Cutaneous nerve fibers participate in the progression of psoriasis by linking epidermal keratinocytes and immunocytes

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Received: 5 December 2021 / Revised: 23 March 2022 / Accepted: 8 April 2022 / Published online: 30 April 2022
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Abstract
Recent studies have illustrated that psoriatic lesions are innervated by dense sensory nerve fibers. Psoriatic plaques appeared to improve after central or peripheral nerve injury. Therefore, the nervous system may play a vital role in psoriasis. We aimed to clarify the expression of nerve fibers in psoriasis and their relationship with immune cells and keratinocytes, and to explore the effect of skin nerve impairment. Our results illustrated that nerve fibers in psoriatic lesions increased and were closely innervated around immune cells and keratinocytes. RNA-seq analysis showed that peripheral sensory nerve-related genes were disrupted in psoriasis. In spinal cord hemi-section mice, sensory impairment improved psoriasiform dermatitis and inhibited the abnormal proliferation of keratinocytes. Botulinum toxin A alleviated psoriasiform dermatitis by inhibiting the secretion of calcitonin gene-related peptide. Collectively, cutaneous nerve fibers participate in the progression of psoriasis by linking epidermal keratinocytes and immunocytes. Neurological intervention may be a new treatment strategy for psoriasis.

Keywords Psoriasis · Sensory nerve · Spinal cord hemi-section model · Botulinum toxin A

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| BTX-A        | Botulinum toxin A |
| CGRP         | Calcitonin gene-related peptide |
| DEG          | Differential expression genes |
| DRG          | Dorsal root ganglion |
| ELISA        | Enzyme linked immunosorbent assay |
| FC           | Fold change |
| GEO          | Gene Expression Omnibus |
| HE           | Hematoxylin–eosin staining |
| IF           | Immunofluorescence |
| IFN          | Interferon |
| IHC          | Immunohistochemistry |
| IL           | Interleukin |
| IMQ          | Imiquimod |
| NS           | Normal saline |
| PASI         | Psoriasis Area Severity Index |
| PGP9.5       | Protein gene product 9.5 |
| qRT-PCR      | Real-Time Quantitative Reverse Transcription PCR |

Introduction
Psoriasis is a chronic immune-mediated inflammatory disease mediated by T cells and dendritic cells [1]. The pathogenesis of psoriasis has not been completely clear. Most researches focus on the immune-related mechanism [2], few researchers on the role of neurons and neurotransmitters as mediators. However, psychosis or mental abnormal can induce onset of psoriasis [3]. Nerve damage can play an important role in skin diseases. For example, negative emotions contribute to skin diseases such as alopecia areata and vitiligo [4]. However, there are limited researches reporting the role of neural factors in the occurrence and development of psoriasis.

As early as 1965, histopathological study had shown increased number of nerve fibers, Schwann cells and other nerve cells in the epiderma of psoriasis plaques [5, 6]. Till 2016, 12 cases reported that after central or peripheral nerve injury (such as cerebrovascular accident, trauma, polio, etc.), the psoriasis plaques in neurological dysfunction skin...
were improved or got completely remission [7]. These case reports provide clinical evidence for the involvement of the nervous system in the pathogenesis of psoriasis. Taken together, these factors shed a light on the role of nerve endings in psoriasis.

The peripheral nervous system can induce protective nociceptive nerve reflexes and release neurotransmitters to resist external dangers. More evidence supports the hypothesis of the two-way neuroimmune communication in body homeostasis [8, 9]. Immune cells express multiple receptors that respond to neuropeptides, regulating local antibacterial responses [10]. For example, after \( C. \) \( albicans \) infection, calcitonin gene-related peptide (CGRP) released by skin neurons stimulates the production of IL-23 by dermal conventional type-2 dendritic cells (cDC2) resulting in Type-17 immunity [11]. Skin peripheral nerves in the skin can also release neuropeptides leading to inflammatory skin diseases. In atopic dermatitis, the initiation of allergic response activates substance p (SP)-producing TRPV1+ nociceptors and then triggers mast cell degranulation via MRGPRB2 [12].

