



**HAL**  
open science

## Interleukin-7/Interferon axis drives T-cell and salivary gland epithelial cell interactions in Sjögren's syndrome

Elodie Rivière, Juliette Pascaud, Alexandre Virone, Anastasia Dupré, Bineta Ly, Audrey Paoletti, Raphaèle Seror, Nicolas Tchitchek, Michael Mingueneau, Nikaïa Smith, et al.

### ► To cite this version:

Elodie Rivière, Juliette Pascaud, Alexandre Virone, Anastasia Dupré, Bineta Ly, et al.. Interleukin-7/Interferon axis drives T-cell and salivary gland epithelial cell interactions in Sjögren's syndrome. *Arthritis & rheumatology*, 2020, 73 (4), pp.631-640. 10.1002/art.41558 . pasteur-03036519

**HAL Id: pasteur-03036519**

**<https://pasteur.hal.science/pasteur-03036519>**

Submitted on 2 Dec 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright



MRS. ELODIE RIVIÈRE (Orcid ID : 0000-0003-1010-3081)

DR. AUDREY PAOLETTI (Orcid ID : 0000-0001-8979-385X)

PROF. RAPHAËLE SEROR (Orcid ID : 0000-0002-5523-1856)

DR. GAETANE NOCTURNE (Orcid ID : 0000-0001-6809-0733)

Article type : Full Length

**Running head:** IL-7 – IFN axis in Sjögren's syndrome

**Interleukin-7/Interferon axis drives T-cell and salivary gland epithelial cell interactions in Sjögren's syndrome.**

Elodie Rivière, MD, PhD<sup>1,2\*</sup>, Juliette Pascaud, BSc<sup>1\*</sup>, Alexandre Virone, MD<sup>1</sup>, Anastasia Dupré, MD<sup>1</sup>, Bineta Ly, BSc<sup>1</sup>, Audrey Paoletti, PhD<sup>1</sup>, Raphaèle Seror, MD, PhD<sup>1</sup>, Nicolas Tchitchek, PhD<sup>1</sup>, Michael Mingueneau, PhD<sup>3</sup>, Nikaïa Smith, PhD<sup>4</sup>, Darragh Duffy, PhD<sup>4</sup>, Lydie Cassard, BSc<sup>5</sup>, Nathalie Chaput, PhD<sup>5</sup>, Sabrina Pengam, BSc<sup>6</sup>, Vanessa Gauttier, PhD<sup>6</sup>, Nicolas Poirier, PhD<sup>6</sup>, Xavier Mariette, MD, PhD<sup>1\*\*</sup>, Gaetane Nocturne, MD, PhD<sup>1\*\*</sup>

<sup>1</sup>Université Paris-Saclay, INSERM, CEA, Centre de recherche en Immunologie des infections virales et des maladies auto-immunes; AP-HP. Université Paris-Saclay, Hôpital Bicêtre, Rheumatology department, 94270, Le Kremlin Bicêtre, France, <sup>2</sup>Arthritis R&D, France, <sup>3</sup>Immunology Research, Biogen, Cambridge, United States, <sup>4</sup>Immunobiology of Dendritic Cells laboratory, INSERM U1223/Institut Pasteur, Paris, France, <sup>5</sup>Université Paris-Saclay, Institut Gustave Roussy, Inserm, CNRS, Analyse moléculaire, modélisation et imagerie de la maladie cancéreuse, Laboratoire d'Immunomonitoring en Oncologie, F-94805, Villejuif, France, <sup>6</sup>OSE Immuno-therapeutics, Nantes, France.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/ART.41558](https://doi.org/10.1002/ART.41558)

This article is protected by copyright. All rights reserved

\*the 2 junior authors have equally participated to the work

\*\* the 2 senior authors have equally participated to the work

**Corresponding author:** Dr Gaetane Nocturne, Department of Rheumatology, Université Paris-Sud, AP-HP, Hôpitaux Universitaires Paris-Sud, INSERM U1184, 78 rue du Général Leclerc, 94275 Le Kremlin Bicêtre, France, phone: +33 1 45 21 37 58, fax : +33 1 45 21 37 57, e-mail : gaetane.nocturne@aphp.fr

**Financial support:** This study was supported by the Labex in Research on Medication and Therapeutic Innovation (LERMIT) (ANR10), the Fondation pour la Recherche Médicale DEQ20150934719 and an unrestricted grant (UPSud/SAIC N 97731) from Biogen to Université Paris-Sud, and the Innovative Medicines Initiative 2 Joint Undertaking (JU) (NECESSITY grant agreement No 806975). This JU received support from the European Union's Horizon 2020 research and innovation program and EFPIA. E.R. is the recipient of a PhD fellowship from Arthritis Fondation Courtin, Arthritis R&D (CIFRE 2016/1406). The Laboratoire d'Immunomonitoring en Oncologie is supported by SIRIC SOCRATE 2.0 (INCa-DGOS-INSERM\_12551).

**Disclosure of potential conflict of interest:** Elodie Rivière, Juliette Pascaud, Alexandre Virone, Anastasia Dupré, Bineta Ly, Audrey Paoletti, Raphaële Seror and Nicolas Tchitchek had no financial disclosures. Michael Migueneau is employed by Biogen. Nikaïa Smith, Darragh Duffy, Lydie Cassard, Nathalie Chaput had no financial disclosures. Sabrina Pengam, Vanessa Gauttier and Nicolas Poirier are employed by OSE Immuno-therapeutics, a company owning IL-7R antagonists. Xavier Mariette received an honorarium from Servier and research grants from OSE Immuno-therapeutics and from Servier. Gaetane Nocturne had no financial disclosures.

**Number of words:** 4145

## ABSTRACT

**Objective:** Primary Sjögren's syndrome (pSS) is characterized by a lymphocytic infiltration of salivary glands and the presence of an interferon (IFN) signature. Salivary gland epithelial cells (SGECs) play an active role in pSS pathophysiology. The aim of this work was to study the interactions between SGECs and T cells in pSS and the role of the IL-7/IFN axis.

**Methods:** Primary cultured SGECs from pSS and controls were stimulated with poly(I:C), IFN $\alpha$  or IFN $\gamma$ . T cells were sorted from blood and stimulated with IL-7. CD25 expression was assessed by flow cytometry. Salivary gland explants were cultured for 4 days with anti-IL-7R antagonist antibody (OSE-127) and transcriptomic analysis was performed by using nanostring.

**Results:** IL-7 serum level was increased in pSS patients versus controls and was associated with B-cell biomarkers. *IL7R* expression was decreased in T cells from pSS patients versus controls. SGECs stimulated with poly(I:C), IFN $\alpha$ , or IFN $\gamma$  secreted IL-7. IL-7 stimulation increased the activation of T cells, as well as IFN $\gamma$  secretion. Transcriptomic analysis of salivary gland explants showed a correlation between *IL7* and *IFN* expression. Lastly, explants cultured with anti-IL-7R antibody showed decreased IFN-stimulated gene expression.

**Conclusion:** These results suggest an IL-7-IFN $\gamma$  amplification loop involving SGECs and T cells in pSS. IL-7 was secreted by SGECs stimulated with type 1 or type 2 IFN and, in turn, activate T cells that secrete type 2 IFN. An anti-IL-7R antibody decreased the IFN signature in T cells during pSS and could be of therapeutic interest.

