

Gancao Nurish-Yin Decoction Ameliorates Imiquimod-Induced Psoriasis-Like Skin Lesions and Inflammation in Mice

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Research

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Abstract

Background

Psoriasis is an autoimmune skin disease with a high clinical prevalence, which is often poses treatment difficulties. In this study, we tested whether Gancao nurish-Yin (GNY) decoction is effective for treating mice with imiquimod (IMQ)-induced psoriasis-like skin lesions.

Methods

Mice were divided into 4 groups including healthy control group, model group (IMQ-induced psoriasis mice), and two psoriasis induced groups of mice treated with GNY-2.5g/kg and GNY-5g/kg given in the drinking water (n = 8/group). Psoriasis area and severity index (PASI) was used to monitor psoriatic symptoms. H&E staining of dermis and baker's scores were used to evaluate disease severity. Inflammatory cells positive for Gr-1, CD11c, RORyt and TGF- β were counted by using immunohistochemistry or immunofluorescence staining methods. Flow cytometry was used to analyse CD4⁺CD17⁺ Th17 cells, and CD25⁺Foxp3⁺ Tregs within splenocyte cell population. Relative mRNA expression of IL-6, IL-10, IL-17A, IL-22, IL-23, TNF- α , TGF- β and AMPK α 1 in the dermis was was semi-quantified using qPCR.

Results

Compared to model group, GNY decoction treated mice, at both the concentrations, improved morphological features, as demonstrated by PASI scores and decreased levels of hyperproliferating keratinocytes. GNY decoction at a higher concentration significantly decreased Gr-1, CD11c, RORyt and TGF- β positive cells in the dermis as well as the differentiated CD4⁺CD17⁺ Th17 cells, whereas, CD25⁺Foxp3⁺ Tregs were increased in the spleen, mainly with low dose GNY decoction. Furthermore, relative mRNA expression of IL-6, IL-10, IL-17A, IL-22, IL-23, TNF- α , TGF- β and AMPK α 1 within dermis were elevated in mice from IMQ model group, which were significantly reduced in mice treated with GNY decoction at GNY-5g/kg concentration.

Conclusion

Here we demonstrate disease ameliorating effects of GNY decoction in IMQ-induced psoriasis-like skin lesions in mice, which could form basis for considering GNY decoction for treatment of psoriasis patients.

1 Background

Psoriasis is an immune mediated chronic, recurrent, inflammatory, systemic disease induced by a combination of heredity and environmental factors^[1]. It has a high prevalence rate. In Europe and North America, psoriasis prevalence is about 2%^[1], and 7.9% in Denmark^[2]. Besides skin manifestations, psoriasis patients are at increased risk for cardiovascular disease, higher incidence of metabolic syndrome, and psoriatic arthritis^[3]. Despite the use of multiple immunosuppressants and upcoming biologicals, patients are not satisfied with the existing therapies^[4], and the side effects of the current treatment modalities are always being a big concern.

Psoriasis is called bai bi (白皮) in Traditional Chinese Medicine (TCM), which has long been applied and has clear benefits for psoriasis patients^[5]. Based on TCM theory, "heat in the blood stirs up wind", is the most frequently seen syndrome differentiation pattern of common psoriasis used in China. Heat and wind belong to Yang pathy. To antagonize the Yang pathy, TCM practitioners chose herbal medicines with cold or cool properties, which belong to

Yin. Although extracts from an herbal medicine would more easily be recognized by modern researchers, in clinical practice, its a basic principle to combine several herbals as an recognized formula to manage diseases^[6]. Based on TCM prescription method and understanding on the efficacy of botanical ingredients, we designed a formula for psoriasis, and named it as Gancao nurish-Yin (GNY) decoction, which contain the ingredients, as described in Table 1.

Table 1
Ingredients of GNY decoction, flavor and tropism

Botanical plant names (Chinese name)	Flavor and property	Tropism of Channel	Functions
Panax ginseng C. A. Mey (ren shen)	Bitter, warm	Spleen, lungs, heart	Invigorate qi, solidify prostration, promote body fluid, soothe the nerves, and nourish the mind.
Glycyrrhiza uralensis Fisch (gan cao)	Sweet, neutral	Spleen, lungs, heart, stomach	Replenish qi and nourish the middle energy, clear away heat, detoxify, expectorant and relieve cough and pain.
Ziziphus jujuba Mill. (da zao)	Sweet, warm	Spleen, stomach	Nourish the middle energy qi and blood and calm the nerves.
Polygonatum odoratum Mill. Druce (yu zhu)	Sweet, neutral (slightly cold)	lungs, stomach	Nourish yin and moisturize dryness, produce body fluid to quench thirst.
Siraitia grosvenorii (Swingle) C. Jeffrey ex Lu et Z. Y. Zhang (luo han guo)	Sweet, cool	lungs, large intestine	Clearing heat and moisturizing the lungs, improving the sound of the throat, smoothing the bowel
Taraxacum mongolicum Hand.-Mazz. (pu gong ying)	Sweet, cool (cold)	lung, large intestine	Nourishes the lungs and relieves cough, promotes body fluid and quenches thirst, moistens the intestines
Zingiber officinale Rosc. (gan jiang)	Pungent, (heat)	Spleen, stomach, kidney, heart, lung	Warm the middle and dispel cold, regain yang and relieve the pulse, warm the lungs and reduce watery phlegm

There are abundant research on anti-inflammatory functions of the contents of the herbal extracts used in this formula. For example, dihydrostilbenes from liquorice show antioxidant and anti-inflammatory activities through stabilizing the generation of free radicals and by decreasing thromboxane B2 (TxB2) and prostaglandin E2 (PGE2) release from human whole blood samples^[7]. Ginseng berry polysaccharide extract significantly inhibits secretion of interleukin-8 and differentiation of Th1 cells^[8]. In this study, we tested GNY decoction on imiquimod (IMQ)-induced psoriasiform inflammation in mice and subsequent changes in the inflammatory cells and cytokines, And the results could form basis for initiating clinical trials for using GNY decoction for psoriasis patients.

