Research Article

Expression of \( \alpha_q \) Is Decreased in Lymphocytes from Primary Sjögren’s Syndrome Patients and Related to Increased IL-17A Expression

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Primary Sjögren’s syndrome (pSS) is a rheumatic disease characterized by the destruction of salivary and lacrimal glands, and its pathogenesis mechanism remains unclear. \( \alpha_q \) is the \( \alpha \)-subunit of the heterotrimeric Gq protein. Researches demonstrated that \( \alpha_q \) was involved in the pathogenesis regulation of several rheumatic diseases. This study explored the role of \( \alpha_q \) in pSS. \( \alpha_q \) mRNA levels in peripheral blood mononuclear cells (PBMCs) from 39 patients and 26 healthy controls (HCs) were investigated using real-time PCR. IL-17A serum concentrations in 22 pSS patients and 23 HCs were tested by ELISA, and the clinical significance of \( \alpha_q \) was analyzed. The association of \( \alpha_q \) with interleukin-17A (IL-17A) expression was also analyzed in patients with pSS. We showed that \( \alpha_q \) expression in PBMCs from patients with pSS was significantly lower than that in PBMCs from HCs. \( \alpha_q \) expression level was closely associated with pSS disease activity. Furthermore, a negative association was also found in IL-17A and \( \alpha_q \) expression level. These data suggest that \( \alpha_q \) is involved in pSS pathogenesis regulation, possibly due to its regulation of Th17. These results provide new insights into the pSS pathogenesis mechanism involving abnormal Th17 regulation.

1. Introduction

Primary Sjögren’s syndrome (pSS), one of the most common rheumatic diseases, is characterized by dry eyes and dry mouth, due to lymphoplasmocytic infiltration and destruction of lachrymal and salivary glands. In addition to the hypofunction of the salivary and lacrimal glands, other vital organs such as the lungs, kidneys, and liver can also be damaged in patients with pSS [1].

Specific details regarding the etiology of pSS remain unknown. The diagnosis of pSS is always at a relatively late stage when irreversible damages of the glands already existed, and the effective treatment strategies are limited compared with other rheumatic diseases. Intensive studies are trying to unravel the molecular, genetic, and immunological mechanisms for this disease and provide a better understanding about the pathogenesis mechanism of pSS [2]. T helper type 17 (Th17), characterized by the production of IL-17, is a subset of effector T helper cells that are distinct from Th1 and Th2 cells [3]. They have been proven to be the main pathogenic cells in inflammation and autoimmunity [4]. Several studies have indicated that Th17 cells are increased in patients with pSS and involved in the glandular tissue damage of SS [5–7]. However, the presence of Th17 is always found to be associated with the onset of gland destruction; how Th17 is regulated in pSS remains unclear. A study about the mechanism of Th17 regulation in pSS may help us have a better understanding of pSS at an early stage before the onset of gland destruction and may help us explore more treatment targets in pSS.

\( \alpha_q \) is encoded by gene GNAQ; it is the \( \alpha \)-subunit of the heterotrimeric Gq protein. The Gq protein is a member of...
the subfamilies of the heterotrimeric G proteins. There are three subunits in the heterotrimeric G proteins, namely, α, β, and γ. According to the difference of the α-subunits, the G proteins can be classified into four subfamilies including Gαs, Gαi, Gαq/11, and G12/13. Gq is a member of the Gαq/11 subfamily [8]. Gαq is widely expressed in several kinds of cells, including lymphocytes. Gq couples with a wide variety of membrane receptors to effector molecules inside cells. Important roles of Gαq in the immune system have been revealed in recent years, giving us new understanding about the pathogenesis mechanism of rheumatic diseases [9, 10]. Our previous researches demonstrated that Gαq is involved in the pathogenesis regulation of several rheumatic diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), which may be related to its regulation in Th17 differentiation [11, 12]. pSS shares some similarities with the pathogenesis mechanisms of SLE or RA; however, whether Gαq is also involved in pSS pathogenesis remains unknown.

