A cohort study of T helper 17 cell-related cytokine levels in tear samples of systemic lupus erythematosus and Sjögren's syndrome patients with dry eye disease

X. Peng[1], Y. Lu[2], J. Wei[1], T. Lin[1], Q. Lu[1], Q. Liu[1], W.-J. Ting[1]

[1]*Nephrology Centre, The Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, Guangdong;* [2]*Department of Ophthalmology, The Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, Guangdong, China.*

Xiang Peng, MD
Yamei Lu, MD
Jianbo Wei, MD
Tianhui Lin, MD
Qinyan Lu, MD
Qiaonan Liu, MD
Wei-Jen Ting, PhD

Please address correspondence to:
Wei-Jen Ting,
Nephrology Centre,
The Sixth Affiliated Hospital of Guangzhou Medical University,
Qingyuan People's Hospital,
B24 Yinquan South Road,
Guangdong 511518, China.
E-mail: tim_sigeen@hotmail.com

Received on April 22, 2021; accepted in revised form on June 21, 2021.

Clin Exp Rheumatol 2021; 39 (Suppl. 133): S159-S165.

Key words: systemic lupus erythematosus; Sjögren's syndrome, dry eye disease, cytokines

Competing interests: none declared.

ABSTRACT

Objective. *Sjögren's syndrome (SS) is the most common autoimmune disease with dry eye (DE) syndrome and some systemic lupus erythematosus (SLE) patients are also with DE syndrome. The occurrence of immune-related DE disease is closely related to T helper (Th) 17 cells in SS patients, and SLE patients have abnormal levels of multiple Th17 cell-related cytokines in their blood. However, the degree of expression of these cytokines in blood differs from that in tears. We hypothesised that the occurrence of DE symptoms in SLE and SS patients may be related to Th17 cells.*

Methods. *In this study, Th17 cell-related cytokines, including interleukin (IL)-1β, IL-2, IL-4, interferon-γ, IL 6, IL-8, IL-17F, tumour necrosis factor (TNF)-α, IL-21, IL-22, and IL-23 were analysed in tear samples of DE, SLE, and SS patients. Ocular surface examinations for patients with DE symptoms, including tear secretion test (Schirmer I Test, SIT) and tests for ocular surface disease index (OSDI), tear break-up time (BUT), and corneal fluorescein stain (CFS), were performed and compared between the following patient groups: normal healthy people (control group, n=30), patients with simple DE disease (DE group, n=13), SLE patients with DE disease (SLE group, n=17), and SS patients with DE disease (SS group, n=18).*

Results. *The expression of Th17 cell-related cytokines in each tear sample was analysed using Luminex assay. The SIT and BUT scores of the SLE group were lower than those of the control ($p<0.001$) and DE ($p<0.05$) groups. However, SIT, BUT, CFS, and OSDI scores were not significantly different between SLE and SS patients. TNF-α, IL-6, IL-8, and IL-21 levels in tear samples were higher in DE, SLE, and SS patients ($p<0.05$) than in control individuals. IL-2 and IL-4 levels in tear samples of SLE patients were higher than DE ($p<0.001$) but lower than the control ($p<0.001$) group patients. IL-23 levels in tear samples of DE, SLE, and SS patients were all lower than those in the control group ($p<0.001$). SIT, BUT, CFS, and OSDI results showed that the DE symptoms of SLE and SS patients were more severe than those of the DE group.*

Conclusion. *It is known that cytokine expression levels in tears are different from those in blood. Abnormal regulation of the Th17 cell pathway may be related to the occurrence of DE disease in SLE and SS patients, and Th17 cell-related cytokines, such as IL-8 and IL-21, may be potential therapeutic targets for treating SLE or SS DE disease.*

Introduction

Dry eye (DE) syndrome is a multifactor disease of the eye characterised by loss of the steady state of the tear membrane. It is associated with eye symptoms including itching, burning, foreign body sensation, tingling, eye fatigue, sensitivity to light, intermittent blurred vision, or even worse. The current global incidence of DE is 5.5–33.7%, of which women are more affected than men, older people are more affected than young people, and Asians are more affected than people of other ethnicities; the incidence rate of DE among the Chinese is 21–30% (1). Currently, there are a number of clinical tests used to evaluate DE, including tear secretion test (Schirmer I Test, SIT), cornea fluorescent staining (CFS), test for break-up time (BUT), and a DE questionnaire –the Ocular Surface Disease Index (OSDI) (2).

Autoimmune diseases are a common cause of DE (3). Some studies have found that DE symptoms, signs, and eye surface inflammation in patients

with combined autoimmune diseases tend to be more severe than those in patients with simple DE. Furthermore, the effectiveness of treatments of such autoimmune patients is poor, and the prognosis is not ideal (4). Autoimmune diseases that cause DE include desiccation syndrome, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), spinal arthritis (SpA), and Sjögren's syndrome (SS). SLE is a complex autoimmune disease which is not yet fully understood in terms of its causes and pathogenesis. SLE can affect the whole body, including multiple systems and organs. People usually focus on SLE-induced damage to the kidneys, heart, lungs, and other important organs, but SLE patients can also exhibit a variety of eye complications such as corneitis, scleritis, and retinal lesions. Some SLE patients with DE have a fear of light, tingling, and other DE performance issues, the worst of which is visual impairment (5). A study has shown that 60% of patients with SLE develop DE symptoms (6).

