Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Percutaneous splenic nerve stimulation attenuates psoriasis-like dermatitis in rats in an α7nAChR-dependent manner with suppression of cutaneous CGRP/IL-23 signalling

Luying Sha

Bo Wang

Guizhou Medical University

Yudie Ding

Guizhou Medical University

Xiangde Xiao

Guizhou Medical University

Yuanxin Liu

Guizhou Medical University

Qian Zhou

Guizhou Medical University

Li Dong

Guizhou Medical University

Jing Zhou

Guizhou Medical University

Caidie Li

Guizhou Medical University

Youxiao Zhao

Guizhou Medical University

Rongfeng Yang

First People's Hospital of Zunyi

Guilong Wang

15423075490qq.com

Guizhou Medical University

Hong Gao

Affiliated Hospital of Guizhou Medical University

Research Article

Keywords:

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11521024/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Percutaneous splenic nerve stimulation attenuates psoriasis-like dermatitis in rats in an α7nAChR-dependent manner with suppression of cutaneous CGRP/IL-23 signalling

Luying Sha[1], Bo Wang[2,3], Yudie Ding[2], Xiangde Xiao[2], Yuanxin Liu[2,4], Qian Zhou[2,4], Li Dong[2,4], Jing Zhou[2,4], Caidie Li[2], Youxiao Zhao[2], Rongfeng Yang[5], Guilong Wang[2,6], Hong Gao[2,4]

[1]Department of Dermatology, The Fourth People's Hospital of Guiyang, Guiyang, Guizhou, P.R. China

[2]School of Anesthesiology, Guizhou Medical University, Guiyang, Guizhou, P.R. China

[3]Xinhua Town Health Center, Liuzhi Special District, Guizhou, Liupanshui, P.R. China

[4]Department of Anesthesiology, The Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, P.R. China

[5]Department of Anesthesiology, Zunyi First People's Hospital, Zunyi, Guizhou, P.R. China

[6]Department of Anesthesiology, The People's Hospital of Zhijin County, Zhijin, Guizhou, P.R. China

*Luying Sha and Bo Wang contributed equally to this study.

Correspondence: Hong Gao (anesth@gmc.edu.cn); Guilong Wang (1542307549@qq.com).

Abstract

Background Psoriasis is a chronic immune-mediated inflammatory skin disease in which neuro-immune crosstalk is increasingly recognised as a pathogenic driver, and current pharmacological options remain constrained by efficacy, safety, cost and long-term adherence.

Objective To determine whether percutaneous splenic nerve stimulation (PSNS) attenuates imiquimod (IMQ)-induced psoriasis-like dermatitis in rats, and whether the effect depends on α7 nicotinic acetylcholine receptor (α7nAChR)-mediated suppression of the calcitonin gene-related peptide (CGRP)/interleukin-23 (IL-23) axis.

Methods Psoriasis-like dermatitis was induced in Sprague-Dawley rats by daily topical IMQ. Seven groups (n = 6 each) received, concurrently with IMQ, daily PSNS at 0, 1, 10 or 20 mA, or PSNS at 10 or 20 mA preceded by intraperitoneal

α-bungarotoxin (α-BGT, 1 μg/kg); a sham group received petrolatum only. After 10 days, dorsal lesions were scored with the Psoriasis Area and Severity Index (PASI) and skin, liver and kidney were examined histologically (Baker score); serum alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen and creatinine were measured; CGRP, α7nAChR, tumour necrosis factor-α (TNF-α) and IL-23 were quantified by western blotting and ELISA.

Results IMQ produced diffuse erythema, thick scaling and acanthosis, with PASI and Baker scores well above those of sham rats ($P < 0.05$). Stimulation at 1 mA had no measurable effect on any endpoint. At 10 and 20 mA, PASI and Baker scores fell close to sham values, splenic noradrenaline and acetylcholine rose, and splenic TNF-α together with cutaneous CGRP, IL-23 and TNF-α decreased ($P<0.05$); 20mA conferred no additional benefit over 10 mA. α7nAChR protein was up-regulated in skin and spleen at 10 and 20 mA. Pre-treatment with α-BGT abolished the protection and restored cutaneous CGRP, IL-23 and TNF-α, without preventing α7nAChR up-regulation. No hepatic or renal histopathological change, and no serum biochemical abnormality were detected.

Conclusions PSNS attenuates IMQ-induced psoriasis-like dermatitis once a 10 mA intensity threshold is reached, and the protection requires functional α7nAChR. Transcutaneous access to the splenic neuro-immune circuit may offer a non-pharmacological adjunct in psoriasis, pending confirmation in therapeutic-start and chronic models.

Introduction

Psoriasis is a common, relapsing, chronic immune-mediated inflammatory skin disease with a global prevalence of approximately 2%–3%[1]. It may involve any skin area and substantially impairs quality of life and mental health[2]. Its histological hallmarks are abnormal epidermal proliferation, parakeratosis, dermal microvascular dilatation and inflammatory cell infiltration; clinically it presents with erythema, silvery-white scale, skin thickening and intractable pruritus[1,3]. The pathogenesis is incompletely understood, beyond the classical cytokine pathways, other signalling systems such as the adenosine pathway participate in the regulation of psoriatic

inflammation[4]. Psoriasis is currently regarded as the product of genetic susceptibility interacting with environmental triggers, including skin trauma, psychological stress, infection, adverse lifestyle and certain drugs[5]. Abnormal crosstalk between keratinocytes and immune cells is the core event in lesion progression, activated infiltrating immune cells secrete large amounts of pro-inflammatory mediators that drive abnormal keratinocyte proliferation and differentiation and amplify the local inflammatory cascade[6,7].

In recent years, disordered neuro-immune interaction has been implicated in the onset and maintenance of psoriasis. Abnormal neural activation and neuropeptide release mediate cutaneous neurogenic inflammation, aggravating lesion damage and itch[8]. The cholinergic anti-inflammatory pathway is a principal endogenous neuro-immune negative regulator; its core receptor, the α7 nicotinic acetylcholine receptor (α7nAChR), is expressed on cutaneous keratinocytes and on several immune cell populations, where it restrains immune overactivation and pro-inflammatory mediator release and shapes both local and systemic inflammation[9-11]. The reflex is not purely vagal: an intact splenic nerve is required for vagal control of TNF in endotoxaemia[12], acetylcholine-synthesising T cells relay neural signals to α7nAChR-bearing macrophages in the spleen[13], and deletion of α7nAChR abolishes the protective effect of the pathway[14]. α7nAChR activation has been linked to improvement of psoriatic lesions, but its role in psoriasiform dermatitis is incompletely defined[10].

