tp* infection. We evaluated the expression of multiple glucose transporters in monocytes in the presence or absence of *Tp* for 24 h. The results demonstrated a significant upregulation of SLC2 A1 (encoding GLUT1 protein) and SLC2 A9 (encoding GLUT9 protein) expression following *Tp* treatment, with GLUT1 exhibiting the highest expression level (Fig. 4A, B). Therefore, we focus on the study of GLUT1. We showed that *Tp* treatment upregulated GLUT1 protein expression in THP-1 cells (Fig. 4C, Supplementary Figure S3 A-D). Fluorescence microscopy revealed that *Tp* induced high plasma membrane expression of GLUT1. (Fig. 4D). To investigate the role of GLUT1 in *Tp*-stimulated monocytes, monocytes were preincubated with BAY876, a selective inhibitor of GLUT1, for 2 h and then stimulated with *Tp* (MOI of 2:1) for 24 h. GLUT1 inhibition significantly reduced *Tp*-induced glucose uptake, positioning GLUT1 as the main GLUT involved in the *Tp* response (Fig. 4E). Additionally, the expression levels of glycolytic enzyme genes *HK1*, *HK2*, and *LDHA* were significantly decreased in monocytes treated with *Tp*+ BAY876 compared to *Tp* treatment alone (Fig. 4G-I). Consistently, the results also showed that GLUT1 inhibition impaired *Tp*-induced extracellular acidification in monocytes (Fig. 4J). These results demonstrate that GLUT1 plays a crucial role in *Tp*-induced glycolysis in monocytes. Next, we evaluated the role of GLUT1 in *Tp*-induced monocyte cytokine production. *Tp* stimulation induced the secretion of IL-6, IL-8, and CCL2 (Fig. 4K-M). However, treatment with BAY876 significantly reduced the secretion of IL-6 and CCL2 compared to *Tp* treatment (Fig. 4K-M). Taken together, these results indicate that GLUT1-dependent glycolysis is involved in *Tp*-induced IL-6, IL-8, and CCL2 secretion. The high expression of GLUT1 may be an adaptive response of

