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ORIGINAL PAPER

Induction of TNF-α by LPS in Schwann Cell is Regulated by MAPK Activation Signals

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Abstract Mitogen-activated protein kinases (MAPKs) are important mediators of cytokine expression and are critically involved in the immune response. The lipopolysaccharide (LPS) of gram-negative bacteria induces the expression of cytokines and proinflammatory genes via the toll-like receptor 4 (TLR4) signaling pathway in diverse cell types. In vivo, Schwann cells (SCs) at the site of injury may also produce tumor necrosis factor– α (TNF-α). However, the precise mechanisms of TNF-α synthesis are still not clear. The purpose of the present study was to elucidate the underlying molecular mechanisms in the cultured SCs for its ability to activate the MAPKs and TNF-α gene, in response to LPS. Using enzyme-linked immunosorbent assay (ELISA), it was confirmed that treatment with LPS stimulated the synthesis of TNF-α in a concentration- and time-dependent manner. Intracellular location of TNF-α was detected under confocal microscope. Moreover, LPS activated extracellular signal-regulated kinase (ERK1/2), P38 and stress activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) and induced their phosphorylation. LPS-elicited SCs TNF-α production was also drastically suppressed by PD98059 (ERK inhibitor), SB202190 (P38 inhibitor), or SP600125 (SAPK/JNK inhibitor). Additionally, the expression of CD14 and TLR4 was examined by RT–PCR. It was demonstrated that the expression of CD14, TLR4 was crucial for the SCs

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responses to LPS. In conclusion, the results provide novel mechanisms for the response of SCs to LPS stimulation, through MAPKs signaling pathways.

Keywords Lipopolysaccharide · Schwann cell · Tumor necrosis factor-alpha · Mitogen-activated protein kinase

Introduction

Schwann cells (SCs) are glia of the peripheral nervous system (PNS). Besides their roles in myelination, trophic support, and regeneration of axons, SCs exhibit potential for some immune functions, similarly to non-myelinating glia of the central nervous system (CNS). SCs can be induced to produce cytokines and chemokines, express major histocompatibility complex (MHC) class II molecules, adhesion molecules, and serve as antigen presenting cells. They produce chemokines and macrophage inflammatory protein-1α (Orlikowski et al. 2003), which recruit macrophages from the blood vessels and induce local inflammation. In vivo, SCs at the site of injury may also produce tumor necrosis factor-α (TNF-α), as SCs are activated and/or damaged following nerve injury, produce cytokine and neuroactive factors, and have recently been described to possess an increasing number of macrophage-like characteristics (Wagner and Myers 1996). In addition, SCs in the course of experimental autoimmune neuritis, the murine model for the human Guillain–Barré syndrome (GBS), or Wallerian degeneration following an axonal injury are producing TNF-α, interleukin (IL)-1α, and IL-1β (Shamash et al. 2002).

It is known that lipopolysaccharide (LPS) is one of the major constituents of the outer membrane of gram-negative bacteria and LPS recognition and signal transmissions are among the key events in the host–defense reaction toward gram-negative bacteria. Many different cell types such as neutrophils and macrophages can respond to LPS by releasing potent inflammatory cytokines to destroy invading bacteria (Arndt et al. 2004). These cytokines, such as TNF-α, IL-1β, IL-6, interferon (IFN), likely to the neurotoxicity observed in neurodegenerative diseases such as GBS, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS) in PNS inflammation (Czlonkowska et al. 2005).

TNF-α is a pleiotropic cytokine, the regulation of TNF-α gene expression in cells of the monocytic lineage stimulus-dependent and quite complex, involving controls at both transcriptional and post-transcriptional levels. Many studies of the transcriptional regulation of TNF-α have focused on the investigation of transcription factors that bind to the responsive element sites within the TNF-α promoter, such as nuclear factor-κB (NF-κB) (Suzuki et al. 2006), activating protein-1 (AP-1), cAMP response element-binding (CREB) protein, signal transducer and activator of transcription (STAT6) (Tang et al. 2005), and LPS-induced TNF-α factor (LITAF) (Tang et al. 2006). However, the relative contributions of these various regulatory elements in transcriptional activation of the TNF-α gene in SCs are poorly understood.

A variety of signaling pathways have been elucidated in the response to LPS (Diks et al. 2004). LPS binds to toll-like receptor 4 (TLR4) and induces a gene expression program almost identical to that induced by a whole bacteria (Jenner and Young 2005). TLRs are specialized surface molecules that recognize a variety of pathogenic molecules and initiate signaling cascades leading to the activation of the innate immune system (Uematsu and Akira 2006). TLR4 recognizes the majority of bacterial lipoproteins and is expressed on a variety of immune cells, including antigen presenting cells and $CD4^+$ T cells (Caramalho et al. 2003).

