Study of dietary-induced progression of psoriasis-like mice based on gut macrophage polarization

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Abstract. The aim of the present study was to investigate the influence of stimulating food (SF), a Traditional Chinese Medicine term for a high protein, high fat diet, on psoriasis exacerbation. It was hypothesized that SF disposed psoriasis-like aggravation might be related to inflammatory pathways induction via gut dysbiosis. In the present study, mice were fed either an SF or normal diet for 4 weeks. In the last week, their back hair was removed to establish psoriasis-like dermatitis by imiquimod. After sacrifice, blood samples, alimentary tissues and skin lesions were collected and tested by enzyme-linked immunosorbent assay, western blotting, immunohistochemistry and immunofluorescence. Compared with normal diet groups, body weight and blood glucose of SF diet mice were not increased, but they exhibited higher modified Psoriasis Area and Severity Index scores and corresponding epithelial hyperproliferation. Unexpectedly, skin lesions showed abnormal lower protein expressions of Notch and TLR-2/NF-κB p65 signaling pathway, which was attributable to severe skin damage. No difference was observed in the structure and inflammatory cell infiltration of the gut between groups. Instead, macrophage polarization (M1/M2) in the gut of the SF diet group marked by high expression of CD11b (a marker of macrophage, M1) and mild low expression of MRC1 (a marker of macrophage, M2), which resulted in increased TNF-α, decreased IL-10, IL-35, and unchanged IL-17 in serum. Furthermore, serum derived from SF diet mice promoted translocation of NF-κB p65 in HaCaT cells, which indirectly suggested a systemic inflammation. These results suggested that mice fed a continuous SF diet for a time could change gut macrophage polarization, which secretes proinflammatory cytokines into blood circulation. Once transported to skin lesions, these cytokines activate psoriasis tissue resident immune cells and present as psoriasis exacerbation.

Introduction

Psoriasis is a common, chronic, immune-mediated systemic disease characterized by skin lesions, including red plaques, scaly patches, and papules. This serious global disease caused a heavy psychosocial burden on patients and continues to increase in incidence (1). Clinically, evidence has shown that a number of psoriasis patients usually suffer from obesity, insulin resistance, metabolic syndrome, cardiovascular disease and other immune-mediated gut chronic inflammation (2). As observed, psoriasis is refractory and easily relapses and it can be triggered by lifestyle factors (3), such as intake of stimulating food (SF). According to the Traditional Chinese Medicine (TCM), SF is usually defined as foods with high nutrition and energy, particularly animal proteins and fat (red meat such as beef and mutton, fried food and cream), with pungent properties (Chinese liquor, and plants such as pepper, fennel, Chinese ginger, cinnamon, verum, amomum tsao-ko and cardamon) and with high sugar content (dessert and sweetmeats). Long-term high consumption of SF can invoke the onset and/or aggravate the progression of diverse skin diseases, especially psoriasis, eczema and allergic dermatitis (4). It appears that certain dietary patterns, such as SF, can induce gut disorder in susceptible individuals and trigger systemic inflammation via unknown signaling and subsequent production of pro-inflammatory cytokines further exacerbate unstable skin immunity and caused recurrence of psoriasis (5). Such phenomena have been noticed in clinical practice and aroused patients' concern. The present study highlighted patients with inflammatory bowel condition who are usually more susceptible to SF.

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Report on psoriasis and diet first appeared in the 1950s and researchers tried nutrient therapy in psoriasis and designed clinical trials to verify the observational results (6). During the past 60 years, various dietary regimens have been proposed, such as a low-tryptophan diet (7), a low-protein diet (8), a low-fat diet (9), a fasting diet (10), a gluten-free diet (11) and even dietary habits from geographical regions, such as the turkey diet (12) and a Mediterranean diet (13). Additionally, supplementing elements in the diet has also been evaluated, mainly on polyunsaturated fatty acids from marine fish oil (omega-3 and -6; ethyl ester lipids-angiosan) (14), vitamins (A, D, E, B1, B2, B6, niacin, biotin and C) (15,16), micronutrients (selenium, zinc, iron and copper) (17) and other additives (taurine, fumaric acid, arachidonic acid, bile acids and bioflavonoids) (18-20). However, a Western diet (WD) plays a crucial diet pattern in modern societies and growing researches had confirmed that WD intake leads to psoriasis exacerbation and is even related to its comorbidities (21,22).

Gradually, it became noticed that some metabolites of food were higher in the blood plasma of psoriasis, especially increased cholesterol, triglyceride (fatty acids), and glucose, which are closely associated with food intake and metabolic condition (23). Unsurprisingly, the management of metabolic disturbance by 'lifestyle intervention' and weight loss has been proposed (24).

However, the pathophysiologic mechanism behind dietetics and skin lesions remain to be elucidated. There is emerging evidence that diet-driven changes in gut microbiota composition and metabolome, which directly or indirectly affect human health, are particularly obvious in people with in inflammatory bowel diseases (IBD) or metabolic syndrome (25,26). Additionally, a WD induced adipose inflammation characterized by increases in the M1:M2 macrophage ratio and proinflammatory cytokine expression contributes to IBD (27). Unexpectedly, such macrophages also exist in the skin as antigen-presenting cells (APCs) and play key role in defending against cutaneous pathogens (28). In light of evidence that psoriasis is a systemic inflammatory disorder linked to the gut, the present study hypothesized that long-term intake of SF induces unbalance of gut immune, which results in the releasing of inflammatory cytokines in circulation which then interact with unstable immune cells residing in the skin lesions of psoriasis. The present study constructed psoriasis-like mice fed with SF, then gut histology and immune condition, serum cytokines and skin lesions were compared with normal fed mice, thus providing valuable information for the prevention and treatment of psoriasis.