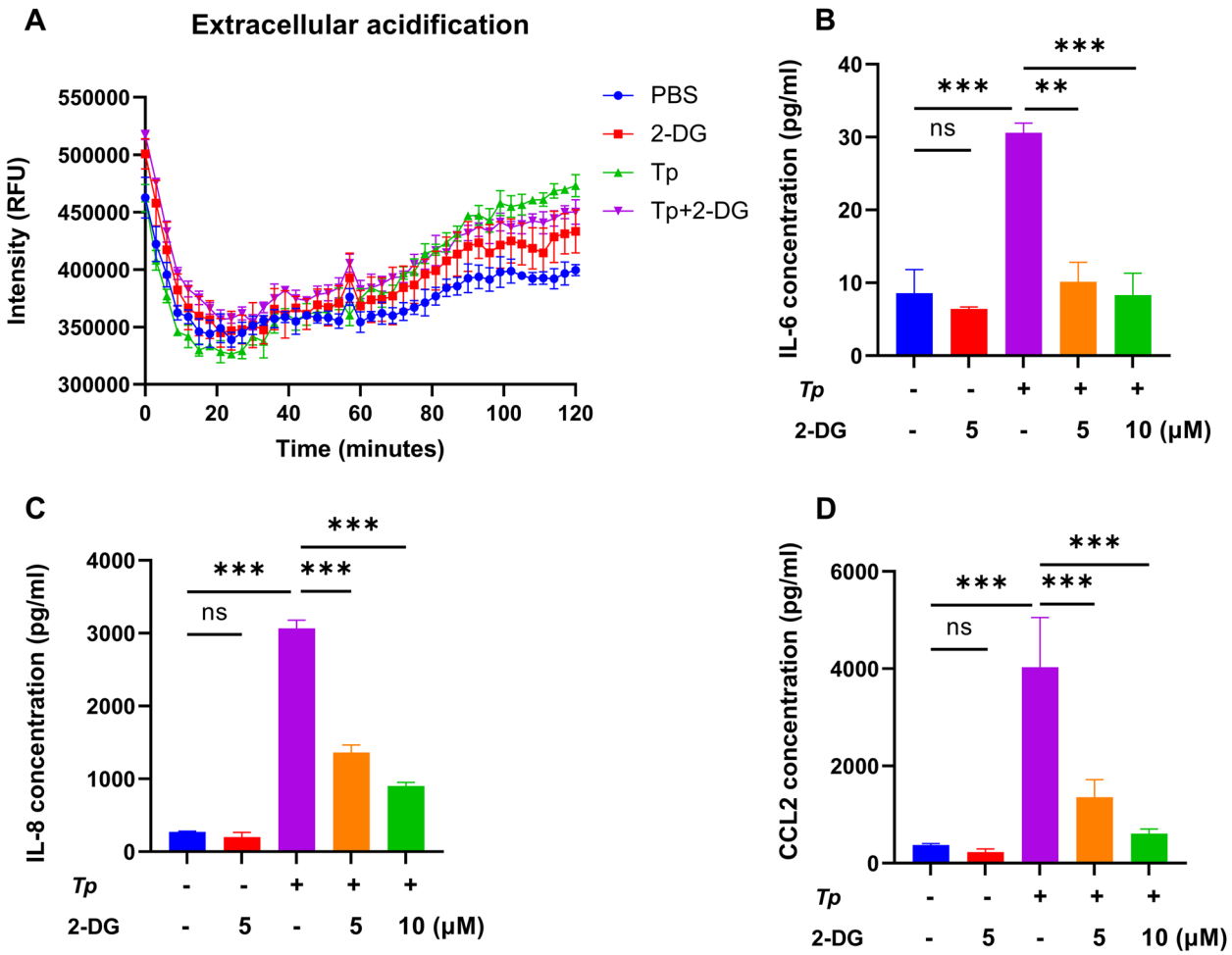

Fig. 3 *Tp*-induced cytokine production is associated with an increase in glycolysis. **A** The glycolysis inhibitor 2-DG (5μM) was pretreated for 2 h and treated with *Tp* (MOI of 2:1) for 24 h. The time course of real-time changes in extracellular acidification after the glycolysis assay. **B**-**D** The glycolysis inhibitor 2-DG (5μM or 10μM) was pretreated for 2 h and treated with *Tp* (MOI of 2:1) for 24 h. **B** IL-6, (**C**) IL-8, and (**D**) CCL2 concentrations in the cell culture supernatants of THP-1 cells were quantified by ELISA. The data are presented as the mean ± SD of three independent experiments. Statistical significance tested by one-way ANOVA test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: no significant

monocytes to *Tp* infection to enhance their pro-inflammatory capacity.

HIF-1α is involved in the regulation of GLUT1-mediated glycolysis and IL-6, IL-8, and CCL2 secretion

HIF-1α is a transcription factor that is a strong inducer of glycolysis and inflammatory responses, playing an essential role in orchestrating the host's immune response to pathogens [33, 34]. However, the role of HIF-1α in the *Tp* infection-induced glycolysis and inflammatory response remains unknown. We found that *Tp* significantly increased HIF-1α mRNA and total protein expression in a concentration-dependent and time-dependent manner (Fig. 5A, B, and Supplementary Figure S4A, B, D, E), and induced its nuclear translocation (Fig. 5C, D, and Supplementary Figure S4C, F). To investigate the role of HIF-1α in *Tp*-induced monocyte glycolysis, we introduced specific si-HIF-1α into THP-1 cells to suppress HIF-1α expression (Fig. 5E). First, HIF-1α inhibition suppressed expressions of glycolytic-related enzyme genes *SLC2 A1, LDHA, HK1,* and *HK2* (Fig. 5F-I), and reduced the protein levels of GLUT1 and LDHA (Supplementary Figure S5A, B). Additionally, we demonstrated that HIF-1α inhibition suppressed the glucose uptake and extracellular acidification induced by *Tp* (Fig. 5J-K). Together, these data suggest that HIF-1α inhibition reduces *Tp*-induced monocyte glycolysis. In addition, our findings indicate a notable reduction in IL-6, IL-8, and CCL2 production following HIF-1α inhibition (Fig. 5L-N).