**Key words:** Sjögren's syndrome, Interleukine-7, T cells, Interferon

## INTRODUCTION

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease characterized by a lymphocytic infiltration of salivary glands. The major components of the salivary gland infiltrate are T and B lymphocytes. Salivary gland epithelial cells (SGECs) can be a driver and also a passive culprit of disease pathophysiology. Autoreactive effector T cells could play a pathogenic role in pSS in different ways, including providing help for promoting B-cell hyperactivity (1). One characteristic of pSS is the presence of an interferon (IFN) gene signature in circulating leukocytes and in salivary glands of pSS patients (2-5). However, the initial trigger responsible for this IFN signature is still not identified, nor is the predominant involvement of type 1 or type 2 IFNs.

Interleukin-7 (IL-7) is a pleiotropic cytokine produced by non-hematopoietic cells, such as stromal and epithelial cells, that plays a central role in T lymphocyte homeostasis. Several observations highlighted the potential role of the IL-7/IL-7R $\alpha$  axis in pSS pathophysiology. The levels of IL-7 and its receptor, IL-7R $\alpha$ , also known as CD127, were found elevated in salivary glands of patients with pSS (6). Bikker *et al.* showed by immunohistochemistry an association between the presence of IL-7R $\alpha$  positive T cells in the salivary glands of patients with pSS, and the severity of sialadenitis and IL-7 expression (7). IL-7 activity might be modulated by the soluble form of its receptor (sIL-7R). Of note, Lundström *et al.* showed a diminished consumption of IL-7 in the presence of sIL-7R $\alpha$  (8). Interestingly, Hillen *et al.* showed an increase of sIL-7R level in serum and salivary gland supernatant of patients with pSS with increased inflammation and decreased salivary output (9). Moreover, the IL-7/IL-7R $\alpha$  axis has been found involved in the formation of ectopic lymphoid structures in salivary glands (10). Interestingly, Jin *et al.* showed that exogenous IL-7 administration accelerated pSS onset in a mouse model, whereas blockade of endogenous IL-7R $\alpha$  signals prevented its development (11). Lastly, IL-7 stimulation of T cells *in vitro* enhanced IL-2, IL-10 and IFN $\gamma$  production which may also play a role in pSS (12,13).

From these findings, we aimed to study the interactions between SGECs and T cells in pSS and the impact of IL-7 in this process by using a fully antagonist anti-IL-7R monoclonal antibody (14).

## MATERIALS and METHODS

### Patients

Serum levels of cytokines and chemokines were assessed in patients from the French multicenter 5-year prospective Assessment of Systemic Signs and Evolution of Sjögren's Syndrome (ASSESS) cohort. In total, 395 patients have been included in this cohort. All patients fulfilled the American–European Consensus Group criteria for pSS (15). The baseline characteristics of the patients were previously described (16). Chemokine levels were also measured in 73 age- and sex-matched controls with symptoms of dry eyes and mouth but without any autoantibody and without lymphocytic infiltrates on minor salivary gland (MSG) biopsy.

MSG biopsies were obtained from consecutive patients referred for suspected pSS to the rheumatology department of Bicêtre hospital, a tertiary reference center for systemic autoimmune diseases. pSS was defined according to the 2016 ACR/EULAR criteria (17) or American–European Consensus Group criteria for pSS (15). The EULAR Sjögren Syndrome Disease Activity Index (ESSDAI) was used to assess pSS activity. Controls presented sicca symptoms without anti-Ro / SSA and anti-La / SSB antibodies and with normal or sub-normal MSG findings (i.e. focus score <1).

T lymphocytes used for *in vitro* experiments were sorted from blood of pSS patients and controls.

The experimental design of the study is presented in **Supp Figure 1**.

### Biological assessment

Serum samples were obtained at enrollment. All biological samples were immediately frozen, stored (-80°C) and shipped to the Centre de Ressources Biologiques of Bichat Hospital, Paris, which has obtained the French Association for Quality Insurance (AFAQ) certification (certification number 2009/34457) according to the norm 96900. Serum markers were assessed centrally and with blinding to any clinical or other biological data. Rheumatoid factor (RF) was assessed by Enzyme-Linked Immunosorbent Assay (ELISA), and C3 and C4 levels by nephelometry (decreased C3 and C4 level defined as < 0.8 and 0.15 g/l, respectively). Beta2-microglobulin and

total Ig levels and kappa and lambda free light chains of Igs were assessed by nephelometry with the Freelite kit (Binding Site, Birmingham, UK). Anti-Ro/SSA and anti-La/SSB antibodies were detected by ALBIA flow cytometry (Addressable laser bead immuno assay) with a Bioplex 2200 (Biorad). The detection was confirmed by the immunodot assay Ana 3b from Euroimmun. CD4 and CD8 T cell counts were determined by flow cytometry. CD4+ T lymphocytopenia was defined by an absolute CD4 count lower than 300 cells/ml.

#### **Assessment of IL-7, CXCL13, CCL19, CXCL10 and IFN levels**

The methods used for assessing IL-7, CXCL13, CCL19, CXCL10 and IFN levels are described in the **supplementary materials**.

#### **RNA-seq of CD4 and CD8 T cells sorted from salivary gland biopsies and blood**

IFN $\alpha$ , IFN $\gamma$  and IFN $\lambda$  gene expression was evaluated by RNA-seq in CD4 and CD8 T cells sorted from salivary gland biopsies and peripheral blood mononuclear cells. The methods of samples collection, cell isolation and RNA-seq are described in **supplementary materials**.

#### **PCR validation of RNA-seq differentially expressed genes**

The methods for PCR validation of RNAseq results are presented in **supplementary materials**.

#### **CD127, ICOS and PD1 assessment in CD4 and CD8 T cells**

Fresh whole blood (100 $\mu$ l) was incubated with fluorochrome-conjugated antibodies for 15 min at room temperature in the dark, followed by 20 min of lysis (Versalyse, Beckman Coulter, Mervue, Galway, Ireland) and washed twice with PBS for surface staining. For Ki67 and FoxP3 staining, cells were fixed and permeabilized after cell surface staining by using the PerFix-nc kit (Beckman Coulter) according to manufacturer instructions. Stained cells were acquired by using a Gallios flow cytometer (Beckman Coulter). Data were analyzed with Kaluza software (Beckman Coulter).

The antibodies used in the experiments are in **Supp Table 2**. The gating strategy is presented in **Supp Figure 2**.

### **Isolation of T lymphocytes and IL-7 stimulation**

Peripheral blood mononuclear cells were isolated from residual apheresis blood from pSS patients or healthy subjects (French blood donors) by Ficoll gradient separation. T lymphocytes were isolated by magnetic-bead negative selection according to the manufacturer's instructions (Pan T cells negative isolation kit, Miltenyi Biotec) to achieve a purity > 70% as assessed by FACS analysis (percentage of CD3+ cells in live cells). T cells were seeded at 1million/mL in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, and penicillin-streptomycin (1X) and stimulated with IL-7 0.1 or 2 ng/mL (Peprotech). T cells were harvested on day 1 or 3 for flow cytometry. Stimulated T lymphocytes were stained with CD3, CD4, CD8, CD127, CD25, or Fixable Viability Dye efluor 780. The antibodies used in the experiments are presented in **Supp Table 2**. Samples were analyzed by using a BD FACS Canto flow cytometer and BD FACS Diva Software (BD, Becton Dickinson, Germany). The results were analyzed with FlowJo10 software.