SS is a chronic inflammatory autoimmune disease that mainly affects the exocrine glands, often accompanied by dry mouth and eyes. Studies of SS have focused on inflammatory factors. It is generally believed that the basic pathological changes in SS involve infiltration of lymphocytes into the exocrine glands, resulting in destruction of the gland structure and secretion dysfunction, with tear and salivary glands most commonly affected. Anti-SS-related antigen A (SSA) antibodies were detected in 65.3% of SLE patients, of whom 29.3% developed DE symptoms (7). Thus, anti-SSA antibodies are biomarkers for the diagnosis of SS. It was thought that DE symptoms in SLE patients were mainly caused by secondary dryness syndrome, and there was a high suspicion of its association with anti-SSA antibodies in SLE patients. However, some reports have suggested that DE symptoms in SLE patients are not related to secondary dryness syndrome, but to the SLE disease itself (8, 9). In patients with lupus without secondary dryness syndrome, the severity of DE symptoms was associated with a high titre of anti-double stranded (ds) DNA antibodies, low level of complement C3 but not complement C4, red blood cell sedimentation, and antinuclear antibodies (ANA) (10). There were no differences in the subjective or objective scores of DE symptoms between patients who tested positive or negative for anti-SSA and anti-SSB antibodies (11, 12).

Infiltration of the eye surface by T cells is a common finding of DE. Th17 cells are a type of $CD4^+$ Th cell. Under normal circumstances, Th17 cells can protect the body by secreting IL-17 to counter attack by extracellular microorganisms. However, overactivation of Th17 cells can lead to the occurrence of a variety of autoimmune diseases and also recurrent episodes of such diseases (13, 14). Overall, Th17 cells secrete a variety of cytokines, such as interleukin (IL)-17A, IL-17F, tumour necrosis factor TNF-α, IL-21, IL-22, and IL-26, that participate in the development of autoimmune and inflammatory diseases (15, 16).

In vitro studies have found that IL-23 can activate the JAK2-STAT3 signalling pathway to amplify and maintain the survival of Th17 cells (17). IL-1-ß and TNF-α can enhance the roles of transforming growth factor-β and IL-6 in the CD4 Th cell differentiation phase, thereby promoting the differentiation of Th17 cells (18). IL-2, IL-4, and interferon (IFN)-γ are negative regulators of Th17 cells, which can impair their secretory function (19). IL-21 can be secreted by Th17 cells, but it is also a key regulatory factor for Th17 cell development (20). In normal conditions, IL-17 and IL-22 both promote neutrophil differentiation, migration, and induction of antimicrobial peptides by stimulating the expression of multiple cytokines and chemokines, thus playing a powerful role in fighting infections by bacteria and fungi (21). However, IL-17 is not always conducive to host defences. Specifically, IL-17 is also involved in inducing inflammation and the development of a variety of autoimmune diseases, and elevated levels are found in SLE, RA, SS, SpA, psoriasis, and inflammatory bowel disease (22). IL-17 not only directly destroys corneal epithelial cells, but also induces the release of other inflammatory factors to increase inflammation and apoptosis, thereby promoting the development of DE (23). IL-22 is also involved in the pathogenic processes of SLE, SS, RA, SpA, and giant cell arteritis (24).

Several studies on cytokines in tears in SS patients have found that the occurrence of immune-related DE disease is closely related to Th17 cells, and SLE patients have abnormal levels of multiple Th17 cell-related cytokines in their blood (25-27). However, the degree of expression of these cytokines in blood differs from that in tears. Thus, we speculated that the occurrence of DE symptoms in SLE and SS patients is related to Th17 cells. Th17 cell-related cytokines (IL-1β, IL-2, IL-4, IFN-γ, IL-6, IL-8, IL-17F, TNF-α, IL-21, IL-22, and IL-23) in tear fluid in SLE and SS patients were evaluated to explore their relationship with DE symptoms and provide valuable information for subsequent exploration of their role in the pathogenesis of DE and the discovery of new therapeutic targets.

Methods

Patients

The protocol used in this study was approved by the Institutional Review Board of The Sixth Affiliated Hospital of Guangzhou Medical University, China (QPH-IRB-A0143). A total of 78 volunteers, including control (n=30), non-connective tissue disease-related simple DE symptoms (n=13), SLE with DE symptoms (n=17), and SS with DE symptoms (n=18) patients, were included during July 2018 to July 2019. The major details of patients included in the study are provided in Table I. The selection criteria of control volunteers were ophthalmologic confirmation of the absence of DE (SIT ≥10 and BUT ≥10) and rheumatologists and immunologists confirmed that none suffered from SLE or SS (negative for anti-SSA, anti-SSB, and anti-dsDNA antibodies). Criteria for the selection of DE patents were SIT <10, BUT <10, and rheumatology immunology confirmed the absence of SLE or SS. Criteria for the selection of SLE with DE patients were SIT <10, BUT <10, and anti-dsDNA (including

Table I. Characteristics in controls and patients.

