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Role of Galphaq in pathogenesis of psoriasis, a new mechanism about the immune regulation in psoriasis

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ABSTRACT

Psoriasis (PSO) is a chronic inflammatory skin disease characterized with skin lesions and abnormal keratinocyte proliferation. The immune dysregulation is involved in the pathogenesis of PSO. However, the detail of immune regulation in PSO is still not very clear. Gαq, the alpha subunit of the Gq protein, played important role in the immune regulation. In this study, we aimed to investigate whether Gαq was involved in the pathogenesis of PSO. We detected the Gαq expression level and analyzed its relationship with test parameters of PSO patients. Furthermore, we used imiquimod to induce PSO mouse model in Gnaq -/- bone marrow (BM) chimeric mice. The inflammatory cytokines and its correlation with Gαq expression were analyzed both in PSO patients and mice. The results showed that the Gαq expression in PSO patients was much lower and negatively correlated with PSO Area and Severity Index (PASI), CRP, cholesterol and low-density lipoprotein. In PSO mice models, the skin lesion and keratinocyte proliferation were much more serious in Gnaq -/- BM chimeric mice. Also, the proportions of Th17 cells in Gnaq -/- BM chimeric mice were much higher than WT mice. Furthermore, the IL-17A and TNF-α in Gnaq -/- chimeric mice were also higher. Moreover, IL-17A and TNF-α in PSO patients were negatively associated with the Gαq expression. Our results indicated that Gαq was involved in the pathogenesis of PSO, and its regulation on Th17 cell differentiation and cytokine production might contribute to part of the mechanism of immune dysfunction of PSO.

1. Introduction

Psoriasis (PSO), one of the most common chronic inflammation diseases, affect almost 3% population worldwide [1] and 0.47% population in China [2]. It is a multisystem disease, which always accompanies with psoriatic arthritis, intestinal and cardiovascular diseases [3]. However, the pathogenesis of PSO is complex and the exact mechanism is still elusive and effective treatment is limited. Recent studies had identified that the interplay of immune cells and the inflammatory cytokines play critical roles in the pathogenesis of PSO [4]. These factors include the Th17/Treg imbalance, the Th1/Th2 homeostasis and the IL-23/IL22/Th17 axis [5]. In addition, other pathways and signaling molecules, such as Th9, Th22, γδT cells, CD8+ T cells and their related cytokines, including TNF-α, IL-9, IFN-γ also involved in the PSO onset and development [6,7]. The researches about the immune imbalance of PSO gave rise to new effective treatment strategies in PSO such as TNF-α blockade [8]. A better understanding of the mechanism of immunological dysfunction in PSO will allow us to find more efficient targets for the treatment of PSO.

Gαq, encoded by GNAQ, belongs to Gαq/11 subfamily of G protein [9]. Our previous studies showed that Gαq play important roles in both innate and adaptive immunity [10]. Gαq regulated the T cells and B cells proliferation, survival and activation [11,12], it also promoted the inflammatory cytokines, such as IL-2, IL-12 and TNF-α production [13]. Moreover, Gαq also involved in the T cell differentiation, such as the Th17/Treg imbalance, the Th1/Th2 homeostasis [14,15]. We also proved that, Gαq expression were much lower in some autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus

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erythematosus (SLE).

As PSO is also an autoimmune diseases characterized by immunological dysfunction, and the Gαq had been proved involved in several autoimmune disease, whether Gαq was involved in PSO and the role of Gαq in immune dysfunction of PSO were still unknown.

In this study, we detected the relative expression of Gαq in PSO patients, and then analyzed the correlation of Gαq expression and test parameters of PSO patients. To further confirm that Gαq participated in the PSO pathogenesis through regulating immune cells, we used the Gαq knock out (Gnaq -/-) bone marrow (BM) chimeric mice, in which the immune system lack Gαq, to produce the PSO-like inflammation. Both of the clinical data and the mouse model data indicated that, Gαq was involved in the pathogenesis of PSO, and it might through regulating the inflammatory cytokines like IL-17 or TNF-α production. The results provided a new insight into the mechanism of the immune regulation of PSO, and the Gαq might be used as a potential target in PSO research and treatment.

2. Material and methods

2.1. Patients and controls

Fifty preliminary diagnosed patients with PSO were recruited from the dermatology department in the First Affiliated Hospital of Xiamen University. Thirty sex and age matched healthy volunteers, who were excluded of autoimmune diseases and other dermatology diseases, were recruited as healthy controls (HCs). This study was performed in the Xiamen key laboratory of rheumatology and clinical immunology, which was approved by the ethics committee of the First Affiliated Hospital of Xiamen University. This study was also informed consent of the patients and the healthy volunteers. The diseases activity was evaluated by the criteria of clinical PSO Area and Severity Index (PASI), which is widely used for monitoring the disease activity of patients with PSO. Blood samples and serum from patients and healthy controls were obtained and stored in −70 degree before use. Peripheral blood mononuclear cells (PBMCs) were isolated by standard density-gradient centrifugation. The Gαq relative expression of PBMC from patients and healthy controls were detected by qualitative real time-PCR (RT-PCR). Test parameters such as CRP and clinical biochemical indexes were obtained from medical record. The demographic and clinical characteristics of PSO patients and HCs were summarized in Table 1.

