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► To cite this version:

Elodie Riviere, Juliette Pascaud, Nicolas Tchitchek, Saida Boudaoud, Audrey Paoletti, et al.. Salivary gland epithelial cells from patients with Sjögren's syndrome induce B-lymphocyte survival and activation. *Annals of the Rheumatic Diseases*, 2020, 79 (11), pp.1468-1477. 10.1136/annrheumdis-2019-216588 . inserm-03150054

HAL Id: inserm-03150054

<https://inserm.hal.science/inserm-03150054>

Submitted on 23 Feb 2021

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1 **Salivary gland epithelial cells from patients with Sjögren's syndrome induce B-lymphocyte**
2 **survival and activation.**

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18 **Number of words:** 3505

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21

22 **ABSTRACT** (248 words)

23 **Objectives:** Primary Sjögren's syndrome (pSS) is characterized by chronic hyperactivation of B-
24 lymphocytes. Salivary gland epithelial cells (SGECs) could play a role in promoting B-
25 lymphocyte activation within the target tissue. We aimed to study the interactions between
26 SGECs from pSS patients or controls and B-lymphocytes.

27 **Methods:** Patients had pSS according to 2016 EULAR/ACR criteria. Gene expression analysis of
28 SGECs and B-lymphocytes from pSS and controls isolated from salivary gland biopsies and
29 blood was performed by RNA-seq. SGECs from pSS and controls were co-cultured with B-
30 lymphocytes sorted from healthy donor blood and stimulated. Transwell and inhibition
31 experiments were performed.

32 **Results:** Gene expression analysis of SGECs identified an upregulation of interferon signaling
33 pathway and genes involved in immune responses (*HLA-DRA*, *IL7*, *BAFFR*) in pSS. Activation
34 genes *CD40* and *CD48* were upregulated in salivary gland sorted B-lymphocytes from pSS
35 patients. SGECs induced an increase in B-lymphocyte survival, which was higher for SGECs
36 from pSS patients than controls. Moreover, when stimulated with Poly(I:C), SGECs from pSS
37 patients induced higher activation of B-lymphocytes than those from controls. This effect
38 depended on soluble factors. Inhibition with anti-BAFF, anti-APRIL, anti-IL6-R antibodies
39 JAK1/3 inhibitor, or hydroxychloroquine had no effect, conversely to leflunomide, BTK or PI3K
40 inhibitors.

41 **Conclusions:** SGECs from patients with pSS had better ability than those from controls to
42 induce survival and activation of B-lymphocytes. Targeting a single cytokine did not inhibit this
43 effect, whereas, leflunomide, BTK or PI3K inhibitors partially decreased B-lymphocytes viability
44 in this model. This gives indications for future therapeutic options in pSS.

45

46 **Key words:** Sjögren's syndrome, salivary gland epithelial cells, B lymphocytes, RNA-seq, co-
47 culture

48 **Key Messages:**

49 - Primary Sjögren's syndrome (pSS) is characterized by a chronic hyperactivation of B
50 lymphocytes, and salivary gland epithelial cells (SGECs) could be involved in pSS
51 pathophysiology.

52 - RNA-seq analysis of SGECs sorted from salivary glands showed upregulated IFN signaling
53 pathway, *HLA-DR*, *IL7* and *BAFFR*. RNA-seq analysis of B lymphocytes sorted from salivary
54 glands showed upregulated *CD40* and *CD48*.

55 - Survival and activation of B lymphocytes was higher with SGECs from pSS patients than
56 controls.

57 - SGECs interact with B lymphocytes mainly by secretion of soluble factors. Targeting a single
58 cytokine is not sufficient to inhibit this effect, but leflunomide, BTK or PI3K inhibitors were
59 successful, which suggests future therapeutic options in pSS.

60

61

62

63 **INTRODUCTION**

64 Primary Sjögren's syndrome (pSS) is a systemic auto-immune disorder characterized by a
65 lymphocytic infiltrate of exocrine glands. Patients present sicca, fatigue, and pain, and in 30%
66 to 40% of them, systemic manifestations including involvement of skin, joints, neurological
67 system, lung or kidney[1]. B-lymphocyte hyperactivation is a hallmark of the disease[2], as
68 illustrated by the presence of serum auto-antibodies, hypergammaglobulinemia, increased
69 levels of free light chains, and increased risk of B-cell lymphoma. The peri-epithelial tropism
70 of the lymphocytic infiltration is specific to pSS and justifies the term auto-immune
71 epithelitis[3].

72 Several lines of evidence support that salivary gland epithelial cells (SGECs) are not only the
73 target of autoimmunity in pSS patients but may also play a role in the pathology. They could
74 act as non-professional antigen-presenting cells by expressing co-stimulatory molecules such
75 as CD80 and CD86 [4]. In salivary glands from pSS patients, CyTOF immunophenotyping
76 showed a significant up-regulation of HLA-DR in SGECs associated with disease activity [5].
77 SGECs could be involved in local B-lymphocyte activation as they can secrete B-cell activating
78 factor (BAFF) [6]. Of note, plasma cells are detected in salivary gland tissue in close contact
79 with CXCL12-expressing ductal and acinar epithelium, which suggests potential interactions
80 between these cells [7].

81 We hypothesized that SGECs could play a supportive role in B-lymphocyte survival and
82 activation. We first focused on gene expression analysis of purified cellular populations sorted
83 from salivary gland biopsies, then developed an in vitro co-culture model including SGECs and
84 B lymphocytes to characterize the cross-talk between both.

85

86 **METHODS**

87 **Patients**

88 Minor salivary gland (MSG) biopsies were obtained from consecutive patients referred for
89 suspected pSS to the Rheumatology Department of Bicêtre Hospital, a tertiary reference
90 center for systemic auto-immune diseases. pSS was defined according to the 2016 ACR/EULAR
91 criteria [8] and controls were patients presenting sicca symptoms without anti-Ro/SSA or anti-
92 La/SSB antibodies and with normal or sub-normal MSGs (i.e., focus score <1). MSG biopsies
93 were used for transcriptomic analysis of sorted cells and co-culture experiments. Peripheral
94 blood mononuclear cells (PBMCs) used for transcriptomic analysis were isolated from whole
95 blood of pSS patients and healthy blood donors. B lymphocytes sorted from blood of healthy
96 donors were used for the co-culture experiments.

97 A total of 42 pSS patients and 62 controls were included in the study, among whom 26 pSS
98 patients and 27 controls had an MSG biopsy. The characteristics of the patients and controls
99 are presented in **Table 1**. Experimental design showing the number of patients included in
100 each experiment is in **Figure S1A**.

101 The study received approval from the local ethics committee, and informed consent was
102 obtained from all patients and control subjects. Patients or the public were not involved in the
103 design, or conduct, or reporting, or dissemination plans of our research.

104 Methods of RNA-sequencing, primary cultures of SGECs [9] and co-cultures of SGECs and B
105 lymphocytes, FACS analysis and assays are described in online **supplementary** text.

106 **Statistical methods**

107 *Statistical analysis of of RNA-seq*

108 Statistical analyses were performed using the DESeq2 package. Comparisons were performed
109 between pSS patients and controls in each cell sub-types based on a non-parametric *t*-test.
110 Cells sorted from biopsies and from blood in four pSS patients (with matched samples
111 available) underwent paired comparison by non-parametric paired *t*-test. p-value < 0.05 was
112 considered statistically significant to identify differentially expressed genes.

113 *Statistical analysis of co-culture experiments*

114 The percentage of surviving B lymphocytes was calculated for each culture condition as the
115 difference between the percentage proportion of alive B lymphocytes co-cultured with SGECs
116 and the percentage of alive B lymphocytes cultured alone. The same strategy was used to
117 assess the percentage of activated B lymphocytes (CD38+ or CD48+ B-lymphocyte percentages
118 and mean fluorescence intensity [MFI]). Statistical analyses were performed with Prism
119 software using Mann-Whitney (unpaired data) and Wilcoxon (paired data) tests.

120 **RESULTS**

121 **SGECs from pSS patients over-express interferon (IFN)-induced genes, *HLA-DRA*, Interleukin**
122 **7 (*IL7*) and BAFF receptor (*BAFFR*)**

123 Differential gene expression analysis of SGECs from 5 pSS patients versus 4 controls revealed
124 494 genes differentially expressed, 280 upregulated and 214 downregulated. *IFI6*, *VGLL2* and
125 *ZNF879* were the most significantly upregulated genes, and *CCL22* was one of the most
126 significantly downregulated genes ($-\log_{10}(\text{p-value}) > 4$) (**Figure 1A**). The identified
127 transcriptional differences were associated with disease status as indicated by the distinct
128 clustering of pSS and control samples in the multidimensional scaling (MDS) plot shown in
129 **Figure 1B**. Functional enrichment analysis of differentially expressed genes highlighted an
130 over-representation of the IFN signaling pathway, B-cell development pathway and IL7
131 signaling pathway ($-\log(\text{p-value}) = 3.52, 2.89$ and 2.51 , respectively) (**Table S3**). Several IFN-
132 induced genes were up-regulated, including *IFI6*, *OAS2*, *STAT1* and *BST2* (bone marrow stromal
133 cell antigen 2) (**Figure 1C**). Among the 280 upregulated genes in pSS SGECs, 18 were IFN-
134 induced genes according to the Interferome database; 8 were induced by type I and II; 9 were
135 induced by type I, II and III; and 1 was induced by type I only. We also observed an upregulation
136 of *HLA-DRA* expression, coding for the alpha chain of the DR major histocompatibility complex,
137 and *IL7* expression in SGECs from pSS patients versus controls ($p = 0.04$, $p = 0.02$, respectively)
138 (**Figure 1C and Table S4**). Of note, for all these genes, the highest gene expression values were
139 obtained in anti-SSA positive patients who also had the highest salivary gland infiltration.
140 These results were confirmed by RT-PCR, statistically significant for *BST2* and *IFI6* and with a
141 trend for *HLA-DRA*, *OAS2*, *STAT1* and *IL7* (**Figure S3**). Lastly, an up-regulation of *BAFFR*
142 expression was observed in SGECs from pSS patients versus controls ($p = 0.01$) with RNA-seq

143 **(Figure 1C)** and confirmed by flow cytometry of unstimulated SGEs, with no change after
144 Poly(I:C) stimulation **(Figure 1D and E)**.

145

146 **B-lymphocyte activation is observed at the transcriptomic level in salivary gland biopsies** 147 **and in blood**

148 When comparing gene expression in sorted B lymphocytes from salivary glands of pSS patients
149 and controls, we detected 620 differentially expressed genes, 397 upregulated and 223
150 downregulated. The most significantly up-regulated genes were *IGHG1* and *HADH* and the
151 most significantly down-regulated genes were *MT-RNR2-L12*, *MARCO*, *S100A9*, *ELF4* and *ITGA1*
152 ($-\log_{10}(p\text{-value}) > 5$) **(Figure 2A)**. The identified differentially expressed genes were associated
153 with the disease status as indicated by the distinct clustering of pSS and control samples in the
154 MDS plot **(Figure 2B)**. Several genes involved in B-lymphocyte activation and differentiation
155 such as *CD40*, *CD48*, and *CD22* ($p=0.02$, $p=0.01$ and $p=0.048$, respectively) were upregulated
156 in pSS patients versus controls **(Figure 2C)**. *CD40* and *CD48*, but not *CD22* upregulation was
157 confirmed by RT-PCR **(Figure S3)**. The IFN-induced gene, *OAS2*, and IFN γ receptor 2 (*IFNGR2*)
158 were also upregulated in B-lymphocytes from pSS patients versus controls ($p=0.04$ and $p=0.03$,
159 respectively), but not confirmed by RT-PCR. The absence of RT-PCR confirmation may be due
160 to a lack of power.

161 In sorted B lymphocytes from blood, 516 genes were differentially expressed between pSS
162 patients and controls, 359 upregulated and 157 downregulated. Functional enrichment
163 pathway analysis highlighted an over-representation of EIF2 signaling and IFN signaling
164 pathways as well as a role for JAK signaling **(Table S3)**. *TLR7* and its downstream signaling
165 molecule *IRF7* were upregulated in pSS; additionally, *IL6*, involved in B lymphocyte growth, was

166 upregulated (**Table S4**). The qPCR results showed a trend for a higher expression of TLR7, IRF7
167 and IL6 in pSS compared to controls (**Figure S3C**).

168 The paired comparison between B lymphocytes from salivary glands and from blood in four
169 pSS patients revealed the upregulated genes *CD138*, a plasma cell marker; *RANK*; and *IL6*.
170 Moreover, *CCR10*, which is the receptor for CCL28, a mucosal homing chemokine, was
171 upregulated in B lymphocytes sorted from biopsies versus blood (**Table S4**). These results were
172 confirmed for *RANK* and *IL6* by RT-PCR ($p=0.03$ and $p=0.03$).

173

174 **SGECs increase B-lymphocyte survival and activation**

175 To analyze the potential interaction between SGECs and B lymphocytes, we used 5-days co-
176 cultures of SGECs from pSS patients or controls with B lymphocytes from healthy donors with
177 or without IFN α , IFN γ or poly(I:C) stimulation. We observed an increase of B cells viability
178 under all stimulation conditions ($p<0.001$) (**Figure 3A and B**). The increase in B-lymphocyte
179 survival was higher in the poly(I:C) than in the IFN α or IFN γ stimulated co-cultures (**Figure 3A**
180 **and Figure S4**). Conversely, poly(I:C) did not drive survival of B lymphocytes cultured alone
181 (**Figure 3A and B**). More importantly, survival of B lymphocytes was higher in co-culture with
182 SGECs from pSS patients (stimulated or not with poly(I:C)) than in co-culture with SGECs from
183 controls ($p<0.05$) (**Figure 3C**).

184 Co-cultures of SGECs with B lymphocytes also increased B-lymphocyte activation, as assessed
185 by increased expression of CD38 on B lymphocytes under all stimulation conditions (**Figure 3D**
186 **and E**), with a difference between SGECs from patients and controls after poly(I:C) stimulation

187 **(Figure 3F and H)**. Poly(I:C) had a minor impact on the percentage and the MFI of CD38 on B
188 lymphocytes cultured alone **(Figure 3E and 3G)**.

