

Gasdermin D-Mediated Neutrophil Pyroptosis drives Inflammation in Psoriasis

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eLife Assessment

This is a **valuable** study regarding the role of gasdesmin D in experimental psoriasis. The study contains **solid** evidence for such a role, involving neutrophils, from murine models of skin inflammation, as well as correlative data of elevated gasdermin D expression in human psoriatic skin. The findings will be of interest to researchers trying to unravel pathways of skin inflammation.

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Abstract

Psoriasis is a multifactorial immune-mediated inflammatory disease. Its pathogenesis involves abnormal accumulation of neutrophils and T-cell related abnormalities. Pyroptosis is a type of regulated cell death associated with innate immunity, but its role in psoriasis is unclear. In this study, we found that *gasdermin D* (*Gsdmd*) is higher in human psoriatic skin than that in normal skin, and in imiquimod-induced psoriasis-like mouse skin, the expression of *Gsdmd* was most significantly altered in neutrophils and *Il1b* was also mainly expressed in neutrophils. Immunohistochemical staining of serial sections of skin lesions from psoriasis patients and healthy control also showed that GSDMD expression is higher in psoriasis lesion, especially in neutrophils. *Gsdmd* deficiency mitigates psoriasis-like inflammation in mice. GSDMD in neutrophils contributes to psoriasis-like inflammation, while *Gsdmd* depletion in neutrophils attenuates the development of skin inflammation in psoriasis and reduces the release of the inflammatory cytokines. We found that neutrophil pyroptosis is involved in and contributes to psoriasis inflammation, which provides new insights into the treatment of psoriasis by targeting neutrophil pyroptosis.

1. Introduction

Psoriasis is an autoimmune disease that involves multiple systems and manifests primarily as plaques and scaling. The incidence of psoriasis displays significant variation across populations, with estimates ranging from 0.47% in China to 8.5% in Norway, and 3.0% among adults in the United States[1–3]. The etiology of psoriasis is multi-factorial and involves a complex interplay of genetic, environmental, infectious and lifestyle factors. Previous studies have suggested that the pathogenesis of psoriasis is related to the involvement of various cells, including immune cells, keratinocytes and stromal cells, and the underlying mechanism is complex[4]. The participation of immune cells remains a research focus. Most biologics utilized to treat psoriasis presently target the immune pathway. Current studies suggest that the interleukin 23 (IL-23)/T-helper 17 (Th17) immune axis is the primary immune pathway involved in psoriasis pathogenesis[5]. Th17 cells play a significant role in the pathogenesis of many autoimmune and inflammatory diseases. Th17-derived proinflammatory cytokines, such as IL-17A, IL-17F, and IL-22 are critical in the development of psoriasis[6]. Studies have shown that IL-17A contributes to both an adaptive immune circuit involving specific T-cell subsets and an innate axis between keratinocytes and neutrophils[7]. Except for Th17 cells, Neutrophils within the skin are a hallmark feature of psoriasis. Neutrophils accumulate in the dermis and epidermis, resulting in the formation of Kogoj pustules and Munro microabscesses [8]. Neutrophils are increased in the skin and blood of patients with psoriasis,[9] and promote psoriasis by secreting proinflammatory mediators and proteases through degranulation or forming neutrophil extracellular traps[10–12]. Nevertheless, the mechanisms by which neutrophils promote and exacerbate inflammation in psoriasis remain under investigation.

Pyroptosis is a type of regulated cell death triggered by the inflammasome and mediated by the gasdermin family[13]. Gasdermin D (GSDMD) is currently the most extensively researched member of the gasdermin family. After perturbations of extracellular or intracellular homeostasis related to innate immunity, the classical and non-classical pathways activate caspase 1 and caspase 4/5/11 (caspase 4/5 in humans and caspase 11 in mice)[14], and the activated caspases cleave GSDMD into its N-terminal and C-terminal fragments[15]. The N-terminus of GSDMD forms a transmembrane pore, which releases inflammatory mediators [16, 17] and results in proinflammatory cell death[18]. In recent years, there has been a focus on the role of pyroptosis in infections, tumors, and autoimmune diseases[19–22] due to its potential to cause inflammatory diseases when occurring excessively.

While previous studies have showed that the expression of GSDMD is increased in lesions of patients with psoriasis[23], and the pyroptosis of keratinocytes plays a role in inflammation seen in psoriasis[24]. However, the exact contribution of immune cell pyroptosis to the development of psoriasis remains unclear. Therefore, our study aimed to investigate the role of GSDMD-mediated immune cell pyroptosis in psoriasis.

2. Methods

2.1 Experimental animals

All mice were of the species *Mus musculus* (C57BL/6). All experiments were performed with female mice (6–8 weeks old). The *Gsdmd*^{−/−} mice were provided by Dr. Feng Shao (National Institute of Biological Sciences, Beijing, China). The *Gsdmd*^{fl/fl} mice were generated using conditional gene targeting methods as described previously[25]. All mice were kept in a barrier facility, and all

animal experiments were conducted in accordance with the procedure approved by the Ethical Review Committee for Laboratory Animal Welfare of the Nanjing Medical University (IACUC-2203023).

2.2 Psoriasis-like mouse model

Psoriasis-like mice were induced by 5% imiquimod (IMQ) cream as previously described [26]. Mice received topical application of 32.5 mg 5% IMQ to the shaved dorsal skin of a limited area (2*3 cm²) for 4 days, and control mice were given the same dose of Vaseline for 4 days. The skin was examined every day, and the dorsa were photographed. The mice were sacrificed by cervical dislocation on day 5, and their skin and serum were collected for the next experiment.

2.3 Western blot

Mice back skin tissues were lysed in lysis buffer solution [150 mM NaCl, 10 mM tris (pH 7.4), 5 mM EDTA, 1 mM EGTA, and 0.1%NP-40] supplemented with 1 mM phenylmethylsulphonyl fluoride, and complete protease inhibitor “cocktail” [Sigma-Aldrich]), followed by tissue homogenization and incubation for 60 min at 4°C. The lysates were centrifuged for 10 min at 14,000 g, and supernatants were denatured with SDS buffer, and boiled for 10 min. Proteins were separated by SDS–polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were immunoblotted with primary antibodies and proteins detected with appropriate secondary anti-rabbit antibody conjugated to fluorescence. Immunoreactivity was visualized by the Odyssey Imaging System (LI-COR Biosciences).

2.4 Enzyme-linked immunosorbent assay

Homogenized the mouse back skin tissues (the method is the same as before), and collected the supernatant. Supernatant were collected and measured for the level of IL-1 β (DY401) and IL-6 (DY406) according to manufacturer’s instructions by sandwich enzyme-linked immunosorbent assay (ELISA) (R&D System).

2.5 Immunofluorescence and Immunohistochemistry staining

For immunofluorescence, the mice skin tissues were collected, fixed in 4% buffered formaldehyde, and embedded in paraffin. Tissue sections were incubated at 4°C overnight with primary antibody to GSDMD (Abcam, ab219800, 1:300) and Ly6G (BDscience, Cat551459, 1:100). Slides were then incubated with indicated secondary antibodies. TUNEL staining was carried out with staining kit (Servicebio). The nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich).