Therefore, exploring the role of peripheral nervous system will help us better understand the pathogenesis of psoriasis. Herein, we defined the distribution pattern of nerve fibers in the lesional skin of psoriasis by immunohistochemistry, combined with the analysis of the results of psoriasis bulk skin RNA-seq in the GEO database. Furthermore, in the mouse model of psoriatic-like dermatitis, hemisection of the spinal cord and intervention of type A botulinum toxin were used to further confirm the role of the nervous system in the occurrence and development of psoriasis.

**Materials and methods**

**Patients**

The skin biopsies used in this project were from 4 psoriasis patients and 4 healthy volunteers in the dermatology clinic. After local anesthesia using 2% lidocaine, an incisional biopsy with 6 mm diameter was performed with a scalpel. Biopsies of the lesional skin from psoriasis patients and normal skin from healthy volunteers were taken. The collection and processing experiments were approved by the Institutional Review Board of the Second Affiliated Hospital, Zhejiang University School of Medicine. Written and informed consent was obtained from all psoriasis patients and healthy controls.

**Mice**

Female C57BL/6 wild-type mice at 6 weeks old were purchased from Shanghai SLAC Laboratory Animal Co., Ltd., and bred in the Animal Experiment Center of the Second Affiliated Hospital, Zhejiang University School of Medicine. All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine.

**Imiquimod (IMQ) induced psoriasis-like mouse model**

Imiquimod cream (Sichuan Mingxin Pharmaceutical Co., Ltd.) was used to induce psoriasis-like dermatitis in mice. The mice were depilated on the dorsal skin and then 62.5 mg 5% imiquimod cream was topically applied for 6 consecutive days. The sham controls were treated with the same dose of base ointment.

**Co-culture of mouse dorsal root ganglion (DRG) neurons and keratinocytes**

The method of culturing keratinocytes is the same as described before [13]. Skin samples from mouse were incubated with 0.5% dispase (Gibco) at 37 °C for 2 h. Then the epidermis was peeled off and incubated with 0.25% trypsin (Gibco) for 10 min, neutralize by 10% FBS (Gibco), centrifuged at 1000 rpm/min for 10 min and then cultured in keratinocyte medium (Millipore). Mouse L4-L6 DRG was taken, digested with 0.1% collagenase (Sigma) and 0.25% trypsin for 30 min, neutralized with 10% FBS and centrifuged at 1000 rpm for 10 min. The precipitate was mixed with keratinocyte culture medium, and added to the keratinocyte culture flask. Nerve growth factor (Sigma) and B-27 supplement (Gibco) were added for neuron incubation. After 4–5 days, cells were used for immunofluorescence detection.

**Spinal cord hemisection model**

The mouse was anesthetized with 1% sodium pentobarbital. The T10 thoracic spinous process was used as surface landmark. Under microscope observation and with the spinal cord anterior and posterior veins as the boundary, microsurgery scissors were used to cut the left half of the spinal cord. Then the muscle and skin were sutured layer by layer. The effectiveness of the mouse model of spinal cord injury was evaluated by trunk imbalance and paralysis of the ipsilateral hind limb and pain and temperature sensory disturbance on the contralateral side of the injury.

**Botulinum toxin A (BTX-A) injection**

Normal saline was applied to formulate BTX-A (Allergen) dry powder into 0.005 U/μl solution. The back of the mice was depilated. Four evenly distributed injection points were selected on the back, and 50 μl of BTX-A of working
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Immunofluorescence (IF) and Immunohistochemistry (IHC)

Skin biopsy was embedded in OCT (Sakura Finetek), sectioned by freezing microtome (Leica), fixed in ice acetone for 15 min and blocked with 10% BSA (Sigma) for 1 h. DRG was also put in OCT immediately after isolation and then blocked with 10% BSA without fixation. Tissues were stained with rabbit monoclonal anti-keratin 14 antibody (1:500, Abcam), mouse monoclonal anti-βIII-tubulin antibody (1:200, Abcam), rabbit monoclonal anti-CGRP antibody (1:200, CST), rabbit monoclonal anti-TRPV1 antibody (1:200, Alomone), rabbit monoclonal anti-PG9.5 antibody (1:200, Abcam), rabbit monoclonal anti-CD113 antibody (1:400, Abcam), rabbit monoclonal anti-CD11b antibody (1:200, Abcam), rabbit monoclonal anti-CD11b antibody (1:200, Abcam) or rabbit monoclonal anti-Nav1.8 antibody (1:200, Abcam) in PBS with 5% FBS for 1 h, and then incubated with Alexa Fluro 488 or 555-conjugated secondary antibody (1:2000, Invitrogen) for 1 h. Nuclei were counterstained with DAPI (1:5000, Roche). Images were pictured by Leica DM5500B. In skin section IF, cells surrounded by nerve fibers were counted. In IF of DRG, ImageJ was used for semi-quantitative statistics.

