Contents lists available at ScienceDirect

International Immunopharmacology

Tp47 induces cell death involving autophagy and mTOR in human microglial HMO6 cells

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ARTICLE INFO

ABSTRACT

Background: Tp47 can induce immune cells to produce numerous inflammatory factors, some of which can trigger autophagy. Increased autophagy has a dual effect on cell survival. However, whether Tp47 induces autophagy in microglia is unknown.
Objective: To evaluate the potential role of Tp47 in microglia.
Methods: After treatment with Tp47, autophagy-related proteins were assessed in HMO6 human microglial cells by flow cytometry, Western blotting and immunofluorescence. Cell death was assessed by flow cytometry and trypan blue staining. Changes in mTOR pathway proteins were explored by using Western blotting.
Results: After treatment with Tp47, a gradual increase in total LC3 expression was observed as a dose- and time-dependent accumulation of its active form, LC3-II ($P < 0.05$), but P62 expression was downregulated ($P < 0.05$). Moreover, microglial mortality gradually increased in a dose- and time-dependent manner. 3-Methyladenine (3-MA), a specific inhibitor of PI3KC3, reversed autophagy and cell death. The mortality rate of HMO6 microglial cells treated with Tp47 was approximately $13.7 \pm 2\%$, and the basal expression of p-mTOR, p-p70s6k and p-S6 in these cells was significantly downregulated by Tp47. Moreover, the mortality rate of microglia was significantly reduced after mTOR agonist intervention.
Conclusion: In human microglial HMO6 cells, Tp47 induces autophagy- and mTOR pathway-dependent cell death.

1. Introduction

Syphilis remains the most common sexually transmitted disease; neurosyphilis is one of the most destructive clinical types of syphilis and is difficult to control. The high prevalence of syphilis leads to a continuous increase in the incidence of neurosyphilis. Approximately 70% of patients with syphilis show cerebrospinal fluid abnormalities, and 25–40% of untreated or partially treated patients may develop neurosyphilis [1]. Neurosyphilis can occur at any stage of syphilis, and its most common form is asymptomatic neurosyphilis following the invasion of the central nervous system (CNS) by *Treponema pallidum* [2]. Asymptomatic cases may create diagnostic problems and become a potential source of infection, leading to incorrect therapeutic decision, causing some scholars to speculate that the incidence of neurosyphilis may be higher than reported.

Asymptomatic neurosyphilis patients have high concentrations of Tp47, the membrane protein of *Treponema pallidum*, in the cerebrospinal fluid [3]. Membrane proteins are recognized as *Treponema pallidum* virulence factors. Among these proteins, Tp47 is the most abundant in *T. pallidum* and shares no sequence homology with any other bacterial or eukaryotic protein [4], which typically form the active center of penicillin-binding protein. Lewis et al. has been reported the Tp47 antigen may be a potential marker for active syphilis disease as presented in all stages of non-treated syphilis [5,6]. Study has found that Tp47 - the abundant membrane immunogens of *T. pallidum* - is proteolipids, which may serve to explain their extraordinary immunogenicity [7]. Timothy et al. also found that Tp47 caused immune cells such as macrophages to produce numerous inflammatory factors, such as TNF-α and IL-8 [8], and these inflammatory factors can trigger autophagy [9].

Autophagy is an evolutionarily conserved process that plays a major role in various stimulus responses, such as cellular responses to host defence, starvation, cell survival and cell death [10]. This process of self-digestion is widely believed to exert a dual effect— cytoprotection and cytotoxicity—on cell survival [11]. Moreover, accumulating studies have shown that autophagy is involved in the processing of pathogenic bacteria by microglia [12].

Microglia, the resident macrophages within the CNS, play an important role in maintaining brain microenvironment homeostasis by continuously surveying tissue damage [13]. Mirco et al. found that neurosyphilis patients showed cortical susceptibility-weighted imaging hypointensity, unlike patients with other infectious or inflammatory diseases of the CNS. The authors speculated that the cortical low-density signalling in patients with neurosyphilis may be associated with abnormal microglial function [14].

Studies have found that lipopolysaccharide (LPS) can cause microglia to produce numerous inflammatory factors and induce autophagy in cells and that excessive autophagy stimulates the autophagic death of cells [15]. Tp47 is abundant in patients with neurosyphilis [4,16], prompting the question of whether Tp47 can induce autophagy in microglia. The mechanism of the relationship between Tp47 and important immune defences in microglial cells of the nervous system is still unclear. In this study, we aim to evaluate the potential role of Tp47 in microglia and to investigate whether autophagy is involved in the processing of pathogenic Tp47 by microglia.

2. Materials and methods

2.1. Preparation of the recombinant T. pallidum Tp47 protein

Endotoxins were removed from the recombinant *T. pallidum* Tp47 protein (Boson Biotech Co., Ltd., Xiamen, China) with an EtEraserTM Endotoxin Removal Kit (Chinese Horseshoe Crab Reagent Manufactory, Ltd., Xiamen, China). Tachypleus amebocyte lysate (Chinese Horseshoe Crab Reagent Manufactory, Ltd., Xiamen, China) was used to detect endotoxins in the Tp47 preparation, which was found to have < 0.05 endotoxin units (EUs)/mL.

