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Rapid communication

High circulating levels of interleukin-18 in patients with primary Sjögren’s syndrome is associated with disease activity

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Introduction

Sjögren’s syndrome (SS) is an autoimmune disease mainly targeting exocrine glands and manifests in dryness of mouth (xerostomia) and eyes (xerophthalmia). This disease can present alone as primary SS (pSS) or associated with other connective tissue diseases as secondary SS [1]. Besides circulating autoantibodies and inflammatory cell infiltration in exocrine glands, abnormal cytokine production is also a hallmark of pSS. Some evidences indicate that IL-18 might play a role in the pathogenesis of SS. In 2004, Bombardieri et al. reported that IL-18 is elevated systemically and locally in pSS than in healthy controls, providing the first link between IL-18 and pSS. The subsequent studies confirmed the finding and further suggested an association between IL-18 and the production of IgG1 and IL-17 [2,3]. Furthermore, Delaleu et al. showed in the mouse model of pSS that the serum levels of IL-18 are higher in the susceptible mouse strain than resistant strain [4]. Taken together, this evidence suggests a role of the IL-18 in the development of pSS.

Interleukin-18 (IL-18), also known as interferon-γ inducing factor, is proinflammatory cytokine belonging to the IL-1 superfamily [1]. IL-18 functions by means of binding to the IL-18 receptor complex which is comprised of IL-18Rα (IL-18R1) chain as the extracellular signaling domain and IL-18β chain as an adaptor molecule. The IL-18Rα acts as the signal chain that alone binds to IL-18 with low affinity, but when recruiting the binding chain IL-18β, it can combine with IL-18 tightly. There are two circulating natural antagonists of IL-18, including IL-18-binding protein (IL-18BP) with a high affinity binding with IL-18 and soluble form of IL-18R1 (sIL-18R1) with a low IL-18 binding affinity [5]. The IL-18-IL-18R complex mediated signaling regulates T cell immunity by promoting Th1 and Th17 response and
consequently plays an important role in infection and inflammation [6,7]. Moreover, there is an increasing body of evidence showing that IL-18 may also play a role in autoimmunity. For example, increased the circulating levels of IL-18 have been observed in many autoimmune diseases, such as rheumatoid arthritis (RA), Systemic Lupus Erythematosus (SLE) and idiopathic thrombocytopenic purpura (ITP) [8,9,16,17]. In addition, the levels of IL-18BP and sIL-18R1 have been also shown to be increased in SLE [8] and RA respectively [9].

Although previous results suggest a role of IL-18 in pSS, the mechanism behind the role is not clear. The elevated circulating level of IL-18 has been observed in pSS patients, the levels of IL-18BP and sIL-18R1 in pSS have not been investigated. Also, whether the elevated IL-18 levels are associated with pSS clinical phenotypes such as disease activity and disease duration is not clear. In this study we investigated the association between the circulating levels of IL-18, IL-18BP and sIL-18R1 and pSS as well as its subphenotypes.

We recruited 35 pSS patients (5 males and 30 females) from the First Affiliated Hospital of Xiamen University (Table 1). The mean age of the patients at the time of the study was 43.46±12 years. All patients were diagnosed according to the standards defined by criteria of the American–European Consensus Group in 2002 [10]. All patients underwent serological evaluations, including C3 levels, C4 levels, total IgG levels, anti-SSA and anti-SSB autoantibodies. Histological evaluation was also performed to detect the inflammatory cell infiltration which was presented as focus score of salivary gland tissue. Disease activity was determined according to the standard of European League Against Rheumatism (EULAR) and presented as Sjögren's syndrome disease activity index (ESSDAI) [11]. ESSDAI scores of 0–7 were defined as mild disease, whereas scores of above 7 were defined as active disease.

The main clinical and laboratory characteristics of pSS patients are presented in Table 1. Age-
and gender-matched healthy controls were recruited from the Xiamen University Hospital.

Sera were prepared freshly from peripheral blood samples of patients and controls and stored at -80°C until use. This study was approved by the ethics committee of Xiamen University.