Slides were dried and mounted using ProLong Antifade mounting medium (Beyotime Biotechnology). Slides were visualized using a Nikon 50i fluorescence microscope. For immunohistochemical staining, human skin tissue samples were obtained from the Department of Dermatology, Jiangsu Provincial People’s Hospital. Our study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital), Ethics Number: 2020-SRFA-101, and informed consent was obtained from all study participants. Sections were blocked and incubated with primary antibodies to GSDMD (Abcam, ab215203, 1:1000) and CD66b (Biolegend, Cat305102, 1:80) after heat-induced antigen retrieval. Slides were then incubated with horseradish peroxidase-conjugated secondary antibodies. Diaminobenzidine was used for detection. Images were captured with a Nikon 50i microscope. Images were processed using ImageJ 1.53c and AdobePhotoshopCS6 software.

2.6 Flow cytometry (FCM) and Fluorescence Activated Cell Sorting (FACS)

For FCM, skin tissue was digested into single cell suspensions with collagenase P (ROCHE, 1mg/mL), DNaseI (ROCHE, 100 g/ml), and HAase (ABSIN, 10 µg/ mL) at 37°C for 1 hour. Single cell suspensions were stained with anti-CD45-Alexa Flour 700 (eBioscience, 1:400), anti-Ly6G-eFlour 450 (eBioscience, 1:400), and FVD eFlour 506 (eBioscience,1:1000) for Flow cytometry (Thermo). After the mice were euthanized, the long bones were separated and the bone marrow was aspirated with a sterile syringe. Single cell suspension was stained with anti-CD45-Alexa Flour 700 (eBioscience, 1:400), anti-CD11b-Percp-cy5.5 (eBioscience, 1:400) and anti-Ly6G-eFlour 450(eBioscience, 1:400) for FACS analysis (Thermo), CD45⁺FVD⁻CD11b⁺Ly6G⁺ neutrophils were obtained by FACS. All flow cytometry analyses were performed on an Attune NxT Flow Cytometer (Thermo Fisher Scientific), and data were analyzed using FlowJo 10 software.

2.7 Neutrophil depletion

An injection of anti-Ly6G antibody (Selleck, 200 µg) was given on day-1 and day2 during continuous IMQ application to deplete neutrophil in mice.

2.8 Statistical analysis

Data were analyzed using Prism 8 (GraphPad, San Diego, CA, USA). Comparisons between two groups were analyzed by using unpaired Student's t-test. All data are presented as the mean ± SEM and are representative of at least three independent experiments. $P < 0.05$ was considered indicative of statistical significance. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p \leq 0.0001$, ns $p > 0.05$.

3. Results

3.1 The GSDMD-mediated pyroptosis is activated in psoriasis

By searching the GEO database, we found that *Gsdmd* is higher in human psoriatic skin than that in normal skin, and the similar trend was seen in IMQ-induced psoriasis model mice compared to controls (**Fig. 1a**). Immunohistochemical staining of serial sections of skin lesions from psoriasis patients and healthy control also showed that GSDMD expression is higher in psoriasis patients (**Fig. 1b**). To further elucidate the presence of pyroptosis in psoriasis, we induced a psoriasis-like mouse model by applying IMQ (**Fig. 1c**). The lesions of the mice were collected for western blotting, our results indicated that the IMQ-treated group not only has elevated expression of caspase 1 and GSDMD relative to controls, but also shows functional cleavage of 30KD GSDMD N fragment (**Fig. 1d-e**). Hence, the activation of pyroptosis-associated molecules was found in psoriasis lesions.

3.2 GSDMD deficiency mitigates psoriasis-like inflammation in mice

Given the particular elevation of GSDMD levels in psoriasis, we questioned whether GSDMD contributed to psoriasis pathogenesis. We treated WT and GSDMD deficient mice with IMQ to induce psoriasis (**Fig. 1c**). We found that the psoriasis area and severity index (PASI)scores were significantly lower in *Gsdmd*^{-/-} mice than WT after 4 days of IMQ application (**Fig. 2a-b**). Moreover, indicators to assess the severity of psoriasis, including acanthosis, parakeratosis, and microabscess formation as well as infiltration of leukocytes, were less pronounced in *Gsdmd*^{-/-} mice compared with WT (**Fig. 2c**). We verified the absence of GSDMD in the skin of *Gsdmd*^{-/-} mice by western blotting and found that after induction of psoriasis in *Gsdmd*^{-/-} mice, the

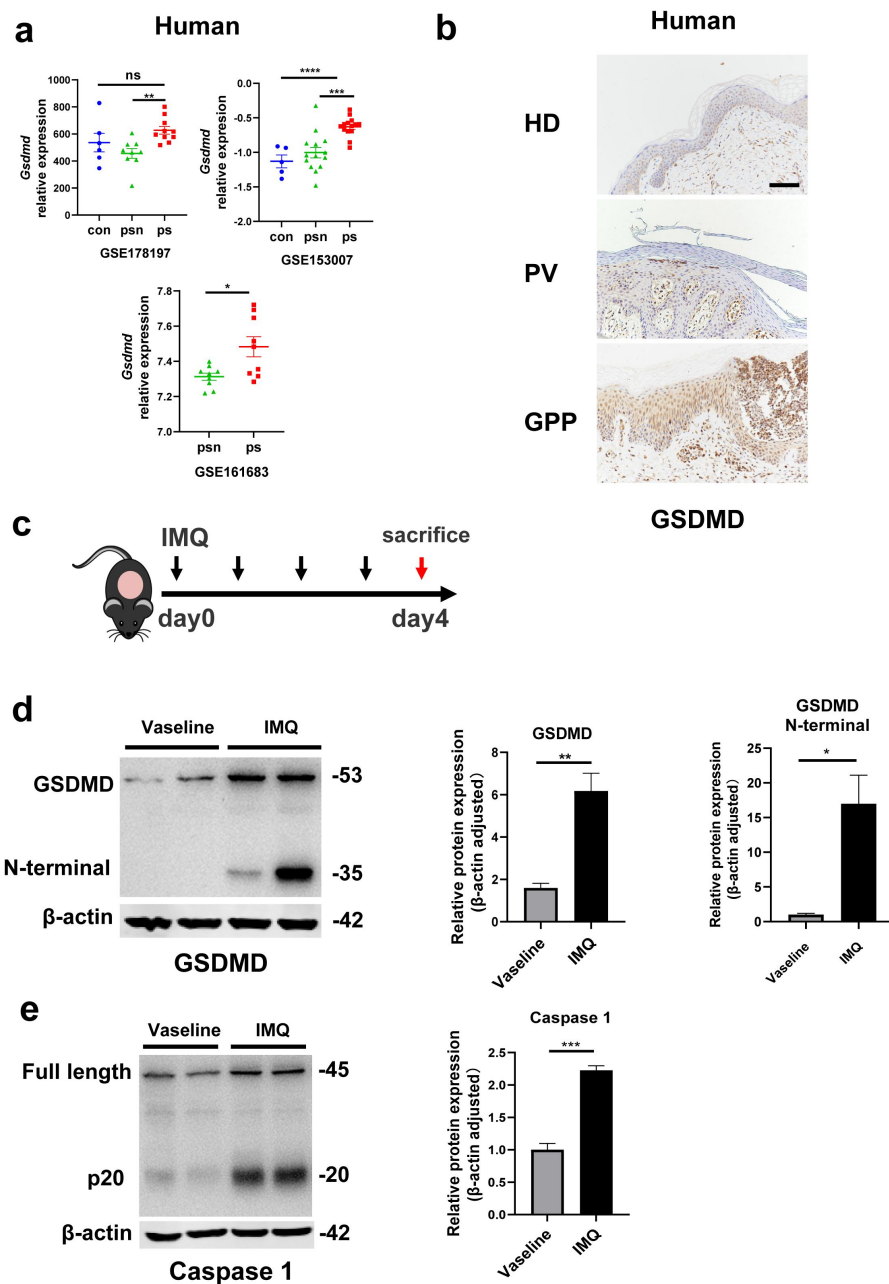


Figure 1.