Materials and methods

Preparation of SF. According to the Chinese cooking culture, SF usually consists of several basic ingredients, such as high in fat and energy (butter, yolk, dried whole power and soybean oil added in SF formulations; and beef and mutton providing protein content in subsequent research), sugar (honey water), pungent tastes (Chinese liquor and soybean oil infused with plant spices) and less abundant in fibrin. Based on normal diet ingredients (Table I), SF was produced by Xietong Biotech, except for additives in soybean oil (obtained from TCM pharmacies). In theory, long-term intake of SF (Table II) could induce gut inflammation in mice.

Psoriasis-like animal model and experimental design. Male BALB/c mice (n=18, aged 5-6 weeks, body weight 16.5±0.4 g) were obtained from Xipuer-BiKai (Shanghai, China), housed at the Zhejiang Chinese Medical University Laboratory Animal Research Center and randomly divided into three groups (normal control, psoriasis, psoriasis + SF). Mice in the psoriasis + SF group were fed SF and the other groups had free access to standard chow and water. At the third week, their back skin hair was removed by shaver and depilatory cream. Mice were topically administered 62.5 mg of 5% imiquimod cream (Aldara; 3M Health Care Ltd.) three times every other day and the control mice were treated with Vaseline. If signs of abnormal coat condition and excessive scratching, or weight loss was >10%, the mice were sacrificed with 70% volume displacement per min of 100% carbon dioxide. The indicator of mortality was loss of heartbeat. Mice were euthanized when their psoriatic lesions were spontaneously alleviated 1 week later. After sacrifice, skin lesions and alimentary tract tissue samples and blood were collected for further analysis. All experimental procedures were performed in compliance with the guidelines set by the Zhejiang University of Traditional Chinese Medicine Animal Care and Use Committee (approval no. IACUC-20190408-12).

Scoring severity of skin lesion under dermascopy. Mouse skin lesions were scored based on the modified Psoriasis Area and Severity Index (mPASI) scores, which were comprised of scale (scores 0-4), erythema (scores 0-4) and infiltration (scores 0-4). From day 0 and days 1, 3, and 6 after establishment of psoriasis-like dermatitis, images of lesions captured by dermascopy (HEINE mini 3000 LED Dermatoscope; HEINE Optotechnik GmbH & Co. KG) were evaluated for scoring on a scale from 0-4: None, 0; slight, 1; moderate, 2; marked, 3; very marked, 4. The level of erythema was scored using a scoring table with red taints. The thickness of the skin lesion was measured by a digital caliper. The cumulative score (erythema plus scaling plus thickening) was marked as a measure of the severity of psoriatic lesions (scale 0-12).

Tissue preparation and immunohistochemistry (IHC). Back skin lesion tissue and alimentary tract tissue (stomach, jejunum, ileum and colon) of each mouse were fixed with 10% neutral buffered formalin separately at 4°C for 1 h, and then transferred to room temperature for 12 h. Briefly, the above samples of each mouse were processed through a paraffin embedding protocol, with dehydration through a series of alcohol-water solutions beginning with 75% and up to absolute alcohol, clearing (Histoclear), embedded in paraffin wax and sectioned at 5 μm using a Leica RM2145 microtome (Leica Microsystems, Inc.). Slides were stained with hematoxylin and eosin (H&E) Leica Autostainer XL Protocol Sheet, each step in the protocol was accurately timed and room temperature was used for all steps and solutions. The slides were stained in hematoxylin solution for 3 min, and in working eosin solution for 1 min. For IHC, slides of sections from the above paraffin-embedded tissues were incubated with anti-rabbit CD11b antibody (cat. no. ab133357; Abcam; 1:500) and MRC1 antibody (cat. no. abs141228; Absin Bioscience Inc. 1:500) overnight at 4°C, followed by incubation with biotinylated secondary antibody Goat anti-Rabbit

Table I. Normal dietary ingredient composition (% by weight) of mice.

Ingredient	g/kg diet
Casein, 30 Mesh	200
L-Cystine	3
Corn starch	397
Maltodextrin 10	132
Sucrose	100
Cellulose	50
Soybean oil (no additives)	70
t-butylhydroquinone	0.014
Mineral Mix S10022M	35
Vitamin Mix V10037	10
Choline Bitartrate	2.5
Total	1,000

Table II. The proportion of constituents of 'stimulating food'

Supplement regimen	Proportion
Normal diet of mice	60%
Soybean Oil (with additives[a])	10%
Yolk	15%
Butter	5%
Dried whole milk powder	7%
Sugar	3%
Drinking water[b]	ad libitum

[a]Crushed plant spices (fennel 3 g, Chinese ginger 5 g, radix angelicae 4 g, Amomum tsao-ko 4 g, cardamon 5 g and pepper 3 g) in 20% volume dose of oil, simmered for 20 min. [b]20% honey water and 20% alcoholic beverage (Chinese liquor), taken alternately (every other day).

IgG H&L (HRP; cat. no. ab205728; Abcam; 1:1,000) for 1 h at room temperature and development with 3,3'-diaminobenzidine. Samples were scanned by NanoZoomer Slide Scanner (Hamamatsu Photonics K.K.), five random high-power field images (magnification, x20) of each slide were captured and CD11b- and MRC1-positive parts were counted using ImageJ software version 2.0 (National Institutes of Health).

ELISA. Blood samples from each mouse were centrifuged at 1,000 x g at 4°C to obtain serum. ELISA kits for mouse interleukin (IL)-17 (cat. no. MM-0170M1), IL-35 (cat. no. MM-0904M1), IL-10 (cat. no. MM-0176M2) and TNF-α (cat. no. MM-0132M1) were purchased from Jiangsu Meimian Industrial Co. The expression levels in the blood serum of mice were measured according to the manufacturer's instructions.

