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The HMGB1 signaling pathway activates the inflammatory response in Schwann cells

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Abstract

Schwann cells are not only myelinating cells, but also function as immune cells and express numerous innate pattern recognition receptors, including the Toll-like receptors. Injury to peripheral nerves activates an inflammatory response in Schwann cells. However, it is unclear whether specific endogenous damage-associated molecular pattern molecules are involved in the inflammatory response following nerve injury. In the present study, we demonstrate that a key damage-associated molecular pattern molecule, high mobility group box 1 (HMGB1), is upregulated following rat sciatic nerve axotomy, and we show colocalization of the protein with Schwann cells. HMGB1 alone could not enhance expression of Toll-like receptors or the receptor for advanced glycation end products (RAGE), but was able to facilitate migration of Schwann cells. When Schwann cells were treated with HMGB1 together with lipopolysaccharide, the expression levels of Toll-like receptors and RAGE, as well as inflammatory cytokines were upregulated. Our novel findings demonstrate that the HMGB1 pathway activates the inflammatory response in Schwann cells following peripheral nerve injury.

Key Words: *nerve regeneration; peripheral nerve injury; sciatic nerve transection; Schwann cells; HMGB1; migration; inflammatory response; TLRs; peripheral nerves; DAMPs; cytokines; neural regeneration*

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Introduction

Peripheral nerve injuries can result in the loss of sensory and motor function and painful neuropathies. Multiple factors, including trauma, diseases, disorders and toxins, can damage nerves (Coleman, 2005; Nave et al., 2007; Blakemore and Franklin, 2008). A variety of changes occur following nerve crush injury or axotomy, such as degeneration of detached axon segments, dedifferentiation of myelinating and ensheathing Schwann cells, recruitment of immune cells, and initiation of axonal regrowth (Navarro et al., 2007). This complex but orchestrated cellular process is under the control of intracellular and extracellular signaling mechanisms in tissues (Navarro et al., 2007). The peripheral nervous system (PNS) has a defense mechanism to alert the immune system following injury. Schwann cells and immune cells respond to injury by expressing various innate pattern recognition receptors, including Toll-like receptors (TLRs) and the receptor for advanced glycation end products (RAGE) (Goethals et al., 2010). Ligands of endogenous origin released from injured cells or of exogenous origin derived from microbial organisms are able to interact with TLRs and/or RAGE on these cells to trigger immune responses. The signaling pathways in immune cells that regulate the immune response have been extensively investigated (Yanai et al., 2009). However, it remains unclear how Schwann cells respond to nerve injury and how the subsequent inflammatory signaling reactions are triggered. TLR3, TLR4 and TLR7 are constitutively expressed by unstimulated Schwann cells, and the expression of TLR1 is upregulated in injured nerves (Goethals et al., 2010). This suggests that Schwann cells may function as sentinels for the innate immune response in the PNS.

The endogenous damage-associated molecular pattern (DAMP) molecules at sites of tissue damage are important triggers of the sterile inflammatory response by binding to TLRs and RAGE (Rubartelli et al., 2007). Many DAMPs are nuclear or cytosolic proteins, and are released from cells after tissue injury (Rubartelli and Lotze, 2007). One of the key DAMP molecules is high mobility group box 1 (HMGB1, amphoterin), which is passively released from necrotic cells

or is actively secreted by neurons and many other cell types upon stimulation (Lotze and Tracey, 2005; Rauvala and Rouhiainen, 2007; Dong et al., 2013). HMGB1 was initially identified as a nuclear protein that participates in nucleosome stabilization, gene transcriptional regulation, and neurite outgrowth (Merenmies et al., 1991; Lotze and Tracey, 2005; Stros, 2010; Yang et al., 2013). Extracellular HMGB1 has been demonstrated to induce the production of inflammatory cytokines by activating macrophages, monocytes and dendritic cells (Yanai et al., 2009). In addition, the protein is able to promote cell migration and cellular differentiation in non-inflammatory settings (Rouhiainen et al., 2004; Yang et al., 2007; Chitanuwat et al., 2013; Wang et al., 2014). Schwann cells play a pivotal role in nerve regeneration in the PNS (Jessen et al., 2008; Kim et al., 2013; Toy and Namgung, 2013; Whalley, 2014). However, little information is available on the role of HMGB1 in inflammatory signaling in Schwann cells. In the present study, we examined the expression of HMGB1 in the axotomized sciatic nerve, and investigated its role in the inflammatory response in Schwann cells.

