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IMMUNOPATHOLOGY

Interleukin-15 as a potential new target in Sjögren's syndrome-associated inflammation

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Summary

IL-15 is a key regulatory cytokine that shares many biological properties with IL-2. Recently, it has been shown that IL-15 could be up-regulated in T cell-mediated inflammatory disorders, such as rheumatoid arthritis and inflammatory bowel diseases. However, the role and expression of IL-15 in the inflammatory autoimmune disease Sjögren's syndrome (SS) has not been investigated. In the present study we evaluated the expression of IL-15 mRNA and protein in minor salivary gland (MSG) biopsy specimens and in human salivary gland epithelial cell (SGEC) cultures obtained from patients with primary SS (pSS) and compared their expression with that seen in normal healthy control subjects. IL-15 gene and protein analysis revealed that SGEC are able to produce IL-15. Results obtained demonstrated that the number of IL-15^{+} cultured SGEC was significantly higher in cells derived from patients with pSS in comparison with SGEC from healthy subjects; similar results were obtained for IL-15 immunoreactivity by using immunohistochemistry that revealed a strong expression both in acinar and in ductal cells from pSS MSG. These studies could provide a rational basis to determine whether IL-15 could be a good candidate for anti-cytokine therapy in chronic inflammatory pSS diseases.

Key words: Salivary gland; epithelial cells; Sjögren's syndrome; IL-15; inflammation; autoimmunity.

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INTRODUCTION

Sjögren's syndrome (SS) is a systemic autoimmune disease that presents with sicca symptomatology of mucosal surfaces, mainly dry mouth and dry eyes.[1] There is often systemic involvement (extra glandular manifestations), lymphoma is a recognised complication[2] and SS is one of the most prevalent autoimmune diseases.[3] The expression 'autoimmune epithelitis' has been proposed as alternative term for SS since as it is considered as an epithelial cell-specific autoimmune disease.[4] SS is classified as primary (pSS) when it occurs in the absence of another underlying rheumatic disorder.[3] A great deal of knowledge has been gained regarding the circumstances leading to cytokines-chemokines production by epithelial cells, and some evidence suggests that minor salivary gland (MSG) inflammation in pSS is associated with increased production by human salivary gland epithelial cells (SGEC) of pro-inflammatory cytokines which might even be the apex of the inflammatory cascade.[5–10] Immunoregulatory cytokines have been demonstrated to have a very important role in the inflammation linked to autoimmune conditions, and in particular IL-15, which has a key role for the modulation of lymphocyte homeostasis as well as natural killer T-cell development, and has been demonstrated to have an important role in the processes that lead to an abnormal inflammatory response.[11,12] IL-15, a 14 kDa glycoprotein mainly produced by macrophages as well as non-lymphoid cells, is a key regulatory cytokine which supports the homeostasis between innate and adaptive immunity.[12,13] IL-15 is a member of a cytokine family that exerts its function via the common γ chain and the IL-2 receptor (IL-2R)-β chain. IL-2 and IL-15 bind to common T-cell surface receptors comprised of unique alpha (IL-2Rα or IL-15Rα) and shared β/γ chain subunits.[13] Although IL-2 and IL-15 are structurally related they have distinct roles with regard to apoptosis and T-cell survival; in fact, ligation of this receptor by IL-2 can lead to apoptosis whereas IL-15 ligation seems to favour cell survival.[13] Functionally, IL-15 plays its role via JAK/ STAT and Ras/MAPK signalling pathways and preventing cell death by a fine balance between pro- and anti-apoptotic signals that involves the activation of the PI3K pathway.[13] As IL-15 expression is strictly regulated at multiple distinct levels, including transcription, translation, and intracellular trafficking,[11] removal of these negative control mechanisms results in increased IL-15 production, which may predispose to the risk of excessive autoreactive T-cell survival and abnormal lymphocyte activation, thus leading to the development of autoimmune or chronic inflammatory diseases.[11] Indeed, IL-15 overexpression is associated with an array of immune mediated disorders, such as inflammatory bowel disease,[14] coeliac disease,[15,16] and rheumatoid arthritis.[17] Little information is available on the levels of IL-15 expression in plasma samples of pSS patients,[18] and unfortunately no data are present in the literature on the exact role of IL-15 in salivary gland pathogenesis or a possible relation between IL-15 production and pSS disease activity. On the basis of this background, in this study we examined the expression of IL-15 in salivary glands from patients with pSS as well as in normal control subjects. Because the expression of IL-15 is regulated at multiple steps, including

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transcriptional and translational controls,[11] we examined both the gene and protein expression in salivary gland biopsy specimens from pSS patients and in pSS SGEC cultures.