189 The effect of SGECs from pSS patients on B-lymphocyte viability and CD38 expression was
190 increased by poly(I:C) but not IFN stimulation. Thus, we wondered whether Toll-like receptor
191 3 (TLR3) signaling might differ between patients and controls. However, on looking at the
192 poly(I:C) signaling pathways in SGECs, we found no difference between pSS and controls in
193 expression of *TLR3*, *TRAF3*, *IRF3*, *RIG1* and *MDA5* (data not shown). However, *PKR* and *IRF7*
194 were upregulated but not significantly in SGECs from pSS patients versus controls **(Figure 1C)**.
195 Of note, no expression of *TLR3* in B lymphocytes sorted from salivary gland biopsies and from
196 blood was detected.

197 After co-culture with SGECs, the proportion of CD27+ B lymphocytes was increased, and
198 notably switched memory B lymphocytes defined as CD27+ IgD- cells on co-culture with
199 poly(I:C) stimulation **(Figure S5A and S5B)**. The co-culture with SGECs did not achieve
200 differentiation of B lymphocytes into antibody-secreting cells, as supported by the lack of
201 increase in immunoglobulins (IgM and IgG) production in the co-culture supernatants **(Figure**
202 **S6A and S6B)**.

203 To sum up, the co-culture experiments demonstrated that SGECs from pSS patients had a
204 higher ability than SGECs from controls to increase survival and activation of B lymphocytes.

205

206 **Interactions between SGECs and B lymphocytes require mostly soluble factors**

207 To determine whether the survival and activation of B lymphocytes required direct cell contact
208 or not, we performed transwell experiments. In absence of stimulation, the transwell

209 separation did not change the percentage of surviving B lymphocytes co-cultured with SGECs,
210 which suggests that B-lymphocytes survival mostly depended on soluble factors (**Figure 4A**).
211 Similar results were obtained for B-lymphocyte activation as assessed by CD38, CD48, and
212 CD40 markers (**Figure 4B-4F**). However, under poly(I:C) stimulation, part of the effect of SGECs
213 on B-lymphocyte survival and activation was reduced with transwell separation, which
214 suggests the additional role of cell contact in this specific condition (**Figure 4A, C, D and E**).

215 Then, in order to determine which factors could play a role in the interactions between SGECs
216 and B lymphocytes, we tested successively potential involved factors (including BAFF, APRIL,
217 IL6) and signaling pathways (including JAK, BTK, PI3K and pathways involved in B lymphocytes
218 activation and proliferation) by adding specific inhibitors in the co-cultures. In spite of
219 detection of numbers of cytokines in the supernatant of co-cultures (**Figure 5A, B, C, D**),
220 especially in pSS patients, none of the tested anti-cytokines (belimumab, anti-APRIL antibody,
221 tocilizumab) was effective for decreasing B-lymphocyte survival and activation by SGECs.
222 Likewise, tofacitinib was unsuccessful (**Figure 5I and J**). Several chemokines were detected in
223 co-cultures supernatants, which suggests a potential role for homing factors secreted by SGECs
224 supporting B-lymphocyte survival and activation. CXCL10, also known as IFN γ -inducible
225 protein 10 (IP-10), and CXCL13 were detected in SGECs cultures and co-cultures supernatants
226 (**Figure 5E and 5G**). At day 5, CXCL12 was detected at very low levels in the supernatant of only
227 3 pSS SGECs co-cultures (**Figure 5F**) but not in control SGECs co-cultures. CXCL12 was detected
228 in SGECs cultures supernatants at day 1 and day 2 (**Figure 57**). Lastly, CCL28, another
229 chemokine involved in mucosal homing was detected in co-culture supernatant, with no
230 difference between pSS patients and controls (**Figure 5H**).

231 Given these results, we tried to target multiple immunological pathways at the same time by
232 using BTK and PI3K inhibitors. BTK was targeted by adding ibrutinib, a BTK inhibitor, in the co-
233 cultures. Ibrutinib decreased B-lymphocyte viability in co-culture ($p < 0.05$), but this effect was
234 lost in the presence of poly(I:C) (**Figure 5I and J**). LY294002, a PI3K inhibitor, decreased B-
235 lymphocyte viability in co-culture ($p < 0.05$) (**Figure 5I**). This effect was maintained but not
236 significantly with poly(I:C) stimulation (**Figure 5J**). LY294002 could also decrease co-cultured
237 B-lymphocyte activation, as assessed by the decrease in CD38 MFI (**Figure 5K**). Of note, there
238 was no effect of the leflunomide, the BTK inhibitor and the PI3K inhibitor on SGEC viability as
239 compared with the control condition (DMSO). LY294002, but not leflunomide or ibrutinib also
240 affected B-lymphocyte viability when B lymphocytes were cultured alone (**Figure S8**).

241 Given some recent clinical results [10], we also looked at the effect of the association of
242 leflunomide and hydroxychloroquine. Leflunomide decreased B-lymphocyte viability in co-
243 culture, but this effect was lost in the presence of poly(I:C) (**Figure 5I and J**).
244 Hydroxychloroquine alone or combined with leflunomide had no effect on co-cultured B-
245 lymphocyte viability (**Figure 5I**).

246

247 **DISCUSSION**

248 Transcriptome analysis of sorted cells from salivary-gland biopsies showed an increased IFN
249 signature in SGECs and a higher activation of tissue and circulating B lymphocytes in pSS
250 patients than controls. Using a co-culture model, SGECs from pSS patients were more efficient
251 to promote survival and activation of B lymphocytes than SGECs from controls. These effects
252 were mainly mediated by soluble factors. Targeting a single cytokine (BAFF, APRIL, IL6 and
253 JAK1/3) did not inhibit this effect, whereas, leflunomide, BTK or PI3K inhibitors partially
254 decreased B-lymphocytes viability induced by SGECs. A schematic representation of this
255 hypothesis is presented in **Figure S9**. Important roles for B lymphocytes, SGECs and IFN have
256 been suggested by previous studies in pSS pathophysiology. This work brings new elements to
257 better characterize the interactions between these actors. Moreover, the results we obtained
258 in this model could have therapeutic consequences by suggesting that directly targeting B
259 lymphocytes activation pathways would be more efficient than targeting a single cytokine.
260 However, our model with only two cellular actors is over-simplified and we cannot eliminate
261 that, in vivo, anti-cytokine therapy might have an indirect action on microenvironment.

262 RNA-seq analysis of sorted SGECs demonstrated an IFN signature within these cells. The IFN
263 signature is well known in PBMCs and salivary gland biopsies of pSS patients[11,12] ; however,
264 this is the first time that this IFN signature has been demonstrated in sorted SGECs. The chronic
265 expression of endogenous retroviral (ERV) sequences could explain the over-representation of
266 the IFN signaling pathway in SGECs from pSS patients[13]. Overexpression of *BST2*, a
267 transmembrane protein that inhibits viral particles budding from infected cells, could suggest
268 an overexpression of some ERVs. A recent work highlighted increased mRNA and protein levels
269 of *BST2* in salivary glands from pSS patients[14]. However, in our study, *BST2* overexpression

270 was only the consequence of IFN overexpression since we did not find a differential expression
271 of coding ERV between MSG from pSS patients and controls (*data not shown*).

272 SGECs could act as non-professional presenting cells by expressing HLA-DR but also adhesion
273 and co-stimulatory molecules [15]. We observed an upregulation of *HLA-DRA*, which codes for
274 one subunit of HLA-DR. This observation agree with previously published works showing an
275 aberrant expression of HLA-DR by SGECs[16], which can be induced by a type II IFN
276 stimulation[17].

277 Type I and II IFN are also able to induce BAFF production by SGECs [6]. BAFF could represent
278 the link between the innate and adaptive immunity in pSS. BAFFR is the most specific receptor
279 of BAFF and is mainly present on B lymphocytes. By using RT-PCR and immunofluorescence,
280 Lahiri et al. showed that BAFFR was expressed by SGECs [18] and that this receptor was
281 involved in SGECs survival owing to an autocrine effect of BAFF. We showed a differential
282 expression of BAFFR on SGECs between pSS and controls. Thus, the BAFF cytokine could play
283 a dual role, having a direct effect on B-lymphocyte survival and also supporting SGECs survival,
284 which in turn may have a feeder effect on B lymphocytes.

285 B lymphocytes sorted from salivary glands from pSS patients overexpressed activating genes,
286 as expected based on the pSS pathophysiology. Of note, the co-culture between SGECs and B
287 lymphocytes increased the expression of activation markers such as CD38, CD48 and CD40 as
288 compared with B lymphocytes cultured alone. CD40, a co-stimulatory pathway receptor
289 essential for germinal center reactions seems of particular interest [19-21]. Moreover, a phase
290 2 therapeutic trial of an anti-CD40 antibody recently yielded positive results [22]. In our study,
291 CD48, which may enhance the CD40-mediated activating signal for B lymphocytes[23], was
292 upregulated in pSS versus controls. This result was congruent with a work describing soluble

293 CD48 as a biomarker of pSS which was correlated with the biological domain of the ESSDAI
294 [24].

295 B lymphocytes sorted from blood overexpressed TLR7 and the downstream signaling molecule
296 IRF7. Interestingly, the upregulation of IRF7 was confirmed by Jin-Lei Sun et al. who recently
297 performed RNAseq analysis of B lymphocytes sorted from blood comparing 3 pSS patients to
298 3 controls (log2FC = 1.39 and p-value = $8.59 \cdot 10^{-7}$) (**Figure S10**) [25]. Moreover, Imgenberg-
299 Kreuz et al. showed in a RNA-seq analysis of B-lymphocytes sorted from blood an upregulation
300 of *TLR7* as well as *IRF7* in pSS compared to controls (FC = 2.8, p-value= $5.09 \cdot 10^{-5}$ and FC=2.8,
301 p-value= $5.09 \cdot 10^{-5}$, respectively) [26].

302 Previously, co-culture experimental models with SGECs used allogenic PBMCs and failed
303 because of allogenic reactions[17]. In our co-culture model, we used sorted allogenic B
304 lymphocytes, avoiding the T-lymphocyte allogenic reaction. Moreover, the effect of SGECs on
305 B-lymphocyte survival and activation was maintained even with transwell separation that
306 would have prevented antigen presentation. Co-culture with a human salivary gland cell line
307 and tonsillar B lymphocytes revealed increased apoptosis in epithelial cells [27]. This model
308 using a cancer cell line was more artificial than primary cultured SGECs from patients or
309 controls. One limitation of this model is that we used B-lymphocytes sorted from blood, which
310 might have different characteristics than B-lymphocytes sorted from biopsies. However, the
311 use of B-lymphocytes sorted from biopsies would have been not technically feasible, due to
312 the low number of B-lymphocytes present in salivary gland biopsies.

313 In our co-culture model, the ability of SGECs from pSS patients to increase B-lymphocyte
314 survival was increased by poly(I:C) rather than $IFN\alpha$ or $IFN\gamma$ stimulation. The transcriptomic
315 analysis showed a higher expression of *PKR* and *IRF7*, although not significant, in SGECs from

316 pSS patients than controls, whereas the expression of other downstream signaling factors of
317 TLR3 were similar. Thus, poly(I:C) stimulation could mimic a viral trigger and signal through
318 several pathways inducing a positive feedback loop.

319 We hypothesized that SGECs could provide a favorable environment enhancing B-lymphocyte
320 survival and activation. We observed an increase in memory B-lymphocyte differentiation in
321 co-cultures; however, the SGECs did not achieve differentiation of B-lymphocytes in antibody-
322 secreting cells. Thus, this co-culture model remains partial, and other actors such as CD40L,
323 IL2, IL4, IL21, as showed by McCarthy, using single-cell Nojima cultures could be required [28].
324 T follicular helper lymphocytes might also be required to obtain differentiated plasma cells.

325 By analogy with bone-marrow plasma cells niches, in which several survival factors including
326 BAFF, APRIL, IL6 and CXCL12 are required [29], we assessed BAFF, APRIL, IL6, CXCL10, CXCL12,
327 CXCL13, and CCL28 in co-culture supernatants. All were present in co-culture supernatant and
328 could participate to survival and activation of B lymphocytes, CXCL12 at very low level and
329 BAFF only after poly(I:C) stimulation, which agrees with our previous studies [6, 30]. Of note,
330 CXCL12 was detected in the SGECs supernatants at day 1 and day 2 of culture, but not at day
331 5 (**Figure S7**). This chemokine could be involved in B lymphocytes recruitment and organization
332 of the tertiary lymphoid structures in the salivary glands [31]. We did not use any specific
333 inhibitor of the CXCL12 signaling but this could be of interest. Our experiments with inhibitory
334 molecules showed that a single pathway inhibition (anti-BAFF, anti-APRIL, anti-IL6R antibody)
335 was not sufficient. Even tofacitinib, which targets numbers of pro-inflammatory cytokines, was
336 not successful. Leflunomide, a pyrimidine synthesis inhibitor that inhibit B-lymphocyte
337 proliferation and cell cycle progression [10, 32], as well as PI3K and BTK inhibitors (targeting at
338 the same time CXCL13, BAFF and IL6 signaling [33]) decreased B-lymphocyte viability in co-

339 cultures. Lastly, PI3K was the only inhibitor to maintain an inhibitory effect under poly(I:C)
340 stimulation, which suggests that in a local IFN-rich environment, such as pSS salivary glands,
341 PI3K inhibition might be the most efficient option.

342 In conclusion, the analysis of SGECs and B lymphocytes sorted from salivary glands from pSS
343 patients showed an up-regulation of several genes and pathways that could be involved in
344 their interactions. Our co-culture model showed that SGECs from pSS patients had better
345 ability than controls to stimulate survival and activation of B lymphocytes. This effect was
346 mediated mostly by soluble factors. Targeting a single cytokine did not inhibit this effect,
347 whereas, leflunomide, BTK or PI3K inhibitors partially decreased B-lymphocytes viability in this
348 model. These findings may give indications for the choice of future therapeutic options in pSS.

349

350 **Competing interests:** A. T., N.A. and M. M. are employed by Biogen. X.M. received an
351 honorarium for consultancy advice on Sjögren's syndrome from BMS; GSK; Novartis and
352 Servier and a research grant from Servier. The rest of the authors declare that they have no
353 relevant conflicts of interest.