### **Primary cultures of SGECs and stimulation**

Primary cultures of SGECs were established from MSGs as previously described (18). After 2-3 weeks of culture, cells at 70–80% confluence were dissociated with 0.125% trypsin-EDTA. Cell suspension was suspended in basal epithelial medium and added at 80 000 cells/cm<sup>2</sup> to a 6-well collagen type I plate (Institut de Biotechnologies, Reims, France) coated and incubated at 37°C and 5% CO<sub>2</sub> in a humidified atmosphere. The basal epithelial medium was changed at day 1 to remove non-adherent epithelial cells. The epithelial origin of cultured cells was confirmed as routine by staining with monoclonal antibodies against CD326 (Miltenyi Biotec, Paris, France) an epithelium-specific marker. Stimuli were added in the medium: poly (I:C) 30 µg/mL (Invivogen), IFN-α 600 UI/mL (Roferon-A, Roche), IFN-γ 5 ng/mL (Sigma Aldrich) or IFN-λ (IL28) 25 ng/ml (Peprotech). Supernatants and SGECs were harvested after 72 hours and frozen (-80°C).



## qPCR

Total RNA from 24-hours stimulated and unstimulated SGEs was extracted by using the RNeasy Minikit (Qiagen) according to the manufacturer's specifications. Contaminating DNA was removed by using RNase-free DNase set (Qiagen) according to the manufacturer's instructions. A 1  $\mu$ g amount of RNA was used to produce cDNA with the First strand synthesis kit (Sigma Aldrich). The quantification of mRNA expression was determined by Taqman real-time PCR according to the manufacturer's instructions (Taqman, Life Technologies) with the TaqMan Gene Expression Master Mix (Life Technologies) using the CFX96 (Biorad). The level of *IL7* was normalized to that of the endogenous *GAPDH*. Calculation of mRNA expression levels was performed using the comparative Ct ( $\Delta\Delta$ Ct) method. Data analyses were performed using CFX Biorad Manager software.

## Culture of salivary gland explants

Each MSG was cut into two parts and cultured for 4 days in 200  $\mu$ L RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, and penicillin-streptomycin (1X) with OSE-127 humanized anti-human CD127 monoclonal antibody (anti-IL-7R) (OSE Immunotherapeutics, Nantes (14)) or control isotype (Ultra LEAF purified human IgG4 Isotype control recombinant (Biolegend)). After 4 days, supernatant was separated from the explants. Supernatant was centrifugated in order to isolate cells that escaped from the explant (pellet cells) and the explant itself (explant cells) (**Figure 5A**). The pellet and explant cells were collected in the RLT buffer of the RNeasy Mini kit (Qiagen) supplemented with beta-mercaptoethanol at 1% and frozen (-80°C) before RNA extraction.

## Nanostring gene expression

RNA from salivary gland biopsies (cells or explants) was extracted by using the FastPrep system. Gene expression was quantified with the NanoString nCounter platform with 15 to 50 ng total

RNA, according to the type of sample, in the nCounter Human Immunology Panel\_V2 (NanoString Technologies). The code set was hybridized with RNA overnight at 65°C. RNA transcripts were immobilized and counted by using the NanoString nCounter Sprint. Normalized expression data were analyzed with nSolver software. The statistical analysis of data did not involve multiple hypothesis testing.

### **Statistical analysis of IL-7 serum level and correlations**

Categorical variables are reported as number (percentage) and were compared by chi-square or when appropriate, Fisher's exact test. Quantitative variables are reported as median (interquartile range) or mean (SD) and were compared by using Mann-Whitney test. For correlation analyses between two quantitative variables, Spearman's correlation coefficients were calculated. In univariate analyses, the correlation/association between disease activity, serum chemokine levels, serum B cell biomarkers, and IL-7 serum level was assessed by Spearman's correlation coefficient for continuous data and Mann-Whitney U test for categorical data. All variables with p-value <0.05 in univariate analysis or  $R \geq 0.20$  were entered into a multivariate model to identify the factors independently associated with IL-7 serum level. Variables were selected by using backward selection. Statistical analyses were performed using SAS 9.3 statistical software (SAS Inst., Cary, NC).

### **Statistical analysis of RNA-seq profiles of sorted cells**

Reads were first quality control-filtered and trimmed by using trimmomatic (19). Paired reads were aligned to the Ensembl human reference genome (V38.79) (20) by using STAR software (v2.5.0c) (21). Statistical analyses involved the DESeq2 package (22). A cut-off of  $p < 0.05$  was used to define differentially expressed genes. The Interferome v2.01 database (23) was used to identify and characterize IFN-induced genes. Functional enrichment analysis of differentially expressed genes was performed for genes with absolute fold change value  $\geq 1.5$ , by using ingenuity pathway analysis software (Qiagen). The statistical analysis of data did not use multiple hypothesis testing.

### **Study approval**

The study received approval from the local ethics committee, and informed consent was obtained from all patients and controls.

## RESULTS

### Patients

The characteristics of patients included in the ASSESS cohort for serum cytokine evaluation were previously described (16). The characteristics of patients and controls included in the current study are presented in **Table 1** and the experimental design of the study is presented in **Supp Figure 1**.

### IL-7 serum level is increased in pSS and IL-7R expression on CD4 and CD8 T cells is decreased in pSS

Patients with pSS showed higher serum IL-7 level than controls: 5.47 ng/ml (3.33, 9.08) (median (interquartile range)) versus 3.03 ng/ml (1.90, 5.76);  $p < 0.0001$  (**Figure 1A**). Serum IL-7 level was positively correlated with B-cell activation markers, IFN-induced chemokines and disease activity markers (**Figure 1B**). A univariate analysis of clinico-biological parameters associated with IL-7 serum levels identified an association with anti-SSA and anti-SSB antibodies, RF positivity, lymphopenia, low C4 level and past or current lymphoma (**Figure 1C**). On multivariate analysis, serum IL-7 level was associated with anti-SSA antibody positivity, serum level of CXCL13, RF positivity, high  $\kappa$  light-chain and low C4 level (**Figure 1C**). Supporting this increase in serum IL-7 level, we observed a decrease of IL-7R (CD127) expression on CD4 and CD8 T cells from pSS versus controls ( $p < 0.05$  and  $p < 0.01$ , respectively) (**Figure 2A**), this result potentially reflecting IL-7R internalization after IL-7 binding (25). Of note, IL-7R expression was decreased on CD4 and CD8 T cells from pSS and controls cultured after IL-7 stimulation (**Supp Figure 3**). IL-7R MFI did not differ between pSS and controls CD4 and CD8 T cells (data not shown). Nor did *IL7R* gene expression differ between pSS and control at the transcriptomic level (**Supp Figure 4**).

The comparison of PD1 expression on CD8 and CD4 T cells between CD127+ and CD127- T cells showed an increased expression of PD1 on CD127- CD8 and CD127- CD4 T cells from pSS patients ( $p < 0.05$  and  $p < 0.0001$ , respectively) (**Figure 2B**). Also, ICOS expression was increased on CD127- CD4 T cells as compared with CD127+ CD4 T cells from pSS patients ( $p < 0.001$ ) (**Figure 2C**).

### **SGECs are a source of IL-7**

We previously showed that SGECs sorted from pSS and control salivary glands, expressed *IL7* RNA and that this expression was higher in SGECs from pSS patients than controls (24). Given this result, we hypothesized that SGECs could be a source of IL-7 in pSS. Primary cultured SGECs from pSS patients and controls stimulated with poly(I:C), IFN $\alpha$ , IFN $\gamma$ , and IFN $\lambda$  could secrete IL-7 (**Figure 3A**). *IL7* mRNA expression was confirmed by quantitative RT-PCR (**Figure 3C**). The protein levels of IL-7 after IFN $\alpha$ , IFN $\lambda$  and poly(I:C) stimulation were higher in SGECs from pSS patients versus controls (**Figures 3B**).