MATERIALS AND METHODS

Patient selection

MSG biopsies were obtained from 20 pSS patients (20 female, mean age 48 ± 15 years, mean disease duration 24 ± 18 months), fulfilling the American–European Consensus Group criteria for pSS.[19] All patients showed the clinical symptoms of dry eyes and mouth, a positive Schirmer's test (less than 5 mm wetting of a strip of filter paper per 6 minutes) and Rose Bengal staining with the presence of at least one of the following autoantibodies: anti-Ro/SSA, anti-La/SSB, or both. The clinical and laboratory features of pSS patients enrolled in the study are detailed in Table 1. The patients all gave their written consent, the study was approved by the local Ethical Review Committee and the experiments were conducted according to the tenets of the Declaration of Helsinki. Fifteen healthy subjects (female, mean age 46 ± 12 years) awaiting removal of salivary mucoceles from the lower lip were considered as control group. Healthy subjects had no sicca symptoms; they were advised about the investigational nature of the study and none refused to participate. MSGs were harvested from the lower lip under local anaesthesia through normal mucosa, according to the explant outgrowth technique.[20] All MSG tissue sections from pSS patients had been evaluated by an oral pathologist and a focus score ≥1 was found. MSG tissue sections derived from from pSS and healthy control subjects were fixed in formalin, embedded in paraffin and stained with haematoxylin and eosin (H&E). MSG sections from pSS patients presented a proportion of epithelial cells comparable with that revealed in healthy MSG sections. MSG sections from pSS patients showed the lymphocytic infiltration that is the histological hallmark of SS.

Microdissection and primary explant cultures of human salivary gland epithelial cells

MSG samples, after removal, were divided into two portions. One portion was immediately analysed to obtain primary cultures of human SGEC or to extract RNA and proteins. The second piece was processed for morphological and immunohistochemical analysis. Microdissection and collagenase (Worthington Diagnostic Division, USA) digestion procedure was used to obtain the SGEC cultures, which were then re-suspended in McCoy's 5a modified medium supplemented with 10% fetal bovine serum, 1% antibiotic solution, 2 mM L-Glutamine, 20 ng/mL epidermal growth factor (EGF; Promega, USA), 0.5 μg/mL insulin (Novo, Denmark) and incubated at 37°C, 5% CO_2 in air. SGEC cultures were fed every 5 days with fresh culture medium and when they reached 70–80% confluence, were used for the experimental procedure. SGEC were routinely subcultured for a total of four passages during which all experimental procedures were completed. To reduce and eliminate the contamination of fibroblast in the SGEC cultures, treatment with 0.02% EDTA was employed. The epithelial origin of cultured cells was routinely confirmed by immunohistochemistry using specific monoclonal antibodies against epithelial-specific markers that include different cytokeratins and epithelial membrane antigens as previously described.[21]

Table 1 Characteristics of healthy subjects and pSS patients enrolled in this study

Feature	pSS (n = 20)	Controls (n = 15)
General		
Age, mean ± SD years	48 ± 15	46 ± 12
Female/male (n)	20/0	15/0
Disease duration, mean ± SD months	24 ± 18	NA
Histological (MSG biopsy)		
Biopsy focus score ≥1 (%)	100	0
Clinical		
Lymphadenopathy (%)	10	0
Lymphoma (%)	1	0
Arthritis (%)	55	0
Vasculitis (%)	20	0
Lung fibrosis (%)	10	0
Renal involvement (%)	5	0
Myositis (%)	15	0
Haematological disorder (%)	35	0
Laboratory		
Anti-SSA (%)	85	0
Anti-SSB (%)	55	0
ANA (%)	65	0
Rheumatoid factor (%)	30	0
Hypergammaglobulinaemia (%)	65	0
Cryoglobulinaemia (%)	5	0

MSG, minor salivary gland; NA, not applicable; pSS, primary Sjögren's syndrome; SD, standard deviation.

RNA extraction and quantitative TaqMan real-time PCR

Total RNA was isolated from human cultured healthy and pSS SGEC using TRIzol reagent and following the manufacturer's protocol (Invitrogen, USA). RNA quality was analysed by gel electrophoresis to confirm the integrity of the RNA preparations and then was treated with DNase I (GIBCO, Life Technologies, USA) prior to reverse transcription with Moloney murine leukaemia virus reverse transcriptase (GIBCO) in the presence of RNaseOUT (GIBCO). One tenth of the cDNA preparation for each PCR reaction was used. Master mix and Taqman gene expression assays for β-2 microglobulin (part no. 4326319E; β2M, control gene) and target genes IL-15 were obtained from Applied Biosystems (USA). Taqman qPCR amplifications were performed as duplex reactions with assays for the test and control in the same well. The result of the relative increase in reporter fluorescent dye emission was analysed by an ABI PRISM 7700 sequence detector (Applied Biosystems). Relative changes in gene expression between pSS and healthy controls samples were determined using the $2^{-\Delta\Delta Ct}$ method. Final values were expressed as fold of induction.