Western blotting

Skin biopsies were grinded to powder in mortar and lysed with RIPA buffer. Lysates were boiled with 5× Loading buffer for 10 min. Protein samples were separated with SDS-PAGE gel and immunoblotted with mouse monoclonal anti-βIII-tubulin antibody (1:1000, Abcam), rabbit monoclonal anti-CGRP antibody (1:1000, CST), rabbit monoclonal anti-TRPV1 antibody (1:1000, Alomone), rabbit monoclonal anti-Nav1.8 antibody (1:1000, Abcam), followed by an incubation with a secondary antibody. The immunoreactive bands were detected using ECL Substrate (Thermo Scientific).

Immunoelectron microscopy

The psoriatic lesional skin from psoriasis patients was placed in 4% paraformaldehyde (Sangon Biotech). Skin slices were treated with 1% hydrogen peroxide for 15 min and then incubated with goat serum (Beyotime) at room temperature for 1 h. Then the sample slice was incubated with the anti-βIII-tubulin antibody (1:200, Abcam) for 24 h at 4 °C. After washed with PBS for 3 times, the sample was incubated with colloidal gold-labeled antibody solution for 1 h at room temperature and washed with ddH2O. Picture was taken with Tecnai G2 Spirit 120 kV cryo-electron microscope.

qRT-PCR

RNA was isolated from DRG with Trizol (Thermo). Nanodrop2000 (Thermo) was used for RNA concentration measurement. SuperScript II reverse transcriptase (Invitrogen) was used for cDNA synthesis. RT-PCR was conducted using SYBR Green Master Mix (Applied Biosystems) in QuantStudio 5 real-time system (Applied Biosystems). Data were presented as relative expression of the target gene to β-actin as housekeeping gene using the 2−ΔΔCT method, analyzed by qBase Plus 2 software.

The following primer sets were used: β-actin, forward 5′-GTCATTTTCAATATGAGATGCCG-3′ and reverse 5′-GCTATACCTCCTCCCTGTGG-3′, il-17rc, forward 5′-TCCACATGTATGAGTTGA-3′ and reverse 5′-AGCTTGAACGATCCATAGCC-3′, ifn-αr1 forward 5′-TGCTGACACCTACAAACC-3′ and reverse 5′-TCAGGCACCTTTGACTGCAA-3′, il-17rc, forward 5′-ATGCCTGTGTCCTGTCCT-3′ and reverse 5′-TTCTAGTGTAGTCAGGGTC-3′.

ELISA

A 6 mm skin punch was taken from each mouse and quickly transferred to a 12-well cell culture plate containing 500 μl DMEM (Gibco). The skin was incubated on a 32 °C shaker for 30 min. Supernatant was used for CGRP ELISA. The protein expression levels of CGRP secreted into the supernatants of cultured skin sheets from mice were quantified via ELISA kits (Cusabio) following the manufacturer’s instructions.

RNA sequence reanalysis from GEO

The transcriptome data were obtained from the GSE121212 and GSE53552 in Gene Expression Omnibus (GEO) database [14, 15]. Read counts were normalized by TMM, and the VOOM transformation was used to model the mean–variance relationship. Bayes linear model in the limma package was used to analyze DEGs.
Statistical analysis

Statistical analyses were performed using GraphPad Prism6. Analyses were carried out using One-way ANOVA. P value < 0.05 was considered statistically significant.