The GSDMD-mediated pyroptosis is activated in psoriasis.

a) Expression of GSDMD in patients with psoriasis patients and healthy people from GSE178197, GSE161683 and GSE153007.

b) Representative immunohistochemical staining of GSDMD in sections of skin tissue from healthy individuals and patients with psoriasis (n=3-5, each group). Scale bar =100 μ m.

c) Schematic representation of the IMQ-induced psoriasis mouse model.

d) Representative images and statistical analysis of western blot analysis showing the expression of GSDMD and its N-terminal fragments in dorsal skin of WT mice treated with vehicle or IMQ at day 4 (n=4).

e) Representative images and statistical analysis of western blot analysis showing the expression level of pro-caspase1 and caspase1 in dorsal skin of WT mice treated with vehicle or IMQ at day 4.

psn, psoriasis non-lesional skin; ps, psoriasis skin; con, control; HD, healthy donor; GPP, generalized pustular psoriasis; IMQ, imiquimod; WT, wild-type. Error bars show mean $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P $\leq$ 0.0001. Data are representative of three independent experiments for (b, d, e).

expression and activation of caspase 1, an upstream molecule of pyroptosis, were decreased (**Fig. 2d**). Levels of the pro-inflammatory cytokines in protein and mRNA were also examined in the skin using ELISA and quantitative PCR, respectively. Our results showed that the levels of inflammatory cytokines in psoriasis such as IL17a, TNF- α , IL6, IL-1 β and IL18 were substantially higher in the WT mice than in the *Gsdmd*^{-/-} mice (**Fig. 2e-f**). These results demonstrated that the absence of GSDMD ameliorated IMQ-induced psoriasiform dermatitis in mice.

3.3 Neutrophils undergo GSDMD mediated pyroptosis in psoriasis

Previous studies have shown that pyroptosis occurs in psoriasis[24, 27]. Moreover, neutrophils are critical to the pathogenesis of psoriasis, which is usually characterized by a high accumulation of neutrophils both in lesions as well as in blood[28]. Therefore, we wanted to clarify whether neutrophils undergo pyroptosis in psoriasis. Firstly, we searched the GEO database and found that psoriasis patients' neutrophils showed higher expression of GSDMD (**Fig. 3a**). Serial sections of skin lesions from psoriasis patients were immunohistochemically stained with CD66b and GSDMD antibodies. We further found that GSDMD was prominently expressed in neutrophils, especially in Munro's microabscesses, which were indicated by CD66b in the patients' skin of both psoriasis vulgaris and pustular psoriasis (**Fig. 3b**). Through database search and analysis, we found that in psoriasis-like mouse skin, the expression of *Gsdmd* was most significantly altered in neutrophils (**Fig. 3c**). Then, we carried out immunofluorescent analysis of GSDMD in the psoriatic skin tissues of WT mice and GSDMD-deficient mice used as a negative control. Lymphocyte antigen 6 (Ly6G), a classical neutrophil marker[29, 30], was found to be mainly expressed in neutrophils in the skin of IMQ-induced psoriasis-like mice (S1a). Therefore, we chose Ly6G as a marker for mouse neutrophils in our experiments. In line with GEO data, immunofluorescence staining showed that GSDMD was more strongly expressed in the mice skin of IMQ applied group than control, including the significant localization of GSDMD in neutrophils (**Fig. 3d**). GSDMD is an effector molecule that mediates pyroptosis. We then found that, compared to the control group, neutrophils infiltrating in IMQ-induced psoriasis-like tissue display a higher expression of pyroptosis-related genes through analyzing the publicly available single-cell transcriptomic data (GSE165021) (**Fig. 3e**). These results highlight the role of neutrophil pyroptosis in the progression of psoriasis. To further confirm that these neutrophils underwent pyroptosis, we used flow cytometry to stain PI to confirm neutrophil pyroptosis and performed TUNEL staining on mouse skin after IMQ application to exclude other cell deaths such as apoptosis (**Fig. 3f**, S1b), these data suggest that GSDMD-mediated pyroptosis exists in neutrophils infiltrating into psoriatic lesions as shown more PI staining but not change in TUNEL staining in neutrophils.

3.4 GSDMD depletion in neutrophils attenuates the development of skin inflammation in psoriasis

To investigate the specific effect of neutrophils in psoriasis, we bred *Gsdmd*^{fl/fl}; *S100a8*^{cre/+} (cKO) mice to make conditionally knockout GSDMD in neutrophils. There are many previous studies using *S100a8*-Cre mice to knockout neutrophil-specific genes[31, 32]. And we found that *S100a8* is mainly expressed in neutrophils and slightly in macrophages in psoriasis-like mouse skin (S1c). Therefore, we believe that the knockout effect of GSDMD in cKO mice on other cells is quite small. We isolated bone marrow-derived neutrophils and demonstrated by immunoblotting that GSDMD expression was reduced in cKO neutrophil (**Fig. 4a**). Then we applied IMQ to the cKO and control mice (*Gsdmd*^{fl/fl}; *S100a8*^{+/+}) mice on a continuous basis for 4 days to induce psoriasis. Our results showed that after induction of psoriasis, cKO mice had milder disease than controls, as evidenced by reduced PASI scores and the apparent remission of skin changes shown by HE staining, including acanthosis, parakeratosis, and infiltration of leukocytes (**Fig. 4b-d**). And the lower levels of inflammatory cytokines, including IL-17a, TNF- α , IL-6, IL-1 β and IL-18, were also showed in cKO mice (**Fig. 4e-f**). Notably, although neutrophil-specific deletion of