Materials and Methods

Animals

A total of 75 adult male Sprague-Dawley (SD) rats, each weighing ~180 g, and 20 postnatal day 1–3 (P1–P3) rats were used in this study. Adult rats were anesthetized by an intraperitoneal injection of complex narcotics (85 mg/kg chloral hydrate, 42 mg/kg magnesium sulfate, 17 mg/kg sodium pentobarbital). A 1-cm-long segment of sciatic nerve was then resected at the site just proximal to its division into the tibial and common peroneal nerves, and the incision sites were then closed. Proximal and distal segments of the nerve (0.5 cm in length) were collected at 0 and 6 hours, and at 1, 4, 7 and 14 days after injury. The experiments were approved by the Animal Care and Use Committee of Nantong University of China and Jiangsu Province Animal Care Ethics Committee (Approval No. SYXK (Su) 2007-0021).

Immunohistochemistry

The sciatic nerve was harvested, post-fixed and sectioned at 10 μm. Sections were incubated with rabbit anti-HMGB1 (1:1,000; Abcam, Shanghai, China) and mouse monoclonal anti-S100 (1:500; Sigma-Aldrich, St. Louis, MO, USA) at 4°C overnight. The sections were then incubated with Cy3-labeled goat anti-rabbit IgG (1:800; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or FITC-labeled goat anti-mouse IgG (1:200; Santa Cruz) at 4°C overnight, followed by three PBS washes. The sections were stained with 5 μg/mL Hoechst 33342 dye (Sigma-Aldrich) for 15 minutes, mounted in fluorescence mounting medium, and photographed under a confocal laser scanning microscope (TCS SP5, Leica Microsystems, Mannheim, Germany).

Western blot analysis

Protein was extracted from proximal and distal segments using the Tissue Total protein extraction kit (CytoMol Corporation, San Francisco, CA, USA) with the addition of 1 mM phenylmethylsulfonyl fluoride (PMSF). Protein concentrations were determined by the Bradford method to equalize loading (Kruger, 1994). Protein samples were electrophoretically separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). After incubation for 1 hour with 5% nonfat dry milk in Tris-buffered saline with Tween-20 (TBS-T), the membrane was probed with rabbit anti-HMGB1 antibody diluted in blocking buffer (1:10,000) overnight at 4°C. After extensive washing with TBS-T, the membrane was incubated with IRDye 800-conjugated secondary antibody diluted in blocking buffer (1:40,000; LI-COR Biosciences, Inc.) for 1 hour at room temperature. The images were scanned with an Odyssey Infrared Imaging System (LI-COR Biosciences, Inc.), and the optical density (OD) was analyzed using Quantity One software (v4.5.0, Bio-Rad).

Schwann cell culture and treatment

Primary Schwann cells were isolated from neonatal rat sciatic nerve or dorsal root ganglia (DRG) taken from P1–P3 pups, as previously described (Weinstein and Wu, 2001). Briefly, the skin was sterilized with 70% ethanol, and the animals were killed by decapitation. The sciatic nerve and DRG were carefully removed and dissociated with collagenase and trypsin. Cells were plated onto poly-L-lysine-coated culture plates in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and maintained in an incubator at 37°C in a 5% CO_2 incubator. After 24 hours, medium was replaced with DMEM/10% FBS supplemented with 10 μM Ara-C. Forty-eight hours later, the medium was replaced with DMEM/10% FBS supplemented with 2 μM forskolin and 50 ng/mL β-heregulin (HRG) to induce Schwann cell proliferation. When the Schwann cells reached 90–95% confluence, they were purified using anti-Thy1.1 antibody on ice for 2 hours, followed by incubation in complement for an additional 1 hour at 37°C. Schwann cells were cultured in DMEM/F12 with or without 1 mM dibutyryl cyclic AMP (db-cAMP) (Sigma-Aldrich) for 3 days to induce differentiation. Purity of the Schwann cells was determined by antibodies against S100. For siRNA transfection, Schwann cells were transfected with siRNAs (Ribobio, Guangzhou, China) using Lipofectamine RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions.

Cell proliferation assay

Schwann cell proliferation was assessed using a 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay. Schwann cells were plated in poly-L-lysine-coated 96-well plates and incubated for 24 hours in the medium. Cells were then treated with or without 0.5 and 1 μg/mL recombinant HMGB1 protein (R&D) containing 50 μM EdU (Guangzhou RiboBio Co, China) and incubated for 24 hours at 37°C. The cells were fixed with 4% formaldehyde in PBS for 30 minutes. After labeling, the Schwann cells were assayed using Cell-Light

