

The IL33/ST2 axis in Sjogren syndrome in relation to disease activity

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Abstract. – OBJECTIVE: Primary Sjogren's Syndrome (pSS) is a systemic autoimmune disorder characterized by infiltration of the exocrine glands leading to secretory insufficiency. Despite the progress made in understanding the pathogenesis of the SS, many aspects remain to be clarified. Interleukin-33 (IL33) is a recently discovered cytokine, belonging to IL-1 superfamily. IL33 and its soluble receptor ST2 were implied in a number of immune and in autoimmune diseases pathogenesis. In this work, we analyzed expression of IL33 and ST2 in Sjogren's syndrome.

PATIENTS AND METHODS: Serum IL-33 and soluble ST2 were analyzed using commercial ELISA kit in 15 pSS, 9 patients with Systemic Lupus Erythematosus and 9 controls.

RESULTS: We found significant hyperexpression of sST2 in sera of SS patients and SLE patients compared to healthy subjects ($p = 0.04$ and $p = 0.07$, respectively). In pSS, sST2 levels in pSS positively correlated with activity index SSDAI ($r = 0.662$, $p = 0.007$). In SLE, we found positive correlation between ST2 and SLEDAI 2K ($r = 0.685$, $p = 0.04$). Circulating levels of IL-33 were detectable in 2 of 15 SS patients, in 2 SLE patients and in 1 of control subjects.

CONCLUSIONS: We found an hyperexpression of sST2 in pSS and SLE patients with a possible immune modulatory role, because of a substantial suppression of circulating IL33. In our pSS and SLE cohort, sST2 levels were in correlation with disease activity indices.

Key words:

Interleukin-33, ST2, Sjogren's syndrome, Disease activity index.

Introduction

Primary Sjogren's Syndrome (pSS) is a systemic autoimmune disorder characterized by focal lymphocytic infiltration of the exocrine glands, mainly salivary and lacrimal ones, leading to secretory insufficiency^{1,2}. However, dis-

ease progression may extend to other exocrine glands and any mucosal surface³. Despite the progress made in understanding the pathogenesis of the pSS, many aspects remain to be clarified^{4,5}. Crescent data suggest a crucial role of innate immunity and epithelial cells damage in early events of disease, through the upregulation of toll-like receptors, the secretion of damage associated molecular pattern molecules and the activation of the type I interferon (IFN) system (the IFN signature)^{6,7}. Concerning adaptive immunity, disturbance in number and function of double negative T cells, T regulatory cells and follicular T helper cells (Tfh) has been reported. B cells hyperactivity is the hallmark of SS leading to hypergammaglobulinaemia, autoantibody production, disturbances of B cell subpopulations, formation in the salivary glands and an increased risk of developing B cell lymphoma⁸.

Interleukin (IL)-33 is a recently discovered IL-1 family member and is a ligand of a receptor consisting of two molecules: IL-1 receptor related protein (IL-1R1 or ST-2) and IL-1 receptor accessory protein (IL-1RAcP), requiring for signal transduction. IL-33 can bind trans-membrane ST2 leading to transduction cascade or soluble ST2 (sST2) with decoy receptor function^{9,10}. The biologic function of IL-33/ST2 system is complex. ST2 is expressed by a number of cells as lymphocytes in particular Th2 cells, macrophages, mast cells and innate lymphoid cells. IL-33 can be secreted by epithelial cells in response to cell injury driven by infections or allergens, acting as alarmin¹¹. IL-33 is involved in immune responses in barrier tissue as skin, gut, bronchial mucosa. Depending on immunological context, IL-33/ST2 plays a role in innate and adaptive immune responses activation or in immunoregulation^{12,13}. Crescent data suggest an involvement of IL-33/ST2 system in pathogenesis of autoimmune diseases, as systemic sclerosis, rheumatoid arthri-

tis and systemic lupus erythematosus (SLE)¹⁴. Recent works described high levels of IL-33 and sST2 in pSS^{15,16}. To further understand its role in pSS, we investigated IL-33/ST2 expression in pSS in relation to disease activity, in a cohort of patients with low disease activity.