The best described of which is the pathway leading to mitogen-activated protein kinases (MAPKs) activation through TLR4. MAPKs are important mediators of cytokine expression; in particular, p38 and extracellular signal-regulated kinase (ERK) play a key role in LPS-induced signal transduction pathways leading to cytokine synthesis. Recently, an adaptor molecule homologous to myeloid differentiation factor 88 (MyD88) has been shown to be involved in the activation of the MAPKs by TLR4. In a fashion similar to that of MyD88 knockout mice, Tirap knockout mice demonstrate delayed activation of the MAPKs after LPS exposure (Schaefer et al. 2005). These findings suggest the presence of pathways independent of MyD88 and Mal/Tirap, which lead to the activation of the MAPKs after LPS stimulation.

In our experiments, we investigated the signaling mechanism by which the activation of SCs CD14, TLR4, and MyD88 regulated TNF-α synthesis via ERK, stress activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and P38MAP kinase, and specific inhibitors of MAPK subgroups could block SCs expression of TNF-α involving transcriptional control mechanism.

Materials and Methods

SC Culture and Treatment

SCs were cultured from excised dorsal root ganglion (DRG), brachial plexus, and sciatic nerves from SD rat as described previously. Briefly, cells were cultured with dulbecco's modified eagle's medium (DMEM) containing 10% calf serum. In order to reduce the number of dividing fibroblasts, SCs cultures were treated with monoclonal antibody anti-thy 1.1 and a rabbit complement (Brockes et al. 1979). Fibroblast contamination was minimal after this stage (~1.5%). For cell culture use, LPS (*Escherichia coli*, 0127:B8, Sigma) should be reconstituted by adding cell culture medium to a vial and swirling gently until the powder dissolves. The LPS concentration was 1 μg/μl. SCs cultures were primed for indicated times or indicated concentrations with LPS. In some experiments, PD98059, SB202190, and SP600125 were applied 1 h prior to LPS stimulation.

Detection of Intracellular and Extracellular TNF-α by ELISA

The concentrations of TNF-α released in the culture medium and present in cell lysates (i.e., intracellular) were quantified by specific rat TNF-α enzyme-linked immunosorbent assay (ELISA) according to the instruction of the manufacturer.

Cell Death Assay

For quantitative analysis of cell viability, 10 μl of WST-8 (Cell Counting Kit-8, CCK-8, Dojindo, Japan) solution was added to each well, and, after incubation at 37°C for 3 h in a humidified CO_2 incubator, absorbance at 450 nm was monitored with a microplate reader (ELx800™ Absorbance Microplate Reader, USA). The cell viability was calculated by the normalization of optical densities (OD) to those of control cells incubated with vehicle only. The terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) assay kit was used to detect apoptotic cells (Roche, Germany). Immunohistochemistry of TUNEL-positive cells was completed by anti-fluorescein antibody Fab fragments, conjugated with alkaline phosphatase. After substrate reaction, stained cells can be analyzed under light microscope.

RNA Isolation and Reverse Transcriptase PCR (RT-PCR) Analysis

Total cytoplasmic RNA of SC was extracted using Trizol extraction kit according to the manufacturer's protocol. Total RNA was reverse-transcribed using ThermoScript reverse transcriptase PCR (RT–PCR) system (Invitrogen, USA). Primer sequences used in this report were listed as follows: TNF-α primers: sense, CGTCGTAGCAAACCACCAAG; antisense, CACAGAGCAATGACTCCAAAG; TLR4 primers: sense, GACATCCCTTATTCAACCA; antisense, TTTGTCTCCACAGCCACC; CD14 primers: sense, TGCTCGGCTTGTT GCTGTT; antisense, AACTTGGAGGGTCGGGAAT; MyD88 primers: sense, AGCCTTGTTA GACCGTGAG; antisense, GCAGATAGTGATGAACCGTAG. The GAPDH was used as an internal control and was detected using the following primers: sense, TGATGACATCAAGAAGGTGGTGAAG; antisense, TCCTTGGAGGCCATGTGGGCCAT. PCR was performed in a Mastercycler personal with the following parameters: TNF-α, CD14, and MyD88 (denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min each step), 26 cycles for TNF-α, 30 cycles for MyD88, 29 cycles for CD14; 30 amplification cycles for TLR4 (94°C for 45 s, at 58°C for 45 s, 72°C for 1 min); 24 cycles for GAPDH (94°C for 45 s, at 55°C for 45 s, 72°C for 1 min). The signal intensities of RT–PCR products were quantified by calculating the integrated volume of the band with a computing laser densitometer.

Immunoblot Analysis

After appropriate stimulation, cell lysates were obtained by scratching cell in a lysate buffer. Protein were loaded into wells of a 10% acryl/bisacrylamide gel, and after separation, proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. After saturation in Tris-Buffered Saline Tween-20 (TBST) containing 5% milk, primary antibody and secondary horseradish peroxidase-conjugated antibody diluted in TBST were sequentially added to and incubated with the membranes for overnight and 2 h, respectively. Revelation was obtained by enhanced chemiluminescence (ECL).