2 Methods

2.1 Animals

Eight weeks old BALB/c female inbred mouse strains were used in the experiments, which were purchased from Southern Medical University Experimental Animal Center and maintained in a pathogen-free animal house (approval

numbers, CUMS-2018-0062-03). Mice were kept on environment with 12 hour light/dark cycles, water and food were taken randomly.

2.2 IMQ induced psoriasis

Mice were divided into 4 groups (control group, model group and two treatment groups viz., GNY-2.5 g/kg and GNY-5 g/kg groups) with 8 mice in each group. Each mouse from all the groups except the control group received a daily topical dose of 30 mg of imiquimod (5% aldera cream, Mingxinlidi Laboratory, China) on the shaved area in the back for 4 days to induce psoriasis lesions. All the mice applied with imiquimod developed skin inflammation.

2.3 Preparation of GNY decoction

The ingredients of GNY decoction are as follows: Licorice (*Glycyrrhiza uralensis* Fisch, gan cao), Changbai mountain Ginseng (*Panax ginseng* C. A. Mey, ren shen), monk fruits (*Siraitia grosvenorii*, Swingle and *C. Jeffrey ex Lu et Z. Y. Zhang*, luo han guo), dandelion (*Taraxacum mongolicum* Hand.-Mazz., pu gong ying), Solomon's seal (*Polygonatum odoratum* Mill. Druce, yu zhu), ginger (*Zingiber officinale* Rosc., gan jiang), and jujube (*Ziziphus jujuba* Mill., da zao) with a ratio of 12 : 9 : 9 : 12 : 6 : 9 : 6 : 18. The raw herbal medicines were boiled twice for 1 h and then diluted using ddH₂O to get the concentrations of 2.5 g/kg and 5 g/kg.

2.4 Treatment and scoring protocols

Mice in the control group and model groups were given double distilled water (ddH₂O), while mice in the GNY-2.5 g/kg and GNY-5 g/kg groups received different concentrations of GNY decoction in the drinking water. On day 10, when the manifestations of psoriasis-like lesions in all the groups were subsided, we applied IMQ for the second time and the treatment was continued for 3 more consecutive days (Fig. 1A). We used PASI (Psoriasis area and severity index) scored to monitor psoriasis symptoms as follows: erythema (0–4), scales (0–4) and thickness (0–4) were scored. 0, no symptoms; 1, mild; 2, moderate; 3, severe; 4, very severe. Skin thickness was measured in a specified area using Vernier calipers (Neill-Lavielle, Kentucky, USA).

2.5 Histology

At the ended of psoriasis period (day 14), mice were put into anesthesia by intramuscularly injection of Zoletil 50 (Virbac, France, 20 mg/kg) and sacrificed by neck broken method. The skin samples were collected for histopathological analysis. For paraffin embedding, tissues were fixed in 4% methanol at room temperature overnight. Before performing standard dehydration and paraffin embedding procedures, tissues were washed in PBS for three times. Skin sample was prepared for paraffin sections (Leica, Solmas, Germany) and tissues were cut (8 μm) using a paraffin slicer. The sections were methanol-fixed and processed for H&E staining by following the steps: deparaffinization, dehydration and staining with hematoxylin solution (Phygene, Shanghai, China) or 0.2% eosin. Images were acquired using an eclipse upright optical microscope digital camera (Nikon Ci-E, Tokyo, Japan). Epidermal thickness was measured by selecting 10 random regions from skin sections of each mouse using 10 × magnification. We used baker's scores to judge pathological manifestations of psoriasis^[9]: Munro's small abscess was found in the stratum corneum, 2.0 point; hyperkeratosis, 0.5 point; hypokeratosis, 1.0 point; granular layer thinning or disappearing in the epidermis 1.0 point; the spinous layer thickening, 1.0 point; skin protuberances and undulations, 0.5. 1.0 and 1.5 points according to the severity; infiltration of mononuclear or multinucleated cells in the dermis according to the severity, 0.5, 1.0 and 1.5 points; 0.5 point for the upper mastoid, and 0.5 point for telangiectasia.