2.2. Realtime-PCR

Total mRNA was extracted from PBMC of PSO patients and HCs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the instructions. The mRNA was transcribed to cDNA using script cDNA synthesis kit (Roche, Indianapolis, IN, USA). Quantitative PCR were performed with FastStar University SYBR Green Master (Roche) according to the manufacture's instructions. The relative expression of mRNA was normalized to the expression levels of β-Actin with the $2^{-\Delta\Delta Ct}$ method. The following primers were designed for testing the human genes: β -Actin: 5′-GTG GGG CGC CCC AGG CAC CA-3′ and 3′-CTC CTT AAT GTC ACG CAC GAT TTC-5′; Gαq: 5′-GTT GAT GTG GAAG AAG GTG TCT A-3′ and 3′-GTA GGC AGG TAG GCA GGG T-5′.

Table 1
Baseline demographics of PSO patients and healthy controls. Date was presented as mean ± SEM.

	PSO (n = 42)	HC (n = 40)
Age, median (range) years	44.9(16–74)	31.3(22–62)
Sex, no. of men/no. of women	27/16	29/11
Arthritis	6(42)	–
PASI score (mean ± SD)	2.762 ± 1.512	–

2.3. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of IL-17A and TNF-α in serum of patients were measured using commercially available ELISA kits according to the manufacturer's instructions (R&D, Minneapolis, USA). Absorbance at 450 nm was measured with a microplate reader.

2.4. Animals

C57BL/6J(B6) and Gnaq -/-mice were purchased and bred in Xiamen University animal facilities (Xiamen, China). 6 to 8 weeks age female mice were used in this study. All experimental procedures were approved by the animal care and use committee of XiaMen University. In order to figure out whether the roles of Gαq in PSO were through regulating immune system, we used Gnaq -/-BM chimeric mice in this study. We generated BM chimeric mice as method previously described [16,17]. Briefly, chimeric mice were generated by reconstituting lethally irradiated C57BL/6 recipient mice with Gnaq -/-BM or with WT BM as control. Mice were irradiated with a split dose of 800 Rads and then receipted with 1–2 × 10^6 BM cells from Gnaq -/- or WT mice. The Gαq of hematopoietic system and immune system in Gnaq -/-chimeric mice were knocked out, but it did not affected the Gαq expression in skin or other systems.

2.5. Imiquimod-induced PSO-like inflammation in mice

To further explore the functions and mechanisms of Gαq in PSO, we produced the PSO-like inflammation mouse model through treating with imiquimod (IMQ) as previously described. The model group of WT chimeric mice and Gnaq -/-chimeric mice were daily treated with available IMQ cream (5%) and the control group was treated with same volume of Vaseline [18–20]. 6 mice per group were used in this model and the experiment repeated 3 times independently. PASI scores were used to evaluate the severity of inflammation of the back skin. Thickening, scaling, erythema and furfuration were scored from 0 to 4; 0 means no change; 1 means slight; 2 means moderate; 3 means serious; 4 means very marked. All mice were sacrificed after the 6 days of IMQ or Vaseline treatment. Serums from mice were collected and analyzed the inflammation cytokine by luminex multiplex assays (Merck Millipore, Darmstadt, Germany) according to manufacture's protocol. Skin samples from back lesions were collected and fixed in 4% formalin and embedded in paraffin. Tissue Sections (5um) were prepared and stained with hematoxylin and eosin (HE). Representative areas were analyzed for histological changes using a light microscope with a 20× objective. The thickness of the keratinocytes was calculated with Image J software (USA).

2.6. Flow cytometry

To prove Gαq regulation role in T cells subset participated in immune dysfunction in PSO, the single cell suspensions harvested from spleen cells of mice were collected after the mice sacrificed. Cells were incubated with PMA (10 ng/ml, eBioscience, San Diego, CA, USA) and BFA (10 μg/ml, eBioscience) at 37 °C and 5% CO_2 for 4 h then harvested and washed twice with PBS and stained with PE-CY7-anti CD4 (eBioscience) or PE-CY5.5- anti CD25 (eBioscience) for 30 min at 4 °C. Cells then fixed permeabilized with Fixation/Permeabilization Solution Kit (eBioscience) for 1 h at 4 °C and then stained with FITC-anti-IL-17A (eBioscience) or PE- anti- Foxp3 or FITC- anti- IFN-γfor another 30 min at 4 °C. CD4+ IL-17A+ cells were identified as Th17 cells, CD25+ Foxp3+ cells were identified as Tregs. CD4 + IFN-γcells were identified as Th1 cells, CD4+ IL-4+ cells were identified as Th2 cells. Cells were analyzed by flow cytometry (Cytomics FC 500; Beckman Coulter, Fullerton, CA, USA) to evaluate the proportion of Th17, Tregs and Th1 and Th2. Results were analyzed and presented with CXP software (Beckman Coulter).