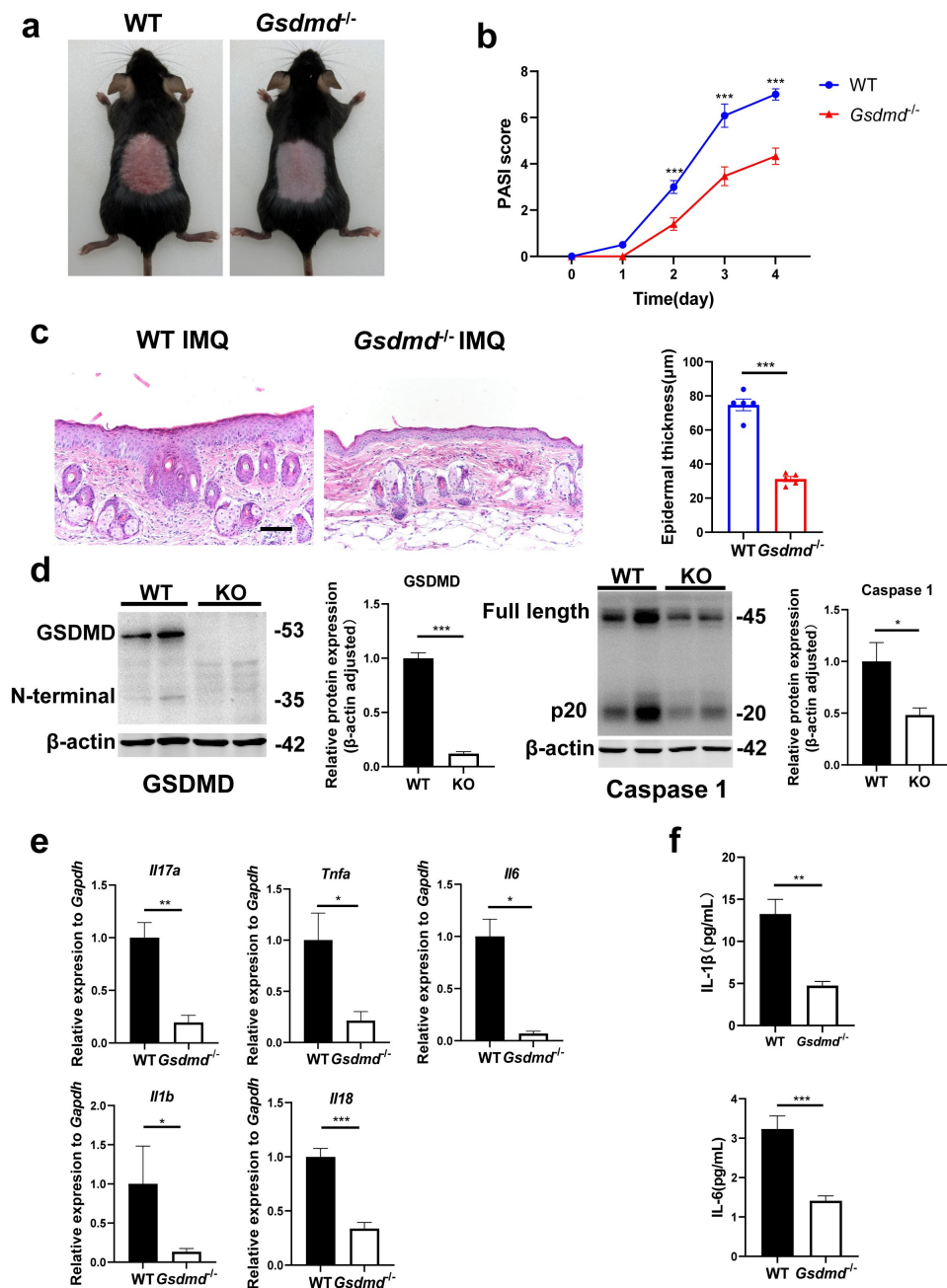


Figure 2.

GSDMD deficiency mitigates psoriasis-like inflammation in mice.

- a)** Macroscopic phenotypic representation of the dorsal skin in WT and GSDMD-KO mice treated with IMQ at day 4.
- b)** Disease severity of psoriasis induced by IMQ in mice as assessed by PASI score (n=10-12).
- c)** Representative images and statistical analysis of hematoxylin and eosin staining of the dorsal skin in WT and GSDMD-KO mice treated with IMQ at day 4 (n=5). Scale bar =100μm.
- d)** Representative images and statistical analysis of western blot analysis showing the expression level of GSDMD and caspase1 in dorsal skin of WT and GSDMD-KO mice treated with IMQ at day 4 (n=4).
- e)** Quantitative PCR analysis of the relative mRNA expression of proinflammatory cytokines in the dorsal skin of WT and GSDMD-KO mice treated with IMQ at day 4 (n = 4). Data were normalized to a reference gene, Gapdh.
- f)** ELISA analysis of IL-6 and IL-1β per 1 mg of the dorsal skin from WT and GSDMD-KO mice treated with IMQ at day 4 (n=5).
- IMQ, imiquimod; WT, wild-type; KO, knockout; PASI, psoriasis Area and Severity Index. Error bars show mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. Data are representative of three independent experiments for (a, c, d).

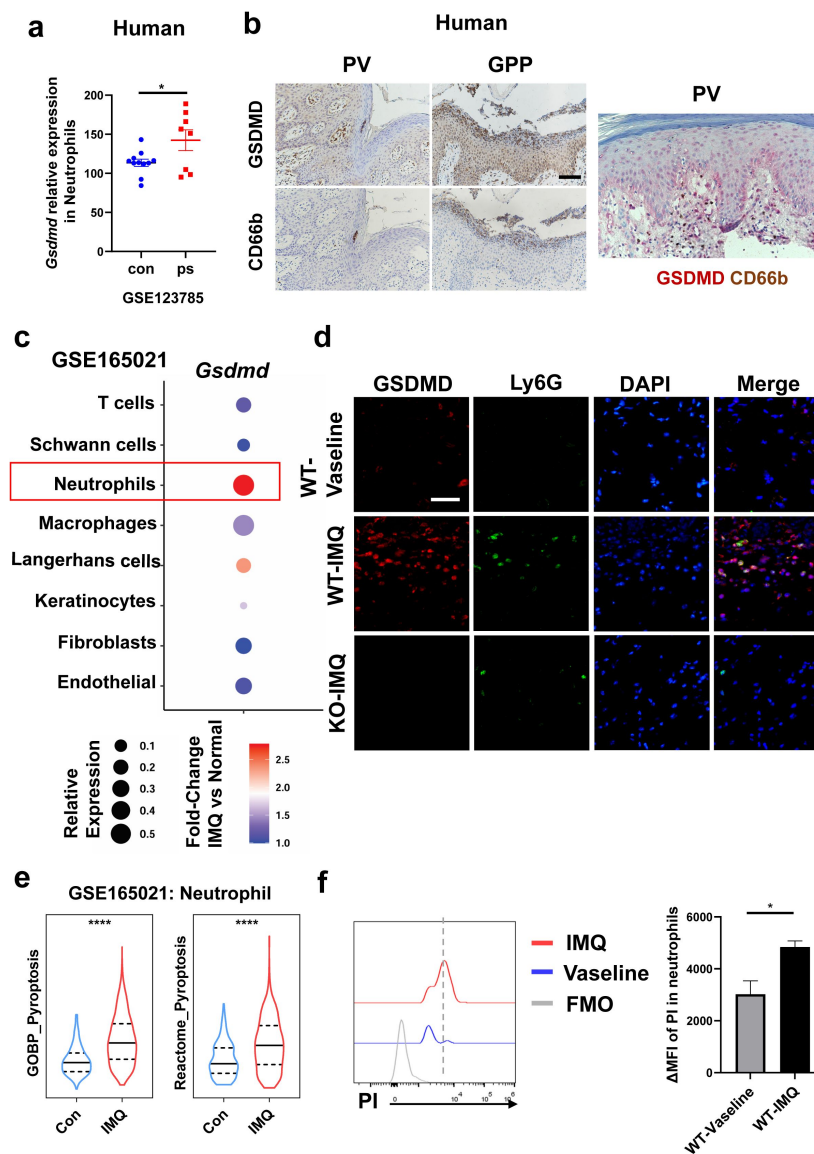


Figure 3.

Neutrophils undergo GSDMD mediated pyroptosis in psoriasis.