354 **Contributorship:** E.R. participated in designing research studies, conducting experiments,
355 acquiring data, analyzing data and writing the manuscript. J.P. participated in designing
356 research studies, conducting experiments, acquiring data and analyzing data. N.T. participated
357 in analyzing data and writing the manuscript. S.B. participated in designing research studies,
358 conducting experiments and acquiring data. A.P., B.L. and A.D. participated in designing
359 research studies, conducting experiments and acquiring data. H.C., A.T. and N.A. participated
360 in conducting experiments and acquiring data. B.J. participated in acquiring data, analyzing
361 data and writing the manuscript. M.M. participated in designing research studies, acquiring
362 data, analyzing data and writing the manuscript. G.N. and X.M. participated in designing
363 research studies, analyzing data and writing the manuscript.

364 **Acknowledgements:** We thank C. Le Pajolec, Assistance Publique-Hôpitaux de Paris, Hôpitaux
365 Universitaires Paris-Sud, Le Kremlin-Bicêtre, France; E. Berge, Rheumatology, Université Paris
366 Sud, Le Kremlin Bicêtre, France who participated in the recruitment of patients and V. Libri,
367 Center for Translation Research, Institut Pasteur, Paris, France, for her expertise and help with
368 the Biomark experiments. We thank Odile Heidmann, Kevin De Azevedo and Thierry
369 Heidmann, Unité des Rétrovirus Endogènes et Éléments Rétroïdes des Eukaryotes Supérieurs,
370 UMR 8122 CNRS, Institut Gustave Roussy, Villejuif, France for expertise and work on the
371 endogenous retroviral sequences analysis. We thank Pascal Schneider, Department of
372 Biochemistry, University of Lausanne, Switzerland for providing a human APRIL blocking

373 antibody.

374 **Funding sources:** Support was obtained from: the Labex in Research on Medication and
375 Therapeutic Innovation (LERMIT) (ANR10), the Fondation pour la Recherche Médicale
376 DEQ20150934719, an unrestricted grant from Biogen to Université Paris-Sud (UPSud/SAIC N
377 97731) and the Innovative Medicines Initiative 2 Joint Undertaking (JU) (NECESSITY grant
378 agreement No 806975). This JU received support from the European Union's Horizon 2020
379 research and innovation program and EFPIA. E.R. is the recipient of a PhD fellowship from
380 Arthritis Fondation Courtin, Arthritis R&D (CIFRE 2016/1406).

381 **Ethical approval:** The study received approval from the local ethics committee, and informed
382 consent was obtained from all participants.

383 **Data availability statement:** Data are available on reasonable request. All data relevant to the
384 study are included in the article or uploaded as online supplementary information. Additional
385 data are available on reasonable request.

386 **Patients and public involvement:** Patients or the public were not involved in the design, or
387 conduct, or reporting, or dissemination plans of our research.

388

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473 **Table1: Characteristics of the patients with primary Sjögren’s syndrome (pSS) and controls.**

	RNA-seq experiments				SGECs used for co-cultures experiments (including transwell and inhibition experiments)	
	SGECs and B lymphocytes sorted from biopsies		B lymphocytes sorted from blood		pSS (n=18)	control (n=21)
	pSS (n=9)	control (n=4)	pSS (n=16)	control (n=7)		
Age (years)	51 (47-71)	64 (55-66)	55 (47-68)	61 (46-82)	52 (42-71)	56 (47-70)
Female sex, no (%)	8 (89)	4 (100)	15 (94)	7 (100)	16 (88)	18(86)
Pathologic Shirmer, n (%)	1 (12.5)	2 (50)	2 (33)	NA	14 (82)	9 (56)
Pathologic salivary flow, n(%)	7 (87.5)	3 (75)	6 (86)	NA	15 (88)	7 (44)
Focus score ≥ 1, n (%)	4 (44)	0 (0)	3 (50)	NA	12(66)	0 (0)
SSA, n (%)	7 (78)	0 (0)	13 (81)	NA	13 (72)	0 (0)
ESSDAI	2 (0-4.5)		0.5 (0-2.75)		2 (1-4)	

474 Data are expressed as median (25th-75th percentiles), unless otherwise specified. All percent-
 475 ages are calculated for patients with available data. SGECs, salivary gland epithelial cells; SSA,
 476 Sjögren’s-syndrome-related antigen; ESSDAI: EULAR Sjögren Syndrome Disease Activity Index;
 477 NA: data not available.

478 **Figure legends:**

479 **Figure 1: Salivary gland epithelial cells (SGECs) sorted from salivary gland biopsies express**
480 **interferon (IFN)-induced genes, *HLA-DRA*, *BAFFR*, *PKR* and *IRF7*.** **A:** Volcano plot of
481 differentially expressed genes in sorted SGECs from patients with primary Sjögren's syndrome
482 (pSS) (n=5) versus controls (n=4). The horizontal red dashed line indicates the cutoff for
483 significance at $p < 0.05$ and the vertical lines for fold change (FC) in expression ≥ 1.5 and ≤ -1.5
484 **B:** Multidimensional scaling (MDS) representation of the similarities between transcriptomic
485 profiles. Each dot in the representation corresponds to a transcriptomic profile, and the
486 distances between the dots are proportional to the transcriptomic distances computed based
487 on the list of 494 differentially expressed genes. **C:** Normalized counts of genes expressed by
488 SGECs in controls (HC) and pSS patients. Results of RNA-seq of sorted cells: IFN-stimulated
489 genes (*IFI6*, *OAS2*, *STAT1*, *BST2*), *HLA-DRA*, *IL7*, *BAFFR*, *PKR* and *IRF7*. P-values were determined
490 by using DESeq2 software. **D:** Flow cytometry example of BAFFR expression in unstimulated
491 alive SGECs and isotype control from a pSS patient. **E:** Expression of BAFFR evaluated by flow
492 cytometry on alive SGECs with or without poly(I:C) stimulation. Statistical analysis was
493 performed using the Mann-Whitney test, one star corresponds to a p-value ≤ 0.05 . SGECs from
494 controls are in green, SGECs from pSS patients are in orange.

495

496 **Figure 2: B lymphocytes sorted from salivary gland biopsies from pSS overexpress activation**
497 **and differentiation genes as compared with controls.** **A:** Volcano plot of differentially
498 expressed genes in sorted B lymphocytes from pSS patients (n=9) versus controls (n=4). The
499 horizontal red line indicates the cutoff for significance at $p < 0.05$ and the vertical lines $FC \geq 1.5$
500 and ≤ -1.5 . **B:** MDS representation of similarities between transcriptomic profiles. Each dot

501 corresponds to a transcriptomic profile and distances between the dots are proportional to
502 the transcriptomic distances computed based on the 620 differentially expressed genes. **C:**
503 Normalized counts of *CD40*, *CD22*, *CD48*, *OAS2*, and *IFNGR2* in B lymphocytes sorted from
504 biopsies from controls (HC) and in pSS patients. P-values were determined by using DESeq2.

505

506 **Figure 3. SGECs from pSS patients increase B-lymphocyte survival and activation. A:**
507 Representative dot plot of B-lymphocyte viability stained with Live/Dead (Fixable Viability Dye)
508 cultured alone and or co-cultured with SGECs with or without poly(I:C). **B:** Percentage of alive
509 B lymphocytes on day 5, cultured alone or co-cultured with SGECs with or without IFN α , IFN γ
510 or poly(I:C). **C:** Difference between the percentage of alive B lymphocytes co-cultured with
511 SGECs from pSS patients and controls and the percentage of alive B lymphocytes cultured
512 alone on day 5. **D:** Representative dot plot of B lymphocytes cultured alone and co-cultured
513 with SGECs with or without poly(I:C) and stained with for CD38 and CD27. **E:** Percentage of
514 CD38+ B lymphocytes at day 5 in all conditions of culture and stimulation. **F:** Difference
515 between the percentage of CD38+ B lymphocytes in co-culture with SGECs and the percentage
516 of CD38+ B lymphocytes cultured alone at day 5. **G:** Mean fluorescence intensity (MFI) of CD38
517 in B lymphocytes cultured alone with or without poly(I:C), or B lymphocytes co-cultured with
518 SGECs with or without poly(I:C). **H:** Difference between the MFI for CD38 for B lymphocytes
519 co-cultured with SGECs or cultured alone on day 5.

520 * p-value ≤ 0.05 , ** p-value ≤ 0.01 and *** p-value ≤ 0.001 .

521

522 **Figure 4. Increases of B-lymphocyte viability and activation were mainly due to soluble**
523 **factors.** B lymphocytes were examined on day 5 with and without transwell, with and without

524 poly(I:C), in co-cultures with SGECs from pSS patients and controls. **A:** Percentage of alive B
525 lymphocytes. **B:** Percentage of CD38+ B lymphocytes **C:** CD38 MFI of B lymphocytes **D:**
526 Percentage of CD48^{high} B lymphocytes. **E:** CD48 MFI of B lymphocytes. **F:** CD40 MFI of B * p-
527 value ≤0.05.

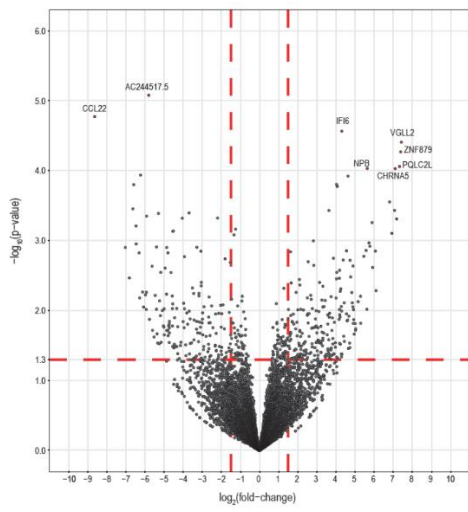
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529 **Figure 5: Assessment of soluble factors in co-cultures supernatants and inhibition**
530 **experiments. A:** Level of APRIL in the supernatants of B lymphocytes cultured alone, SGECs
531 from pSS and controls cultured alone and SGECs co-cultured with B lymphocytes. **B:** Dosage of
532 APRIL in supernatants of SGECs cultured alone or co-cultured with B lymphocytes (after
533 subtraction of APRIL level in supernatants of B lymphocytes cultured alone). **C:** Level of IL6 in
534 supernatants of B lymphocytes cultured alone, SGECs from pSS and controls cultured alone
535 and SGECs co-cultured with B lymphocytes. **D:** Level of IL6 in supernatants of SGECs cultured
536 alone or co-cultured with B lymphocytes (after subtraction of IL6 level in supernatants of B
537 lymphocytes cultured alone). **E, F, G and H:** Level of CXCL10 (**E**), CXCL12 (**F**), CXCL13 (**G**) and
538 CCL28 (**H**) in supernatants of B lymphocytes cultured alone, SGECs from pSS and controls
539 cultured alone and SGECs co-cultured with B lymphocytes. Dashed line represents the lower
540 limit of detection of the assay. **I, J:** Viability of B lymphocytes co-cultured with SGECs and
541 different inhibitors relative to the condition without the inhibitor and without poly(I:C) (**I**), and
542 with poly(I:C)(**J**). **K:** Effect of leflunomide, ibrutinib (BTK inhibitor) and LY294002 (PI3K
543 inhibitor) on CD38 MFI of B lymphocytes cultured alone and in co-culture with SGECs. * p-
544 value ≤0.05, ** p-value≤0.01 and *** p-value≤0.001.

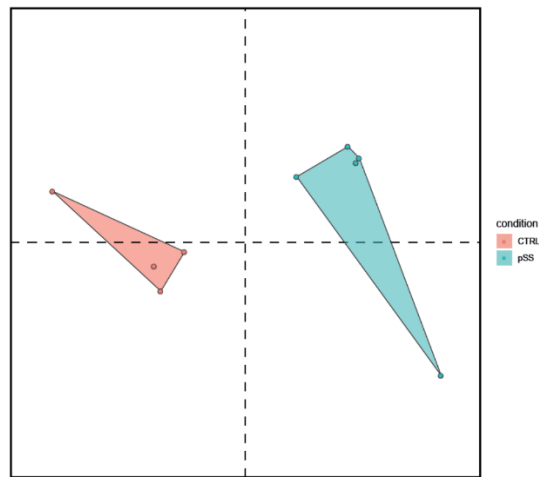
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547 **Figure 1 A**

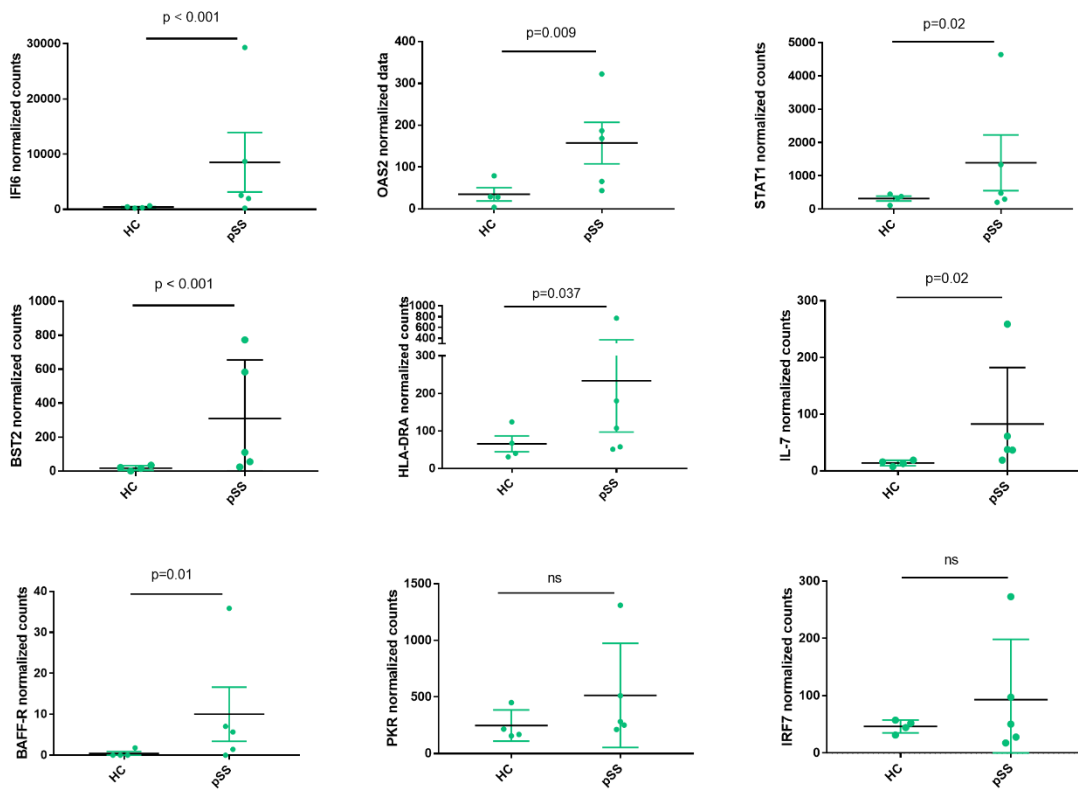


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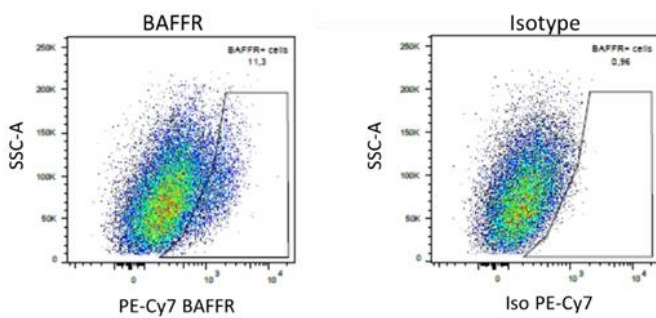
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549 **C**