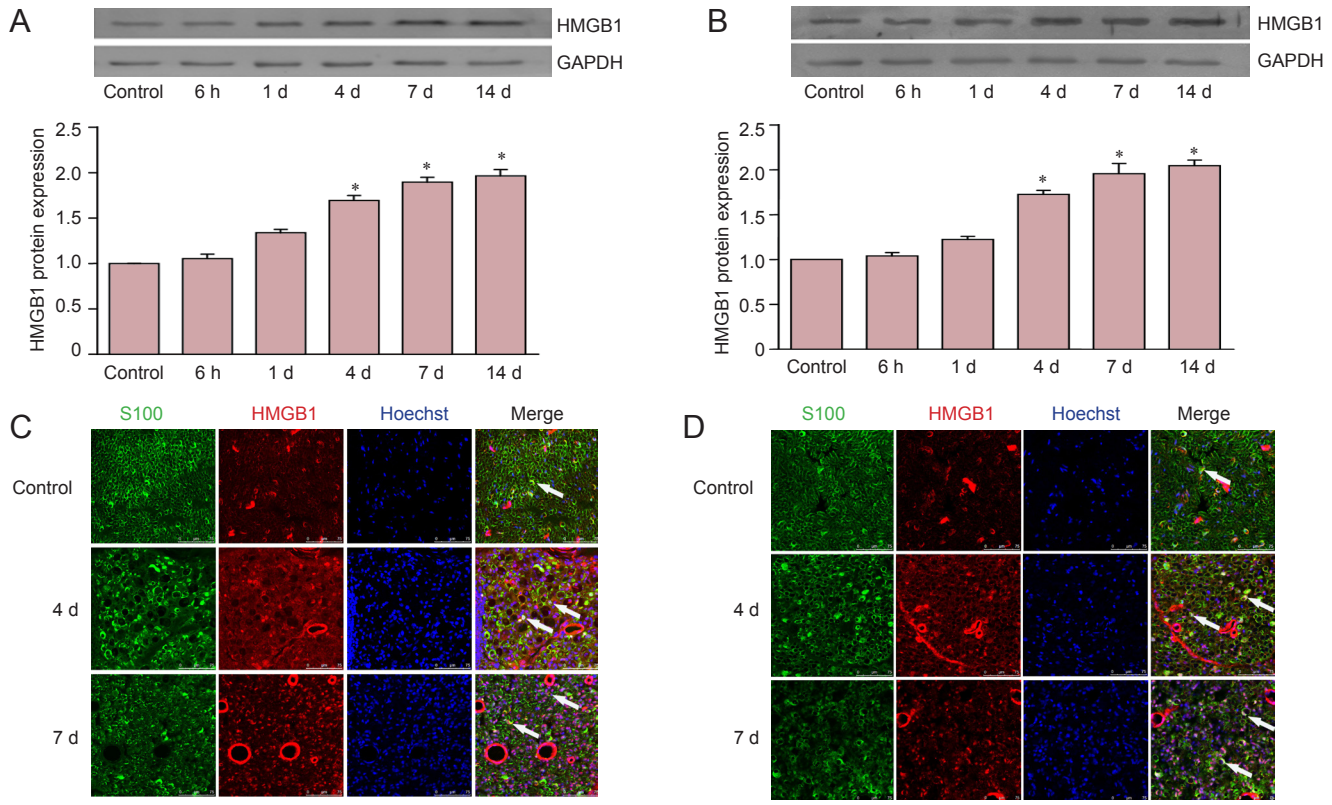

Figure 1 The expression of HMGB1 in proximal and distal segments of axotomized sciatic nerves.
(A, B) Western blot analysis of HMGB1 in proximal (A) and distal (B) segments at 0 and 6 h, and at 1, 4, 7 and 14 d following sciatic nerve axotomy. GAPDH was used as an internal control. The experiments were performed in triplicate ($n = 10$). Error bars represent the standard deviation (*$P < 0.05$, *vs.* control). (C, D) Colocalization of HMGB1 (red) with S100-positive cells (green) in the proximal (C) and distal (D) segments of sciatic nerves at 0, 4 and 7 d. Arrows show the colocalization of HMGB1 with S100-positive cells. Scale bars: 75 μm in C and D. HMGB1: High mobility group box 1; h: hours; d: day(s).