- a)** Expression of GSDMD in neutrophils in psoriasis patients and healthy people from GSE123785.
- b)** Representative immunohistochemical staining of CD66b and GSDMD in two consecutive sections of skin tissue from patients with psoriasis vulgaris or generalized pustular psoriasis (left); representative immunohistochemical staining of CD66b (brown) and GSDMD (red) in sections of skin tissue from patients with psoriasis vulgaris (n=3-5, each group). Scale bar =100μm.
- c)** Dot plot of *Gsdmd* expression in each cell type in the skin of IMQ-induced psoriasis-like mice (GSE165021).
- d)** Representative immunofluorescence of GSDMD (red), Ly6G (green) and nuclear (blue) in dorsal skin of WT mice treated with vehicle, WT mice treated with IMQ and GSDMD-KO mice treated with IMQ at day 5. Scale bar =50μm.
- e)** ssGSEA analysis showed the expression of pyroptosis-related genes in neutrophils infiltrating in control and IMQ-induced psoriasis-like tissue (GSE165021).
- f)** Representative and statistical graphs of the mean fluorescence intensity of propidium iodide in neutrophils from skin of WT mice as detected by flow cytometry (n=3).
- ps, psoriasis skin; con, control; PV, psoriasis vulgaris; GPP, generalized pustular psoriasis; IMQ, imiquimod; WT, wild-type; MFI, mean fluorescence intensity; PI, propidium iodide; MFO, Fluorescence Minus One. Error bars show mean ± SEM. *P < 0.05, ****P < 0.0001. Data are representative of three independent experiments for (b, d-e).

GSDMD mice showed a reduction in psoriasis-like symptoms after induction, the reduction was not as pronounced as in GSDMD full knockout mice, suggesting that additional cellular pyroptosis may also be involved in psoriasis pathogenesis. To further investigate neutrophilic pyroptosis in psoriasis, we detected the expression of GSDMD and its N-terminal fragments by WB in the psoriatic skin of WT mice, *Gsdmd*^{-/-} mice and cKO mice. We found that after psoriasis induction, the GSDMD N-terminus was only weakly expressed in the skin of cKO mice, whereas it was strongly expressed in WT mice (**Fig 4g**), suggesting that GSDMD-mediated neutrophil pyroptosis is one of the important components of cell pyroptosis in psoriasis. In addition, we used Ly6G antibody to eliminate neutrophils in cKO mice and control mice. It was found that the difference in lesions between the two groups was abolished after neutrophil depletion (**Fig 4i**, S2a), indicating that GSDMD in neutrophil plays an important role in the pathogenesis of imiquimod-induced psoriasis-like lesions in mice.

Considering that pyroptosis is a form of regulated cell death that results in inflammation, its effects can be linked to the release of inflammatory cytokines through membrane pores formed by GSDMD. While neutrophils do undergo pyroptosis, they may exhibit resistance to this cytolytic effect, leading to sustained cytokine release. IL-1 β , a significant inflammatory cytokine of pyroptosis, has a crucial function in the pathogenesis of psoriasis. The IL-1 β -IL-1R pathway promotes the ailment by regulating IL-17-producing cells in the dermis and stimulating keratinocytes to magnify inflammatory cascades. Therefore, it is hypothesized that the occurrence of neutrophil pyroptosis in psoriasis may activate inflammatory pathways downstream by releasing cytokine IL-1. In order to further verify our hypothesis, we analyzed publicly available single cell sequencing data. We found that in the skin of psoriasis-like mice, *Il1b* and *Il6* were mainly expressed in neutrophils, while *Il17a* was mainly expressed in T cells, *Il18* was mainly expressed in macrophages and fibroblasts, and *Tnfa* was mainly expressed in macrophages and T cells (S2b). This suggests that neutrophil pyroptosis may affect the secretion of cytokines such as IL-1 β and IL-6 by neutrophils, thereby affecting the functions of other immune cells such as T cells and macrophages, forming a complex inflammatory network in psoriasis and participating in the pathogenesis of psoriasis.

4 Discussion

In this study, we dissected the role of GSDMD protein, a key pyroptosis executioner, in the context of psoriasis. We found that GSDMD protein was activated in the skin of psoriasis, and that GSDMD in neutrophils promotes psoriasis inflammation through pyroptosis and the release of cytokines. Research on pyroptosis has tended to focus on infections, tumors, and other acute inflammations. Pyroptosis is not only involved in the regulation of sepsis, but also mediates sepsis-related organ damage[33]. This means that while moderate pyroptosis promotes clearance of pathogens, excessive pyroptosis can lead to uncontrolled inflammatory responses and promote the onset and development of sepsis[33–35]. Moreover, pyroptosis promotes severe pancreatitis and associated lung injury through the release of cytokines IL-1 β and IL-18[36], and is implicated in acute myocardial injury and spinal cord injury[37, 38]. In addition, GSDMD induced pyroptosis of antigen presenting cells in tumor microenvironments impairs antigen presentation in response to anti-PD-L1 treatment[22]. Only in recent years, studies have explored the function of pyroptosis in psoriasis. Iwona Flisiak et al. found that GSDMD expression was significantly increased in the skin of psoriasis patients, suggesting that it may be involved in the pathogenesis of psoriasis[23]. Xu Chen et al. reported that GSDMD-mediated keratinocyte pyroptosis promotes excessive proliferation and abnormal differentiation induced by the immune microenvironment in psoriatic skin inflammation, thus contributing to the pathogenesis of psoriasis[24]. In addition, there are some studies on pyroptosis-related proteins upstream of GSDMD, such as NLPR3 inflammasome and AIM2 inflammasome, which are involved in and promote psoriasis through downstream inflammatory cytokines[27, 39, 40].

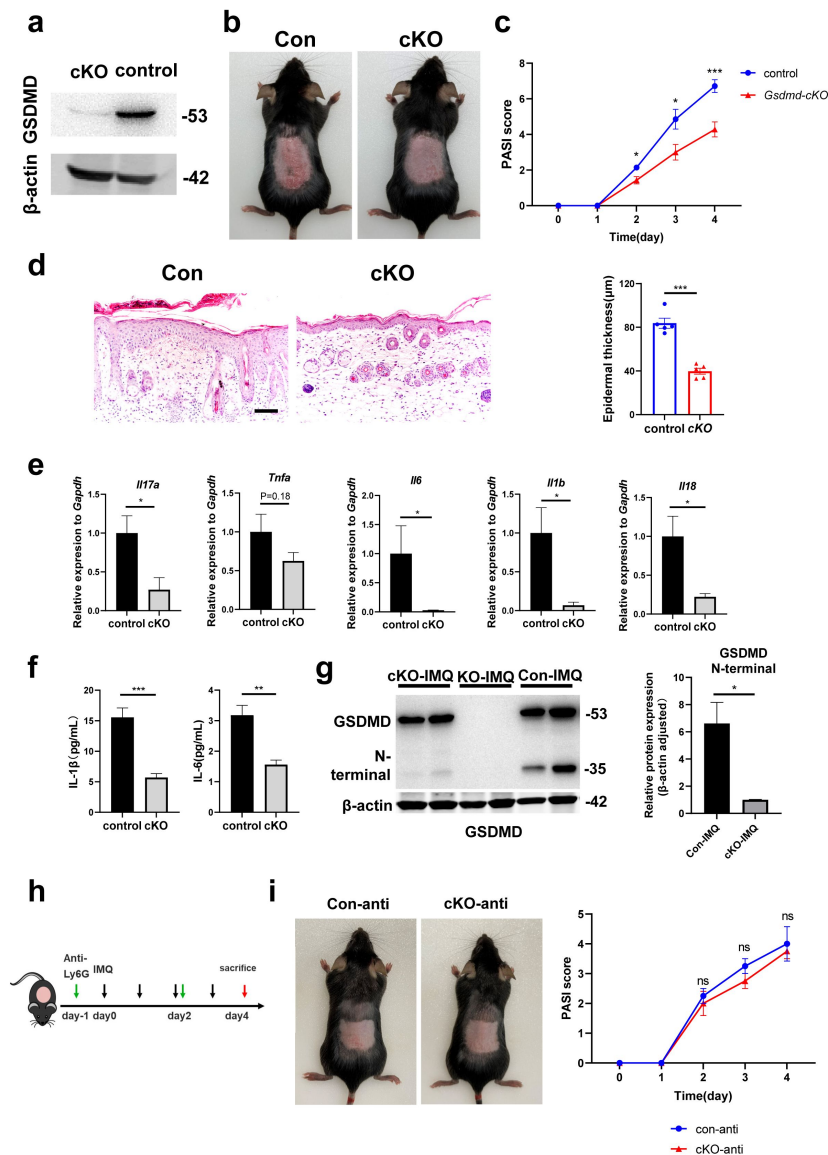


Figure 4.