Immunocytochemistry

The cells were fixed with 4% formaldehyde for 30 min, then treated with 0.1% TritonX-100/PBS for 5 min, and incubated with phosphate buffered saline (PBS) containing 3% normal goat serum for 1 h. The cells were incubated overnight at 4°C with polyclonal anti-TNF-α antibody (Santa Cruz, USA), rabbit anti-p-ERK, p-P38, p-SAPK/JNK (cell signal, 1:500), and monoclonal anti-S-100 (Sigma, USA). After the cells were rinsed with PBS, they were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-goat (TNF-α) or rabbit IgG (p-ERK, p-P38, p-SAPK/JNK) in blocking solution and tetramethyl rhodamine isothiocyanate (TRITC)-labeled anti-mouse IgG (Jackson, USA) to visualize monoclonal antibody (S-100). The cells were rinsed and mounted onto slides, which were then analyzed and imaged by confocal laser scanning microscopy.

Results

LPS Induces the Expression of TNF-α mRNA and Protein in SCs

SCs were treated with various concentrations of LPS (1, 10, 100 μg/ml) for 2 h and we found that LPS-induced TNF-α biosynthesis in a concentration-dependent manner (Fig. 1A).

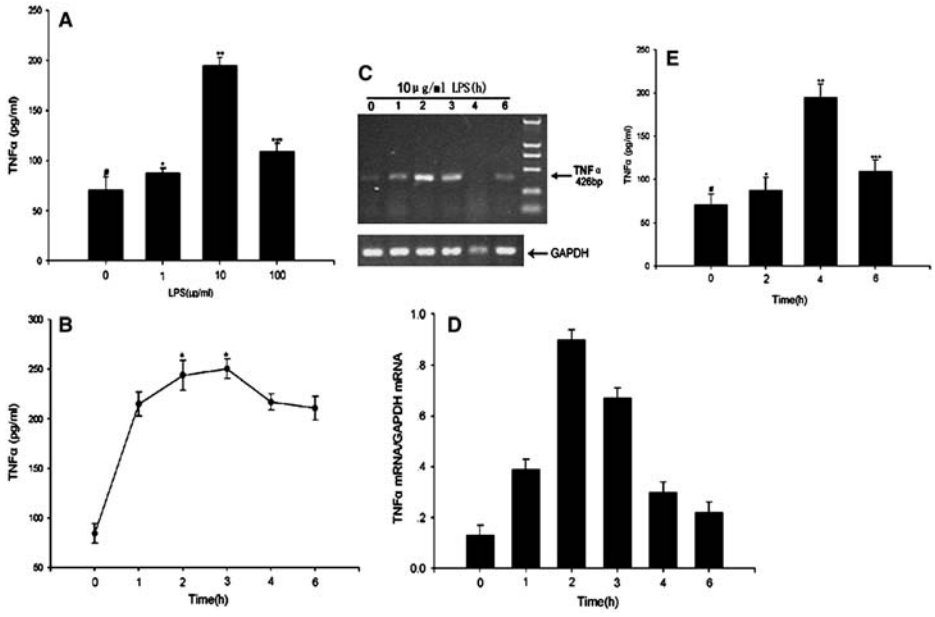


Fig. 1 LPS induces the expression of TNF-α mRNA and protein in cultured SCs. LPS induces TNF-α synthesis in a (**A**) concentration- and (**B**) time-dependent manner. (A) Cultures were untreated or treated with various concentrations of LPS for 2 h in triplicate. Data were expressed as mean ± SEM of the maximum response observed. *Significant difference between untreated cultures and cultures treated with 10 μg/ml LPS ($n = 3$; $^{**}P < 0.01$); 100 μg/ml ($n = 3$; $^{***}P < 0.05$). (**B**) Cultures were treated with 10 μg/ml LPS for various times (0, 1, 2, 3, 4, 6 h) in triplicate. *Significant difference between untreated cultures and cultures treated for 3 h ($n = 3$; $^{*}P < 0.05$). (**C**) Time-course of TNF-α mRNA expression in LPS cultured SCs. (**D**) Integrated band densities were obtained by scanning using a densitometer. Plotted mean ± SEM of three independents experiments. (**E**) LPS induces TNF-α release in a time-dependent manner. TNF-α release and total protein in each well were determined as described in Experimental Procedures. Cultures were treated with 10 μg/ml LPS for various times (0, 2, 4, 6 h) in triplicate. Data were collected from three independent experiments and expressed as mean ± SEM of the percentage of the maximum response observed at 4 h treatment

Significant biosynthesis was observed at 10 μg/ml LPS ($^{**}P < 0.01$). Of note, treatment with 100 μg/ml LPS appeared to induce less TNF-α biosynthesis than treatment with 10 μg/ml LPS; this may reflect a loss of cell viability or numbers at the highest LPS concentration. We also conducted time course studies at 10 μg/ml LPS. Significant TNF-α biosynthesis was observed at 1 h ($P < 0.01$) and the maximum response occurred at following 2–3 h ($^{*}P < 0.001$) induced by 10 μg/ml LPS (Fig. 1B). But there was no significant difference between the response at 2 h and 3 h. Exposure of the cells to LPS also induced the expression of TNF-α mRNA as well as TNF-α protein (Fig. 1C, D). The kinetic profile of the expression of TNF-α mRNA by RT–PCR analysis revealed a time-dependent induction of TNF-α mRNA by LPS. TNF-α mRNA was detected as early as 1 h, peaked at 2 h, and then started to decline by 3 h. To detect the release of TNF-α from the LPS-treated SCs, we analyzed the supernatant of medium at indicated times. Significant TNF-α release was observed at 2 h ($^{*}P < 0.01$) and the maximum release occurred following 4 h treatment ($^{**}P < 0.001$) (Fig. 1E). Analysis through immunofluorescent staining also showed a cytoplasmic staining of TNF-α in the SCs after stimulation with LPS. TNF-α was significantly co-localized with S-100 (SCs marker) in the LPS treated cytoplasm of SCs (Fig. 2b, d). In nonstimulated SCs, TNF-α was scarcely