2.6 Immunohistochemistry and Immunofluorescence

Skin samples were harvested at the end of psoriasis period (day 14) and 5 mice from each group were used. Tissues were embedded in optimal cutting temperature (OCT) compound (Sakura Finetek Inc, California, USA), snap-frozen overnight at -80°C. Before embedding, skin samples were dehydrated with buffer containing 30% sucrose in PBS overnight and with 30% sucrose-PBS:OCT in a ratio of 1:1 gradually for 4 h. The tissues were sectioned (7 µm) using a cryostat and frozen at -20°C until used. For immunohistochemistry, the frozen sections were dehydrated with different concentrations of ethanol and fixed in acetone for 10 min and, 3% hydrogen peroxide and 5% goat serum were used as blocking agents. Inflammatory cells were stained with biotin-anti-mouse Ly6C/6G, CD11c from Biolegend (California, USA). Streptavidin-HRP or goat anti rabbit IgG (Yeasen, Shanghai, China) was used as secondary reagent. Sections were developed with DAB (Vector Laboratories, California, USA) solution and counter stained with hematoxylin before visualization under the microscope (Leica, Solmas, Germany).

For immunofluorescence, skin samples were embedded and sliced similarly as described above. Then the sections were fixed with acetone on ice for 10 min and immersed in PBS for 15 min. PBST containing 2% BSA (Biofroxx, Karlsruhe, Germany) was used for blocking for 45 min. Slides were incubated with rabbit mAb to TGF-β (Affinity Biosciences, Jiangsu, China) or RoR γt (Abcam, Cambridge, UK) for 60 min at room temperature. Then fluorescent labelled anti-rabbit secondary antibodies were added and incubated for 60 min at room temperature (Biotime, Nanjing, China). DAPI (Biotime) solution was used for staining the nuclei. After each step, slides were washed with PBST for 3 times with an incubation period of 5 min in the solution every time. Pictures were taken using a confocal microscope (Cannon, Tokyo, Japan).

2.7 Flow cytometry

To explore the levels of Th17 and Treg cells in mice from each group, spleen cells were harvested at the end of the experimental period. Single cells were prepared by grinding and incubated with antibodies against specific cell surface markers on ice for 30 min in dark. The following antibodies were used: Anti-mouse CD4⁺-APC, CD4⁺-PE, CD25⁺-APC, Foxp3⁺-BV570, IL-17A⁺-PE (BD Biosciences, New Jersey, USA). Acuri C6 or LSR II (BD Biosciences) was used to acquire data and analysis was done using Flow Jo software version 7.6 (Tree Star, San Carlos, USA).

2.8 RNA isolation and RT-qPCR

Total RNA was isolated from the skin using Trizol (Invitrogen, California, USA) extraction procedure, and dissolved in RNase-free RQ-DNAse water (Promega, Madison, WI). The mRNA samples were reverse-transcribed with the PrimeScript RT reagent Kit with gDNA Eraser (Thermo Scientific, Cambridge, UK) by following manufacturer's instructions. Each qRT-PCR reaction was performed using SYBR Premix Ex Taq II Tli RNaseH Plus (Takara biotech, Osaka, Japan) using a LightCycler 96 (Roche, Basel, Switzerland) thermocycler. β-actin was used for quantitative control. Transcript levels were calculated relative to control and the relative fold inductions were calculated using the 2^{-ΔΔCt} algorithm. The primer sequences for different genes used are given below:

β-actin (ACCGTGAAAAGATGACCCAG, GTACGACCAGAGGCATACAG)

TNF-α (ACGCTCTTCTGTCTACTGAACT, ATCTGAGTGTGAGGGTCTGG)

IL-6

(GAGAAAAGAGTTGTGCAATGGC,CCAGTTTGGTAGCATCCATCAT)

**IL-10 (CTGCTAACCGACTCCTTAATGC,
GCTCCACTGCCTTGCTCTTAT)**

TGF- β (ATCTCGATTTTTACCCTGGTGGT,CTCCCAAGGAAAGGTAGGTGATAGT)

AMPKA (TGTGGCTGGGTGTGTAAA,GGCTGTGTGTGGCATTTC)

IL-17A

(CCCCTAAGAAACCCCCACG,TAAAGTCCACAGAAAAACAAACACG)

IL-17E (ACAGGGACTTGAATCGGGTC,TGGTAAAGTGGGACGGAGTTG),

IL-17F (GTCAGGAAGACAGCACCA,AGCCAACTTTTAGGAGCA) ,

IL-22 (CATGCAGGAGGTGGTACCTT,CAGACGCAAGCATTCTCAG),

IL-23-P19

(AGCAACTTCACACCTCCCTAC,ACTGCTGACTAGAACTCAGGC)

The experiments were repeated three times to confirm the results.

2.9 Statistical analysis

The data were analyzed using GraphPad Prism 8 software and the results are presented as mean \pm SEM. Two-tailed unpaired Student's t test was used for comparisons between 2 groups, and one-way analysis of variance with Bonferroni or Newman–Keuls correction was used for multiple comparisons. Probability values ≤ 0.05 were considered as significant. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ and NS, not significant. Error bars depict SEM with a 95% confidence interval.

3 Results

3.1 GNY decoction improved clinical manifestations and alleviated hyperproliferation of keratinocytes in IMQ-induced psoriasis

Experimental scheme was shown in Fig. 1A. After 4 days of applying IMQ on the shaved back of BALB/c mice, the skin showed typical erythema, scaling, and increased thickness. The average PASI score increased over the course of IMQ treatment, suggesting progression of skin lesions in the model group, and also in GNY decoction treatment groups. However, the erythema and scale scores in both GNY-2.5 g/kg and GNY-5 g/kg groups were less than model group and subsided earlier. After second IMQ stimulation, GNY decoction treatment effects were more obvious with more smooth skin, shallow erythema and sparse scales. Treatment effects were more obvious in GNY-5 g/kg group than GNY-2.5 g/kg group (Fig. 1B–C). H&E staining of the skin lesions in both the treatment (GNY-2.5 g/kg and GNY-5 g/kg) groups showed a significant decrease in pathological parameters as evidenced by less epidermal thickening, acanthosis, parakeratosis, residual condensation of nuclei in the stratum corneum. The epidermal thickness and pathological scores in the model group were higher than control and GNY decoction treated groups (Fig. 1D - F).