GSDMD depletion in neutrophils attenuates the development of skin inflammation in psoriasis.

- a)** Representative images of western blot showing the expression of GSDMD in bone marrow-derived neutrophils of cKO and isotype control mice.
- b)** Macroscopic phenotypic representation of the dorsal skin in control and GSDMD-cKO mice treated with IMQ at day 4.
- c)** Disease severity of psoriasis induced by IMQ in mice as assessed by PASI score (n=7).
- d)** Representative images and statistical analysis of hematoxylin and eosin staining of the dorsal skin in control and GSDMD-cKO mice treated with IMQ at day 4 (n=5). Scale bar = 100μm.
- e)** Quantitative PCR analysis of the relative mRNA expression of proinflammatory cytokines in the dorsal skin of control and GSDMD-cKO mice treated with IMQ at day 4 (n = 4). Data were normalized to a reference gene, Gapdh.
- f)** ELISA analysis of IL-6 and IL-1β per 1 mg of the dorsal skin from control and GSDMD-cKO mice treated with IMQ at day 4(n=5).
- g)** Representative images and statistical analysis of western blot analysis showing the expression level of GSDMD and its N-terminal fragments in dorsal skin of WT, cKO and *Gsdmd*^{-/-} mice treated with IMQ at day 4 (n=4).
- h)** Schematic representation of the use of anti-Ly6G antibody in the IMQ-induced psoriasis mouse model.
- i)** Macroscopic phenotypic representation and PASI score of cKO mice and control mice treated with IMQ and Ly6G antibody. cKO, conditional knockout. Error bars show mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. Data are representative of three independent experiments for (a, b, d, g, i).

Consistent with earlier research, our study found that the expression of GSDMD and its upstream molecule caspase 1 was upregulated in the skin of psoriasis patients and psoriasis-like mice, indicating that the pyroptosis pathway is activated in psoriasis skin lesions. Knockout of GSDMD *in vivo* is effective in significantly ameliorating IMQ-induced psoriasis-like lesions and reducing the level of skin inflammation, which also notably reduces the expression of the pyroptosis-inducing inflammatory cytokine IL-1 β . In our study, we found that the upregulated GSDMD signal was more significant in neutrophils in both human and animal tissues. In addition, specific knockout of GSDMD in neutrophils *in vivo* can also yield results consistent with GSDMD KO mice, although not as obvious as in KO mice. Our study is for the first time demonstrating that GSDMD-mediated neutrophil pyroptosis in psoriasis is involved in psoriatic inflammation and may affect the inflammatory network in psoriasis. Considering the role and early recruitment of neutrophils in psoriasis[28 [↗](#), 41 [↗](#)], it is readily accepted that neutrophils act as innate immune cells in response to changes in the microenvironment to initiate pyroptosis signaling and release cytokines to engage and maintain psoriasis inflammation. IL-1 β released by pyroptosis may amplify the inflammatory cascade through the IL-1 β -IL-1R pathway via direct regulation of dermal IL-17-producing cells and stimulation of keratinocytes[42 [↗](#)], thereby contributing to skin inflammation and psoriasis. In addition, the unique resistance of neutrophils to pyroptosis[43 [↗](#)] makes it reasonable for them to continuously release cytokines after activation of the pyroptosis pathway and further stimulate skin inflammation.

Our research primarily focuses on the role of neutrophil pyroptosis in psoriasis, this does not conflict with existing reports indicating that KC cell pyroptosis also contributes to disease progression[24 [↗](#)]. Both studies underscore the significant role of GSDMD-mediated pyroptotic signaling in psoriasis, and the consistent involvement of KC cells and neutrophils further emphasizes the potential therapeutic value of targeting GSDMD signaling in psoriasis treatment. Nevertheless, combining previous studies and our findings, we can believe that GSDMD-mediated pyroptosis, which is a dynamic biological process, particularly neutrophil pyroptosis, is involved in the inflammation of psoriasis, especially in the early stage. This provides a potential basis for the possible use of drugs targeting pyroptosis, especially drugs targeting neutrophil pyroptosis, in the treatment of psoriasis. Dimethyl fumarate is a type of medicine widely used to treat psoriasis in Europe[44 [↗](#), 45 [↗](#)]. The latest research proves that dimethyl fumarate can inhibit the function of GSDMD and thereby inhibit pyroptosis-related inflammation[46 [↗](#)].

There is some limitation in our study such as the lack of exploration of upstream signaling involved in neutrophil pyroptosis along with its specific molecular mechanisms. Although we observed significantly increased GSDMD in neutrophils in pustular psoriasis, we were constrained to studying the established PV animal model due to the current absence of a mature GPP animal model. This represents a limitation of our study. Nevertheless, our study showed that neutrophil pyroptosis is involved in and contributes to psoriasis inflammation, indicating potential avenues and foundations for novel psoriasis treatment strategies.

Additional information

Funding sources

This work was supported by the National Natural Science Foundation of China (82073439 and 82373475).

Conflicts of Interest

None declared.

Declarations

Ethics approval and consent to participate

All mice were kept in a barrier facility, and all animal experiments were conducted in accordance with the procedure approved by the Ethical Review Committee for Laboratory Animal Welfare of the Nanjing Medical University (IACUC-2203023). Human skin tissue samples were obtained from the Department of Dermatology, Jiangsu Provincial People's Hospital, which was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital), Ethics Number: 2020-SRFA-101, and informed consent was obtained from all study participants.

Consent for publication

All authors agreed with the submission of this manuscript to *eLife* and consent for publication was obtained.

Availability of data and material

The data and materials are available from the corresponding authors upon reasonable request.

Competing interests

None declared.

Funding

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Authors' contributions

Jian Liu: Data curation, Investigation, Methodology and Writing - original draft YuYing Jiang and ZiYue Diao: Data curation, Investigation and Methodology DanDan Chen and RuiYuan Xia: Methodology

BingWei Wang, Shuo Yan and ZhiQiang Yin: Conceptualization, Project administration, Supervision and Writing - review & editing

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Reviewer #1 (Public review):

Summary:

Recommendations for the authors In this study, Liu, Jiang, Diao et.al. investigated the role of GSDMD in psoriasis-like skin inflammation in mice. The authors have used full-body GSDMD knock-out mice and Gsdm floxed mice crossed with the S100A8- Cre. In both mice, the deficiency of GSDMD ameliorated the skin phenotype induced by the imiquimod. The authors also analyzed RNA sequencing data from the psoriatic patients to show an elevated expression of GSDMD in the psoriatic skin.

Strengths:

It has the potential to unravel the new role of neutrophils.

Comments on revisions:

The authors have addressed the majority of comments and concerns and highlighted the potential limitations wherever not possible.

<https://doi.org/10.7554/eLife.101248.2.sa2>

Reviewer #2 (Public review):

Summary:

The authors describe elevated GSDMD expression in psoriatic skin, and knock-out of GSDMD abrogates psoriasis-like inflammation.

