IL-34 is overexpressed in the inflamed salivary glands of patients with Sjögren’s syndrome and is associated with the local expansion of pro-inflammatory CD14^{bright}CD16^{+} monocytes

Francesco Ciccia¹, Riccardo Alessandro², Vito Rodolico³, Giuliana Guggino¹, Stefania Raimondo², Carla Guarnotta³, AnnaRita Giardina¹, Guido Sireci², Giuseppina Campisi⁴, Giacomo De Leo² and Giovanni Triolo¹

Abstract

Objectives. To investigate the expression of IL-34 in labial salivary glands (LSGs) of patients with primary SS (p-SS) and its role in inducing a pro-inflammatory monocyte phenotype.

Methods. LSG biopsies were obtained from 20 patients with p-SS and 10 patients with non-Sjögren’s sicca syndrome (n-SS). The expression of IL-34, IL-1β, TNF-α, IL-17 and IL-23 was assessed by real-time PCR. IL-34 expression was also investigated in LSGs by immunohistochemistry. The frequencies of sub-populations of CD14^{+} monocytes were evaluated by flow cytometry among isolated mononuclear cells from peripheral blood and salivary glands from both patients and controls. The role of recombinant IL-34 on isolated peripheral blood mononuclear cells was also evaluated.

Results. IL-34 m-RNA was overexpressed in the inflamed salivary glands of p-SS and associated with increased expression of TNF-α, IL-1β, IL-17 and IL-23p19. The increased expression of IL-34 was confirmed by immunohistochemistry in paraffin-embedded salivary glands from p-SS patients. IL-34 expression was accompanied by the expansion of pro-inflammatory CD14^{bright}CD16^{+} monocytes in the salivary glands. In vitro stimulation of peripheral blood mononuclear cells with IL-34 induced the expansion of both CD14^{+}CD16^{−} cells and CD14^{bright}CD16^{+} cells in p-SS and non-SS subjects.

Conclusion. IL-34 seems to be involved in the pathogenesis of salivary gland inflammation in p-SS.

Key words: interleukin-34, Sjögren’s syndrome, monocytes.
IL-34 has also recently been demonstrated to be overexpressed in the synovial tissue of patients with RA where, acting as a downstream effector of TNF-α and IL-1β, it has been found to promote macrophage differentiation and proliferation in synovial tissue, contributing to inflammation and bone erosions [11–13].

Over recent years, several studies have emphasized the crucial role of monocytes/macrophages in the expansion and organization of inflammatory infiltrate in several autoimmune diseases as well as in p-SS [14–17]. Different populations of monocytes have been observed in peripheral blood. In particular, based on CD14 and CD16 expression, human peripheral blood monocytes have been divided into a major CD14<sup>bright</sup>CD16<sup>−</sup> population and two minor CD14<sup>bright</sup>CD16<sup>+</sup> and CD14<sup>dim</sup>CD16<sup>−</sup> subpopulations [18]. These monocyte subpopulations are known to display distinct phenotypes, functions and behaviour in inflammatory human diseases. CD14<sup>bright</sup>CD16<sup>−</sup> are considered pro-inflammatory cells based on the higher expression of pro-inflammatory cytokines as well as their ability to promote the expansion of the Th17 cell population [19]. Conversely, CD14<sup>dim</sup>CD16<sup>−</sup> monocytes represent a subset that patrois blood vessels with high migratory but limited phagocytic potential [20]. Migration of monocytes in the tissues is driven by cells of connective tissue origin through the release of chemokines, such as CSF-1 [21, 22]. The fate of monocytes (whether pro- or anti-inflammatory) appears to be dependent on a tightly regulated balance between stromal chemokines and pro-inflammatory cytokines (such as IL-34), the latter playing an important role in inducing a pro-inflammatory phenotype.

IL-34 expression and monocyte subpopulations have not yet been studied in patients with p-SS. The objective of this study was to investigate the expression of IL-34 in labial salivary glands (LSGs) of patients with p-SS and its role in modulating monocyte phenotype.