Protein extraction and western blotting. Proteins of skin tissue were extracted with Enhanced RIPA lysate (cat. no. AR0102; Wuhan Boster Biological Technology, Ltd.) mixed with complete Protease Inhibitor Cocktail (Roche Life Science). Total protein content in the tissue lysates were determined by a bicinchoninic acid assay kit (cat. no. AR0146; Wuhan Boster Biological Technology, Ltd.) and mixed with loading buffer when subjected to SDS-PAGE on Bolt 10% Bis-Tris Plus Gels (Invitrogen; Thermo Fisher Scientific, Inc.). Proteins were transferred onto a PVDF membrane (MilliporeSigma), and then incubated with primary antibodies specific for NF-κB p65 (cat. no. 6956), phospho-NF-κB p65 (cat. no. 3033), Toll-like receptor-2 (cat. no. 2229S), IKBα (cat. no. 9242), Jagged-1 (cat. no. 70109s), Notch-1 (cat. no. 3608s; all CST; 1:1,000), Hes-5 (cat. no. ab25374; Abcam; 1:1,000) and anti-β-actin (cat. no. BM0627; Wuhan Boster Biological Technology, Ltd.; 1:2,000) overnight at 4°C and then followed by incubation with horseradish peroxidase-conjugated secondary anti-mouse or anti-rabbit IgG (cat. nos. 7076 and 7074; CST; 1:2,000) at room temperature for 2 h. The blots were visualized by Beyo ECL Star (cat. no. P0018AS; Beyotime Institute of Biotechnology) with Bio-Rad Gel Doc XR+ and quantified with Image Lab software (Bio-Rad Laboratories, Inc.). The relative expression of proteins in each band was quantified using ImageJ v 1.8.0 (National Institutes of Health) and normalized to β-actin as an internal loading control.

Cell culture and immunofluorescence (IF). HaCaT cells (human immortal keratinocytes) were obtained from the Chinese Academy of Sciences Kunming Cell Bank (cat. no. KCB 200442YJ) with cell line authenticated by STR profiling, cultured with DMEM containing 10% fetal bovine serum and penicillin (100 U/ml)/streptomycin (100 mg/ml; Gibco; Thermo Fisher Scientific, Inc.) at 37°C and 5% CO_2. Prior to performing IF, HaCaT cells were cultured with adding serum (filter sterilization) from the aforementioned three groups of mice on glass slides for 1, 3 and 6 h separately. At the end of culture, cells were harvested and fixed with 4% paraformaldehyde for 15 min at room temperature. Then, IF experiments were performed according to the manufacturer's protocol. Briefly, cells were permeabilized for 10 min, and then incubated with primary antibodies against NF-κB p65 (cat. no. 6956; CST; 1:1,000) at 4°C overnight. Then cells were incubated with secondary anti-mouse IgG antibodies conjugated to Alexa Fluor 594 (cat. no. 8890; CST; 1:1,000). Nuclei were counterstained with DAPI obtained from Abcam.

Statistical analyses. Data analyses was performed using SPSS 20.0 (IBM Corp.) and GraphPad Prism version 6.0 (Dotmatics). The data were presented as the mean ± standard error of the mean and each experiment was performed in triplicate independently. Comparisons with the other two groups were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

SF diet and normal diet did not induce prediabetes in mice. Impaired fasting blood glucose was recognized as an indicator of prediabetes in mice. After 30 days of regular diet and SF feeding, mice in the SF-disposed group did not exhibit overweight and blood glucose disorders or features of obesity
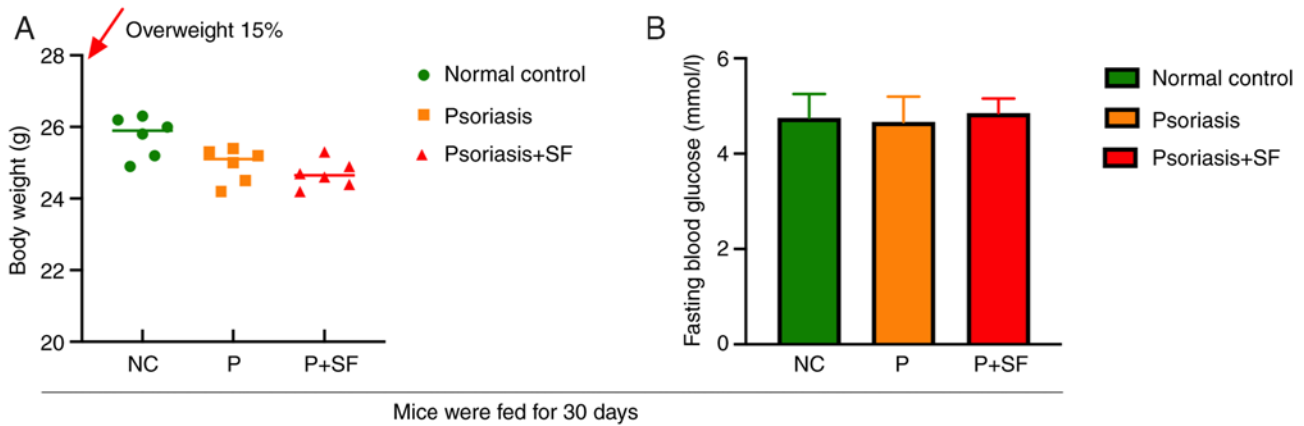
Figure 1. SF diet did not induce prediabetes in mice after 30 days. (A) Body weight of mice after fed different diets for 30 days. (B) Fasting blood glucose values of mice on the 30th day. One-way ANOVA was adopted to compare the differences between these groups. Groups marked with different letters are significantly different (P<0.05). SF, stimulating food; NC, normal control; P, psoriasis; P + SF, psoriasis + SF.

or even emaciation (Fig. 1). This result suggested that an SF diet may induce harmful irritating factors other than energy metabolic disorders during this phase and that their gut perturbations were more similar to the inflammatory 'stressed state'.