The interleukin-23 (IL-23)/Th17 axis is the canonical immune core of psoriasis, and its sustained activation drives lesion progression; IL-23 and IL-17 are elevated in lesions and in peripheral tissue and correlate with disease severity[15]. Calcitonin gene-related peptide (CGRP) is a major cutaneous neuropeptide that mediates neurogenic inflammation and itch and promotes IL-23 secretion by dermal dendritic cells[16-18], positioning it as the intermediate coupling neural activation to downstream IL-23 immunity. Targeted regulation of CGRP-driven IL-23 signalling is therefore a plausible entry point for neuro-immune intervention in psoriasis.

Current treatments remain constrained. Topical agents, immunosuppressants and

biologics each fall short on efficacy, safety, cost or long-term maintenance, and intolerance or poor adherence is common, leaving room for adjunctive strategies[1,5,19]. Bioelectronic medicine has advanced rapidly, and non-invasive splenic neuromodulation — notably ultrasound stimulation of the spleen — produces clear anti-inflammatory effects in inflammatory disease models such as rheumatoid arthritis[20-22]. Whether percutaneous electrical stimulation over the splenic projection area can modify psoriasis, and whether its effect is intensity-dependent, has not been reported.

Here we used an IMQ-induced rat model of psoriasiform dermatitis to examine the effects of percutaneous splenic nerve stimulation (PSNS) at 0, 1, 10 and 20 mA on skin phenotype, histopathology and neuro-immune inflammation, and used α-bungarotoxin (α-BGT) to test whether α7nAChR is required. We show that PSNS acts above a 10 mA intensity threshold and that its protection depends on functional α7nAChR.

Materials and Methods

1. Animals and Ethics

Specific Pathogen-Free (SPF) healthy male Sprague-Dawley (SD) rats, 8-10 weeks old, weighing 280-360 g, were purchased from Henan Skobes Bio-Technology Co., Ltd. (Production License No. SCXK [Yu] 2025-0005). The rats were housed in the animal facility of the Translational Medicine Center of Guizhou Medical University at an ambient temperature of 22-24°C, relative humidity of 45%-50%, under a 12h/12h light/dark cycle, with free access to food and water. All rats were adaptively fed for 1 week before formal experiments. This experiment strictly followed the Guide for the Care and Use of Laboratory Animals and the 3Rs (Replacement, Reduction, and Optimization) principles. Animal housing, handling, and euthanasia complied with animal welfare requirements. The experimental protocol was approved by the Experimental Animal Ethics Committee of Guizhou Medical University (Approval No. 2602110).

2. Main Reagents and Instruments

5% imiquimod cream (Senren Pharmaceutical Co, China, Batch No.20260301);

α-bungarotoxin (TargetMol, China, Cat. No.TP2077), dissolved in sterile saline at a dosage of 1 μg/kg; rabbit anti-CHRNA7 antibody (Proteintech, China, Cat. No. 82848-3-RR), rabbit anti-CGRP antibody (Bioswamp, China, Cat. No. HA722991), mouse anti-GAPDH antibody (Bioswamp, China, Cat. No.EM1101); HRP-conjugated goat anti-rabbit IgG secondary antibody (Proteintech, China, Cat. No. RGAR001), goat anti-mouse IgG secondary antibody (Proteintech, China, Cat. No. RGAM001); IL-23 ELISA kit (Shanghai Enzyme-linked Biotechnology, China, Cat. No. ml003152); TNF-α ELISA kit (Shanghai Enzyme-linked Biotechnology, China, Cat. No. ml002859); RIPA lysis buffer (Servicebio, China, Cat. No.G2002), PMSF (Servicebio, China, Cat. No.G2008), phosphatase inhibitor (Servicebio, China, Cat. No. G2007), BCA protein assay kit (Servicebio, China, Cat. No.G2026); albumin biochemical kit (Solarbio, China, Cat. No. BC5835), total protein biochemical kit (Nanjing Jiancheng, China, Cat. No. A045-2), ALT biochemical kit (Solarbio, China, Cat. No. BC1555), AST biochemical kit (Solarbio, China, Cat. No. BC1565), BUN biochemical kit (Nanjing Jiancheng, Cat. No.C013-2-1), CRE biochemical kit (Nanjing Jiancheng, China, Cat. No.C011-2-1); electrical stimulator (Beijing Yaoyangda Medical Equipment Co, Ltd., Model KD-2B). Self-adhesive electrodes (specification 40 mm × 40 mm, manufacturer: Beijing Yaoyangda Medical Equipment Co, Ltd.).

3. Grouping and intervention

After adaptive feeding, the rats were randomly divided into 7 groups (n=6 per group) using a random number table: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. In the sham group the dorsal skin was shaved and an equal amount of petrolatum was applied daily, and electrodes were positioned over the splenic projection area (approximately the left 10th–12th intercostal spaces; Figure 1) without current output. In all other groups, 100 mg of 5% IMQ cream was applied evenly to the shaved dorsal area once daily（1.5× 1.5 cm and 44.44 mg/cm²)[23], and percutaneous splenic stimulation was delivered concurrently for 30 min daily through self-adhesive electrodes (40×40 mm; 0.63 mA/cm² at 10 mA) placed over the same projection area, at 10 Hz, 0.2 ms pulse width

and 0, 1, 10 or 20 mA. Rats were sedated with 1.5%–2.0% sevoflurane in all groups.In the two α-BGT groups, α-bungarotoxin (1 μg/kg) was injected intraperitoneally 30 min before stimulation[24]. Modelling, stimulation and antagonist administration ran concurrently for 10 days. the protocol is preventive rather than treatment-starting. Body weight, general condition and any erythema or burn at the electrode site were recorded daily. Dorsal lesions were photographed and PASI-scored independently by two researchers blinded to group allocation, and the mean was used.