Patients

Twenty patients with p-SS (18 women and 2 men, median age 55 years, range 29–67 years) diagnosed on the basis of the American-European Consensus Group criteria for p-SS [23] were consecutively included in the study. The presence of other underlying autoimmune diseases or HCV infection was carefully excluded. All patients underwent serology evaluation that included tests for the presence of ANAs, anti-SSA/Ro, anti-SSB/La, RF and levels of CRP and ESR. All patients were naive to the use of immunosuppressive drugs. The clinical characteristics of these patients are shown in Table 1. Ten patients (all women, median age 58 years, range 31–70 years) with sicca symptoms who did not fulfil the SS classification criteria and without histopathological or serological evidence of p-SS were also considered as controls (non-SS, n-SS). The study was approved by the local hospital ethics committee (the ethics committee of the AOUP Paolo Giaccone). Informed patient consent was obtained from all the patients and controls enrolled.

LSG biopsies

LSG biopsies were obtained, after informed consent, from p-SS and n-SS, during routine diagnostic procedures. Biopsy specimens were fixed in 10% buffered formalin and placed in RNA or RPMI. At least four salivary gland lobules were used for histological evaluation. Paraffin-embedded sections stained with haematoxylin and eosin were studied by the same pathologist (V.R.), using a microscope equipped with an ocular micrometer. Evaluation of the presence of lymphocytic infiltrates and/or foci was performed as previously described [24]. A focus was defined as an aggregate of ≥ 50 lymphocytes. The focus score was reported as the number of foci per 4 mm<sup>2</sup> of tissue, up to a maximum of 12 foci. A biopsy focus score > 1 was observed in all p-SS patients, whereas the control group invariably displayed a focus score < 1.

RNA isolation and quantitative real-time PCR

Salivary gland biopsy specimens were stored in RNAlater solution (Applied Biosystems, Foster City, CA, USA). Each sample was lysed in a tissue homogenizer and RNA was extracted using the commercially available illustra RNAspin Mini Isolation Kit (GE Healthcare, Little Chalfont, UK), according to manufacturer’s instructions. Total RNA was reverse-transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystem). Samples were stored at −20°C until use. For quantitative TaqMan real-time PCR, master mix and TaqMan gene expression assays for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) control for IL-34 (Hs01050926_m1), IL-1β (Hs00174079_m1), TNF-α (Hs00174128_m1), IL-17 (Hs00174383_m1) and IL-23p19 (Hs00372324_m1) were obtained from Applied Biosystems. Samples were run in triplicate using the Step-One Real-Time PCR system (Applied Biosystem). Relative changes in gene expression between n-SS and p-SS samples were determined using the ΔΔC<sub>t</sub> method. Levels of the target transcript were normalized to a GAPDH endogenous control, constantly expressed in both groups (ΔC<sub>t</sub>). For ΔΔC<sub>t</sub> values, additional subtractions were performed between p-SS samples and n-SS ΔC<sub>t</sub> values. Final values were expressed as fold of induction.

Immunohistochemical staining for IL-34

Tissue samples were fixed in 10% buffered formalin, dehydrated in ethanol and paraffin-embedded according to the routine technique. Immunohistochemical analysis was performed on 3-μm-thick paraffin-embedded sections as previously described [25]. Briefly, sections were exposed to rabbit polyclonal anti-IL-34 antibody (Abcam plc, Cambridge, UK), dilution 1:250, for 1 h at 25°C or unconjugated rabbit immunoglobulins (negative control) for 1 h at 25°C. Staining was detected using the Novolink Polymer Detection System (Novocastra Laboratories, Newcastle upon Tyne, UK) according to the manufacturer’s instructions and counterstained with aqueous haematoxylin. For the evaluation of IL-34 immunoreactions, tissues were examined for evidence of
staining with the aid of the NIH ImageJ software (http://rsbweb.nih.gov/ij/), as previously described [25]. Only ductal epithelial cells were counted regardless of intensity of staining. For each case, a minimum of 10⁵ cells was counted; ImageJ software was then able to automatically generate the percentage of ductal epithelial cells stained with IL-34 antibody, which was regarded as labelling index (LI); results were reported as the mean ± S.E.M.