Parameter	C	DE	SLE	SS
n	30	13	17	18
Age	33.7 ± 9.6	35.2 ± 11.7	34.1 ± 9.3	41.6± 12.6
Gender				
Male	15 (50.0%)	3 (23.1%)	1 (5.9%)	3 (16.7%)
Female	15 (50.0%)	10 (76.9%)	16 (94.1%)	15 (83.3%)
Co-morbidities				
AIDS	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Sarcoidosis	0 (0%)	0 (0%)	0 (0%)	0 (0%)
IgG4-RD	0 (0%)	0 (0%)	0 (0%)	0 (0%)
RA	0 (0%)	0 (0%)	0 (0%)	3 (16.7%)
Allergies	0 (0%)	0 (0%)	3 (17.6%)	0 (0%)
Asthma	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CKD	0 (0%)	3 (23.0%)	5 (29.4%)	0 (0%)
COPD	0 (0%)	0 (0%)	0 (0%)	0 (0%)
DM	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)
Dyslipidaemia	0 (0%)	5 (38.5%)	3 (17.6%)	1 (5.6%)
Hypertension	0 (0%)	1 (7.7%)	2 (11.8%)	1 (5.6%)

Co-morbidity was identified within 3 years before index date.
AIDS: acquired immunodeficiency syndrome; IgG4-RD: IgG4 related disease; RA: rheumatoid arthritis; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus.

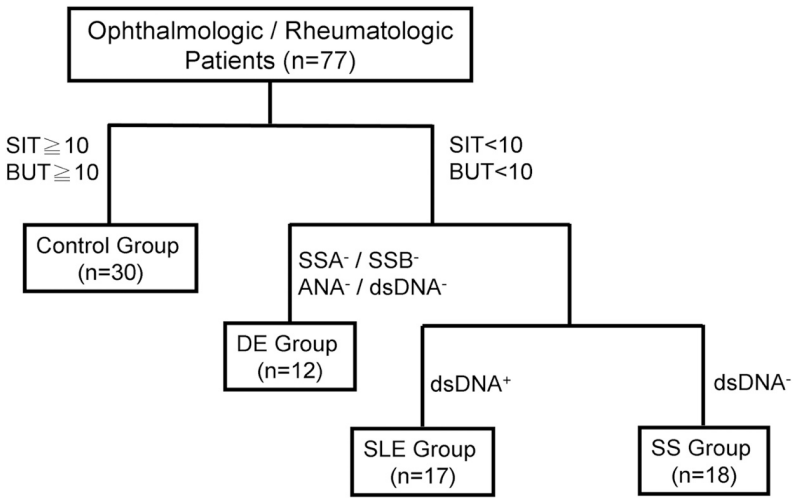

Fig. 1. Flow chart for the participations grouping in this study.

anti-Smith positive) antibody positive (anti-SSA antibody negative SLE patients were not included in this study). Criteria for the selection of SS with DE patients were SIT <10, BUT <10, and anti-dsDNA or anti-Smith antibodies negative (anti-SSA antibody negative SLE patients were not included in this study). The flow chart for the participation grouping in this study is presented in Figure 1.

OSDI determination

OSDI is a subjective questionnaire that measures the severity of DE disease based on the participants' responses to symptomatic questions. It includes 12 questions about visual function, eye symptoms, and environmental triggers during the previous week. Scoring was as follows: less than 25 points, mild; 25–45 points, moderate, and greater than 45-points, severe (28).

SIT analysis

In the absence of an anaesthetic, tear fluid test papers were folded back 5 mm to the end and placed in the outer one-third of the patient's double lower eyelid conjunctiva sac. With the other end naturally drooping, the patient was asked to gently close their eyes. After 5 min, the paper was removed and its wet length measured. The average of the SIT results of two eyes was then calculated.

BUT analysis

A drop of 2% fluorescein solution was placed into the conjunctiva sac of the subject's eyes. The patient was told to blink several times, then look forward while preserving the open eye state. With continuous observation under a microscope, the time from the last time the patient began to blink to the first black spot of the corneal surface was recorded. The average of the BUT results of the subject's eyes was then calculated.

CFS analysis

A fluorescein sodium filter paper was placed into the lower eyelid of the patient who was then instructed to blink so that the fluorescein was distributed over the full eye surface. The cornea was observed after about 2 min under cobalt light of the slit lamp. The cornea becomes a tender green colour at breakage sites, while the intact epithelium is not coloured. The cornea was divided into four quadrants and scored according to the methods published by Bron *et al*. (29). The total CFS score of the subject's eyes was then calculated.

Tear collection assay

All participants were informed of the procedure and tear fluid samples were collected with sterile capillary glass tubes. The total volume of tear fluid was 5 μL from the left and right eyes, the tear samples were diluted 1:4 (v/v) with a 0.1% bovine serum albumin in phosphate-buffered saline solution. All samples were stored immediately at -80°C until further analysis.