In this study, we investigated the expression of Gαq in patients with pSS and analyzed the association of Gαq expression and the clinical characteristics of patients with pSS. We then studied the level of IL-17A in the serum of pSS patients with both PBMCs and sera) matched for sex and age were included in this study. pSS patients were diagnosed based on the criteria of systemic Sjogren’s syndrome [13]. Data such as sex, age, history, clinical manifestations, laboratory findings, and treatment strategy on patients with pSS were collected from the patients’ medical records. This study was approved by the medical ethics committee of the First Affiliated Hospital of Xiamen University. The basic clinical characteristics of the pSS patients and HCs are summarized in Table 1.

2. Materials and Methods

2.1. Patients and Controls. 39 patients diagnosed with pSS (22 pSS patients with both PBMCs and sera) and 40 healthy controls (HCs, 17 HCs with samples of PBMCs, 14 HCs with samples of sera, and 9 HCs with samples of both PBMCs and sera) matched for sex and age were included in this study. Patients with pSS were all from the outpatient and inpatient departments of Rheumatology and Clinical Immunology, the First Affiliated Hospital of Xiamen University. The patients with pSS were diagnosed based on the criteria of the American College of Rheumatology [13]. Data such as sex, age, history, clinical manifestations, laboratory findings, and treatment strategy on patients with pSS were collected from the patients’ medical records. This study was approved by the medical ethics committee of the First Affiliated Hospital of Xiamen University. The basic clinical characteristics of the pSS patients and HCs are summarized in Table 1.

2.2. Peripheral Blood Mononuclear Cell Isolation. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples of HCs and pSS patients using standard density gradient centrifugation. The heparinized blood samples were centrifuged with the addition of Ficoll-Paque Plus (Eppendorf, GER). Total RNA of PBMCs was isolated using TRIzol™ Reagent (Invitrogen, USA). RNA was then reverse transcribed following the manufacturer’s instructions. The concentration of RNA was determined with a Nanodrop ND1000 spectrophotometer.

2.3. RNA Extraction and Quantification of Transcripts Using Real-Time Polymerase Chain Reaction. Total RNA of PBMCs of patients and HCs was extracted by TRIzol (Invitrogen, USA) according to the manufacturer’s instructions. Briefly, total RNA was reverse-transcribed first to cDNA using reverse transcription reagent kits (Roche, CH) following the manufacturer’s instructions. The reaction conditions were as follows: 50°C for 60 mins, then 85°C for 5 min, and finally 4°C for 5 min. The mRNA expression levels of GAPDH and Gαq were investigated by real-time quantitative PCR (RT-PCR) using SYBR Green (Roche, CH). A 10 μL SYBR Green RT-PCR reaction mixture containing 2 μL of cDNA, 0.2 μL of sense or antisense primer, and 7.6 μL ddH2O was used. Quantitative PCR was performed according to the manufacturer’s instructions (ABI7500, USA). The PCR reactions were amplified as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles at 95°C for 15 s and 60°C for 1 min, and 95°C for 1 min to denature. GAPDH expression level was used to normalize the mRNA expression level of

| Table 1: Demographic and clinical characteristics of pSS patients. |
|---------------------------------------------------------------|
| **Number, N** | **Health controls** | **P** |
| Number, N | 39 | 40 | NS |
| Age (years), mean ± SD | 46.1 ± 8 | 48 ± 9 | NS |
| Sex, M/F | 3/36 | 4/36 | NS |
| Disease duration (years), mean ± SD | 2.7 ± 1.2 | |
| Clinical manifestations, N (%) | |
| Oral dry | 27 (69) | 0 (0) |
| Eye dry | 24 (62) | 0 (0) |
| Arthritis | 14 (36) | 0 (0) |
| Pulmonary domain | 12 (31) | 0 (0) |
| Autoimmune hemolytic anemia | 13 (43) | 0 (0) |
| Lymphopenia | 5 (13) | 0 (0) |
| Renal involvement | 12 (31) | 0 (0) |
| Nervous system involvement | 2 (5) | 0 (0) |
| ESSDAI score, mean (range) | 3 (0–10) | |
| Active disease (ESSDAI ≥ 4) | 9 | |
| Serological features, N (%) | |
| ANA | 31 (79) | 0 (0) |
| Anti-SSA/Ro | 33 (85) | 0 (0) |
| Anti-SSB/La | 21 (54) | 0 (0) |
| Anti-SSA/Ro and anti-SSB/La | 20 (51) | 0 (0) |
| Low serum C3 | 19 (49) | 0 (0) |
| High serum IgG | 16 (41) | 0 (0) |
| CRP | 22 (56) | 0 (0) |
| ESR | 27 (69) | 0 (0) |
3.1. Expression of Gaq Was Decreased in Patients with pSS. To explore the role of Gaq in pSS, we first investigated the Gaq expression level in patients with pSS. As an imbalance in lymphocytes is a main factor in pSS pathogenesis, we collected PBMCs from patients with SS and HCs and analyzed the mRNA expression of Gaq in PBMCs. As shown in Figure 1, we found that the mRNA expression of Gaq in PBMCs was significantly lower in patients with SS compared with that in HCs. These data suggest Gaq might have a potential role in the regulation of SS pathogenesis (Figure 1).