Western blot analysis of IL-15 expression

Cell lysates were obtained through the incubation with lysis buffer [1% (v/v) Triton X-100, 20 mM Tris-HCl, 137 mM NaCl, 10% (v/v) glycerol, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 μM leupeptin hemisulfate salt, 0.2 U/mL aprotinin] for 30 min on ice. The lysates (40 μg protein per lane) were electrophoresed on a sodium dodecyl sulfate (SDS)-polyacrylamide gel and then transferred to a nitrocellulose membrane. For gel, membrane saturation and blot was used a blot buffer [20 mM Tris/150 mM glycine, pH 8, 20% (v/v) methanol]. The blot conditions were the following: 200 mA (constant amperage), 200 V for 110 min. Then, the blot was blocked by phosphate-buffered saline (PBS) pH 7.2 with 0.1% (v/v) Tween 20, 5% w/v non-fat dried milk for 1 h, washed three times with 0.1% (v/v) Tween 20-PBS 1x (T-PBS) and the membranes were incubated for 90 min with mouse anti-human IL-15 monoclonal Ab (mAb) (R&D Systems, USA; cat no. MAB247, 0.1 μg/mL) and for 30 min with the relative secondary antibody-HRP conjugates (Santa Cruz Biotechnology, USA). Proteins recognised by the antibodies were revealed using chemoluminescence luminal reagent (Santa Cruz Biotechnology) according to the protocol. Immunoblots were then stripped with stripping buffer (Thermo Scientific, USA) for 20 min, washed three times × 10 min in 0.1% PBS/Tween. As a control for equal protein loading, Western blot for β-actin (Santa Cruz Biotechnology; cat no. sc-130300, 0.25 μg/mL) was performed.

Immunohistochemical analysis for IL-15 expression in MSG

Paraffin embedded sections of 5 μm thickness were stained with H&E for the histological evaluation of the presence of lymphocytic infiltrates and/or foci. A focus was defined as an aggregate of ≥50 lymphocytes and the focus score was reported as the number of foci per 4 mm^2 of tissue. The sections were deparaffinised, dehydrated and washed in Tris-buffered saline (TBS) (Sigma, USA) containing 0.1% BSA. Endogenous peroxidase activity was quenched by incubating the slides in a solution of 700 μL H_2O_2 (30%) in 70 mL methanol. The sections were pre-treated with pepsin (0.4%) for 30 min at 37°C. Blocking was performed with normal serum. After incubation with the primary antibody against IL-15 (R&D Systems; cat no. MAB247), at room temperature for 60 min, the slides were washed in TBS buffer, the HRP-conjugated secondary antibody (Santa Cruz Biotechnology) was added and

the slides were incubated at room temperature for 30 min. Afterwards the slides were incubated with diaminobenzidine tetrahydrochloride (DAB) (Sigma) as substrate. Negative controls without primary antibody were included in each experiment to verify antibody specificity. The images were captured under the Nikon Eclipse 80i light microscope (Nikon, Japan).

Flow cytometry

Expression of IL-15 was analysed by incubating the cells for 30 min at 4°C in the dark at optimal concentrations of antibodies. Intracellular detection of IL-15 was performed using the mouse anti-human-Phycoerythrin (PE) conjugated antibody (R&D Systems, cat no. IC2471P). The protein expression was analysed by a Becton Dickinson (BD, Germany) FACSCanto II flow cytometer and BD FACS diva software according to manufacturer instructions. Values were given as percentages of positive cells, and the mean fluorescence intensity (MFI) was reported.

Statistical analysis

Experimental values were expressed as mean ± SE, and the differences in means for paired observations were analysed by Student's t-test; we subjected the results of measurements from all sets to an analysis of variance (ANOVA). p values < 0.05 were considered statistically significant.

RESULTS

Differential expression of the IL-15 gene and protein between healthy subjects and pSS patients

Inflammatory cytokines lead to systemic and exocrine manifestations that characterise pSS and an exacerbated expression of these molecules is able to alter the glandular integrity and functionality dramatically. Following the discovery of pro-inflammatory cytokines accumulation in functionally and structurally damaged areas of the salivary glands and its pathogenic significance in this autoimmune disease,[5] we assessed the gene and protein expression levels of IL-15 in SGEC derived from pSS patients and healthy subjects. Quantitative real-time PCR was employed. The results showed that IL-15 mRNA levels were detected in both healthy and pSS subjects with significantly increased expression of IL-15 mRNA in pSS SGEC (Fig. 1A). The IL-15 mRNA copy number in pSS cells was about 2–3-fold higher than in control cells, revealing an up-regulation of IL-15 gene expression ($p < 0.01$) in diseased cells.