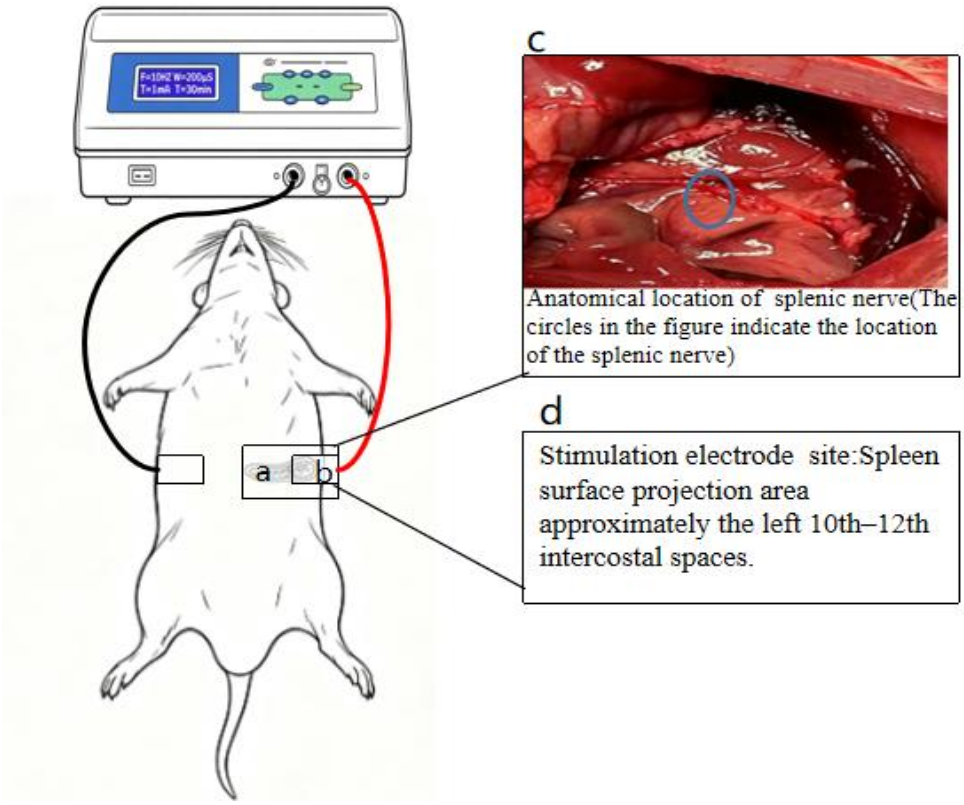


Figure 1 Schematic diagram of splenic nerve stimulation. The stimulation site is the body-surface projection of the spleen, approximately the left 10th–12th intercostal spaces. (a) Surface projection of the rat spleen; (b) stimulation electrode applied over the splenic projection area; (c) rat spleen and splenic nerve after laparotomy, with the blue circle indicating the splenic nerve; (d) annotated stimulation site, approximately between the left 10th and 12th ribs.

4. Haematoxylin–eosin staining and Baker scoring

After 10 days of intervention, all rats were euthanized under deep sevoflurane anaesthesia followed by exsanguination via the abdominal aorta. Dorsal skin lesions, liver and kidney were harvested and fixed in 4% neutral buffered formalin, dehydrated, paraffin-embedded and sectioned. Haematoxylin–eosin (HE) staining was performed and histopathological changes were assessed under light microscopy, with

five randomly selected fields per section. Skin damage was semi-quantified with the Baker score (Munro microabscess 2.0; hyperkeratosis 0.5; parakeratosis 1.0; thinning or loss of the granular layer 1.0; acanthosis 1.0; elongation of rete ridges 0.5/1.0/1.5 for mild/moderate/severe; dermal mononuclear or polymorphonuclear infiltration 0.5/1.0/1.5 for mild/moderate/severe; papillary oedema 0.5; capillary dilatation 0.5). Scoring was performed independently by two researchers blinded to group allocation, and the mean was used.

5. Enzyme-linked immunosorbent assay

Dorsal skin lesion tissue and spleen tissue were rinsed with pre-cooled phosphate-buffered saline (PBS), homogenised in PBS (1:9,w/v) on ice and centrifuged at 5 000 × g for 15 min; the supernatant was collected. TNF-α and IL-23 were measured in skin, and noradrenaline, TNF-α and acetylcholine in spleen, by ELISA according to the manufacturer's instructions. Concentrations were normalised to total protein.

6. Liver and kidney function

Blood (3 mL) was collected from the abdominal aorta, left at room temperature for 60 min and centrifuged at 5 000 × g for 15 min to obtain serum. Serum albumin (ALB), total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine (CRE) were measured with commercial kits according to the manufacturer's instructions.

7. Western blotting

Dorsal skin lesion tissue and spleen tissue were homogenised in lysis buffer (RIPA:PMSF:phosphatase inhibitor = 100:1:1; 10 μL per 1 mg tissue). After mincing, grinding beads were added and homogenisation was performed three times for 30 s with 10 s intervals until no tissue fragments were visible. Lysates were kept on ice for 30 min, centrifuged at 15 000 × g for 15 min and the supernatant collected. Protein concentration was determined with a BCA kit. Proteins were separated on 10% gels (stacking 80 V, separating 120 V) and transferred to PVDF membranes (0.2 μm) at a constant current of 400 mA for 40 min at low temperature. Membranes were blocked in rapid blocking buffer for 30 min at room temperature and incubated overnight at

4 °C with primary antibodies (rabbit anti-CHRNA7 1:3 000, rabbit anti-CGRP 1:1 000, mouse anti-GAPDH 1:5 000). After three 10-min washes, membranes were incubated with HRP-conjugated secondary antibodies (1:5 000) for 1 h at room temperature, washed again and visualised by ECL. Band intensities were quantified by densitometry in ImageJ, normalised to GAPDH and expressed relative to the sham group.

8. Statistical analysis

Data were analysed in GraphPad Prism 10.6.0. Normality (Shapiro–Wilk) and homogeneity of variance (Levene) were tested first. Variables satisfying both assumptions are expressed as mean ± SD and compared by one-way ANOVA with Tukey's post hoc test. Comparisons of interest were pre-specified as each PSNS group versus PSNS-0 mA, and each α-BGT group versus the corresponding PSNS group. $P < 0.05$ was considered statistically significant.

Results

1. Regulation of splenic nerve activity by different intensities of PSNS

Splenic noradrenaline, acetylcholine and TNF-α were measured in the four stimulated groups to confirm that the stimulus engaged the splenic neuro-immune circuit (Figure 2). Splenic noradrenaline was higher at 10 and 20 mA than at 0 and 1 mA ($P < 0.0001$), whereas 1 mA did not differ from 0 mA ($P > 0.05$). Splenic acetylcholine followed the same pattern, rising sharply at 10 and 20 mA relative to 0 and 1 mA ($P < 0.0001$). Splenic TNF-α fell at 10 and 20 mA relative to 0 mA ($P < 0.0001$) and was unchanged at 1 mA ($P > 0.05$). Thus 10 and 20 mA, but not 1 mA, engaged splenic neuro-immune activity.