2.2. Cell culture and treatment

HMO6 (Columbia University) cells, an immortalized human microglial cell line, were grown in Dulbecco's modified Eagle's medium (DMEM, HyClone, Logan, Utah, USA) with high glucose supplemented with 10% heat-inactivated foetal bovine serum (FBS, Biological Industries Ltd., Kibbutz Beit Haemek, Israel) and incubated at 37 °C with 5% CO_2. The cells used for the tests were assessed for viability using trypan blue (Sigma) under an Olympus (USA) microscope; only cells with > 95% viability were used in the experiments.

Cells treated with PBS were used as the control. Tp47 was used alone at the following concentrations: 1) 100 μg/mL, 2) 150 μg/mL, or 3) 200 μg/mL. Concurrently, the autophagy and mortality of microglia were observed by adding the inhibitor 3-methyladenine (3-MA) (1 mM) dissolved in PBS used to pretreat the cells for 1 h. 3-MA was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Flow cytometry test

Cells were seeded in a 6-well plate, and after different durations (0, 6, 12, 24 and 48 h), the cells were collected and washed twice with PBS, suspended in binding buffer and incubated with FITC-conjugated annexin V and propidium iodide (PI) (Sigma) for staining at room temperature for 15 min in the dark. Apoptotic cells were quantified by flow cytometry (BD Biosciences, San Diego, USA). Cells were plated in 3-cm dishes and treated with Tp47 (200 μg/mL) or an equal amount of PBS for 24 h. Then, the cells were fixed and lysed. Mouse anti-human LC3-APC (Cell Signalling Technology, Beverly, MA, USA) was used as a

marker for the staining of autophagy-related proteins. Flow cytometry was performed using a BD Biosciences FACSCanto II flow cytometer (BD Biosciences).

2.4. Western blot (WB) analysis

Cells were treated with 200 μg/mL Tp47 in a 6-well plate. Then, cells were collected after 24 h for WB analysis. WB analysis was performed according to the method previously described in the literature [17], and 20 μg of total protein per lane was loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad). After blocking using 5% nonfat milk for 1 h, membranes were incubated overnight at 4 °C with the primary antibodies (rabbit anti-human LC3, rabbit anti-human P62, rabbit anti-human β-actin, rabbit anti-p-mTOR, rabbit anti-p70s6k and rabbit anti-p-S6) and the secondary antibody (an anti-rabbit horseradish peroxidase-conjugated antibody) at dilutions of 1:1000 and 1:5000, respectively. The images were quantified using ImageJ software. Antibodies against LC3, P62, mTOR, p70s6k, S6 and β-actin were purchased from Cell Signalling Technology (Beverly, MA, USA).

2.5. Fluorescence confocal microscopy test

Cells were seeded on coverslips in 12-well plates. After 24 h of growth, cells were transiently transfected with the pmCherry-EGFP-LC3 plasmid with Lipofectamine 3000 transfection reagent according to the manufacturer's protocol. After 72 h, the cells were treated with Tp47. After treatment for 24 h, the cells were viewed under an LSM700 confocal laser scanning microscope (Zeiss, Oberkochen, Germany). The percentage of cells with more than five LC3 puncta, which were considered autophagic vacuoles, was quantified. Lipofectamine® 3000 was purchased from Life Technologies, and pmCherry-EGFP-LC3 was obtained from MiaoLingBio.

2.6. Statistical analysis

The data are expressed as the means ± standard errors of the mean (SEMs) of at least three independent experiments. Statistical significance between two groups was determined using Student's *t*-test (paired *t*-test for clinical samples and unpaired t-test for all other samples). For comparisons of multiple groups, one-way ANOVA followed by a Bonferroni-Dunn test was used. Statistical analyses were performed using PRISM version 6.0 (GraphPad Software, San Diego, CA). A *P* value of < 0.05 was considered statistically significant.