Phenotypic analysis of peripheral blood and tissue monocytes

Salivary gland tissues from all p-SS and n-SS subjects were extensively washed in saline buffer and enzymic digestion was performed using collagenase (1.5 mg/ml; Life Technologies, Carlsbad, CA, USA) in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% inactivated fetal calf serum (FCS, Sigma, St Louis, MO), 2 mM L-glutamine, 20 mM HEPES and 100 U/ml penicillin/streptomycin with or without recombinant IL-34 (R&D Systems, Minneapolis, MN, USA). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂. Accordingly to Lin et al. [9], cells were stimulated with 2 ng/ml of IL-34 and harvested 72 h later, and the surface expression of CD14 and CD16 on monocytes was determined by flow cytometry.

Statistical analysis

Analyses were performed using GraphPad Prism software (version 5.0, GraphPad Software, La Jolla, CA, USA). Multiple groups were analysed using one-way analysis of variance and pairwise comparisons using the Mann–Whitney U test, with Bonferroni’s correction to adjust significance levels for multiple comparisons where appropriate. Correlation analyses were performed using Spearman’s rank correlation coefficient. P < 0.05 was considered significant.

Results

IL-34 expression in the salivary glands of p-SS patients

The expression of IL-34 in the salivary glands from patients with p-SS and n-SS was assessed by real-time PCR and immunohistochemistry. By real-time PCR analysis, we found a significant up-regulation of IL-34 m-RNA in the inflamed salivary glands of p-SS patients compared with n-SS (2.5-fold increase, P = 0.0013) (Fig. 1A). The overexpression of IL-34 was confirmed by immunohistochemical analysis. IL-34 positivity was detected only in ductal epithelial cells and among infiltrating periductal mononuclear cells in salivary glands from patients with p-SS (Fig. 1C and D) compared with n-SS (Fig. 1B). Staining for IL-34 was not observed in ductal epithelial cells located in areas without cell infiltrates (data not shown). No IL-34 expression was observed in the acinar cells (Fig. 1C and D). In the 30 LSG specimens investigated, the LIs ranged from 0% to 91.3% (mean 41.37 ± 34.19%). Generally, ductal epithelial cells showed intense cytoplasmatic positivity in p-SS (Fig. 1C and D), whereas weak cytoplasmatic expression was found in few ductal epithelial cells for only 2 of the 10 controls (data not shown). No significant differences for the mean value of IL-34 LIs were found for age or sex (data not shown).

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristic of patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-SS (n = 20)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
</tr>
<tr>
<td>Disease duration, months (range)</td>
</tr>
<tr>
<td>ANA, % of patients</td>
</tr>
<tr>
<td>Anti-Ro and/or anti-La antibodies, % of patients</td>
</tr>
<tr>
<td>RF, % of patients</td>
</tr>
<tr>
<td>ESR mm/h, mean (s.d.)</td>
</tr>
<tr>
<td>CRP, mg/l, mean (s.d.)</td>
</tr>
</tbody>
</table>

penicillin/streptomycin with or without recombinant IL-34 (R&D Systems, Minneapolis, MN, USA). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂. Accordingly to Lin et al. [9], cells were stimulated with 2 ng/ml of IL-34 and harvested 72 h later, and the surface expression of CD14 and CD16 on monocytes was determined by flow cytometry.

Statistical analysis

Analyses were performed using GraphPad Prism software (version 5.0, GraphPad Software, La Jolla, CA, USA). Multiple groups were analysed using one-way analysis of variance and pairwise comparisons using the Mann–Whitney U test, with Bonferroni’s correction to adjust significance levels for multiple comparisons where appropriate. Correlation analyses were performed using Spearman’s rank correlation coefficient. P < 0.05 was considered significant.