Long-term SF feeding exacerbates imiquimod-induced psoriasis-like lesions. Compared with the normal control, the application of Aldara induced psoriasis-like skin lesions in BALB/c mice in dermascopic features and pathological manifestations. Such skin lesions were estimated by mPASI scores (Fig. 2A). Psoriasis-like skin lesions on the 3rd day after Aldara irritation were most typical and then started to resolve spontaneously. Comparison of erythema, scaling, thickening and mPASI scores were highly consistent and higher in the psoriasis + SF group. Additionally, compared with skin lesion pathology of normal control and psoriasis group, there was significant thickening of stratum corneum and epidermis, along with inflammatory cell infiltration and neoangiogenesis in dermis, without distinctive Munro microabscess (neutrophil accumulation at the stratum corneum) or Kogoj spongy microabscess (neutrophilic aggregates in the epidermal stratum spinosum) (Fig. 2B), which represent distinctive features for psoriasis although not often present, only being observed in pustular psoriasis.

The pathology of the gut (from the greater gastric curvature of stomach, jejunum and ileum to colon) were analyzed. The result (Fig. 2C) indicated that there were no significant differences in the distribution and construction of the gut mucosal features and inflammatory infiltrations between SF-disposed group and other groups.

Correlation of macrophage polarization in the gut tract and skin lesions. Skin and gut tract tissues from each mouse were paraffin embedded and sectioned to confirm the correlation of gut and skin. The quantification analysis of IHC staining (Fig. 3) showed that the macrophage polarization (M1/M2) in the gut tract of the psoriasis + SF group was different from that in the normal control and psoriasis groups (both without SF). In the normal control, MRC1 + macrophages (marker of M2 macrophage; anti-inflammatory cells) were enriched throughout the whole gut tract and skin, being especially distinct in the basal part of gastric glands and around the hair

follicle, while M1 macrophages marked by CD11b (pro-in inflammatory cells) had generally a lower expression. Such condition also presented in the psoriasis group, excepting an increased expression of CD11b in the dermis. In the psoriasis + SF group, a decreased expression of MRC1 was observed in the stomach, followed by an increased expression levels of CD11b in the gut tract and dermis, excepting only the colon part. The expression levels of CD11b in skin lesions were positively associated with their expression in the gut tract, especially the stomach, jejunum and ileum. It indicated that SF may promote M1 macrophage activation. Notably, epidermis of several mice in the SF disposed group showed a severe injury which almost reach to a spongiotic dermatitis and necrotic foci.

Cytokines in blood serum of mice and their effect on nuclear translocation of HaCaT cell. Cytokines released by tissue cells (mainly by immune cells) are important for signal transduction. In the SF predisposed group (Fig. 4A), the TNF-α (higher; P=0.007)/IL-10 (lower; P=0.047) ratio was unbalanced, which mediated a chronic inflammation condition. Additionally, IL-35, which is usually an anti-inflammatory cytokine in the gut (28), was also decreased mildly (P=0.084) in the SF predisposed group. No significant result was observed in serum level of IL-17 in psoriasis-like dermatitis mice.

To demonstrate the influence of such serum cytokines on skin, HaCaT cells were stimulated by serum from aforementioned three groups of mice. After culturing for 1 h, IF showed no difference in NF-κB p65 translocation in cells. However, after 3 and 6 h, NF-κB p65 was significantly activated by serum from SF-disposed mice, although there was no obvious activation in the other groups (Fig. 4B). Thus, it was hypothesized that cytokines (more TNF-α and less IL-10) from the serum of SF-disposed mice mediated translocation of NF-κB p65 in HaCaT cells.