Strengths:

The study is well conducted with transgenic mouse models. Using mouse-models with GSDMD knock-out showing abrogating inflammation, as well as GSDMD fl/fl mice without neutrophils having a reduced phenotype.

My major concern would be the involvement of other inflammasome and GSDMD bearing cell types, esp. Keratinocytes (KC), which could be an explanation why the experiments in Fig 4 still show inflammation.

Comments on revisions:

The authors have sufficiently addressed my questions.

<https://doi.org/10.7554/eLife.101248.2.sa1>

Author response:

The following is the authors' response to the original reviews.

eLife Assessment

This is a potentially interesting study regarding the role of gasdesmin D in experimental psoriasis. The study contains useful data from murine models of skin inflammation,

however the main claims (on neutrophil pyroptosis) are incompletely supported in its current form and require additional experimental support to justify the conclusions made.

We sincerely appreciate the positive assessment regarding the significance of our study, as well as the valuable suggestions provided by the reviewers. We have included new data, further discussions and clarifications in the revised manuscript to adequately address all the concerns raised by the reviewers and better support our conclusions.

Public Reviews:

Reviewer #1 (Public review):

Summary:

In this study, Liu, Jiang, Diao et.al. investigated the role of GSDMD in psoriasis-like skin inflammation in mice. The authors have used full-body GSDMD knock-out mice and Gsdm floxed mice crossed with the S100A8- Cre. In both mice, the deficiency of GSDMD ameliorated the skin phenotype induced by the imiquimod. The authors also analyzed RNA sequencing data from the psoriatic patients to show an elevated expression of GSDMD in the psoriatic skin.

Overall, this is a potentially interesting study, however, the manuscript in its current format is not completely a novel study.

Strengths:

It has the potential to unravel the new role of neutrophils.

Weaknesses:

The main claims are only partially supported and have scope to improve

We thank the reviewer for the positive evaluation of the interest and potential of our work. In response to reviewers' suggestions, we have added new content, including additional data and discussions, to further demonstrate the important role of GSDMD-mediated neutrophil pyroptosis in the pathogenesis of psoriasis, thereby enhancing the completeness of our research.

Reviewer #2 (Public review):

Summary:

The authors describe elevated GSDMD expression in psoriatic skin, and knock-out of GSDMD abrogates psoriasis-like inflammation.

Strengths:

The study is well conducted with transgenic mouse models. Using mouse-models with GSDMD knock-out showing abrogating inflammation, as well as GSDMD fl/fl mice without neutrophils having a reduced phenotype.

I fear that some of the conclusions cannot be drawn by the suggested experiments. My major concern would be the involvement of other inflammasome and GSDMD bearing cell types, esp. Keratinocytes (KC), which could be an explanation why the experiments in Fig 4 still show inflammation.

Weaknesses:

The experiments do not entirely support the conclusions towards neutrophils.

We appreciate the reviewers' positive evaluation regarding the application of our mouse models. We also thank the reviewers for insightful comments and suggestions that can improve the quality of our work. Addressing these issues has significantly strengthened our conclusions. Our responses to the above questions are as follows.

Specific questions/comments:

Fig 1b: mainly in KC and Neutrophils?

In Figure 1b, we observed that GSDMD expression is higher in the psoriasis patient tissues compared to control samples. As the role of GSDMD in keratinocytes during the pathogenesis of psoriasis has already been explored[1], we focused our study on GSDMD in neutrophils. In response to the comments, we have added co-staining results of the neutrophil marker CD66b and GSDMD in the revised manuscript (see new Figure 3b in the revised manuscript). This addition further substantiates the expression of GSDMD in neutrophils within psoriasis tissue.

Fig 2a: PASI includes erythema, scaling, thickness and area. Guess area could be trick, esp. in an artificial induced IMQ model (WT) vs. the knock-out mice.

In our model, to accurately assess the disease condition in mice, we standardized the drug treatment area on the dorsal side (2*3 cm). Therefore, the area was not factored into the scoring process, and we have included a detailed description of this in the revised manuscript.

Fig 2d: interesting finding. I thought that CASP-1 is cleaving GSDMD. Why would it be downregulated?

Regarding the downregulation of CASP in GSDMD KO mouse skin tissue, existing studies indicate that GSDMD generates a feed-forward amplification cascade via the mitochondria-STING-Caspase axis [2]. We hypothesize that the absence of GSDMD attenuates STING signaling's activation of Caspase.

Line 313: as mentioned before (see Fig 1b). KC also show a strong GSDMD staining positivity and are known producers of IL-1b and inflammasome activation. Guess here the relevance of KC in the whole model needs to be evaluated.

Our research primarily focuses on the role of neutrophil pyroptosis in psoriasis, this does not conflict with existing reports indicating that KC cell pyroptosis also contributes to disease progression[1]. Both studies underscore the significant role of GSDMD-mediated pyroptotic signaling in psoriasis, and the consistent involvement of KC cells and neutrophils further emphasizes the potential therapeutic value of targeting GSDMD signaling in psoriasis treatment. We have expanded upon this discussion in the revised manuscript.

Fig 4i - guess here the conclusion would be that neutrophils are important for the pathogenesis in the IMQ model, which is true. This experiment does not support that this is done by pyroptosis.

To address the question, we analyzed the publicly available single-cell transcriptomic data (GSE165021) and found that, compared to the control group, neutrophils infiltrating in IMQ-induced psoriasis-like tissue display a higher expression of pyroptosis-related genes (see new

Figure 3e in the revised manuscript). These results strengthen our conclusions about the role of neutrophil pyroptosis in the progression of psoriasis.

Recommendations for the authors:

Reviewer #1 (Recommendations for the authors):

Specific Comments:

- *Figure 1: Micro abscesses would already be dead, which would likely reflect as non-specific staining. Authors should consider double staining (e.g., GSDMD+Ly6G).*

We thank the reviewer for the useful suggestion. We have added co-staining results of the neutrophil marker CD66b and GSDMD in the revised manuscript (see new Figure 3b in the revised manuscript). This addition further substantiates the expression of GSDMD in neutrophils within psoriasis tissue.

- *Figures 1 b, c, and d do not have the n number for representative experiments and images.*

We apologize for our oversight. We have added the relevant information in the revised manuscript and have reviewed and corrected the entire text.

- *What is the difference between psoriasis patients in Figure 1 versus Figure 3 as the staining patterns are different? It is difficult to interpret from Figure 1 that expression is limited to neutrophils. Authors should consider double staining (e.g., GSDMD+Ly6G). How many samples were stained to draw this conclusion?*

We thank the reviewer for the suggestion. In Figure 1b, we observed that GSDMD expression is higher in the psoriasis patient tissues compared to control samples. We have added co-staining results of the neutrophil marker CD66b and GSDMD in the revised manuscript (see new Figure 3b in the revised manuscript). For each staining group, we examined samples from 3-5 patients to draw the conclusion.

- *Figure 2: GSDMD deficiency mitigates psoriasis-like inflammation in mice has been shown before (PMID#37673869). The paper showed that the GSDMD was mainly expressed in keratinocytes. What is the view of the authors on it and how does this data correlate with the data presented in this manuscript by the authors?*

Consistent with previous studies[1], we observed increased expression of pyroptosis-related proteins in psoriatic lesions. However, our research focused specifically on the role of neutrophil pyroptosis in psoriasis, this does not conflict with existing reports indicating that KC cell pyroptosis also contributes to disease progression. Both studies underscore the significant role of GSDMD-mediated pyroptotic signaling in psoriasis, and the consistent involvement of KC cells and neutrophils further emphasizes the potential therapeutic value of targeting GSDMD signaling in psoriasis treatment. We have expanded upon this discussion in the revised manuscript.