observed in the cytoplasm (Fig. 2b0, d0). Although there were few fibroblasts among our acquired SCs, they did not express TNF-α (Fig. 2d, arrows).

Effects of LPS on Cell Viability

The cytotoxic effects of LPS on mouse leukocytes have been examined. In addition, LPS was cytotoxic for macrophages (Peavy et al. 1978). Cytotoxic factor can be triggered by LPS, but a low LPS concentration is less toxic. However, cytotoxic of LPS at high concentrations has not been reported in SCs. Therefore, we examined the cytotoxic of LPS in SCs. During experiments, we first observed the effect of various concentrations of LPS (1, 10, and 100 μg/ml) on cell viability for 3 h or 24 h. Interestingly, higher doses of LPS (100 μg/ml) did result in loss of cell viability either 3 h or 24 h, and OD decrease rapidly (Fig. 3A). Apoptotic cell death was also assessed at 24 h by TUNEL. We treated the SCs at 100 μg/ml LPS for 24 h. There was a significant increase in the number of apoptotic cells in LPS-treated cells (Fig. 3B), as a high concentration and a long time treatment of LPS had a predominant cytotoxic effect. These results may elucidate that treatment with 100 μg/ml LPS appears to induce less TNF-α biosynthesis than treatment with 10 μg/ml LPS.

LPS Modulates the Expression of CD14, TLR4, and MyD88 in SCs

The cellular recognition of and response to LPS stimulation depend on the interplay of CD14 and TLR4/MyD88 compounds. Our results showed that SCs exhibited a relatively high level of expression of TLR4 mRNA and MyD88 mRNA after treatment of LPS. However, SC expressed CD14 mRNA no matter they were treated with LPS or not (Fig. 4A, B). These data demonstrate that SCs bind to LPS by cell membrane LPS receptors such as CD14 and TLR4 and then activate the MAPK signaling pathway.

LPS Activates the MAPKs and Effect of MAPKs Inhibitors on LPS-induced Phosphorylated MAPKs in SCs

The activation of MAPKs is also important to transmit LPS-induced signals in many cell types. As illustrated in Fig. 4C, the activities of ERK, P38, and SAPK/JNK were significantly increased in the LPS-treated SCs. Specifically, significant phosphorylation of P38 and SAPK/JNK was observed at 30 min, followed by a gradual decline reaching basal levels after 45 min, and the maximum response occurred again following persistent stimulation of LPS for 120 min. These indicate that TNF-α, which synthesis in SCs, has the ability to induce its own biosynthesis through p38 and SAPK/JNK pathway in SCs. Therefore, utilizing a longer treatment time can activate the phosphorylation of P38 and SAPK/JNK due to autocrine effects of TNF-α itself.

To detect the effect of PD98059, SB202190, and SP600125 on the inhibition of phosphorylated ERK (pERK), phosphorylated P38 (pP38), and phosphorylated JNK (pJNK) activity in SC, respectively, SCs were pretreated with the PD98059, SB202190, and SP600125 for 1 h and stimulated with 10 μg/ml LPS for 30 min. As shown in Fig. 5, the inhibited effect of pERK, pP38, and pJNK were displayed by using 50 μM PD98059, 20 μM SB202190, and 30 μM SP600125 and the inhibited effect of pERK, pP38, and pJNK was striking by using 70 μM PD98059, 40 μM SB202190, and 50 μM SP600125 in SCs, respectively.

Analysis with immunofluorescent staining also showed a cytoplasmic staining for pERK, pP38, and pSAPK/JNK in the SCs after stimulation with LPS. As illustrated in Fig. 6a″, c″, and e″, the phosphorylated-ERK, P38, and SAPK/JNK were significantly co-localized with