Cytokine assays

Using fluorescent-coded microbead-conjugated monoclonal antibodies against IL-1β, IL-2, IL-4, IFN-γ, IL 6, IL-8, IL-17F, TNF-α, IL-21, IL-22, and IL-23, the levels of these cytokines in tear samples were determined. Fluorescence coding of the antibodies was identified by laser scanning using a Luminex200 liquid suspension chip system (Luminex, Seattle, WA, USA). Cytokine concentrations in each tear sample were calculated according to fluorescence intensity standard curves using Luminex200 IS v. 2.1 software.

Statistical analyses

All data were analysed statistically using SPSS v. 25.0 software (IBM, Aromak, NY, USA). Measurement data subject to a normal distribution are expressed as means ± standard deviation. Differences between groups were determined with a one-way analysis of variance. Multiple comparisons used the least significant difference test, and correlation analyses used the Pearson method. Measurement data that were not normally distributed are represented by median and quarter-position spacing [M (Q25, Q75)]. Differences between these groups were determined with the Kruskal-Wallis test, and the correlation analysis was based on the Spearman method. Differences with $p<0.05$ were considered statistically significant.

Results

Ophthalmologic analyses

The SIT and BUT results of DE patients were decreased significantly compared with those of healthy control individuals ($p<0.001$). DE patients with autoimmune diseases had less tear secretion and shorter tear BUT ($p<0.05$) than simple DE patients. There were no significant differences in either the SIT or BUT results between the SLE and SS patients (Table II). Using the CFS score, the healthy control subjects did not have abnormal corneal staining, while DE patients had abnormal positive corneal staining, though the overall CFS scores were not significantly different between the four groups of subjects in this study. Comparing the OSDI questionnaire scores, differences between the groups of subjects were statistically significant ($p<0.001$). Among them, the OSDI score of DE patients was significantly higher than that of the healthy control group (Table II).

Rheumatologic analyses

Anti-ANA, anti-dsDNA, anti-SSA, and anti-SSB antibody levels in control and DE group participants were not detectable or present at non-meaningful levels (Table III). In the SLE and SS groups, especially SLE patients, anti-ANA, anti-dsDNA, anti-SSA, and anti-SSB antibody levels were significantly increased. However, the C3 and C4 expression levels were only reduced in SLE group patients.

Table II. Ophthalmological analysis.

Parameter	C	DE	SLE	SS
SIT (mm/5min)	14.85±2.70	6.77±1.36*	3.46±1.05*#	5.03±2.46*#
BUT (s)	14.02±1.36	6.09±1.76*	4.22±1.96*#	4.45±2.07*#
CFS (score)	0.00 (0.00, 0.00)	0.00 (0.00, 1.00)	0.00 (0.00,2.00)	0.00 (0.00,0.00)
OSDI (score)	5.00 (0.00, 5.00)	20.80* (14.00, 27.70)	25.50* (17.65, 35.90)	24.35* (19.58, 35.20)

SIT, BUT values are average ± standard deviation ($\chi\pm s$), CFS, OSDI values are median and quarter spacing [M (Q25,Q75)].
*$p<0.05$, compared to the control group; #$p<0.05$, compared to SDE.

Table III. Rheumatologic analysis.

Parameter	C	DE	SLE	SS
anti-ANA (AU/ml)	N.D.	N.D.	558.88 ± 373.37	260.93 ± 128.43
anti-dsDNA (IU/ml)	N.D.	N.D.	446.76 ± 401.18	47.11 ± 35.91
151. anti-SSA (AU/ml)	N.D.	N.D.	209.12 ± 110.26	21 ± 94.17
anti-SSB (AU/ml)	N.D.	N.D.	49.01 ± 33.79	19.00 ± 7.82
C3 (g/L)	1.12 ± 0.32	1.25 ± 0.26	0.53 ± 0.20*#	1.10 ± 0.21
C4 (g/L)	0.25 ± 0.06	0.30 ± 0.08	0.10 ± 0.05*#	0.40 ± 0.23

N.D.: the value presents no meaningful or no detectable results.
Anti-ANA, anti-dsDNA, anti-SSA, anti-SSB, C3, C4 values are average ± standard deviation ($\chi\pm s$).
*$p<0.05$, compared to the control group; #$p<0.05$, compared to DE.

Cytokine levels in tear samples

The concentrations of TNF-α ($p<0.05$), IL-6 ($p<0.001$), IL-8 ($p<0.001$), and IL-21 ($p<0.001$) were increased significantly in DE, SLE, and SS patients compared with those in the control group (Fig. 2). IL-8 ($p<0.001$) and IL-21 ($p<0.001$) were increased more in both the SLE and SS groups than in the DE group (Fig. 2). The concentrations of IL-2 ($p<0.001$) and IL-4 ($p<0.001$) were increased in the DE group and reduced in the SLE and SS groups compared with those in the control group (Fig. 3). The IL-22 ($p<0.001$) level was reduced only in the SLE group, whereas IL-23 ($p<0.001$) expression was reduced in the DE, SLE, and SS groups compared with those in the control group (Fig. 2). The concentration of IFN-γ was slightly increased in the DE ($p<0.05$) group but decreased in the SLE ($p<0.05$) and SS ($p<0.001$) groups (Fig. 4). The concentrations of IL-1β ($p<0.05$) and IL-17F ($p<0.05$) were only slightly increased in the DE group and were not significantly different between the other groups (Fig. 4).