Patients and methods

Study Population

15 patients affected by pSS according to revised American-European classification criteria were enrolled in University Campus Bio-Medico outpatients clinic¹. Moreover 9 patients with SLE and 10 healthy subjects were recruited as control cohort¹⁷. Patients and controls were female. Local Ethics Committee approved the study and informed consent was obtained from all subject enrolled.

Clinical Evaluation and Laboratory Assessment

Medical history comprising disease features and medication were evaluated for pSS and SLE patients. Disease activity index were assessed: Sjogren Syndrome Disease Activity Index (SS-DAI) in pSS and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) 2K in SLE^{18,19}. All subjects enrolled underwent to peripheral blood analysis for complete blood count, liver enzymes, creatinine, blood-urea nitrogen (BUN), coagulation, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), Immunoglobulin G (IgG), IgA, IgM, complement fraction 3 (C3) and complement fraction 4 (C4), lactic dehydrogenase (LDH), protein electrophoresis, antinuclear antibodies (ANA). In pSS and SLE, anti-dsDNA, anti-SSA/Ro, anti-SSB/La, anti-Sm antibodies, 24 hour proteinuria were evaluated.

IL-33/ST2 system determination

IL-33 and sST2 were measured by ELISA according to manufacturer's protocol (R&D Systems, Minneapolis, MN, USA).

Statistical Analysis

IL-33 and sST2 levels between patients and control were compared using Student's *t*-test. Correlation of IL-33 and sST2 with clinical and laboratory variable were calculated using Spearman's correlation test. Statistical analysis was performed by GraphPad Prism 5 (GraphPad Software, Inc, San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

Results

Demographic and disease features of pSS, SLE and healthy subjects were reported in Table I.

We found significant hyperexpression of sST2 in sera of SS patients and SLE patients compared to healthy subjects ($p = 0.04$ and $p = 0.07$, respectively) (Figure 1). In particular, mean sST2 levels were 18980 ± 1668 pg/mL in pSS, 22377 ± 4100 pg/mL in SLE and 13741 ± 1731 pg/mL in control subjects.

Circulating levels of IL-33 were detectable in 2 of 15 SS patients, in 2 SLE patients and in 1 of control subjects.

sST2 levels in pSS positively correlated with activity index SSDAI ($r = 0.662$, $p = 0.007$) (Figure 2). We found a positive correlation between sST2 and SLEDAI 2K among SLE patients ($r = 0.685$, $p = 0.04$) (Figure 3).

Discussion

SS is a complex disease affecting esocrine glands, with a potential systemic involvement and evolution in hematologic malignancies^{1,3}.

Crescent data contributed to clarify significant aspects of pSS pathogenesis in recent years. However, the immunological phenomena involved in disease initiation and progression are still unknown. As a consequence, effective therapies for pSS are not available yet⁴⁻⁸.

IL33/ST2 system is a recently discovered immunologic pathway with a multiplicity of biologic functions. This system is involved in infective and allergic diseases, as a part of mucosal immune response. Moreover, IL33/ST2 seems to have a role in pathogenesis of several autoimmune diseases⁹⁻¹⁴.

Our data demonstrated an hyperexpression of sST2, the soluble form of IL33 receptor, in both pSS and SLE patients. These finding are in line with previous literature data.

Awada et al¹⁵ shown increased levels of IL33 and ST2 in sera and salivary glands of pSS patients. In this work, IL33 serum levels in pSS were detectable in a large number of pSS subjects in contrast to what observed in SLE and in healthy controls. Similar data were reported by Jung et al¹⁶.