Results

IL-34 expression in the salivary glands of p-SS patients

The expression of IL-34 in the salivary glands from patients with p-SS and n-SS was assessed by real-time PCR and immunohistochemistry. By real-time PCR analysis, we found a significant up-regulation of IL-34 m-RNA in the inflamed salivary glands of p-SS patients compared with n-SS (2.5-fold increase, P = 0.0013) (Fig. 1A). The overexpression of IL-34 was confirmed by immunohistochemical analysis. IL-34 positivity was detected only in ductal epithelial cells and among infiltrating periductal mononuclear cells in salivary glands from patients with p-SS (Fig. 1C and D) compared with n-SS (Fig. 1B). Staining for IL-34 was not observed in ductal epithelial cells located in areas without cell infiltrates (data not shown). No IL-34 expression was observed in the acinar cells (Fig. 1C and D). In the 30 LSG specimens investigated, the LIs ranged from 0% to 91.3% (mean 41.37 ± 34.19%). Generally, ductal epithelial cells showed intense cytoplasmatic positivity in p-SS (Fig. 1C and D), whereas weak cytoplasmatic expression was found in few ductal epithelial cells for only 2 of the 10 controls (data not shown). No significant differences for the mean value of IL-34 LIs were found for age or sex (data not shown).

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristic of patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-SS (n = 20)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
</tr>
<tr>
<td>Disease duration, months (range)</td>
</tr>
<tr>
<td>ANA, % of patients</td>
</tr>
<tr>
<td>Anti-Ro and/or anti-La antibodies, % of patients</td>
</tr>
<tr>
<td>RF, % of patients</td>
</tr>
<tr>
<td>ESR mm/h, mean (s.d.)</td>
</tr>
<tr>
<td>CRP, mg/l, mean (s.d.)</td>
</tr>
</tbody>
</table>

penicillin/streptomycin with or without recombinant IL-34 (R&D Systems, Minneapolis, MN, USA). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂. Accordingly to Lin et al. [9], cells were stimulated with 2 ng/ml of IL-34 and harvested 72 h later, and the surface expression of CD14 and CD16 on monocytes was determined by flow cytometry.

Statistical analysis

Analyses were performed using GraphPad Prism software (version 5.0, GraphPad Software, La Jolla, CA, USA). Multiple groups were analysed using one-way analysis of variance and pairwise comparisons using the Mann–Whitney U test, with Bonferroni’s correction to adjust significance levels for multiple comparisons where appropriate. Correlation analyses were performed using Spearman’s rank correlation coefficient. P < 0.05 was considered significant.

Results

IL-34 expression in the salivary glands of p-SS patients

The expression of IL-34 in the salivary glands from patients with p-SS and n-SS was assessed by real-time PCR and immunohistochemistry. By real-time PCR analysis, we found a significant up-regulation of IL-34 m-RNA in the inflamed salivary glands of p-SS patients compared with n-SS (2.5-fold increase, P = 0.0013) (Fig. 1A). The overexpression of IL-34 was confirmed by immunohistochemical analysis. IL-34 positivity was detected only in ductal epithelial cells and among infiltrating periductal mononuclear cells in salivary glands from patients with p-SS (Fig. 1C and D) compared with n-SS (Fig. 1B). Staining for IL-34 was not observed in ductal epithelial cells located in areas without cell infiltrates (data not shown). No IL-34 expression was observed in the acinar cells (Fig. 1C and D). In the 30 LSG specimens investigated, the LIs ranged from 0% to 91.3% (mean 41.37 ± 34.19%). Generally, ductal epithelial cells showed intense cytoplasmatic positivity in p-SS (Fig. 1C and D), whereas weak cytoplasmatic expression was found in few ductal epithelial cells for only 2 of the 10 controls (data not shown). No significant differences for the mean value of IL-34 LIs were found for age or sex (data not shown).
shown). In the patients with p-SS, the IL-34 LIs ranged from 22.3% to 91.3% (mean 52.64 ± 21.37%) (Fig. 1E), while in the control group, it ranged from 0% to 18.2% (mean 3.24 ± 6.81%, P < 0.001) (Fig. 1E). Of relevance, IL-34 LIs in ductal epithelial cells of LSGs from patients with p-SS syndrome correlated significantly with the focus score (r² = 0.8127, P < 0.0001) (Fig. 1F).

TNF-α and IL-1β were significantly overexpressed and correlated with IL-34 expression in the salivary glands of p-SS.