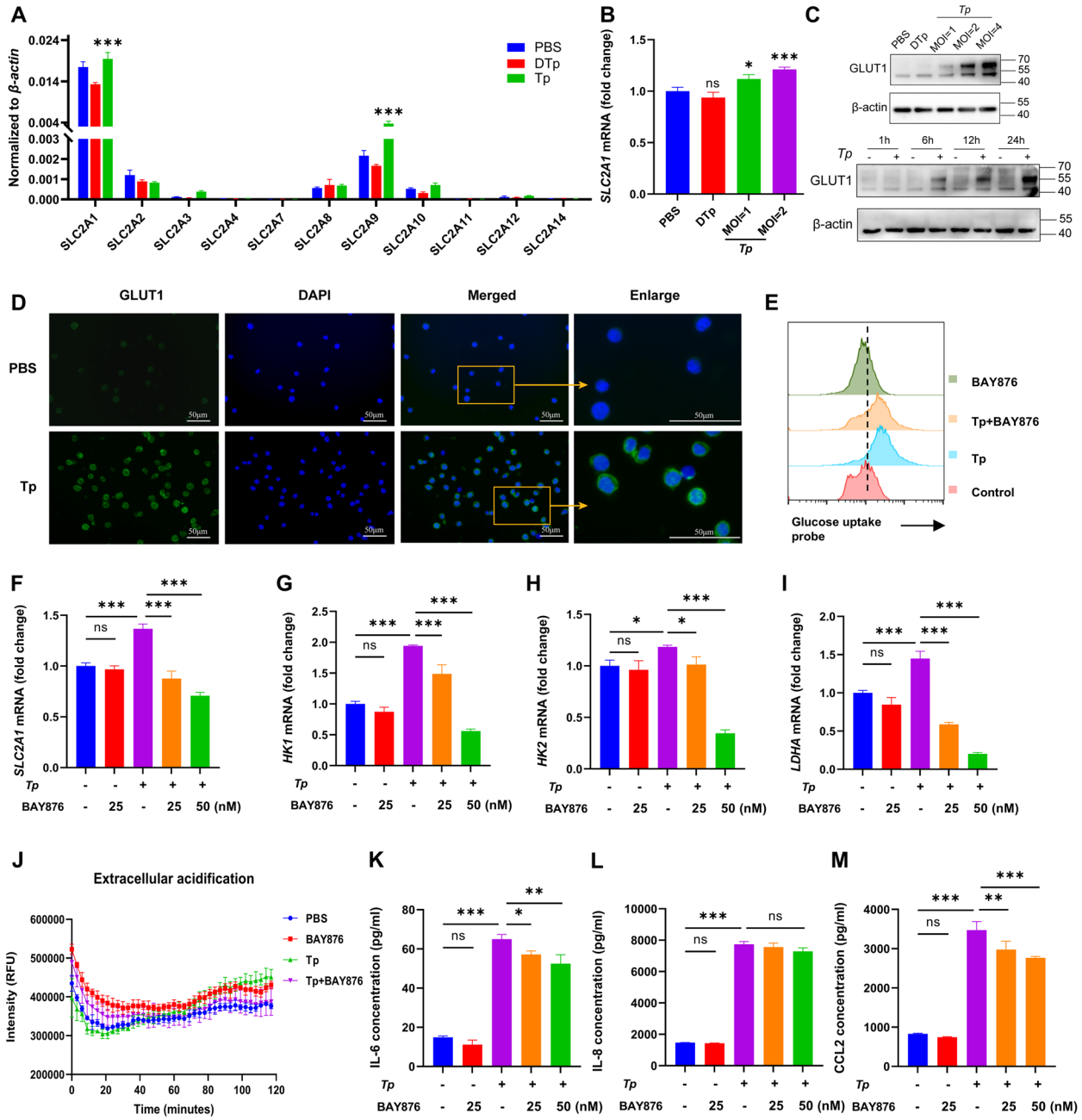

Fig. 4 GLUT1 drives *Tp*-induced cytokine production and increased glycolysis. **A** THP-1 cells were treated with PBS, DTp, and *Tp* (MOI of 2:1) for 24 h. Gene expression of different glucose transporters was measured by qRT-PCR, normalized to β-actin. **B** The mRNA levels of SLC2 A1 after THP-1 cells were treated with PBS, DTp, and *Tp* (MOI of 1:1, 2:1) for 24 h. **C** The expression of GLUT1 protein after THP-1 cells were treated with different concentrations of *Tp* (MOI of 1:1, 2:1, 4:1) for 24 h was measured by Western blot. Expression of GLUT1 protein after THP-1 cells were treated with *Tp* (MOI of 2:1) for different times was measured by Western blot. **D** GLUT1 membrane localization in THP-1 cells treated with PBS or *Tp* was assessed by immunofluorescence microscopy. Yellow arrows indicate the enlargement of some cells. The imaging is representative of three experiments. **F**-**I** THP-1 cells were pretreated with the GLUT1-specific inhibitor BAY876 (25, 50 nM) for 2 h and then cocultured with *Tp* (MOI of 2:1) for 24 h. The mRNA levels of (**F**) SLC2 A1, (**G**) HK1, (**H**) HK2, and (**I**) LDHA were detected by qRT-PCR. **E** Glucose uptake by THP-1 cells was measured by flow cytometry. **J** The time course of real-time changes in extracellular acidification after the glycolysis assay. **K** IL-6, (**L**) IL-8, and (**M**) CCL2 concentrations in the cell culture supernatants of THP-1 cells were quantified by ELISA. The data are shown as the mean ± SD. Statistical significance tested by one-way ANOVA test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: no significant, *n* = 3

