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Treponema pallidum enhances human monocyte migration and invasion by dysregulating the MMP/TIMP balance

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ABSTRACT

Although the infiltration of monocytes into local lesions is an obvious pathological manifestation in the pathogenesis of syphilis, little is known about the role of metalloproteinase (MMP)/tissue inhibitor of metalloproteinases (TIMP) imbalance in the migration/invasion of THP-1 cells induced by *Treponema pallidum (T. pallidum)*. The influence of *T. pallidum* on the invasion and migration of THP-1 cells was evaluated. Changes in the MMP/TIMP balance and the mechanisms underlying the involvement of the MAPK and NF-κB signaling pathways in this process were explored. *T. pallidum* induced the migration/invasion of THP-1 cells and the mRNA and protein expression of MMP-1, MMP-9 and TIMP-1. The mRNA expression of *TIMP-2* was reduced, and the protein expression of TIMP-2 was not changed. The MMP-1/TIMP-1, MMP-1/TIMP-2, MMP-9/TIMP-1 and MMP-9/TIMP-2 ratios were increased. Inhibition of JNK, MEK/ERK, p38 MAPK and NF-κB significantly decreased the MMP/TIMP ratio and ultimately suppressed the migration/invasion of THP-1 cells. These findings revealed that MMP/TIMP imbalances induced by *T. pallidum* enhanced THP-1 cell migration and invasion via MAPK and NF-κB signaling pathway activation, which revealed a novel step in syphilis pathophysiology.

1. Introduction

Syphilis is a sexually transmitted infection caused by the spirochete *Treponema pallidum* (*T. pallidum*) [1]. With a reported annual incidence rate of 16.3% [2], syphilis remains a global public health issue. Although *T. pallidum* has been recognized by the general public for hundreds of years, the study of *T. pallidum* and syphilis pathogenesis is still hindered by the inability to culture *T. pallidum* long term on artificial media [3]. Skin lesions are a major feature among the clinical manifestations caused by *T. pallidum*. The classic histological presentation of primary syphilis consists of proliferating vessels surrounded by spirochetes, macrophages, lymphocytes and plasma cells [4]. In secondary syphilis, mucocutaneous lesions exhibit an infiltrate containing monocytes recruited from the peripheral blood [5]. Additionally, monocytic infiltration with tissue destruction is a clinical manifestation of gummas in tertiary syphilis [6]. Therefore, the migration and invasion of monocytes could play a pivotal role in syphilis pathogenesis, as in other disease states (e.g., cancer, cardiovascular disease and inflammation) [7,8].

Similar to most other biological processes, the migration and invasion of immune cells to sites of inflammation is a complex process involving increased vascular permeability, degradation of the extracellular matrix (ECM) and modulation of cytokine and chemokine activity [9]. The activation of matrix metalloproteinases (MMPs) requires the involvement of inflammatory cells. MMPs are members of a family of zinc-dependent endopeptidases that can degrade ECM macromolecules [10]. The activity of MMPs is specifically inhibited by tissue inhibitors of metalloproteinases (TIMPs) [11]. Monocytes abundantly express MMPs and TIMPs, and the balance between MMP and TIMP expression is important in recruiting immune cells, especially monocytes, to sites of inflammation [12–14].

To our knowledge, little is known about whether an imbalance of MMP/TIMP expression affects the migration or invasion of monocytic cells in syphilis, and the related mechanism is unclear. Previous studies have shown that *T. pallidum* induces dermal fibroblasts to produce MMP-1 and that *T. pallidum* flagellins stimulate MMP-9 and MMP-13 expression in epidermal keratinocytes via the MAPK/NF-κB signaling pathways [15,16]. Therefore, we propose that *T. pallidum* disturbs the MMP/TIMP balance by inducing MMP expression or suppressing TIMP expression in THP-1 cells and that the MMP/TIMP imbalance induces syphilis-associated monocyte migration and invasion. In this study, we evaluated the influence of *T. pallidum* on monocyte migration and invasion. Furthermore, we investigated the altered MMP/TIMP balance and the signaling pathways involved in this process.

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2. Methods and materials

2.1. Propagation of T. pallidum

The *T. pallidum* Nichols strain was kindly provided by Lorenzo Giacani, PhD (University of Washington, Seattle, USA), and was propagated intratesticularly in adult male New Zealand white rabbits, as previously described [17].

2.2. Cell culture

THP-1 cells (ATCC, Manassas, USA) were incubated in RPMI-1640 medium (HyClone, Logan, USA) supplemented with 10% fetal bovine serum (Biological Industries Ltd., Kibbutz Beit HaEmek, Israel), penicillin and streptomycin at 37 °C and 5% CO_2. For stimulation experiments, cells in 24-well plates were transferred into serum-free medium for a starvation period of 1 h. THP-1 cells were stimulated by *T. pallidum* at different multiplicities of infection (MOIs) (*T. pallidum*:cell ratios of 25:1, 50:1, 75:1, and 100:1) for 24 h. PBS-treated monocytes were used as the control cells. Culture media were collected for subsequent experiments.

2.3. In vitro cell migration and invasion assays

Cell migration assays were performed using 8 μm pore size 24-well Transwell plates (Corning Incorporation, NY, USA). THP-1 cells were pretreated with different MOIs (25, 50, 75 or 100) of *T. pallidum* at 37 °C in 5% CO_2 for 24 h in 24-well culture plates. Then, 400 μL of cell culture supernatant was collected and added to the lower chambers of the Transwell plates. A total of 2×10^5 THP-1 cells in 200 μL of serum-free RPMI medium were loaded into the upper chambers of Transwell plates. After incubation for 12 h at 37 °C, flow cytometry was used to count the number of cells in the lower chamber under a high flow rate.