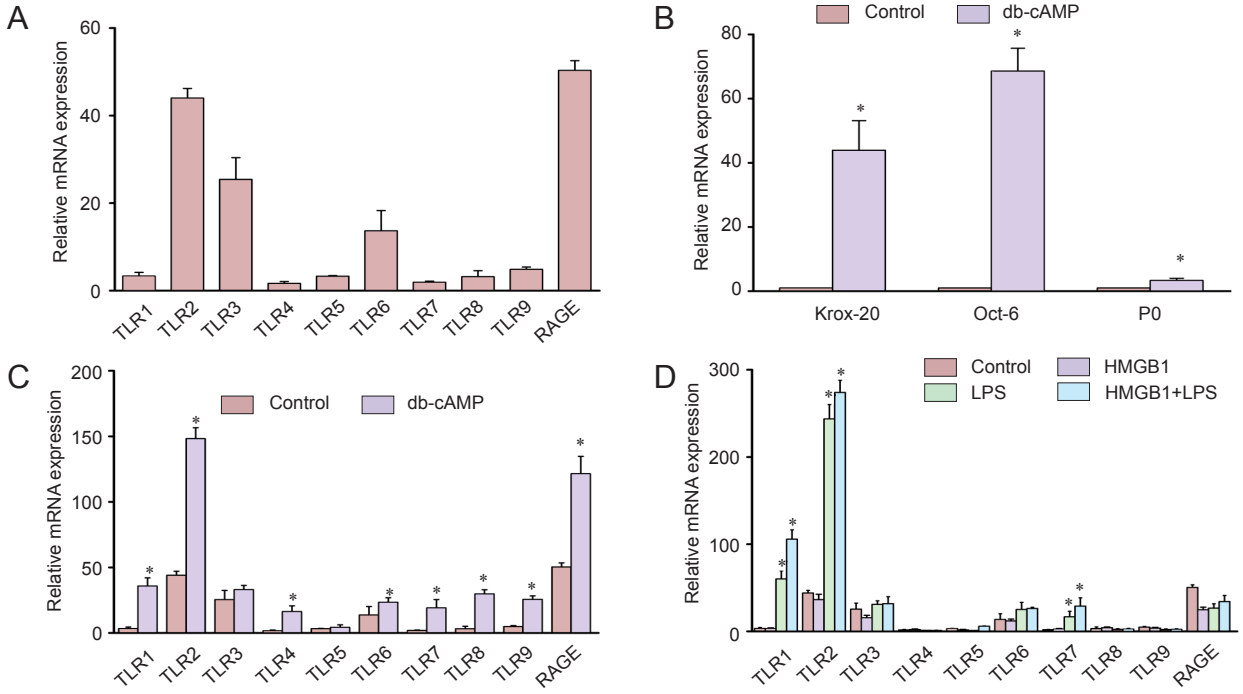

Figure 2 Effects of HMGB1 on mRNA expression levels of TLRs and RAGE in Schwann cells.
(A) Basal expression of TLRs and RAGE in primary Schwann cells. (B) The expression of Krox-20, Oct-6 and P0 after treating Schwann cells with 1 mM db-cAMP for 3 days. (C) The expression of TLRs and RAGE in differentiated Schwann cells induced with db-cAMP. (D) The expression of TLRs and RAGE in Schwann cells after addition of 1 μg/mL HMGB1 and/or 1 μg/mL LPS for 24 hours. The experiments were performed in triplicate. Error bars represent the standard deviation (#$P < 0.05$, db-cAMP *vs.* control or LPS treatment *vs.* control or HMGB1 + LPS treatment *vs.* LPS treatment). HMGB1: High mobility group box 1; TLRs: Toll-like receptors; RAGE: receptor for advanced glycation end products; LPS: lipopolysaccharide.