Expression of the TLR-2/NF-κB p65 and Notch signaling pathways in skin lesions. TLR-2/NF-κB p65 is a classic inflammatory signaling pathway that is highly activated in advanced stage of psoriasis (29). Toll-like receptor-2 (TLR-2) is usually expressed on macrophages to recognize structurally conserved molecules of microbes, also known as pathogen-associated
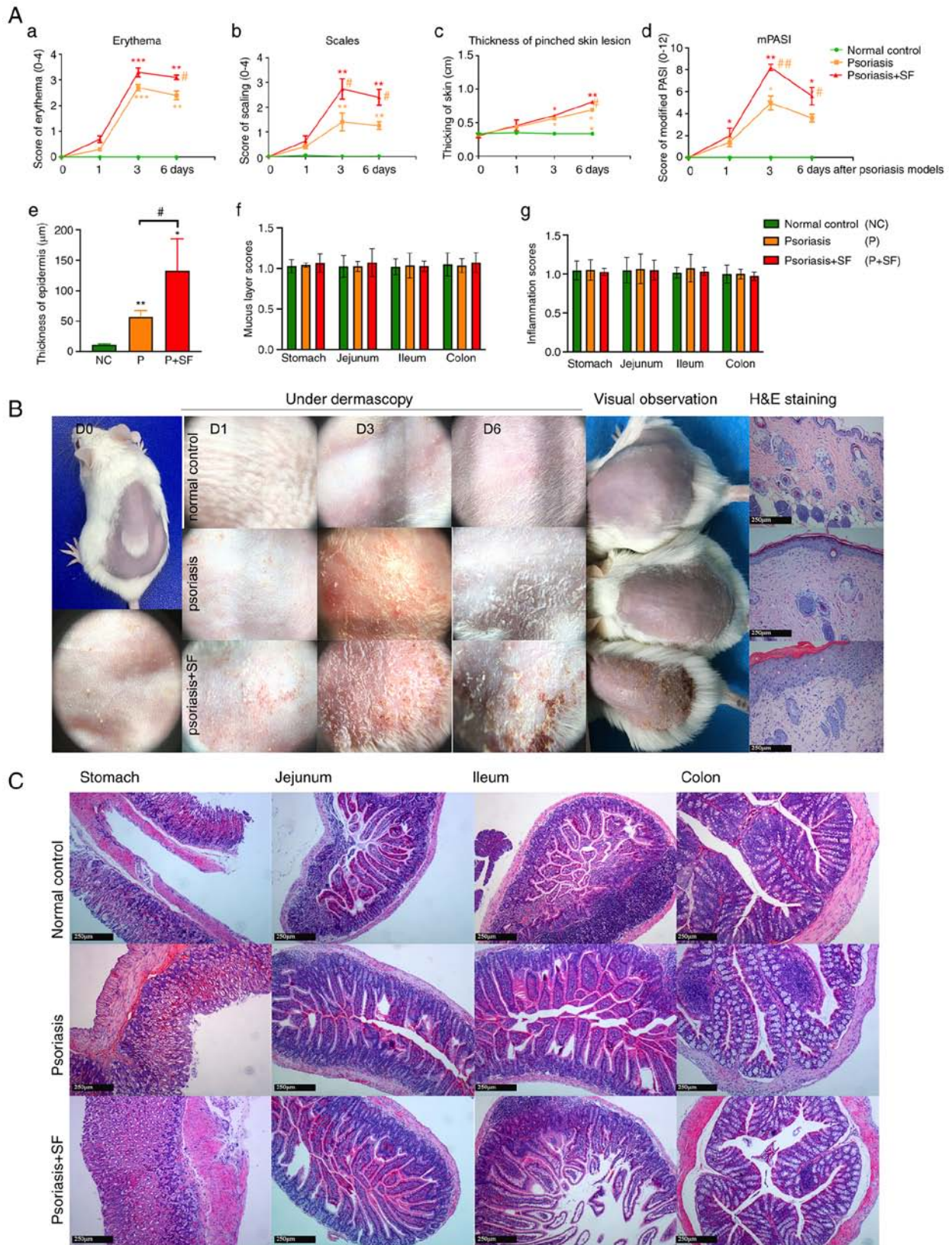
Figure 2. Score index of skin lesions under dermascopy and H&E staining. (A) Scores of (a) erythema, (b) scaling, (c) thickening, (d) mPASI, (e) thickness of epidermis and histological sores of (f) gastrointestinal mucus layer and (g) inflammation of the three groups. The results showed that the SF group had more severe skin lesions, by either mPASI or H&E staining. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. the normal control; $^\#P<0.05$, $^{\#\#}P<0.01$ vs. the psoriasis group. (B) Under skin dermatology, the progression of skin lesions was classified into three stages: D1, Compared with normal control skin (D0), the subcutaneous bleeding point in the psoriasis + SF group was more than that in the psoriasis group; D3, Erythema scaling of the psoriasis + SF group (erythematous covered with thick scales, sporadic blood crust) was more obvious than that of the normal control smooth skin group (erythema with thin silvery scales); D6, Compared with normal control with hair growth skin, skin lesion started to remission in other groups, erythema and scale still obvious in SF disposed group, while only chaff scales were observed in psoriasis group. (C) The effect of food on the gastrointestinal tract. The pathology of the gut tract (magnification, x10) demonstrated that mucosa, luminal contents and even acute inflammatory cell infiltration were not changed during the experimental process. H&E, hematoxylin and eosin; mPASI, modified Psoriasis Area and Severity Index; SF, stimulating food.