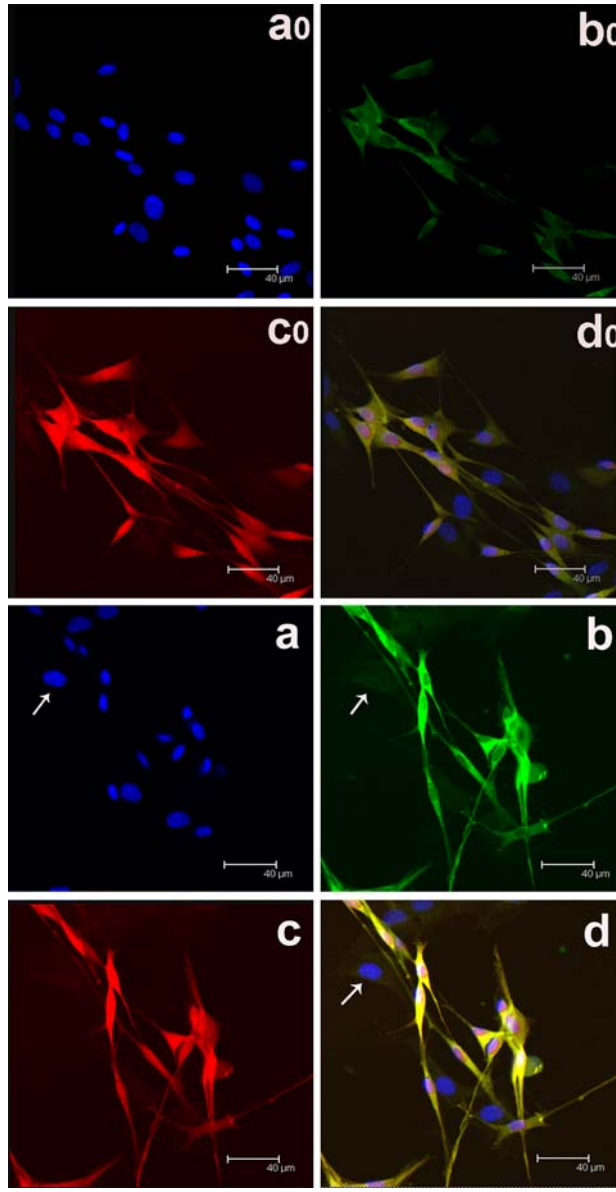


Fig. 2 TNF-α expression in cultured SCs after LPS challenge. Double fluorescence staining is performed using SCs marker S-100 (red) and polyclonal antibody against TNF-α (green). Pseudocolor in yellow indicates colocalization (d). Nucleus of SCs and fibroblasts were stained with Hoechst (a0, a), TNF-α in the cytoplasm of SCs were stained with green fluorescence, LPS induce expression of TNF-α in SCs, but not in some fibroblasts (b, arrow), S-100 positive of SCs were stained with red fluorescence (c0, c). The remaining unstimulated dish was used as a control (a0, b0, c0, d0). Merged images were shown in d0 and d

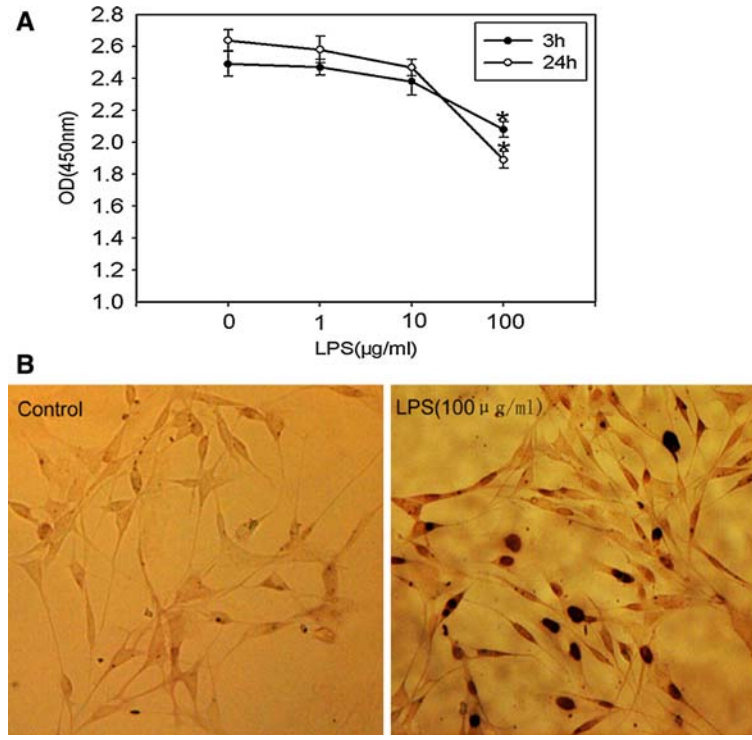


Fig. 3 Effect of LPS on SCs viability. Cell viability was assessed by using a Cell Counting kit (CCK-8). Cells were pretreated without or with 1, 10, and 100 μg/ml LPS for 3 h or 24 h, as indicated. $*P < 0.05$, compared with cultures treated with 10 μg/ml LPS (**A**). Apoptotic cell was detected using TUNEL. Brown-stained nuclei are considered apoptosis in LPS (100 μg/ml)-treated group. There was no significant apoptotic cell in control group (**B**)

S-100 in the LPS treated cytoplasm of SCs. We also used specific inhibitors of MAPKs, and pretreatment cell with these inhibitors weakened the intensity of fluorescence, as shown in Fig. 6b′, d′, and f′.