In view of the observed transcriptional up-regulation of IL-15 gene in pSS patients, we evaluated IL-15 protein expression in pSS SGEC by western blotting (Fig. 1B). The analysis, performed with an affinity-purified anti-human IL-15 monoclonal antibody on healthy and pSS cultured SGEC, yielded a 14 kDa immunoreactive band for IL-15 in the control and pSS samples and the densitometric analysis indicated a clear increase of IL-15 protein expression in SS cell lysates (Fig. 1C). Specificity was proven in controls without the primary antibody, which was deemed negative (not shown). These initial results demonstrate that IL-15 is overexpressed at both mRNA and protein levels in the inflamed salivary glands of patients with pSS.

Immunohistochemistry and flow cytometric analysis confirmed the increased IL-15 expression in the MSG of pSS

Since the findings so far suggested increased expression of IL-15 in cultured pSS SGEC as compared with healthy SGEC, we examined whether increased IL-15 levels could be found within the pSS MSG biopsies. Representative samples of IL-15 immunohistochemical staining are shown in Fig. 2A. Significantly higher expression of IL-15 was observed in pSS compared to controls and each biopsy specimen examined from the 20 pSS patients exhibited similar staining patterns for IL-15. In control subjects, IL-15 expression was rarely detected and observed essentially among cells located closely to ducts (Fig. 2A,B). Conversely,

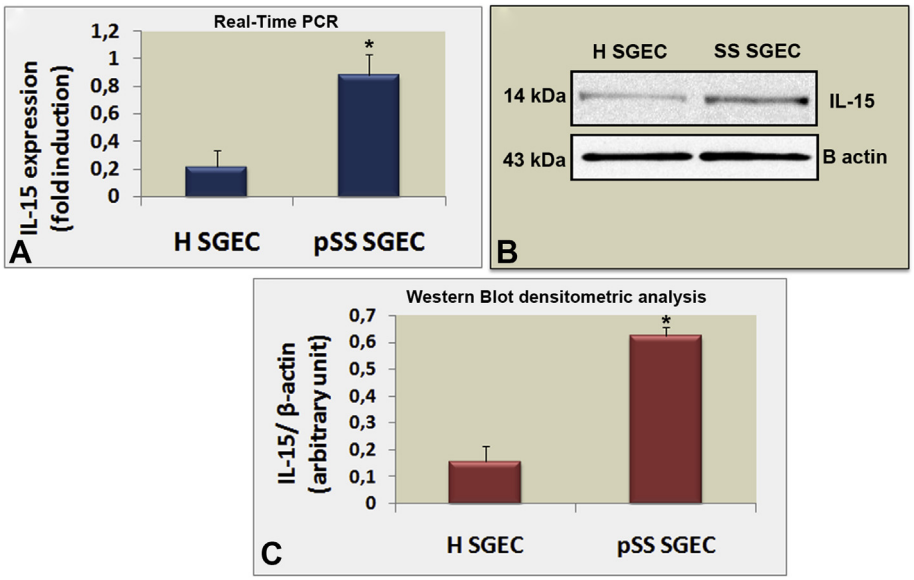

Fig. 1 Detection of IL-15 gene and protein expression in the minor salivary gland (MSG) from healthy subjects and primary Sjögren's syndrome (pSS) patients. Expression of IL-15 mRNA was determined by quantitative real-time PCR in human salivary gland epithelial cell (SGEC) cultures obtained from MSG explants from pSS patients and healthy (H) control subjects (A). Normalised gene expression levels were given as the ratio between the mean value for the target gene and that for the β-2 microglobulin. PCR reactions were performed in triplicate and the data were presented as fold change in gene expression, as explained in the Material and Methods section (mean ± SE of three independent experiments). The protein expression of IL-15 (B) was measured by western blot in protein extract from pSS and H SGEC. The representative western blot analysis reports a strong expression of IL-15 in pSS SGEC in comparison with healthy cells. All results shown are expressed as fold increase compared with the protein lysate of healthy control SGEC and are means ± SE of individual experiments. (C) Densitometric analysis of IL-15, performed against the protein loading control β-actin. Asterisks indicate statistical significance (*$p < 0.01$).

in pSS the expression of IL-15 was observed in inflammatory infiltrates among lymphoid cells (Fig. 2A,C) and a significant acinar and/or ductal epithelial expression of IL-15 was observed in pSS patients (Fig. 2A,C). Microscopic observation revealed, as shown, that diffuse infiltrating mononuclear cells were found in the salivary glands of pSS biopsies (Fig. 2A,C) but not in those of normal subjects (Fig. 2A,B). The flow cytometry results (Fig. 2C), and the evaluation of MFI (Fig. 2B), also confirmed the above reported data; in fact, a significant increase in the levels of IL-15 MFI was observed in pSS SGEC (median MFI = 9926 ± 215), as compared with MFI of healthy subjects (median MFI = 2788 ± 207, $p < 0.01$). These data are highly indicative of a clear over-expression of IL-15 protein in pSS SGEC.