We detected the serum levels of IL-18 and IL18R1, IL-18BP in the patients and control subjects using sandwich ELISA. The free IL-18 was calculated using the method as described previously [12]. The mean ± SD value of IL-18 levels in pSS patients was significantly higher than in controls (119.73±100.06 pg/mL vs 65.03 ± 45.70 pg/mL, P<0.01) (Table 1). When the cut-off level for IL-18 was set as mean+2SD of levels of the 35 control sera, the rate of samples with high IL-18 levels in pSS patients was higher than in controls (28.6% vs 5.7%, P<0.05). The levels of IL-18BP in pSS sera and in control sera were 21.11±10.79 ng/ml and 20.33±4.44 respectively. Significant difference between patients and control was observed neither in the levels nor in the rate of the samples with high IL-18BP levels. Also, no significant difference in the levels of IL-18R1 was observed between pSS patients (310.53±160.00 pg/ml) and controls (262.89±123.18 pg/ml) (Table 1). We then calculated the free IL-18 levels in controls and pSS patients. Similar to the IL-18 levels, the free IL-18 levels in pSS patients were significantly higher than those in controls (31.90±28.25 pg/ml vs 16.83±16.83 pg/ml, P<0.01) (Table 1). Furthermore, the rate of samples with high levels of free IL-18 was also higher in pSS patients than in controls (20% vs 2.86%, P<0.05).

To further investigate the role of the IL-18 in pSS, we evaluated the association of IL-18 with clinical phenotypes of pSS. We firstly investigated whether the levels of IL-18 is associated with disease activity of pSS. We considered ESSDAI scores of 0-7 and ESSDAI scores of >7 representing mild disease and active, disease respectively. As shown in Figure 1A, the serum levels of IL-18 in patients with active disease are significantly higher than in patients with
mild disease (166.12 ± 120.20 pg/mL vs 80.66 ± 57.86 pg/mL, \( P < 0.01 \)). Such significant
difference was also observed in the levels of free IL-18 (40.28 ± 31.45 pg/mL vs 24.85 ± 23.85
pg/mL, \( P < 0.05 \)) (Figure 1B) but not in the levels of IL-18BP or IL-18R1. We next
investigated whether the levels of IL-18 is associated with disease duration. We compared the
levels of IL-18 in patients with disease duration of less than one year with the levels of IL-18
in patients with disease duration of more than one year. The result demonstrated there was no
significant difference in either IL-18 levels or free IL-18 levels between the two patients
groups (Figure 1C, 1D). We also investigated the association of IL-18 with other
subphenotypes of pSS, including anti-SSA, anti-SSB, total IgG levels, C3 levels, C4 levels
and focus score, but no significant difference was observed (supplementary table S1).
Our results demonstrate that the serum levels of IL-18 in pSS patients are significantly higher
than in controls. This finding is in line with previous observations, which show the elevated
circulating levels of IL-18 in pSS patients [2]. In addition, we show that the levels of free
IL-18 which is a biologically relevant index for IL-18 activity is elevated in pSS patients as
compared with controls. Previous studies showed that in pSS IL-18 is produced locally by
acinar cells, intraducts, and CD68 macrophages in salivary glands [2, 13] of pSS patients.
Taken together, these evidences suggest that IL-18 might be involved in the pathogenesis of
pSS.
Maybe the most interesting finding of the present study is that the circulating levels of IL-18
and free IL-18 is associated with disease activity of pSS, with high levels of IL-18 and free
IL-18 in patients with active disease. To our knowledge, this is the first study showing the
association of circulating levels of IL-18 with disease activity of pSS. The levels of IL-18 have
been reported to be associated with the disease activity of other autoimmune diseases such as
SLE and ITP [14, 15]. Taken together, this suggests that the circulating levels of IL-18 might be associated with disease activity of autoimmune diseases in general, indicating a pathogenic role in the development of autoimmune diseases.