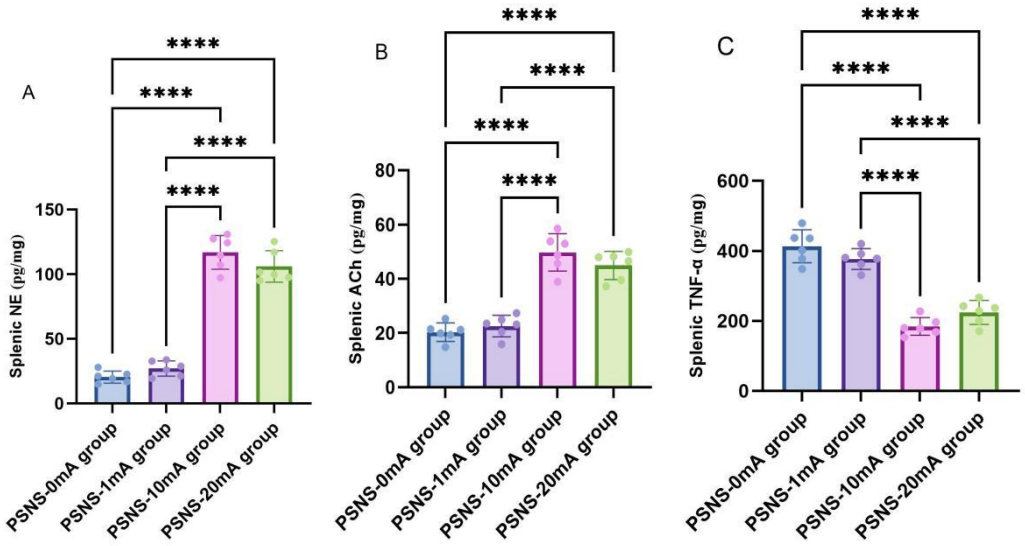


Figure 2 Regulation of splenic nerve activity by PSNS at different intensities (n=6 per group). (A) Splenic noradrenaline; (B) splenic acetylcholine; (C) splenic TNF-α. Groups: PSNS-0 mA, PSNS-1 mA, PSNS-10 mA and PSNS-20 mA. ****P < 0.0001 for the comparisons indicated by the brackets.

2. PSNS attenuates psoriasiform dermatitis above a 10 mA threshold in an α7nAChR-dependent manner

All rats remained in good general condition, with no erythema or burn at the electrode site. The dorsal skin of sham rats was smooth and lesion-free. After 10 consecutive days of IMQ, rats in the PSNS-0 mA group developed diffuse erythema, thick silvery-white scale and marked skin thickening. the typical psoriasiform appearance. Lesion severity then fell with increasing intensity in a stepwise rather than graded manner: PASI scores at 1 mA did not differ from 0 mA ($P > 0.05$), whereas scores at 10 and 20 mA were lower than at 0 mA ($P < 0.05$) and were close to those of sham rats, with no difference between 10 and 20 mA. After α-BGT, PASI scores in both blocked groups returned to PSNS-10 mA and 20 mA group and were higher than in the corresponding PSNS groups ($P < 0.05$), indicating that the protective effect of PSNS was lost when α7nAChR was blocked (Figure 3).

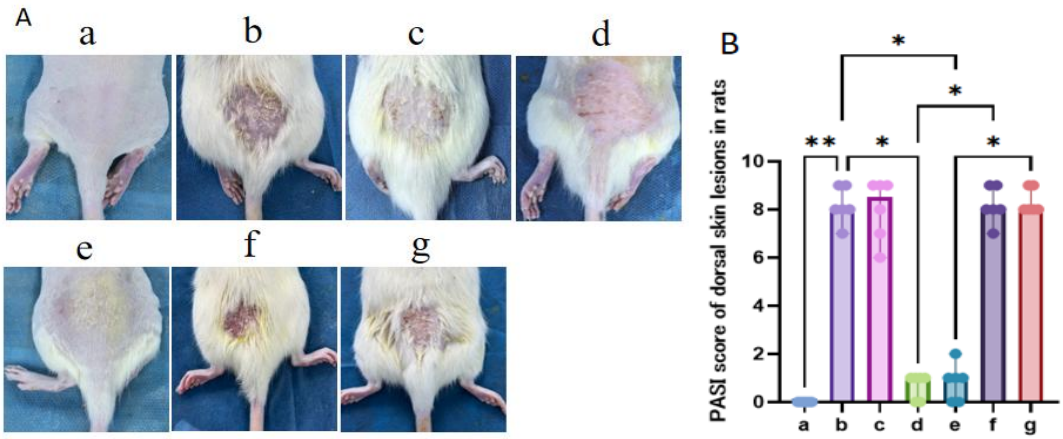


Figure 3 Effects of stimulation intensity on IMQ-induced psoriasis-like lesions, and the effect of α7nAChR blockade (n=6 per group). (A) Dorsal skin appearance; (B) PASI scores. a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. **P < 0.01 versus sham; *P < 0.05 for the remaining bracketed comparisons (versus PSNS-0 mA, and each α-BGT group versus the corresponding PSNS group).

3. α7nAChR mediates the threshold-dependent inhibition of histopathological change by PSNS

HE staining showed a thin, flat epidermis with no obvious dermal inflammatory infiltrate in sham rats. The PSNS-0 mA group displayed the typical psoriasiform picture: hyperkeratosis with marked acanthosis, irregular elongation of rete ridges and abundant inflammatory cells in the superficial dermis. Changes at 1 mA were essentially identical to those at 0 mA. In the PSNS-10 mA and PSNS-20 mA groups, epidermal hyperplasia and dermal infiltration were attenuated, the stratum spinosum was thinner and rete ridges were shortened and blunted, with Baker scores below those of the PSNS-0 mA group ($P < 0.05$). After α-BGT, the histological phenotype and Baker scores in both blocked groups returned to PSNS-10 mA and 20 mA group levels ($P < 0.05$ versus the corresponding PSNS group).

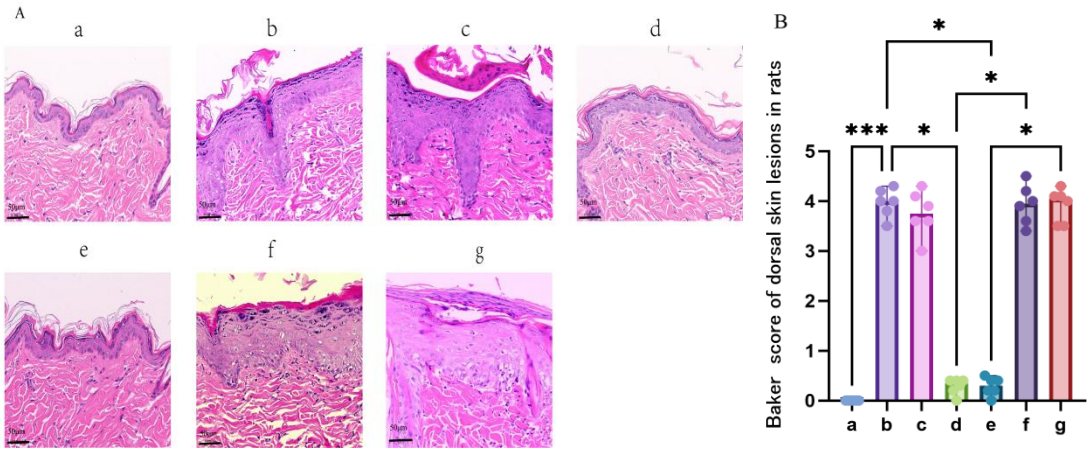


Figure 4 Effects of stimulation intensity on IMQ-induced histopathological change in rat skin and Baker scores (n=6 per group). (A) HE-stained dorsal skin (scale bars, 50 μm); (B) Baker scores. a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. ***P < 0.001 versus sham; *P < 0.05 for the remaining bracketed comparisons (versus PSNS-0 mA, and each α-BGT group versus the corresponding PSNS group).

