Interleukin 17E associates with haematologic involvement and autoantibody production in primary Sjögren's syndrome

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Abstract

Objective

Primary Sjögren's syndrome (pSS) is one of the most prevalent systemic autoimmune diseases characterised by inflammation and tissue damage of exocrine glands, especially salivary or lacrimal gland. IL-17 related immune response is pathogenic with proinflammatory feature in pSS. However, whether IL-17E, an IL-17 family member, is involved in pSS pathogenesis or not, has not been determined.

Methods

Serum levels of IL-17E and IL-17A as comparison in 107 patients with pSS and 42 healthy controls were determined with multiplex cytokine assays. EULAR Sjögren's syndrome disease activity index (ESSDAI) score was calculated. Laboratory parameters were measured by standard laboratory techniques. The inflammatory infiltration of minor labial gland biopsies was graded based on numbers of lymphocyte and quantified by Focus Score (FS). Expression of IL-17E and IL-17A in the biopsy was evaluated with immunohistochemistry.

Results

Significantly elevated IL-17E in pSS patients associated with ESSDAI, haematologic disorders and autoantibody production, including anti-nuclear antibodies (ANA), rheumatoid factor (RF) and anti-SSA antibodies were found. Histopathological features showed that expression of IL-17E was found in labial salivary gland and correlated with lymphocytic infiltration.

Conclusion

IL-17E expression in pSS patients was increased and associated with haematologic disorders, autoantibody production and lymphocytic infiltration in salivary gland. This finding indicated that IL-17E is involved in pSS pathogenesis.

Key words

Sjögren's syndrome, IL-17E, autoantibody, system involvement

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Introduction

Primary Sjögren's syndrome (pSS) is one of the most common rheumatic diseases characteristic of exocrine gland inflammation and destruction. Tissue damages primarily take place in exocrine glands including salivary and lacrimal glands, leading to impaired secretory functions and disease symptoms such as xerostomia and keratoconjunctivitis sicca (1). The pathogenic features of pSS included lymphocyte infiltration and autoantibody production. Although the aetiology of pSS is still elusive, $CD4^+$ helper T cell infiltration into exocrine glands may be one of the primary effectors contributing to tissue damage (2-4). Th17 effector cell is a pivotal subset of $CD4^+$ T cells that contribute to the pathogenesis of pSS, partly by expression of IL-17A (5-7).

IL-17A, commonly referred to as IL-17, is mainly expressed by Th17 cells. It is a leading proinflammatory and pathogenic cytokine in many autoimmune diseases, especially T-cell mediated autoimmune disease (8). There are six members with structural similarity in the IL-17 family, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F. The homology between these IL-17 family members is between 20% to 50% (8), mainly in the C-terminal of these molecular. IL-17E, with the least homology with IL-17A, is involved in Th2 immune responses (9). The IL-17E receptor is a heterodimeric receptor formed by IL-17 receptor A (IL-17RA) and IL-17 receptor B (IL-17RB), and is expressed in Th2 cells, Th9 cells, basophils and group 2 innate lymphoid cells (9-11). Now IL-17E is considered to be one of the vital cytokines in allergic diseases and plays a protective role in helminthic parasite infection (12). Additionally, it might be immunosuppressive in some animal models of autoimmune diseases, including rheumatoid arthritis (13). However, the functions of IL-17E and its clinical relevance in pSS has not been reported. In this study, we determined the levels of IL-17E in serum and labial glands from pSS patients. The clinical significance of IL-17E was analysed. Differential clinical relevance was revealed between IL-17E and IL-17A in pSS, which suggested that, in comparison to IL-17A, IL-17E might play a different role in pSS pathogenesis.

Methods

Patients recruitment

One hundred and seven inpatients were enrolled into this study in the Department of Rheumatology, Peking University People's Hospital, Beijing, China, between January 2017 and December 2018. The pSS diagnosis was established according to the 2002 American-European Consensus Group, with Classification Criteria (14). Forty-two age- and sex-matched healthy controls (HC) were also recruited from the health examination centre of the same hospital, according to their medical records. This study was approved by the Ethics Committee of Peking University People's Hospital (approval no. 2016PHB168-01). All participants signed the consent for this study.