It is known that TNF-α and IL-1β up-regulate the expression of IL-34 [13]. In this regard, we assessed the TNF-α and IL-1β expression levels by real-time PCR in the salivary glands of p-SS patients. Both TNF-α and IL-1β were significantly up-regulated in p-SS compared with n-SS (3-fold and 2-fold increase, respectively, P < 0.001) (Fig. 2A and B) and correlated with IL-34 levels (Fig. 2C and D), suggesting the presence of a local pro-inflammatory milieu capable of inducing IL-34 expression.

Phenotypic analysis of freshly isolated monocytes from LSGs and peripheral blood

We next evaluated the behaviour of the different monocyte subsets in the peripheral blood and salivary glands of p-SS and their relationship with IL-34 expression. Monocytes isolated from the peripheral blood and LSGs of patients with p-SS could clearly be divided into CD14dimCD16+, CD14brightCD16+ and CD14+CD16− populations. Among salivary gland mononuclear cells from p-SS patients only, the CD14brightCD16+ population was significantly expanded (12 ± 0.5 vs 6.6 ± 0.6, P < 0.001) (Fig. 3A, B and E), the expression of CD14 and CD16 never being observed in epithelial cells (Fig. 3G and H).

The number of CD14brightCD16+ monocytes in the inflamed salivary glands appeared to be directly correlated with the expression of IL-34, suggesting a role of IL-34 in the expansion of this monocyte population (r² = 0.9420, P = 0.006) (data not shown). Differently from salivary glands, among PBMCs only the CD14dimCD16+ subset was significantly expanded in p-SS patients compared with controls (32 ± 1.2 vs 15 ± 1.1, P < 0.03) (Fig. 3C, D and F). On the other hand, the frequency of circulating CD14brightCD16+ and CD14+CD16− monocytes did not significantly differ between patients and control subjects (10 ± 1.2 vs 12 ± 1 and 52.8 ± 3.5 vs 60 ± 5.3, respectively) (Fig. 3C, D and F).

IL-34 induces the expansion of CD14brightCD16+ monocytes in isolated PBMCs

IL-34 is known to act as a regulator of monocyte differentiation, proliferation and survival [9], but direct evidence of IL-34 function on the different monocyte subpopulations is missing. We investigated, in vitro, the role of IL-34 on...
classic monocytes CD14⁺CD16⁻, CD14brightCD16⁺ and CD14dimCD16⁺. PBMCs from p-SS patients and n-SS cultured with IL-34 significantly expanded classic CD14⁺CD16⁻ monocytes (Fig. 4A-F). Interestingly, IL-34 stimulation induced a significant reduction of CD14dimCD16⁺ cells (Fig. 4A-F) and expansion of CD14brightCD16⁺ monocytes (Fig. 4A-F) in the PBMCs obtained from both p-SS and n-SS, providing evidence for a role of IL-34 in promoting the differentiation of this subset of pro-inflammatory monocytes.

Salivary gland expansion of CD14brightCD16⁺ is correlated with IL-23 expression

CD14brightCD16⁺ monocytes have been demonstrated to induce a strong Th17 polarization, mainly through the production of IL-23 [19]. According to previous reports, both IL-23 and IL-17 were significantly overexpressed in the inflamed salivary glands of p-SS (Fig. 5A and B) [5, 24] and significantly correlated with the expression levels of IL-34 (Fig. 5C and D).

Discussion

IL-34 is a recently described pro-inflammatory cytokine that specifically acts on the monocyte/macrophage system with a functional overlap with M-CSF, inducing the proliferation of classic CD14⁺CD16⁻ monocytes [9]. In RA, IL-34 is overexpressed in the inflamed synovium (where it is correlated with disease severity) and, acting as a downstream effector of TNF-α and IL-1β, induces osteoclastogenesis and contributes to tissue inflammation and bone erosions [11, 12].

SS is a chronic inflammatory autoimmune disease characterized by the disturbance of cytokine networks and the presence of focal B and T lymphocyte infiltration [26]. A major involvement of monocytes/macrophages and of their mediators in the organization of inflammatory infiltrates has also been hypothesized [15–17].