### **Relationship between IL-7 and type 1 and type 2 IFNs in blood and salivary glands**

IL-7 stimulation (2 ng/mL) of blood T cells increased CD25 expression in CD4 and CD8 T lymphocytes as compared with the unstimulated condition both in pSS patients and control CD4 and CD8 T cells (**Supp Figure 5A and B**). Also, CD25 expression was higher in pSS CD4 T cells on day 1 (**Supp Figure 5C**), and CD8 T cells on day 3 (**Supp Figure 5D**) after stimulation with IL-7 (0.1 ng/mL) as compared with controls ( $p=0.03$ ). IFN $\gamma$  was detected in supernatants from T cells stimulated with IL-7 (2 ng/mL). There was a significant increase of IFN $\gamma$  secretion by IL-7 stimulated T cells from pSS patients, but not from controls (**Figure 4A**). As expected, IL-7 did not stimulate IFN $\alpha$  production in T cells (**Figure 4B**). Of note, in the whole salivary gland tissue, IL-7 expression was correlated with IFN expression: nanostring transcriptomic analysis of salivary gland explants from pSS showed a positive correlation between *IL7* mRNA expression and IFN $\alpha$ 1,  $\alpha$ 2,  $\beta$  and  $\gamma$  gene expression ( $R^2= 0.4, 0.5, 0.6$  and  $0.5$ , respectively) (**Figure 4C**).

### **IFN signaling pathway and IFN $\gamma$ expression are upregulated in pSS T cells**

The comparison of gene expression in sorted CD4 T cells from blood of pSS patients and controls showed 474 differentially expressed genes: 312 up-regulated and 162 down-regulated. Functional enrichment pathway analysis highlighted an over representation of the EIF2 and the

IFN signaling pathways, as well as Th1 and Th2 pathways (**Supp Table 1**). IFN induced genes such as *IFI27*, *IFIT1* and *IFI44L* were among the most significantly up-regulated genes in pSS patients as compared with controls ( $\log_2FC = 3.295$ ,  $3.029$  and  $2.552$ , respectively). Among the 312 upregulated genes in blood CD4 T cells from pSS patients, 198 were IFN regulated genes (type I =25, type II = 46, type I and II = 105 and types I, II and III =22). Of note, there was an up regulation of the *PDCD1* (programmed cell death 1) gene expression ( $\log_2$ Fold change =  $2.667$ ) in pSS patients versus controls.

When comparing gene expression in sorted CD8 T cells from blood of pSS patients and controls, 532 differentially expressed genes were detected: 325 up-regulated and 207 down-regulated. As in CD4 T cells, functional enrichment pathway analysis highlighted an over representation of EIF2 signaling and IFN signaling pathways in CD8 T cells (**Supp Table 1**). IFN induced genes such as *IFI44L*, *IFI44*, *OAS1*, *IFIT3* and *MX1* were up-regulated in pSS patients versus controls ( $\log_2FC = 4.073$ ,  $1.944$ ,  $1.735$ ,  $1.7$  and  $1.542$ , respectively). Among the 325 upregulated genes in blood CD8 T cells from pSS patients versus controls, 42 were IFN regulated genes (type I =1, type I and II = 20 and types I, II and III =21).

CD8 T cells sorted from salivary glands and from blood expressed *IFN $\gamma$*  and *IFN $\lambda$*  genes (**Supp Figure 6**). Interestingly, *IFN $\gamma$*  expression was upregulated in blood CD8 T cells from pSS patients versus controls ( $\log_2FC= 1.809$ ) (**Supp Figure 6**). *IFN $\gamma$*  expression was also detected in CD4 T cells from biopsies and blood. As expected, type I IFN expression (*IFN $\alpha$ 1*,  *$\alpha$ 2*,  *$\alpha$ 17* and  *$\beta$* ) was not detected in CD4 and CD8 T cells from biopsy and blood (*data not shown*).

In sorted CD4 T cells from biopsies of pSS patients and controls, 539 differentially expressed genes were detected: 305 up-regulated and 234 down-regulated. The enrichment analysis identified only 3 overrepresented pathways: 14-3-3-mediated signaling, IGF-1 signaling and Natural Killer Cell signaling (**Supp Table 1**).

In sorted CD8 T cells from biopsies of pSS patients and controls, 373 differentially expressed genes were detected: 207 up-regulated and 166 down-regulated. The enrichment analysis did not identify significant pathways (**Supp Table 1**).

Accepted Article

Lastly, 4 pSS patients had available data at the same time for T cells sorted from biopsy and blood. *IFN* $\gamma$  expression was upregulated in CD4 and CD8 T cells sorted from biopsies versus blood (Log2FC = 3.36, p-value = 0.02 and Log2FC = 3.64, p-value = 0.02, respectively). *IFNL1* was upregulated in CD4 T cells sorted from biopsies versus blood (Log2FC = 6.51, p-value = 0.011).

#### **IL-7R inhibition decreases IFN signature in salivary glands**

We analyzed the transcriptomic modifications induced by adding an anti-IL-7R antibody (OSE-127) to the culture of MSG explants from pSS patients. The pellet cells corresponded to the cells escaped from the explant during the culture, and explant cells corresponded to the explant itself (**Figure 5A**). The analysis of the mRNA signature revealed that inhibition of IL-7 signaling by OSE-127 decreased the IFN gene signature as assessed by decreased expression of *IFITM1* and *Mx-1*, both in explant and pellet cells (**Figure 5B**). Looking specifically at the different IFN subtypes, we observed that OSE-127 decreased the expression of *IFN* $\gamma$  specifically in pellet cells (**Figure 5C**).

## DISCUSSION

In this study, we found that IL-7 level was increased in pSS patients as compared with controls, with higher IL-7 levels in serum from patients and decreased IL-7R expression level in circulating T cells. Also, IL-7 serum level was associated with B-cell biomarkers and IFN related biomarkers in pSS such as anti-SSA antibody and CXCL13. In addition, SGECs were producers of IL-7 upon type 1 and type 2 IFN activation and T cells stimulated with IL-7 secreted IFN $\gamma$ . T cells from patients were more prone to secrete IFN $\gamma$  after IL-7 stimulation than T cells from controls. Finally, blocking the IL-7 pathway with an anti-IL-7R monoclonal antibody was associated with a decrease in IFN related gene expression in salivary gland explants. Given these results, we hypothesized the existence of an IFN-IL-7 axis in pSS. SGECs stimulated with both types of IFN might produce IL-7, which activates T lymphocytes able to secrete IFN $\gamma$  (**Figure 5D**). This vicious circle could be potentially inhibited by an anti-IL-7R antibody.

IL-7 is a key cytokine involved in T lymphocytes homeostasis. The presence of lymphopenia, affecting mainly T-cells, is one of the hallmarks of some systemic autoimmune diseases such as systemic lupus and pSS. In pSS, lymphopenia is included in the biologic criteria used to assess activity in the ESSDAI score and the presence of lymphopenia is associated with risk of lymphoma (26). Circulating levels of IL-7 are increased in response to lymphopenia. An interesting question in pSS is whether the increased IL-7 level is a reaction to lymphopenia or if lymphopoiesis is non-responsive to IL-7 stimulation.