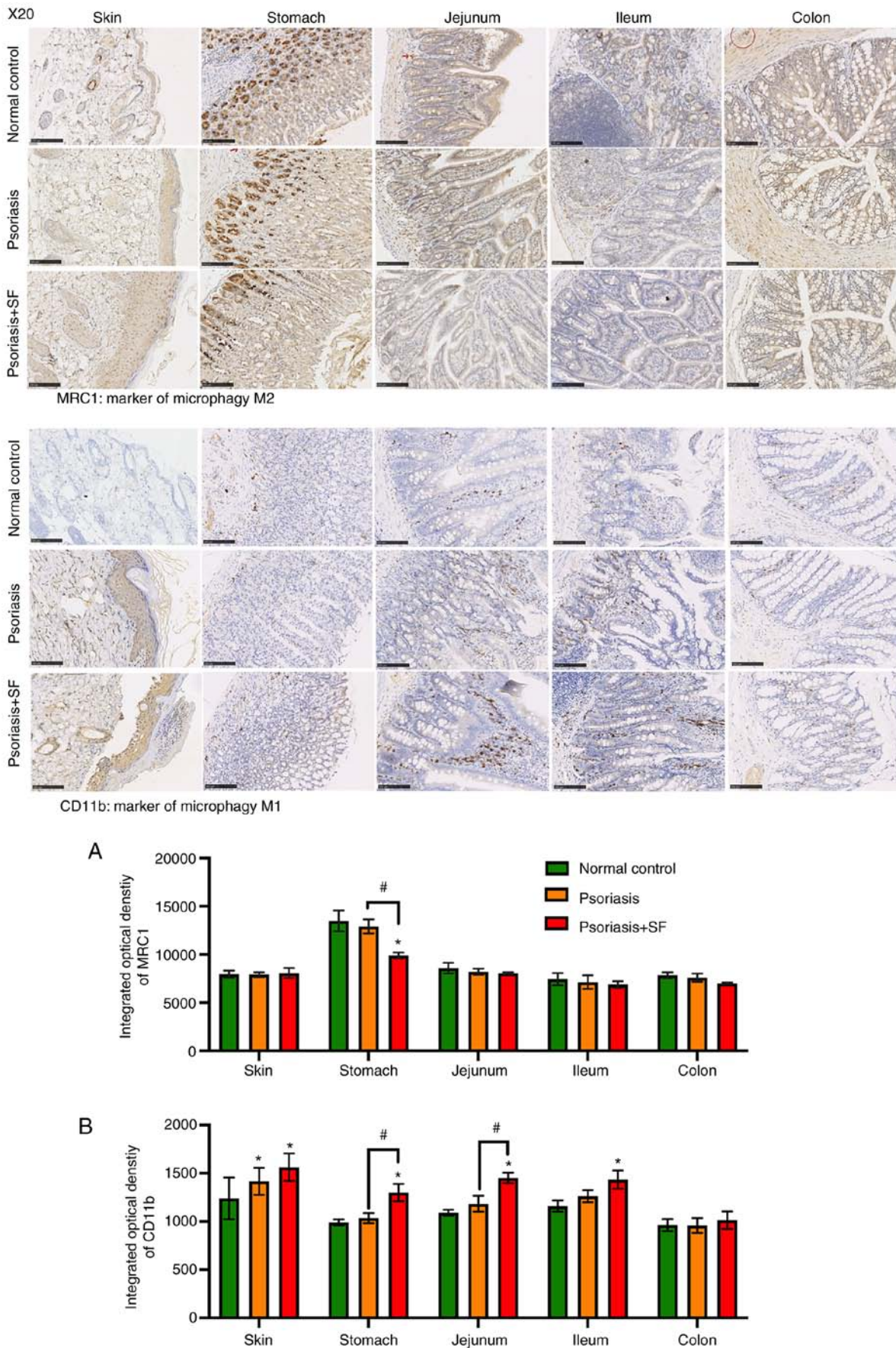
Figure 3. Immunohistochemical detection of CD11b and MRC1 in the skin and gut tract of individual mice. (A) MRC1, as a marker of M2 macrophages, were rich in the gut and skin of the control group but decreased slightly in the SF-predisposed group, apparent in stomach. (B) As a marker of M1 macrophages, CD11b was expressed at low levels in the gut tract and skin of the normal control group but increased significantly in SF mice, especially in the stomach, jejunum, ileum and skin. The dark brown region indicates active zone enrichment of macrophages (*P<0.05 vs. the normal control; $^{\#}$P<0.05 vs. the psoriasis group. SF, stimulating food.
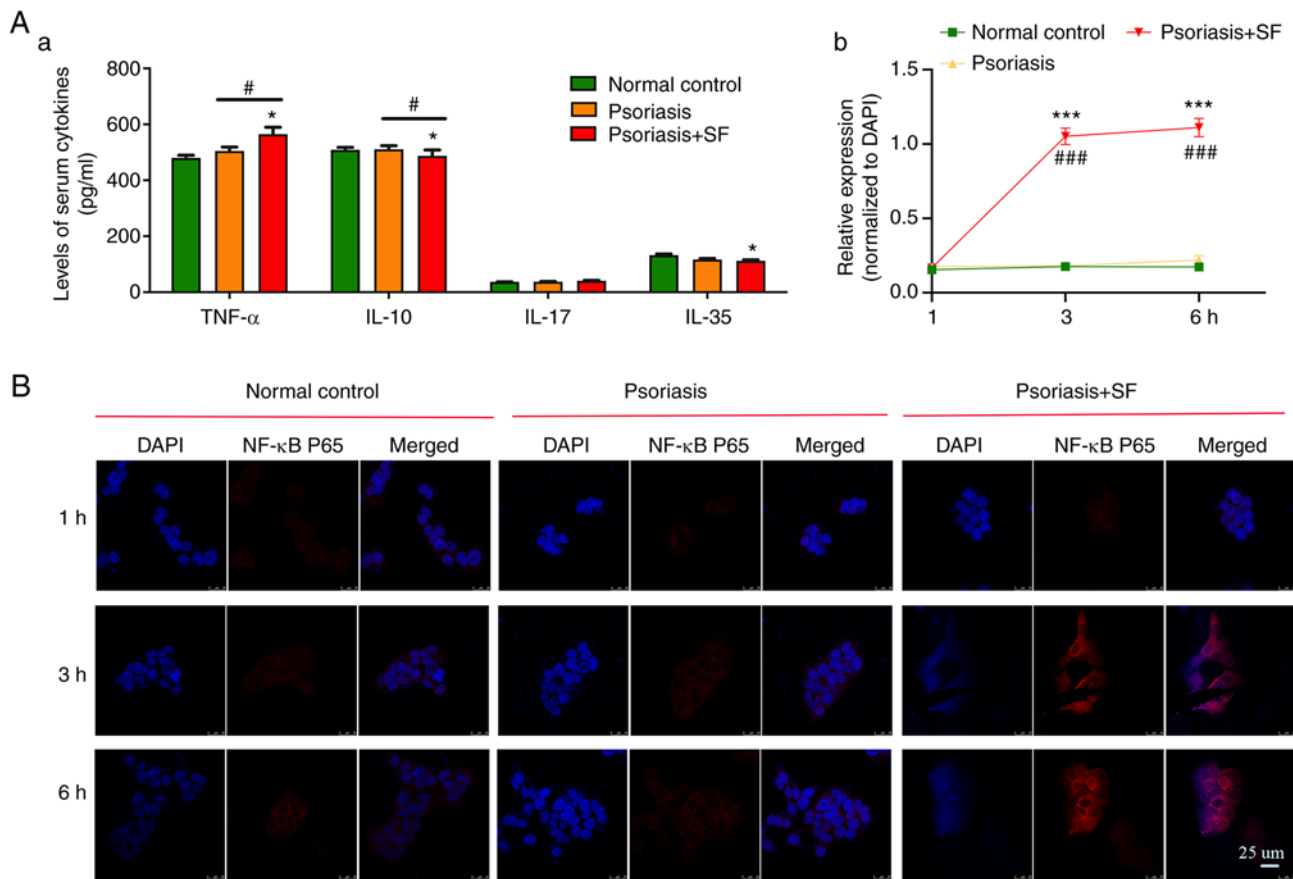
Figure 4. Serum levels of cytokines in the three groups of mice and their effect on translocation of HaCaT cells. (A and a) There was a significant difference in the expression of TNF-α, IL-10, and IL-35 in the SF, while there was no significant difference in the expression of IL-17, which was higher in the SF-disposed group. (B and b) The serum of SF-disposed mice promotes the proliferation of HaCaT cells by activating the transcription of NF-κB p65. HaCaT cells were cultured with serum from different groups of mice over time (1, 3 and 6 h), and whole mounts of HaCaT cells stained for NF-κB p65 (protein, red) and DAPI (cell nuclei distribution, blue) were observed by microscopy. The data are the mean ± SD values ($^*P<0.05$, $^{***}P<0.001$ vs. the normal control; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ vs. the psoriasis group) and were statistically analyzed with one-way ANOVA.