3.2 GNY decoction decreased the expression of Gr-1 and CD11c positive cells

Gr-1 (granulocyte receptor 1) is a myeloid differentiation antigen, a glycosylphosphatidylinositol (GPI)-linked protein expressed on granulocytes and macrophages^[10], which participates in the pathogenesis of autoimmune diseases^[11]. Expressed on monocytes and macrophages, CD11c is characteristically co-expressed with CD1c (BDCA-1). CD1c⁺ dermal myeloid DCs were shown to have a capacity for priming and activating CD4⁺ T cells, and further initiating the flare of psoriasis inflammation^[12]. In this study, we observed that the model mice expressed higher levels of Gr-1 and CD11c than mice in the control group, while GNY decoction reduced their expression in a dose dependant manner (Fig. 2A & B).

3.3 GNY decoction decreased the expression of ROR γ t and TGF- β in the dermis

Studies have elucidated the role of IL-17A-producing helper T cells (Th17) in psoriasis, which could be specifically marked by ROR γ t^[12]. Immunofluorescence was used to detect ROR γ t positive cells. In the dermis of IMQ modeled mice presence of ROR γ t⁺ cells was observed (1–3 cells/section), while they were expressed in both control and GNY decoction treated mice at negligible levels (Fig. 2C). TGF- β promotes Th17 cell development^[13]. Compared to healthy mice, psoriatic mice expressed higher number of TGF- β ⁺ cells. GNY decoction at both the concentrations significantly decreased the expression of TGF- β ⁺ cells (Fig. 2C).

3.4 GNY decoction regulated Treg/Th17 cells

Flow cytometry analysis of T cell subsets of splenocytes showed increased percentage of CD4⁺CD17⁺ Th17 cells, with decreased CD25⁺Foxp3⁺ Tregs in psoriasis mice. Th17 cells are pomotors of autoimmunity and inflammation, while Treg cells inhibit these phenomena and maintain immune homeostasis^[14]. At both the concentrations of GNY decoction differentiated CD4⁺CD17⁺ Th17 cells were significantly decreased, while CD25⁺Foxp3⁺ Tregs in spleen were found to be increased only with the lower dose of GNY decoction (Fig. 3. A & B).

3.5 GNY decoction decreased several inflammatory cytokines and AMPK α 1 in the psoriasis mice.

Complex network of cytokines, cytokine receptors and their signal transduction pathways is elevated in the serum of psoriasis patients^[15]. Among them, we semi-qualified relative mRNA expression level of IL-6, IL-10, IL-17A, IL-22, IL-23, TNF- α , TGF- β and AMPK α 1 in the dermis samples by qPCR. All these genes were elevated in psoriasis mice, and except IL-10 gene, expression of all the other genes were significantly decreased by higher concentration of GNY decoction (Fig. 4).

4 Discussion

The GNY decoction is a TCM formula modified from Gancao Xiexin Decoction (Licorice heat-draining decoction) recorded in Synopsis of Golden Chamber, which was composed by a legendary medical sage called Zhang Zhongjing more than 2,000 years ago. The formula of Gancao Xiexin Decoction was used to treat Huhuo (disease terminology of TCM), which is regarded as the autoimmune disease namely Behcet's disease nowadays^[16]. Apart from Behcet's disease, Gancao Xiexin Decoction was applied and investigated with broad range of indications in China, like rheumatoid arthritis, digestive system diseases (e.g. ulcerative colitis, peptic ulcer), nervous system (e.g.

neurosis) and endocrine system-related gynecological disorders^[17]. However, the original formula contains coptis (*Coptis Salisb.*), which causes the decoction extremely bitter in taste and Pinellia ternate (*Pinellia ternata* (Thunb.) Breit.), which was recorded for mild toxicity in TCM as well as in modern research^[18]. So, we changed the two herbs to dandelion (*Taraxacum mongolicum* Hand.-Mazz.) and Solomon's seal (*Polygonatum odoratum* (Mill.) Druce) as they are having similar nature of flavor, property and functions according to TCM theory.

The GNY decoction showed ameliorating effects on clinical manifestations and keratinocyte proliferation in IMQ-induced psoriasis in this study. Contribution of both non-specific and specific immune reactions in the pathogenesis of psoriasis is well known^[19, 20]. Subsequently, we investigated the therapeutic mechanisms of GNY decoction, involving both non-specific and specific immune reactions and found them to be decreased after treatment. Gr-1, CD11c and ROR- γ t positive cells were lower in the treating groups compared to model group. Granulocytes including neutrophils, eosinophils, and basophils have granules with enzymes released during infections, allergic reactions, asthma and psoriasis^[21], and Gr-1 is a specific marker expressed on granulocytes and macrophages. CD11c is another surface marker mostly expressed on non-specific immune cells like monocytes, dendritic cells, granulocytes, NK cells, and also subsets of T and B cells^[22]. The best known surface antigen for the identification of dermal myeloid DCs is CD11c. Psoriatic skin was reported to have increased numbers of CD11c⁺ myeloid DCs in the dermis, which has an important role in the psoriasis pathogenesis^[23]. Retinoic acid receptor-related orphan receptor gamma-t (ROR γ t) has critical role in the differentiation, maintenance and function of IL-17-producing cells and is a highly attractive target for the treatment of IL-17-mediated autoimmune diseases, particularly psoriasis^[24]. In this study, we observed presence of ROR γ t positive cells only in the dermis of model group, while in health controls and the GNY decoction treated groups negligible levels of ROR γ t positive cells were present.