IL-7 is produced by stromal cells but also by epithelial cells, such as small intestinal epithelial cells (27), or enterocytes (28). We showed that SGECs could produce IL-7 after stimulation with all types of IFNs or a Toll-like receptor 3 agonist. The IFN-IL-7 pathway that we describe could be involved in the organization of ectopic lymphoid structures found in pSS salivary glands. Seo *et al.* demonstrated that IL-7 plays a pivotal role in T follicular helper (Tfh) generation and germinal center formation *in vivo*, because treatment with an anti-IL-7 neutralizing antibody markedly impaired the development of Tfh cells and IgG responses (29). Interestingly, we found more CD127<sup>+</sup> T cells in pSS patients versus controls, probably because of the internalization of CD127, or IL-7R, after IL-7 binding (25). Moreover, we showed that CD127<sup>+</sup> CD4 and CD8 T cells from pSS patients showed increased expression of PD1, and expression of ICOS was higher in CD127<sup>+</sup> CD4 T



cells from pSS patients than CD127<sup>+</sup> T cells. These results suggest that IL-7 could be involved in CD4 T cell differentiation to Tfh, which are PD1<sup>+</sup>, ICOS<sup>+</sup>. Tfh cells are specialized providers of T-cell help to B cells, and are essential for germinal center formation, affinity maturation, and the development of high affinity antibodies and memory B cells. The link between high IL-7 level and increased B-cell biomarkers is intriguing. Since we did not find any expression of IL-7R mRNA on RNAseq of blood or salivary gland B cells (**Supp Figure 4A**), this interaction is indirect. IL-7 plays a pivotal role in Tfh generation and germinal center formation in vivo (29), which might explain the correlation between serum IL-7 level and B-cell biomarkers. Alternatively, since IL-7 level is increased in patients with active pSS, and type I IFN and BAFF levels are also increased in the same subgroup of patients, the correlation between IL-7 and B-cell biomarkers may reflect a parallel augmentation in patients with the most active disease. Conversely, the impact of IL-7 stimulation on CD8 T cells might drive IFN $\gamma$  production. These differences between CD4 and CD8 T cell involvement requires better characterization. Of note, RNA-seq analysis showed higher *IFN $\gamma$*  expression in CD8, but not CD4 T cells from pSS patients versus controls. Moreover, in 4 patients with both blood and biopsy samples, the mRNA expression of *IFN $\gamma$*  and *IFNL1*, was upregulated in T cells sorted from biopsies versus blood. These results support the role of the salivary gland tissue microenvironment and especially the interactions between T cells and SGECs.

One limitation of this study is that we hypothesized that the action of IL-7 was mainly due to its effect on T cells resulting in IFN $\gamma$  secretion. However, IL-7 contributes to arthritis in RAG deficient mice, which lack T and B cells. Thus, cells other than T and B cells might express IL-7R and be sensitive to IL-7 signaling. For example, the presence of IL-7R<sup>+</sup> macrophages was associated with joint inflammation and IL-7 enhanced inflammation and osteoclastogenesis, independently of T and B cells in this mouse model (30). Moreover, innate lymphoid cells (ILCs) express IL-7R and are also involved in IL-7-mediated inflammation (31). Notably, ILC3s play a role in IL-7-mediated lymphoid structure formation. In addition to ILCs, some tissue-resident cells with innate-like properties, such as IL-7R<sup>+</sup> mucosal-associated invariant T cells and IL-7R<sup>+</sup> CCR9<sup>+</sup> T cells could contribute to IFN $\gamma$  secretion. Interestingly, these cells have been found increased in target tissues in several autoimmune diseases, notably the salivary glands of patients with pSS (32).

Lastly, inhibiting this IL-7 stimulation showed interesting results in terms of *IFN* gene expression. Confirmation of the transcriptomic results at the protein level would have been interesting, but IFN dosages in supernatants of explants containing a few cells were not contributive, even when using sensitive techniques such as SIMOA. It was recently demonstrated that the administration of a blocking antibody against the IL-7R $\alpha$  chain to female NOD mice ameliorated pSS characteristics including hyposalivation and leukocyte infiltration of submandibular glands. Moreover, the authors observed a decrease of IFN $\gamma$  producing CD4 and CD8 T cells in the submandibular glands (33). The use of OSE-127, an anti-IL-7R $\alpha$  monoclonal antibody, showed interesting results in non-human primates for controlling skin inflammation despite repeated antigen challenges (34). Also, IFN $\gamma$  level was significantly decreased in humanized mice models of colitis and ex-vivo colon explants culture from ulcerative colitis (35). Of note, no modification of T cell numbers, phenotype, function or metabolism was observed in the peripheral blood in this study.

In conclusion, IL-7 secreted by SGECs under the influence of both IFN types may activate T cells, which in turn secrete IFN $\gamma$ , thus amplifying this vicious circle. Given these results and those already described in the “Sjögren like” NOD mouse model, targeting the IL-7 pathway in pSS could represent an interesting therapeutic option.

**Author contributions:** E.R. participated in designing research studies, conducting experiments, acquiring data, analyzing data and writing the manuscript. J.P. participated in designing research studies, conducting experiments, acquiring data and analyzing data. A.V., A.D., B.L., A.P., R.S.: participated in designing research studies, conducting experiments and acquiring and analyzing data. N.T. participated in analyzing data and writing the manuscript. M.M. participated in designing research studies, acquiring data and analyzing data. N.S., D.D L.C. and N.C. participated in conducting experiments, acquiring and analyzing data. S.P., V.G., N.P. participated in designing research studies, conducting experiments, acquiring and analyzing data. G.N. and X.M. participated in designing research studies, analyzing data and writing the manuscript.

### **Acknowledgements:**

We thank C. Le Pajolec, Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris-Sud, Le Kremlin-Bicêtre, France and E. Berge, Rheumatology, Université Paris Sud, Le Kremlin Bicêtre, France who participated in the recruitment of patients. We thank Vincent Bondet, Immunobiology of Dendritic Cells laboratory, INSERM U1223/Institut Pasteur, Paris, France, who participated in SIMOA experiments.

## REFERENCES

1. Singh N, Cohen PL. The T cell in Sjogren's syndrome: force majeure, not spectateur. *J Autoimmun.* 2012;39(3):229-33.
2. Nezos A, Gravani F, Tassidou A, Kapsogeorgou EK, Voulgarelis M, Koutsilieris M, et al. Type I and II interferon signatures in Sjogren's syndrome pathogenesis: Contributions in distinct clinical phenotypes and Sjogren's related lymphomagenesis. *J Autoimmun.* 2015;63:47-58.
3. Gottenberg J-E, Cagnard N, Lucchesi C, Letourneur F, Mistou S, Lazure T, et al. Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjögren's syndrome. *Proc Natl Acad Sci U S A.* 2006;103(8):2770-5.
4. Khuder SA, Al-Hashimi I, Mutgi AB, Altorok N. Identification of potential genomic biomarkers for Sjögren's syndrome using data pooling of gene expression microarrays. *Rheumatol Int.* 2015;35(5):829-36.
5. Hall JC, Baer AN, Shah AA, Criswell LA, Shiboski CH, Rosen A, et al. Molecular Subsetting of Interferon Pathways in Sjögren's Syndrome. *Arthritis Rheumatol Hoboken NJ.* 2015;67(9):2437-46.
6. Bikker A, van Woerkom JM, Kruize AA, Wenting-van Wijk M, de Jager W, Bijlsma JWJ, et al. Increased expression of interleukin-7 in labial salivary glands of patients with primary Sjögren's syndrome correlates with increased inflammation. *Arthritis Rheum.* 2010;62(4):969-77.
7. Bikker A, Kruize AA, Wenting M, Versnel MA, Bijlsma JWJ, Lafeber FPJG, et al. Increased interleukin (IL)-7R $\alpha$  expression in salivary glands of patients with primary Sjogren's syndrome is restricted to T cells and correlates with IL-7 expression, lymphocyte numbers and activity. *Ann Rheum Dis.* 2012;71(6):1027-33.