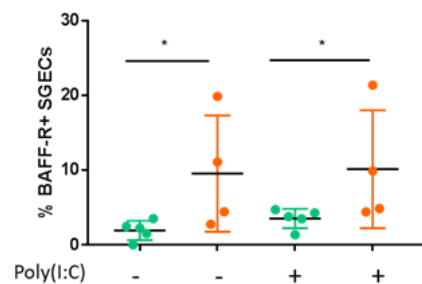
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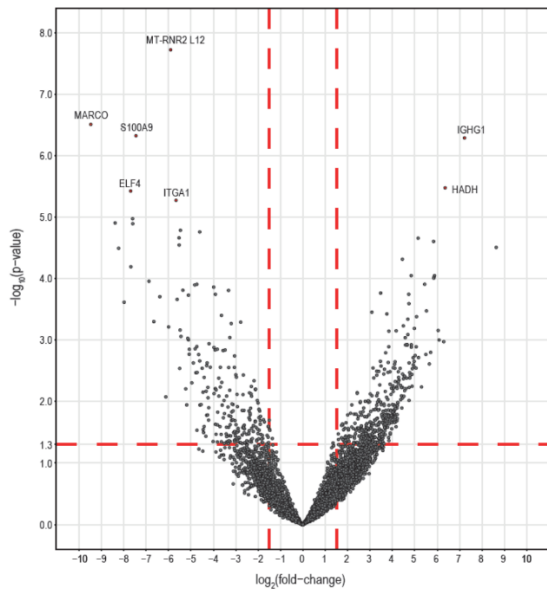


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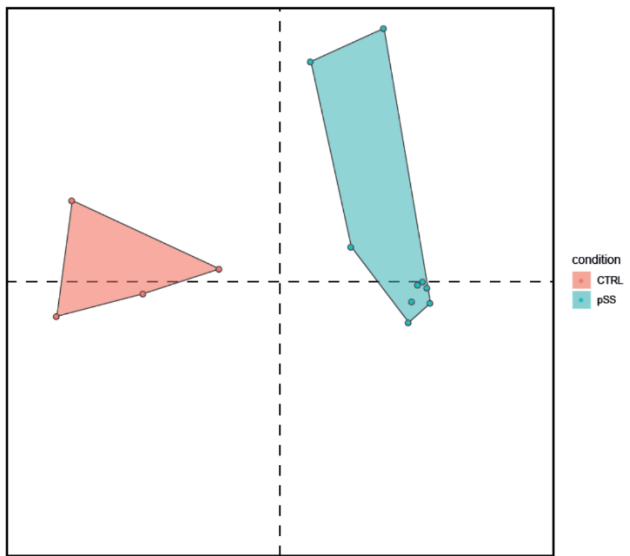
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553 **Figure 2 A**



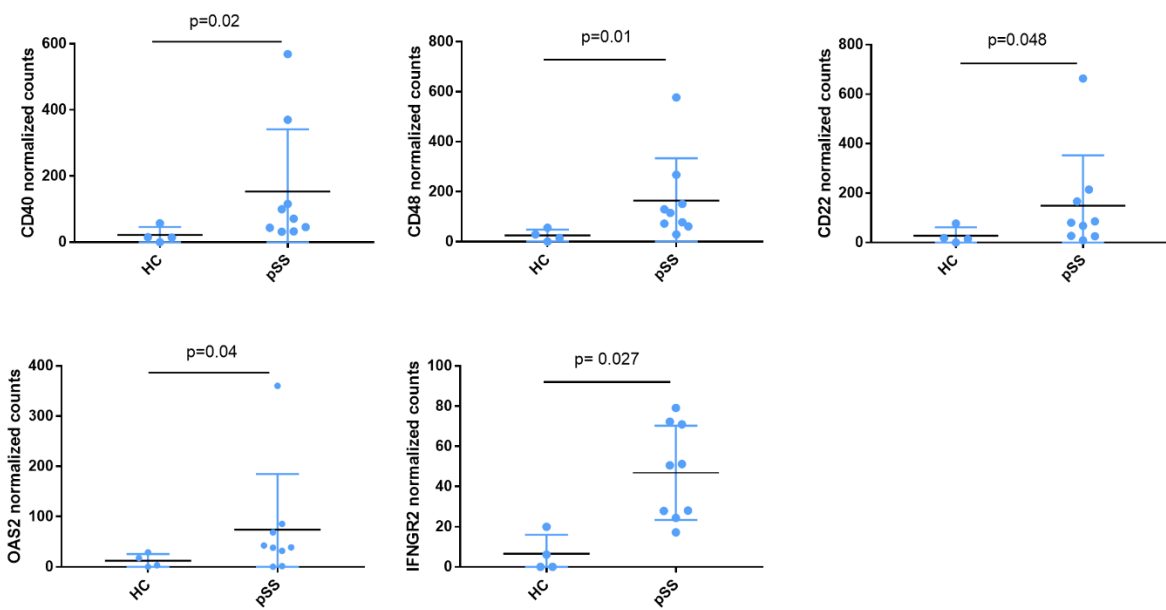
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555 **C**

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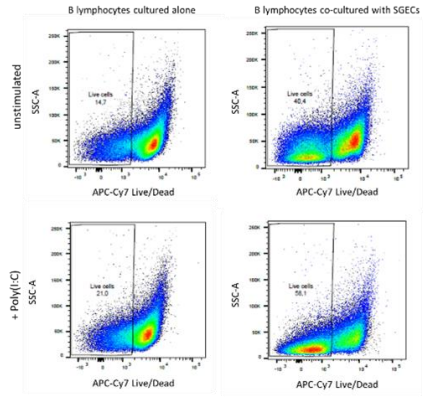
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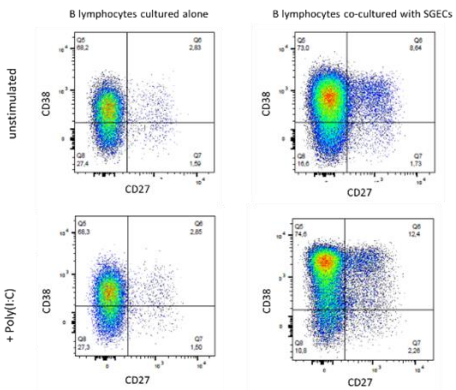
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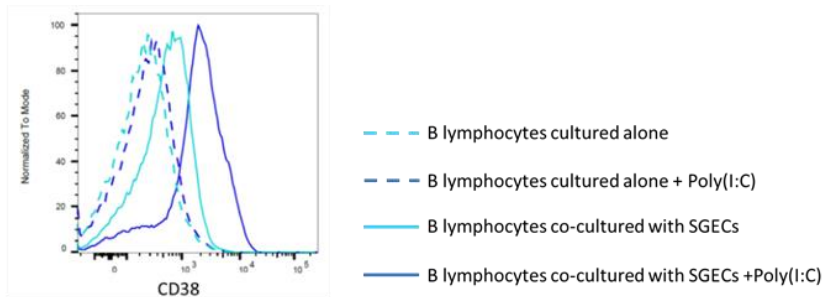
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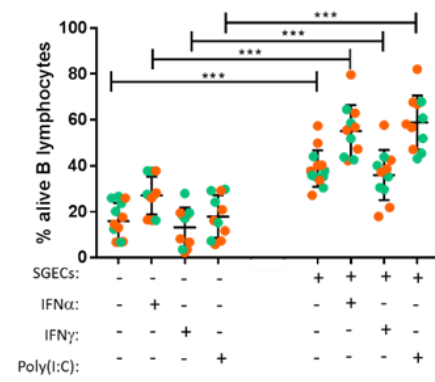
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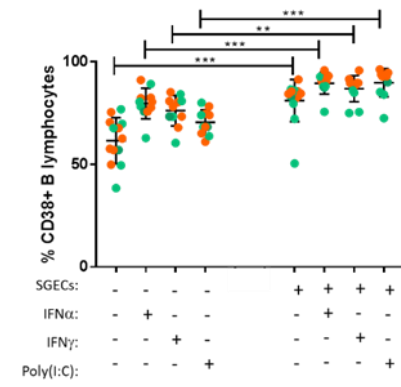
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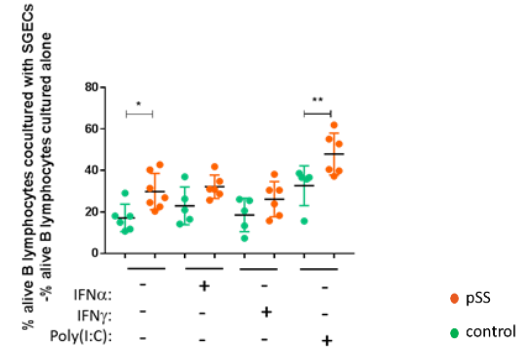
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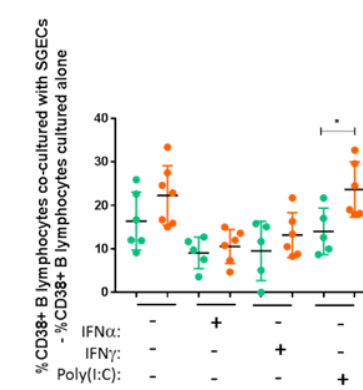
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C



F



H

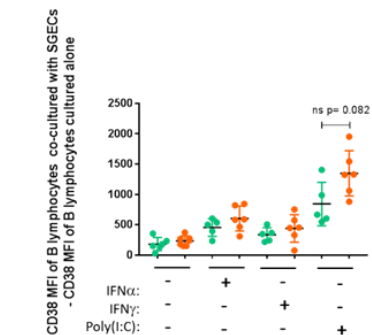
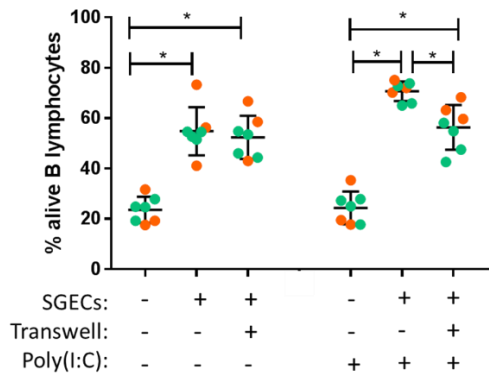
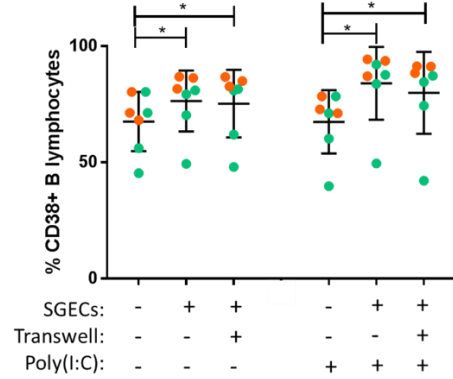