Deregulation of TGF- β signalling was reported in human psoriasis^[25], and TGF- β promotes Th17 cell development^[13]. The expression of TGF- β in IMQ-induced psoriasis was reduced by GNY decoction. Thus, Th17 cells could be one of the important effective targets in treating psoriasis by GNY decoction. Next, we analyzed the percentage of Th17 cells by flow cytometry, which showed significant reduction in this population of cells after treatment with GNY decoction. We also analyzed percentage of CD25⁺Foxp3⁺ Tregs which were proposed to control inflammation in psoriasis^[26]. In our study CD25⁺Foxp3⁺ Tregs were reduced in the model group, and only low but not high concentration of GNY decoction treated mice had significantly increased levels of these cells, which suggests differential effects of components of GNY decoction on this cell population.

Next we focused on IL-17 signalling pathway. We investigated expression changes of the genes at mRNA level for IL-17A and IL-6, IL-10, IL-22, IL-23, TGF- β and TNF- α , which interacted with IL-17^[27-29]. Except for IL-10, all these cytokines were decreased after administration of GNY decoction at higher concentration. We have also tested AMPK/IL-17 pathway. AMPK α 1 was reported to be increased in auto-inflammatory condition^[30]. In this study, the elevated AMPK α 1 mRNA level in psoriatic mice model was decreased after treatment with GNY decoction, and its implications need to be explored further.

Conclusions

Using IMQ induced psoriasis model, we analyzed therapeutic effects of a TCM formula named GNY decoction. Decreased PASI scores demonstrated the effectiveness of GNY decoction, which improved clinical manifestations and alleviated keratinocyte hyperproliferation. IHC, IF, Flow cytometry and qPCR analysis further revealed its

efficiency and mechanisms of action. Thus, this study will form the basis for considering GNY decoction for treatment of psoriasis patients and to design future clinical trials.

Abbreviations

GNY

Gancao nurish-Yin;

IMQ

Imiquimod;

PASI

Psoriasis area and severity index;

H&E

hematoxylin and eosin;

Gr-1

granulocyte receptor 1;

CD

cluster of differentiation;

ROR γ t

Retinoic acid receptor-related orphan receptor gamma-t;

TGF- β

Transforming growth factor-beta;

IL

Interleukin;

TNF- α

Tumor necrosis gactor- α ;

AMPK

Adenosine 5'-monophosphate (AMP)-activated protein kinase;

TCM

Traditional Chinese Medicine;

TxB₂

Thromboxane B₂;

PGE₂

Prostaglandin E₂.

Declarations

Ethics Approval and Consent to Participate

Experiments dealing with mice in this study were conducted in accordance with the the Laboratory Animals Welfare Act of ethics committee, Southern Medical University.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing Interests

All authors declare that they have no conflict of interest.

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Authors' contributions

YC conceived the idea and design of this study, HMW and YC conducted the experiments, analyzed the data and composed the manuscript. YC and HMW list as first co-authors. CHX and KSN reviewed and edited the paper. All authors have read and approved the manuscript.