The Effects of MAPK Inhibitors on TNF-α Synthesis Induced by LPS

It has been demonstrated that LPS can significantly activate ERK, p38, and SAPK/JNK. To assess whether MAPKs play an important role in LPS-induced TNF-α synthesis, we used specific inhibitors of MAPKs cascades: PD98059 (Fig. 7A, B), SB202190 (Fig. 7C, D), and SP600125 (Fig. 7E, F). All the three reagents inhibited TNF-α and TNF-α mRNA production induced by LPS. They exert inhibitory effects in a concentration-dependent manner. These results suggest that LPS stimulation induces the activation of the MAPKs cascade, leading to the synthesis of TNF-α. So it is reasonable to conclude that the production of TNF-α is regulated by ERK, P38, and SAPK/JNK at transcriptional level.

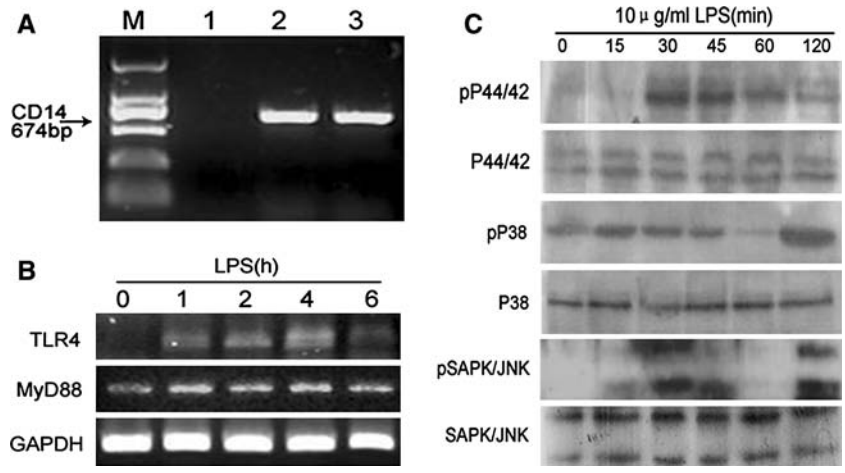


Fig. 4 Activation of MAPKs in LPS-stimulated SCs through binding with CD14, TLR4. (**A**, **B**) Expresses CD14, TLR4, and MyD88 mRNA after treated SCs with 10 μg/ml LPS for 1 h (**A**: lane 1, water, as a negative control; lane 2, SCs; lane 3, SCs with LPS treatment). (**C**) The phosphorylated, total ERK (P44/42), P38, and SAPK/JNK detected by Western blot

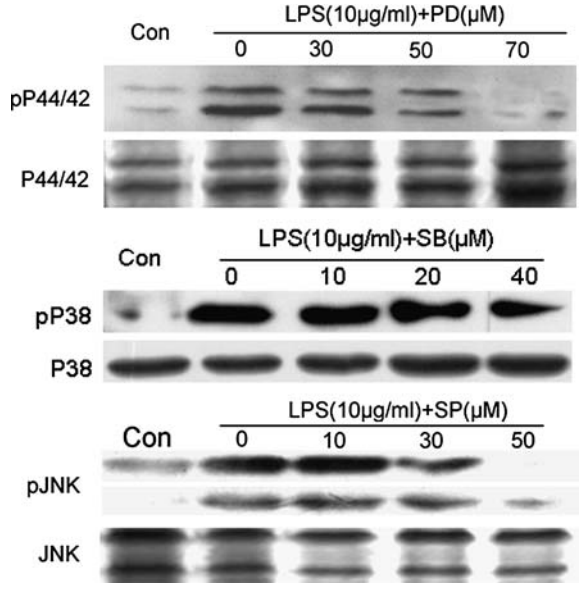


Fig. 5 Inhibition of LPS-induced phosphorylated-ERK (pERK), pP38, and pSAPK/JNK activation by inhibitor of MAPKs in SCs. Cell were treated with LPS in the absence or presence of PD98059 (30, 50, and 70 μM), SB202190 (10, 20, and 40 μM), and SP600125 (10, 30, and 50 μM). Cell extracts were subsequently prepared and subjected to Western blotting using antibody specific for pERK, pP38, pSAPK/JNK and total-ERK (tERK), tP38, and tSAPK/JNK

Discussion

The overall aim of this study is to investigate the way that MAPKs regulate TNF-α processing. We have identified a novel signaling pathway in the LPS-stimulated SCs, which induces the activation of MAPK. From the results presented here, we can draw a general scheme of coordinated events. The immune challenge of SC by LPS, through interaction with CD14 and TLR4 on the surface of the membrane, triggers the intracellular synthesis of TNF-α. CD14 is a

Fig. 6 The immunofluorescence analysis of phosphorylated-ERK (pERK), pP38, and pSAPK/JNK expression after LPS stimulation in SCs. Cell were incubated with LPS for 30 min or pretreated with indicated inhibitor for 1 h. Immunofluorescence reveals that LPS increases pERK, pP38, and pSAPK/JNK-IR extensively in the SCs (a″, c″, e″). Double immunofluorescence reveals that pERK, pP38 and pSAPK/JNK-IR (a, c, e, green) co-localizes with S-100 (a′, c′, e′, red) in the cytoplasm of SCs. Pretreatment cell with indicated inhibitor PD98059 (50 μM), SB202190 (20 μM), and SP600125 (30 μM) weaken LPS-induced activation of ERK, P38, and SAPK/JNK (b″, d″, f″)