Figure 4

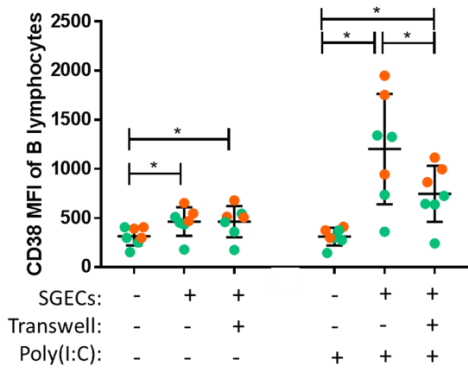
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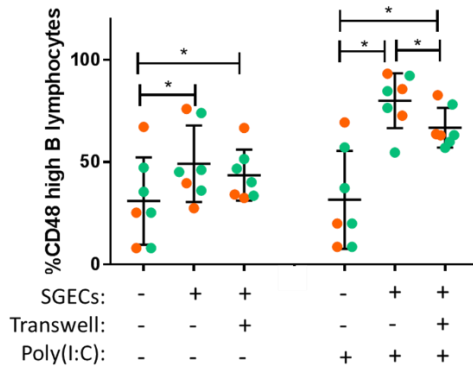
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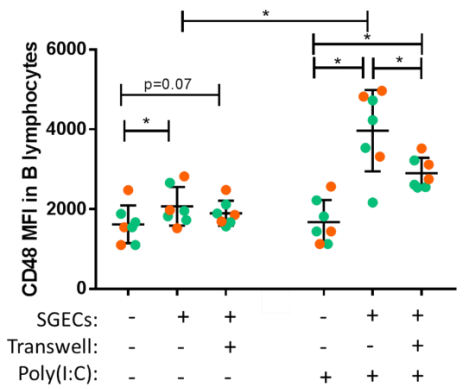
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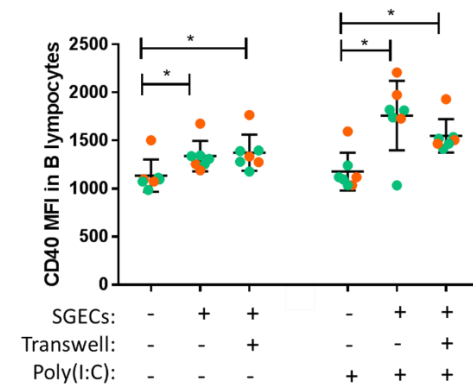
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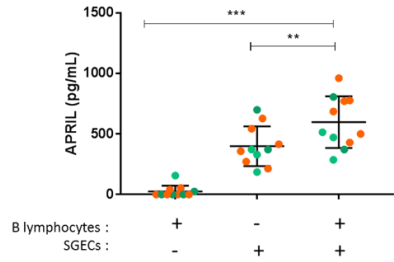
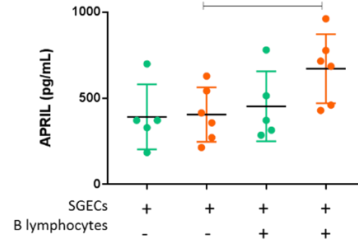
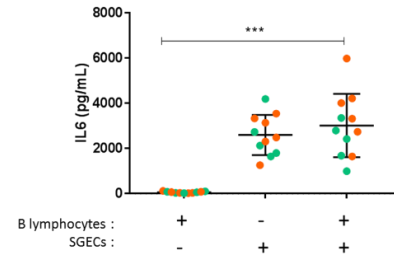
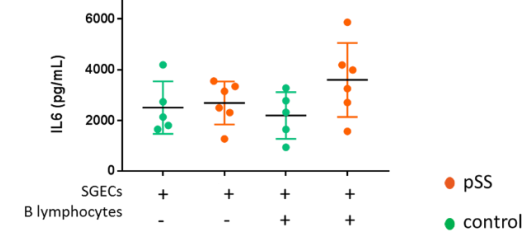
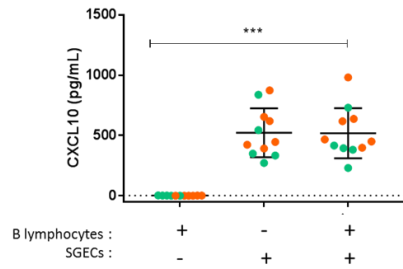
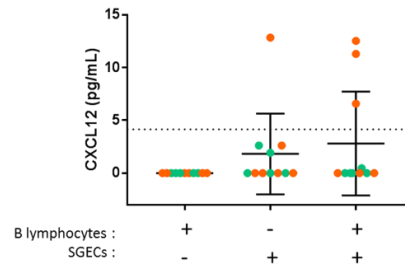
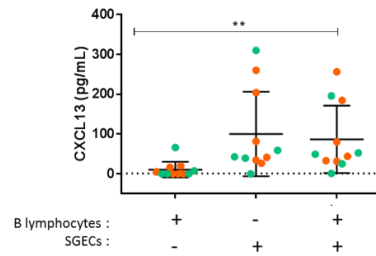
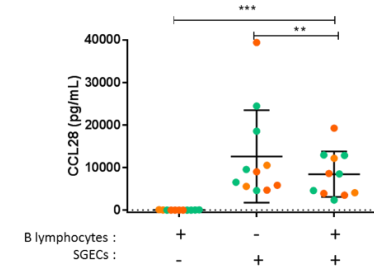
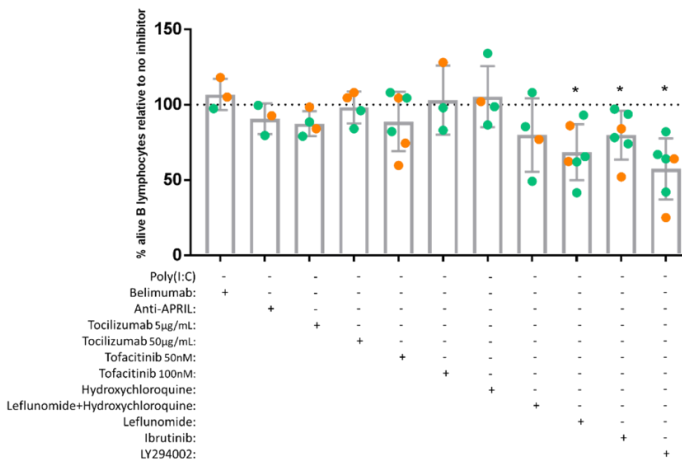
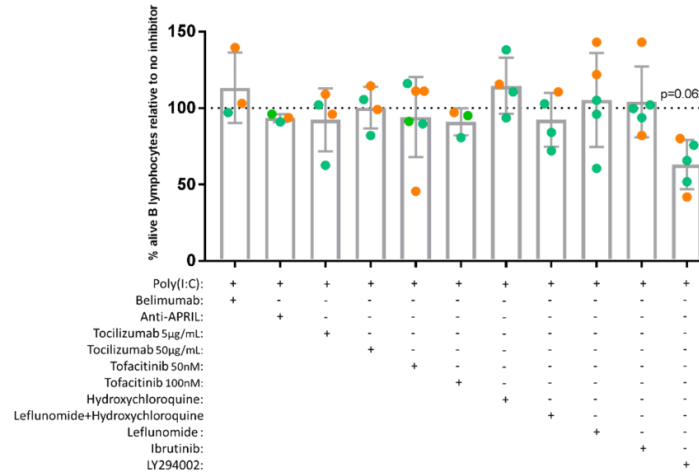
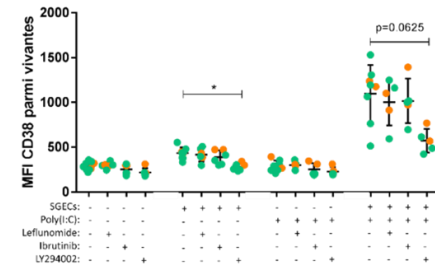
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F



● pSS
● control

A**Figure 5****B****C****D****E****F****G****H****I****J****K**

Supplementary methods:

RNA-sequencing

Cell isolation: One to two minor salivary glands (MSGs) were collected in Dulbecco modified Eagle medium (DMEM) supplemented with antibiotics, sodium pyruvate, nonessential amino acids, and glutamine. Glands were first minced with scissors and enzymatically digested at 37°C under rotating agitation (100 rpm) in DMEM containing 232 U/mL collagenase II (Worthington Biochemical, Lakewood, NJ) and 8 U/mL DNase I (Sigma, St Louis, MO). After 2 washes in calcium- and magnesium-free PBS containing 1 mmol/L EDTA, cellular aggregates were resuspended in 0.5 mL TrypLE Express Enzyme (Gibco, Carlsbad, CA) and incubated for 2 min at 37°C. Enzyme inactivation was achieved by dilution with 4 mL of DMEM and immediately gentle pipetting with a P1000 to facilitate cell dissociation. The resulting cell suspension was washed twice with medium supplemented with 0.8 U/mL DNase I. Peripheral mononuclear cells were isolated by Ficoll gradient separation.

Cell sorting: After isolation, cells were stained with DAPI Pacific Blue (LifeTechnologies) to identify dead cells. After saturation of Fc receptors by incubation with Fcblock (Miltenyi Biotec). CD326 PE+ (Miltenyi Biotec) DAPI- cells [epithelial cells] in salivary gland and CD45+ CD19 PE Cy7+ (Biolegend) DAPI- cells [B lymphocytes], in salivary gland biopsies and in blood were sorted by using a FACS ARIA cell sorter (BD Biosciences). The list of antibodies used is in **Table S1**. Sorted cells were frozen on dry ice and stored at -80°C.

RNA extraction: Samples were thawed on ice and RNA extraction was performed according to the manufacturer's instructions (PicoPure RNA Isolation Kit (Arcturus, Applied Biosystems, Foster City, CA) with the following modifications. Because of low yield of RNA expected for this type of samples, linear acrylamide (Ambion) was diluted in extraction buffer to be spiked-in to

a final concentration of 20 ng/uL. RNA quality control was performed on the Agilent 2100 Bioanalyzer with the RNA 6000 Pico Kit (Agilent).

RNA Sequencing of sorted cells, library Generation and Sequencing methods: The number of B cells sorted from biopsies difference between pSS ad controls ($p=0.02$). To avoid bias due to this initial difference, we performed RNA-seq with a normalized amount of RNA. An amount of 100pg total RNA was used as input for cDNA preparation by using the SMART-Seq v4 Ultra Low Input RNA Kit (Clontech catalog # 634892). Universal Human Reference RNA (UHRR) was included in the experiment as a positive control, and nuclease-free water was used to dilute the samples as a negative control or non-template control (NTC). Using the concentrations derived from the Ribogreen assay, a normalization plate was made with a final concentration of 10.53 pg/uL per sample. cDNA was prepared according to the manufacturer's instructions, except for the additional spike-in of 1uL diluted ERCC RNA Spike-In Mix (ThermoFisher catalog #4456740) in each sample. After subtracting the averaged concentration of the ERCC spiked NTC from the sample concentration from the Bioanalyzer trace, cDNA samples were normalized to 125pg in 5uL per sample. Library preparation used the Nextera XT DNA Sample Preparation Kit (Illumina catalog # FC-131-1096), and the Nextera XT Index Kit v2 Set A (catalog # FC-131-2001) and Set D (catalog # FC-131-2004), according to the manufacturer's instructions. The High Sensitivity D1000 ScreenTape Assay (Agilent catalog # 5067-5585 and # 5067-5584) was used on the Agilent 4200 TapeStation system to check the libraries for base pair size and molarity. All samples were then pooled to a final concentration of 4nM in an DNA LoBind Microcentrifuge Tubes (Eppendorf catalog # 022431048). The High Sensitivity DNA Assay (Agilent catalog # 5067-4626) was used on the Agilent 2100 Bioanalyzer to analyze the pool, which was then adjusted with Buffer EB (Qiagen catalog # 19086). The 12.5pM denatured library with 1% Phix spike-in was run on the MiSeq with MiSeq Reagent Kit v2 (50 cycles)

(Illumina catalog # MS-102-2001), following the manufacturer's instructions. The frequency of identified reads per index was used to adjust individual samples concentration in the pool. The re-adjusted pool was then re-Bioanalyzed and rerun on the MiSeq using the same kit and protocol. The same process of re-adjusting the pool with the new set of MiSeq index data and validation with the Bioanalyzer was performed before running the pool on the Illumina HiSeq 2500 System. The 18pM denatured library with 1% Phix spike-in was clustered on the cBot with the HiSeq PE Cluster Kit v4 (Illumina catalog # PE-401-4001), following Illumina's HiSeq and GAllx Systems Denature and Dilute Libraries Guide, and cBot System Guide, and run on the HiSeq with the HiSeq SBS Kit v4 (Illumina catalog # FC-401-4002), following the HiSeq 2500 System Guide.

Reads were first quality control-filtered and trimmed by using trimmomatic. Paired reads were aligned to the human reference genome (V38.79) by using STAR software (version 2.5.0c). Counting of reads involved use ofHTSeq.

The Interferome v2.01 database was used to identify and characterize interferon (IFN)-induced genes. Functional enrichment analysis of differentially expressed genes was performed for genes with absolute expression fold change ≥ 1.5 , by using Ingenuity Pathway Analysis software (Qiagen). Volcano plot representation were performed by using R software and multidimensional scaling representations were generated using the SVD-MDS method.

Data are available upon reasonable request.

PCR validation of the differentially expressed genes

To confirm the results of RNA-seq on sorted cells, the residual complementary DNA (cDNA) were transferred to a 48.48 Dynamic Array primed chip, and real-time PCR (RT-PCR) was run according to the Fluidigm protocol. The primers tested are in **Table S2**. Data were analyzed by

using Fluidigm RT-PCR software. Relative mRNA expression was determined from normalized Ct values by using HPRT1 as housekeeping gene and the $2^{-\Delta Ct}$ method. To compare means the Mann-Whitney test was applied.

Primary cultures of salivary gland epithelial cells (SGECs)

After 2 to 3 weeks of culture, cells at 70% to 80% confluence were dissociated with 0.125% trypsin-EDTA. Cell suspension was suspended in basal epithelial medium and added at 80 000 cells/cm² to a 6-well collagen type I (Institut de Biotechnologies, Reims, France) coated plate and incubated at 37°C and 5% CO₂ in a humidified atmosphere. The basal epithelial medium was changed at day 1 to remove epithelial cells that were not adherent. The epithelial origin of cultured cells was routinely confirmed by staining with monoclonal antibodies against CD326 (Miltenyi Biotec, Paris) an epithelium-specific marker (**Figure S2A**).

Positive isolation of B lymphocytes for co-culture experiments

Peripheral mononuclear cells were isolated from residual blood of apheresis from healthy subjects (French blood donors) by Ficoll gradient separation. B lymphocytes were isolated by CD19 magnetic bead positive selection according to the manufacturer's instructions (CD19 Microbeads human and Fc-Block, Miltenyi Biotec) to achieve a purity of greater than 80% as assessed by FACS analysis (percentage of CD20+ cells).

Co-cultures of SGECs and B lymphocytes

The primary culture of salivary gland biopsy lasted 2 to 3 weeks until we obtained a cell layer. Then we performed only one passage of the SGECs that were harvested by using trypsin and seeded in wells. After 4 days, when cells were 70% confluent in the wells, we added the B cells.

The coculture lasted 5 days. B lymphocytes (1.5×10^6 cells) were cultured alone or with SGECs in 2mL RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, and penicillin-streptomycin (1X). Stimuli were added in the medium: IFN α 2400 U/mL (Roferon-A, Roche), IFN γ 5ng/mL (Sigma Aldrich) or poly(I:C) 10 μ g/mL (Invivogen). After 5 days, B lymphocytes were harvested for immunostaining and flow cytometry. Culture supernatants were collected for cytokine quantification by multiplex assay or ELISA. Schematic representation of cocultures experimental design is presented in **Figure S1B**.