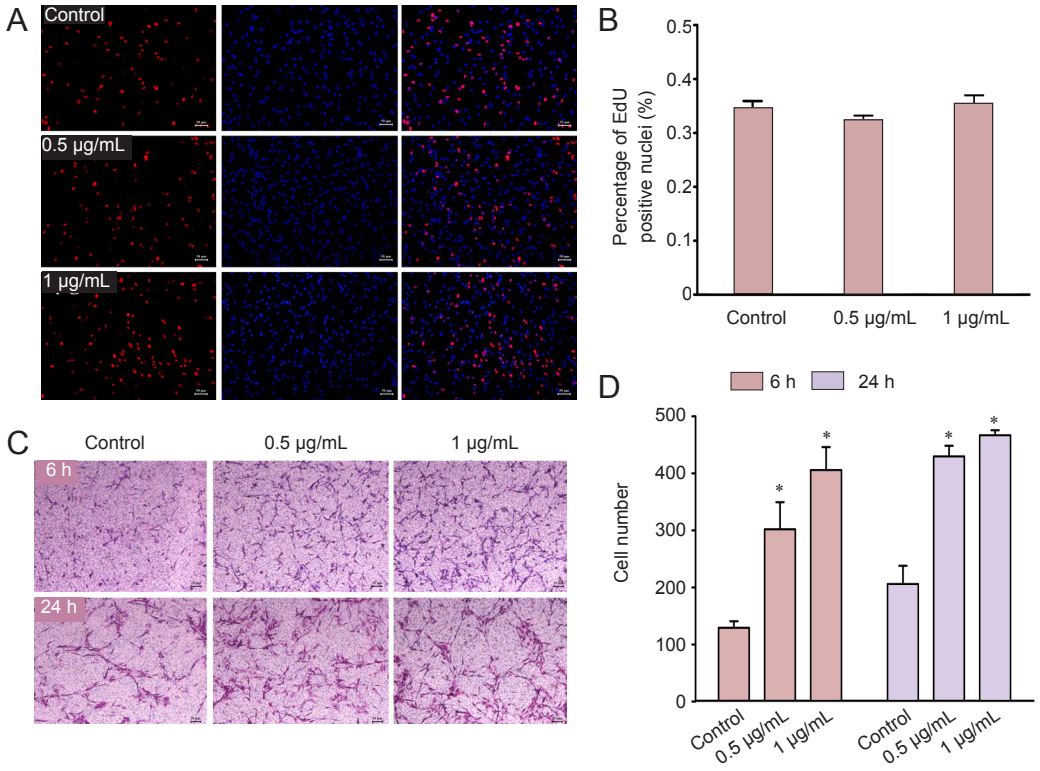

Figure 3 HMGB1 promotes Schwann cell migration but not proliferation.
(A) The proliferation of Schwann cells was assessed using EdU incorporation following treatment with 0 (control), 0.5 or 1 μg/mL recombinant HMGB1 for 24 hours (h). EdU (red) and Hoechst 33342 (blue). (B) Quantification of cell proliferation. (C) The migration of Schwann cells was evaluated with the Transwell assay using treatment with 0, 0.5 or 1 μg/mL recombinant HMGB1 for 6 or 24 hours. (D) Quantification of cell migration. The experiments were performed in triplicate. Error bars represent the standard deviation (*$P < 0.05$, *vs.* control). Scale bars: 50 μm in A and 20 μm in C. HMGB1: High mobility group box 1; EdU: 5-ethynyl-2′-deoxyuridine.