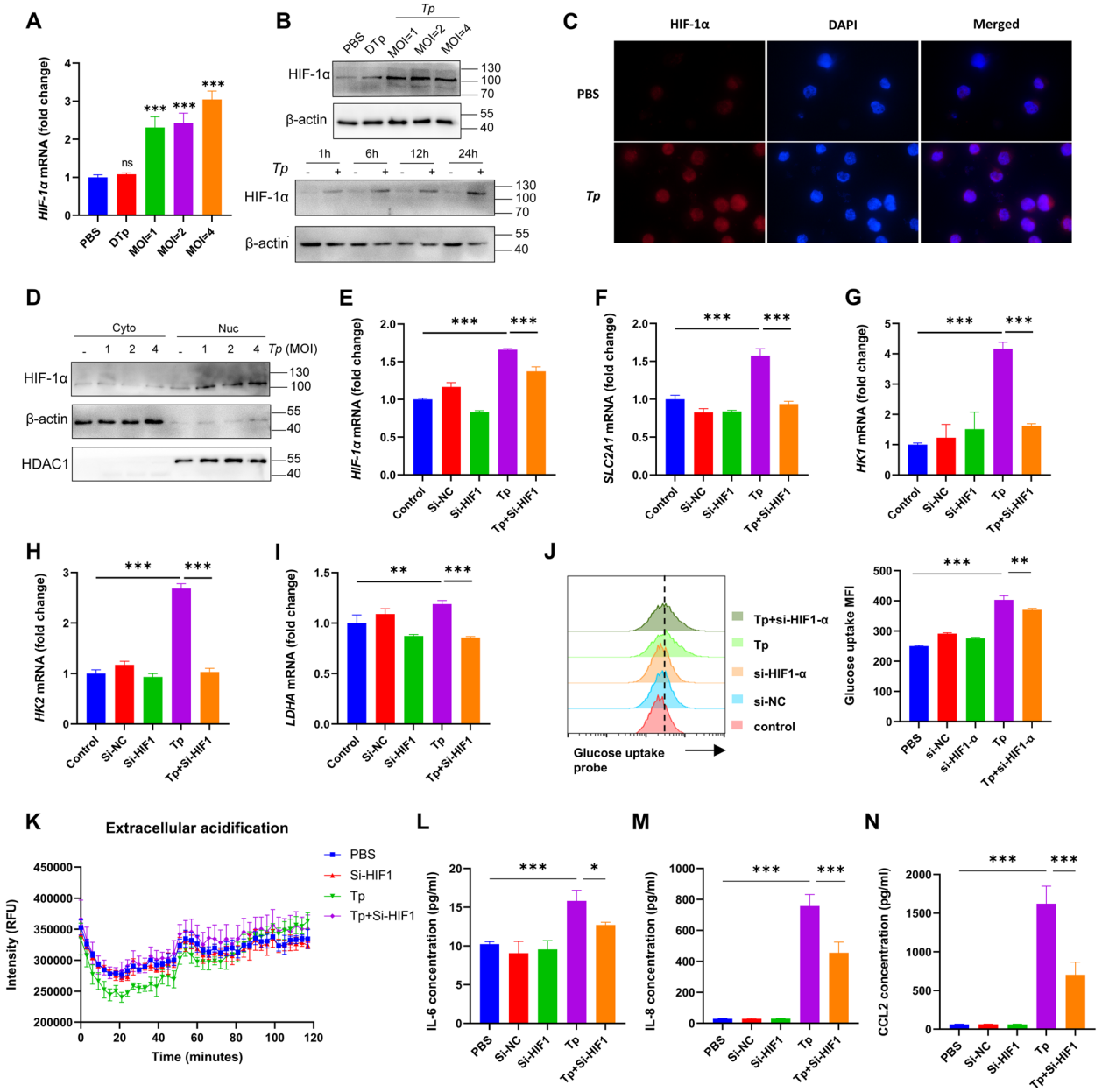

Fig. 5 HIF-1α is involved in the regulation of GLUT1-mediated glycolysis and cytokine secretion after *Tp* treatment of monocytes. **A** The mRNA levels of HIF-1α after THP-1 cells were treated with different concentrations of *Tp* (MOI of 1:1, 2:1, and 4:1) for 24 h. **B** The expression of HIF-1α protein after THP-1 cells were treated with different concentrations of *Tp* (MOI of 1:1, 2:1, and 4:1) for 24 h was measured by Western blot. Expression of the HIF-1α protein after THP-1 cells were treated with *Tp* (MOI of 2:1) for different times was measured by Western blot. **C** Representative images from immunofluorescence analysis of HIF-1α protein accumulation and nuclear localization in THP-1 cells treated by PBS or *Tp*. Nuclei were stained with DAPI. Immunofluorescence was observed and photographed by a fluorescence microscope. **D** Nuclear and cytoplasmic extraction reagents were used to extract the nuclear and cytoplasmic proteins from the cells. Western blot analysis was performed after THP-1 cells were treated with different concentrations of *Tp* (MOI of 1:1, 2:1, and 4:1) for 24 h. β-actin was used as a loading control of cytoplasmic protein (abbreviated as 'Cyto' in the figure), and HDAC1 was used as a loading control of nuclear protein (abbreviated as 'Nuc' in the figure). **E**-**N** THP-1 cells were transfected with Si-NC or Si-HIF-1α for 24 h, and then the transfected THP-1 cells were treated with *Tp* (MOI of 2:1) for 24 h. The mRNA levels of (**E**) HIF-1α, (**F**) SLC2 A1, (**G**) HK1, (**H**) HK2, and (**I**) LDHA were detected by qRT-PCR. **J** The glucose uptake capacity of THP-1 cells was analyzed by flow cytometry. **K** The time course of real-time changes in extracellular acidification after the glycolysis assay. **L** IL-6, (**M**) IL-8, and (**N**) CCL2 concentrations in the cell culture supernatants of THP-1 cells were quantified by ELISA. The data are presented as the mean ± SD of three independent experiments. Statistical significance tested by one-way ANOVA test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: no significant

Taken together, *Tp* increases HIF-1α expression and its nuclear translocation, driving glycolysis and the production of inflammatory cytokines. these data show that *Tp* markedly increases the expression of HIF-1α and promoted its nuclear translocation, which is essential for glycolysis and inflammatory cytokine production.