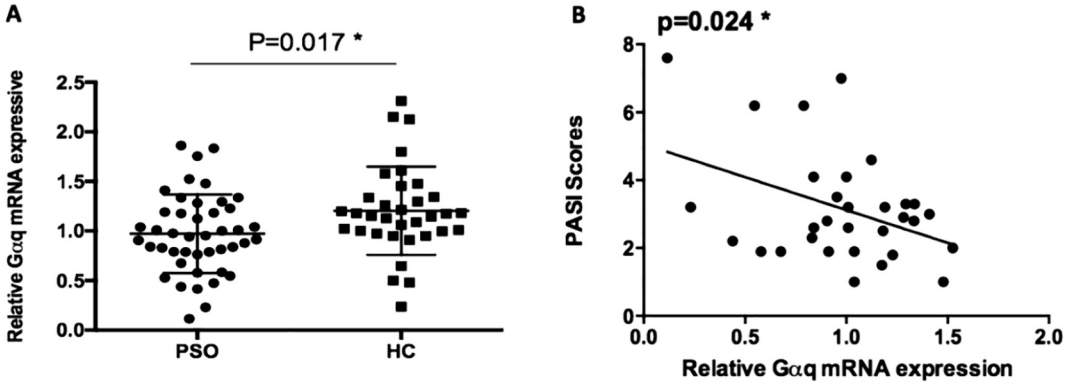

Fig. 1. The relative Gαq mRNA expression was decrease and negative related with PASI scores in psoriasis patients.
The relative Gαq mRNA expression in psoriasis patients and healthy control were detected by Q-PCR. The Gαq expression in psoriasis was much lower than healthy control. Also, The Gαq mRNA expression was significantly negative related with PSAI scores in psoriasis patients. $^{*}P < 0.05$.

2.7. Statistical analysis

SPSS 17.0 software was used for all statics analysis. Data were presented as the mean ± standard deviation. Unpaired Student *t* - tests were used to determine statistically significant differences. A value of $P < 0.05$ was considered significant at the 95% confidence level.

3. Results

3.1. Gαq might be involved in pathogenesis regulation of PSO

To investigate the role of Gαq in PSO, we first detected the expression of Gαq in PSO patients. As immune dysfunction play important roles in the pathogenesis of PSO, we collected the peripheral blood mononuclear cells (PBMCs) from PSO patients and HCs and detected the Gαq mRNA expression by RT-PCR. We found that, the relative expression of Gαq in PSO was significantly lower compared with HCs (Fig. 1A). To further investigate the role of Gαq in PSO, we then evaluated the disease activity with the criteria of clinical PSO Area and Severity Index (PASI). We analyzed the association of Gαq expression in PBMCs with PASI scores. As shown in Fig. 1B, we found that, the expression of Gαq was negatively associated with PASI in PSO patients. The data suggested that low expression of Gαq might regulate the onset and pathogenesis of PSO.

To further assess the clinical significance of Gαq in PSO, we analyzed the association of Gαq expression and test parameters of PSO. Firstly, we analyzed the association of Gαq mRNA expression with the C reactive protein (CRP), which is the inflammation index widely used in the inflammation evaluation. Negative association between Gαq expression and CRP was found, suggesting that Gαq might involve in the inflammation of PSO. Moreover, significant negative associations were found between the Gαq mRNA expression and the cholesterol and low-density lipoprotein, suggesting that Gαq might also participated in the lipid metabolism in PSO. No association were found in others test parameters such as Alanine aminotransferase (ALT), Aspartate transaminase (AST), Urea, Total-bilirubin (TBIL), Direct-bilirubin (DBIL), Uric acid (UA), and triglyceride (TG) (Fig. 2).

3.2. IMQ- induced PSO-like inflammation is significantly attenuated in Gnaq -/- BM chimeric mice, indicating Gαq might be involved in the immune regulation of PSO pathogenesis

IMQ-induced PSO-like inflammation was widely used as a rodent model of PSO. To confirm that whether the role of Gαq in PSO pathogenesis regulation was by its regulation on immune system, we used the Gnaq -/-BM chimeric mice and WT BM chimeric mice to induce PSO-like inflammation. As showed in Fig. 3A, the PSO-like inflammation induced by IMQ was much serious in Gnaq -/-BM chimeric mice than WT BM chimeric mice. Microscopic evaluation of skin sections after 6 days of IMQ treatment was used to evaluate the characteristic changes of PSO lesions, including hyperkeratosis and acanthosis. Skin lesions in Gnaq -/-BM chimeric mice showed more characteristic changes than those of WT mice (Fig. 3B). Cumulative scores (erythema plus scale plus furfuration) of back skin were significantly increased in Gnaq -/-BM chimeric mice at 6 days following IMQ application (Fig. 3C). Epidermal thickness of the skin and the number of proliferated cells in the basal epidermal layer were measured. Epidermal thickness of back skin from Gnaq -/-BM chimeric mice was significantly thicker than that of WT BM chimeric mice (Fig. 3D).