8. Lundström W, Highfill S, Walsh ST, Beq S, Morse E, Kockum I et al. Soluble IL7R $\alpha$  potentiates IL-7 bioactivity and promotes autoimmunity. *Proc Natl Acad Sci U S A*. 2013
9. Hillen MR, Blokland SL, Risselada AP, Bikker A, Lauwerys B R , Kruize A A et al. High soluble IL-7 receptor expression in Sjögren's syndrome identifies patients with increased immunopathology and dryness. *Ann Rheum Dis*. 2016
10. Timmer TCG, Baltus B, Vondenhoff M, Huizinga TWJ, Tak PP, Verweij CL, et al. Inflammation and ectopic lymphoid structures in rheumatoid arthritis synovial tissues dissected by genomics technology: identification of the interleukin-7 signaling pathway in tissues with lymphoid neogenesis. *Arthritis Rheum*. 2007;56(8):2492-502.
11. Jin J-O, Kawai T, Cha S, Yu Q. Interleukin-7 enhances the Th1 response to promote the development of Sjögren's syndrome-like autoimmune exocrinopathy in mice. *Arthritis Rheum*. 2013;65(8):2132-42.
12. Shmarov VA, Malashchenko VV, Meniailo ME, Gazatova ND, Todosenko NM, Melashchenko OB, et al. Direct effects of interleukin-7 on the function of human T cells in vitro. *Eur Cytokine Netw*. 2016;27(4):102-7.
13. van Roon JAG, Glaudemans K, Bijlsma J, Lafeber F. Interleukin 7 stimulates tumour necrosis factor  $\alpha$  and Th1 cytokine production in joints of patients with rheumatoid arthritis. *Ann Rheum Dis*. 2003;62(2):113-9.
14. Belarif L, Mary C, Jacquemont L, Mai HL, Danger R, Hervouet J, et al. IL-7 receptor blockade blunts antigen-specific memory T cell responses and chronic inflammation in primates. *Nat Commun*. 2018;9(1):4483.
15. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*. 2002;61(6):554-8.
16. Gottenberg J-E, Seror R, Miceli-Richard C, Benessiano J, Devauchelle-Pensec V, Dieude P, et al. Serum levels of beta2-microglobulin and free light chains of immunoglobulins are

associated with systemic disease activity in primary Sjögren's syndrome. Data at enrollment in the prospective ASSESS cohort. *PLoS One*. 2013;8(5):e59868.

17. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis*. 2017;76(1):9-16.
18. Dimitriou ID, Kapsogeorgou EK, Abu-Helu RF, Moutsopoulos HM, Manoussakis MN. Establishment of a convenient system for the long-term culture and study of non-neoplastic human salivary gland epithelial cells. *Eur J Oral Sci*. 2002;110(1):21-30.
19. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinforma Oxf Engl*. 2014;30(15):2114-20.
20. Cunningham F, Achuthan P, Akanni W, Allen J, Amode M, Armean I, et al. Ensembl 2019. *Nucleic Acids Res*. 2019;47(D1):D745-51.
21. Dobin A, Davis C, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinforma Oxf Engl*. 2013;29(1):15-21.
22. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
23. Rusinova I, Forster S, Yu S, Kannan A, Masse M, Cumming H, et al. Interferome v2.0: an updated database of annotated interferon-regulated genes. *Nucleic Acids Res*. 2013;41(Database issue):D1040-1046.
24. Rivière E, Pascaud J, Tchitchek N, Boudaoud S, Paoletti A, Ly B, et al. Salivary gland epithelial cells from patients with Sjögren's syndrome induce B-lymphocyte survival and activation. *Ann Rheum Dis*. 2020;annrheumdis-2019-216588.
25. Henriques CM, Rino J, Nibbs RJ, Graham GJ, Barata JT. IL-7 induces rapid clathrin-mediated internalization and JAK3-dependent degradation of IL-7R $\alpha$  in T cells. *Blood*. 2010;115(16):3269-77

26. Nocturne G, Virone A, Ng W-F, Le Guern V, Hachulla E, Cornec D, et al. Rheumatoid Factor and Disease Activity Are Independent Predictors of Lymphoma in Primary Sjögren's Syndrome. *Arthritis Rheumatol Hoboken NJ*. 2016;68(4):977-85.
27. Madrigal-Estebas L, McManus R, Byrne B, Lynch S, Doherty DG, Kelleher D, et al. Human small intestinal epithelial cells secrete interleukin-7 and differentially express two different interleukin-7 mRNA Transcripts: implications for extrathymic T-cell differentiation. *Hum Immunol*. 1997;58(2):83-90.
28. Laky K, Lefrançois L, Lingenheld EG, Ishikawa H, Lewis JM, Olson S, et al. Enterocyte expression of interleukin 7 induces development of gammadelta T cells and Peyer's patches. *J Exp Med*. 2000;191(9):1569-80
29. Seo YB, Im SJ, Namkoong H, Kim SW, Choi YW, Kang MC, et al. Crucial Roles of Interleukin-7 in the Development of T Follicular Helper Cells and in the Induction of Humoral Immunity. *J Virol*. 2014;88(16):8998-9009
30. Kim S-J, Chang HJ, Volin MV, Umar S, Van Raemdonck K, Chevalier A, et al. Macrophages are the primary effector cells in IL-7-induced arthritis. *Cell Mol Immunol*. 2019
31. Hinrichs AC, van Roon JAG. Do IL-7 R+ innate cells orchestrate autoimmune pathology? *Nat Rev Rheumatol*. 2019;15(11):640-2.
32. Blokland SLM, Hillen MR, Kruize AA, Meller S, Homey B, Smithson GM, et al. Increased CCL25 and T Helper Cells Expressing CCR9 in the Salivary Glands of Patients With Primary Sjögren's Syndrome: Potential New Axis in Lymphoid Neogenesis. *Arthritis Rheumatol Hoboken NJ*. 2017;69(10):2038-51.
33. Zhou J, Yu Q. Anti-IL-7 receptor- $\alpha$  treatment ameliorates newly established Sjögren's-like exocrinopathy in non-obese diabetic mice. *Biochim Biophys Acta Mol Basis Dis*. 2018;1864(7):2438-47.

34. Belarif L, Mary C, Jacquemont L, Mai HL, Danger R, Hervouet J, et al. IL-7 receptor blockade blunts antigen-specific memory T cell responses and chronic inflammation in primates. *Nat Commun.* 2018;9(1):4483.
35. Belarif L, Danger R, Kermarrec L, Nerrière-Daguin V, Pengam S, Durand T, et al. IL-7 receptor influences anti-TNF responsiveness and T cell gut homing in inflammatory bowel disease. *J Clin Invest.* 2019;129(5):1910-25.