Transwell and inhibition experiments

Transwell assays were performed with 0.4 μ m pore sized inserts (Falcon Cell Culture Inserts, 6-well plates). B lymphocytes were placed in the upper chamber, and confluent SGECs in the lower in 4 mL of RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, and penicillin-streptomycin (1X). Poly(I:C) at 10 μ g/mL (Invivogen) was added in the medium. After 5 days, B lymphocytes were harvested for immunostaining and flow cytometry. Several cocultures were dedicated to inhibition experiments. Inhibitors or their control were added in the co-culture at day 0: belimumab (GSK) 10 μ g/mL or IgIV at the same concentration as control, tofacitinib (Pfizer) 50 or 100nM or DMSO at the same concentration as control, anti-APRIL (kindly provided by P. Schneider) or anti-EDA at the same concentration as control, tocilizumab (Roche) 5 or 50 μ g/mL or IgIV at the higher concentration as control, ibrutinib (Selleckchem) 1 μ M, LY294002 (Selleckchem) 20 μ M, leflunomide (Selleckchem)100 μ M, hydroxychloroquine (Selleckchem) 10 μ M or DMSO at the same concentration as control. Concentrations of inhibitors were defined according to manufacturers recommendations or, when not available, were based on the serum levels measured in patients.

FACS analysis

To assess viability of B lymphocytes cultured alone or co-cultured with SGECs in the different conditions of stimulation, B lymphocytes were harvested and stained with Fixable viability Dye APC Vio770 (LifeTechnologies, Paisley, UK). Viability was assessed as the percentage of live B lymphocytes out of all events. The activation of B lymphocytes was assessed by CD38 + B lymphocytes percentage and CD38 MFI. CD27, IgD, CD40 and CD48 were also assessed in B lymphocytes. Then, after two PBS washing, adherent SGECs were harvested after dissociation with 0.125% trypsin-EDTA and stained with anti-BAFFR antibody. The list of the antibodies used in experiments is in **Table S1**. 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. The percentage of alive cells was determined as the percentage of unstained cells with the Fixable viability Dye marker. The other percentages of positive cells were determined by comparison with the negative isotypic control. The gating strategy is presented in **Figure S2B**.

Cytokine assays

Cell-free supernatants were collected and assayed for IgG and Ig M production by enzyme-linked immunosorbent assay (ELISA) (IgG total uncoated ELISA and IgM uncoated ELISA, Life Technologies). The dosage of cytokines and chemokines (BAFF, APRIL, IL-6, CXCL10, CXCL12 and CXCL13) was performed using a multiplex assay (Thermo Fisher Scientific). BAFF and CCL28 levels were assessed by ELISA (R&D system and Thermo Fisher Scientific, respectively).

Anti-Ro/SSA antibodies were assessed by using the ALBIA method (Addressable laser bead immuno- assay) with Bioplex 2200 (Biorad). The confirmation was performed by immunodot (Euroimmun Ana3b).

Supplementary figures:

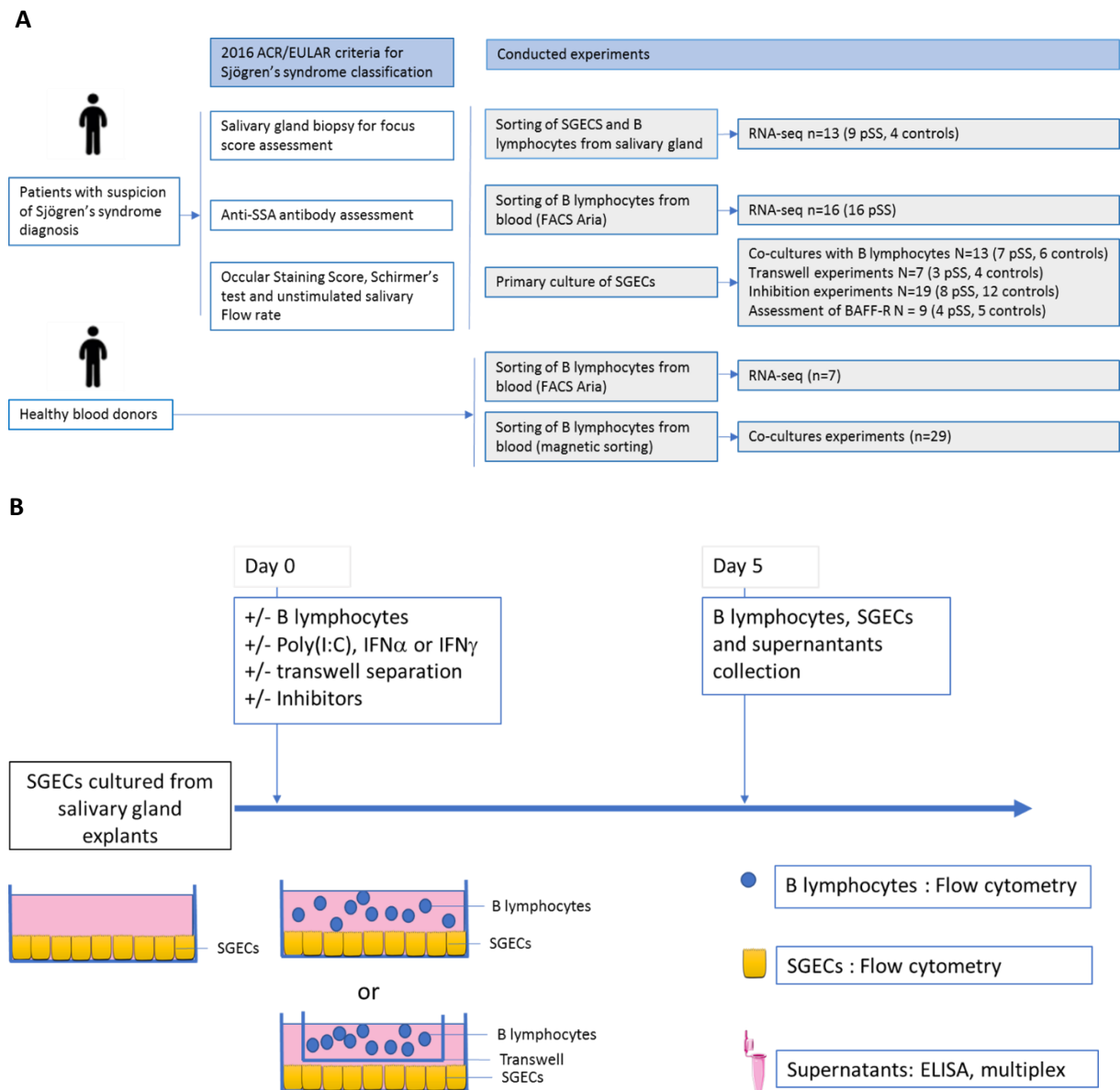


Figure S1: A: Experimental design of the study. SGECs, B lymphocytes sorted from biopsies and pSS B lymphocytes sorted from blood used for RNA-seq experiments as well as SGECs used for co-culture experiments were obtained from patients with suspected pSS. pSS was diagnosed according to 2016 ACR/EULAR criteria, and controls presented sicca symptoms without anti-SSA/SSB antibodies and had normal or sub-normal salivary glands. Control B lymphocytes sorted from blood used for RNA-seq experiments and B lymphocytes used for co-cultures, transwell and inhibition experiments were sorted from healthy blood-donors PBMCs. **B:** Schematic representation of co-culture experiments of SGECs and B lymphocytes.

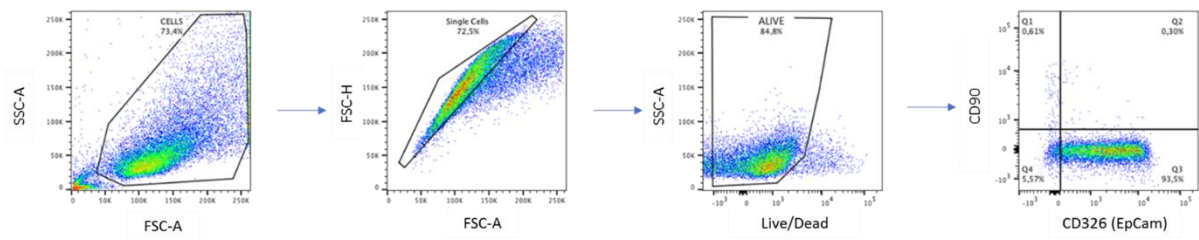
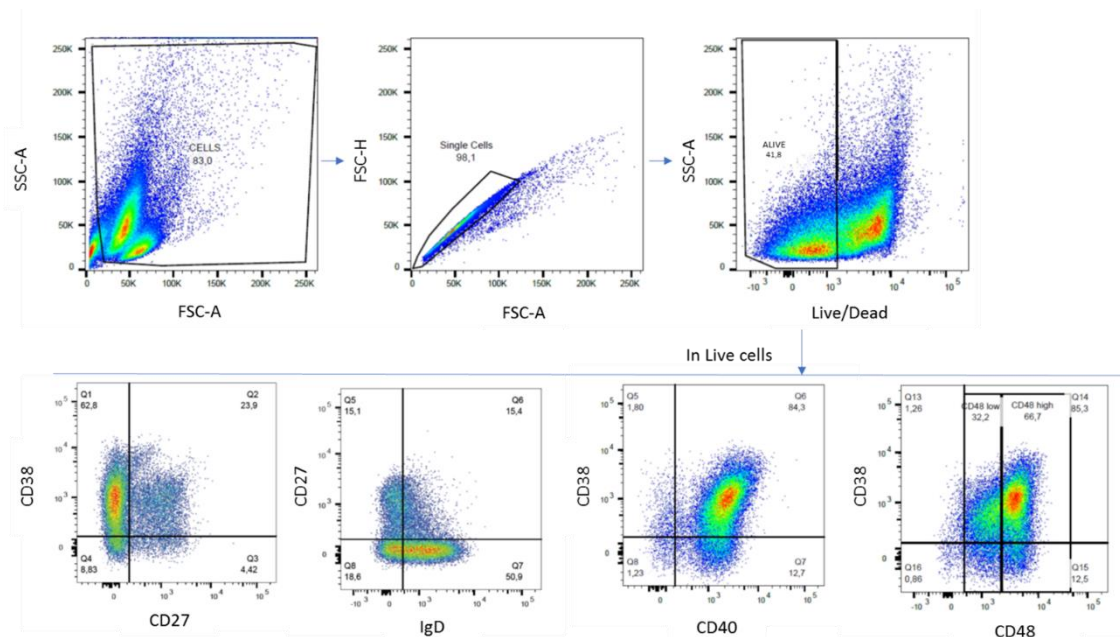
A**B**

Figure S2: A: Gating strategy used to phenotype SGECs. CD326 and CD90 expression on SGECs after 2 weeks of primary culture. **B:** Gating strategy used to phenotype/co-cultured B lymphocytes. Percentages of CD38, CD27, IgD, CD40 and CD48 B lymphocytes were determined among alive B cells. MFI was determined with the same strategy.

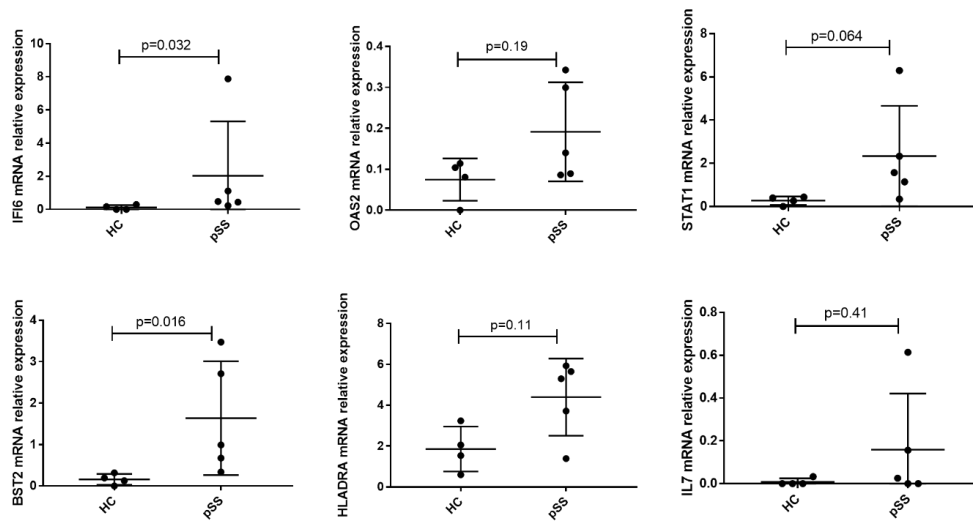
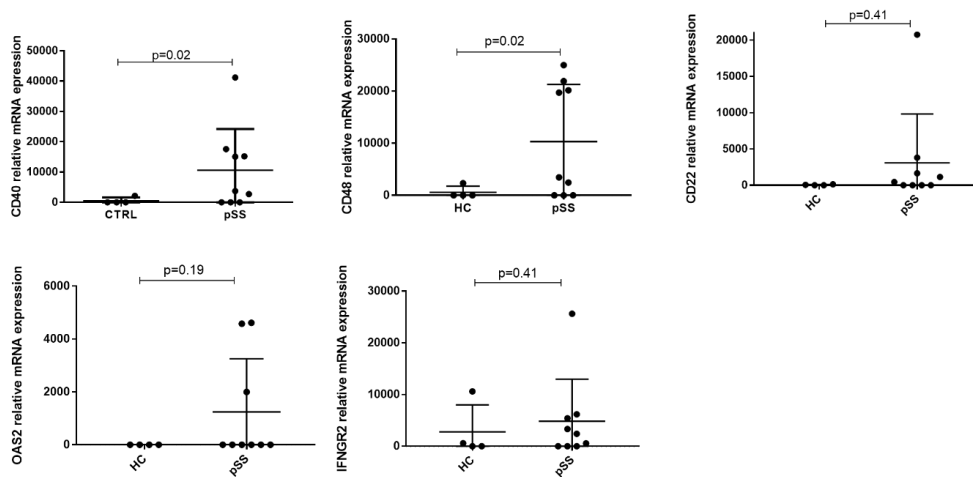
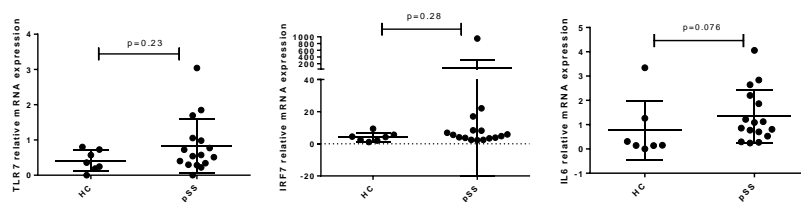
A**SGECs sorted from biopsy****B****B cells from biopsy****C****B cells from blood**

Figure S3: A: Validation of the RNA-seq results by RT-PCR. Relative mRNA expression of *IFI6*, *OAS2*, *STAT1*, *BST2*, *HLA DRA* and *IL-7* in SGECs. **B:** Relative mRNA expression of *CD40*, *CD48*, *CD22*, *OAS2*, and *IFNGR2* in B lymphocytes sorted from salivary gland biopsy. **C:** Relative mRNA expression of *TLR7*, *IRF7* and *IL-6* in B lymphocytes sorted from blood. To compare groups the Mann-Whitney test was applied.