In the study by Awada et al¹⁵ IL33 acted synergistically with IL-12 and IL-23 to promote IFN γ production. Jung et al¹⁶ demonstrated that

Table I. Demographics of study cohort.

	pSS	SLE	Healthy Subjects	<i>p</i>
Number, N	15	9	10	NS
Age, years, mean $\pm$ SD	52.4 $\pm$ 13	50 $\pm$ 11	51 $\pm$ 9	NS
Sex, M/F	0/15	0/9	0/10	NS
Current smokers, N (%)	0 (0)	0 (0)	0 (0)	NS
Kidney disease*, N (%)	0 (0)	0 (0)	0 (0)	NS
Disease duration, years, mean $\pm$ SD	6.6 $\pm$ 3	7.2 $\pm$ 3.5		NS
SSDAI (punti)	2.4 $\pm$ 2.1			
SLEDAI-2K		8 $\pm$ 3.46		
ESR (mm/h), range 0-20, mean $\pm$ SD	18.7 $\pm$ 8.2	19.0 $\pm$ 6.5	12.4 $\pm$ 6.2	0.05 pSS vs CTR
CRP (mg/dl), range 0-5, mean $\pm$ SD	4.2 $\pm$ 2.0	5.3 $\pm$ 3.4	3.2 $\pm$ 2.4	0.3 pSS vs CTR
RF positive, N (%)	8 (53.3)	2 (22.2)	0 (0)	
ANA $\geq$ 1:160, N (%)	15 (100)	9 (100)	0 (0)	
Anti-SSA positive, N (%)	11 (73.3)	3 (33.3)	0 (0)	
Anti-SSB positive, N (%)	7 (46.6)	1 (11.1)	0 (0)	
Anti-dsDNA positive, N (%)	0 (0)	4 (44.4)	0 (0)	
Anti-Sm positive, N (%)	0 (0)	3 (33.3)	0 (0)	
C3 below inferior limit, N (%)	0 (0)	4 (44.4)	0 (0)	
C4 below inferior limit, N (%)	0 (0)	3 (33.3)	0 (0)	
Mean daily prednisone dosage, mg, mean $\pm$ SD	5 $\pm$ 2.3	8.7 $\pm$ 3.5	0	
Oral prednisone therapy, N (%)	11 (73.3)	7 (77.7)	0 (0)	
Antimalarial, N (%)	11 (73.3)	9 (100)	0 (0)	
Azathioprine, N (%)	5 (33.3)	7 (77.7)	0 (0)	
Methotrexate, N (%)	4 (26.6)	2 (22.2)	0 (0)	
Other immunosuppressant, N (%)	0 (0)	0 (0)	0 (0)	

*Kidney disease: clinical renal disease (nephrosic syndrome, nephritic syndrome, refractory arterial hypertension), isolated urinary abnormalities, histology of WHO class III, IV, V or VI SLE glomerulonephritis.

Table legend: M, male; F, female; SSDAI, SS disease activity index; SLEDAI 2K, SLE disease activity index 2000, ESR, erythrocyte sedimentation rate, CRP, C-reactive protein, ANA, antinuclear antibodies, RT, rheumatoid factor.

IL33 production by salivary gland epithelial cells is stimulated by Interferon-gamma (IFN-gamma).

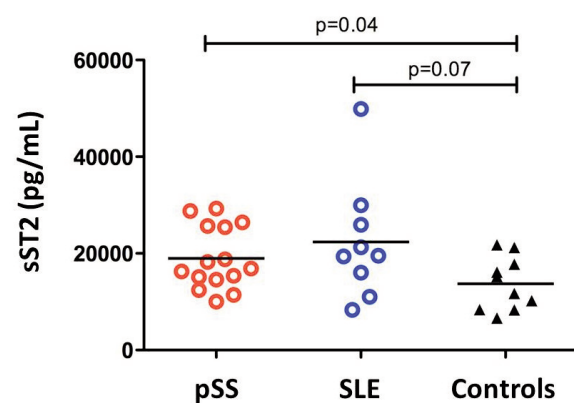


Figure I. Mean levels of soluble ST2 (sST2) in pSS, SLE and healthy controls.

We found detectable circulating IL33 in a minority of pSS and SLE patients. This finding could be explained by the low disease activity in our pSS cohort (mean SSDAI 2.4 point, with only three patients above 4). The increased sST2 levels that we observed in pSS and SLE in relation to the low expression of IL33 could be part of an immune regulatory phenomenon.

Moreover we found that sST2 levels were in relation to disease activity indices SSDAI in pSS and SLEDAI 2K in SLE patients. A relation between IL33/ST system and pSS disease activity was recently described by Jung et al¹⁶.