Invasion assays were performed in an invasion chamber (Corning Incorporation, NY, USA). THP-1 cells were pretreated with different MOIs (25, 50, 75 or 100) of *T. pallidum* at 37 °C in 5% CO_2 for 24 h in 24-well culture plates. Then, 200 μL of different cell culture supernatants was collected. THP-1 cells (2×10^5) were suspended in different culture supernatants in the upper chamber. Then, 500 μL of RPMI-1640 with 10% FBS was added to the lower chamber. After incubation for 24 h at 37 °C, the cell numbers in the lower chamber were counted by flow cytometry.

2.4. MMP and TIMP expression profile analysis in THP-1 cells stimulated by T. pallidum

The expression profile of MMP-related proteins was analyzed using a RayBio Matrix Metalloproteinase Antibody Array (RayBiotech Inc., Norcross, GA, USA) following the manufacturer's instructions. Briefly, THP-1 cells were incubated with *T. pallidum* at an MOI of 100 for 24 h, and cell culture supernatant was then added to the antibody array membranes. The relative expression levels of proteins were analyzed by comparing the signal intensities quantified by densitometry. The signal intensities for MMP proteins were normalized to the PBS-treated samples.

2.5. Detection of the MMP/TIMP balance in the THP-1 cells stimulated by T. pallidum

Cells incubated with *T. pallidum* were harvested for RNA isolation, and conditioned medium was collected for protein level measurement. The mRNA expression levels of *MMP-1, MMP-9, TIMP-1* and *TIMP-2* were assessed by RT-PCR as described previously [18]. The primers used in RT-PCR analyses are listed in Table 1. The levels of MMP-1, MMP-9, TIMP-1 and TIMP-2 proteins in the culture supernatants were measured using ELISA kits (MMP-1, MMP-9 and TIMP-1, Neobioscience Technology Co., Ltd. Guangzhou, China; TIMP-2, Ray-Biotech, GA, USA) according to the manufacturer's protocols, and the MMP-1/TIMP-1, MMP-1/TIMP-2, MMP-9/TIMP-1 or MMP-9/TIMP-2 ratios were calculated.

2.6. Cell viability assay

Cell viability was evaluated using a CCK-8 kit (Dojindo, Dojindo Molecular Technologies, Inc., Kumamoto, Japan). THP-1 cells were seeded into a 96-plate at 10^3 cells per well. After treatment with 10 μmol/L U0126 (a MEK inhibitor), 10 μmol/L PD98059 (an ERK inhibitor), 20 μmol/L SP600125 (a JNK inhibitor), 10 μmol/L SB203580 (a p38 inhibitor), 10 μmol/L BAY 11–7082 (an NF-κB inhibitor) or 100 μmol/L PDTC (an NF-κB inhibitor), 10 μL of the tetrazolium substrate was added to each well of the plate. PBS-treated monocytes were used as the control group. The plates were incubated at 37 °C for 1 h, and then the optical density (OD) was measured at 450 nm using a microplate reader. The cell inhibitory rate was calculated according to the following equation: cell inhibitory rate = [1 − (OD experiment − OD blank) / (OD control − OD blank)] × 100%.

2.7. MAPK signaling pathway activation assay in THP-1 cells stimulated by T. pallidum

THP-1 cells were incubated with *T. pallidum* at an MOI of 100 for various time periods. Cell lysates were collected, and the protein levels of phosphorylated and total ERK1/2, JNK, c-Jun (all from Abcam, Cambridge, USA), MEK1/2, and p38 (both from Cell Signaling Technology, Inc., MA, USA) were measured by Western blot analysis, as described previously [18]. To further confirm that *T. pallidum* induced the activation of MAPK signaling pathways, we pretreated THP-1 cells with 10 μmol/L U0126 (a MEK inhibitor), 10 μmol/L PD98059 (a MEK inhibitor), 20 μmol/L SP600125 (a JNK inhibitor) or 10 μmol/L SB203580 (a p38 inhibitor) for 1 h and then incubated the cells with *T. pallidum* (MOI 100) for 24 h. The protein levels of MMP-1, MMP-9, TIMP-1 and TIMP-2 were measured by ELISA.

2.8. NF-κB signaling pathway activation assay in THP-1 cells stimulated by T. pallidum

THP-1 cell lysates were collected for measurement of the protein level of phosphorylated and total IκB (Cell Signaling Technology, Inc., MA, USA) using Western blot analysis, as described above. To further confirm NF-κB signaling pathway activation following incubation with *T. pallidum*, THP-1 cells were pretreated with NF-κB inhibitors (BAY11-7082, 10 μmol/L; ammonium pyrrolidinedithiocarbamate (PDTC), 100 μmol/L) for 1 h, followed by incubation with *T. pallidum* (MOI 100) for 24 h. The MMP-1, MMP-9, TIMP-1 and TIMP-2 protein levels were then measured by ELISA.

Immunofluorescence staining was used to assess NF-κB p65 nuclear translocation in THP-1 cells, as described previously [19]. THP-1 cells were stimulated as described above for 2 h and were then fixed and permeabilized using 4% formaldehyde containing Triton X-100 (0.1%) for 30 min. The slides were incubated with an anti-NF-κB p65 antibody at 4 °C overnight, followed by incubation with a FITC-conjugated anti-rabbit IgG antibody at room temperature. Finally, the slides were treated with 4′, 6-diamidino-2-phenylindole (DAPI) to stain nuclei and were then observed using an LSM 710 inverted microscope (Carl Zeiss, Heidenheim, Germany).

2.9. Statistical analysis

All experiments were repeated at least three times. Data are expressed as the means ± SEMs unless otherwise indicated and were analyzed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Correlations between the number of migrated or invaded cells and the MMP/TIMP

Table 1
Primers for RT-PCR.

