Size matters: decreased glandular levels of anti-inflammatory short thymic stromal lymphopoietin in primary Sjögren's syndrome

Sirs,

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterised by lymphocytic infiltration of the exocrine glands and dryness of mouth and eyes (1, 2). We recently found decreased protein and mRNA expression of the cytokine thymic stromal lymphopoietin (TSLP) in labial salivary glands (LSG) of patients with pSS compared to non-Sjögren's sicca (nSS) controls, associated with markers of increased disease activity (3). These data contrasted with the data on TSLP in other rheumatic autoimmune diseases, which showed that TSLP plays a pro-inflammatory role (4, 5). This contrast could be related to the expression of different TSLP isoforms.

The presence of a short TSLP isoform (shTSLP) in addition to the most often studied full-length (long) isoform (lTSLP) had been demonstrated before, and it was shown to have antimicrobial properties (6, 7). Interestingly, a recent paper showed that shTSLP also functions as a homeostatic cytokine with anti-inflammatory properties (8). Unlike lTSLP, shTSLP does not bind and signal through the TSLP receptor/IL-7 receptor alpha chain heterodimer and is expressed in steady state, while its expression is decreased upon inflammation in the gut and skin. In contrast, lTSLP triggers pro-inflammatory responses and is induced upon inflammation (7, 8). As the expressed isoform of TSLP seems to be crucial in determining tissue homeostasis, we studied expression of both isoforms separately in the pSS LSG.

Twenty-three pSS patients were diagnosed according to American-European consensus group criteria (9). Thirteen sicca syndrome patients not fulfilling these criteria were defined as nSS (Table I). The institutional review board (TcBio) of the UMC Utrecht approved the use of left-over patient material in anonymous manner. Total RNA was isolated from LSG biopsy samples using AllPrep Universal kit (Qiagen, Valencia, CA USA) and QIAcube system (Qiagen). TSLP gene expression was determined by quantitative real-time PCR using the Quantstudio 12k flex RT-PCR system (LifeTechnologies). Results were normalized to expression of GAPDH. Relative expression was calculated by using the ΔCT method and dividing by a sample in the control group, which was set at 1. T-tests were used to evaluate differences between groups, values of $p<0.05$ were considered statistically significant.

Table I. Patients' characteristics.

	nSS	pSS
N (M/F)	13 (2/11)	23 (1/22)
Age (yr.)	47 ± 13	47 ±14
ESR (mm/hour)	11 ± 6.4	18 ± 17
sIgG (g/L)	10 ± 2.7	17 ± 9.1
LFS (foci/ 4mm^2)	0.1 ± 0.3	2.4 ± 1.6
%IgA+	77 ± 15	48 ± 15
C3 (g/L)	1.3 ± 0.4	1.1 ± 0.2
C4 (g/L)	0.3 ± 0.0	0.2 ± 0.1
ESSDAI	-	1.6 ± 2.8
ANA (pos/neg)	4/9	17/6
SSA (pos/neg)	2/11	17/6
SSB (pos/neg)	0/13	8/15
Anti-inflammatory therapy (pos/neg)	4/5	8/10

nSS: non-Sjögren's Sicca; pSS: primary Sjögren's syndrome; ESR: erythrocyte sedimentation rate; sIgG: serum IgG; LFS: lymphocyte focus score; ESSDAI: EULAR Sjögren's syndrome disease activity index; ANA: anti-nuclear antibodies; SSA: anti-SSA/Ro; SSB: anti-SSB/La; Values are mean ± SD unless stated otherwise. Therapy status data were unavailable for 4 nSS and 5 pSS patients.

In nSS LSG, lTSLP was expressed at very low levels while shTSLP was robustly expressed in all samples and mean shTSLP expression levels were markedly higher (Fig. 1A). lTSLP expression was undetectable in the majority of LSG samples from both groups and no differences in expression were observed. However, shTSLP mRNA was significantly lower in pSS patients as compared to those with nSS. This was also true for total TSLP, in line with previously published results from our group (3) (Fig. 1B). In addition, the expression of shTSLP strongly correlated with that of total TSLP (Fig. 1C).