3.3. The regulation of TH17 cells differentiation might contribute to part of mechanism of the immune regulation of Gαq in PSO

Our data above suggested that Gαq was involved in the immune regulation of PSO. To further investigate the regulation role of Gαq in PSO, we detected the T cell subset in Gnaq -/-BM chimeric mice and WT BM chimeric mice after 6 days of IMQ treatment. The results showed that, the numbers of TH17 cells and Treg cells in IMQ-treated mice increased almost 2 fold than the control group. In addition, the proportion of TH17 cells in IMQ-treated Gnaq -/- BM chimeric mice was significantly higher than IMQ-treated WT BM chimeric mice (Fig. 5A–B). However, the proportion of Treg cells in IMQ-treated Gnaq -/- BM chimeric mice was similar to IMQ-treated WT BM chimeric mice (Fig. 5C–D). Also, the Th1 cells and Th2 cells were detected and analyzed by Flow cytometry. Both Th1 cells and Th2 cells slightly increased after 6 days of IMQ treatment. But the percentage of Th1 and Th2 between Gnaq -/- BM chimeric mice and WT chimeric mice had no differences. (Fig. 5E–H) The data displayed that Gαq regulation role in PSO primarily through regulating the Th17 cells and further proved the roles of Gαq in immune regulation in PSO (Fig. 4).

3.4. Inflammatory cytokines associated with PSO pathogenesis were negatively associated with the Gαq expression both in PSO patients and in Gnaq -/- BM chimeric mice

Our data above suggested that, Gαq was involved in PSO pathogenesis regulation, and it might regulate the Th17 cells proliferation without affecting Th1, Th2 and Treg. To further confirm whether Gαq regulating the function of Th17 cells function, we also detected the IL-17A in serum of Gnaq -/-BM and WT chimeric mice induced by IMQ. As showed in Fig. 5B, the IL-17A in Gnaq -/-BM chimeric mice induced by IMQ was significantly higher than WT BM chimeric mice treatment with IMQ. Also, the proinflammatory cytokine TNF-α, secreted by Th17 cells and other inflammatory cells is considered as an important target