DISCUSSION

The inflammatory reaction characterises the pathogenesis of the autoimmune disease involving both cellular and soluble players. Pro-inflammatory cytokines are at the centre of the pathways resulting in an altered activation of immune cells. Immunoregulatory cytokines have been demonstrated to have a central role for the regulation of lymphocyte homeostasis, and in particular IL-15 has been shown to have an important function in the events that lead to an abnormal inflammatory response.[11] IL-15 mRNA is constitutively expressed by a wide variety of human tissues and cell types, including activated monocytes, macrophages, dendritic cells, osteoclasts and fibroblasts of the spleen, gingiva and skin.[12] Interestingly, the patterns of IL-15 protein and mRNA expression differ greatly depending on the cell type, indicating tight control of protein production at both translational and post-translational levels.

Despite an array of regulatory controls, disordered IL-15 expression has been observed in patients with a series of inflammatory autoimmune diseases. McInnes and colleagues reported abnormalities of IL-15 in rheumatoid arthritis and have suggested that IL-15 might precede TNF-α in the cytokine cascade.[22] Excessive overexpression of IL-15, that perpetuates epithelial damage and promotes the emergence of T cell clonal proliferations, has also been observed in refractory coeliac disease.[16] In addition, there was a resolution of psoriasis after blockade of IL-15 activity in a xenograft mouse model of human psoriasis.[23,24] IL-15 has also been suggested to have a pathogenic role in an array of other chronic inflammatory diseases including sarcoidosis,[25] chronic hepatitis C,[26] ulcerative colitis,[27,28] and inflammatory bowel diseases.[29]