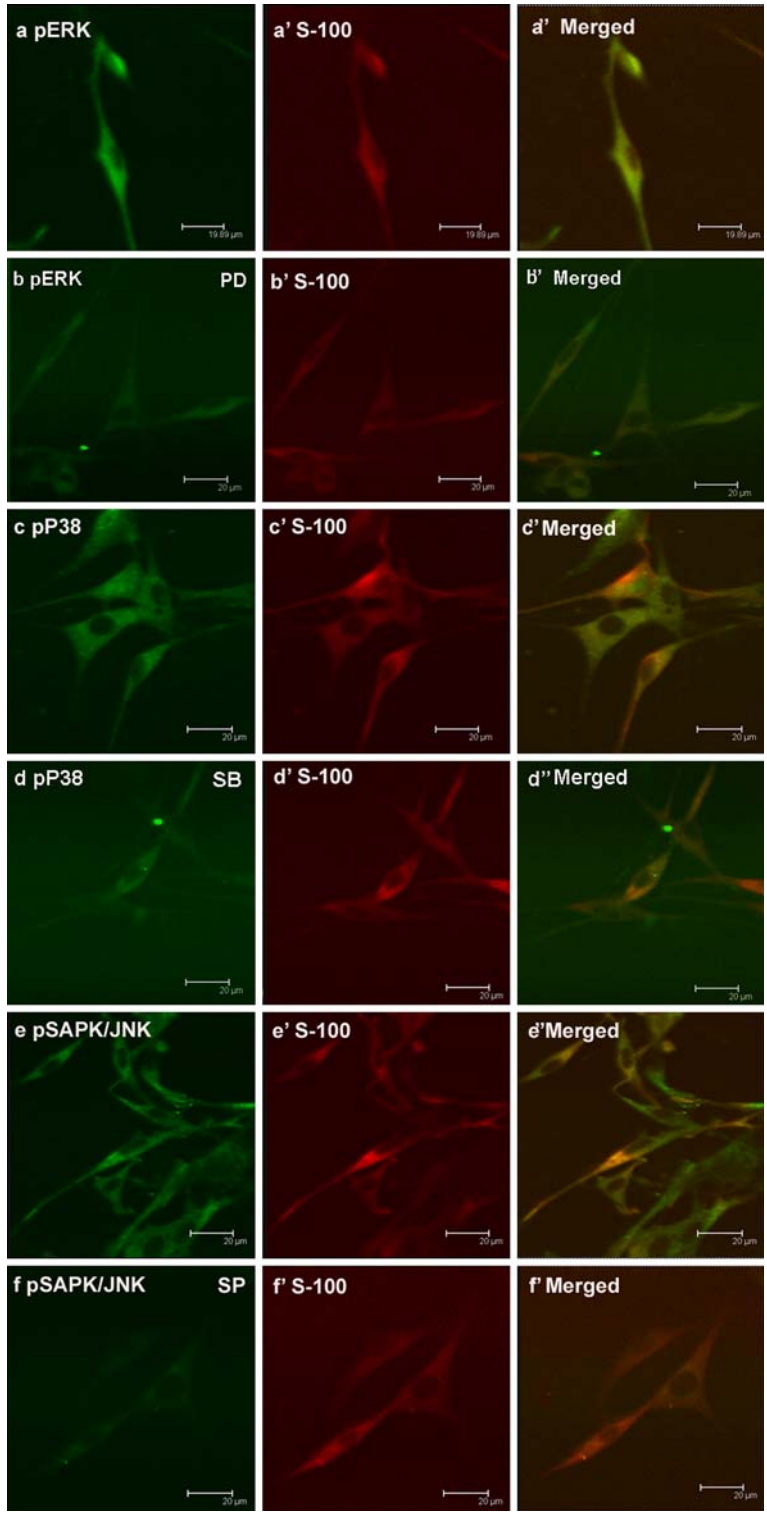
cell surface receptor binding with LPS and transferring signals to TLR4 (Jiang et al. 2005) and the CD14–LPS complex activates TLR4 and MyD88. Upon activation of TLR, MyD88 is recruited to TLR domains and links TLR with the downstream intracellular signaling cascades, leading to the induction of many key cytokines.

Now, we have first demonstrated the expression of CD14, TLR4, and MyD88 mRNA in the rat SCs. TLR4 is specifically activated by the endotoxin LPS and its stimulation leads to the activation of MAPKs, which regulate the expression of TNF-α as well as several other immune response genes. Therefore, cultured SCs express membrane receptors and do possess the needed intracellular mechanism to trigger an innate immune response to bacterial LPS.