Labial salivary gland grading and immunohistochemistry

Small labial salivary gland sections from 27 pSS patients were fixed in formalin and embedded in paraffin. These sections were stained with hematoxylin and eosin (H&E) and were examined by an independent pathologist. The FS is the total number of foci per 4 mm^2 salivary gland tissue (15, 16). A focus is defined as an aggregate of ≥50 lymphocytes. The grading standard is as following: grade 0 (G0): absent, no lymphocyte infiltration; grade 1 (G1): slight infiltration, scattered lymphocytes infiltrating with an aggregate of less than 50 cells; grade 2 (G2): moderate infiltration, focal periductal lymphocytes aggregating in the labial salivary gland, with 50 or more cells per lesion (17-19). IL-17A and IL-17E was stained using an immunohistochemical method (IHC). The primary antibodies for the IHC analysis were polyclonal rabbit anti-human IL-17A and IL-17E (Abcam, UK). A two-step peroxidase staining method was applied to visualise antigen-antibody complexes.

Clinical and laboratory examination

The clinical and serological examinations include, erythrocyte sedimenta-

tion rate (ESR), C-reactive protein (CRP), anti-SSA antibodies, anti-SSB antibodies, antinuclear antibodies (ANA), complement 3 (C3), rheumatoid factor (RF), complement 4 (C4), white blood cell (WBC) count, hemoglobin (Hb), lymphocyte count, erythrocyte count (RBC), granulocyte count, platelet count (PLT), immunoglobulin G (IgG) and immunoglobulin M (IgM). Disease activity of pSS patients was determined by EULAR Sjögren's syndrome disease activity index (ESSDAI) score (17).

Measurement of IL-17A and IL-17E

The serum samples were collected from pSS patients or healthy controls with polypropylene microfuge tubes. These samples were stored at -80°C without repeated thawing and freezing, and were not thawed until measurement. IL-17A and IL-17E in these sera were determined with MILLIPLEX MAP Human Th17 magnetic bead panel kits (Millipore, USA), following instructions from the manufacturer. Data were read on a Luminex-200 machine (Luminex Corporation, USA) and analyzed with MILLIPLEX Analyst 5.1 software (Millipore, USA).

Statistical analysis

The SPSS 22.0 was used for data analysis. Shapiro-Wilk test was performed to evaluate data distribution. Statistical significance between two groups was assessed with Mann-Whitney test, paired *t*-test, χ^2 test. Spearman's rank correlation coefficient was performed to evaluate the correlations. A *p*-value <0.05 is regarded to be significant. In this study, we determined the cut-off values by mean + 2 × standard deviation of IL-17E or IL-17A in healthy controls.

Results

Patients and healthy controls

107 patients with pSS and 42 healthy volunteers were recruited in this study. The patients and healthy controls were matched for age and gender. Table I showed the clinical and demographic characteristics of the patients and controls. The average disease duration was 9.89 years (ranging from 3 months to 35 years). Sixty patients (56.07%) had moderate to high disease activity (ESSDAI ≥5). Positivity of anti-SSA and anti-SSB were found in 67 (62.62%) and 50 (46.73%) pSS patients, respectively.

Table I. Characteristics of the 107 pSS patients and 42 healthy controls.

Clinical characteristics	pSS	Healthy controls	t/χ^2	*p*
Age	55.84 ± 14.49	55.12 ± 13.17	0.191	0.869
Sex (F:M)	103:4	40:2	0.001	1.000
WBC (×10^9/L)	4.80 ± 2.11	-	-	-
Neutrophils (×10^9/L)	3.07 ± 1.90	-	-	-
RBC (×10^{12}/L)	3.75 ± 0.62	-	-	-
Hb (g/L)	116.34 ± 18.31	-	-	-
PLT (×109/L)	144.42 ± 71.07	-	-	-
IgA (g/L)	3.36 + 1.75	-	-	-
IgG (g/L)	19.37 ± 9.43	-	-	-
IgM (g/L)	1.70 ± 1.33	-	-	-
C3 (g/L)*	0.91 ± 0.21	-	-	-
C4 (g/L)*	0.19 ± 0.06	-	-	-
CRP (g/L)	6.76 ± 14.48	-	-	-
ESR (mm/h)	31.53 ± 28.29	-	-	-
RF (IU/mL)*	178.65 ± 295.04	-	-	-
ANA≥1:320**	53.85%	-	-	-
anti-SSA≥200RU/mL	62.62%	-	-	-
anti-SSB≥20RU/mL	46.73%	-	-	-
ESSDAI≥5	56.07%	-	-	-