Gene	Forward Primer (5′ → 3″)	Reverse Primer (5′ → 3′)	Fragment size (bp)
MMP-1	AAAATTACACGCCAGATTTGCC	GGTGTGACATTACTCCAGAGTTG	82
MMP-9	TGCGCTACCACCTCGAACTT	GATGCCATTCACGTCGTCCT	201
TIMP-1	CTTCTGCAATTCCGACCTCGT	ACGCTGGTATAAGGTGGTCTG	79
TIMP-2	AAGCGGTCAGTGAGAAGGAAG	GGGGCCGTGTAGATAAACTCTAT	136
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG	185

ratio were evaluated by Spearman rank correlation analysis. One-way ANOVA was used to compare values among multiple groups. The statistical significance of the differences between the control and experimental groups in the MMP protein array was determined using a paired *t*-test. A 2-tailed *P*-value of < 0.05 was considered significant.

3. Results

3.1. Effects of T. pallidum on THP-1 cell migration and invasion in vitro

Migration and invasion assays were used to measure changes in cell motility. First, we assessed the effects of *T. pallidum* on THP-1 cell migration. As shown in Fig. 1A, the number of migrated cells was appreciably increased at an MOI of 25 ($P \leq 0.05$) and peaked at an MOI of 100 ($P \leq 0.001$), indicating a concentration-dependent pattern.

In addition to assessing increased migration, we evaluated the effects of *T. pallidum* on THP-1 cell invasion. The number of invaded cells was significantly increased at an MOI of 50 ($P < 0.01$) and continued to increase gradually up to an MOI of 100 ($P < 0.001$), indicating a concentration-dependent pattern (Fig. 1B).

3.2. The expression of MMPs and TIMPs in THP-1 cells stimulated by T. pallidum

To analyze the effects of *T. pallidum* on the expression of MMPs and TIMPs in THP-1 cells, we first conducted an MMP array assay (Fig. 2A). *T. pallidum* significantly stimulated the secretion of MMP-1, MMP-9 and TIMP-1 in THP-1 cells relative to that in control cells ($P < 0.01$), but TIMP-2 expression did not appreciably differ between the groups (Fig. 2B). Moreover, the expression of MMP-2, MMP-3, MMP-8, MMP-10, MMP-13 or TIMP-4 was not detected in this assay.

In addition, we further clarified the effect of *T. pallidum* on the expression of MMP-1, MMP-9, TIMP-1 and TIMP-2 in THP-1 cells at the transcriptional and translational levels. *T. pallidum* markedly increased *MMP-1* and *MMP-9* mRNA expression in a dose-dependent manner (Fig. 2C–D). The mRNA expression levels of *MMP-1* and *MMP-9* were significantly increased at an MOI of 75 ($P \leq 0.01$) and peaked at an MOI of 100 ($P \leq 0.001$). In addition, the *TIMP-1* mRNA level was significantly increased at an MOI of 100 ($P \leq 0.01$) (Fig. 2E). In contrast, the *TIMP-2* mRNA level started to decrease at an MOI of 75 ($P \leq 0.05$), with a marked decrease at an MOI of 100 ($P < 0.01$) (Fig. 2F). The protein expression level of MMP-1 was significantly increased at MOIs > 75 ($P < 0.01$) (Fig. 2G), and the protein expression level of MMP-9 was significantly increased at an MOI of 50 ($P < 0.001$) (Fig. 2H), exhibiting a concentration-dependent response. In addition, the TIMP-1 protein expression level in the culture medium began to increase at an MOI of 75 ($P < 0.01$) (Fig. 2I), but the protein expression level of TIMP-2 did not significantly differ among the groups (Fig. 2J).

3.3. T. pallidum enhanced THP-1 cell migration and invasion by dysregulating the MMP/TIMP balance

To further clarify whether *T. pallidum* can disrupt the balance between MMPs and TIMPs, the MMP-1/TIMP-1, MMP-1/TIMP-2, MMP-9/TIMP-1 and MMP-9/TIMP-2 ratios were calculated. Fig. 3A–D showed that the MMP/TIMP ratios were elevated in a dose-dependent manner. Specifically, the MMP-1/TIMP-1 ratio began to increase at an MOI of 75 ($P < 0.01$) and peaked at an MOI of 100 ($P < 0.001$); however, the MMP-1/TIMP-2, MMP-9/TIMP-1 and MMP-9/TIMP-2 ratios began to noticeably increase at an MOI of 50 ($P < 0.001$) and peaked at an MOI of 100 ($P < 0.001$). With increases in the MMP/TIMP ratio, the number of migrating cells also increased.

To explore the effect of the MMP/TIMP imbalance on THP-1 migration and invasion, the relationship between the MMP/TIMP imbalance and migration or invasion was investigated via correlation analysis (Table 2). The number of migrated cells was positively correlated with the MMP/TIMP ratio. Among the ratios, the MMP-9/TIMP-1 ratio had a correlation coefficient of 0.853 ($P < 0.001$), followed by the MMP-1/TIMP-1 ratio, with a correlation coefficient of 0.597 ($P < 0.001$); the MMP-1/TIMP-2 ratio, with a correlation coefficient of 0.44 ($P = 0.004$); and the MMP-9/TIMP-2 ratio with a correlation coefficient of 0.433 ($P = 0.004$). Similar to the number of migrated cells, the number of invaded cells was also positively correlated with the MMP/TIMP ratio. The MMP-9/TIMP-1 ratio had a correlation coefficient of 0.804 ($P < 0.001$), the MMP-1/TIMP-1 ratio had a correlation coefficient of 0.664 ($P < 0.001$), the MMP-1/TIMP-2 ratio had a correlation coefficient of 0.499 ($P = 0.001$), and the MMP-9/TIMP-2 ratio had a correlation coefficient of 0.492 ($P = 0.001$).