Thus, the lower expression of TSLP in pSS LSG is attributable to the shTSLP isoform while lTSLP is almost absent. These data are in line with expression in the gut and skin, where shTSLP is expressed in the steady state and decreases upon inflammation (8). In addition, the identification of shTSLP as the predominant isoform in salivary glands could explain the contrast with the increased TSLP expression in other autoimmune diseases (10), which can be assumed to be mainly lTSLP.

Although lTSLP was only detectable in a small number of patients, this was not associated with any specific clinical features in our analyses. As we previously observed that patients with lower TSLP protein levels had higher markers of disease, including LFS and serum IgG (3), the decrease in shTSLP fits with its described homeostatic anti-inflammatory role (8, 11). Since loss of shTSLP expression contributes to increased proinflammatory cytokine production (8), decreased shTSLP production is likely to promote immunopathology in pSS. Furthermore, as shTSLP functions as an antimicrobial peptide, decreased expression may contribute to loss of barrier function and increased tooth decay and oral infections in pSS patients (1). These data urge for additional research into the role of shTSLP in pSS immunopathology.

M.R. HILLEN, *PhD*[1,2]
F.M. MORET, *PhD*[1,2]
B. GIOVANNONE, *PhD*[2,3]
A.A. KRUIZE *MD, PhD*[1]
T.R.D.J. RADSTAKE, *MD, PhD*[1,2]
J.A.G. VAN ROON, *PhD*[1,2]

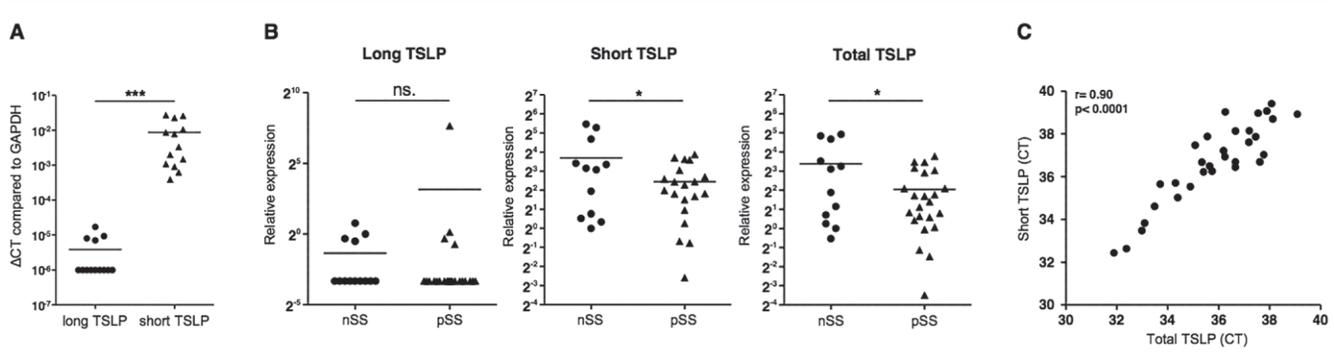

Fig. 1. Short TSLP is the predominant TSLP isoform in salivary glands and is decreased in pSS. Total mRNA was isolated from LSG of pSS and nSS patients and qPCR was performed using three primer sets: One specific for the long TSLP isoform, one specific for the short TSLP isoform, and one that binds a region present in both molecules and thus recognises both isoforms (total TSLP). In salivary glands of nSS patients, short TSLP is expressed at a much higher level than long TSLP is (**A**). Long TSLP expression is below detection limit in most donors in both groups. Expression of short TSLP and total TSLP is decreased in pSS patients as compared to nSS patients (**B**). There is a very strong correlation between the expression of shTSLP and that of total TSLP in all samples (**C**). Samples below detection limit for long TSLP were set at 10^{-6} ΔCt or relative expression of 0.1 for plotting. Means are depicted in dotplots. *and ***indicate *p*-values of $p<0.05$ and $p<0.0001$, respectively.

Letters to the Editors

[1]Department of Rheumatology and Clinical Immunology, [2]Laboratory of Translational Immunology, [3]Department of Dermatology, University Medical Center, Utrecht, the Netherlands.

Please address correspondence to: Dr Joel van Roon, Lundlaan 6, 3508 GA Utrecht, The Netherlands. E-mail: j.vanroon@umcutrecht.nl

Reprints will not be available from the authors.

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