Discussion

Although it is known that SLE and SS occur more frequently in females than in males, the current finding that this is also true of DE patients is interesting (Table I). There are many causes of DE and the pathological mechanisms are complex, but it is generally considered that inflammation is most related to the occurrence of this disorder. The ophthalmology analyses results showed that SIT, BUT, and OSDI were not only increased in DE patients, but also significantly increased in both SLE and SS patients (Table II). DE with SLE or SS co-morbidity seems to enhance DE symptoms. The rheumatologic analyses results showed that anti-ANA, anti-dsDNA, anti-SSA, and anti-SSB antibodies are potentially related to the symptoms of DE in SLE or SS patients, though more evidence is needed to support this contention (Table III).

In tear glands and on the eye surface, infiltration of a large number of lymphocytes and excessive production of inflammatory cytokines can damage the normal nerve stimulation of tear secretion and inhibit the release of neurotransmitters, thereby affecting the qual-

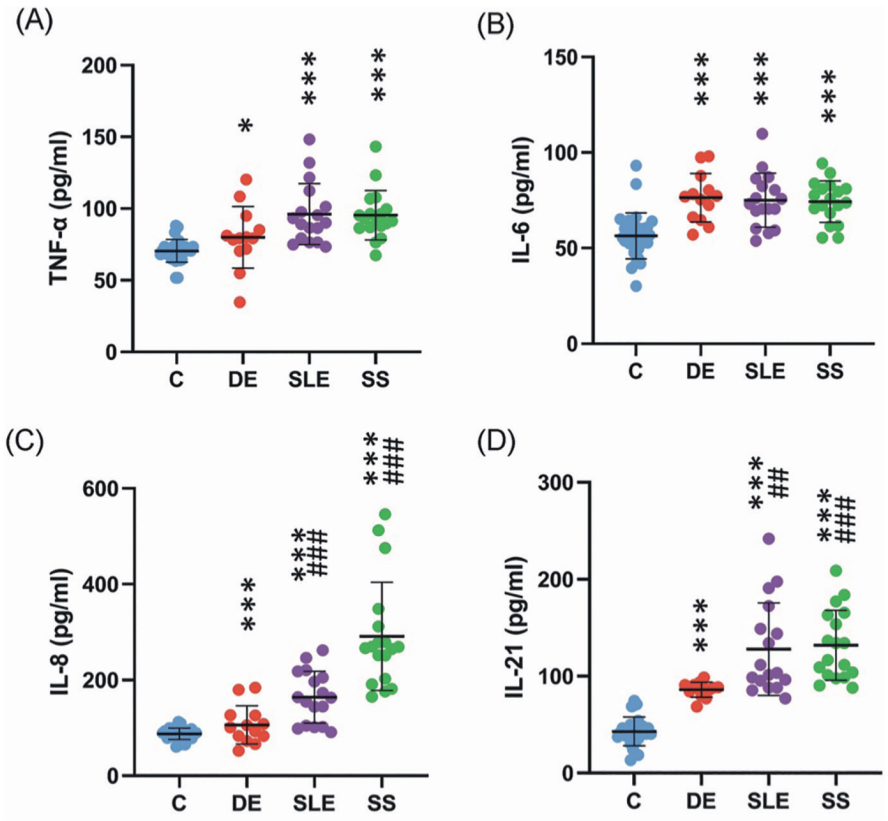

Fig. 2. Concentrations of TNF-α (A), IL-6 (B), IL-8 (C) and IL-21 (D) in tear samples from dry normal control participations and DE, SLE, SS patients were determined by Luminex assay.
*p<0.05; ***p<0.001 compared with control group; ###p<0.001 compared with DE group.