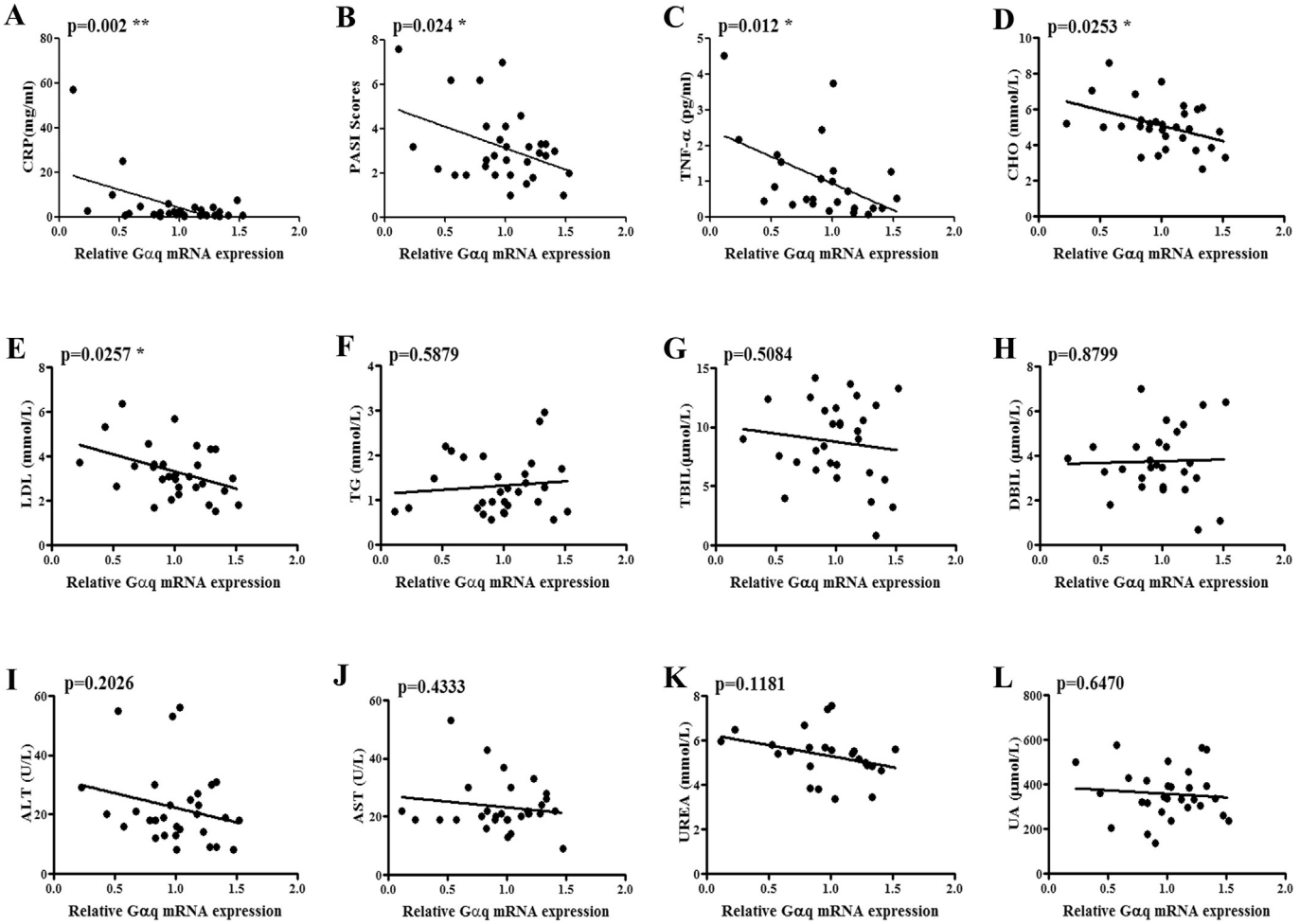

Fig. 2. The correlations of the relative Gαq mRNA expression and test parameters in psoriasis patients.
Significant negative associated were found between Gαq mRNA expression and C reactive protein (CRP), Cholesterol (CHO) and Low-density lipoprotein (LDL). However, the Gαq mRNA expression were not related with other blood biochemical indexes like Alanine aminotransferase (ALT), Aspartate transaminase (AST), UERA, Urea; Total-bilirubin (TBIL), Direct-bilirubin (DBIL), Uric acid (UA) and triglyceride (TG). $^{*}P < 0.05$.

for PSO. We also detected TNF-α level in serum. The results showed that TNF-α in Gnaq -/-BM chimeric mice was also higher than WT chimeric mice. To further confirm the inflammatory cytokines were negatively associated with Gαq expression and it may contribute to the mechanism of how Gαq was involved in PSO pathogenesis regulation. We also tested the levels of the level of TNF-α and IL-17A in serum of PSO patients and analyzed the correlation with Gαq expression. Similar with other researches, we also found that, the IL-17A in serums of PSO patients were much higher than healthy controls (Supplemental data 1). In this study, we found that the Gαq expression negatively related to TNF-α and IL-17A (Fig. 5A). The data of the IMQ-mouse model and patients' sample both suggested that Gαq might involve in the pathogenesis of PSO through regulating some inflammatory cytokines, such as IL-17A or TNF-α.

4. Discussion

In this study, we first reported that Gαq expression in PBMCs of PSO patients were decreased, and it was negatively related to disease activity and some test parameters, such as CRP, CHO and LDL. That indicated that, Gαq gene expression might involve in the pathogenesis of PSO. Then, we further confirm the role of Gαq in PSO using the Gnaq -/-BM chimeric mice to induce the PSO mouse model compared with WT BM chimeric mice. Our study displayed that deficiency of Gαq attenuated the PASI scores of PSO induced by IMQ. We also found Th17 cells in Gnaq -/-BM chimeric mice were significantly higher than WT chimeric mice. Beside Th17 cells, there was no significantly change of Th1, Th2 and Treg between Gnaq -/-BM chimeric mice and WT BM chimeric mice. Also, the IL-17A and the Th17 cells related inflammatory cytokines TNF-α were higher in Gnaq -/-BM chimeric mice after IMQ stimulating. The correlation of Gαq with IL-17A and TNF-α were also proved in patients' samples. Our study revealed the role of Gαq in PSO and might provide a new mechanism about immune dysfunction in PSO.

PSO is a chronic inflammation autoimmune disease characterized by immune dysfunction [5,21]. Our result first displayed the expression of Gαq decreased and negatively related with PASI scores in PSO patients. Also, we found the Gαq expression negatively related with cholesterol and LDL in psoriasis patients. Previous research had proved that cholesterol level and LDL level in serum were negatively related with the PASI scores in PSO patients in China [22]. Moreover, some reports had proved that, the cholesterol in serum was essential for the IL-17 signaling and activating [23,24], and the increasing cholesterol has beneficial effects in inflammation diseases [25,26]. So, the abnormal lipid metabolism may also be regulated by Gαq and participate in the development of PSO. PSO is an immune dysfunction disease. The regulation of immune system in PSO is still unknown. Several important subsets of immune sunsets of immune cells, including Th1/Th2, Th17/Treg cells have been identified to play critical roles in the pathogenesis of PSO [8,27]. The corresponding inflammation cytokines, like TNF-α,