Results

Increased sensory nerve fibers surround epidermal keratinocytes and immune cells in psoriatic lesional skin

To clarify the distribution of nerve fibers, we determined the expression of βIII-tubulin, a cytoskeletal protein that is often used as a neuron marker, by IF in healthy and psoriatic lesional skin. Numerous nerve fibers were detected in the skin and were densest along the basement membrane zone (Fig. 1a, b). Compared with healthy skin, the density of nerve fibers is increased in psoriatic lesional skin. Some cells in the basal layer were closely surrounded by nerve fibers. The number of these cells was statistically different between psoriatic and normal skin (P < 0.001, Fig. 1a). Furthermore, three specific markers of sensory nerve fibers, protein levels of βIII-tubulin, Nav1.8, and PGP9.5, were all significantly upregulated in the psoriatic epidermis as defined by Western blotting (Fig. 1b).

To further establish the relationship between keratinocytes and nerve fibers, immunoelectron microscopy was performed to localize nerve fibers in the psoriatic epidermis. A keratinocyte containing a nuclear structure and transparent keratinous particles is well recognized in Fig. 1c. The βIII-tubulin-positive particles (arrowhead) were distributed around the cell membrane of keratinocytes. Next, we built a co-culture system of keratinocytes and DRG neurons. It is very clear that the DRG neurons extended their neurites around the cell membrane of keratinocytes. Next, we built a co-culture system of keratinocytes and DRG neurons. It is very clear that the DRG neurons extended their neurites toward the keratinocytes like a hand to catch things (Fig. 1d).

CD11b+ DCs and CD103+ tissue-resident memory T (Trm) cells are barely present in normal skin, but in psoriatic epidermis, especially in the basal layer, their number increased, and almost all CD11b+ DCs and CD103+ Trm cells were innervated by dense nerve fibers (Fig. 1e, f, white arrow). These results suggest that in addition to epidermal keratinocytes, increased peripheral nerves in psoriatic lesional epidermis were also in close contact with immune cells.

Disordered sensory nerve-related genes in psoriatic lesions

To further clarify the role of nerves in psoriasis, we conducted an in-depth analysis of related genes in neural pathways from transcriptome data (GSE121212) [14], which includes normal skin, non-lesional, and lesional psoriatic skin. According to single-cell RNA-seq of the DRG [16], 175 genes enriched in the sensory nerve were identified. These genes are involved in various operational components of sensory neurons, including perception, conduction, signal transmission, synaptic regulation, and chronic pain perception. There were 26 differentially expressed genes (DEGs) between normal and psoriatic lesional skin (Fig. 2b, c). DEGs were scattered in every component, as shown in Fig. 2d. Among the upregulated differentially expressed genes, 5-hydroxytryptamine receptor 3A (HTR3A) showed the largest fold change (Fig. 2d). Internexin neuronal intermediate filament protein alpha (INA) encodes α-internexin, a neurofilament protein that promotes the growth of neuronal processes and regulates the expression of other neurofilament proteins.

To explore whether these DEGs will change with the improvement of psoriatic lesions, we analyzed another transcriptome dataset, GSE53552, in the GEO database [15]. This dataset includes 99 paired psoriatic RNA-seq data of non-lesional and lesional full-thickness skin before and after (baseline, 15 days, 43 days) an IL-17 receptor monoclonal antibody, brodalumab, treatment at different doses (control, 140 mg, 350 mg, 700 mg). The RNA level of INA in the leional skin was higher than that in the non-lesional area, but decreased after brodalumab treatment, especially in the high-dose (700 mg) group. On day 43, the leional INA was close to the non-lesion level (Fig. 2f).

Sensory neurons and cytokine receptors IFN-αR1, IFN-αR2, and IL-17RC are increased in the DRG of IMQ-treated mice

IMQ-induced psoriasis-like skin inflammation [17] was included to explore whether the peripheral nervous system was abnormally activated in psoriasis (Fig. 3a–d) [18]. First, we used DRG to detect the expression of neuron subtypes. Transient receptor potential V1 (TRPV1) is a transient receptor potential cation channel that is expressed in the central and peripheral nervous systems and is responsible for the conduction of pain and itching [19]. If showed that the fluorescence intensity of Nav1.8, TRPV1, and neuropeptide CGRP in the DRG of psoriasis-like dermatitis was markedly increased compared with that in control mice (Fig. 3e, f). Immune cells communicate with neurons via cytokine receptors. Thus, we next used qRT-PCR to detect the expression of cytokine receptors ifn-αr1, ifn-αr2, and il-17rc at the gene level (Fig. 3g–j). IFN-α plays an indispensable role in the initial stage of psoriasis after skin injury [20], and ifn-α receptors r1 and r2 mRNA were significantly upregulated after IMQ induction (Fig. 3g, h). The mRNA level of il-17rc, a core factor in the pathogenesis of psoriasis,
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was also significantly upregulated (Fig. 3j). We suggest that the neuro-immune interaction might be enhanced by the increased expression of these cytokine receptors in neurons.