*1 patient did not have the data of C3 and C4; **3 patients did not have the data of ANA.

Serum level of IL-17E was significantly increased and associated with haematologic involvement in pSS

The serum IL-17E was significantly elevated in pSS patients comparing to healthy controls (Fig. 1) 36.11±40.01 pg/ml *vs*. 23.48±4.44 pg/ml, *p*=0.04), while there was no statistical significance between the two groups (10.40±3.40 pg/ml *vs*. 10.29±1.87 pg/ml, *p*=0.87).

Multi-organ involvement is characteristic of pSS. Haematologic involvement is common in patients with active pSS, which often manifests as decreased leukocyte number in peripheral blood (18). The correlation between clinical and laboratory phenotypes and serum IL-17E and IL-17A in pSS patients were shown in Table II, serum level of IL-17E was significantly associated with decreased WBC (r=-0.226, *p*=0.019) and neutrophils (r=-0.209, *p*=0.030). It was also associated with decreased C4 (r=-0.281, *p*=0.004) (Fig. 2 E-G). In comparison, serum IL-17A was only inversely correlated with C3 (r=-0.316, *p*=0.001) (Table II) (20).

We further analysed the incidence of organ involvement in pSS patients with increased serum IL-17E and those with normal IL-17E levels (Table III). There was no significant difference in lung involvement and arthritis between patients with increased serum IL-17E and patients with normal IL-17E levels (Table III). Patients with increased serum IL-17E had decreased C4 (0.17±0.05g/L *vs*. 0.19±0.07g/L, *p*=0.027) and neutrophils (2.52±1.22×10^9/L *vs*. 3.29±2.08×10^9/L, *p*=0.049). Decreased white blood cell count was also found in patients with higher IL-17E levels, but did not reach statistical significance (*p*=0.094, Table III). In comparison, decreased C3 was observed in patients with higher serum IL-17A (0.81±0.12g/L *vs*. 0.91±0.24g/L, *p*=0.029) and increased incidence

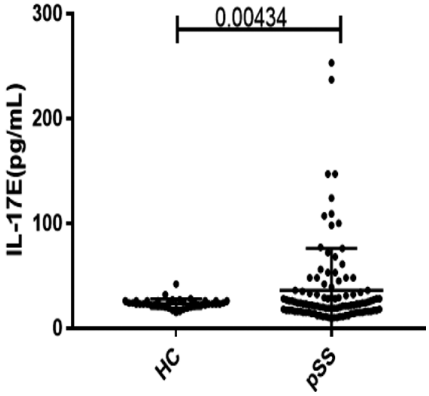

Fig. 1. Serum levels of IL-17E and IL-17A between pSS and HC.
Serum IL-17E levels were not significantly elevated in pSS patients *versus* HC.

Table II. Correlation of IL-17A (ref. 20), IL-17E with clinical and laboratory features from pSS patients.