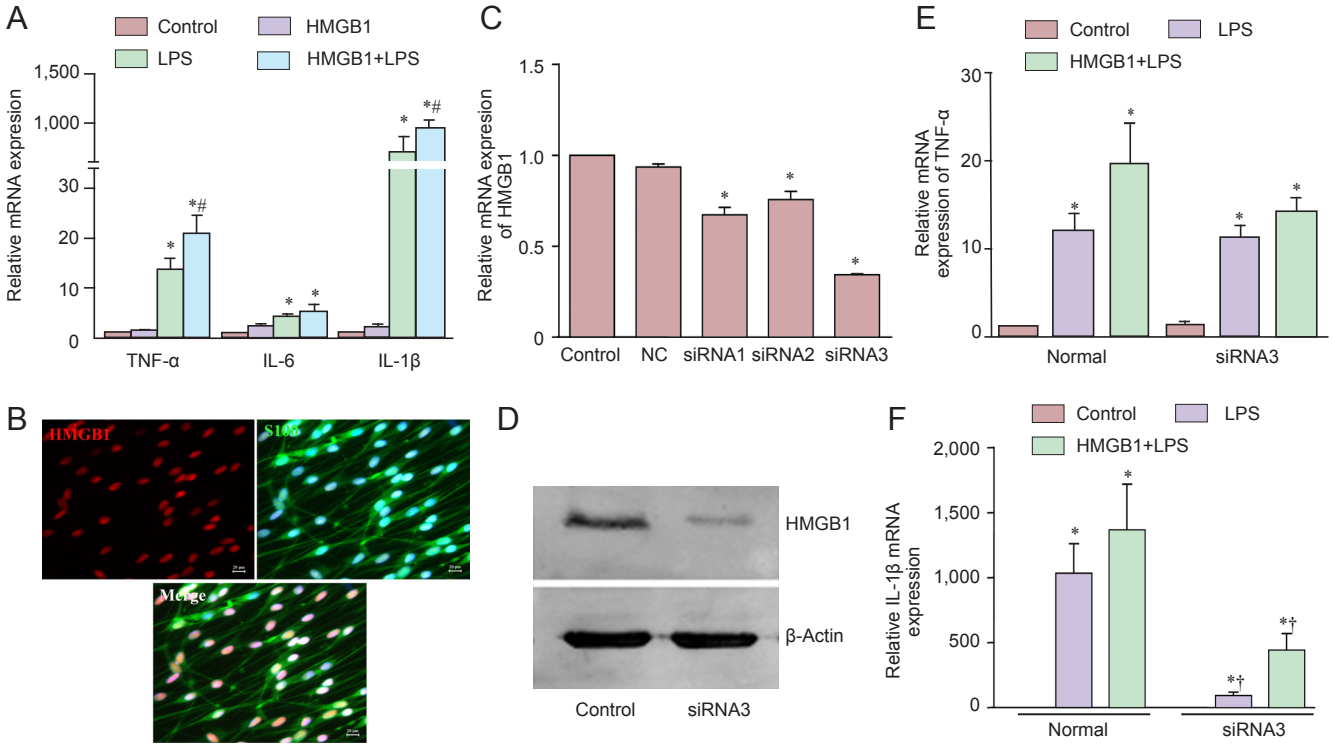

Figure 4 The effects of HMGB1 on the expression of inflammatory cytokines in Schwann cells.
(A) The expression of TNF-α, IL-1β and IL-6 in Schwann cells after addition of 1 μg/mL HMGB1 and/or 1 μg/mL LPS for 24 hours. (B) HMGB1 was localized in the nucleus of Schwann cells. Red fluorescence: HMGB1; green fluorescence: S100. (C, D) Knockdown efficiencies of three different siRNA oligonucleotides for HMGB1 measured by real-time PCR and western blot analysis after treatment for 48 hours. siRNA3 had the highest efficiency and was used for subsequent knockdown experiments. (E, F) Expression of TNF-α and IL-1β by Schwann cells transfected with siRNA3 following stimulation with 1 μg/mL LPS or 1 μg/mL LPS + 1 μg/mL HMGB1 for 24 hours. The experiments were performed in triplicate. Error bars represent the standard deviation (*$P < 0.05$, *vs.* control; #$P < 0.05$, *vs.* LPS treatment; †$P < 0.05$, *vs.* normal). Scale bar: 20 μm in (B). HMGB1: High mobility group box 1; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; IL-6: interleukin-6; LPS: lipopolysaccharide.

Table 1 Primer sequences for quantitative PCR

	Forward primer	Reverse primer
Gapdh	AAG TTC AAC GGC ACA GTC AAG	CCA GTA GAC TCC ACG ACA TAC TCA
TLR1	CAG TTT CTG GGA TTG AGC GGT T	TAA TGT GCT GAA GAC ACT TGG GAT C
TLR2	TCA TTA GAG TTC TTA GAC CTC AGC G	AGA GTC AGC AAA ATC TCA GCA GTT
TLR3	ATT GGC AAG TTA TTC GTC CTC CTC	AGA GAT TCT GGA TGC TTG TGT TTG A
TLR4	TCA CAA CTT CAG TGG CTG GAT TTA T	TCC TGT CAG TAC CAA GGT TGA GAG C
TLR5	GCT CCG TGC CTT GGA CAT AAC	TAG CAG TGA ATT GGG GTA CAT GC
TLR6	CAA CCT TAT TGA ATC TGA CCC TCC	CCC TGC TTA TGC TCT CAG TTA TCG
TLR7	TCC TTG GGT TTC GAT GGT ATC CT	AGA GAT GCT TGG TAT GTG GTT GAT G
TLR8	GAG ACT CTG ACA CGC TTG GAC TTA T	AGC ACA TGA AGG TGA GGA AAA TAC T
TLR9	CTG GTG CTG AAG GAC AGT TCT CTC	GCT GGT TTT GTT GAT GCT CTC GTA
RAGE	TGA ACT CAC AGC CAA TGT CCC TAA	CGA AGC GTG AAG AGT CCC GT
Krox20	CCG TCG GTG ACC ATC TTC	CCA TGT AAG TGA AGG TCT GGT T
Oct-6	CAA GCA ACG ACG CAT CAA	CGG TCT CCT CCA GCC ACT
P0	GGT GTT GGG AGC CGT GAT	GCA TAC AGC ACT GGC GTC T
TNF-α	CAA ACC ACC AAG CGG AG	GGT ATG AAA TGG CAA ATC G
IL-1β	CGT CCT CTG TG A CTC GTG G	TCG TTG CTT GTC TCT CCT T
IL-6	TTC CAG CCA GTT GCC TTC TTG	GGT CTG TTG TGG GTG GTA TCC TC

TLR: Toll-like receptor; RAGE: receptor for advanced glycation end products; TNF-α: tumor necrosis factor-α; IL: interleukin.

EdU DNA Cell Proliferation Kit (RiboBio) according to the manufacturer's protocol. Analysis of Schwann cell proliferation (ratio of EdU^+ Schwann cells to the total number of Schwann cells) was performed using images of randomly selected fields obtained on a DMR fluorescence microscope (Leica Microsystems, Bensheim, Germany). Assays were performed three times using triplicate wells.