molecular patterns (PAMPs) and then activates the immune cell response, such as activation of NF-κB p65 and subsequent effector molecules to mediate psoriasis relapse. Moreover, TLR activation can activate canonical and non-canonical Notch signaling. In psoriatic skin lesions, Notch signaling molecules have been reported to be highly expressed with diffused mode (30). The results of western blotting (Fig. 5) demonstrated that TLR-2/NF-κB p65 signaling and Notch signaling were activated in psoriasis lesions. Protein expressions of Jagged-1 (a ligand for Notch receptor), Notch-1 and TLR-2, NF-κB p65 and its phosphorylated NF-κB p65 were upregulated significantly in the psoriasis group, while abnormally downregulated in the SF group.

Discussion

The major finding of the current study is that SF could aggravate the progression of psoriasis, which may occur by inducing a chronic inflammatory gut tract with unbalanced polarization of macrophages (M1/M2) and production of proinflammatory cytokines. Once these cytokines are released into blood circulation and transported to skin lesions, they easily activate the primary abnormal immune cells of skin lesions and cause an inflammatory response. Finally, the disease is exacerbated

after certain food intake. Future research should explore the role of serum cytokines of psoriasis in crosstalk to resident immune cells of skin lesions, such as Langerhans cells (31) and CD8+ tissue-resident memory T cells (TRMs). Therefore, the potential efficacy of preventative measures against gut inflammation should be assessed and could be of great value.

According to the skin lesion of psoriasis and PASI scores, guidelines of different countries have recommended detailed therapeutic regimens. While alternative options for the treatment of psoriasis with various comorbidities are still being sought. In Asia, a 15-year nationwide population-based cohort study of Korea showed that atherosclerotic cardiovascular risk increased in psoriasis (32). In the US, results from the National Health and Nutrition Examination Survey also indicated that an increased risk of psoriatic mortality is partially mediated by an increased prevalence of cardiovascular disease, diabetes and cancer (33). In TCM, the body condition of all individuals who suffer from psoriasis has been taken into consideration more than mere skin lesions. Based on the constitution of patients, suggestions as to what type of food in certain nature and taste should be avoided have been given to patients. As mentioned earlier, it amount to one thing, metabolic disorder, which is closely related to lifestyle, particularly what one eats. Data from real world concerning psoriasis gradually
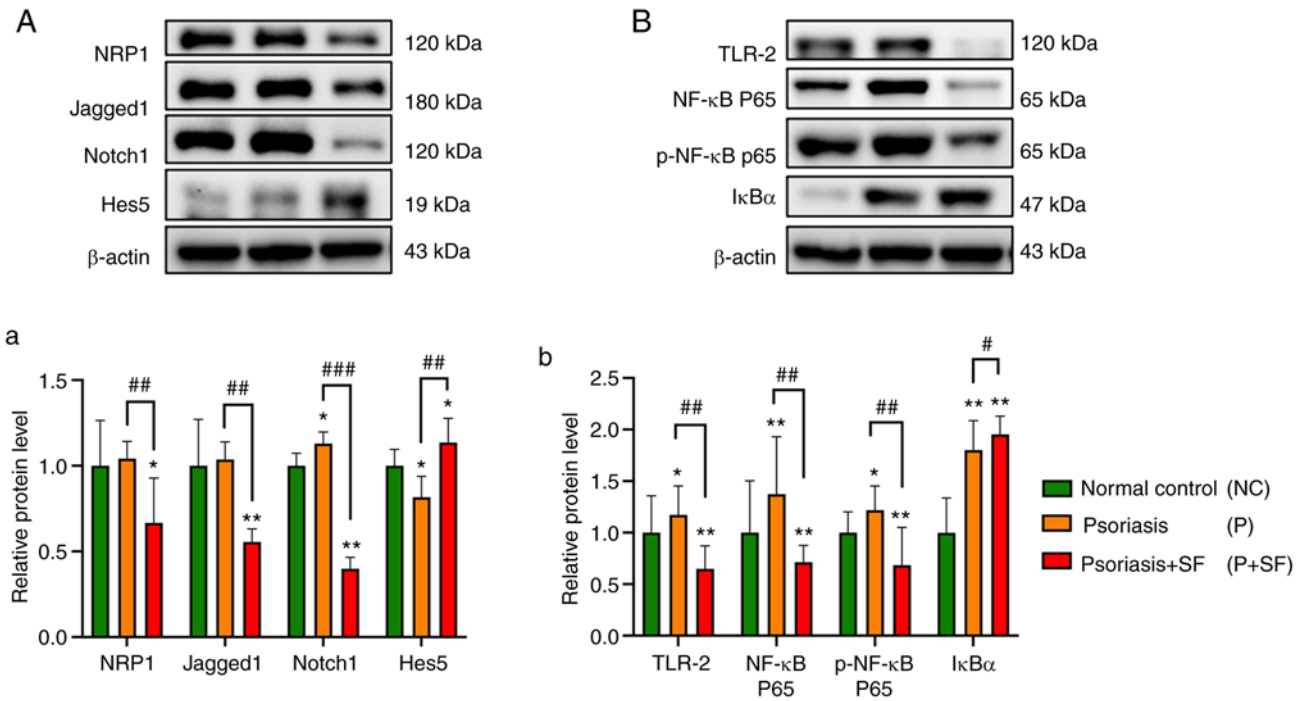
Figure 5. Protein expressions of Jagged1/Notch1/HES5 and TLR-2/IκB-α/NF-κB p65 signaling pathway on skin lesions of imiquimod-induced psoriasis model. (A and a) Western blot analysis of Notch signaling and NRP1 in skin lesions. The mammalian Notch signaling pathway consists of four Notch receptors (Notch 1, 2, 3, and 4) and five Notch ligands (Jagged 1, and 2, and Delta-like 1, 3 and 4). HES5 is an important downstream target gene of Notch signaling pathway. NRP1 is involved in keratinocyte hyperproliferation of psoriasis accompanied by inflammation activated angiogenesis. (B and b) Western blot analysis of TLR-2/IκB-α/NF-κB p65 signaling in skin lesions. Values are expressed as mean ± SD (*P<0.05, **P<0.01 vs. the normal control; $^#$P<0.05, $^{##}$P<0.01, $^{###}$P<0.001 vs. the psoriasis group) and were statistically analyzed with one-way ANOVA. NC, normal control; P, psoriasis; P + SF, psoriasis + SF; SF, stimulating food; p-, phosphorylated.

underlined the importance of lifestyle changes. To clarify the underlying mechanisms of SF on the progression of psoriasis, preliminarily tests were performed.