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Figures

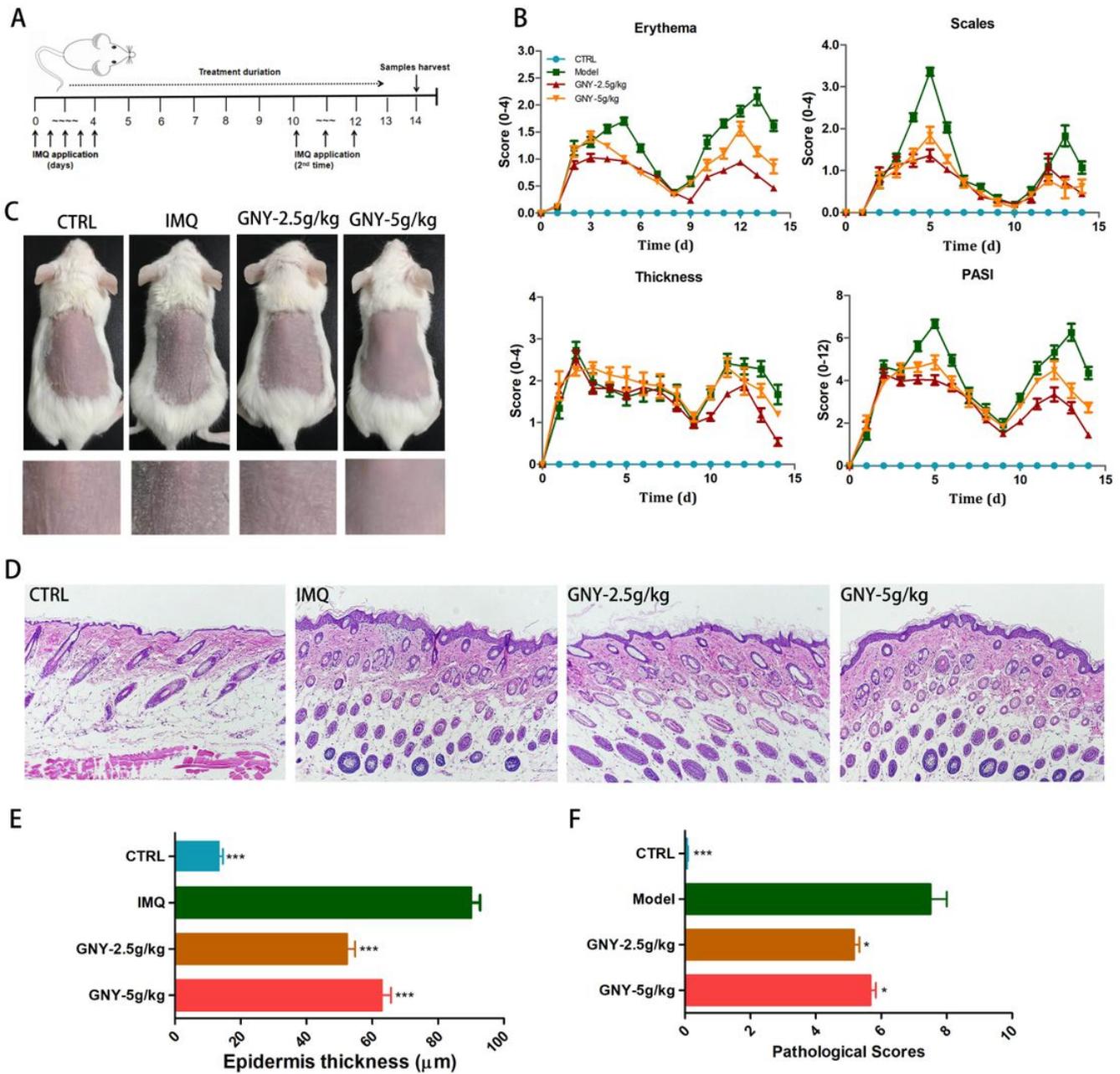


Figure 1

GNY decoction ameliorated IMQ-induced psoriasis in mice. (A) Flow diagram of experimental scheme. (B - C) Erythema, scaling and thickness of the back skin was scored daily using a scale from 0 to 4. The cumulative score is depicted. Data were presented as mean \pm SD ($n = 8$ mice). BALB/c mice were daily treated with IMQ on the shaved back for 4 days except for the control group. Mice treated with GNY decoction at both 2.5g/kg and 5g/kg concentration provided in the drinking water showed lower scores of erythema, scaling and PASI. After second IMQ stimulation, similar effects of GNY decoction were observed. (D - F) H&E staining of the skin lesions in the model group showed increased epidermal thickening, acanthosis, parakeratosis, residual condensation of nuclei in the stratum corneum, representing typical characteristics of psoriasis-like skin lesions. GNY decoction treatment

ameliorated the pathological features of the skin lesions *, $p < 0.05$ and ***, $p < 0.001$ treatment groups vs model group.