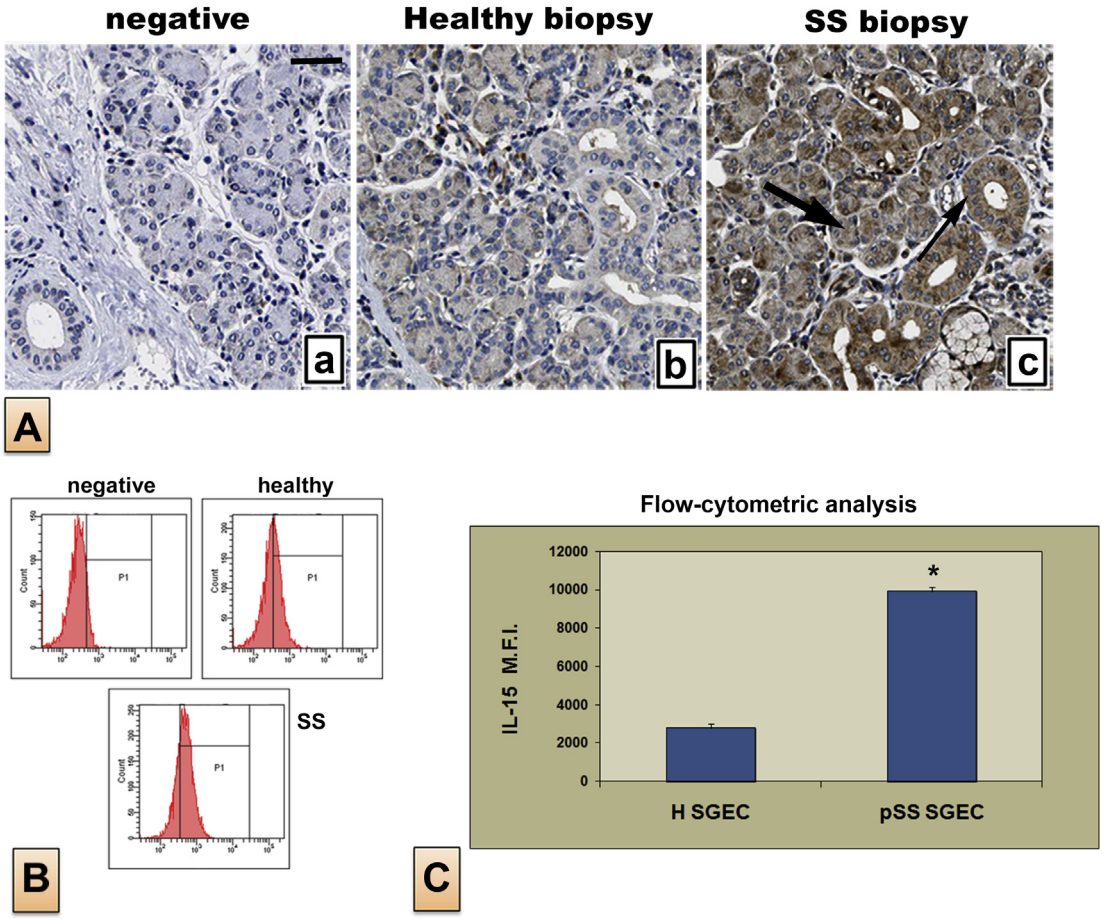

Fig. 2 Expression of interleukin-15 protein in the minor salivary gland (MSG) of primary Sjögren's syndrome (pSS) patients determined by immunohistochemistry (A) and flow-cytometric analysis (B,C). Explanted MSGs from pSS patients were fixed and embedded in paraffin. Sections were cut to 5 µm and stained with anti-human IL-15 primary antibody. Negative controls were performed through the staining with secondary antibody alone (A,a). A weak positive staining for IL-15 protein (A,b) in ductal and acinar cells from healthy biopsies was detected. On the contrary, pSS biopsies show a strong cytoplasmic positivity in ductal and acinar cells (A,c). Brown staining shows positive immunoreaction; blue staining shows nuclei. Bar = 20 µm. Large arrows indicate acinar cells and small arrows indicate intercalated duct cells. (B,C) Levels of IL-15 expression on healthy (H) and pSS SGEC were measured by flow cytometry and expressed as mean fluorescence intensity (MFI). These experiments were repeated twice with similar results.

The role of IL-15 in pSS is still largely unexplored; Szodoray *et al.* measured the amount of IL-15 in the plasma samples of SS patients, demonstrating a higher expression in comparison with healthy subjects,[18] but to date, the function of IL-15 in the healthy salivary gland and under pSS disease conditions remains unexplored. In this study we evaluated the expression of IL-15 in human MSG tissue and in primary cultured SGEC obtained from patients with pSS as well as from normal subjects. Different techniques were employed to demonstrate and provide the first direct evidence that IL-15 is expressed both at the mRNA and protein level in human salivary gland epithelial cells. These results increased the list of epithelial cells from various tissues that have been documented to produce IL-15 mRNA and/or protein, including kidney epithelial cell lines,[30] epidermal skin cells and keratinocytes,[31] fetal skin,[32] retinal pigment epithelium[33] and intestinal epithelial cells.[34] Furthermore, from our study it is clear that salivary glands from pSS patients showed an intensified epithelial expression of IL-15, as demonstrated also from the experiments conducted on the primary pSS epithelial cells culture. These observations seem to be consistent with the role that IL-15 plays in the immune system.[13] First, it is known that IL-15 is involved in the proliferation and survival of T cells, and it occupies a key role in the development of numerous autoimmune diseases.[35] Moreover, IL-15 enhances the proliferation of B-lymphocytes and their differentiation into plasma cells,[36] and it is known that disturbances in B cell homeostasis, including their role in ectopic germinal tissue in lacrimal and salivary glands, are major characteristics of SS and SS-like disease.[3] Obviously, it remains unclear whether in pSS, IL-15 facilitates the recruitment and activation of T and B cells, and in addition, if IL-15 plays a role in the perpetual production of other pro-inflammatory cytokines, thereby contributing to the pathophysiology of exacerbation of pSS. Our emerging understanding of the IL-15 role in pSS is providing the scientific basis for the development of various approaches for the IL-15-targeted treatment of this autoimmune disease. Furthermore, considering the recent observation that IL-15 transgenic mice develop CD8/NK lymphomas and leukaemia,[37] and that a significant percentage of pSS patients develop lymphoma,[8] we suggest that the introduction of strategies that inhibit IL-15 action might prove to be of great value in the treatment of the pSS inflammatory autoimmune disorder and in the knowledge of how continuous B-cell activation and inflammation may lead to lymphomagenesis in pSS patients.