**Figure legend:**

**Figure 1: IL-7 serum level in pSS patients and controls. A: ELISA** assessment of IL-7 in serum from 372 patients with pSS (ASSESS cohort) and 73 controls. Statistical analysis involved using Mann-Whitney test. \*\*\*\*  $p < 0.0001$ . **B:** Correlation between IL7 serum level and B cells activation markers, IFN-induced chemokines and disease activity markers in pSS patients. **C:** Association between clinico-biological parameters and IL-7 serum level on univariable and multivariable analysis in pSS patients. RF, rheumatoid factor

**Figure 2: The percentage of IL-7R+ CD4 and CD8 T cells is decreased in pSS patients versus controls and levels of PD1 and ICOS are higher in CD127- than CD127+ CD4 T cells.** Expression of **A:** CD127 in CD4 and CD8 T cells sorted from blood in pSS patients (n=15) and controls (n=12), **B:** PD1 in CD127+ and CD127- CD4 and CD8 T cells in pSS patients (n=15), and **C:** ICOS in CD127+ and CD127- CD4 and CD8 T cells in pSS patients (n=15). Data are mean (SD). Statistical analysis involved using Mann-Whitney test for unpaired data and Wilcoxon test for paired data. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ . Controls are colored in green and pSS patients are colored in orange.



**Figure 3: A:** Secretion of IL-7 by salivary gland epithelial cells (SGECs). ELISA of IL-7 secretion by SGECs under different stimulation conditions (poly(I:C) 30 $\mu$ g/mL, IFN $\alpha$  5ng/mL, IFN $\gamma$  600 UI/mL or IFN $\lambda$  25 ng/mL) after 3 days in **A** : pSS patients and controls combined **and B:** S pSS patients and controls. qPCR analysis of mRNA level of *IL7* in SGEC under different conditions of stimulation relative to unstimulated conditions in **C:** pSS patients and controls combined and **D:** pSS patients and controls relative to unstimulated condition (qPCR). Data are mean (SD). Statistical analysis involved using Mann-Whitney test for unpaired data and Wilcoxon test for paired data. \*\*\*\* p<0.0001.

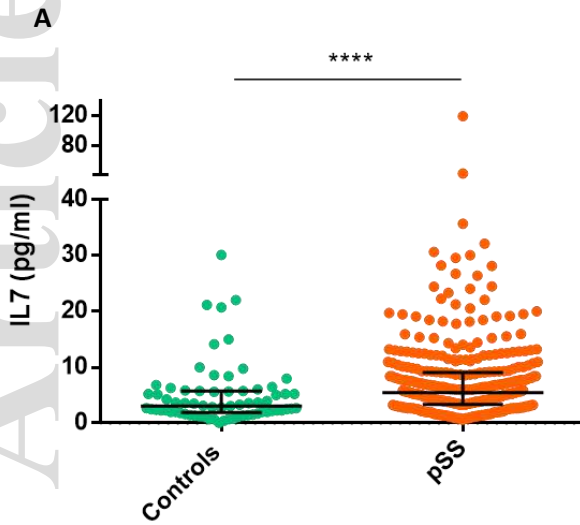
**Figure 4: Association between IL-7 and IFN in blood and salivary glands.** Detection of IFN $\gamma$  protein (**A**) and IFN $\alpha$  protein (**B**) in supernatants from T cells stimulated with IL-7 0.1 and 2 ng/mL, for 3 days, sorted from pSS patients and controls blood. Data are mean (SD). Statistical analysis involved using Wilcoxon test. **C:** Correlation between *IL7* gene expression and *IFN $\alpha$ 1*, *IFN $\alpha$ 2*, *IFN $\beta$*  and *IFN $\gamma$*  gene expression in salivary gland explant cells from pSS (n=9). Statistical analysis involved using Spearman test. \*\* p<0.01.

**Figure 5 A:** Schematic representation of the protocol used for nanosting experiments. **B:** Volcano plot representation of differentially expressed genes after treatment with anti-IL-7R monoclonal antibody (OSE-127) compared to control isotype in explant and pellet cells. **C:** Effect of anti-IL-7R monoclonal antibody (OSE-127) on *IFN* mRNA expression in pellet cells and in explants cells compared with control isotype. \* p<0.05. **D:** Simplified schematic representation of relationship between SGECs and T cells via the IL-7-IFN axis.

**Table 1 : Characteristics of primary Sjögren’s syndrome patients (pSS) included in the study**

	CD127 and PD1 assessment of T cells	RNAseq of biopsy sorted cells	RNAseq of blood sorted cells	T cells stimulated with IL-7	Primary cultured SGECs for IL-7 dosage	Salivary gland explants for nanostring
	pSS (n=15)	pSS (n=9)	pSS (n=16)	pSS (n=12)	pSS (n=5)	pSS (n=9)
<b>Median age (min-max)</b>	59(40-59)	51 (47-71)	55 (47-68)	65 (38-88)	50(40-64)	41(38-52)
<b>Female sex, no (%)</b>	13(86)	8 (89)	15 (94)	12 (100)	4(80)	9(100)
<b>Focus score <math>\geq</math> 1, no (%)</b>	4/4(100)	4 (44)	3/6 (50)	NA	4(80)	5(55)
<b>SSA antibodies, no (%)</b>	10(71)	7 (78)	13 (81)	9 (75)	4(80)	8(88)
<b>Median ESSDAI (min-max)</b>	5(2-8)	2 (0-4.5)	1 (0-2.75)	3 (0-15)	3 (2-12)	2 (1-4)

SGECs, salivary gland epithelial cells; ESSDAI, EULAR Sjögren Syndrome Disease Activity Index

**B**

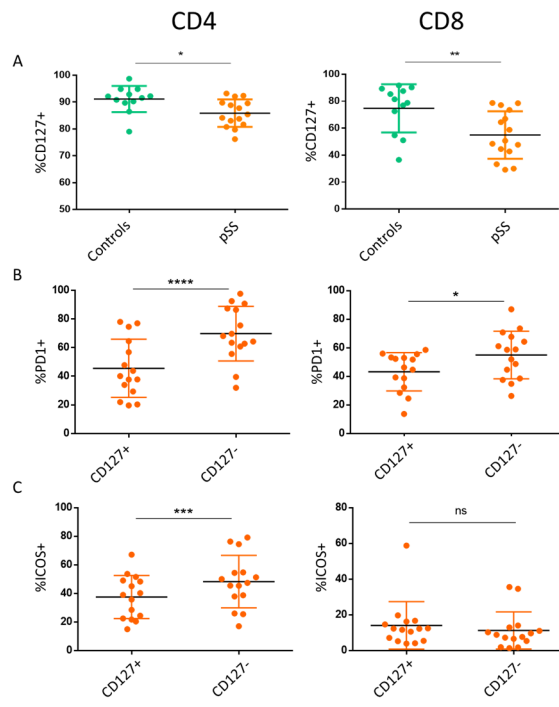
IL-7 serum level is correlated to B cells activation markers, IFN-induced chemokines and disease activity markers

Parameters	Spearman correlation coefficient	p-value
CXCL13	r=0.35	<0.0001
CCL19 (MIP-3b)	r=0.20	<0.0001
CXCL10 (IP-10)	r=0.23	0.0002
Gammaglobulines	r=0.27	<0.0001
Total IgG	r=0.28	<0.0001
Kappa Light Chain	r=0.30	<0.0001
Lambda Light Chain	r=0.28	<0.0001
$\beta$ 2microglobuline	r=0.27	<0.0001
Rheumatoid Factor	r=0.32	<0.0001
Level of lymphocytes	r= -0.28	<0.0001
C4 level	r= -0.22	<0.0001