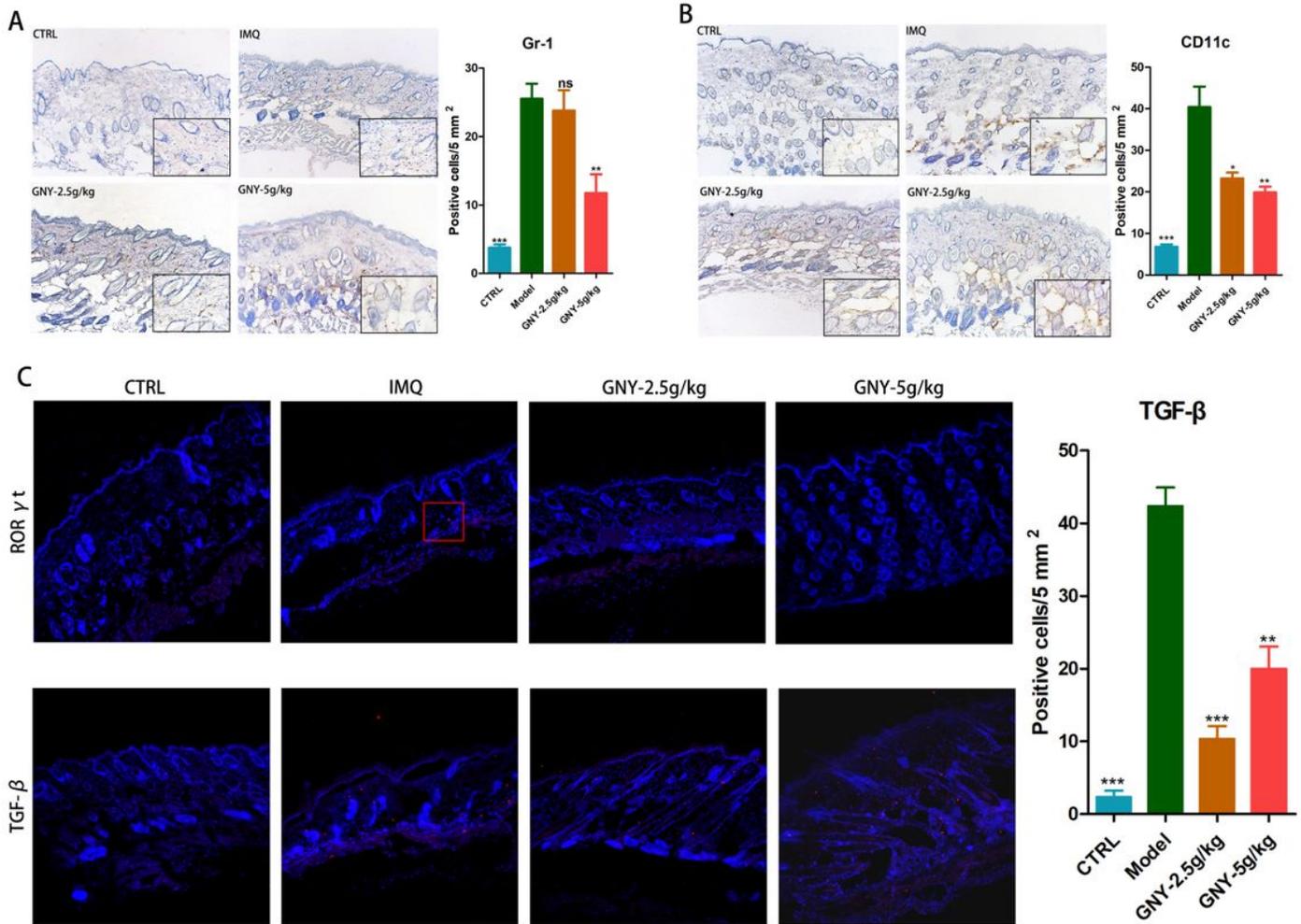


Figure 2

GNY decoction inhibited both non-specific and specific cells in IMQ-induced psoriasis. (A-B) Psoriasis mice expressed higher levels of Gr-1 and CD11c positive cells than mice in the control group, while GNY decoction reduced their expression in a dose dependant manner (n = 8/group). (C) ROR γ t positive cells were observed by immunofluorescence in the dermis of psoriatic mice (1-3 cells/section), while they were at negligible levels in both control and GNY decoction treated mice. Compared to healthy control mice, psoriatic mice expressed higher number of TGF- β + cells. GNY decoction at both the concentrations decreased the expression of TGF- β (n = 8/group). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ treatment groups vs Model group.