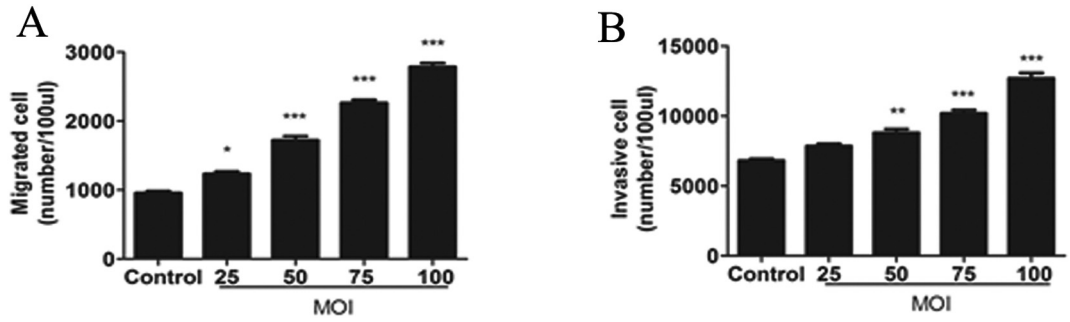

Fig. 1. *T. pallidum* altered the migration and invasion abilities of THP-1 cells
A, Transwell migration assay in THP-1 cells; B, Transwell invasion assay in THP-1 cells. One-way ANOVA was used to compare values among multiple groups. (*, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$).

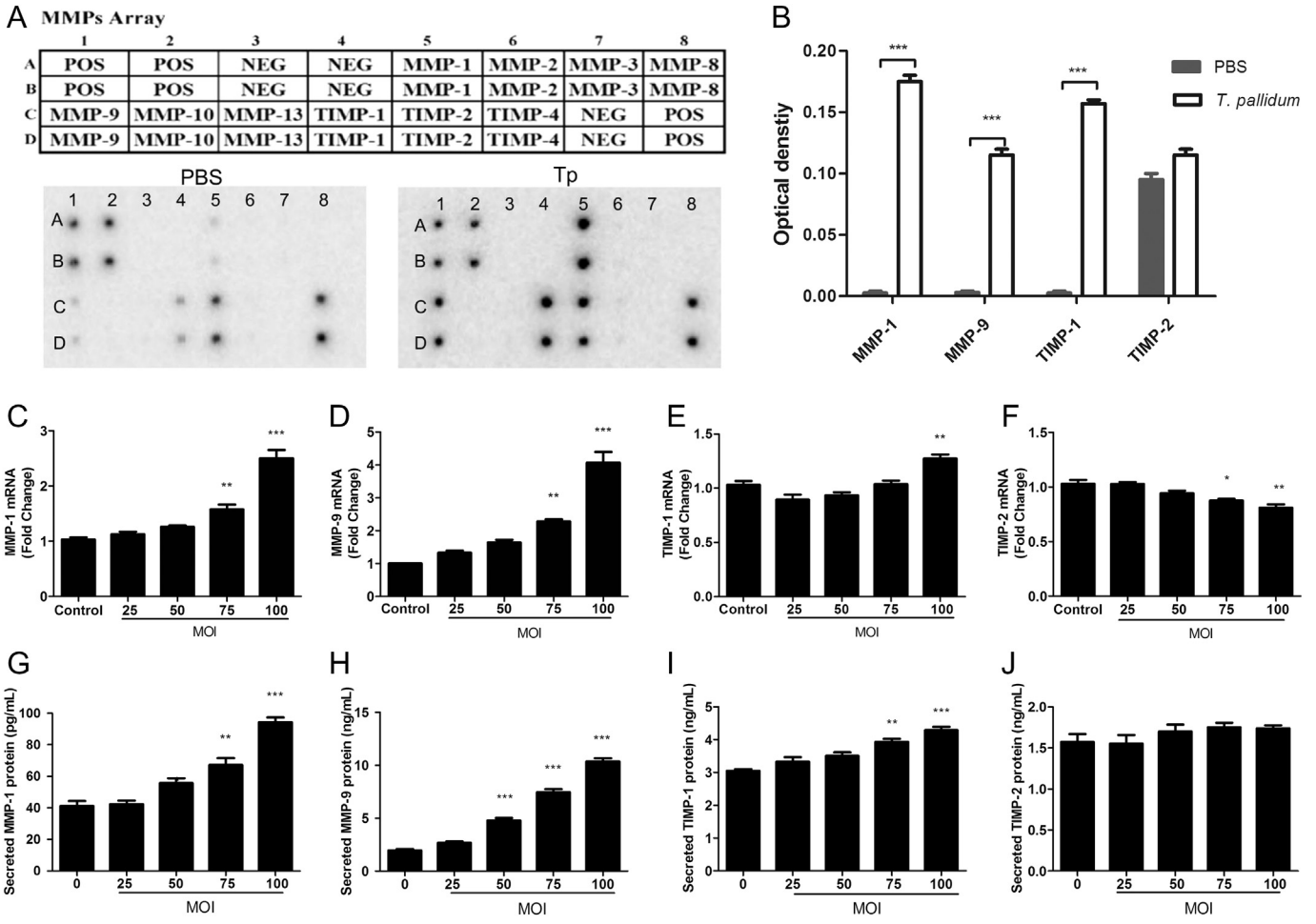

Fig. 2. The expression of MMPs and TIMPs in THP-1 cells stimulated by *T. pallidum*

A and B, The expression profile of MMPs and TIMPs, as determined by the Human MMP Array Kit; C–D, mRNA expression levels of MMP-1 and MMP-9; E–F, mRNA expression levels of TIMP-1 and TIMP-2; G–H, protein expression levels of MMP-1 and MMP-9; I–J, protein expression levels of TIMP-1 and TIMP-2. The statistical significance of the differences between the control and experimental samples in the MMP array was analyzed using a paired *t*-test. One-way ANOVA was used to compare values among multiple groups. (**, $P < 0.01$ and ***, $P < 0.001$).

POS: Positive control; NEG: Negative control; MMP-1: Matrix metalloproteinase-1; MMP-9: Matrix metalloproteinase-9; TIMP-1: Tissue inhibitor of metalloproteinases-1; TIMP-2: Tissue inhibitor of metalloproteinases-2.