3. Results

3.1. Tp47 induces autophagy in microglia

LC3 is reliably associated with autophagosomes and is widely used to assess autophagy. After treatment of the cells with Tp47 at concentrations of 0, 100, 150, and 200 μg/mL for 24 h, the relative fold changes in LC3-II expression were 1.0 ± 0.1%, 2.2 ± 0.5%, 2.3 ± 0.3% and 3.2 ± 0.6%, respectively; the corresponding relative fold changes in P62 expression were 2.5 ± 0.3%, 2.0 ± 0.2%, 1.3 ± 0.3% and 1.1 ± 0.2%. The immunoblot showed a gradual increase in total LC3 expression by the dose-dependent accumulation of its active form, LC3-II ($P < 0.05$), but P62 expression was downregulated ($P < 0.05$) (Fig. 1A). Next, we observed the autophagy of cells treated with Tp47 in a kinetic evaluation. The relative fold changes in LC3-II expression at 0 h, 6 h, 12 h, and 24 h after treatment with 200 μg/mL Tp47 were 1.0 ± 0.1%, 2.1 ± 0.4%, 2.5 ± 0.7% and 3.5 ± 0.8%, respectively. Under the same conditions, the relative fold changes in P62 expression were 2.8 ± 0.1%, 2.5 ± 0.4%, 2.0 ± 0.2% and 1.2 ± 0.1%. LC3-II expression increased with time, and P62
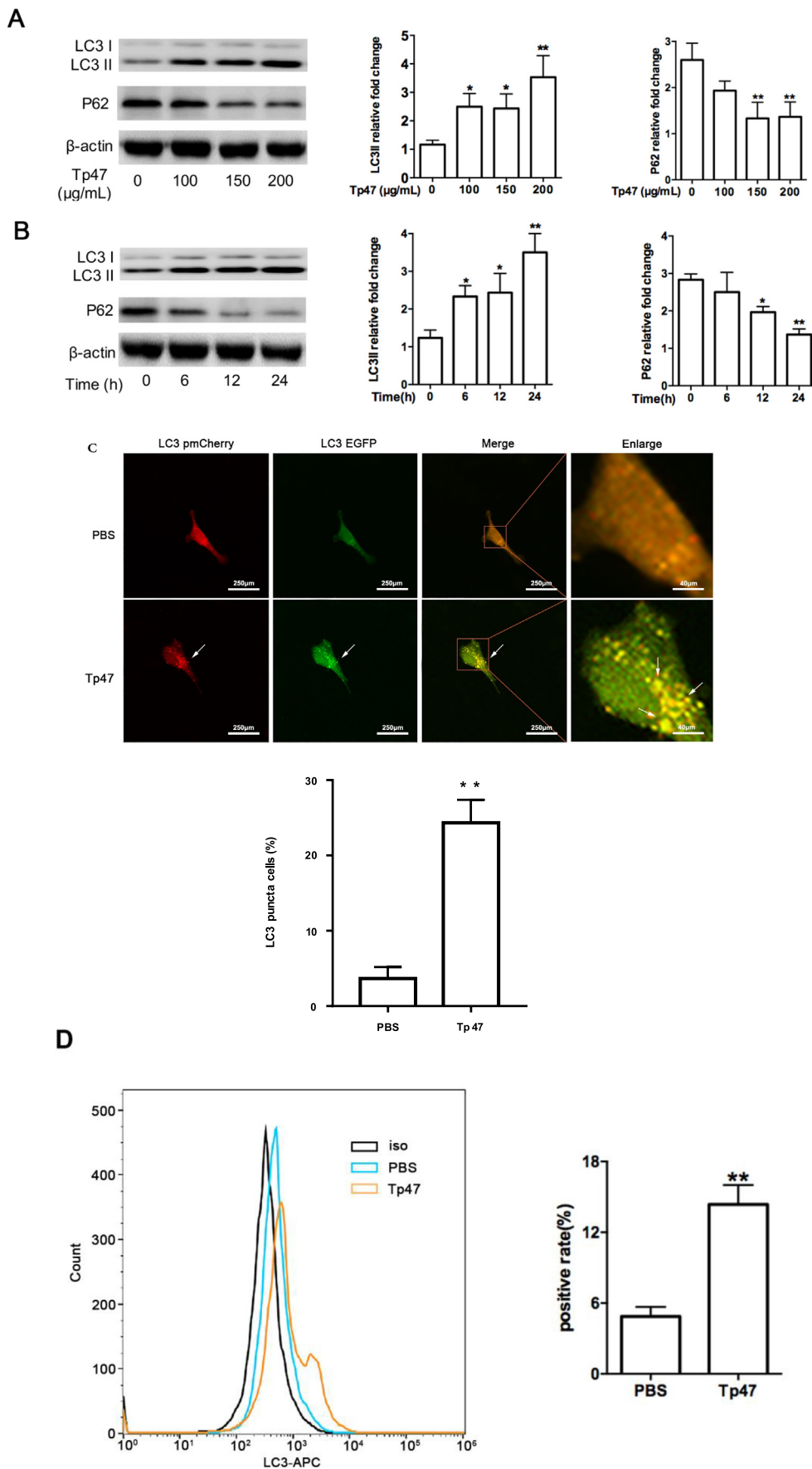
A

B

C

D

expression decreased with increasing autophagic flow ($P < 0.05$). Compared with the LC3-II levels at 0 h, the LC3-II levels increased significantly after treatment for 12 h and 24 h, while the P62 levels were significantly decreased (Fig. 1B).

To visualize the formation of autophagic vacuoles, expression vectors for the green and red fluorescent proteins mCherry-EGFP-LC3 (pmCherry-EGFP-LC3) were used, and the presence of pmCherry-EGFP-LC3-labelled structures was examined. Our results showed an increase in pmCherry-EGFP-LC3 puncta, which represented autophagic vacuoles, after Tp47 treatment relative to that in the control cells (Fig. 1C).

To further demonstrate the occurrence of autophagy, we used flow cytometry to detect the expression of the autophagy-related protein LC3 in the cytoplasm. The percentage of LC3 positive cells among the Tp47-induced cells was significantly higher than that among the control cells (14.8 ± 0.2% vs. 5.1 ± 0.3%, $P < 0.01$, Fig. 1D).