**Fig. 1** Location and expression of nerves in psoriasis lesions. a IF of βIII-tubulin in healthy and psoriatic lesional skin (left). Qualification of innervated cells (right), scale bar = 100 µm. b Western blotting of βIII-tubulin, Nav1.8, PGP9.5 and β-actin in the epidermis of normal skin (N) and psoriatic lesion (P). c Immunoelectron microscopy to detect βIII-tubulin in psoriatic epidermis, scale bar = 2 µm. d IF to detect the localization of mouse keratinocytes (K14, red) and DRG neurons (βIII-tubulin, green), scale bar = 50 µm. e IF of CD11b (green) and βIII-tubulin (red) in psoriatic lesion, white arrow marked CD11b+ cells, scale bar = 50 µm. f IF of CD103 (red) and βIII-tubulin (green) in psoriatic lesion, white arrow marked CD103+ cells colocalized with nerve fibers, scale bar = 100 µm

**IMQ-induced psoriatic dermatitis was attenuated on the sensory impairment side of spinal cord hemisection mice**

In recent years, a number of cases have reported that after central nervous system injury, such as cerebrovascular accident, spinal cord injury, polio, etc., psoriatic plaques in the
**a** GSE121212

**b**

**c**

**d**

| Function                  | Up                               | Down                          |
|--------------------------|----------------------------------|-------------------------------|
| Sensory perception       | TRPM8, TRPA1                      | TRPC3, TRPM2                  |
| Conductive channel       | INA, NDEL1, TMEM38B, KCNS3        | CACNA2D1, KCNT2, CACNA1H, CACNA2D2, KCNMB4, KCNK2, SCN9A |
| Neurotransmission        | PCSK1N, CCL2, SLC17A7             | GAL                           |
| Presynaptic regulation   |                                  | GRIA4, GLRB, GABRB3           |
| Chronic pain             | HTR7, HTR3A                      | LPAR1, PTGIR                  |

**e**

**f**
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Fig. 2 Gene expression of sensory nerves in skin. a The top two principal components for the samples in the cohort. b Volcano plot detecting proteins by FC and P value. c Venn diagram showing the overlap between DEGs. d List of DEGs in different functions of sensory nerve. e Up-regulated DEGs and down-regulated DEGs. f tRNA gene expression at baseline, 15 days and 43 days after treatment with control substance, 140 mg, 350 mg and 700 mg doses of Brodalu-mab. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, one-way ANOVA. (Dataset from GSE121212)

neurological dysfunction area were improved or completely relieved [21]. We established a mouse model of spinal cord hemi-section (Fig. 4a) to simulate Brown–Séquard syndrome, a common type of spinal cord injury caused by a lesion on half of the spinal cord, the clinical manifestations of which were pain and temperature sensory disturbance on the contralesional side, and dyskinesia (paralysis of hind limbs) on the ipsilesional side [22]. After IMQ induction, the ipsilesional side showed a phenotype similar to that of wild-type mice, while psoriasis-like dermatitis was not obvious on the contralesional side (Fig. 4b), including mild erythema, slight induration, and fine scales. Taking the midline of the back as the boundary, there is a clear contrast between the skin on the contralesional and ipsilesional sides (Fig. 4b). On the 4th and 6th day of modeling, the PASI score of the contralesional side was significantly lower than that of the ipsilesional side (Fig. 4c).