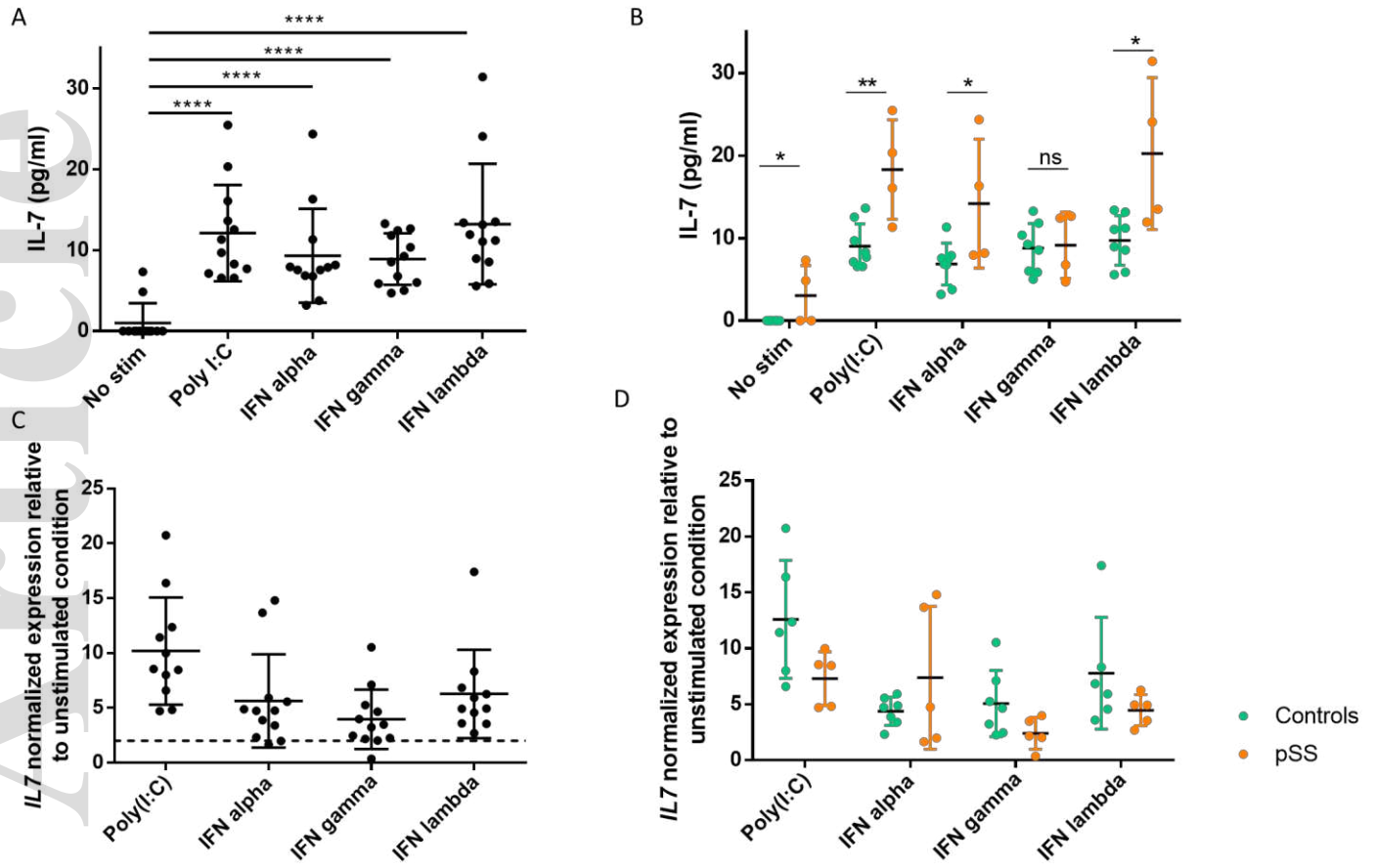
**C**

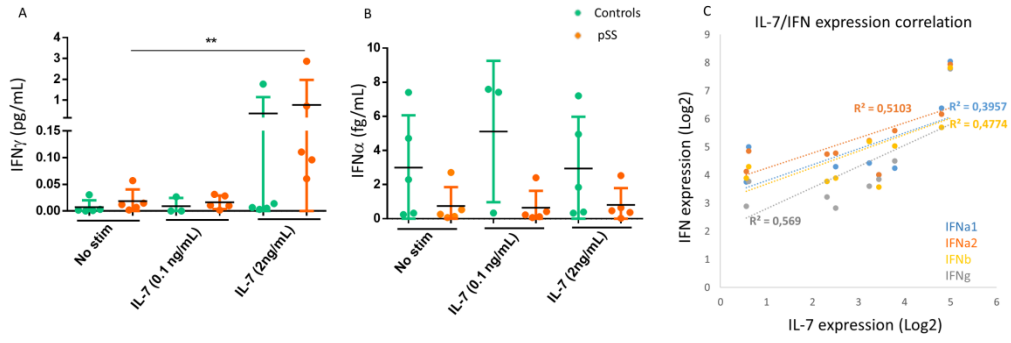
Clinico-biological parameter associated with IL-7 serum level in univariate analysis and multivariate analysis

Parameter	p-value
<b>UNIVARIATE ANALYSIS</b>	
Anti-SSA	p<0.0001
Anti-SSB	p<0.0001
RF	p<0.0001
Lymphopenia	p=0.0002
Low C4	p<0.0001
Lymphoma	p=0.005
<b>MULTIVARIATE ANALYSIS</b>	
Anti-SSA	p=0.019
CXCL13	p=0.043
Rheumatoid Factor	p=0.003
kappa Light Chain	p=0.024
Low C4	p=0.024

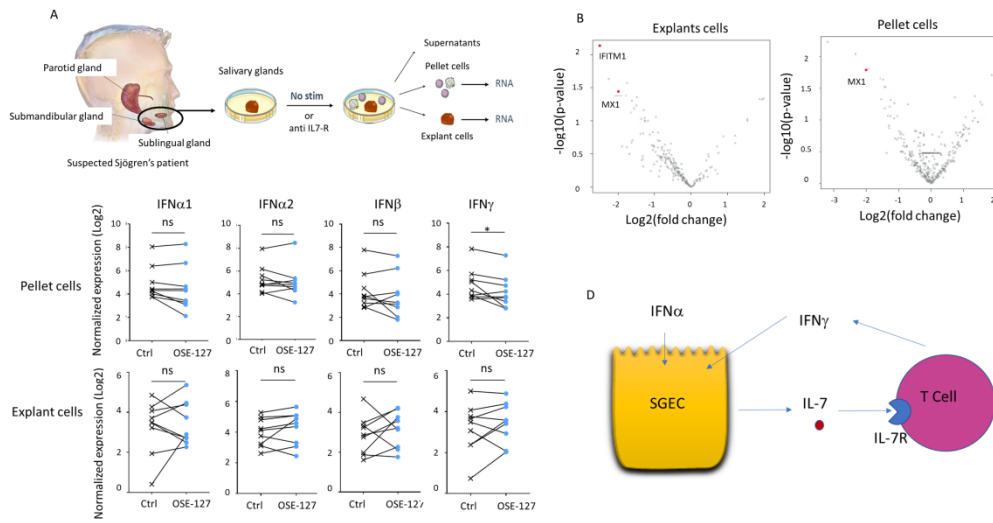


art\_41558\_f2.tiff





art\_41558\_f4.tiff



art\_41558\_f5.tiff