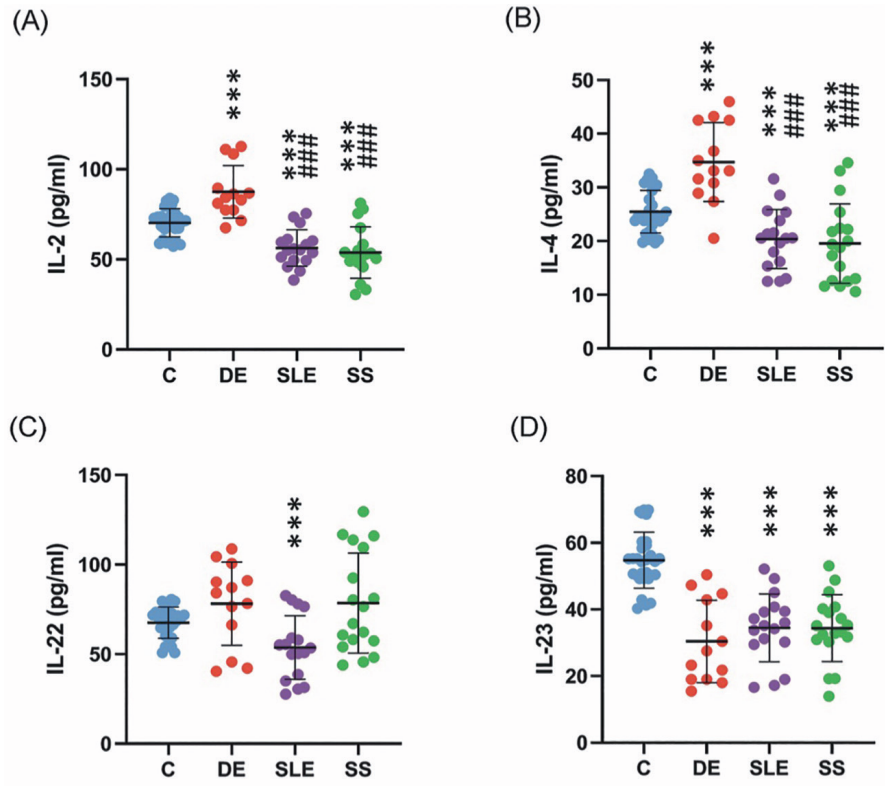

Fig. 3. Concentrations of IL-2 (A), IL-4 (B), IL-22 (C) and IL-23 (D) in tear samples from dry normal control participations and DE, SLE, SS patients were determined by Luminex assay.
*p<0.05; ***p<0.001 compared with control group; ###p<0.001 compared with DE group.

ity and quantity of tear secretion (26). These cytokines play different and important roles in complex immune networks and affect each other, and some have become important targets for the treatment of autoimmune diseases. In the current study, increases in the levels of some cytokines, such as TNF-α, IL-6, IL-8, and IL-21, were potentially related to the development of DE symptoms in DE patients, and may have been involved in the enhancement of these symptoms in SLE and SS patients (Fig. 2). TNF-α, IL-1α, IL-6, IL-8, IL-12, and macrophage inhibitory protein-1α were also reported previously to be related to SS with DE symptoms, and their levels might correlate with inflammation around the eyes (26).

There have been many drugs used to reduce or block TNF-α-mediated signals for the treatment of SS, such as infliximab and adalimumab (30, 31). Whether these drugs can effectively relieve DE needs more research. High IL-6 expression was reported as a downstream result of inflammation associated with activation of the JAK-STAT pathway, and correlated to SS (32). The use of tofacitinib, as an anti-inflammatory treatment in SS, was also suggested in the same report. Enhanced release of the proinflammatory cytokines, IL-8 and TNF-α, will augment inflammatory reactions and can cause cell damage (33). IL-8 also has a strong chemotactic effect on T cells and neutrophils. The penetration and activation of T lymphocytes can damage lacrimal glands and ocular surface tissues through cytotoxic and apoptotic mechanisms (34). IL-21 has been linked to autoimmune diseases, and its levels are increased in the peripheral blood and tissues of SLE and SS patients (35). In the current work, IL-8 and IL-21 levels were enhanced more in SLE and SS than DE patients. This suggested that IL-8 and IL-21 may be related to the development of DE symptoms that were exacerbated by other co-factors in SLE and SS patients. Unfortunately, tolerance and low bioavailability appear to reduce the value of IL-21 as a therapeutic target in some clinical trials (36).

IL-2, IL-4, IL-22, and IL-23 expression was increased in DE patients but

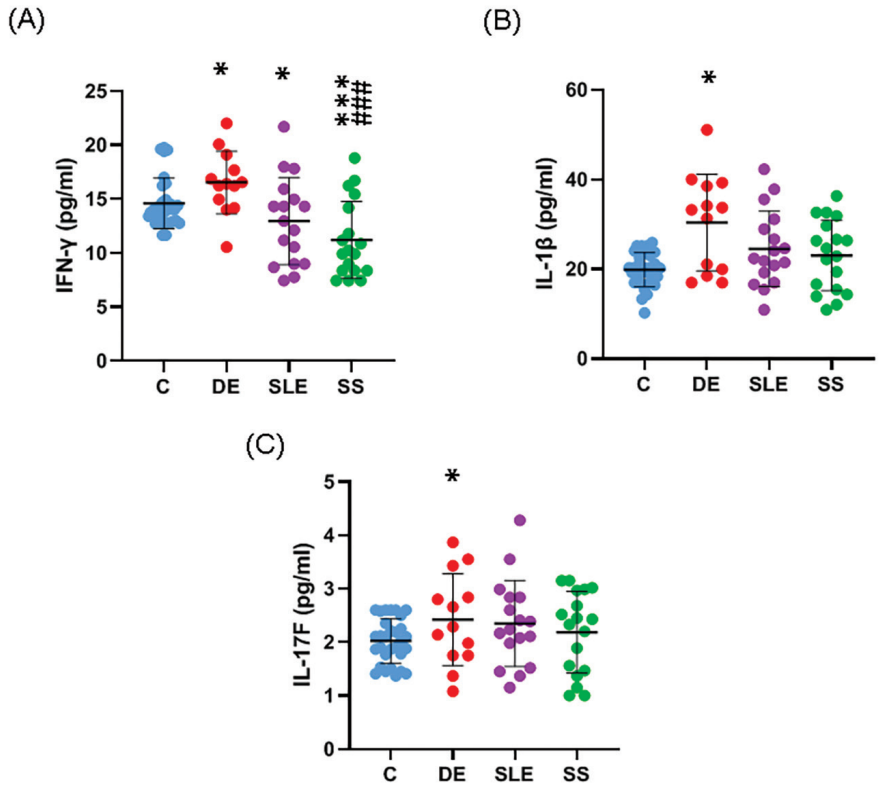

Fig. 4. Concentrations of IFN-γ (A), IL-1β (B) and IL-17F(C) in tear samples from dry normal control participations and DE, SLE, SS patients were determined by Luminex assay.
*p<0.05; ***p<0.001 compared with control group; ###p<0.001 compared with DE group.