Disturbance of the cytokine network is one hallmark of pSS [1, 16]. pSS has long been thought to be associated with Th1-related cytokine, e.g. IFN-γ dysregulation, while recent studies further showed that Th17-related cytokines are also associated with pSS. Since IL-18 is able to enhance both the Th1 and Th17 response [3, 16], this might be a possible mechanism of the role of IL-18 in the pathogenesis of pSS. The pathogenic role of IL-18 has been shown in many mouse models of autoimmune diseases [17]. For example, IL-18 deficiency (IL-18−/− mice) suppressed the production of the pathogenic antibody and prevent the mice from the development of disease in a murine model of myasthenia gravis [18]. This makes the IL-18 a favorite molecule of therapeutic target, and so far many approaches of neutralizing endogenous IL-18 have been developed, including neutralizing antibodies to IL-18, IL-18 receptor blocking antibodies, IL-18BP, and caspase-1 inhibitors [16]. Those therapeutic approaches have been shown to be effective in modulating the severity of many experimental models of many autoimmune diseases [16]. The association of IL-18 with disease activity of pSS demonstrated in this study encourages that the IL-18-targeting therapeutic approaches could be also evaluated in pSS and its experimental models.

In summary, our results show the levels of IL-18, but not IL-18BP or sIL-18R1 is higher in pSS patients than in controls. Furthermore, the levels of IL-18 in patients with active disease are significantly higher than in patients with mild disease. To our knowledge, this is the first study that shows an association between IL-18 levels and the disease activity of pSS.
Acknowledgements

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Conflict of interest

None
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### Table 1 Characteristics of pSS patients and control participants

| Participant characteristics | Control (n=35) | pSS (n=35) |
|-----------------------------|---------------|------------|
| Mean age, yr                | 44.43±12.88   | 43.46±12   |
| Sex, n(%)                   |               |            |
| male                        | 5             | 5          |
| female                      | 30            | 30         |
| xerophthalmia (%)           |               | 19 (54.3)  |
| xerostomia (%)              |               | 23 (65.7)  |
| Anti-Ro/SSA(%)              |               | 31 (88.6)  |
| Anti-La/SSB(%)              |               | 14 (40)    |
| ANA(%)                      |               | 28 (80)    |
| Anti-Ro52(%)                |               | 29 (82.9)  |
| RF(%)                       |               | 20 (57.1)  |
| C3↓ (%)                     |               | 15 (42.9)  |
| C4↓ (%)                     |               | 14 (40)    |
| IgG↑ (%)                    |               | 21 (60)    |
| IgA↑ (%)                    |               | 3 (8.6)    |
| IgM↑ (%)                    |               | 3 (8.6)    |
| Focus score(1-6)            |               | 20 (57.1)  |
| CRP↑ (%)                    |               | 9 (25.7)   |
| Disease duration(Year)      | 2.71±3.18     |            |
| ESSDAI(>0)                  | 9.3±6.8       |            |
| Immunosuppressive drug(%)   | 7 (20)        |            |
| Hydroxychloroquine(%)       | 24 (68.6)     |            |
| Corticosteroids (%)         | 27 (77.1)     |            |
| IL-18 (pg/mL)               | 65.03±45.70   | 119.73±100.06§ |
| IL-18BP (ng/mL)             | 20.33±4.44    | 21.11±10.79 |
| IL-18R1 (pg/mL)             | 262.89±123.18 | 310.53±160.00 |
| Free IL-18 (pg/mL)          | 16.83±12.31   | 31.90±28.25§ |

*note: data are presented as mean ±SD.*

*Compared with healthy controls, P<0.01, by Mann-Whitney Test,*
Figure 1. Association of IL-18 with disease activity of pSS. The associations of disease activity with IL18 (A) and free IL-18 (B) and the association of disease duration with IL18 (C) and free IL-18 (D) are presented. The disease activity is presented as ESSDAI, where the ESSDAI scores of 0-7 were regarded as mild disease and the ESSDAI scores of >7 were regarded as active disease. The significance was determined using the Mann-Whitney U-test. Significant $P$ values are indicated.