In addition, the diameter of the inguinal lymph nodes on the ipsilesional side was larger than that on the contralesional side, suggesting that the skin inflammation on the sensory impairment side was relatively reduced (Fig. 4f). A large part of the nerves in the skin are sensory nerves [23]. IF showed that the number of βIII-tubulin-positive neurons in the dermis and epidermis of the contralesional side was reduced (Fig. 4f). Abnormal proliferation of keratinocytes is one of the typical pathological features of psoriasis [24]. We used immunohistochemistry (IHC) to detect the proliferation marker Ki67 in spinal cord hemisectioned mice after IMQ induction. The number of Ki67-positive keratinocytes on the contralesional side was significantly reduced (Fig. 4g). Our results illustrated that IMQ-induced psoriatic dermatitis was attenuated on the sensory impairment side of spinal cord hemisection, possibly by inhibiting inflammation and proliferation of keratinocytes.

Subcutaneous injection of BTX-A alleviated imiquimod-induced psoriasis-like dermatitis

BTX-A is a neurotoxin that can inhibit the release of acetylcholine and other neurotransmitters and block nerve conduction [25]. Therefore, BTX-A was applied to pretreat mice to determine whether the inhibition of neurotransmitters can alleviate or block psoriasis inflammation. We administered a single subcutaneous injection of BTX-A to the backs of these mice and started to induce psoriasis-like dermatitis in mice with imiquimod for 5 consecutive days. Compared with the vehicle group, the mice treated with BTX-A showed a dramatically alleviated phenotype, which was manifested by a reduction in scales and skin thickening (Fig. 5a, b). The PASI score of BTX-A-treated mice was lower than that of the control group (P < 0.05, Fig. 5c). The epidermal thickness measured by H&E staining also decreased after BTX-A injection (Fig. 5d, e).

A previous report showed that the number of PGP9.5-positive sensory nerve fibers and neuropeptides secreted by psoriasis skin lesions was increased, especially CGRP, which acts as a bridge between the nervous and immune systems in various skin infections and atopic dermatitis [26]. PGP9.5-positive nerve fibers in the skin of mice after BTX-A injection was reduced compared with the control group (Fig. 5f). Subsequently, we measured the concentration of CGRP in the supernatant from tissue-cultured skin by ELISA. The results showed that the CGRP secreted by the skin of mice induced with IMQ was much higher than that in the vehicle group (Fig. 5g). After BTX-A pre-treatment, the CGRP concentration was markedly lower than that of the IMQ group, and there was no statistical difference between the two groups (Fig. 5g).

Next, immunofluorescence of DRG was performed to further define the effect of BTX-A. CGRP-positive neurons in the imiquimod model showed that approximately 40% of the DRG neurons were CGRP-positive. However, after BTX-A pre-treatment, there were less than 10% CGRP-positive neurons in the DRG (Fig. 5h). These data showed that subcutaneous injection of BTX-A can reduce cutaneous nerve fibers and thus alleviate IMQ-induced psoriasiform dermatitis.

Discussion

The important role of the neuroimmune microenvironment in skin diseases has recently attracted attention [27]. Communication and cooperation between the nervous and immune systems is an indispensable part of the body’s homeostasis [8, 9]. Abnormal neuroimmune communication may not only concern psoriasis but also diseases with different immune responses spectrum, such as atopic dermatitis [28].

Whether nerve fibers increase or decrease in psoriasis remains controversial. One of the reasons for this is the difference in the detection methods [29, 30]. Our results showed that the distribution of nerve fibers in the epidermis of psoriasis was denser, as determined by IF and Western blotting. Peripheral sensory nerve endings can participate in immune regulation by communicating with a variety of immune cells [31, 32]. We found that in psoriasis, nerve endings were in close contact with CD11c+ DC and CD103+ Trm cells. CD103 in dermis was reported to play a pivotal role in the development of skin autoimmune diseases such as psoriasis.
role in the development of psoriasis by controlling the function of cDCs [33]. Whereas, in epidermis, CD103+ T cells were mostly CD8+ memory T cells that are associated with a progressive clinical course of psoriasis [34]. In addition, nerve endings in the epidermis are distributed around and innervated keratinocytes. Thus, the peripheral nervous system in the skin may link both cutaneous immune cells and keratinocytes and participate in the disease process of psoriasis.