reduced in SLE and SS patients (Fig. 3). In the 1990s, several studies revealed IL-2 as a major survival factor of regulatory T cells in the periphery (37). Moreover, low-dose IL-2 administration was shown to be a promising treatment for SLE (38). In the current work, IL-2 was highly expressed only in DE patients, suggesting that IL-2 may cause simple DE autoimmune symptoms. Th1 and Th17, among various subtypes of T helper cells, can trigger the JAK-STAT signalling pathways and control the production of several Th-17-related cytokines, such as IFN-γ, IL-4, IL-17, IL-22, IL-23, and IL-22, that mediate downstream inflammation (39). Interestingly, IL-4 expression in tear samples from each group was similar to IL-2. IL-22 expression was only reduced in SLE patients, while IL-23 expression was reduced in DE, SLE, and SS patients (Fig. 3). The Th-17-related cytokine, IFN-γ, is known to be related to autoimmune problems, and its expression differed in tear samples of SLE and SS patients (Fig. 4). This suggests that IFN-γ directly or indirectly modulates the different mechanisms of DE symptoms in SLE and SS patients.

This study has some limitations. First, only the protein in tear fluid samples was tested. In addition, because all patients were recruited before any clinical treatment, there was a lack of control of parameters before and after treatment. Finally, only patients in Guangdong, China, who do not represent the entire human population, were tested. Nevertheless, there are several important conclusions that may be inferred from this study; specifically, the up-regulation of IL-8 and IL-21 in tear samples, and the downregulation of IL-2 and IL-4, may aggravate DE symptoms due to immune diseases.

In conclusion, an increased IL-6 and decreased IL-23 concentration in tears may be the main factors causing DE symptoms, and these symptoms may be less associated with immune diseases. However, whether the above-mentioned Th17 cell-related cytokines can be used as targets for the treatment of DE requires further research.

Acknowledgements

We thank Guangzhou Medical University for technical support.

References

1. ZHANG X, M VJ, QU Y *et al*.: Dry eye management: targeting the ocular surface microenvironment. *Int J Mol Sci* 2017, 18: 1398.
2. AYYILDIZ T, SEZGIN FM: The effect of ocular demodex colonization on Schirmer test and OSDI scores in newly diagnosed dry eye patients. *Eye Contact Lens* 2020, 46: S39-S41.
3. VEHOF J, UTHEIM TP, BOOTSMA H, HAMMOND CJ: Advances, limitations and future perspectives in the diagnosis and management of dry eye in Sjögren's syndrome. *Clin Exp Rheumatol* 2020; 38 (Suppl. 126): S301-9.
4. WANNG H, WANG PB, CHE T *et al*.: Analysis of clinical characteristics of immune-related dry eye. *J Ophthalmol* 2017; 2017: 8532397.
5. BOWMAN SJ: Primary Sjögren's syndrome. *Lupus* 2018; 27: 32-5.
6. RESCH MD, MARSOVSZKY L, NEMETH J, BOCSKAI M, KOVACS L, BALOG A: Dry eye and corneal Langerhans cells in systemic lupus erythematosus. *J Ophthalmol* 2015; 2015: 2543835.
7. CHEN A, CHEN HT, HWANG YH, CHEN YT, HSIAO CH, CHEN HC: Severity of dry eye syndrome is related to anti-dsDNA autoantibody in systemic lupus erythematosus patients without secondary Sjögren syndrome: A cross-sectional analysis. *Medicine* (Baltimore) 2016; 95: e4218.
8. CARSONS SE, PATEL BC: Sjögren Syndrome. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan. PMID: 28613703.
9. BEN-ELI H, SOLOMON A, AFRAMIAN DJ *et al*.: Serological and hematological characteristics of Sjögren's syndrome and dry eye syndrome patients using a novel immune serology technique. *PLoS One* 2020; 15: e0244712.
10. TARVIN SE, O'NEIL KM: Systemic lupus erythematosus, Sjögren syndrome, and mixed connective tissue disease in children and adolescents. *Pediatr Clin North Am* 2018; 64: 711-37.
11. GIRARD BC, ABDELLAOUI M, DE SAINT SAUVEUR G, HUANG A, LÉVY P: Blepharospasm, dry eye and extractable nuclear antigen antibodies. *J Fr Ophtalmol* 2020; 43: e211-5.
12. LI Z, FU T, LI L *et al*.: Prevalence, severity, and predictors of dry eye and dry mouth in Chinese patients with primary Sjögren syndrome. *Clin Rheumatol* 2018; 37: 2971-9.
13. CHAUHAN SK, DANA R: Role of Th17 cells in the immunopathogenesis of dry eye disease. *Mucosal Immunol* 2009; 2: 375-6.
14. STEVENSON W, CHAUHAN SK, DANA R: Dry eye disease: an immune-mediated ocular surface disorder. *Arch Ophthalmol* 2012; 130: 90-100.
15. YANG XO, PAPPU BP, NURIEVA R *et al*.: T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity* 2008; 28: 29-39.
16. HO AW, GAFFEN SL: IL-17RC: a partner in