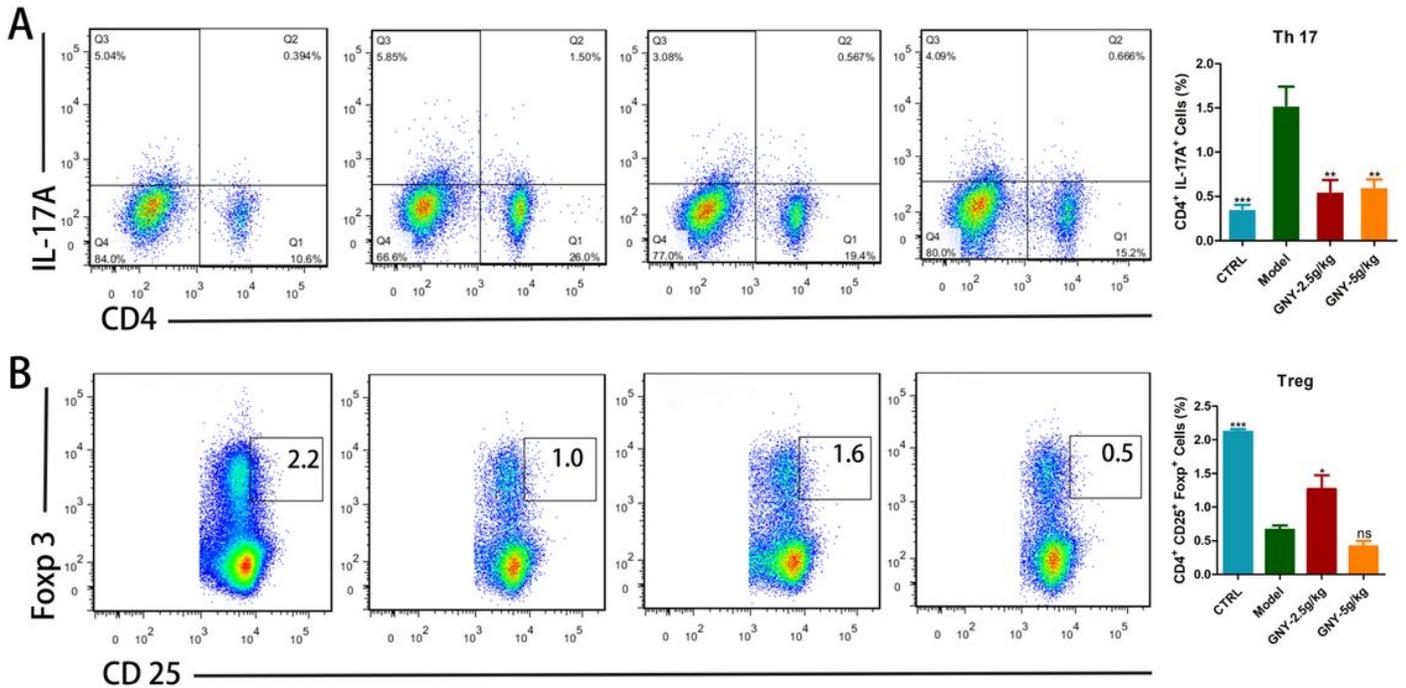


Figure 3

GNy decoction regulated Treg/Th17 cells in the spleen. Flow cytometry analysis of T cell subsets in the spleen of model mice showed an increased percentage of CD4+CD17+Th17 cells but with a decreased CD25+Foxp3+ Tregs. (A) At both the concentrations of GNy decoction the differentiated CD4+CD17+Th17 cells were decreased. Whereas (B) CD25+Foxp3+ Tregs were decreased only after treatment with lower concentration of GNy decoction. *, p < 0.05; **, p < 0.01; ***, p < 0.001 treatment groups vs Model group.

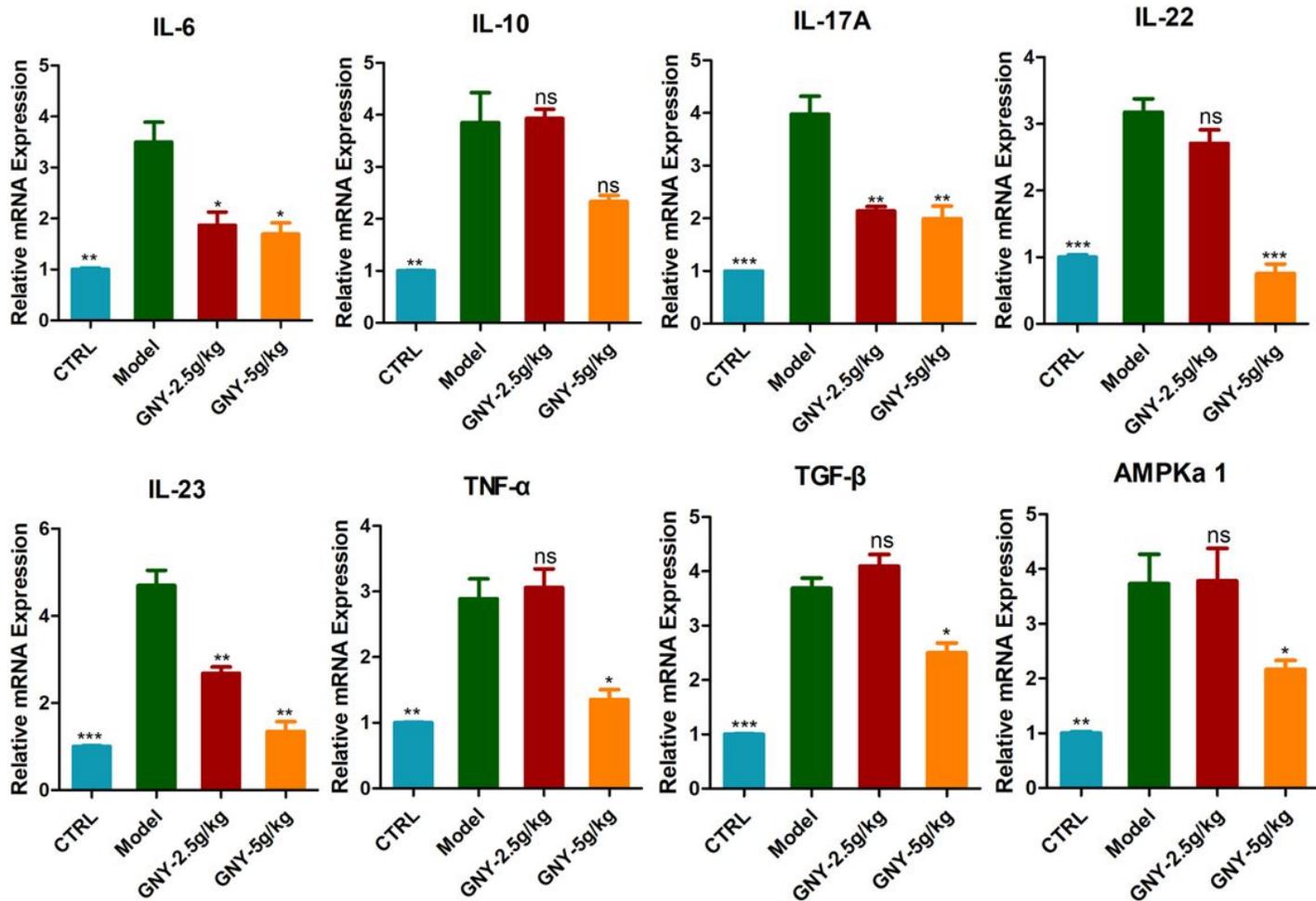


Figure 4

GNY decoction decreased several inflammatory cytokines and AMPK α 1 in the dermis of psoriasis mice. Relative mRNA expression of IL-6, IL-10, IL-17A, IL-22, IL-23, TNF- α , TGF- β and AMPK α 1 genes were elevated in the mice from model group. Except IL-10 gene, expression of all the other genes were significantly decreased by higher concentration of GNY decoction. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ treatment groups vs Model group.