In the present study, BALB/c mice were administered an SF diet every day to construct 'unhealthy gut' type. After psoriasis-like dermatitis modeling, skin lesions in the SF-disposed group significantly worsened. During these processes, SF tended not to increase the body weight and impact on defecation of the mouse as was expected. Additionally, the ethology of mice showed that SF-disposed mice were more irritable and like to bully one another. Thus, it was hypothesized that SF-induced aggravated lesions might mediated by unknown response in the gut.

In the present study, SF-induced pathology of the gut tract and its corresponding skin lesions were evaluated. The mucosal immune system of the gastrointestinal tract plays a unique role in the balance of commensal microbes and resident immune cells. Among them, accumulating evidence suggests that an overwhelming load of nutrients and their metabolites disturbs the polarization of macrophages, which are closely related to inflammatory response (34). The data of the present study showed an increased M1 macrophages in gut tract and skin lesions of the SF group, which was marked by elevated expression of CD11b. Subsequently, alterations in the serum levels of cytokines (more TNF-α, and less IL-10, IL-35) suggested a low gradation systemic inflammation due to activation of M1 macrophages. However, as key cytokine in the progression of psoriasis, IL-17 levels were not enhanced as expected. At present, researchers have found IL-17 levels were higher both in serum and lesions of psoriasis patients, such just reported in patients who suffered from serious advanced psoriasis and with higher PASI score (35,36). In vivo, skin lesions of psoriasis-like dermatitis were different from patients both in pathogenesis and PASI. Thus, such differences of serum cytokines in mice were probably induced by food intake, which further confirmed the influence of food on gut tissue.

Mechanistically, unbalanced macrophages caused gut local immune response disorder and caused aforementioned cytokines release, which induced mild systemic inflammation of SF diet mice. Once these cytokines were translocated to skin and interacted with local immune cells, progression of primary skin lesions of psoriasis occurred, present as lesions worsening in mPASI scores and skin histology. Therefore, NF-κB p65 nuclear transcription of HaCaT cells was promoted significantly by serum from SF diet mice and was highly associated with inflammation-induced proliferation of epidermis.

The present study found that unstable resident immune cells in primary skin lesions was the reason for interval or sequential therapy of calcipotriol or Daivobet (37) even in the stable stage, which has been highly recommended in guidelines of psoriasis (38). In the pathogenesis of psoriasis, a signal transduction pathway that connects PAMPs recognition to NF-κB activation is involved in the progression of psoriasis. TLRs, especially TLR-2, build a bridge between innate immunity and autoimmunity. The present study revealed that TLR-2/NF-κB p65 and Notch signaling were activated in psoriasis-like dermatitis but overinhibited by SF, which was inconsistent with aforementioned findings. There are several possibilities to account for this. As mentioned in the section

on IHC, some skin lesions in SF-disposed mice showed necrotic foci and spongiotic condition. Normal expression of NF-κB p65 and Notch are indispensable for skin development and homeostasis (39,40). Taken together, it was hypothesized that such an unusual downregulation of the aforementioned proteins could be attributed to serious skin damage triggered by cytokines from gut or negative feedback regulation of mice.

The national epidemiological survey of psoriasis in 1984 showed that psoriasis prevalence was higher in North China than South China and more frequent in men compared with women (41). Cases of psoriasis flare following some risk factors, cold weather in mild-high latitudes and more consumption of red meat (beef and mutton, often considered as common kinds of SF) are more likely to be the demographic and epidemiological drivers (42). As is well-known, men have more social intercourse and unhealthful dietary behaviors when exposed to urban food environment (43). It is recommended that patients should avoid taking SF when suffering from an acute skin inflammation. Although the present study offered new insights on the role of SF in psoriasis relapse and preliminarily explored its possible mechanism, effects of diet on female mice and small number of experimental mice should be investigated. To further investigate influence of the common kinds of SF on the progress of psoriasis, key laboratory of meat processing and quality control on SF diets with added beef and mutton and the aforementioned limits should be employed.

At present, diet-induced intestinal homeostasis, gut microbiota and their metabolites are still unknown in psoriasis. Future studies in other fields of medicine, 16S rRNA amplicon sequencing would be valuable in determining how microbes respond to diet. Otherwise, microbiota metabolites in stool could be identified by gas chromatographic mass spectrometry, including l-aspartic acid, cholestan-3-ol (5β, 3α), campesterol, lactic acid, and butyrate, which have been associated with lipogenesis and inflammation. However, these effects on psoriasis remain to be elucidated and should be further studied.

SF is one of the danger factors responsible for the progression of psoriasis and can change the gut barrier and metabolite of the patient. Then, damaging substances from the gut are released into the blood circulation and cause low-level systemic inflammatory response, thereby exacerbating local unstable TRMs or APCs in primary skin lesions. Consequently, it induces the progression of the advanced stage of psoriasis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Conceptualization and visualization was by FX. Methodology and investigation by WZ and YW. FX and XY confirm the authenticity of all the raw data. FX and XY analyzed the data, revised the draft and revised the figures and tables. Funding acquisition and work modification was by YC. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experimental procedures were performed in compliance with the guidelines set by the Zhejiang Chinese Medical University Laboratory Animal Research Center (approval no. IACUC-20190408-12).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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