Clinical manifestations and laboratory features	IL-17A		IL-17E	
	Spearman r	*p*	Spearman r	*p*
WBC	-0.133	0.173	**-0.226**	**0.019**
Neutrophils	-0.087	0.371	**-0.209**	**0.030**
RBC	-0.036	0.713	0.070	0.476
Hb	-0.070	0.473	0.021	0.832
PLT	0.036	0.710	-0.026	0.788
IgA	-0.041	0.672	0.133	0.172
IgG	-0.028	0.778	**0.343**	**0.001**
IgM	0.108	0.269	**0.296**	**0.002**
C3	**-0.316**	**0.001**	-0.185	0.058
C4	-0.150	0.130	**-0.281**	**0.004**
CRP	-0.123	0.222	-0.076	0.433
ESR	-0.004	0.964	0.056	0.567
RF	**0.256**	**0.013**	**0.495**	**<0.001**
anti-SSA	-0.046	0.636	**0.202**	**0.037**
anti-SSB	-0.147	0.130	-0.007	0.940

of lymphadenectasis (7(50.00%) *vs*. 17(18.28%), *p*=0.008) (Table III). This result further supported that elevated serum IL-17E was associated with decreased leukocytes in pSS patients, which was a common manifestation of pSS haematologic involvement.

IL-17E expression was associated with pSS disease severity

The pSS patients with higher IL-17E also showed increased disease activity with a marginal significance in comparison to the patients with normal serum IL-17E levels (ESSDAI: 5.48±1.81 *vs*. 4.55±2.37, *p*=0.052). In patients with elevated IL-17A, the increase of pSS disease activity was not so significant (5.50±2.28 *vs*. 4.72±2.24, *p*=0.229).

The salivary expression of IL-17E and IL-17A were evaluated by positive staining area in immunohistochemistry (IHC) images. The positive staining area of IL-17E expression was significantly higher than that of IL-17A (G0: 0.22±0.06 *vs*. 0.07±0.01, *p*=0.04; G1: 0.93±0.25 *vs*. 0.35±0.16, *p*=0.07; G2: 2.15±0.83 *vs*. 0.38±0.14, *p*=0.049; Fig. 3 I). We further divided 27 labial salivary gland biopsy samples from pSS patients into three groups according to the degree of lymphocyte infiltration (Grading standard is shown in Methods). The result showed that IL-17A and IL-17E shared similar tissue expression patterns in the inflammatory gland. Both cytokines distributed differently in G1 and G2 groups. In G1 group, IL-17A and IL-17E were expressed on lymphocytes around acinar and ductal epithelial cells, while in G2 group, they were mainly expressed around the lymphoid infiltration focus (Fig. 3 A-F). The percentage of positive area of IL-17E and IL-17A increased with the density of lymphocytes infiltration (Fig. 3 G-I). Then we further quantified the inflammatory infiltrate of minor salivary gland by the focus score (FS). The staining area of IL-17E was positively correlated with the FS (r=0.628, *p*=0.001). While the expression of IL-17A in the gland also show an increase trend with the increase of FS, there was no statistical significance (r=0.196, *p*=0.393) (Fig. 3 J and K). These results indicated that IL-17E expression were correlated with the severity of the salivary gland inflammation in pSS.

Serum level of IL-17E was associated with autoantibody production in pSS patients

Serum IL-17E was positively correlated with anti-SSA antibodies (r=0.202, *p*=0.037), RF (r=0.495, *p*<0.001),the levels of total IgG (r=0.343, *p*=0.001) and IgM (r=0.296, *p*=0.002) in patients with pSS (Table II, Fig. 2 A-D). In comparison to IL-17E, serum IL-17A was also associated with RF with lower significance (r=0.256, *p*=0.013), but did not show significant association with IgG, IgM and other pSS related autoantibodies (Table II) (20). It appeared that increased serum IL-17E was associated with more types of pSS autoantibodies in comparison to IL-17A.

A cut-off value of 31.36 pg/ml was determined as the mean value plus two times standard deviation (mean+2×S.D) of the healthy control subjects to distinguish pSS patients with increased serum IL-17E levels and patients with normal serum IL-17E comparable to healthy people. Patients with elevated serum IL-17E showed significantly increased RF (Table III, 279.91±265.44 IU/mL *vs*. 136.02±298.08 IU/mL, *p*=0.020), anti-SSA antibodies (1164.16±680.24 RU/ml *vs*. 733.05±720.15 RU/ml, *p*=0.005), ANA (1:320(1:160,1:320) *vs*. 1:160(1:40,1:320), *p*=0.037), total IgG (21.57±7.13g/L *vs*. 18.47±10.13g/L, *p*=0.025) and IgM (2.00±1.31g/L *vs*. 1.55±1.32g/L, *p*=0.006). By comparison, the pSS patients with elevated serum IL-17A only showed higher RF levels (360.74IU/mL±372.44IU/mL *vs*. 155.90±277.98IU/mL, *p*=0.019) (20). This result further supports the positive correlation between IL-17E and autoantibody production in pSS development.