3.2. Expression of Gaq Was Negatively Associated with Disease Activity in Patients with pSS. To further investigate the specific role of Gaq in pSS pathogenesis regulation, we then analyzed the disease activity of patients with pSS with different expression levels of Gaq. The EULAR Sjögren’s syndrome disease activity index (ESSDAI) is now widely used to evaluate pSS disease activity [14]. We analyzed the relation of Gaq mRNA expression in PBMCs with the ESSDAI in patients with pSS. As shown in Figure 2, we found the expression level of Gaq mRNA was negatively related to the ESSDAI in patients with pSS. This suggested that Gaq negatively regulates the pathogenesis of pSS and that a low level of Gaq might contribute to the disease onset of pSS.

3.3. Clinical Significance of Gaq in Patients with pSS. We then analyzed the association of the expression of Gaq with the clinical characteristics of patients with pSS to explore the clinical significance of Gaq in pSS. The expression of Gaq was significantly lower in pSS patients with arthritis than that in those without arthritis (Figure 3(a)), suggesting that Gaq might be involved in the pathogenesis of arthritis. However, no differences in Gaq expression between pSS patients with and without interstitial lung disease (ILD), dry eyes, and dry mouth were found (Figures 3(b)–3(d)). We further analyzed the association of Gaq mRNA expression with other clinical characteristics, including lymphocyte count in peripheral blood, urinary protein, hemoglobin (HB) level, platelet (PLT) level, complement 3 (C3) level, C-reactive protein (CRP) level, immunoglobulin G (IgG) level, anti-SSA titer, and anti-SSB titer (Figure 4). A significant negative association was found between Gaq mRNA expression and IgG level, whereas no association was found for the others.
These data suggest that Gaq plays a role in the negative regulation of the immune reaction.

3.4. Gaq Expression Was Negatively Related to IL-17A Concentration, Which May Contribute to the Mechanism by Which Gaq Is Involved in pSS Pathogenesis Regulation. Our data suggest that Gaq is involved in pSS pathogenesis regulation, but the mechanism remains unclear. Th17 is a key factor in pSS pathogenesis, and our previous researches demonstrated that Gaq inhibited the differentiation of Th17 [11]. We further investigated the correlation between Gaq and Th17 in patients with pSS. We first investigated the level of IL-17A in the serum of patients with pSS and HCs. We found that the concentration of IL-17A was significantly higher in patients with pSS than in HCs (Figure 5(a)). We then analyzed the correlation of Gaq mRNA expression in PBMCs from patients with pSS with IL-17A. A negative correlation was found between Gaq mRNA expression and IL-17A expression (Figure 5(b)). These data suggested that Gaq might be a factor involved in Th17 regulation in patients with pSS.