Sensory neurons in the skin are mainly responsible for identifying pathogens, uploading danger signals, and mobilizing immune responses. Under these conditions,
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sensory neurons can activate the type 17 immune responses [35]. The density of PGP 9.5-positive nerve was found engaged in itch occurring in psoriasis patients. Moreover, PGP9.5 expressed by keratinocytes was increased in itchy skin lesions [36]. Similarly, our results also showed that the expression of PGP9.5 increased in psoriatic lesions. And in IMQ-induced psoriasis-like mouse model, the expression of PGP9.5 decreased after BTX pre-treatment.

Fig. 4 IMQ-induced psoriasiform dermatitis was attenuated on the sensory impairment side of spinal cord hemi-section mouse. a Schematic diagram of spinal cord hemi-section operation. b Mouse phenotype on days 0, 3, and 6 after IMQ induction. Red dashed line marked midline of the ipsilesional side and contralesional side. e PASI score of each side. d HE staining and the thickness of the epidermis (μm), scale bar=100 μm. e Gross map and diameter of inguinal lymph nodes. f IF of βIII-tubulin (green), scale bar=100 μm. g IHC staining of Ki67 (left) and Ki67-positive keratinocytes counts (right). *P < 0.05, **P < 0.01, one-way ANOVA.
TRPV1+ sensory neurons, often co-expressing Nav1.8, have been confirmed to play a role in regulating barrier immunity in local skin infections [11]. TRPV1+ neurons can also mediate psoriasiform dermatitis in mice through IL-23 [32]. The increased number of TRPV1 and CGRP-positive neurons in the DRG might account for IMQ-induced skin inflammation.

Sensory nerve fibers can secrete neuropeptides in the skin to regulate the local immune responses. Among them, CGRP has a key regulatory function in skin immunity and

![Image](image.png)
inflammation. CGRP-containing nerves are intimately associated with epidermal Langerhans cells (LCs) by regulating the antigen-presenting capability of Langerhans cells [37]. In addition, the immune system can transmit signals to neurons through cytokines, activate neurons to cause itching, pain, and other sensations, and transmit signals to the central nervous system [38]. Our data showed that in the mouse DRG, receptors of IFN-α and IL-17 increased, suggesting that the strengthened neuro-immune communication may aggravate the local inflammatory response.

Skin nerve denervation prior to the application of IMQ could attenuate the induction of psoriasiform dermatitis [39]. Using the spinal cord hemisection model, we found that the performance of psoriasiform dermatitis in mice on the sensory impairment side was also significantly improved. Recent studies have found that in the pathogenesis of psoriasis, neuropeptides may be involved in promoting the proliferation of keratinocytes [40, 41]. On the sensory impairment side, the proliferation marker Ki67 in epidermal keratinocytes was significantly reduced. Combined with the dense innervation of keratinocytes by nerve endings, it may directly inhibit the abnormal proliferation of keratinocytes. After pre-treatment with BTX-A, IMQ-induced psoriasiform dermatitis in mice was attenuated. Previous studies have found that botulinum toxin type B (BTX-B) can ameliorate psoriasiform dermatitis in mice [42]. The dose used here was half that of BTX-B (1 unit), and the results showed a more significant difference. It has been reported that the number of CGRP-positive nerves in the skin of psoriasis lesions has increased significantly, which is also confirmed by our aforementioned results. Nevertheless, CGRP-positive neurons and the secretion of CGRP in skin tissue culture both decreased after BTX-A pre-treatment. Furthermore, our results showed that BTX-A improved psoriasiform dermatitis by inhibiting CGRP secretion.

In summary, our study suggests that cutaneous nerve fibers communicate with immune cells and keratinocytes and are abnormally activated in psoriasis. Neurological intervention, especially blocking the secretion and transmission of neurotransmitters, may be a new treatment strategy for psoriasis.

Author contributions Conceptualization was performed by X-YM and S-QC. Data curation and formal analysis and writing were performed by S-QC, X-YC and Y-ZC. Supervision and validation were performed by YZ, B-XY and Z-YW. Review and editing were performed by FX, Y-ZH and Y-XZ.

Funding This work was supported by the National Natural Science Foundation of China (Grant No. 81930089).

Data availability The datasets analyzed during the current study are available in the GEO repository. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse121212 and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse53552.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval The study was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine. All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent to publish No individual information, image or video was included in this study.

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