TNF-α is the most important cytokine in the process of immune regulation and inflammation. The precise mode of action of MAPK in SCs TNF-α production is not yet known. It has been reported that gram-negative bacterial LPS is a potential inducer of TNF-α gene expression, that the transcriptional activation of TNF-α is regulated in an NFκB-dependent manner in monocytes and macrophages. Based on our results, the production of TNF-α was significantly increased in SCs treated with LPS and ERK, P38, and SAPK/JNK mediated TNF-α synthesis through transcriptional regulation. Moreover, TNF-α is a pleiotropic cytokine that can cause either beneficial or detrimental properties through its proinflammatory and proapoptotic effects in various cell types (Aggarwal 2003). It is still not entirely clear whether SCs protect or harm neurons and whether TNF-α is beneficial or toxic. Recent reports indicate that the dual actions of TNF-α are mediated via different TNF receptors (TNFR), with the TNFR1 eliciting neurotoxic effects and the TNFR2 eliciting neuroprotection (Yang et al. 2002). Interestingly, these two receptors have been shown to have similar specific roles in oligodendrocytes, with TNFR1 being implicated in demyelination and TNFR2 in remyelination (Arnett et al. 2001).

Traditionally, it seems that PNS is protected by the blood–nerve barrier from bacterial assault. However, the PNS is not completely sheltered from bacteria. As a prototypical example, *Mycobacterium leprae*, the causative agent of leprosy, acts specifically on SCs to induce their apoptosis and causes nerve damage. *M. leprae* has a remarkable affinity to SCs, the molecular basis of which has been elucidated recently: *M. leprae* binds specifically to the G domain of the extracellular matrix protein laminin-2, which ligates to α/β-dystroglycan receptor-complexes on myelinating SCs (Ng et al. 2000). Thus, *M. leprae* has illustrated the interactions between matrix- and cytoskeletal-linked glycoproteins to target and infect SCs. Very interestingly, SCs express TLR2, which seems to be responsible for the ability of *M. leprae* to induce SCs apoptosis (Oliveira et al. 2003). The infancy of the knowledge of the mammalian TLR family as key molecules of the innate immunity probably narrow our vision of the relevance of these molecules in SCs. However, further studies are still needed to determine whether TLRs are expressed on SCs in vivo, as they are regarded as important items of the panoply used by SCs to regulate local inflammatory responses.

In summary, we demonstrated that SCs responded to LPS stimulation, involving the activation of MAPK activity and TNF-α expression, induction of CD14, TLR4, and MyD88. More studies are needed to clarify whether the transcriptional activation of TNF-α is regulated in an NF-κB or AP-1-dependent manner. It is notable that our investigation was carried out by cell


a pERK
a' S-100
a' Merged
b pERK
PD
b' S-100
b' Merged
c pP38
c' S-100
c' Merged
d pP38
SB
d' S-100
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e pSAPK/JNK
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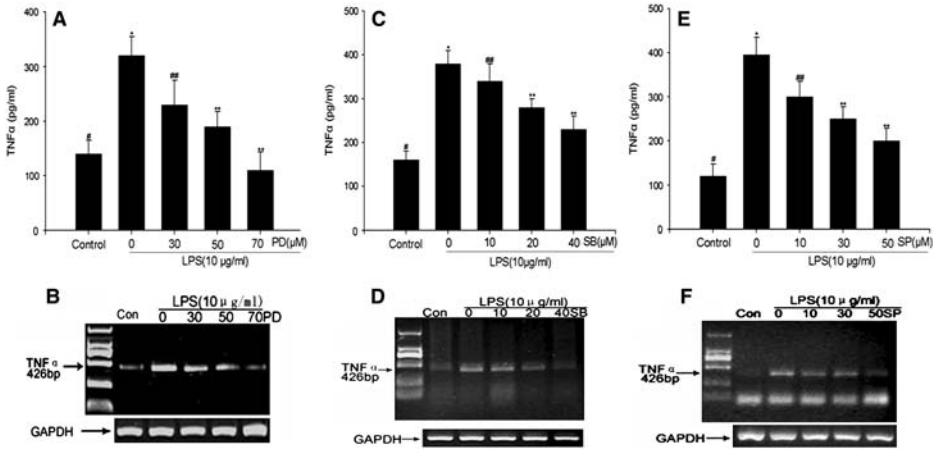

Fig. 7 Effects of inhibitors of MAPKs on TNF-α synthesis in SCs. The cells were pretreated with various concentrations of PD98059 (30, 50, and 70 μM), SB202190 (10, 20, and 40 μM), and SP600125 (10, 30, and 50 μM) for 60 min and then stimulated with LPS for 2 h. The intracellular TNF-α (**A**, **C**, **E**) and TNF-α mRNA (**B**, **D**, **F**) contents were measured by ELISA and RT–PCR, respectively

culture in vitro. However, in vivo, the results should be further confirmed, ideally with human SCs, in order to signify the importance of SCs response to inflammation of peripheral nerve after injury. Activation of MAPKs pathways might be a precondition for the inducement of TNF-α expression. Reducing the biosynthesis of TNF-α and other cytokines by blocking the cell signal pathway may give us a new strategy against inflammatory and immune reaction after injury of peripheral nerve.

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