4. PSNS regulates α7nAChR in skin and spleen and suppresses cutaneous CGRP in an α7nAChR-dependent manner

Compared with sham rats, α7nAChR protein was higher in both skin and spleen of PSNS-0 mA rats ($P < 0.05$), and cutaneous CGRP was also higher ($P < 0.05$). None of these differed between 1 mA and 0 mA ($P > 0.05$). At 10 and 20 mA, α7nAChR was further up-regulated in both tissues while cutaneous CGRP was suppressed ($P < 0.05$). In the two α-BGT groups, α7nAChR remained at the level reached with stimulation alone, antagonist treatment did not reverse receptor up-regulation whereas cutaneous CGRP returned to PSNS-10 mA and 20 mA group levels ($P < 0.05$ versus the corresponding PSNS group; Figures 5–7). Functional blockade therefore abolished the suppression of CGRP without preventing the increase in receptor protein.

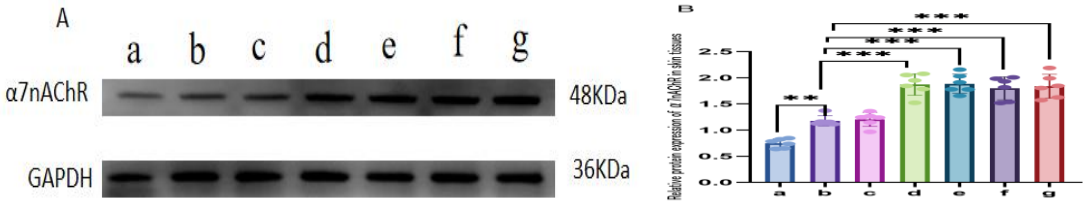


Figure 5 α7nAChR protein in rat skin after PSNS at different intensities (n=6 per group). (A) Representative immunoblot; (B) relative band intensity normalised to GAPDH. a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10

mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. **P < 0.01 versus sham; ***P < 0.001 for the remaining bracketed comparisons.

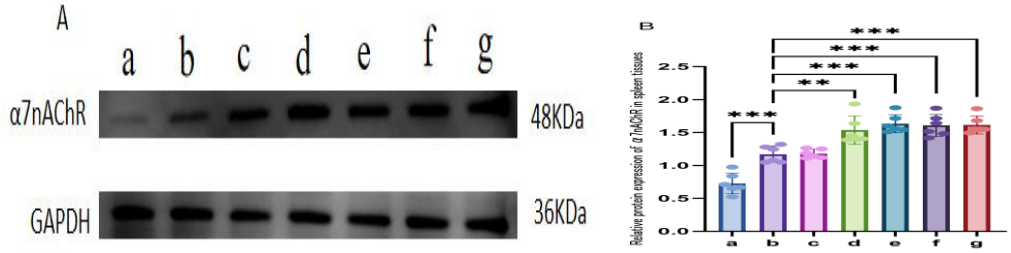


Figure 6 α7nAChR protein in rat spleen after PSNS at different intensities (n=6 per group). (A) Representative immunoblot; (B) relative band intensity normalised to GAPDH. a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. **P < 0.01 and ***P < 0.001 for the comparisons indicated by the brackets.

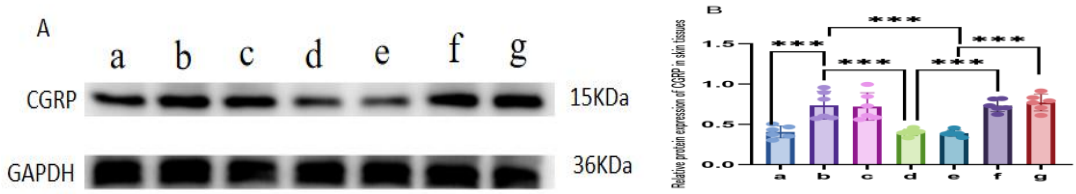


Figure 7 CGRP protein in rat skin after PSNS at different intensities (n=6 per group). (A) Representative immunoblot; (B) relative band intensity normalised to GAPDH. a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. ***P < 0.001 for the comparisons indicated by the brackets.

5. Blocking α7nAChR reverses the down-regulation of cutaneous inflammatory mediators by PSNS

ELISA showed that cutaneous TNF-α and IL-23 were higher in PSNS-0 mA rats than in sham rats (P < 0.001) and were unaffected at 1 mA (P > 0.05). At 10 and 20 mA both cytokines were lower than in the PSNS-0 mA group (P < 0.001). After α-BGT, both returned towards PSNS-10 mA and 20 mA group levels and differed from the corresponding PSNS groups (P < 0.05), indicating that the down-regulation of cutaneous inflammatory mediators by PSNS requires α7nAChR (Figure 8).

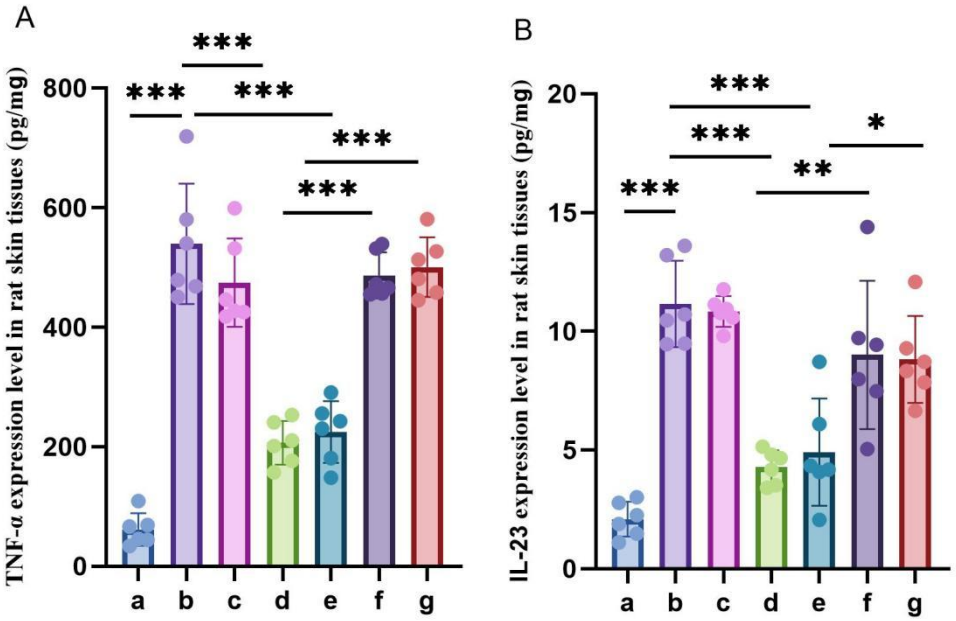


Figure 8 Effects of stimulation intensity on TNF-α and IL-23 in IMQ-treated rat skin (n=6 per group). (A) TNF-α; (B) IL-23. a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. ***P < 0.001 versus sham; *P < 0.05, **P < 0.01 and ***P < 0.001 for the remaining bracketed comparisons.