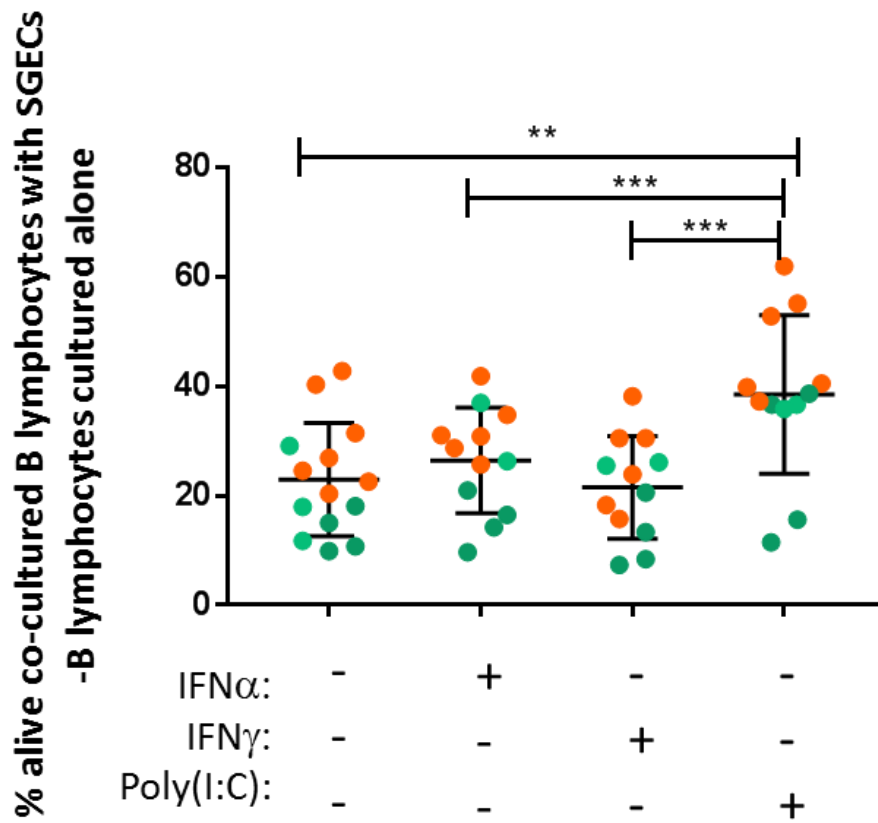


Figure S4: Difference between the percentage of alive B lymphocytes on co-culture with SGECs and the percentage of alive B lymphocytes cultured alone on day 5 stimulated with IFN α , IFN γ or poly(I:C). **P<0.01, ***P<0.001

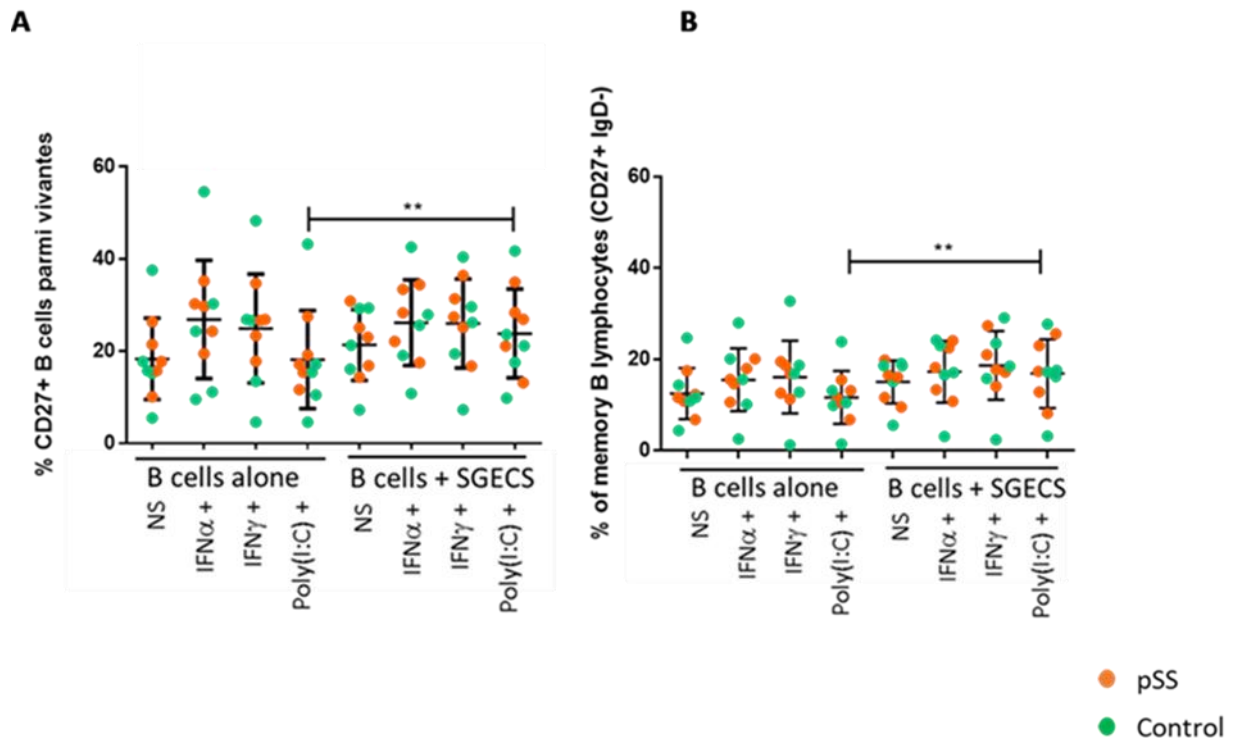
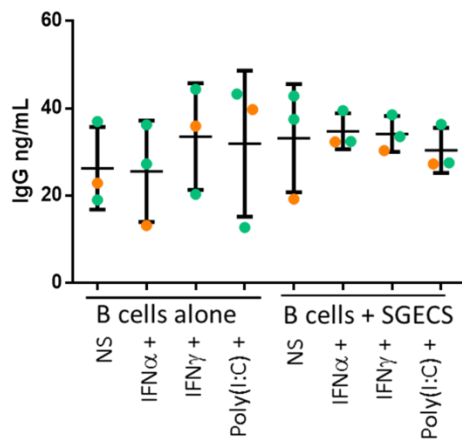
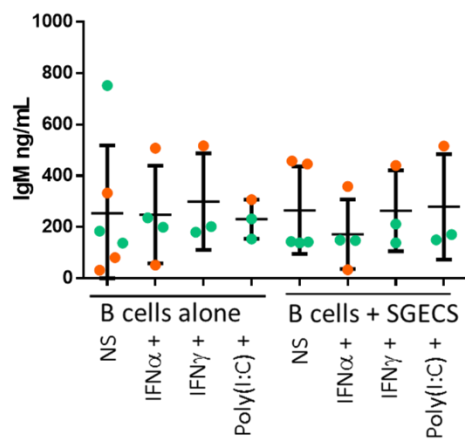


Figure S5: A: Percentage of memory B lymphocytes (CD27+) on day 5 under all conditions of culture and stimulation. **B:** Percentage of switched memory B lymphocytes (CD27+ IgD-) on day 5 under all conditions of culture and stimulation. **P<0.01

A**B**

● pSS
● Control

Figure S6: Level of IgG (A) and IgM (B) in supernatant of B lymphocytes cultured alone and co-cultured with SGECs for 5 days.

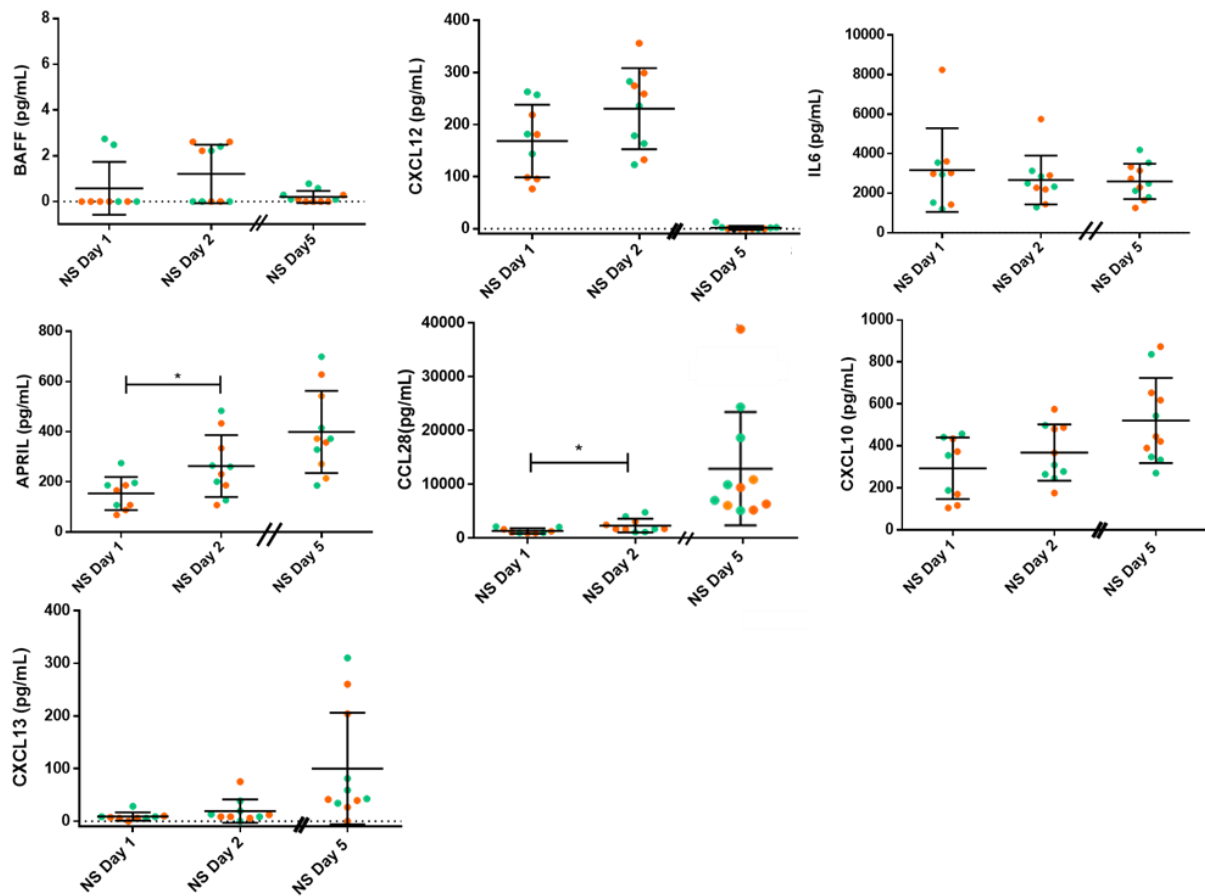


Figure S7 : Levels of BAFF, CXCL12, APRIL, IL-6, CCL28, CXCL10 and CXCL13 in supernatants from cultured SGECS from 5 pSS and 5 controls at day 1 and day 2. The results obtained at day 5 are also reported on the graphs (similar to the results presented on Figure 5). Controls are in green and pSS are in orange. NS : not stimulated.

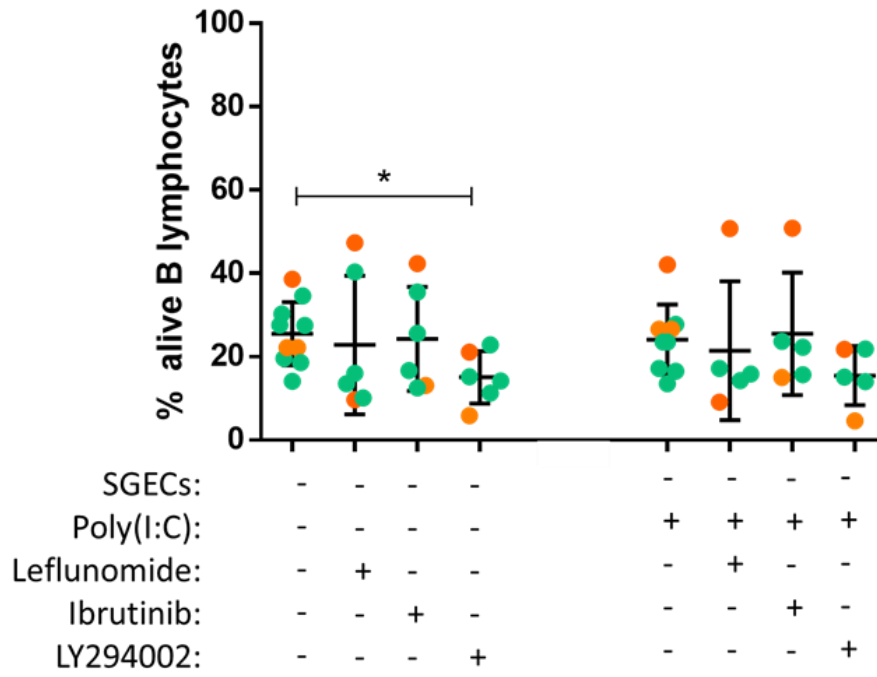


Figure S8 : Percentage of alive B lymphocytes cultured alone after 5 days in the presence of leflunomide, ibrutinib (BTK inhibitor) or LY294002 (PI3K inhibitor). * p<0.05.