Transwell migration assay

The effect of HMGB1 on Schwann cell migration was evaluated using transwell chambers with 8-μm pores (Costar; Corning Inc., NY, USA). The cells (2×10^4 cells/mL) in serum-free DMEM were plated onto the upper chamber, and 0, 0.5 or 1 μg/mL recombinant HMGB1 was added to the lower chamber containing 600 μL DMEM with 10% fetal bovine serum (complete medium). Schwann cells were allowed to migrate at 37°C in 5% CO_2 for 6 or 24 hours. Cells on the upper surface of the membrane were removed with cotton swabs, and those adhering to the bottom surface of each membrane were stained with 0.1% crystal violet, imaged, and counted using a DMR inverted microscope (Leica Microsystems). Assays were performed three times using triplicate wells.

RNA extraction and quantitative real-time RT-PCR

Total RNA from Schwann cells was extracted using Trizol (Invitrogen). First-strand cDNA was synthesized using the Omniscript Reverse Transcription Kit (QIAGEN, Valencia, CA, USA). Quantitative real-time RT-PCR (qPCR) was performed on a LightCycler Real-Time PCR System (Roche, Penzberg, Germany) using FastStart Essential DNA Green Master Mix (Roche) according to the manufacturer's instructions. The reaction mixture contained 10 μL of 2× FastStart SYBR Green qPCR Master Mix, 1 μL of primers, 1 μL of cDNA sample, and 7 μL of RNase/DNase-free water. The three-step fast cycle protocol was conducted. Relative gene expression levels were validated and normalized to Gapdh (internal reference). Primer sequences are listed in **Table 1**. All primers were validated for specificity and amplification efficiency. All PCR reactions were done in triplicate.

Statistical analysis

Data are presented as the mean ± SEM. Statistical significance of differences between groups was analyzed by Student's *t*-test with SPSS 15.0 (SPSS, Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

Results

HMGB1 was upregulated at the site of injury following sciatic nerve axotomy

We first examined the changes in expression of HMGB1 in the injured PNS. Western blot analysis showed that HMGB1 was upregulated in both the proximal and distal segments at 0 and 6 hours, and at 1, 4, 7 and 14 days following sciatic nerve axotomy, with expression increasing over time (**Figure 1A**, **B**). Immunohistochemistry revealed that HMGB1 colocalized with S100-positive cells (**Figure 1C**, **D**), suggesting that the cells expressing HMGB1 were Schwann cells. These results indicate that HMGB1 is possibly involved in the regulation of axonal injury responses by Schwann cells.

HMGB1 activated innate pattern recognition receptors expressed by Schwann cells

The TLR family and RAGE are a major class of pattern recognition receptors involved in the initiation and regulation of both innate and subsequent adaptive immune responses (Beutler, 2004; Iwasaki and Medzhitov, 2004). Real-time PCR results demonstrated that primary Schwann cells display basal expression of all nine TLRs and RAGE, with a notably high abundance of TLR2, TLR3 and RAGE, and moderate

levels of TLR6 (**Figure 2A**). To examine their expression in differentiated Schwann cells, we induced primary Schwann cells (5×10^4 cells/mL) with 1 mM db-cAMP for 3 days. Upregulation of the transcription factors Krox20 and Oct-6 and of myelin structural protein P0 demonstrated successful differentiation with this method (**Figure 2B**). All eight TLRs and RAGE were significantly upregulated in differentiated Schwann cells, except for TLR3 and TLR5 (**Figure 2C**), suggesting that phenotypically distinct Schwann cells have correspondingly distinct roles in innate immunity.

To evaluate the effects of HMGB1 on inflammatory signaling in Schwann cells, primary Schwann cells were treated with 1 μg/mL recombinant HMGB1 (R&D, Minneapolis, MN, USA) and/or 1 μg/mL LPS (Sigma-Aldrich) for 24 hours. HMGB1 alone did not change the expression of the TLR family or RAGE in Schwann cells, whereas TLR1, TLR2 and TLR7 were upregulated following treatment with LPS (**Figure 2D**). Addition of HMGB1 to the LPS-treated culture significantly augmented the expression of TLR1, TLR2 and TLR7 (**Figure 2D**), suggesting that HMGB1 and LPS synergistically induce the expression of innate pattern recognition receptors in Schwann cells.

HMGB1 promoted Schwann cell migration, but not proliferation

Given that Schwann cells basally express RAGE, TLR2 and other TLR family members, we conjectured that HMGB1 can activate these receptors to affect downstream signaling. The HMGB1/RAGE signaling pathway has been found to regulate cell proliferation, survival, differentiation and migration *via* the activation of NF-κB, AP-1, CREB and STAT3 (Sorci et al., 2013). Therefore, we examined the proliferation and migration of Schwann cells following treatment with 0, 0.5 or 1 μg/mL recombinant HMGB1 protein for 24 hours. EdU incorporation assay showed that HMGB1 did not affect the proliferation of Schwann cells (**Figure 3A**, **B**). However, the protein was able to promote Schwann cell migration measured using Transwell chambers (**Figure 3C**, **D**). These data indicate that HMGB1 is able to promote Schwann cell migration to the site of injury.