3.4. The MAPK signaling pathway is involved in the T. pallidum-induced MMP/TIMP imbalance

To explore the mechanism by which *T. pallidum* induces an MMP/TIMP imbalance, we first evaluated the role of the MEK/ERK, JNK and p38 pathways by Western blot analysis. Fig. 4A showed that MEK phosphorylation was stimulated by *T. pallidum* in a time-dependent manner, exhibiting a significant response at 5 min ($P < 0.05$) and then declining within 30 min ($P < 0.001$); in addition, ERK phosphorylation exhibited a significant response at 5 min ($P < 0.01$) and then declined within 60 min ($P < 0.001$). JNK phosphorylation increased significantly at 5 min ($P < 0.001$) and then gradually declined until 30 min ($P < 0.001$), and c-Jun phosphorylation peaked at 10 min ($P < 0.001$) and began decreasing substantially at 60 min ($P < 0.01$). However, p38 phosphorylation began increasing at 5 min ($P < 0.001$), peaked at 60 min ($P < 0.001$) and then moderately decreased at 120 min ($P < 0.001$).

To determine the role of the MAPK signaling pathway in the *T. pallidum*-induced MMP/TIMP imbalance and in THP-1 cell migration and invasion, MAPK inhibitors were used. As shown in Fig. S1A, the cell viability was not significantly different between the MAPK inhibitors and the control group. After pretreatment with the MAPK inhibitors, cell migration and invasion stimulated by *T. pallidum* significantly diminished to levels similar to those of the control cells ($P < 0.01$) (Fig. 4B and C). Fig. 4D–G showed that after pretreatment with MAPK inhibitors, the MMP-1/TIMP-1, MMP-1/TIMP-2, MMP-9/TIMP-1 and MMP-9/TIMP-2 ratios in the culture medium were significantly lower than those in cells stimulated by *T. pallidum* ($P < 0.05$) but did not differ from those in control cells. When the MMP/TIMP imbalance was corrected, the numbers of migrating and invading cells were significantly reduced, suggesting that the migration and invasion abilities of monocytes were affected by the MMP/TIMP imbalance.

3.5. The NF-κB signaling pathway is essential for the T. pallidum-induced MMP/TIMP imbalance

To explore the mechanism by which *T. pallidum* induces an MMP/TIMP imbalance, we next evaluated the role of the NF-κB pathway by Western blot analysis. Fig. 5A showed that after treatment with *T. pallidum*, phosphorylation of IκBα began at 5 min ($P < 0.01$) and peaked at 60 min ($P < 0.001$). Total IκBα degradation began at 15 min ($P < 0.001$), reaching a minimum protein level at 30 min ($P < 0.001$) and then gradually increasing. In addition, treatment with *T. pallidum* led to a significant increase in the NF-κB immunofluorescence signal in the nucleus, suggesting a high level of NF-κB translocation from the cytoplasm into the nuclear areas in monocytes stimulated by *T.*