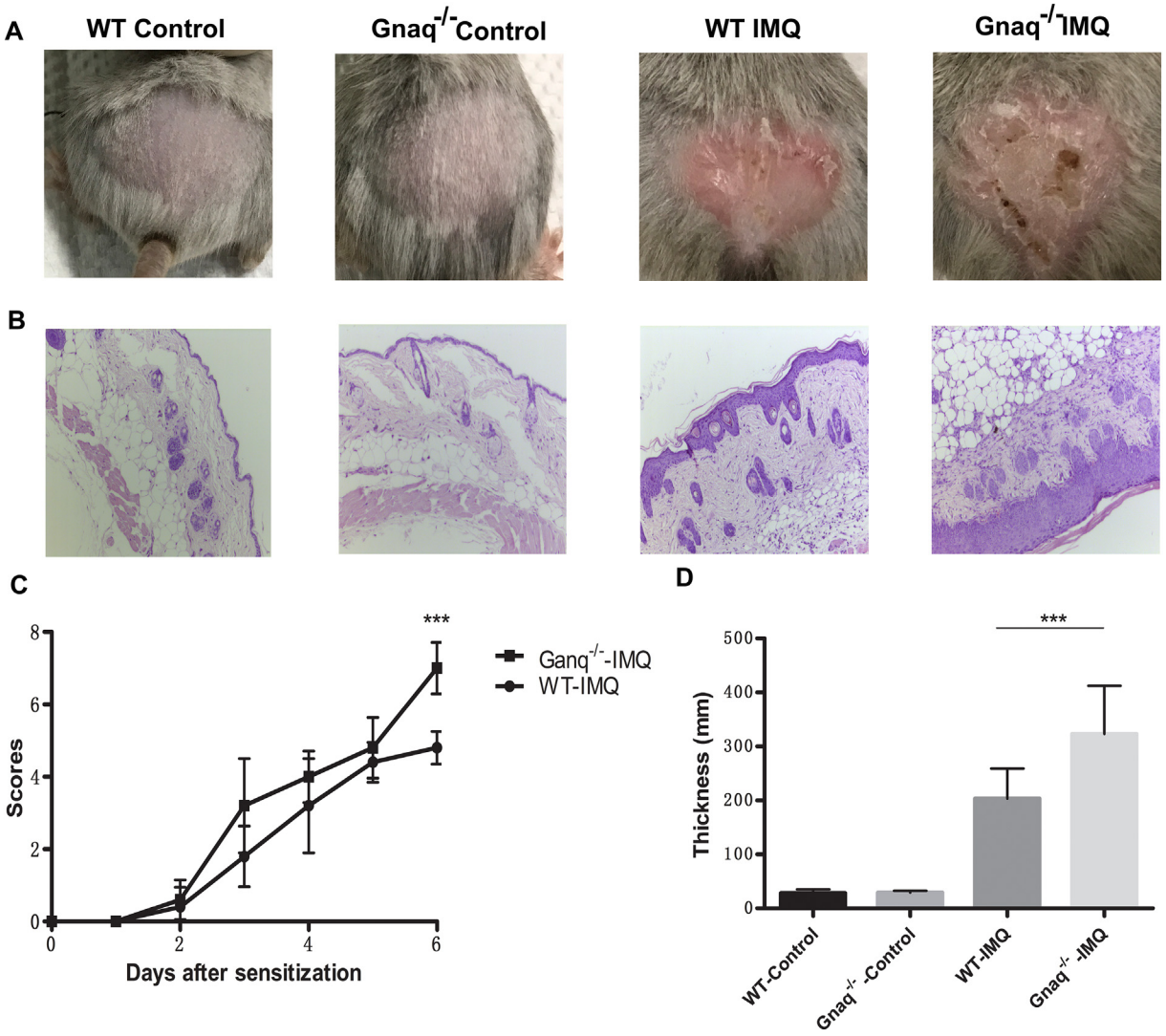

Fig. 3. Imuqiumod (IMQ) induced psoriasis-like inflammation is attenuated in Gnaq-/- chimeric mice than WT chimeric mice.
(A) Phenotypic presentation of back skin of WT and Gnaq-/- chimeric mice after 6 days stimulated with 5% IMQ. (B) Histologic changes of IMQ-induced psoriasis-like inflammation were evaluated with microscopic. (C) Cumulative scores of back skin were calculated following the IMQ treatment. The scores of the Gnaq -/- BM chimeric mice were significantly higher than WT BM chimeric mice at 6 days of IMQ treatment. (D) Epidermal thickness of back skin in Gnaq -/- BM chimeric mice was also much thicker than of WT BM chimeric mice. (n = 6 mice/group)*$P < 0.05$.