HMGB1 and LPS co-activated the expression of inflammatory cytokines by Schwann cells

We next investigated whether HMGB1 can induce inflammatory responses by Schwann cells. Schwann cells were treated with 1 μg/mL recombinant HMGB1 alone or in combination with 1 μg/mL LPS for 24 hours. The expression of the inflammatory cytokines TNF-α, IL-1β and IL-6 remained unchanged after treatment with recombinant HMGB1 protein. In comparison, LPS treatment significantly upregulated the expression of these cytokines (**Figure 4A**). When Schwann cells were co-stimulated with HMGB1 and LPS for 24 hours, the expression of TNF-α, IL-1β and IL-6 significantly increased (**Figure 4A**). These results indicate that extracellular HMGB1 promotes the expression of inflammatory cytokines by Schwann cells in combination with LPS treatment. HMGB1 was distributed in the nucleus of Schwann cells (**Figure 4B**). To examine whether the intracellular protein is involved in the regulation of inflammatory signaling, HMGB1 siRNA oligonucleotides were transfected into primary Schwann cells. One of three siRNA oligonucleotides tested, siRNA3, with a higher efficiency in reducing expression was used for the assay (**Figure 4C**, **D**). The expression of TNF-α was found to be not significantly different between siRNA knockdown and control when Schwann cells were stimulated with LPS or LPS + HMGB1 (**Figure 4E**). However, the expression of IL-1β was greatly downregulated after Schwann cells were transfected with HMGB1 siRNA3 (**Figure 4F**). These results indicate that HMGB1 and LPS co-activate the expression of inflammatory cytokines.

Discussion

Upon nerve injury, Schwann cells are activated and release various proinflammatory cytokines and chemokines, leading to the recruitment of macrophages and the phagocytosis of myelin debris (Goethals et al., 2010). The activation of Schwann cells following nerve injury relies on the TLR receptors on the cell surface, and loss of TLR2 or TLR3 attenuates the production of inflammatory cytokines (Goethals et al., 2010). Schwann cells are immune competent cells, and act as sentinel cells in the PNS. The differential expression of TLRs in undifferentiated versus differentiated Schwann cells suggests that these cells have distinct immunological roles during normal PNS development and injury.

Although several studies have examined the activation of Schwann cells using LPS or necrotic neuronal cells, it remains unclear how DAMPs activate the inflammatory response in Schwann cells. HMGB1, a ubiquitously expressed proinflammatory cytokine, contributes to the pathogenesis of diverse inflammatory and infectious disorders, including CNS disease (Ulloa and Messmer, 2006; Kim et al., 2008; Hayakawa et al., 2010). It seems that HMGB1 regulates the production of inflammatory cytokines in various cell types. HMGB1 promotes the release of inflammatory cytokines by macrophage (Yang et al., 2010). However, in human peripheral blood mononuclear cells and synovial fibroblasts, HMGB1 together with LPS or the TLR9 ligand CpG-ODN, but not on its own, enhances proinflammatory cytokine production (Hreggvidsdottir et al., 2009). In the present study, we show that HMGB1 recruits Schwann cells by promoting their migration, and that with LPS, it co-activates the expression of innate pattern recognition receptors. Our novel findings indicate that the HMGB1 signaling pathway activates the inflammatory response in Schwann cells, which contributes to the immunological reaction following PNS injury.

Following injury to the PNS, a variety of DAMPs and alarmins, including HMGB1, uric acid, heat shock proteins and ATP, are released from dying cells during necrosis (Chiu et al., 2012). They elicit inflammatory responses by binding with various receptors on immune cells and other types of cells. Schwann cells have been shown to basally express receptors for other DAMPs, such as P2X7 receptors, which bind ATP (Luo et al., 2013). Therefore, it appears that the

inflammatory response in Schwann cells is triggered not only by HMGB1, but also by other DAMPs. Further studies are needed to clarify the contribution of the various signaling pathways to Schwann cell activation following PNS injury.

In conclusion, HMGB1 colocalizes with Schwann cells, and expression is upregulated following sciatic nerve axotomy. HMGB1 promotes the migration of Schwann cells. Furthermore, it induces the expression of TLRs, RAGE and inflammatory cytokines by these cells when combined with LPS.

Author contributions: *LLM and FL conducted experiments and performed the statistical analysis of the data. YJW and HHS were responsible for tables and figures. HBX, ZWZ and QZ provided technical assistance. YJW designed the study and wrote the paper. All authors approved the final version of this paper.*

Conflicts of interest: *None declared.*

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