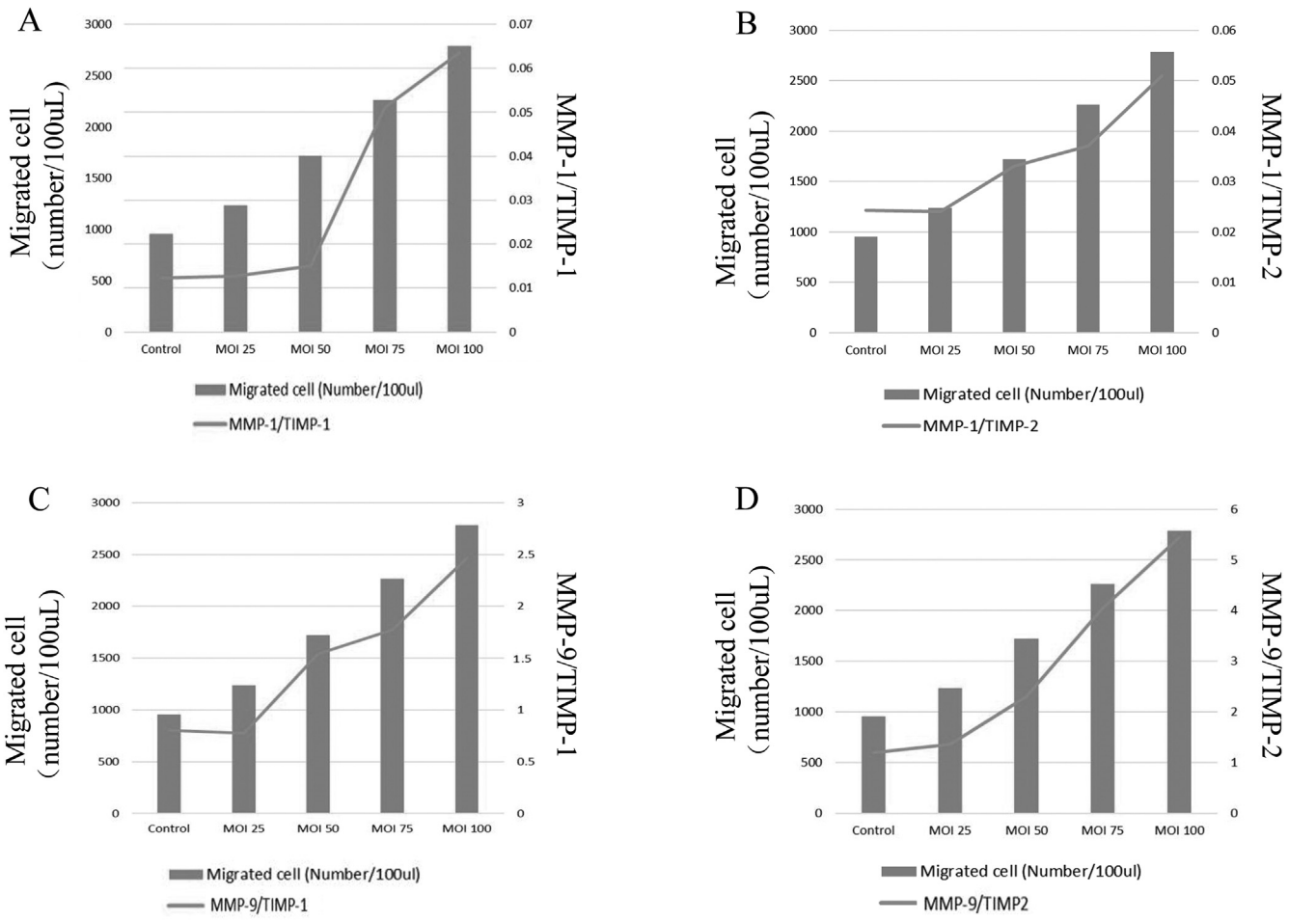

Fig. 3. *T. pallidum* induced an imbalance of MMP and TIMP expression in THP-1 cells
A, MMP-1/TIMP-1 ratio; B, MMP-1/TIMP-2 ratio; C, MMP-9/TIMP-1 ratio; D, MMP-9/TIMP-2 ratio. The values are the means of triplicate trials; one-way ANOVA was used to compare values among multiple groups. **, $P < 0.01$ and ***, $P < 0.001$.

Table 2
Correlation analysis is between the number of invasive and migrating cells and MMP/TIMP ratio.

	Migrated cell number		Invasion cell number	
	correlation	*p* value	correlation	*p* value
MMP-1/TIMP-1	0.597	< 0.001	0.664	< 0.001
MMP-1/TIMP-2	0.44	0.004	0.499	0.001
MMP-9/TIMP-1	0.853	< 0.001	0.804	< 0.001
MMP-9/TIMP-2	0.433	0.004	0.492	0.001

pallidum. As shown in Fig. S1B, the cell viability was not significantly different between the NF-κB inhibitors and the control group. When cells were pretreated with NF-κB inhibitors, the nuclear p65 immunofluorescence signal was appreciably attenuated (Fig. 5B).

To determine the role of the NF-κB signaling pathway in the *T. pallidum*-induced MMP/TIMP imbalance and in cell migration and invasion, NF-κB inhibitors were used. After pretreatment with NF-κB inhibitors, cell migration and invasion stimulated by *T. pallidum* were significantly reduced ($P \leq 0.01$) (Fig. 5C and D). As shown in Fig. 5E–H, the *T. pallidum*-induced increase in the MMP-1/TIMP-1, MMP-1/TIMP-2, MMP-9/TIMP-1, and MMP-9/TIMP-2 ratios in the culture medium was significantly decreased by BAY11–7082 ($P < 0.001$) and PDTC ($P < 0.01$) relative to that in cells stimulated by *T. pallidum* but did not differ significantly from that in control cells. These results indicated that the MMP/TIMP imbalance might promote cell migration and invasion. Moreover, when the MMP/TIMP imbalance was corrected, migration and invasion were suppressed.

4. Discussion

Leukocyte migration and invasion into sites of infection play a crucial role in disease states, including asthma, infectious disease and atherosclerosis [9]. As leukocytes, monocytes are recruited by cancer cells into the tumor from the circulation and then differentiate into macrophages, which promote the invasion and metastasis of carcinoma cells [20]. Similarly, monocyte cell infiltration is a major event in the pathogenesis of syphilis, and monocyte-derived macrophages play a central role in the clearance of *T. pallidum* [21]. In our study, *T. pallidum* was found to induce the migration and invasion of THP-1 cells in vitro, suggesting that monocyte migration and invasion might be crucial for triggering the inflammatory response during *T. pallidum* infection and for eradicating this pathogen.

The balance between the activities of MMPs and TIMPs mediates both normal and pathological events, such as wound healing, tissue remodeling, angiogenesis, invasion, and metastasis [22–24]. Previous investigators have reported that in atherosclerotic plaques, highly invasive macrophages express high levels of MMP-14 and lower levels of TIMP-3 [25]. Additionally, an imbalance in MMPs and TIMPs is involved in disruption of the blood-brain barrier in HIV-1-associated neurocognitive disorders [26]. In our study, MMP-1 and MMP-9 expression levels were significantly increased in THP-1 cells stimulated by *T. pallidum*. MMP-1 is a collagenase that cleaves fibrillar collagens [27].