Conclusions

We found an hyperexpression of sST2 in pSS and SLE patients with a possible immune modulatory role, because of a substantial suppression of circulating IL33. In our pSS and SLE cohort,

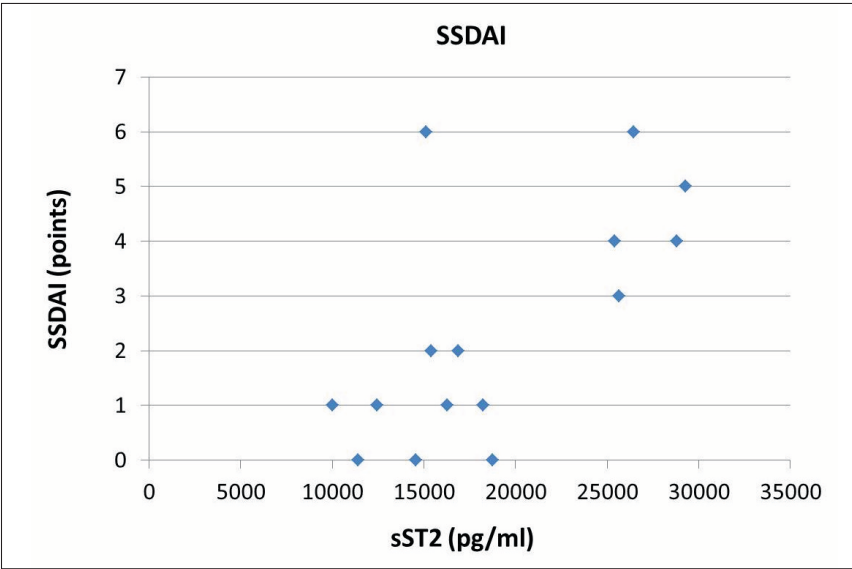
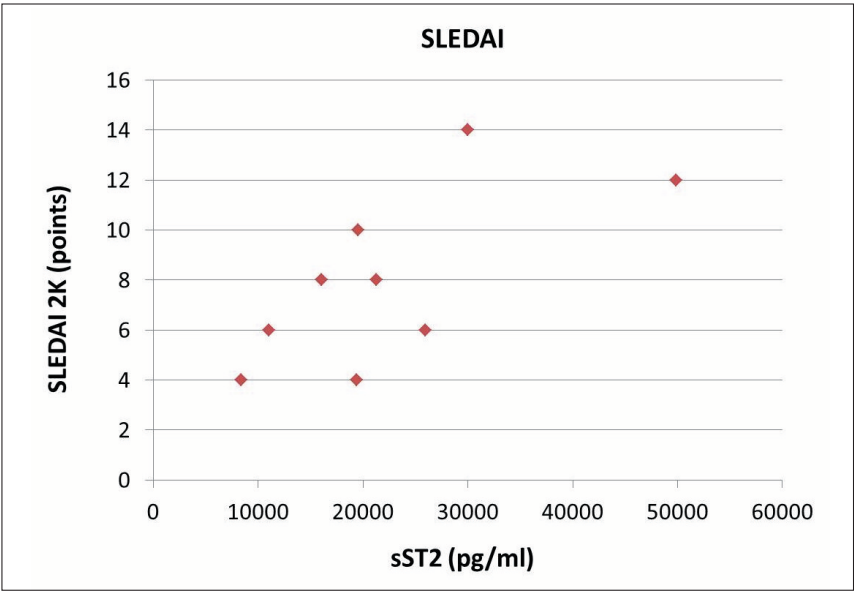


Figure II. Correlation between serum sST2 and SSDAI in pSS.

Figure III. Correlation between serum sST2 and SLEDAI 2K in SLE.



sST2 levels were in correlation with disease activity indices.

This study confirms the importance of proinflammatory cytokines in autoimmune diseases as previously demonstrated^{20,21}.

Further studies are required to explore sST2 immune regulatory action, in order to evaluate the possibility of therapeutic implications.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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