Analysis of HIF-1α expression and glycolytic activity in syphilis patients

To determine whether HIF-1α-mediated glycolysis is also involved in syphilis patients, we analyzed PBMC transcription levels in syphilis patients with inflammatory pathological manifestations ($n = 8$) and healthy controls ($n = 14$) (Fig. 6A). These data are consistent with in vitro findings that HIF-1α expression is significantly upregulated in syphilis patients (Fig. 6B). In syphilis patients with inflammation, glycolytic pathway genes such as *HK1*, *HK2*, *SLC2 A1*, and *LDHA* were significantly upregulated, while *LDHB* showed no significant changes (Fig. 6C-G). To further validate the in vivo findings, we performed in vitro CD14 + monocyte stimulation experiments. We isolated $CD14^+$ monocytes from normal controls and stimulated $CD14^+$ monocytes with different concentrations of *Tp* for 24 h. Compared with the PBS control group, the expression of HIF-1α and LDHA was significantly up-regulated after *Tp* stimulation of $CD14^+$ monocytes (Fig. 6H, I). And promote glucose uptake in cells (Fig. 6J). The upregulation of HIF-1α in monocytes may represent an adaptive response to *Tp* infection, enhancing glycolysis to meet the increased energy demands required to sustain a robust pro-inflammatory response (Fig. 7).

Discussion

Monocytes are crucial in initiating and sustaining an effective inflammatory response against pathogens through the secretion of diverse inflammatory cytokines. However, excessive inflammation can contribute to tissue damage and chronic disease. In syphilis pathogenesis, monocyte infiltration and subsequent inflammatory cytokine secretion represent prominent pathological features [35, 36]. However, the role of *Tp* in inducing monocyte cytokine secretion and the underlying mechanisms are poorly understood. In this study, we investigated 27 cytokines associated with *Tp* infection, providing a comprehensive perspective that can elucidate the complex mechanisms by which *Tp* influences monocyte inflammatory responses, immune function, and cell growth. Our findings demonstrate that *Tp* enhances the pro-inflammatory response of monocytes through glycolysis. Further results indicate that *Tp* induces IL-6, IL-8, and CCL2 secretion by enhancing glycolysis. Mechanistically, *Tp* promotes HIF-1α nuclear translocation, which upregulates GLUT1 expression and increases glucose uptake, thereby regulating glycolysis and inducing the secretion of IL-6, IL-8, and CCL2. In conclusion, our findings not only expand the understanding of *Tp*'s role in monocyte immune responses but also shed light on the mechanism of glycolysis in *Tp*-induced inflammation. These insights offer valuable guidance for advancing the diagnosis and treatment of syphilis.

Cytokines are crucial components of the immune system, playing significant roles in host antimicrobial defense and the pathophysiology of syphilis. IL-6 plays a crucial role in immune responses and acute-phase reactions, with elevated levels linked to persistent syphilis infection and immune regulation [37]. Meanwhile, IL-8 and CCL2 are key chemokines that recruit neutrophils and monocytes, respectively, contributing to local inflammation and immune defense against bacterial infections, including syphilis [38]. Collectively, these cytokines orchestrate a pro-inflammatory milieu that shapes the immune response against *Tp*. Their dysregulation may contribute to the persistent inflammation and tissue pathology observed in syphilis [39]. Previous studies have demonstrated that *Tp* and its associated proteins can stimulate the secretion of CCL2, IL-6, and IL-8 by immune cells, including mononuclear macrophages [39–42]. However, the mechanism by which live *Tp* triggers the inflammatory response in monocytes remains unclear. In this study, we found that *Tp* induces the secretion of IL-6, IL-8, and CCL2. By focusing on these cytokine secretion levels, we aimed to gain a deeper understanding of the immune response mechanisms of monocytes in syphilis.

There is growing evidence that alterations in cellular metabolic processes can promote functional changes in immune cells, such as cell proliferation, cell differentiation, and inflammatory responses [43]. During peak inflammation, immune cells preferred glycolysis as an energy source to meet the increased energy requirements of activated immune cells [11]. After infection with SARS-CoV-2, monocytes regulate their metabolism and become highly glycolytic, which aggravates inflammatory responses to COVID-19 [25, 34]. Metabolic reprogramming plays an important pathological role in various infectious diseases; however, its role in *Tp* infection remains unclear. In this study, we observed that monocyte metabolism shifts towards glycolysis following *Tp* stimulation. Our targeted metabolomics assay showed that *Tp* increased monocyte glycolytic pathway-related metabolites such as pyruvate and lactic acid. In contrast, DTp had no significant effect on glycolytic metabolic pathways in monocytes. This difference may be attributed to DTp primarily functions as an antigen that activates the immune system and