3.2. Tp47 induces microglial cell death in a dose- and time-dependent manner

We evaluated the effect of Tp47 on human microglial cell survival. After treatment of the cells with Tp47 at concentrations of 0, 100, 150, and 200 μg/mL for 24 h, the cell death rates were 2.3 ± 1.0%, 2.9 ± 0.3%, 5.9 ± 0.3% and 10.8 ± 2.0%, respectively, resulting in a significant increase in cell death at the concentration of 200 μg/mL ($P < 0.05$) (Fig. 2A). Then, the time-course analysis showed that the mortality of microglia at 0 h, 6 h, 12 h, 24 h and 48 h after treatment with 200 μg/mL Tp47 was 2.0 ± 0.3%, 4.2 ± 0.4%, 8.6 ± 1.9%, 11.8 ± 2.0%, and 19.3 ± 3.0%, respectively. The kinetic evaluation of annexin V vs. PI staining using flow cytometry indicated that 200 μg/mL Tp47 significantly increased microglial cell death at 12 h post treatment (Fig. 2B).

Next, we used trypan blue to further confirm microglial activity after stimulation with 0, 100, 150, or 200 μg/mL Tp47 for 24 h. As shown in Fig. 2C, the mortality of microglia at these concentrations was 2.0 ± 0.9%, 3.0 ± 0.8%, 5.6 ± 1.9% and 11.3 ± 2.3%, respectively. When microglia were treated with 200 μg/mL Tp47, the rate of cell death was significantly increased compared to that in control cells ($P < 0.05$) (Fig. 2C). In addition, after microglia were incubated with Tp47 at a concentration of 200 μg/mL for 0 h, 6 h, 12 h, 24 h or 48 h, the mortality of microglia was 2.0 ± 0.6%, 4.5 ± 0.4%, 9.6 ± 1.7%, 12.8 ± 2.0%, and 18.3 ± 3.0%, respectively. Cell death levels significantly increased at 12 h post treatment. ($P < 0.001$) (Fig. 2D), similar to the above flow cytometry results.

3.3. Autophagy is required for Tp47-induced microglial cell death

To further confirm the occurrence of autophagy, we pretreated microglial cells with 3-MA, a specific inhibitor of PI3KC3, for 1 h before treatment with Tp47. After treatment of the microglial cells with 200 μg/mL Tp47 for 24 h, the relative fold change in LC3-II expression was significantly decreased and that of P62 expression was significantly increased compared with the LC3-II and P62 expression in microglial cells not pretreated with 3-MA (2.8 ± 0.7% vs. 1.7 ± 0.2% and 0.7 ± 0.1% vs. 1.8 ± 0.3%, respectively) (Fig. 3A).

To further confirm this observation, HMO6 cells were transfected with an mCherry-EGFP-LC3 plasmid, which was a useful assay to monitor autophagy flux. The green fluorescence signal (EGFP) was quenched by the low pH inside the lysosomal lumen, whereas the red signal (mCherry) exhibited more stable fluorescence in acidic conditions, in cells treated with Tp47 alone or in combination with 3-MA. Fluorescent microscopic analyses showed that although Tp47 noticeably increased the formation of LC3 puncta, an indicator of autophagic vacuoles, pretreatment with 3-MA appreciably reduced the formation of LC3 puncta. This result demonstrated that Tp47-induced autophagy could be partially inhibited by 3-MA (Fig. 3B).

Next, by flow cytometry, we examined the cell death rate after treatment with autophagy inhibitors. The Tp47-induced microglial cell death was partly blocked by 3-MA; the mortality rate of microglia decreased from approximately 13.8 ± 0.9% to 8.1 ± 0.7% ($P < 0.05$) (Fig. 3C).

3.4. Basal expression of p-mTOR, p-p70s6k and p-S6 in HMO6 is significantly downregulated by Tp47

Our results revealed that the basal phosphorylation levels of mTOR, p70s6k and S6 gradually decreased over time in microglial cells treated with Tp47. As shown in Fig. 4A, the relative ratios of p-mTOR/mTOR after Tp47 treatment for 0 h, 6 h, 12 h and 24 h were 1.0 ± 0.2, 0.9 ± 0.1, 0.65 ± 0.1, 0.35 ± 0.1; those of p-p70s6k/p70s6k were 1.1 ± 0.1, 0.86 ± 0.15, 0.66 ± 0.1, 0.39 ± 0.1; and those of p-S6/S6 were 1.1 ± 0.09, 0.82 ± 0.06, 0.64 ± 0.08, 0.26 ± 0.02, respectively. The relative fold change in the p-mTOR/mTOR, p-p70s6k/p70s6k and p-S6/S6 ratios after treatment with Tp47 for 12 h and 24 h were significantly lower than those at 0 h ($P < 0.05$). Next, cells were pretreated with salidroside, an agonist of mTOR, for 1 h before treatment with Tp47. After treatment of the microglial cells with 200 μg/mL Tp47 for 24 h, the p-mTOR/mTOR ratio was higher than that in microglial cells not pretreated with salidroside (0.7 ± 0.2 vs. 0.4 ± 0.1, $P < 0.05$). The quantification of the staining intensity is shown on bar graphs (Fig. 4B). Furthermore, we used flow cytometry to test the effect of mTOR agonist treatment on Tp47-induced cell death. When Tp47 was applied to microglia, the microglia mortality rate was approximately 13.7 ± 2%. However, adding salidroside 1 h before Tp47 treatment decreased the microglial mortality rate to 9.9 ± 1%. Thus, the mortality rate of microglia was significantly reduced after mTOR agonist intervention. (Fig. 4C).