The clear intensified epithelial expression of IL-15 in salivary glands from pSS patients, therefore, could be explained considering that several important studies have revealed the inherent capacity of SGEC to induce and promote chronic inflammatory reactions, as corroborated by the constitutive or inducible expression of various molecules implicated in innate and acquired immune responses. This fact strongly indicates the operation of intrinsic activation mechanisms in the epithelia of pSS patients and further supports the active participation of these cells in the pathogenesis of the disorder. In this respect, it can be argued that SGEC, through the IL-15 production, along with other pro-inflammatory cytokines and chemokines, could exert their chemoattractive function for leukocytes, driving their compartmentalisation into lymphoid structures, and therefore priming and contributing to both the initiation and the ongoing amplification of the inflammatory autoimmune response. On the other hand, it is plausible to maintain that IL-15 could be a general survival factor for SGEC. In fact, although initially IL-15 was identified as a survival factor in T cells, it is now clear from recent studies that IL-15 is a survival factor for a broader array of cell types. IL-15 promotes cell survival in haematopoietic cells[38–40] as well as in non-haematopoietic cells, since IL-15 resulted in anti-apoptotic behaviour in fibroblasts[41] and keratinocytes.[24] Moreover, *in vivo* studies demonstrated the capacity of IL-15 to suppress apoptosis in hepatocytes.[42] Further clarification is then required to establish whether all the events which can potentially be initiated and/or maintained by IL-15 result in chronic inflammation in an abnormal immune response, and in tissue damage or, on the contrary, in the enhanced survival of pSS SGEC.

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References

1. Sjögren H. About keratoconjunctivitis sicca: keratitis filiformis with hypofunction of the lacrimal glands. *Acta Ophthalmologica* 1933; 11: 1–151.
2. Kassan SS, Moutsopoulos HM. Clinical manifestations and early diagnosis of Sjögren syndrome. *Arch Intern Med* 2004; 164: 1275–84.
3. Ramos-Casals M, Tzioufas AG, Font J. Primary Sjögren's syndrome: new clinical and therapeutic concepts. *Ann Rheum Dis* 2005; 64: 347–54.
4. Moutsopoulos HM. Sjögren's syndrome: autoimmune epithelitis. *Clin Immunol Immunopathol* 1994; 72: 162–5.
5. Roescher N, Tak PP, Illei GG. Cytokines in Sjögren's syndrome. *Oral Dis* 2009; 15: 519–26.
6. Lisi S, Sisto M, Lofrumento DD, *et al*. GRO-α/CXCR2 system and ADAM17 correlated expression in Sjögren's syndrome. *Inflammation* 2013; 36: 759–66.
7. Lisi S, D'Amore M, Sisto M. ADAM17 at the interface between inflammation and autoimmunity. *Immunol Lett* 2004; 162: 159–69.
8. Mavragani CP, Moutsopoulos HM. Sjögren's syndrome. *Annu Rev Pathol* 2014; 9: 273–85.
9. Lisi S, Sisto M, D'Amore M, Lofrumento DD. Co-culture system of human salivary gland epithelial cells and immune cells from primary Sjögren's syndrome patients: an in vitro approach to study the effects of Rituximab on the activation of the Raf-1/ERK1/2 pathway. *Int Immunol* 2015; 27: 183–94.
10. Sisto M, Lisi S, D'Amore M, Lofrumento DD. The metalloproteinase ADAM17 and the epidermal growth factor receptor (EGFR) signaling drive the inflammatory epithelial response in Sjögren's syndrome. *Clin Exp Med* 2015; 15: 215–25.
11. Tagaya Y, Bamford RN, DeFilippis AP, Waldmann TA. IL-15: a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. *Immunity* 1996; 4: 329–36.
12. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Ann Rev Immunol* 1999; 17: 19–49.
13. Zambricki E, Shigeoka A, Kishimoto H, *et al*. Signaling T-cell survival and death by IL-2 and IL-15. *Am J Transplant* 2005; 5: 2623–31.
14. Sakai T, Kusugami KK, Nishimura H, *et al*. Interleukin 15 activity in the rectal mucosa of inflammatory bowel disease. *Gastroenterology* 1998; 11: 1237–43.
15. Maiuri L, Ciacci C, Auricchio S, *et al*. Interleukin 15 mediates epithelial changes in celiac disease. *Gastroenterology* 2000; 119: 996–1006.
16. Mention JJ, Ahmed MB, Begue B, *et al*. Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. *Gastroenterology* 2003; 125: 730–45.