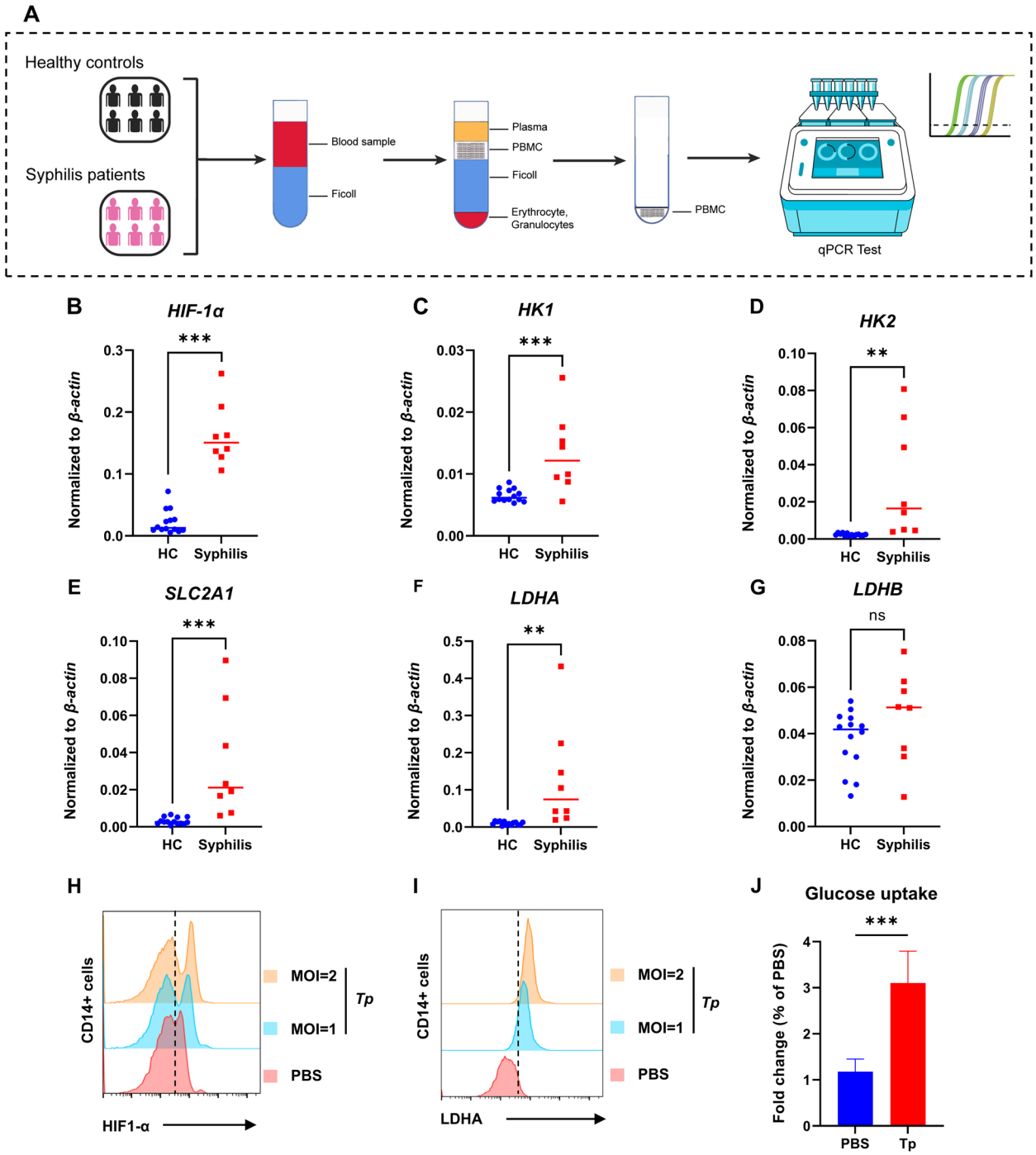

Fig. 6 Expression analysis of PBMCs from syphilis disease patients. **A** Peripheral blood samples were collected from healthy controls and syphilis patients with obvious inflammatory pathological manifestations. Mononuclear cells were isolated using a lymphocyte separation solution, and total RNA in the cells was extracted for qRT-PCR detection. Gene expression in (**B**) HIF-1α, (**C**) HK1, (**D**) HK2, (**E**) SLC2 A1, (**F**) LDHA, and (**G**) LDHB in healthy controls (HC; $n = 14$) versus syphilis patients ($n = 8$). *Tp* promoted HIF-1α expression and increased glycolysis in CD14 + monocytes. Normal peripheral blood CD14 + monocytes were separated. CD14 + monocytes were divided into the PBS group and the live *Tp* (MOI of 1:1, 2:1) group and treated for 24 h, respectively. Flow cytometry was used to detect (**H**) HIF-1α and (**I**) LDHA protein expression. **J** A glucose uptake kit was used to detect the glucose uptake capacity of cells after CD14 + monocytes were treated with *Tp* (MOI of 2:1) for 24 h. The data are presented as the mean ± SD. Statistical significance tested by unpaired, two-tailed Student's t test. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns: no significant