4. Discussion

In this study, we report for the first time that Gaq mRNA expression in lymphocytes was decreased in patients with pSS and that the expression of Gaq was closely related to disease activity. Gaq mRNA expression was negatively associated with IL-17A levels in the serum of patients with pSS. Our study revealed that Gaq plays a role in pSS pathogenesis regulation, providing a new mechanism for how Th17 cells are regulated in pSS.

pSS is a disorder in which dry eyes and dry mouth occur as a manifestation of immune dysregulation [15, 16]. Dry eyes and mouth are often the first symptoms of SS and may indicate the involvement of other organs, including the salivary glands, lungs, and kidneys. Roughly 5–10% of patients with SS develop lymphoma [17]. Treatment strategies for pSS are relatively limited because the irreversible destruction of the salivary glands is always present when pSS is diagnosed. Studies on the pathogenesis mechanism of pSS are needed to clarify the early stages of pSS. Th17 cells have been shown to be a factor in pSS pathogenesis. IL-17 KO mice were shown to be completely resistant to SS induction, and the adoptive transfer of Th17 cells induced the presence of SS symptoms in immunized IL-17 KO mice rapidly, proving the crucial role of Th17 in pSS pathogenesis [18]. Increased levels of IL-17 were also found in the serum as well as the salivary glands of patients with pSS [6, 19]. Consistent with previous studies, we also found that IL-17A was increased in the serum of patients with pSS, confirming the crucial role of Th17 cells in pSS pathogenesis. However, how the abnormal Th17 cells are regulated in pSS remains unclear. Studies on the regulation mechanism of Th17 cells in pSS can help to better understand the stage before the onset of Th17 cell upregulation and the destruction of salivary glands and help to develop better management strategies for pSS in its early stages.
Ga q is the α-subunit of the Gq protein encoded by gene GNAQ [8]. Our previous studies confirmed the vital role of Ga q in several aspects of immune regulation such as dendritic cell trafficking, B cell selection, and T cell activation [9, 10, 20]. By using Gnaq−/− chimeric mice by reconstituting lethally irradiated C57BL/6J recipient mice with Gnaq−/− bone marrow, we also revealed the role of Ga q in the pathogenesis of autoimmune disease. Autoimmunity with multiorgan involvement and arthritis can spontaneously develop in Gnaq−/− chimeric mice [10]. In previous studies, the expression of Ga q was shown to decrease in lymphocytes from patients with RA and SLE and closely related to disease activity, indicating the role of Ga q in RA and SLE pathogenesis regulation [11, 12, 21, 22]. Thus, these studies revealed a new mechanism for autoimmune disease pathogenesis regulation.

This study demonstrated the role of Ga q in pSS. We found that the expression of Ga q was also decreased in lymphocytes from patients with pSS and that the expression level of Ga q was closely associated with the disease activity of pSS, presence of arthritis, and high level of IgG. Our previous studies demonstrated that Gnaq−/− chimeric mice spontaneously developed inflammatory arthritis [10], and the expression of Ga q was shown to be decreased in lymphocytes from RA patients, suggesting that Ga q is involved in the regulation of inflammatory arthritis development. pSS shares some similarities with RA regarding the pathogenesis mechanism and is often coexisted with RA [23]. Our studies have shown that Ga q is decreased in both patients with pSS and patients with RA, suggesting that Ga q might contribute to the overlap in the pathogenesis mechanisms of pSS and RA. Furthermore, we found that Ga q expression was lower in patients with pSS with arthritis compared with that in those without arthritis, suggesting that a low expression level of Ga q may be used as a predictor for the presence of arthritis in pSS. However, prospective studies are needed to confirm the ability of the Ga q level to predict arthritis in pSS.

It was demonstrated that Gaq could negatively regulate Th17 differentiation in our previous studies, and a negative association was found between the expression of Gaq and IL-17A in patients with RA [11]. In this study, we also found a negative association between Gaq and IL-17A, revealing a
novel regulation mechanism for the abnormal Th17 levels in patients with pSS. This represents a potential new research target for the early stages of pSS.

In conclusion, this study showed that Gaq expression is involved in pSS pathogenesis regulation, possibly because of its regulation of Th17 cells. These results provide a new mechanism for pSS pathogenesis regarding abnormal Th17 cell regulation.

Data Availability
The data used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Conflicts of Interest
The authors declare that they have no conflict of interests.

Authors’ Contributions
Yuechi Sun, Ying Wang, and Shiju Chen contributed equally to this study.

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