17. Waldmann TA. Targeting the interleukin-15 system in rheumatoid arthritis. *Arthritis Rheum* 2005; 52: 2585–8.
18. Szodoray P, Alex P, Brun JG, Centola M, Jonsson R. Circulating cytokines in primary Sjögren's syndrome determined by a multiplex cytokine array system. *Scand J Immunol* 2004; 59: 592–9.
19. Vitali C, Bombardieri S, Jonsson R, *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: 554–8.
20. Sens DA, Hintz DS, Rudisil MT, Sens MA, Spicer SS. Explant culture of human submandibular gland epithelial cells: evidence for ductal origin. *Lab Invest* 1985; 52: 559–67.
21. Kapsogeorgou EK, Dimitriou ID, Abu-Helu RF, Moutsopoulos HM, Manoussakis MN. Activation of epithelial and myoepithelial cells in the salivary glands of patients with Sjogren's syndrome: high expression of intercellular adhesion molecule-1 (ICAM.1) in biopsy specimens and cultured cells. *Clin Exp Immunol* 2001; 124: 126–33.
22. McInnes IB, Leung BP, Sturrock RD, Field M, Liew FY. Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-alpha production in rheumatoid arthritis. *Nat Med* 1997; 3: 189–95.
23. Villadsen LS, Schuurman J, Beurskens F, *et al*. Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft-mouse model. *J Clin Invest* 2003; 112: 1571–80.
24. Ruckert R, Asadullah K, Seifert M, *et al*. Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis? *J Immunol* 2000; 165: 2240–50.
25. Agostini C, Trentin L, Facco M, *et al*. Role of IL-15, IL-2, and their receptors in the development of T cell alveolitis in pulmonary sarcoidosis. *J Immunol* 1996; 157: 910–8.
26. Allison RD, Katsounas A, Koziol DE, *et al*. Association of interleukin-15-induced peripheral immune activation with hepatic stellate cell activation in persons coinfected with hepatitis C virus and HIV. *J Infect Dis* 2009; 200: 619–23.
27. Yamamoto-Furusho JK, De-León-Rendón JL, Alvarez-León E, *et al*. Association of the interleukin 15 (IL-15) gene polymorphisms with the risk of developing ulcerative colitis in Mexican individuals. *Mol Biol Rep* 2014; 41: 2171–6.
28. Kirman I, Nielsen OH. Increased numbers of interleukin-15-expressing cells in active ulcerative colitis. *Am J Gastroenterol* 1996; 91: 1789–94.
29. Liu Z, Geboes K, Colpaert S, *et al*. IL-15 is highly expressed in inflammatory bowel disease and regulates local T cell-dependent cytokine production. *J Immunol* 2000; 164: 3608–15.
30. Grabstein KH, Eisenman J, Shanebeck K, *et al*. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science* 1994; 264: 965–8.
31. Mohamadzadeh M, Takashima A, Dougherty I, *et al*. Ultraviolet B radiation up-regulates the expression of IL-15 in human skin. *J Immunol* 1995; 155: 4492–6.
32. Kawai K, Suzuki H, Tomiyama K, *et al*. Requirement of the IL-2 receptor beta chain for the development of Vgamma3 dendritic epidermal T cells. *J Invest Dermatol* 1998; 110: 961–5.
33. Kumaki N, Anderson DM, Cosman D, Kumaki S. Expression of interleukin-15 and its receptor by human fetal retinal pigment epithelial cells. *Curr Eye Res* 1996; 15: 876–82.
34. Reinecker HC, MacDermott RP, Mirau S, Dignass A, Podolsky DK. Intestinal epithelial cells both express and respond to interleukin 15. *Gastroenterology* 1996; 111: 1706–13.
35. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nat Rev Immunol* 2006; 6: 595–601.
36. Armitage RJ, Macduff BM, Eisenman J, Paxton R, Grabstein KH. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *J Immunol* 1995; 154: 483–90.
37. Malamut G, El Machhour R, Montcuquet N, *et al*. IL-15 triggers an antiapoptotic pathway in human intraepithelial lymphocytes that is a potential new target in celiac disease-associated inflammation and lymphomagenesis. *J Clin Invest* 2010; 120: 2131–43.
38. Dooms H, Desmedt M, Vancaeneghem S, *et al*. Quiescence-inducing and antiapoptotic activities of IL-15 enhance secondary CD4+ T cell responsiveness to antigen. *J Immunol* 1998; 161: 2141–50.
39. Tagaya Y, Burton JD, Miyamoto Y, *et al*. Identification of a novel receptor/signal transduction pathway for IL-15/T in mast cells. *EMBO J* 1996; 15: 4928–39.
40. Girard D, Paquet ME, Paquin R, *et al*. Differential effects of interleukin-15 (IL-15) and IL-2 on human neutrophils: modulation of phagocytosis, cytoskeleton rearrangement, gene expression, and apoptosis by IL-15. *Blood* 1996; 88: 3176–84.
41. Bulfone-Paus S, Bulanova E, Pohl T, *et al*. Death deflected: IL-15 inhibits TNF-alpha-mediated apoptosis in fibroblasts by TRAF2 recruitment to the IL-15Ralpha chain. *FASEB J* 1999; 13: 1575–85.
42. Bulfone-Paus S, Ungureanu D, Pohl T, *et al*. Interleukin-15 protects from lethal apoptosis in vivo. *Nat Med* 1997; 3: 1124–8.