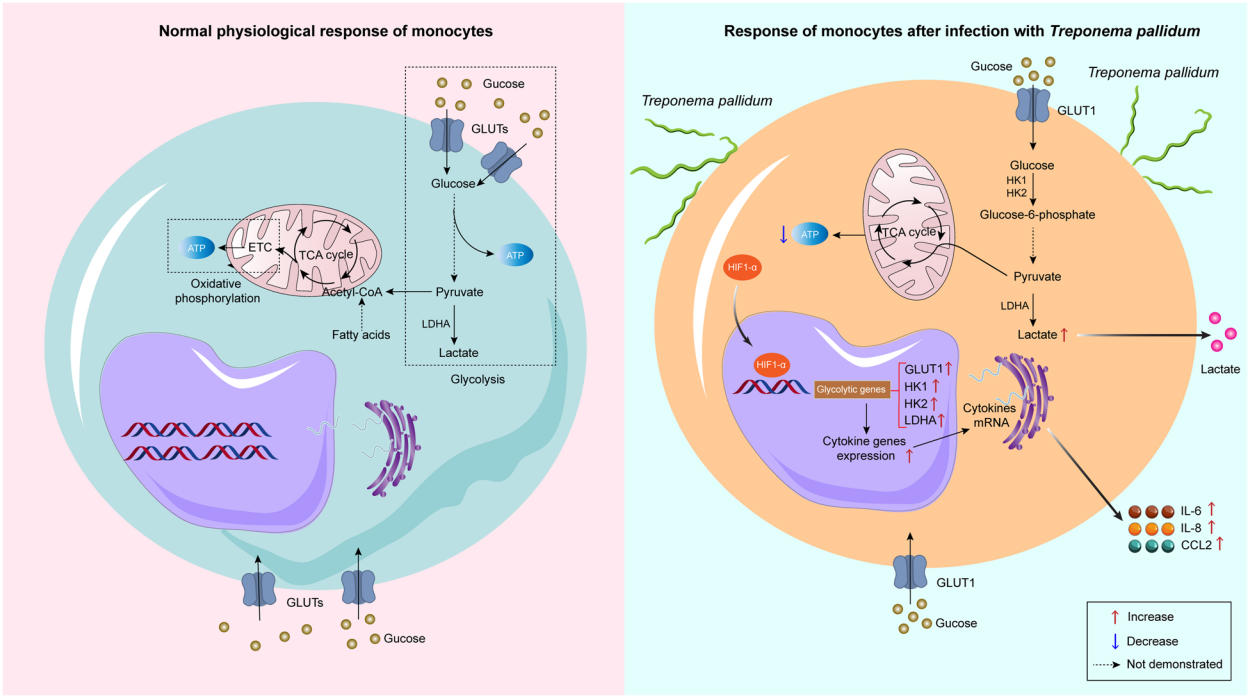

Fig. 7 HIF1-α regulates GLUT1-mediated glycolysis, leading to increased monocyte cytokine production following *Treponema pallidum* infection. Under normal physiological conditions, monocytes uptake glucose through various glucose transporters and metabolize it via glycolysis and mitochondrial respiration to maintain cellular homeostasis. Following *Tp* infection, there is an induction of HIF-1α expression in the nucleus of monocytes, leading to the upregulation of GLUT1, LDHA, and other glycolytic enzyme genes, thereby enhancing monocyte glycolysis and subsequently promoting monocyte cytokine secretion

induces nonspecific immune responses, without triggering significant metabolic changes in monocytes. Furthermore, our results suggest that the *Tp*-induced enhancement of monocyte glycolysis may be related to the upregulated expression of key glycolytic enzymes, including HK1, HK2, and LDHA. Previous research indicates that metabolites and enzymes involved in glycolysis, including HK-1 and HK-2, can regulate IL-1β production in macrophages [44]. These metabolic changes indicate that *Tp* upregulates glycolytic enzyme expression, leading to increased levels of pyruvate and lactic acid, which further drives the shift of monocyte metabolism towards glycolysis. To further validate the robustness of our findings, we performed pathway enrichment analysis using the Metabolite Set Enrichment Analysis (MSEA) method with the Pathway-Associated Metabolite Sets (SMPDB) library. The results highlighted glycolysis as the most significantly enriched pathway, indicating that *Tp* infection induces glycolytic reprogramming in monocytes. This consistent evidence suggests that enhanced glycolytic flux supports inflammatory cytokine production. Specifically, we observed increased GLUT1 and LDHA expression, which is known to promote the secretion of pro-inflammatory cytokines such as IL-6, IL-8, and CCL2 [31]. These findings align with previous reports linking glycolytic upregulation to cytokine release in infectious and inflammatory conditions [15].

Glucose is a critical nutrient component for inflammatory macrophages [45]. Pro-inflammatory immune cells are powered by glycolysis, which relies on high levels of glucose uptake. In this study, these data indicated a significant increase in glucose uptake by monocytes following *Tp* stimulation. Most cells take up glucose by promoting membrane diffusion, a process mediated by the glucose transporters (GLUTs) family of membrane transporters. Here, we found that glucose uptake in monocytes is primarily associated with GLUT1 expression on the cell membrane. We identified for the first time the key role of GLUT1 in *Tp*-induced monocyte cytokine responses. We demonstrated that transcriptional and protein expression of GLUT1 is upregulated in monocytes after *Tp* stimulation. In addition, our data indicated that *Tp*-induced glucose uptake favored glycolytic activity, which was consistent with the metabolic reprogramming of cells to aerobic glycolysis. Previous studies have found that monosodium urate and calcium pyrophosphate crystals increase GLUT1 plasma membrane expression and glucose uptake, leading to the transformation of macrophage metabolism to aerobic glycolysis [46]. More

importantly, after treatment with BAY876, an inhibitor of GLUT1, *Tp*-induced monocyte secretion of IL-6 and CCL2 was significantly reduced. The results were similar to those observed in microglia, where GLUT1 inhibitor treatment reduced glucose uptake and extracellular acidification rates in naive microglia and inhibited the upregulation of inflammatory cytokines TNF-α, IL-1β, IL-6, and CCL2 in LPS- and IFNγ-induced microglia [31]. The upregulation of GLUT1 provides additional glucose uptake, ensuring sufficient energy supply for the pro-inflammatory response. Collectively, GLUT1-mediated glycolysis plays a key role in *Tp*-induced cytokine secretion in monocytes, but its upstream regulatory mechanism remains unclear.