4. Discussion

Tp47 is a membrane protein that is highly immunogenic and is widely used in the diagnosis of *Treponema pallidum* [5]. Although there is a high concentration of Tp47 protein in the cerebrospinal fluid of patients with neurosyphilis [16], neurosyphilis patients often present with no symptoms of infection, i.e., asymptomatic neurosyphilis [18]. However the brain tissue specimens of patients with neurosyphilis are difficult to obtain due to ethical concerns and many other reasons; What is more, the animal model of Treponema pallidum is New Zealand rabbits, and there are few antibodies to rabbits on the market, which largely limits the development of syphilis research. Studies have found that microglia participate in the pathogenesis of neurosyphilis [19]. Microglial cells manage the innate and adaptive immune responses not only during CNS repair but also during various pathological processes [20]. However, the role of Tp47 in microglia is unclear. Recently, an observation showed that active microglia can clear pathogenic bacteria via autophagy, thereby promoting tissue repair and regulating the response to pathogens [21].

In our study, we found that Tp47 promoted autophagy in microglia. Our results showed that the protein level of LC3-II gradually increased and that of P62 decreased with the therapeutic concentration of Tp47 and extended incubation durations. This result suggested that autophagy occurred in Tp47-treated HMO6 cells in a dose- and time-dependent manner. LC3 is reliably associated with autophagosomes and is widely used to assess autophagy. LC3 often exhibits a molecular conversion from cytosolic LC3-I into its enzymatic LC3-II counterpart, which is bound to the membrane of autophagosomes during autophagy [11]; the autophagy receptor P62 then interacts with LC3-II at the forming autophagosome and is degraded by autophagy, which makes P62 another useful marker of autophagy [22]. Autophagy is a fundamental catabolic process that degrades cellular contents and recycles damaged organelles [23]. According to its different mechanisms, autophagy can be divided into macroautophagy, microautophagy and
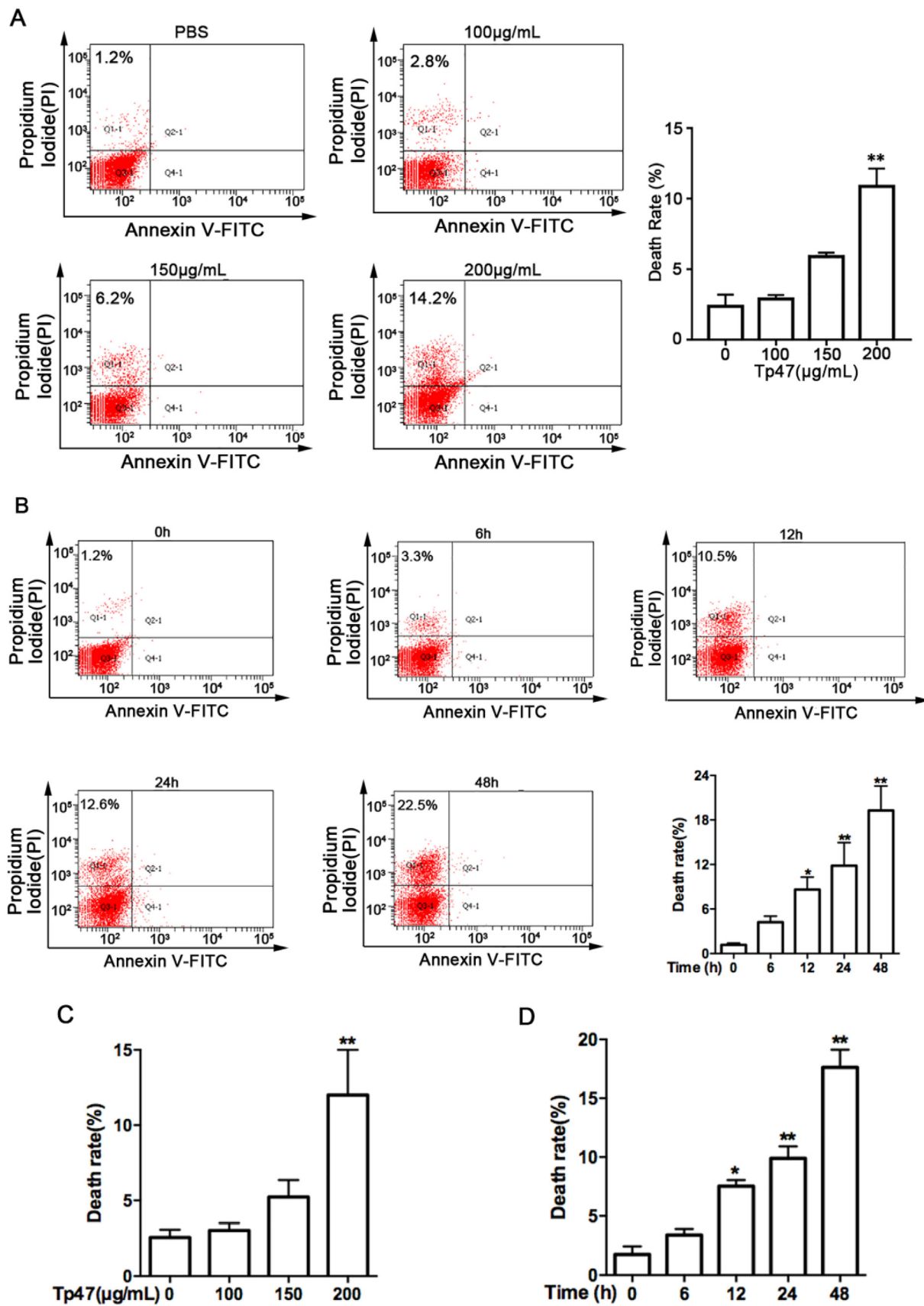
Fig. 2. Tp47 induced HMO6 human microglial cell death in a dose- and time-dependent manner.