Discussion

In the current study, we examined IL-17E and IL-17A levels in the serum from pSS patients. The results showed that IL-17E was more significantly increased in serum from pSS patients than IL-17A. Differential clinical relevance of IL-17E and IL-17A were shown in pSS patients, indicating potential different roles of these two IL-17 family members in pSS.

Increased IL-17A levels were identified in pSS patient body fluids including serum, tears and saliva (5-6, 19), which indicated that IL-17 axis might induce pathogenic effects in pSS. A previous study showed that the disease durations were longer in pSS patients with increased IL-17A and these patients showed less prevalent parotid gland swelling than those with normal IL-17A levels (19). IL-17A was also reported to be associated with decreased C3 and increased RF in pSS patients (20). In this study, we repeated the same analysis in the same cohort as the IL-17A control group, which is basically consistent with previous findings (20). Besides,

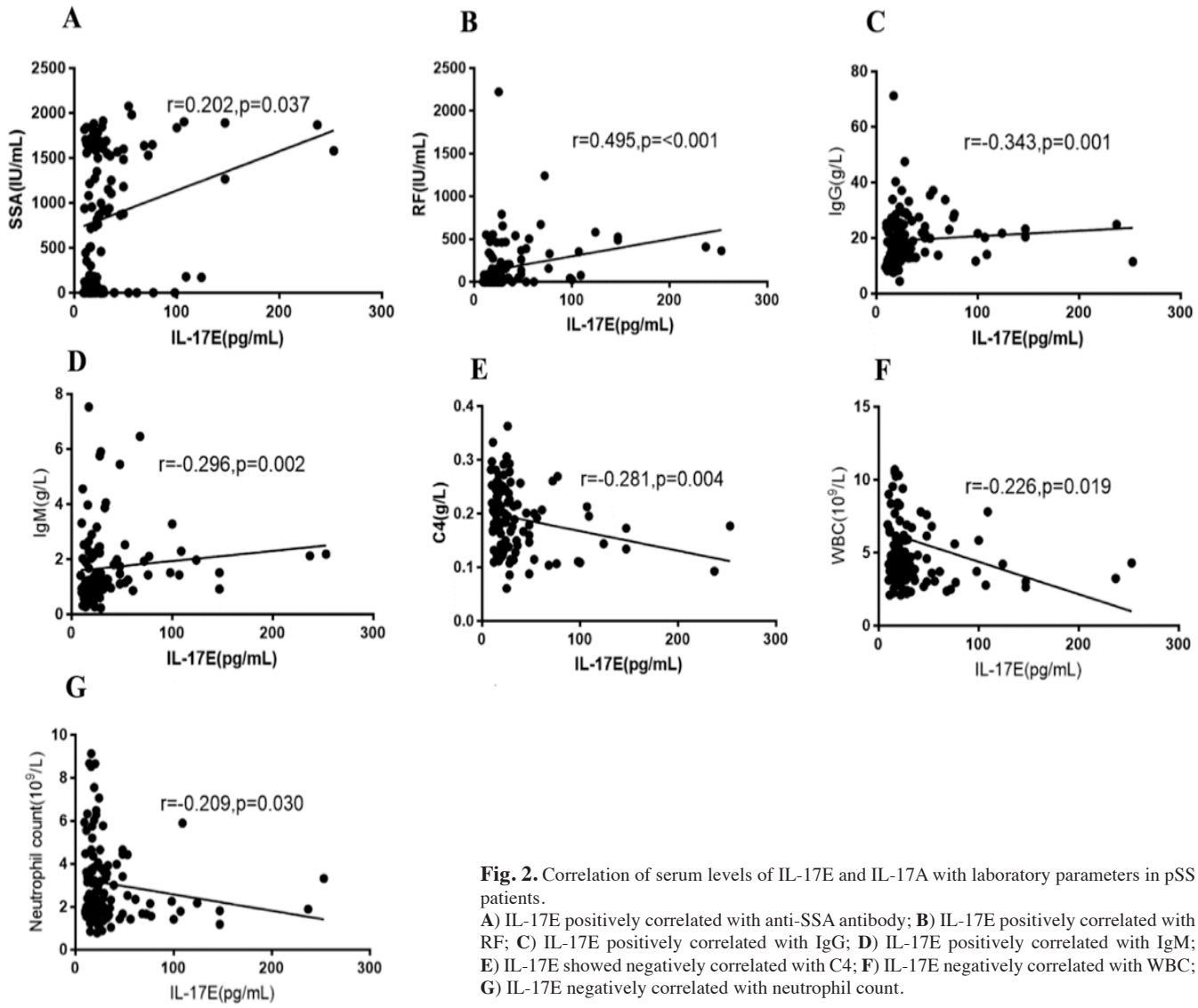

Fig. 2. Correlation of serum levels of IL-17E and IL-17A with laboratory parameters in pSS patients.
A) IL-17E positively correlated with anti-SSA antibody; **B**) IL-17E positively correlated with RF; **C**) IL-17E positively correlated with IgG; **D**) IL-17E positively correlated with IgM; **E**) IL-17E showed negatively correlated with C4; **F**) IL-17E negatively correlated with WBC; **G**) IL-17E negatively correlated with neutrophil count.

Table III. Clinical and laboratory characteristics of pSS patients with the higher or lower levels of IL-17A (ref. 20) and IL-17E.

Clinical and laboratory parameters	IL-17A				IL-17E			
	<13.8pg/mL (n=93)	≥13.8pg/mL (n=14)	t/u/χ^2	*p*	<31.36pg/mL (n=76)	≥31.36pg/mL (n=31)	t/u/χ^2	*p*