- *Figure 3d: It is unclear if the IF shows an epidermal or dermal area. As shown by authors in other figures (human psoriatic skin), do authors observe more GSDMD in the micro abscess, which is localized in the epidermis? The authors should also show the staining of GSDMD/Ly6G in the whole skin sample.*

The region we presented for immunofluorescence staining corresponds to the dermis of the mice, as we did not observe typical neutrophil micro abscesses similar to those in human

psoriasis in the epidermis of IMQ-induced classical psoriasis vulgaris (PV) model. Therefore, we have only shown the staining in the dermal area.

• *Figure 3e: PI staining also represents necrotic cells and TUNEL staining would not represent just apoptotic cells. It is unclear how the authors conclude an ongoing pyroptosis in neutrophils. A robust dataset is needed to provide evidence supporting neutrophil pyroptosis in the IMQ-challenged mice.*

We thank the reviewer for the valuable suggestion. GSDMD is the effector protein of pyroptosis. To further confirm that cells are undergoing pyroptosis, it is necessary to morphologically stain the GSDMD N-terminal protein. Although there is currently no GSDMD N-terminal fluorescent antibody available, we detected the cleaved N-terminus of GSDMD by WB in mouse psoriasis-like skin tissue, and its increased expression suggested increased cell pyroptosis (see new Figure 1d in the revised manuscript). Moreover, we analyzed the publicly available single-cell transcriptomic data (GSE165021) and found that, compared to the control group, neutrophils infiltrating in IMQ-induced psoriasis-like tissue display a higher expression of pyroptosis-related genes (see new Figure 3e in the revised manuscript). These results strengthen our conclusions about the role of neutrophil pyroptosis in the progression of psoriasis.

• *Figure 4: The authors did not clarify the reason for choosing D4 over the usual D7 for the imiquimod experiment. S100A8-Cre is also reported in monocytes and granulocytes/monocyte progenitors. And, the authors also show the expression in macrophages and neutrophils, but in the text, only neutrophils are mentioned. The authors should state the results in the text as well to avoid misrepresentation of the data.*

We thank the reviewer for the useful suggestion. We have repeated many times of experiments in our previous studies and observed that the IMQ-induced mouse psoriasis model showed the obvious signs of self-resolution after Day 4 even with continuing topical IMQ application, thus we chose 4 days over 7 days for the imiquimod experiment, which are consistent with many other studies[3, 4].

Many studies use S100A8-Cre mice for neutrophil-specific gene knockout[5, 6]. Moreover, we used Ly6G antibody to eliminate neutrophils in GSDMD-cKO mice and control mice. It was found that the difference in lesions between the two groups was abolished after neutrophil depletion, indicating that neutrophil pyroptosis plays an important role in the pathogenesis of imiquimod-induced psoriasis-like lesions in mice. As the database analysis results showed that macrophages have slight expression of *S100a8*, according to the suggestion of the reviewer, we have added a more precise description in the revised manuscript.

• *Figure S2a: Ly6G antibody reduced the ly6G positive, but also negative cells compared to PBS. If this is correct, what is the explanation, and how this observation has been considered for concluding results?*

Neutrophils play an important role in regulating inflammatory responses, and their deletion can reduce the overall inflammatory level in the body, which also results in a decrease in other non-neutrophil cells. However, this change does not affect our conclusions. Our results show that after the deletion of neutrophils, there is no difference in the pathological manifestations between the cKO group and the control group. This further that GSDMD in neutrophil plays an important role in the pathogenesis of imiquimod-induced psoriasis-like lesions in mice.

• *The conclusion in Figure 4i is incorrect as Ly6G administration had an effect on the wt, so it shows neutrophils play a role, but not neutrophil pyroptosis.*

- 321 "It was found that the difference in lesions between the
- 321 two groups was abolished after neutrophil depletion (Fig4i, S2a), indicating that
- 322 neutrophil pyroptosis plays an important role in the pathogenesis of
- 323 imiquimod-induced psoriasis-like lesions in mice"

Our results show that after the deletion of neutrophils, there is no difference in the pathological manifestations between the cKO group and the control group. This further indicates that the lower disease scores observed in cKO mice, in the absence of neutrophil deletion, depend on the presence of neutrophils. In the revised manuscript, we have changed the statement to "It was found that the difference in lesions between the two groups was abolished after neutrophil depletion (Fig4i, S2a), indicating that GSDMD in neutrophil plays an important role in the pathogenesis of imiquimod-induced psoriasis-like lesions in mice"

- *The effect of LyG Ab: reduced PASI in the wt, but the effect on the ko remains the same. What are the other molecular changes observed? What was the level of neutrophils in the wt and the S1A008Cre GsdmDfl/fl mice under steady state and how are they change upon imiquimod challenge? A complete profiling of the immune cells is needed for all the experiments.*

As demonstrated by the results, the deletion of neutrophils did not significantly alter the pathological phenotype of cKO mice. We believe that this outcome precisely highlights the crucial role of GSDMD in regulating neutrophil inflammatory responses.

- *Figure S2b: The authors conclude that IL-1b in the imiquimod skin is mainly expressed by neutrophils, but the analysis presented in the figure does not support this conclusion. Both neutrophils and macrophages are majorly positive for IL-1b, with some expression on Langerhans and fibroblasts. No n numbers are provided for the experiment*

As we discussed in the manuscript, we speculate that neutrophil pyroptosis may release cytokines, which in turn activate other cells to secrete cytokines, forming a complex inflammatory network in psoriasis. This may suggest that neutrophil pyroptosis may be involved in the pathogenesis of psoriasis by affecting the secretion of cytokines such as IL-1b and IL-6 by neutrophils, thereby affecting the function of other immune cells such as T cells and macrophages.

We have added the n number in the revised manuscript.

- *For clarity and transparency, a list of antibodies with the associate clone and catalogue number should be provided or integrated into the method text.*

We thank the reviewer for the useful suggestion. We have added the associate clone and catalogue number of antibodies used in the method text of revised manuscript.

Reviewer #2 (Recommendations for the authors):

- Fig 3b: psoriasis and pustular psoriasis have a different pathophysiology (autoimmune vs. autoinflammatory). Neutrophils are centrally important for GPP for the cleavage of IL-36. Guess as not further referred to pustular psoriasis in the paper, that comparison is rather deviating from the story.*

In Figure 3b, we stained for GSDMD and CD66b in both plaque psoriasis (PV) and generalized pustular psoriasis (GPP), not to compare the expression differences between the two types of

psoriasis, but rather to demonstrate that significant GSDMD expression is present in neutrophils in different types of psoriasis. Unfortunately, due to the lack of a well-established animal model for GPP, we were only able to conduct studies using the established PV animal model. We acknowledge this limitation in our research. In our revised manuscript, we have added the following explanation in the discussion section: “Although we observed significantly increased GSDMD in neutrophils in pustular psoriasis, we were constrained to studying the established PV animal model due to the current absence of a mature GPP animal model. This represents a limitation of our study.”

In summary, we appreciate the Reviewer’s comments and suggestions. We feel that the inclusion of new data addresses the concerns in a comprehensive manner and adds further support to our original conclusions. We hope you will now consider the revised manuscript worthy of publication in *eLife*.

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