HMO6 (5×10^5 cells per well) cells were seeded in 12-well plates for 12 h and were then incubated with the indicated concentrations of Tp47 for 24 h or with 200 μg/mL Tp47 for the indicated durations. (A) and (B) Cells were stained with annexin V-FITC/PI to analyse cell apoptosis by flow cytometry. (C) and (D) Trypan blue dye was used to stain the cells and analyse the death ratio. Data from three independent tests are presented as the means ± SEMs compared with untreated cells. *$P < 0.05$, **$P < 0.001$.
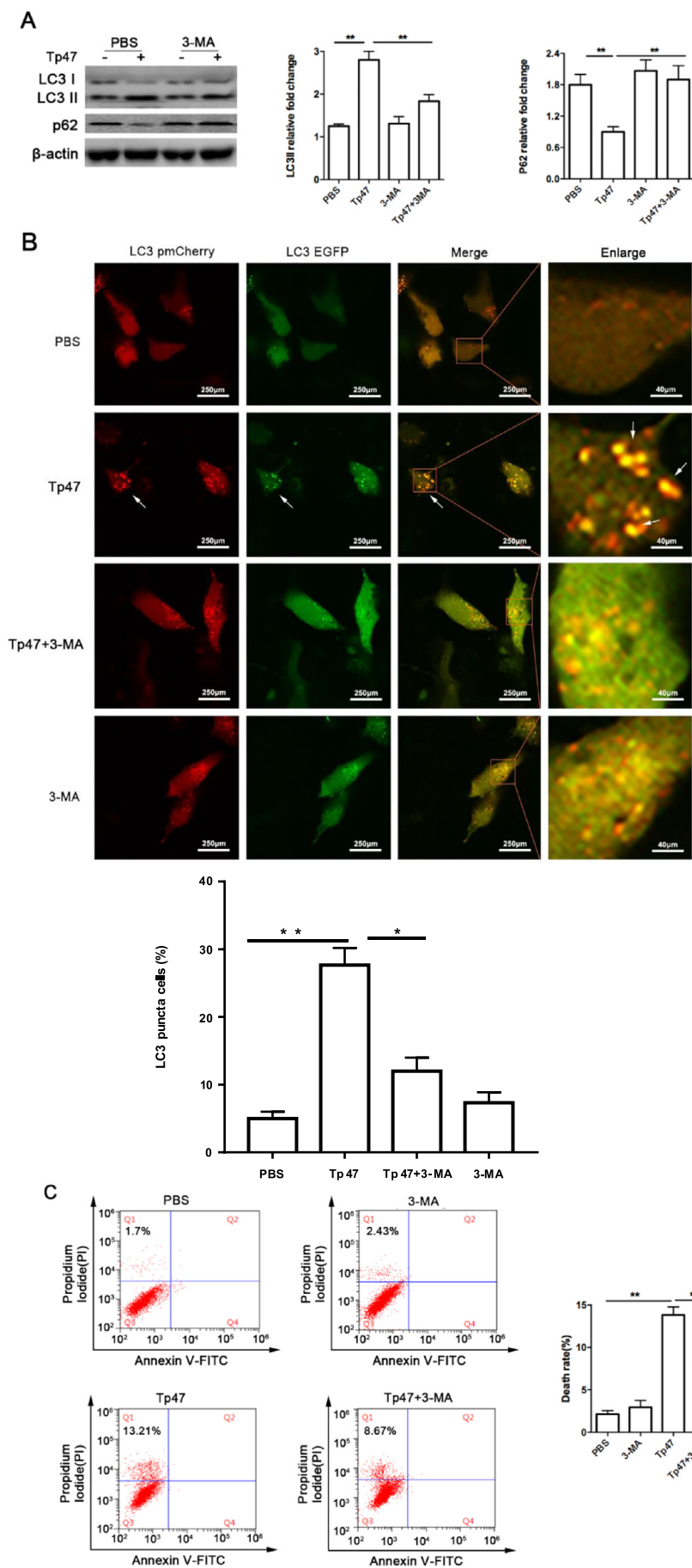
Fig. 3. Autophagy was required for Tp47-induced HMO6 cell death.