6. Regulation of splenic TNF-α by PSNS depends on α7nAChR

Splenic TNF-α was higher in PSNS-0 mA rats than in sham rats ($P < 0.05$) and was unaffected at 1 mA ($P > 0.05$). At 10 and 20 mA it was lower than in the PSNS-0 mA group ($P < 0.001$). After α-BGT, splenic TNF-α rose again relative to the corresponding PSNS groups ($P < 0.001$), indicating that the splenic effect of PSNS also depends on α7nAChR (Figure 9).

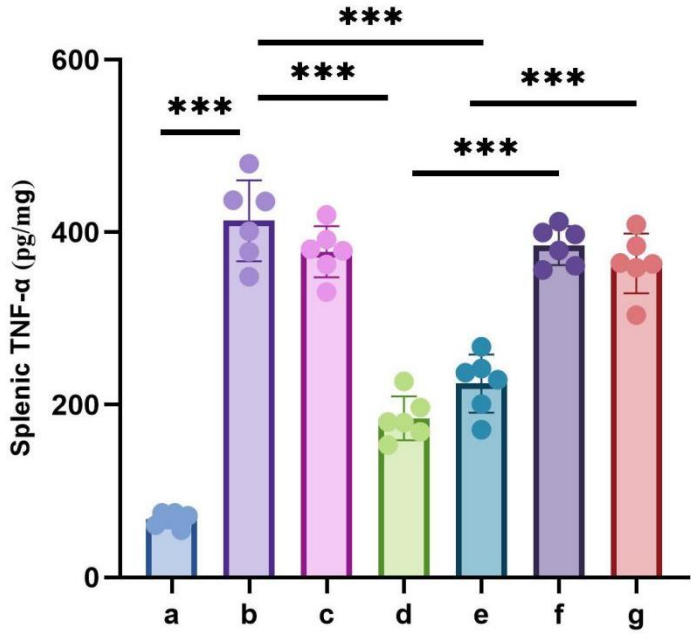


Figure 9 Effect of stimulation intensity on TNF-α in the spleen of IMQ-treated rats (n=6 per group). a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT. ***P < 0.001 versus sham; *P < 0.05, **P < 0.01 and ***P < 0.001 for the remaining bracketed comparisons.

7. **Biosafety of repeated PSNS**

Serum ALT, AST, BUN and CRE did not differ among groups (P > 0.05; Table 1), and HE staining showed no obvious degeneration, necrosis or inflammatory infiltration in liver or kidney in any group (Figure 10). Continuous 10-day PSNS at the intensities tested therefore produced no detectable hepatic or renal injury.

Table 1 Comparison of serum biochemical indicators in rats among different stimulus intensities of percutaneous splenic nerve stimulation ($\bar{x} \pm s$, n = 6)

Indicator	Groups						
	Sham	PSNS-0 mA	PSNS-1 mA	PSNS-10 mA	PSNS-20mA	PSNS-10mA+α-BGT	PSNS-20mA+α-BGT
ALB（g/L）	38.58±5.98	39.66±9.09	33.46±6.11	39.88±6.02	40.77±5.32	38.37±3.26	39.46±3.23
ALT（U/L）	65.39±10.80	66.88±19.13	66.14±21.74	75.80±16.20	71.34±18.49	66.14±25.29	83.97±27.97
AST（U/L）	146.00±24.10	159.60±56.93	144.30±25.44	117.40±18.91	153.60±30.20	164.20±37.96	140.20±28.22
TP（g/L）	58.37±5.90	57.74±10.76	57.32±7.83	65.91±8.91	58.20±6.66	63.46±4.88	60.13±5.43
CRE（μmol/L）	47.47±7.93	46.29±4.87	45.39±5.69	46.64±6.29	46.79±6.09	46.03±5.69	45.65±5.42
BUN（mmol/L）	7.15±0.94	7.27±0.71	7.19±0.68	7.10±0.85	7.41±0.81	6.98±0.78	7.01±0.59

Note: ALB, albumin; TP, total protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRE, serum creatinine; BUN, blood urea nitrogen. Data are mean±SD (n=6 per group); no significant differences among groups (P > 0.05).

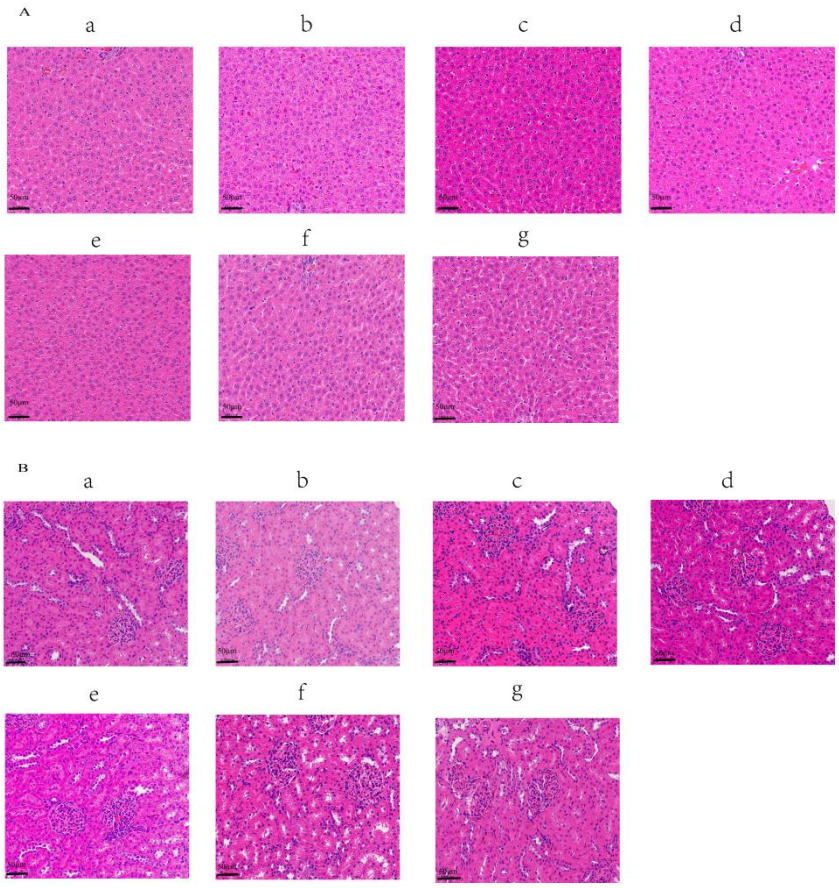


Figure 10 Liver and kidney histopathology in IMQ-treated rats under PSNS at different intensities (n = 6 per group). (A) Liver (scale bars, 50 μm); (B) kidney (scale bars, 50 μm). a–g: sham, PSNS-0 mA, PSNS-1 mA, PSNS-10 mA, PSNS-20 mA, PSNS-10 mA+α-BGT and PSNS-20 mA+α-BGT.