and IL-17 were also proved to promote the PSO progress [28–31]. These cytokines mediate effects on keratinocytes to amplify PSO inflammation. So, biological therapy targeted at the pathogenesis cytokine, such as the IL-17A antibodies and TNF- α antibodies were widely used in PSO [27,29,32,33]. However, how the abnormal IL-17 and TNF-α were regulated in PSO was still unclear. Study about regulation mechanism of the immune dysfunction in PSO can help us investigate the pathogenesis of PSO better and may give us a new insight about PSO. In our study, we had proved that Gαq participated in the pathogenesis of PSO, and it negatively associated with the Th17 cells differentiation and the inflammatory cytokines IL-17A and TNF-α secretion both in mouse model and patients. The results might provide a new mechanism of the immune dysfunction in PSO. However, beside Th17 cells, IL-17A and TNF-α, many other immune cells, such as dendritic cells, macrophage and cytokines, like IL-22, IL-23 also participated in the development of PSO. Whether the Gαq regulated the other immune cells and cytokines still need further researches.

The expression of Gαq has been detected in a variety of autoimmune diseases, such as SLE, RA and Sjogren's syndrome (SS). Previous studies had showed that Gαq can directly interact with its effectors such as PLC-β and p63RhoGER and activates its cell signaling, including p38, ERK and intercellular calcium mobilization [34]. Our previous studies had displayed Gαq signal activation regulated the immune cells differentiation, migration, survival and function and participated the immune regulation of autoimmune diseases [10–12,34]. Emerging data suggest a potential pathogenic role of p38 MAPK, ERK1/2 and calcium in the development of PSO [35,36]. Inhibition of ERK and p38 kinase can alleviate the PSO inflammation by targeting the inflammatory cytokines secretion [35,37]. In this study, we had proved Gαq role in PSO both in patients' sample and in IMQ-induced PSO-like inflammation. The exact mechanism of Gαq in PSO and the cell signal pathway involved in PSO is still unclear and needs further research. The study of Gαq in PSO might give a new target to further investigate the cell signal involved in the pathogenesis of PSO.

In conclusion, we first proved the Gαq participated in the onset and development of PSO and it might be a new mechanism about the immune dysfunction factor in PSO. Moreover, Gαq might be a new potential target in the research of the immune dysfunction of PSO. The specific agonist of Gαq might be a new way to treat PSO in future. The studies may help to understand the immune response in PSO better and