Lymphadenectasis	17 (18.28%)	7 (50.00%)	**7.037**	**0.008**	16 (21.05%)	8 (25.81%)	0.286	0.593
Splenomegaly	9 (9.68%)	2 (14.29%)	0.003	0.954	7 (9.21%)	4 (12.90%)	0.048	0.826
Joint involvement	12 (12.90%)	0	0.945	0.331	8 (10.53%)	4 (12.90%)	0	0.987
Lung involvement	55 (59.14%)	5 (35.71%)	2.711	0.100	40 (52.63%)	11 (35.48%)	2.595	0.107
WBC (×10⁹/L)	4.87 ± 2.16	4.37 ± 1.70	0.817	0.426	5.02 ± 2.22	4.27 ± 1.70	-1.691	0.094
Neutrophils (×10⁹/L)	3.12 ± 1.97	2.71 ± 1.41	0.760	0.449	3.29 ± 2.08	2.52 ± 1.22	**-1.928**	**0.049**
RBC (×10¹²/L)	3.75 ± 0.63	3.75 ± 0.64	0.016	0.988	3.70 ± 0.65	3.86 ± 0.55	1.180	0.241
Hb (g/L)	117.03 ± 17.07	111.72 ± 25.40	1.012	0.314	115.96 ± 18.07	117.26 ± 19.17	0.332	0.741
PLT (×10⁹/L)	143.69 ± 72.26	149.29 ± 64.79	-0.274	0.785	148.07 ± 73.70	135.48 ± 64.43	-0.830	0.409
IgA (g/L)	3.31 ± 1.70	3.64 ± 2.10	-0.656	0.513	3.32 ± 1.82	3.45 ± 1.61	0.348	0.728
IgG (g/L)	19.20 ± 9.64	20.45 ± 8.18	-0.459	0.647	18.47 ± 10.13	21.57 ± 7.13	**1.652**	**0.025**
IgM (g/L)	1.62 ± 1.31	2.07 ± 1.45	-1.117	0.267	1.55 ± 1.32	2.00 ± 1.31	**2.774**	**0.006**
C3 (g/L)	0.91 ± 0.24	0.81 ± 0.12	**-2.113**	**0.029**	0.91 ± 0.25	0.87 ± 0.16	0.854	0.395
C4 (g/L)	0.19 ± 0.07	0.16 ± 0.07	1.225	0.223	0.19 ± 0.07	0.17 ± 0.05	**-2.205**	**0.027**
RF (IU/mL)	155.90 ± 277.98	360.74 ± 372.44	**2.390**	**0.019**	136.02 ± 298.08	279.91 ± 265.44	**2.363**	**0.020**
ANA	1:320 (1:80,1:320)	1:160 (1:80.1:320)	0.134	0.893	1:160 (1:40,1:320)	1:320 (1:160,1:320)	**2.089**	**0.037**
anti-SSA (RU/mL)	886.27 ± 727.74	644.12 ± 768.29	1.081	0.282	733.05 ± 720.15	1164.16 ± 680.24	**-2.892**	**0.005**
anti-SSB (RU/mL)	266.82 ± 518.60	187.25 ± 383.67	0.513	0.609	266.59 ± 541.27	237.54 ± 410.39	0.272	0.786
ESSDAI	4.72 ± 2.24	5.50 ± 2.28	1.210	0.229	4.55 ± 2.37	5.48 ± 1.81	1.967	0.052