IL-17 signaling and beyond. *Semin Imm unopathol* 2010; 32: 33-42.
17. VELDHOEN M, HOCKING RJ, ATKINS CJ, LOCKSEY RM, STOCKINGER B: TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006; 24: 179-89.
18. BI Y, YANG R: Direct and indirect regulatory mechanisms in TH17 cell differentiation and functions. *Scand J Immunol* 2012; 75: 543-52.
19. NOACK M, MIOSSEC P: Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev* 2014; 13: 668-77.
20. KORN T, BETTELLI E, OUKKA M, KUCHROO VK: IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; 27: 485-517.
21. WANG B, LIM JH, KAJIKAWA T *et al*.: Macrophage β2-integrins regulate IL-22 by ILC3s and protect from lethal citrobacter rodentium-induced colitis. *Cell Rep* 2019; 26: 1614-26.
22. FARAH IZATI A, WONG KK, CHE MARAINA CH: IL-23/IL-17 axis in the pathogenesis and treatment of systemic lupus erythematosus and rheumatoid arthritis. *Malays J Pathol* 2020; 42: 333-47.
23. WANG HX, CHU S, LI J *et al*.: Increased IL-17 and IL-21 producing TCRαβ+CD4-CD8- T cells in Chinese systemic lupus erythematosus patients. *Lupus* 2014; 23: 643-54.
24. PAN HF, LI XP, ZHEN SG, YE DQ: Emerging role of interleukin-22 in autoimmune diseases. *Cytokine Growth Factor Rev* 2013; 24: 51-7.
25. LIM SA, NAM DH, LEE JH, KWOK SK, PARK SH, CHUNG SH: Association of IL-21 cytokine with severity of primary Sjögren syndrome dry eye. *Cornea* 2015; 34: 248-52.
26. ZHAO H, LI Q, YE M, YU J: Tear Luminex analysis in dry eye patients. *Med Sci Monit* 2018; 24: 7595-602.
27. LIU R, GAO C, CHEN H, LI Y, JIN Y, QI H: Analysis of Th17-associated cytokines and clinical correlations in patients with dry eye disease. *PLoS One* 2017; 12: e0173301.
28. MIDORIKAWA-INOMATA A, INOMATA T, NOJIRI S *et al*.: Reliability and validity of the Japanese version of the Ocular Surface Disease Index for dry eye disease. *BMJ Open* 2019; 9: e033940.
29. BRON AJ, BENJAMIN L, SNIBSON GR: Meibomian gland disease. Classification and grading of lid changes. *Eye* (Lond) 1991; 5: 395-411.
30. GINSBER S, ROSNER I, SLOBODIN G *et al*.: Infliximab for the treatment of refractory polyarteritis nodosa. *Clin Rheumatol* 2019; 38: 2825-33.
31. BERKHOUT LC, L'AMI MJ, RUWAARD J *et al*.: Dynamics of circulating TNF during adalimumab treatment using a drug-tolerant TNF assay. *Sci Transl Med* 2019; 11: eaat3356.
32. BARRERA MJ, AGUILERA S, CASTRO I *et al*.: Tofacitinib counteracts IL-6 overexpression induced by deficient autophagy: implications in Sjögren's syndrome. *Rheumatology* (Oxford) 2021; 60: 1951-62.
33. SUN KH, YU CL, TANG SJ, SUN GH: Monoclonal anti-double-stranded DNA autoantibody stimulates the expression and release of IL-1beta, IL-6, IL-8, IL-10 and TNF-alpha from normal human mononuclear cells involving in the lupus pathogenesis. *Immunology* 2000; 99: 352-60.
34. XIA L, ZHANG S, ZHOU J, LI Y: A crucial role for B and T lymphocyte attenuator in preventing the development of $CD4^+$ T cell-mediated herpetic stromal keratitis. *Mol Vis* 2010; 16: 2071-83.
35. LONG D, CHEN Y, WU H, ZHAO M, LU Q: Clinical significance and immunobiology of IL-21 in autoimmunity. *J Autoimmun* 2019; 99: 1-14.
36. IGNATENKO S, SKRUMSAGER BK, MOURITZEN U: Safety, PK, and PD of recombinant anti-interleukin-21 monoclonal antibody in a first-in-human trial. *Int J Clin Pharmacol Ther* 2016; 54: 243-52.
37. KLATZMANN D, ABBAS AK: The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. *Nat Rev Immunol* 2015; 15: 283-94.
38. BOYMAN O, SPRENT J: The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol* 2012; 12: 180-90.
39. HE H, GUTTMAN YASSKY E: JAK inhibitors for atopic dermatitis: an update. *Am J Clin Dermatol* 2019; 20: 181-92.