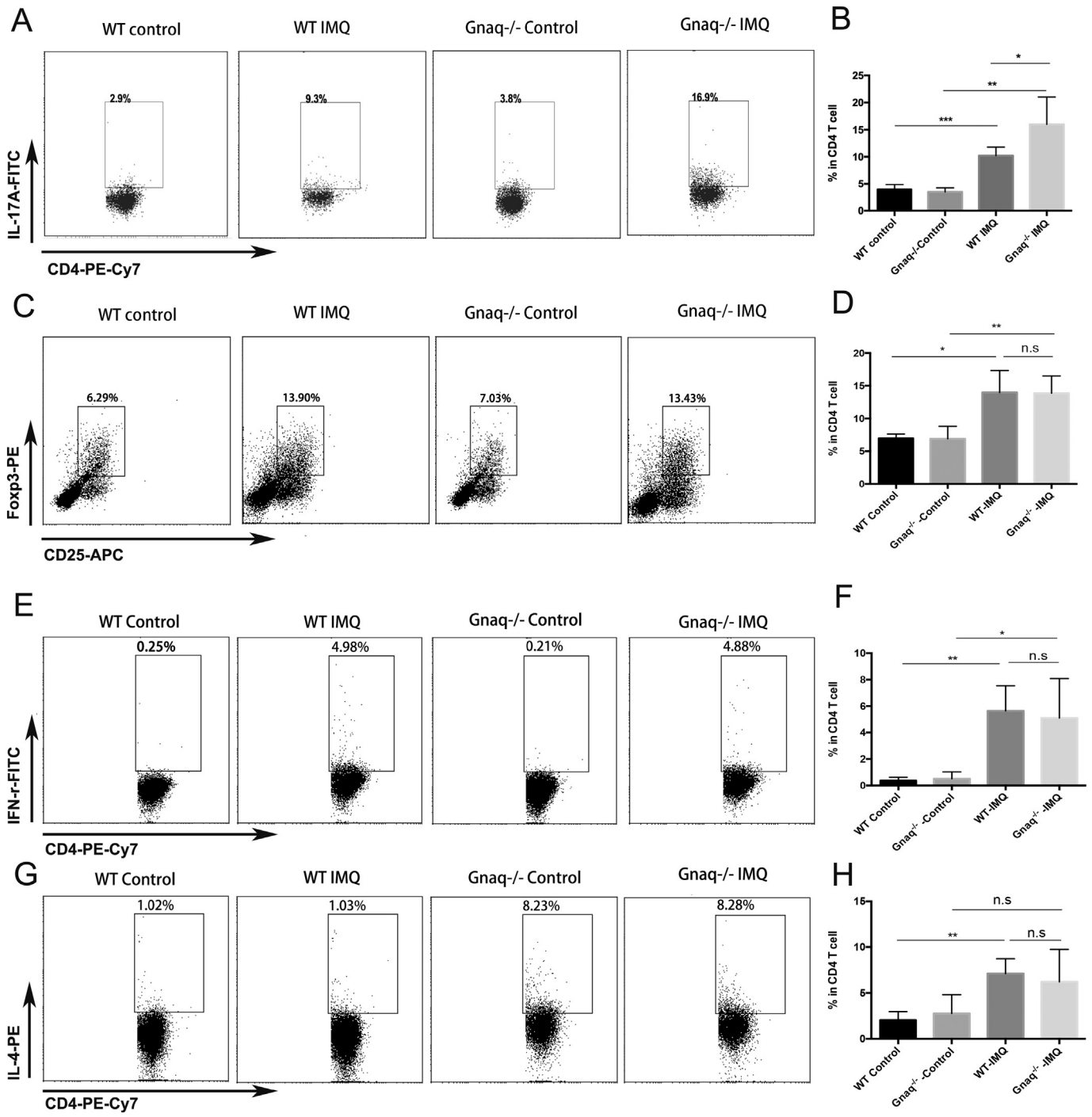

Fig. 4. Th17 cells were significantly increased in Gnaq-/- chimeric mice when treatment with IMQ.
Mice spleens cells were isolated and stimulated with PMA and BFA for 4 h. Proportions of T subsets (Th1, Th2, Th17 and Treg cells) were detected by flow cytometry. Each subset of T cells increased when stimulated by IMQ both in WT and Gnaq-/- chimeric mice. However, besides Th17 cells in Gnaq-/- chimeric mice were much higher than WT mice, the percentage of Th1, Th2 and Treg cells were similar in Gnaq-/- chimeric mice and WT mice. $^{*}P < 0.05$.

provide a new target.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2018.12.054.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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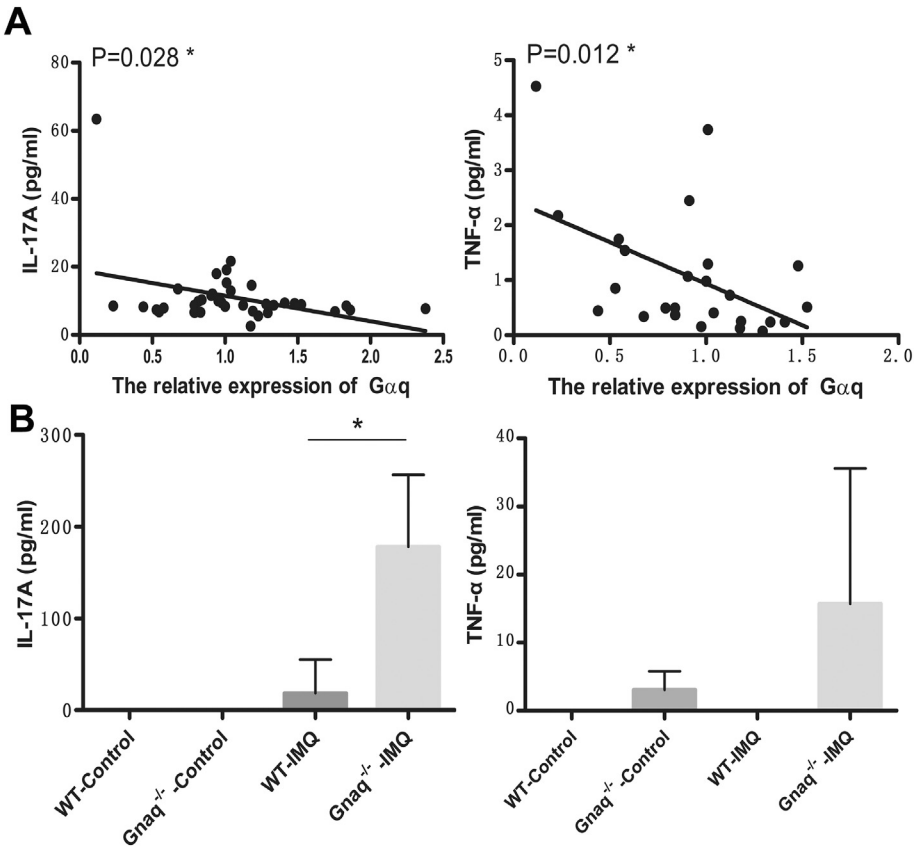

Fig. 5. The inflammatory cytokines were negatively associated with the Gαq expression both in PSO patients and in Gnaq -/- BM chimeric mice.
The inflammatory cytokines associated with PSO pathogenesis, like IL-17A and TNF-α, were negatively associated with Gαq expression (A). Also, the IL-17A in Gnaq -/- BM chimeric mice was much higher after treatment with IMQ (B). $^{*}P < 0.05$,$^{**}P < 0.01$.

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