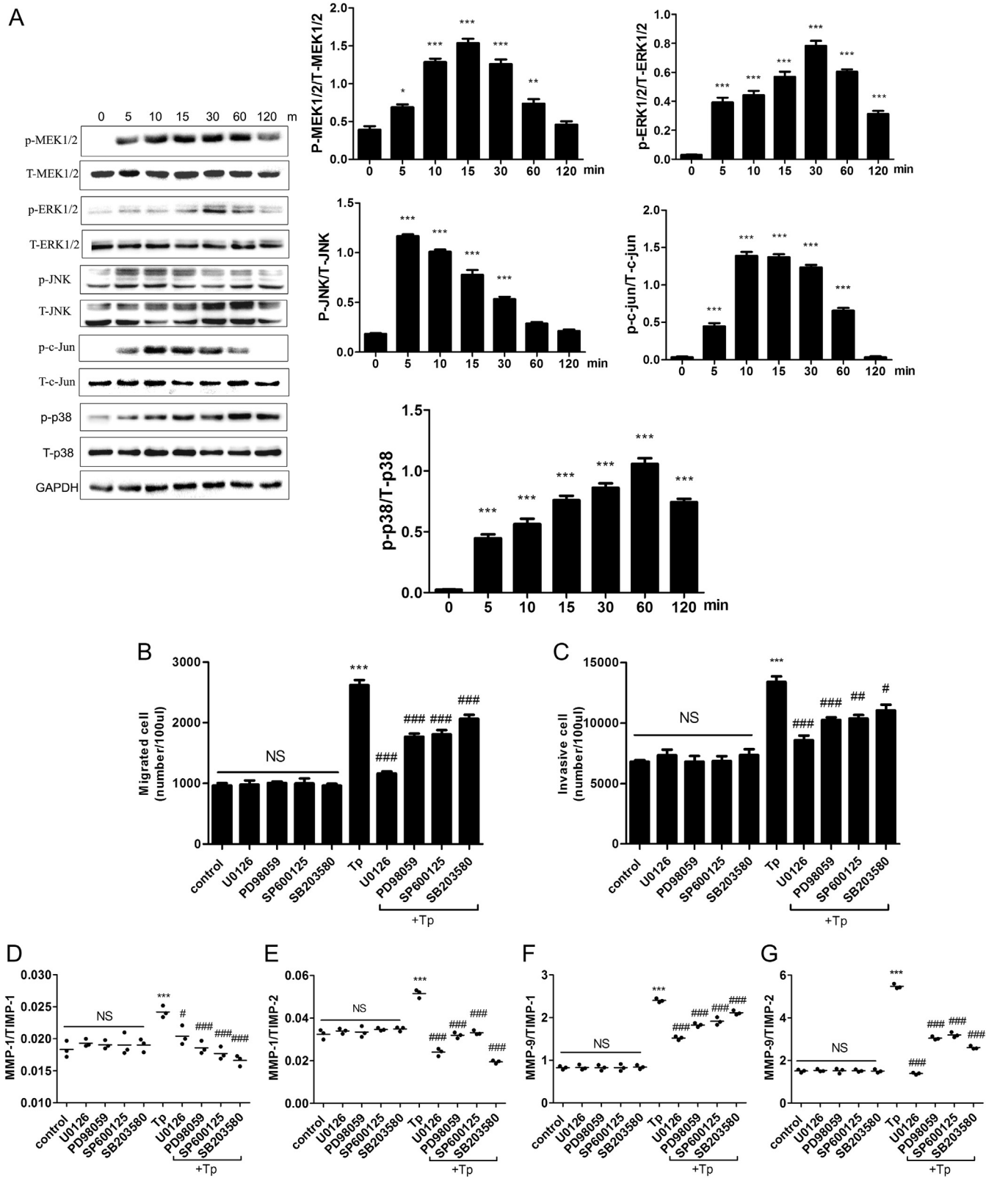

Fig. 4. *T. pallidum* activated the MAPK signaling pathway and promoted an MMP/TIMP imbalance in THP-1 cells

THP-1 cells were pretreated with 10 μmol/L U0126, 10 μmol/L PD98059, 20 μmol/L SP600125 or 10 μmol/L SB203580 for 1 h and then treated with *T. pallidum* (MOI 100) for 24 h. A, Time course of MAPK signaling pathway activation, as determined by Western blot analysis; B, migration of THP-1 cells; C, invasion of THP-1 cells; D, MMP-1/TIMP-1 ratio; E, MMP-1/TIMP-2 ratio; F, MMP-9/TIMP-1 ratio; G, MMP-9/TIMP-2 ratio. One-way ANOVA was used to compare values among multiple groups *, $P < 0.01$, ***, $P < 0.001$ and NS compared with the control group. $^{\#}$, $P < 0.05$, $^{\#\#}$, $P < 0.01$, and $^{\#\#\#}$, $P < 0.001$ compared with the *T. pallidum* group.

Tp: *T. pallidum*; NS: no significance.