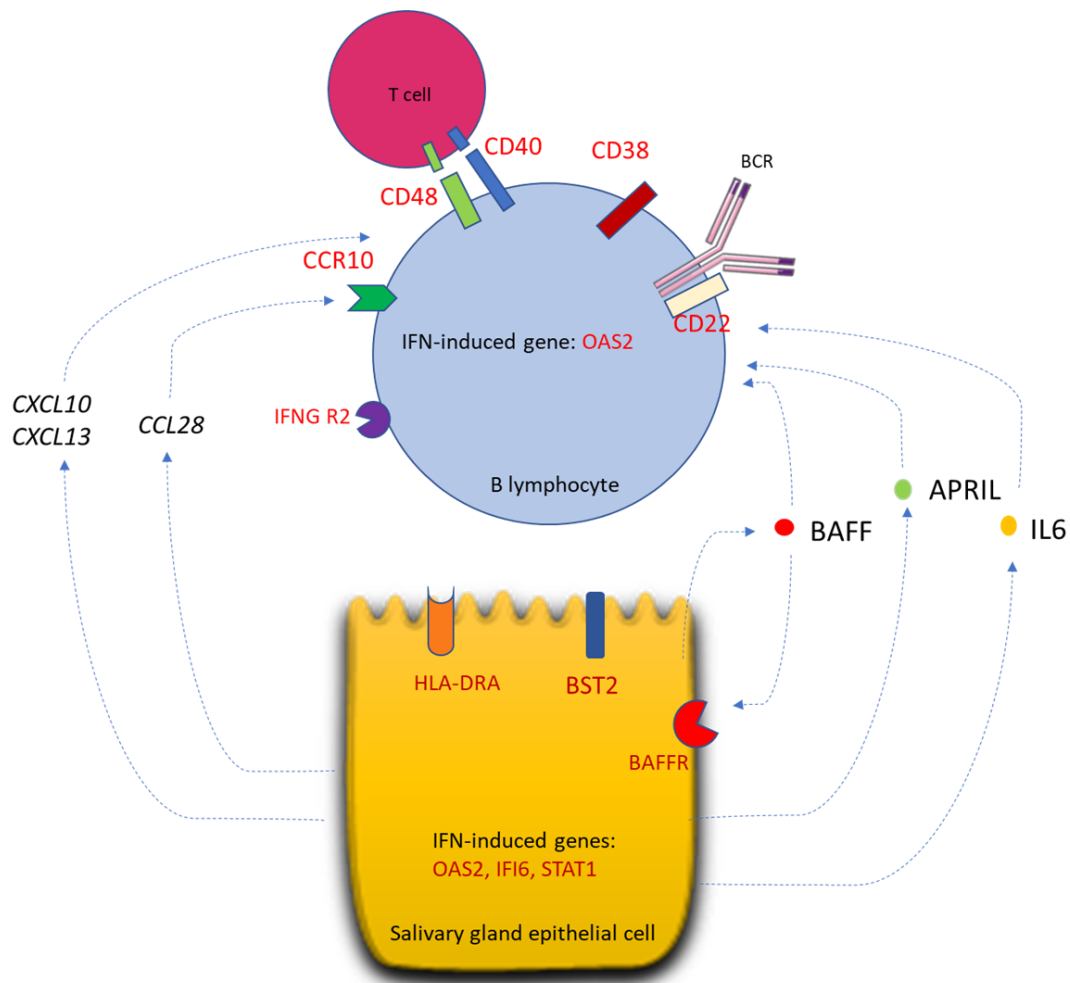


Figure S9: Figure 8: Schematic representation of hypothesis for mechanisms of interaction between SGECs and B lymphocytes. Summary of the genes and pathways that were highlighted by transcriptome analysis (CD40, CD48, CD22, CCR10, IFNGR2 and OAS2 for B cells ; HLA-DRA, BST2, BAFFR, OAS2, IFI6, STAT1 for SGECs) or flow cytometry (CD38, CD48 for B cells ; BAFFR for SGECs) or measured in SGEC supernatant (CXCL10, CXCL13, CCL28, APRIL, IL-6 and BAFF after poly(I:C) simulation). SGECs secrete cytokines and chemokines potentially involved in B-lymphocytes activation or homing.

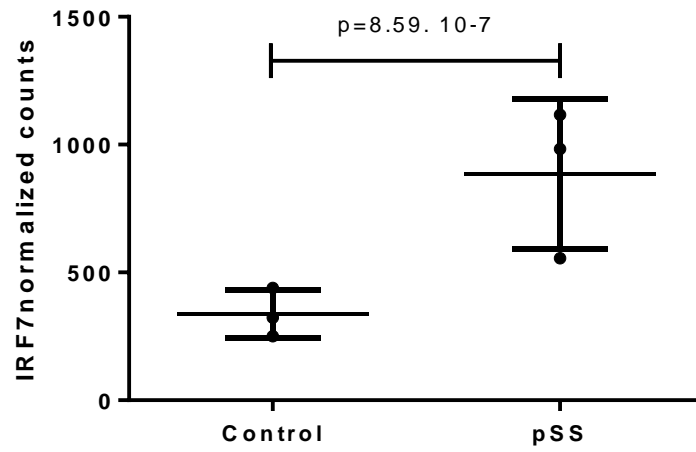


Figure S10: Normalized counts of IRF7 in the RNAseq dataset from Jin-Lei Sun et al. in B lymphocytes sorted from blood from 3 controls and 3 pSS patients

Supplementary tables:

Table S1: List of antibodies used for FACS experiments on co-cultured cells

ANTIBODY	CONJUGATED FLUOROCHROME	CLONE	TYPE OF ANTIBODY	SOURCE
Sorting of SGECs and B lymphocytes from salivary gland biopsies				
496-diamidino-2-phenylindole dihydrochloride (DAPI)	Pacific Blue			Life Technologies
CD45	APC Vio770	HI30	mouse, monoclonal	BioLegend
CD19	PE Cy7	HIB19	mouse, monoclonal	BioLegend
CD326	PE	HEA-125	mouse, monoclonal	Miltenyi Biotec
Co-culture experiments				
Fixable viability Dye	APC Vio770			LifeTechnologies
anti-CD20	PerCpVio700	REA780	recombinant human IgG1	Miltenyi Biotec
anti-CD38	APC	REA572	recombinant human IgG1	Miltenyi Biotec
anti-CD27	PE	REA499	recombinant human IgG1	Miltenyi Biotec
anti-IgD	PEVio770	REA740	recombinant human IgG1	Miltenyi Biotec
anti-CD40	PE	REA733	recombinant human IgG1	Miltenyi Biotec
anti-CD48	PEVio770	REA426	recombinant human IgG1	Miltenyi Biotec
anti-CD326	PE	REA764	recombinant human IgG1	Miltenyi Biotec
anti-HLA-DR	FITC	REA805	recombinant human IgG1	Miltenyi Biotec
anti-BST2	APC	REA202	recombinant human IgG1	Miltenyi Biotec
anti-BAFFR	PEVio770	REA1115	recombinant human IgG1	Miltenyi Biotec
REA-control	PerCpVio700	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	APC	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	PE	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	PEVio770	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	FITC	REA293	recombinant human IgG1	Miltenyi Biotec

Table S2: List of primers used for RT-PCR validation of RNA-seq results with Biomark technology purchased from Fluidigm

Target	Assay Name	Forward Primer	Reverse Primer	Design RefSeq
BST2	BST2_90198_i2	ACATTAAACCATAAGCTTCAGGAC	GCGATTCTCACGCTTAAGAC	NM_004335.N
CCL28	CCL28_90212_i1	GAGCTGATGGGATTGTGAC	TTGGCAGCTTGCACTTCA	NM_001301875.N
CCR10	CCR10_90205_i0	GGACGGAGGCCACAGA	AGTGGCTCAGCCGAGTAT	NM_016602.N
CD22	CD22_59645_i9	CTGCCTCGCCATCCTCA	TGGCTGTGTCTCTTCC	NM_001771.3
CD40	CD40_55716_i2	ACTGCCACCAGCACAATAC	TACAGTGCCAGCCTTCTCA	NM_152854.2
CD48	CD48_86944_i0	ATTCGTGTCTGGCTCTGGAA	CGGAGACCACGGTCATATGTA	NM_001256030.N
PKR/EIF2AK2	EIF2AK2_90200_i13	GCGAACAAGGAGTAAGGGAA	AGAGGTCCACTTCTTTCCA	NM_002759.N
HLA-DRA	HLA-DRA_54516_i0	CGCTCAGGAATCATGGGCTA	CGCCTGATTGGTCAGGATTCA	NM_019111.4
HPRT1	HPRT1_5093_i5	GCTTTCCTTGGTCAGGCAGTA	ACTTCGTGGGGTCTTTTCCAC	NM_000194.2
IFI16	IFI16_26848_i8	GTGAATGGGGTGTGAGGTAC	CACCACTTCCATCTCCCTGTA	NM_005531.2
IFI6	IFI6_90203_i1	TGCTACCTGCTGCTTCA	TCAGGGCCTCCAGAACC	NM_022873.N
IFNGR2	IFNGR2_87575_i2	GTGGCCCTGAGCAATAGCA	TGTCGGCCGTAACCATTTA	NM_001329128.N
IL6	IL6_12521_i3	AGAGCTGTGCAGATGAGTACAA	GTTGGGTGAGGGGTGGTTA	NM_000600.3
IL7	IL7_60520_i1	ATTGAAGGTAAGATGGCAAACA	TCATTATTCAGGCAATTGCTACC	NM_000880.3
IRF7	IRF7_25580_i5	GGCAGAGCCGTACCTGTCA	ACCGTGCGGCCCTTGTGA	NM_004031.2
OAS2	OAS2_83324_i0	TGGTGAACACCATCTGTGAC	CCATCGGAGTTGCCTCTTAA	NM_001032731.N
SDC1 (CD138)	SDC1_90204_i2	AAGATGGCTCTGGGGATGAC	GAGCTGCGTGCCTTCCA	NM_001006946.N
STAT1	STAT1_56036_i14	ATGCTGGCACCAGAACGAA	GCTGGCACAATTGGGTTTCAA	NM_007315.3
TLR7	TLR7_67595_i1	TCTTCAACCAGACCTCTACATCC	AGCCCAAGGAGTTTGGAAA	NM_016562.3
TNFRSF13C (BAFFR)	TNFRSF13C_90207_i1	CCCCGACGGAGACAAG	CTGTGGCATCAGAGATTCCC	NM_052945.N
TNFRSF11A (RANK)	TNFRSF11A_90208_i1	TGGGACGGTGTGTAACAAA	CAGGGCAGACATACACTGTCA	NM_003839.N

Table S3: Pathways over-represented in salivary gland epithelial cells (SGECs) sorted from salivary glands and B lymphocytes sorted from blood, analysis performed with Ingenuity pathway analysis software

Pathway	-log p-value	Genes
<i>SGECs sorted from salivary gland</i>		
Primary Immunodeficiency Signaling	4.08	<i>PTPRC, BTK, IGHG1, CD8A, TAP1, TNFRSF13C</i>
Interferon Signaling	3.52	<i>IFIT3, OAS1, IFI6, STAT1, TAP1</i>
B Cell Development	2.89	<i>PTPRC, HLA-DRA, CD86, IL7</i>
Role of JAK2 in Hormone-like Cytokine Signaling	2.73	<i>STAT5A, IRS1, SH2B3, STAT1</i>
IL7 Signaling Pathway	2.51	<i>STAT5A, SLC2A1, IRS1, IGHG1, STAT1, IL7</i>
<i>B lymphocytes sorted from blood</i>		
EIF2 Signaling	8,6	<i>PIK3CA,RPLP1,RPL3,RPS23,RPLP2,EIF4G3,EIF4G1,RPL28,PPP1CC,RPL8,EIF4G2,RPL13,UBA52,PIK3C3,RPS9,RPL10,RPS25,RPS2,EIF2AK2,RPS3,RPS12,RPL18,RPL13A,RPL41,RPLP0,RPS14</i>
Interferon Signaling	7,02	<i>IFIT3,OAS1,MX1,TYK2,IFI6,IFI35,STAT2,IFNAR2,STAT1,IFITM1</i>
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	4,24	<i>IFIH1,PIK3CA,IRF7,OAS1,OAS2,TICAM1,PIK3C3,TLR7,MAVS,PRKCH,NFKB2,IL6,CCL5,EIF2AK2</i>
Activation of IRF by Cytosolic Pattern Recognition Receptors	3,98	<i>IFIH1,IRF7,MAVS,IKBKE,STAT2,IL6,NFKB2,ADAR,STAT1</i>
mTOR Signaling	3,35	<i>PIK3CA,RPS23,VEGFB,EIF4G3,EIF4G1,RHOG,EIF4G2,PIK3C3,RPS9,RPS6KB2,RPS25,PRKCH,RPS2,RPS12,RPS3,RPS14</i>
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	3,13	<i>TYK2,STAT2,NFKB2,IFNAR2,STAT1</i>

Table S4: Fold change and p-value for differential gene expression between pSS patients and controls in SGEs and B lymphocytes sorted from salivary gland biopsies and from blood.

Gene Symbol	Gene ID	log2 fold-change	p-value
SGECs from salivary gland			
<i>HLA-DRA</i>	ENSG00000204287	1.84	0.04
<i>IFI6</i>	ENSG00000126709	4.32	2.7 10 ⁻⁵
<i>STAT1</i>	ENSG00000115415	2.13	0.02
<i>BST2</i>	ENSG00000130303	4.08	<0.01
<i>BAFFR</i>	ENSG00000159958	4.94	0.01
<i>IL7</i>	ENSG00000104432	2.56	<0.01
<i>OAS2</i>	ENSG00000111335	2.18	<0.01
B lymphocytes from salivary gland			
<i>CD48</i>	ENSG00000117091	2.59	0.01
<i>CD22</i>	ENSG00000012124	2.29	.048
<i>CD40</i>	ENSG00000101017	2.64	0.02
<i>IFNGR2</i>	ENSG00000159128	2.28	0.03
<i>OAS2</i>	ENSG00000111335	2.91	0.04
B lymphocytes from salivary gland			
<i>TLR7</i>	ENSG00000196664	1.40	<0.01
<i>IRF7</i>	ENSG00000185507	0.76	0.04
<i>IL6</i>	ENSG00000136244	1.54	<0.01
Salivary gland vs blood B lymphocytes in pSS			
<i>CD138</i>	ENSG00000115884	6.92	9.6 10 ⁻⁵
<i>IL6</i>	ENSG00000136244	3.05	<0.01
<i>CCR10</i>	ENSG00000184451	2,73	<0.01
<i>RANK</i>	ENSG00000141655	6.14	3.7 10 ⁻⁵