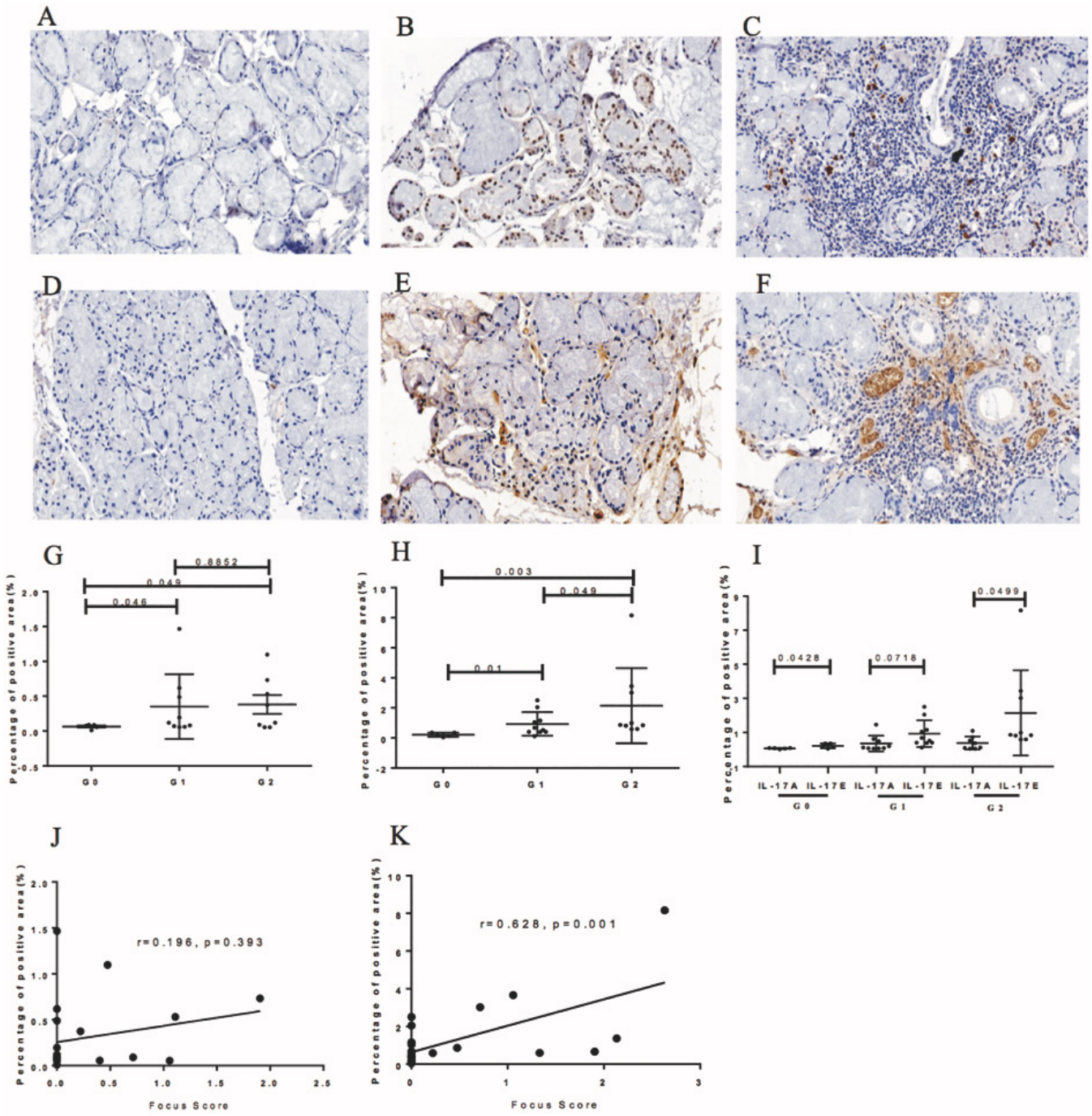

Fig. 3. IL-17E and IL-17A expression in labial salivary glands in patients with pSS.
A) IL-17A did not express in G0 group of patients with pSS (×400). **B**) IL-17A expressed in G1 group of patients with pSS (×400). **C**) IL-17A expressed in pSS patients with G2 (×400). **D**) IL-17E did not express in G0 group of patients with pSS (×400). **E**) IL-17E expressed in G1 group of patients with pSS (×400). **F**) IL-17E expressed in G2 group of patients with pSS (×400). **G**) Percentage of positive area of IL-17A in different groups of pSS patients and normal control samples. **H**) Percentage of positive area of IL-17E in different groups of pSS patients and normal control samples. **I**) Percentage of positive area of IL-17A and IL-17E in different groups of pSS patients and normal control samples.)) Percentage of positive area of IL-17A in different Focus Score of pSS patients. **K**: Percentage of positive area of IL-17E in different Focus Score of pSS patients.

we also revealed that IL-17A was associated with higher incidence of lymph node enlargement. In pSS mouse models, IL-17A was also found to be associated with inflammatory cell infiltration. It is likely that IL-17A upregulates certain chemokines which could mediate chemotaxis of inflammatory cells and their further infiltration into involved tissues (21), leading to the inflammation in lymph nodes.

IL-17E, also known as IL-25, induces production of Th2 related cytokines. Previous studies mainly concentrated on its pathogenic effects on allergic lung inflammation (12, 22) and psoriasis (23) and its protective role in parasite infection (24, 25), rheumatoid arthritis (26) and colitis (27). In these autoimmune conditions, IL-17E was reported to play an anti-inflammatory

role in disease development. Surprisingly, we found that IL-17E was more significantly elevated in patients with pSS and correlated with increased autoantibody production, such as higher levels of RF, ANA and anti-SSA, and increased total IgG and IgM in serum. Haematological involvement was observed, including decreased leukocytes, decreased neutrophils. Pathogenic roles of IL-17A in pSS were identified in early studies, which did not find obvious association between IL-17A and extra-glandular involvement and other clinical manifestations (28). However, it is still elusive whether the other IL-17 family members are involved in pSS pathogenesis. In this study, we revealed that IL-17E was involved in the multisystem involvement and immune activation in pSS for the first time. We also found that the expression of IL-17E in labial salivary gland increased as the lymphocytic infiltration became more severe. This is the first study that reveals the clinical relevance of IL-17E in pSS.

In summary, our study revealed and compared the clinical relevance of IL-17E and IL-17A, which suggested different members of IL-17 family might play different roles in disease onset and development of pSS. It is also probable that IL-17E might be induced to act as a negative regulator against excessive inflammation during the pathogenic process of pSS. The exact mechanisms of such discrepancy will be investigated in future studies, which might further exploit the roles and potential therapeutic values of IL-17 family cytokines in pSS.

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