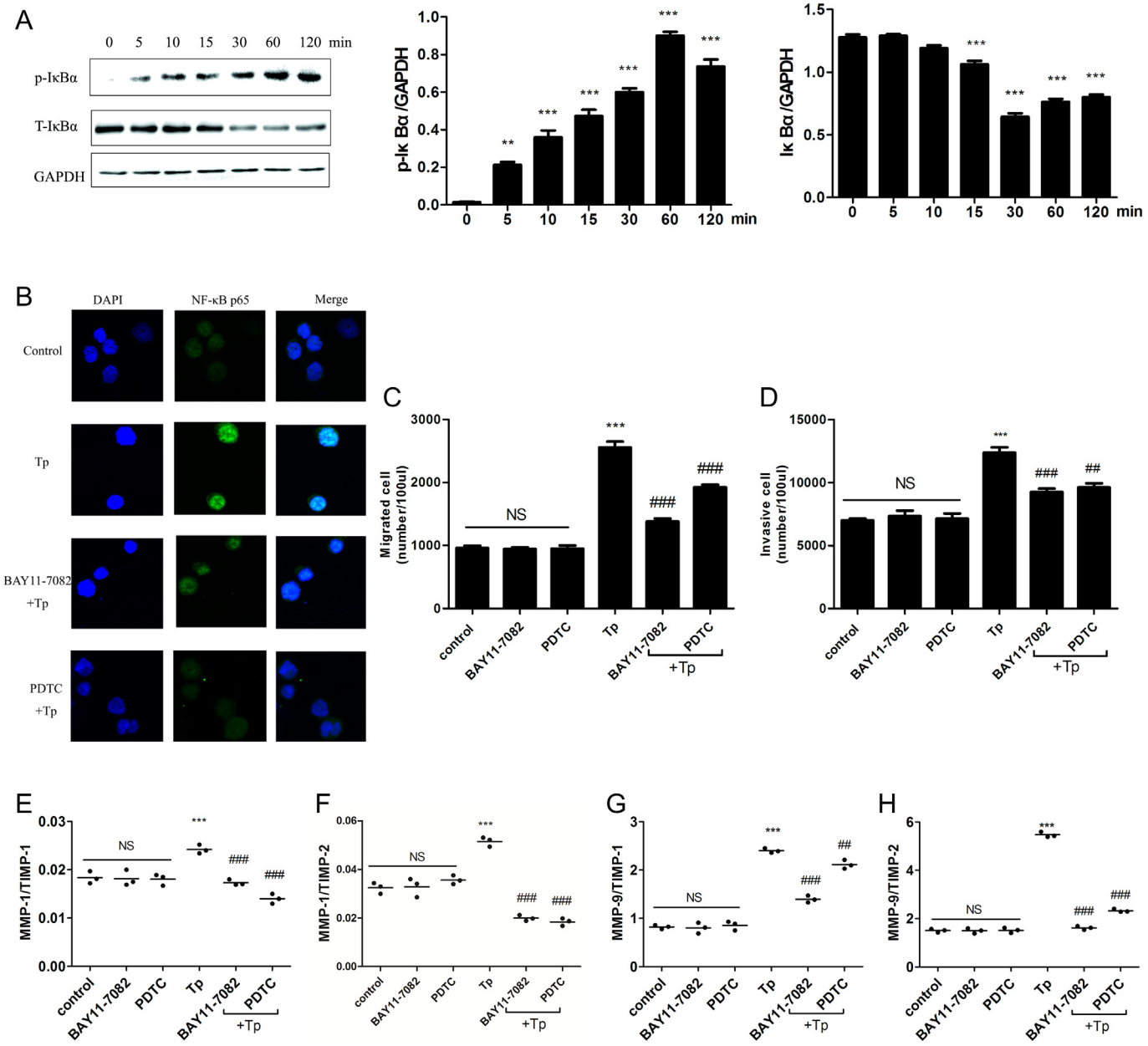

Fig. 5. *T. pallidum* induced NF-κB signaling pathway activation to disrupt the MMP/TIMP balance in THP-1 cells
THP-1 cells were pretreated with NF-κB inhibitors (BAY 11–7082, 10 μmol/L; PDTC, 100 μmol/L) for 1 h and then treated with *T. pallidum* (MOI 100) for 24 h. A, Time course of NF-κB pathway activation; B, NF-κB p65 nuclear translocation; C, migrated cells; D, invaded cells; E, MMP-1/TIMP-1 ratio; F, MMP-1/TIMP-2 ratio; G, MMP-9/TIMP-1 ratio; H, MMP-9/TIMP-2 ratio. One-way ANOVA was used to compare values among multiple groups. **, $P < 0.01$, ***, $P < 0.001$ and NS compared with the negative control group. ##, $P < 0.01$, and ###, $P < 0.001$ compared with the *T. pallidum* group.
TP: *T. pallidum*; NS: no significance.

MMP-9 is a gelatinase that cleaves denatured collagen, particularly type IV collagen, which constitutes the major component of basement membranes [28]. In addition, MMPs are not only required for the infiltration of inflammatory monocytes but also involved in driving tissue damage in tuberculosis [29,30]. Similarly, monocytes expressed high MMP-1 and MMP-9 levels induced by *T. pallidum*, which might destroy the ECM and cause tissue damage.

MMPs are tightly controlled by molecules that regulate their expression and by specific inhibitors called TIMPs [31]. TIMP-1 expression was appreciably elevated in monocytes stimulated by *T. pallidum*, but TIMP-2 expression did not significantly change. The MMP-1/TIMP-1, MMP-9/TIMP-1, MMP-1/TIMP-2 and MMP-9/TIMP-2 ratios were significantly increased, indicating an imbalance between MMPs and TIMPs. In addition, the migration and invasion abilities of monocytes were clearly enhanced, a phenomenon related to the increased MMP/TIMP ratios.

An interesting finding in our study was that TIMP-1 expression increased, but TIMP-2 expression did not change. Previous studies have reported that TIMP-1 preferentially inhibits MMP-7, MMP-9, MMP-1 and MMP-3, whereas TIMP-2 is also a more effective inhibitor of MMP-2 [32]. Hence, elevated TIMP-1 expression might be considered feedback regulation for the increased MMP levels in THP-1 cells stimulated by *T. pallidum*, but its elevation was below the level of MMPs. On the other hand, TIMP-2 expression in THP-1 cells stimulated by *T. pallidum* did not increase, probably because the expression of MMP-2 did not change. These results suggest that an imbalance of MMP/TIMP induced

by *T. pallidum* enhanced monocyte migration and invasion into inflammatory sites and promoted inflammatory progression.

The MAPK signaling pathway and NF-κB activation play a key role in mediating MMP and TIMP expression [33] and regulating the migration and invasion of cells by modulating the expression of MMPs and TIMPs [34,35]. Previous studies have shown that *T. pallidum* flagellins stimulate not only MMP-9 and MMP-13 expression but also IL-6 expression via MAPK/NF-κB signaling pathways [16]. Our study also showed that the MAPK signaling pathway and NF-κB activation are involved in the MMP/TIMP imbalance induced by *T. pallidum*. Signaling pathway inhibitors significantly alleviated the MMP/TIMP imbalance induced by *T. pallidum* and confirmed the activation of the MAPK and NF-κB signaling pathways, ultimately affecting monocyte migration and invasion.

Our study has several limitations. First, only certain MMPs were detected by the MMP antibody array. Second, monocyte migration and invasion are complex processes, many aspects of which remain to be studied. Third, the maintenance of the MMP/TIMP balance is an intricate process involving several interrelated factors, and future animal experiments could allow a more comprehensive understanding of the role of the MMP/TIMP imbalance in the process of *T. pallidum* infection.

Taken together, the results of this research showed that *T. pallidum* causes an imbalance in MMPs/TIMPs in THP-1 cells and promotes monocyte migration and invasion through the MAPK and NF-κB signaling pathways. Understanding both the role of the MMP/TIMP imbalance in *T. pallidum* infection and the mechanisms relevant to monocyte infiltration furthers our understanding of syphilis pathogenesis.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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