Discussion

Three emerge three findings from our study. First, transcutaneous stimulation over the splenic projection area reduced IMQ-induced skin inflammation, and the response was threshold-like rather than graded: 1 mA was inert, 10 mA produced near-complete protection and 20 mA added nothing. Second, the protection was accompanied by a shift in splenic neurochemistry (noradrenaline and acetylcholine rose while splenic TNF-α fell) consistent with recruitment of the splenic cholinergic anti-inflammatory circuit. Third, α-bungarotoxin abolished the protection without reversing receptor up-regulation, placing functional α7nAChR downstream of the stimulus and upstream of cutaneous CGRP/IL-23.

The threshold behaviour is consistent with published charge–response relationships for splenic nerve stimulation, in which catecholamine release and the resulting

anti-inflammatory effect appear only above a recruitment threshold and saturate thereafter[24-26]. Below that threshold the spleen releases too little noradrenaline to engage the pathway. For translation this implies that 10 mA may already be sufficient, and that escalating intensity buys little while increasing the risk of off-target recruitment.

Mechanistically, the present data place CGRP downstream of α7nAChR. CGRP is released mainly from cutaneous sensory endings and is up-regulated in psoriatic lesions[16,27], where it drives IL-23 production by dermal dendritic cells, Th17/γδT differentiation and the release of TNF-α[18,28]. In our model CGRP, IL-23 and TNF-α all rose with IMQ and fell with 10–20 mA PSNS, and α-BGT restored all three. We did not measure the intermediate steps: splenic choline acetyltransferase, cell-specific α7nAChR or circulating mediators, so the chain from splenic cholinergic signalling to cutaneous CGRP remains inferential rather than demonstrated. Two routes are plausible: a humoral route, in which reduced circulating TNF-α or altered catecholamine tone lowers sensory neuron excitability, and a local route, in which systemically administered α-BGT also acts on α7nAChR within the skin. Distinguishing them will require intrasplenic or splenic-nerve-localised blockade, or splenic denervation.

α7nAChR was higher in both skin and spleen of IMQ-treated rats than in sham rats, in keeping with the endogenous counter-regulatory response described in other inflammatory models[29].α7nAChR is up-regulated in keratinocytes and macrophages within psoriatic lesions, selective agonists alleviate psoriasiform inflammation and restore the keratinocyte proliferation–differentiation balance, and antagonists aggravate lesions[10]. The present data suggest that, although the organism mounts an α7nAChR-mediated counter-response to inflammatory insult, its magnitude is insufficient to halt progression, and exogenous amplification of the pathway is protective. Because α-BGT occupies the receptor without changing its abundance, the persistence of high α7nAChR in the antagonist groups indicates that PSNS continued to drive receptor expression while the receptor itself was functionally silent.

This study evaluated the in vivo biosafety of PSNS. Liver and kidney are core organs for assessing a new intervention, and after 10 consecutive days of stimulation at the intensities tested neither histopathology nor serum ALT, AST, BUN and CRE revealed injury, providing preliminary safety data for translation. As a physical intervention, PSNS also avoids hepatic first-pass metabolism and the cumulative organ toxicity of long-term pharmacotherapy, a potential advantage for a disease requiring prolonged management. Two caveats apply: the exposure was only 10 days, so the safety of longer stimulation and the emergence of stimulation tolerance remain untested; and the stimulated skin, chest wall and spleen were not examined histologically, so local thermal or mechanical effects cannot be formally excluded.

This study has several limitations. First, efficacy was established only in the acute IMQ model; whether PSNS retains activity in chronic relapsing models is unknown. Second, stimulation began on the first day of IMQ application, so the protocol is preventive rather than therapeutic, and a treatment-start design is required before clinical claims can be made. Third, no off-target stimulation control was included: current delivered over the splenic projection area must traverse skin, chest wall and peritoneum, so a contribution from somatic afferent or local muscular activation cannot be excluded, and a group stimulated at a non-splenic site with identical parameters is needed. Fourth, α-BGT was given systemically, so the blockade cannot be assigned to the spleen; intrasplenic or splenic-nerve-localised blockade, or splenic denervation, would be required. Fifth, no α-BGT-alone group was included; although α7nAChR antagonists can themselves aggravate psoriasiform lesions[10], this confounder cannot be fully excluded. Sixth, downstream Th17 effectors such as IL-17 were not measured, and the steps between splenic cholinergic signalling and cutaneous CGRP were not examined. Finally, splenic weight and splenic histology were not reported, although the spleen was both the stimulation target and an immune organ responding to IMQ.

In summary, percutaneous stimulation over the splenic projection area attenuates IMQ-induced psoriasiform dermatitis in rats once a 10 mA intensity threshold is reached; the protection requires functional α7nAChR and is accompanied by

suppression of cutaneous CGRP/IL-23 signalling. These findings provide preliminary preclinical support for transcutaneous access to the splenic neuro-immune circuit as a non-pharmacological adjunct in psoriasis. Confirmation in therapeutic-start and chronic models, and direct demonstration of the splenic-to-skin link, are the next steps.

Funding

This work was supported by Science and Technology Fund Project of Guizhou Provincial Health Commission (gzwkj2025-044).