(A) Cells were pretreated with 3-MA (1 mM) for 1 h followed by treatment with Tp47 (200 μg/mL for 24 h). The data in the bar graph are the relative abundance of the LC3-II and p62 proteins in three experiments. (B) Autophagosomes were viewed by using a confocal fluorescence microscope. The arrows indicate the presence of LC3 puncta in cells. Scale bar, 250 μm. Quantification of cells containing LC3 puncta in HMO6 cells (C) HMO6 cells incubated with 3-MA for 1 h at 37 °C were cultured in the presence or absence of Tp47 for 24 h and were then costained with annexin V-FITC/PI and analysed by flow cytometry. The data in the bar graphs are the means ± SEMs of 3 separate experiments. *$P < 0.05$, **$P < 0.001$ vs. unstimulated cells.
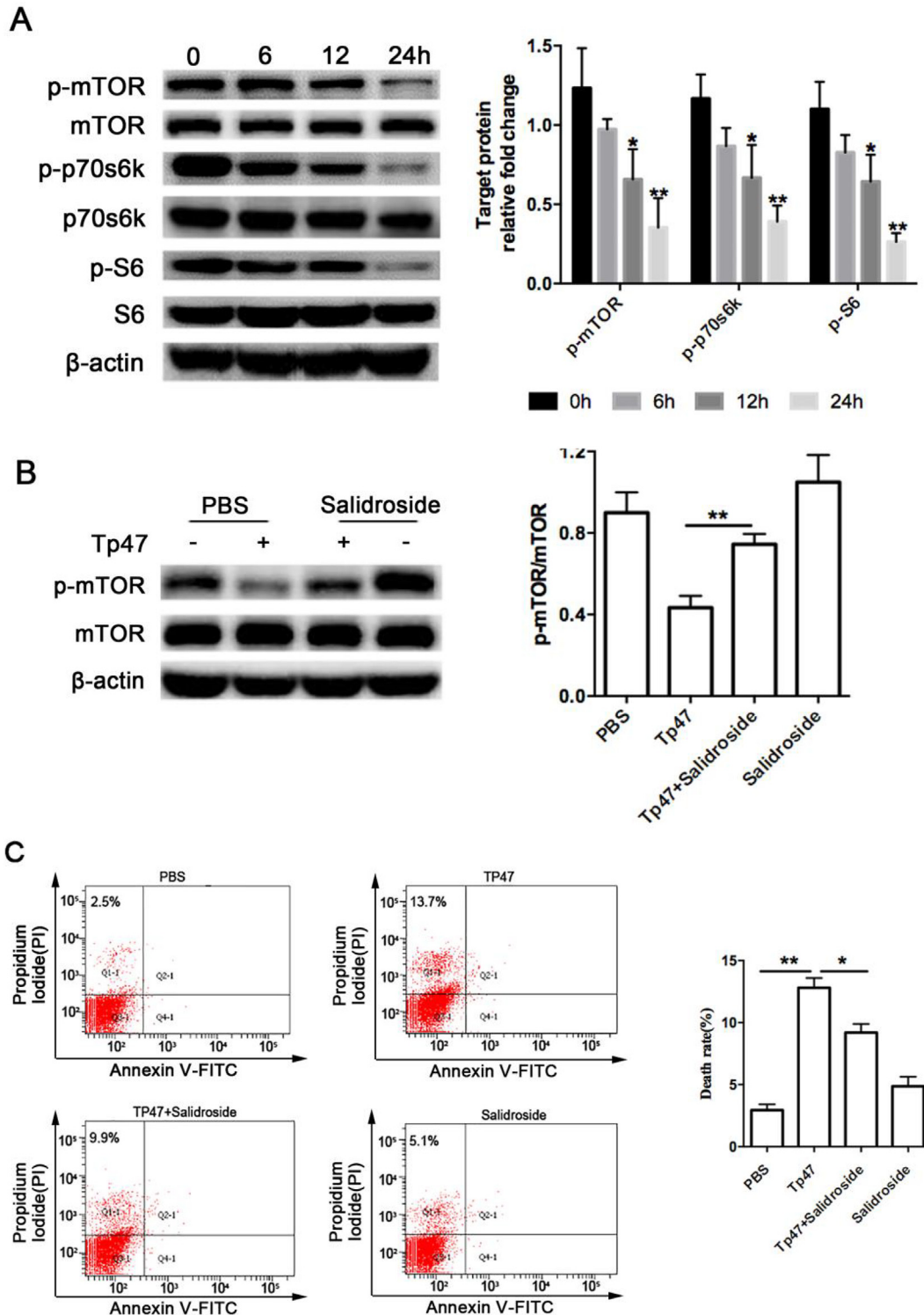
Fig. 4. Analysis of p-mTOR, p-p70s6k and pS6 expression levels in HMO6 cells.
(A). Cells were treated for various durations with Tp47 alone, and the expression of p-mTOR, p-p70s6k, p-pS6, and β-actin expression was assessed via Western blot analysis. (B) and (C) HMO6 cells were pretreated with salidroside (10 μg/mL) for 1 h prior to treatment with Tp47 (200 μg/mL) for a 24 h. p-mTOR protein levels were determined by immunoblotting, and cell viability was measured by flow cytometry. *$P < 0.05$, **$P < 0.01$.