HIF-1α is a key metabolic and inflammatory regulator that plays an important role in various infectious diseases [18]. In this study, we were surprised to find that *Tp* can not only significantly up-regulate the transcription of HIF-1α in monocytes but also promote the expression of HIF-1α in the nucleus. The nuclear translocation of HIF-1α is a crucial step for activating downstream glycolysis-related genes and driving the pro-inflammatory response. Previous studies have suggested that HIF-1α may be regulated by two mechanisms. Under hypoxic conditions, the activity of prolyl hydroxylase (PHDs) is inhibited, resulting in an increase in the HIF-1α protein level [47]. Under normal oxygen conditions, bacterial or viral infection can up-regulate the mRNA and protein levels of HIF-1α and enhance the transcription of HIF-1α target genes [15, 25]. In line with this, HIF-1α regulates the transition of monocyte metabolism from oxidative phosphorylation to aerobic glycolysis after Borrelia infection [15]. This is because HIF-1α, as a transcription factor, is a metabolic regulatory switch. It was found that the GLUT1 promoter contains a binding site for HIF-1α, and HIF-1α can induce the expression of GLUT1 [48]. Similar to other pathogens like Borrelia, HIF-1α acts as a major regulator of glycolysis and pro-inflammatory responses in *Tp* infection. Our results indicate that HIF-1α drives *Tp*-induced pro-inflammatory cytokine secretion by regulating GLUT1 and glycolysis [49]. In addition, several drugs reduce inflammation and inhibit disease progression by inhibiting HIF-1α expression, suggesting that HIF-1α is an important new therapeutic target for controlling inflammation [50, 51]. Therefore, further exploration of the regulatory mechanisms of HIF-1α may provide new insights and targets for the treatment of syphilis and other chronic inflammatory diseases.

Despite providing valuable insights into the role of HIF-1α-mediated glycolysis in *Tp*-induced immune responses, this study has several limitations. First, while we employed pharmacological inhibitors (BAY876) and siRNA-mediated knockdown to investigate the role of GLUT1 and HIF-1α, a complete genetic knockout model would provide more definitive causal evidence. However, due to the highly unstable nature of HIF-1α under normoxic conditions, establishing a stable knockout system is technically challenging. Future studies may explore alternative genetic approaches, such as CRISPR-based knockout models, to further validate these findings. Second, while our study focused on IL-6, IL-8, and CCL2 as key cytokines in *Tp*-induced inflammation, we acknowledge that other inflammatory mediators, such as TNF-α and IL-1β, also play important roles in syphilis pathogenesis. Expanding cytokine profiling would provide a broader perspective on *Tp*-induced immune responses. Additionally, as this study relied primarily on in vitro models (THP-1 cells and primary human monocytes), it may not fully replicate the complexity of immune responses in vivo. Syphilis is a systemic infection, involving interactions between multiple immune cell types and tissues, which cannot be completely modeled in cell culture. Although mouse and rabbit models exist, they each have inherent challenges—mouse models do not fully mimic human syphilis, and rabbit models lack essential immunological tools. Furthermore, due to limited clinical sample availability and donor variability, we were unable to conduct extensive parallel experiments in primary monocytes. However, we recognize the importance of validation in a broader patient population and plan to continue collecting clinical samples. Larger-scale clinical studies will be necessary to confirm the observed upregulation of HIF-1α and glycolytic genes, assess their pathophysiological significance, and explore their potential as biomarkers for syphilis diagnosis and disease progression. Lastly, the exact mechanism by which *Tp* interacts with monocytes remains to be further explored. Specifically, whether *Tp* is internalized by monocytes through phagocytosis or activates immune responses via pattern recognition receptors (PRRs) remains unclear.

These limitations imply that our understanding of how *Tp* induces glycolysis and cytokine responses in monocytes remains incomplete, especially considering the potential involvement of multiple signaling pathways. In conclusion, we used a luminex detection approach to describe the cytokine responses of monocytes induced by *Tp*. Our present study results demonstrate that HIF-1α, involved in the regulation of GLUT1-mediated glycolysis in monocyte cells, is crucial for the induction of cytokines by *Tp*. In this study, we present new insights into the effect of glycolysis on host monocyte inflammation following *Tp* infection, providing novel evidence for understanding the pathogenesis of syphilis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-025-02211-1.

Supplementary Material 1

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Authors' contributions
Conceptualization: SX, ZPL, FJZ Methodology: SX, ZPL, FJZ Investigation: SX, SBH, JCY, Visualization: SX, ZPL, Supervision: FJZ, XHZ, Writing—original draft: SX, Writing—review & editing: SX, ZPL, FJZ, JCY, XD, HY, TL All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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