Author Contributions

Conceptualization: Hong Gao, Guilong Wang. Methodology and investigation: Luying Sha, Bo Wang, Yudie Ding, Xiangde Xiao, Yuanxin Liu, Qian Zhou, Li Dong, Jing Zhou, Caidie Li, Youxiao Zhao, Rongfeng Yang. Formal analysis: Luying Sha, Bo Wang. Writing – original draft: Luying Sha. Writing – review and editing: Hong Gao, Guilong Wang. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. Guo J, Zhang H, Lin W, Lu L, Su J, Chen X. Signaling pathways and targeted therapies for psoriasis. Signal Transduct Target Ther. 2023;8(1):437. doi:10.1038/s41392-023-01655-6
2. Singh S, Taylor C, Kornmehl H, Armstrong AW. Psoriasis and suicidality: a systematic review and meta-analysis. J Am Acad Dermatol. 2017;77(3):425-440.e2. doi:10.1016/j.jaad.2017.05.019
3. Yan J, Ye F, Ju Y, et al. Cimifugin relieves pruritus in psoriasis by inhibiting TRPV4. Cell Calcium. 2021;97:102429. doi:10.1016/j.ceca.2021.102429

4. Giraulo C, De Palma G, Plaitano P, Cicala C, Morello S. Insight into adenosine pathway in psoriasis: elucidating its role and the potential therapeutical applications. Life Sci. 2024;357:123071. doi:10.1016/j.lfs.2024.123071
5. Prema SS, Shanmugamprema D. Systemic psoriasis: from molecular mechanisms to global management strategies. Clin Rev Allergy Immunol. 2025;68(1):79. doi:10.1007/s12016-025-09089-4
6. Ortiz-Lopez LI, Choudhary V, Bollag WB. Updated perspectives on keratinocytes and psoriasis: keratinocytes are more than innocent bystanders. Psoriasis (Auckl). 2022;12:73-87. doi:10.2147/PTT.S327310
7. Park S, Jang J, Kim HJ, Jung Y. Unveiling multifaceted roles of myeloid innate immune cells in the pathogenesis of psoriasis. Mol Aspects Med. 2024;99:101306. doi:10.1016/j.mam.2024.101306
8. Popescu MN, Beiu C, Iliescu MG, et al. Botulinum toxin use for modulating neuroimmune cutaneous activity in psoriasis. Medicina (Kaunas). 2022;58(6):813. doi:10.3390/medicina58060813
9. Aidan Falvey, Christine N Metz, Kevin J Tracey, et al. Peripheral nerve stimulation and immunity: the expanding opportunities for providing mechanistic insight and therapeutic intervention. Int Immunol. 2022 Jan 22;34(2):107-118. doi: 10.1093/intimm/dxab068.
10. Chen Y, Lian P, Peng Z, et al. Alpha-7 nicotinic acetylcholine receptor agonist alleviates psoriasis-like inflammation through inhibition of the STAT3 and NF-κB signaling pathway. Cell Death Discov. 2022;8(1):141. doi:10.1038/s41420-022-00943-4
11. Keever KR, Yakubenko VP, Hoover DB. Neuroimmune nexus in the pathophysiology and therapy of inflammatory disorders: role of α7 nicotinic acetylcholine receptors. Pharmacol Res. 2023;191:106758. doi:10.1016/j.phrs.2023.106758

12. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. Nature. 2003;421(6921):384-388. doi:10.1038/nature01339
13. Rosas-Ballina M, Ochani M, Parrish WR, et al. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. Proc Natl Acad Sci U S A. 2008;105(31):11008-11013. doi:10.1073/pnas.0803237105
14. Rosas-Ballina M, Olofsson PS, Ochani M, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science. 2011;334(6052):98-101. doi:10.1126/science.1209985
15. Menter A, Krueger GG, Paek SY, et al. Interleukin-17 and interleukin-23: a narrative review of mechanisms of action in psoriasis and associated comorbidities. Dermatol Ther (Heidelb). 2021;11(2):385-400. doi:10.1007/s13555-021-00483-2
16. Zhang X, Cao J, Zhao S, et al. Nociceptive sensory fibers drive interleukin-23 production in a murine model of psoriasis via calcitonin gene-related peptide. Front Immunol. 2021;12:743675. doi:10.3389/fimmu.2021.743675
17. Zhang J, Zhao S, Xing X, et al. Effects of neuropeptides on dendritic cells in the pathogenesis of psoriasis. J Inflamm Res. 2023;16:35-43. doi:10.2147/JIR.S397079
18. Kashem SW, Riedl MS, Yao C, et al. Nociceptive sensory fibers drive interleukin-23 production from CD301b+ dermal dendritic cells and drive protective cutaneous immunity. Immunity. 2015;43(3):515-526. doi:10.1016/j.immuni.2015.08.016
19. Radu A, Tit DM, Endres LM, et al. Naturally derived bioactive compounds as precision modulators of immune and inflammatory mechanisms in psoriatic conditions. Inflammopharmacology. 2025;33(2):527-549. doi:10.1007/s10787-024-01602-z

20. Pavlov VA, Tracey KJ. Bioelectronic medicine: preclinical insights and clinical advances. Neuron. 2022;110(21):3627-3644. doi:10.1016/j.neuron.2022.09.003
21. Cotero V, Fan Y, Tsaava T, et al. Noninvasive sub-organ ultrasound stimulation for targeted neuromodulation. Nat Commun. 2019;10(1):952. doi:10.1038/s41467-019-08750-9
22. Zachs DP, Offutt SJ, Graham RS, et al. Noninvasive ultrasound stimulation of the spleen to treat inflammatory arthritis. Nat Commun. 2019;10(1):951. doi:10.1038/s41467-019-08721-0
23. Parmar KM, Jagtap CS, Katare NT, Dhobi M, Prasad SK. Development of a psoriatic-like skin inflammation rat model using imiquimod as an inducing agent. Indian J Pharmacol. 2021;53(2):125-131. doi:10.4103/ijp.IJP_506_19
24. Wang Z, Liu T, Yin C, et al. Electroacupuncture pretreatment ameliorates anesthesia and surgery-induced cognitive dysfunction via activation of an α7-nAChR signal in aged rats. Neuropsychiatr Dis Treat. 2021;17:2599-2611. doi:10.2147/NDT.S322047
25. Mughrabi IT, Gerber M, Jayaprakash N, et al. Voltammetry in the spleen assesses real-time immunomodulatory norepinephrine release elicited by autonomic neurostimulation. J Neuroinflammation. 2023;20(1):236. doi:10.1186/s12974-023-02902-x
26. Jin X, Wang X, Sun J, et al. Subthreshold splenic nerve stimulation prevents myocardial ischemia-reperfusion injury via neuroimmunomodulation of proinflammatory factor levels. Int Immunopharmacol. 2023;114:109522. doi:10.1016/j.intimp.2022.109522
27. Guyot M, Simon T, Panzolini C, et al. Apical splenic nerve electrical stimulation discloses an anti-inflammatory pathway relying on adrenergic and nicotinic receptors in myeloid cells. Brain Behav Immun. 2019;80:238-246. doi:10.1016/j.bbi.2019.03.015

28. Riol-Blanco L, Ordovas-Montanes J, Perro M, et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. Nature. 2014;510(7503):157-161. doi:10.1038/nature13199
29. Pu W, Su Z, Wazir J, et al. Protective effect of α7 nicotinic acetylcholine receptor activation on experimental colitis and its mechanism. Mol Med. 2022;28(1):104. doi:10.1186/s10020-022-00532-2