molecular chaperone-mediated autophagy. Macroautophagy (commonly referred to simply as autophagy) involves a double- or multi-membrane-bound structure, which is termed the autophagosome or autophagic vacuole. The vacuolar membrane (referred to as the autophagosome) fuses with the lysosome to deliver the contents into the organelle lumen, where they can be degraded, and the resulting macromolecules are recycled [24]. Under normal physiological conditions, cells preserve a low basal rate of autophagy to maintain homeostasis [25]. However, autophagy is increased in response to various stimuli and stresses. Moreover, recent studies show that autophagy a crucial weapon in the host cell defence against bacterial and viral invasion. For example, the HIV proteins TAT, NEF and ENV are involved

in stimulating autophagy [26]. However, intracellular pathogens, including *Mycobacterium tuberculosis* (Mtb), have specialized anti-autophagy adaptations that allow them to block, prevent, or elude autophagic elimination [27]. In our study, we dynamically observed the formation and degradation processes of autophagosomes, i.e., autophagic flow, by immunofluorescence. The formation of puncta of the mCherry-EGFP-LC3 fusion protein was observed to be significantly increased after Tp47 treatment. mCherry-EGFP-LC3 is a well-characterized marker for visualizing autophagosomes. However, the exact mechanism regulating the autophagy induced by Tp47 has not yet been clarified. Like LPS, Tp47 is highly immunogenic and induces immune cells to produce large amounts of inflammatory factors [28], which serve an important trigger of autophagy. For example, the cytokines IFNγ and TNF generally upregulate autophagy in target cells, and IL1β can activate autophagy through a mechanism similar to that of LPS [29,30].

Although autophagy is considered a cytoprotective process under most conditions, increasing numbers of researchers have correlated increased autophagy with cell death under some conditions [31]. This process of self-digestion is widely believed to have a dual effect—cytoprotection and cytotoxicity—on cell survival. Evidence suggests that autophagy promotes either cell survival or cell death depending on the type of cell, the particular environment, the presence of a stimulus, and the duration and intensity of the stimulus [11]. An increasing number of studies have shown that pathogens can cause autophagy and even autophagic death in vivo. Recently, the autophagic-lysosomal compartment has been shown to be involved in the initiation of programmed cell death, namely, autophagic cell death (ACD) [32]. Large cytosolic autophagic vacuoles from accumulated autophagosomes, which are marked by LC3 labelling, are the most prominent characteristics of ACD. Based upon our observations and these concepts, we hypothesized that autophagy may determine microglial HMO6 cell fate under exposure to Tp47; we therefore examined the survival of these cells in the context of robust autophagy induction by Tp47. The results showed that cell death increased with autophagy and that this process was reversed by autophagy inhibitors, indicating that the induction of autophagy was involved in the mechanism of Tp47-induced microglial death and demonstrating that Tp47 stimulation may trigger autophagic cell death in microglial cells. However, the mechanism by which Tp47 induces autophagic death in microglia is unclear. Iribarren et al. demonstrated that some immunogenic substances, such as LPS and peptidoglycan, can cause autophagic death in some cells, and autophagic death mechanisms are involved in the means by which some anticancer drugs clear tumour cells. The prolonged and excessive activation of microglia can lead to changes in function and even evolve into autophagic death [33]. Moreover, the pathogenesis of *Treponema pallidum* has been found to involve cell death [34]. The Tp47 protein may play an important role in the mechanism of cell death caused by *Treponema pallidum*.

Another question is whether the mechanism by which Tp47 causes microglial autophagy death is associated with the mTOR pathway. Given the vital role of the mTOR pathway in regulating cell survival/death/autophagy in response to external stimuli, we investigated the effect of Tp47 on mTOR pathway protein expression in HMO6 microglial cells. Our results demonstrated that Tp47 decreased the basal phosphorylation level of mTOR. Salidroside, an agonist of mTOR, can improve muscle nutrition by increasing mTOR, p-mTOR, and MyHC expression [35]. However, the cell mortality was not significantly reduced after using the mTOR agonist (salidroside) compared with that seen with inhibition by 3-MA using autophagy as an indicator. Thus, Tp47 may cause cell death via multiple mechanisms involving autophagic cell death and the inhibition of the mTOR pathway. The mTOR pathway is an important signalling pathway that regulates autophagy. In the initial stage of autophagy, mTOR activity is inhibited by upstream genes, thereby activating the downstream target gene Ulk1 [36]. Activation of Ulk1 induces the formation of the Atg complex and completes the construction of the basic autophagosome structure. In addition, the complex promotes the conversion of LC3-I to LC3-II, and LC3-II is then transported to the cell membrane via Atg7 and Atg3 to complete the formation of autophagosomes [37]. Our research demonstrated that Tp47 induces autophagic death in microglia. This finding further clarifies the mechanisms underlying the immune escape and long-term chronic infection of *Treponema pallidum*, thus providing a basis for the clinical treatment of syphilis.

In conclusion, we demonstrated that autophagy was induced in human microglial cells by Tp47 and that cell death occurred after Tp47 treatment. This mechanism of death involves autophagy and the mTOR pathway, which is itself involved in the mechanism of autophagic death. Further investigations will augment our understanding of the role of autophagy in pathogenesis and the management of neurosyphilis.

Acknowledgements

Conflicts of interest

The authors declare that they have no competing interests.

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