The present study demonstrates that IL-34 is overexpressed at m-RNA levels in the salivary glands of patients with p-SS. Analysis of IL-34 expression by immunohistochemistry confirmed, at protein level, IL-34 overexpression in patients’ salivary glands and showed a direct correlation between IL-34 expression and the lymphocytic focus score. IL-34 was expressed exclusively in the ductal epithelial cells located in areas with cell infiltrates and in mononuclear cells located within periductal inflammatory foci. Neither acinar cells nor isolated inflammatory cells showed IL-34-positive staining. IL-34 overexpression was correlated with increased salivary gland expression of IL-1β and TNF-α cytokines and accompanied by the expansion of CD14brightCD16⁺.
monocytes. In in vitro experiments, IL-34 was observed to expand this subset of pro-inflammatory monocytes.

Monocytes develop initially in the bone marrow and emigrate into peripheral blood, providing routine immune surveillance [27]. In humans, peripheral blood monocytes may be divided into at least two main subsets: a CD14+CD16+ monocyte population and, to a lesser extent, a subset of monocytes that expresses CD16 with variable expression of CD14 [18]. CD14+CD16+ monocytes are in fact subdivided into two subpopulations (CD14brightCD16+ and CD14dimCD16+) according to the level of CD14 expression. These cells exhibited distinct phenotype and function [18]. CD14dimCD16+ monocytes appear to be essentially involved in the patrolling of blood vessels and are characterized by high migratory but limited phagocytic potential [20]. Conversely, CD14brightCD16+ monocytes have been demonstrated to produce high levels of pro-inflammatory cytokines [19].

In this study, in agreement with a previous report [28], we confirm that CD14dimCD16+ monocytes are increased in the peripheral blood of p-SS and demonstrate for the first time that salivary glands of p-SS are characterized by the selective expansion of the CD14brightCD16+ population. In contrast to salivary glands, in the peripheral blood of p-SS patients CD14dimCD16+ predominated. From the migratory capacity of this subset of monocytes one might speculate that these cells, having migrated in the salivary glands of p-SS patients, could differentiate into the pro-inflammatory CD14brightCD16+ subset under the stimulation of local specific immunological triggers. In our study, increased IL-34 expression was correlated with the expansion of CD14brightCD16+ monocytes, suggesting that IL-34 may promote a monocytic pro-inflammatory phenotype. In agreement with this hypothesis, we demonstrated a significant positive correlation between the number of CD14brightCD16+ monocytes and the local expression of IL-34. In this regard, in addition to the
demonstrated expansion of CD14⁺CD16⁻ monocytes in response to IL-34 stimulation, in our study IL-34 was also capable of significantly expanding in vitro the CD14brightCD16⁺ monocyte subset. In RA peripheral blood, CD14brightCD16⁺ monocytes have been found to be expanded and to be actively involved in the expansion of the highly pathogenic Th17 subset of lymphocytes [19]. In our study, salivary gland expansion of CD14brightCD16⁺ cells was accompanied by increased expression of IL-23 and IL-17, with a direct correlation between IL-23/IL-17 and IL-34 expression, suggesting the existence of an immunological link between IL-34 and the IL-23/Th-17 axis in the salivary glands of p-SS.

In conclusion, the present study provides evidence of increased IL-34 expression in the inflammatory foci and in the ductal epithelial cells of inflamed salivary glands of p-SS. Although functional studies are clearly required to confirm the role of IL-34 in the pathogenesis of p-SS, the finding that IL-34 overexpression is accompanied by salivary gland expansion of the pro-inflammatory CD14brightCD16⁺ population of monocytes suggests a role for IL-34 in the modulation of immune inflammatory pathways in p-SS.

**Rheumatology key messages**

- IL-34 seems to be involved in the pathogenesis of salivary gland inflammation in p-SS.
- IL-34 could be involved in the expansion of a pro-inflammatory subset of monocytes in the salivary glands of p-SS.
- Targeting IL-34 represents a potential therapeutic approach to p-SS.

**Funding:** This study was supported by a grant from Ministero della Università e della Ricerca Scientifica of Italy.

**Disclosure statement:** The authors have declared no conflicts of interest.
Fig. 5 IL-23p19 and IL-17 expression in the salivary glands of patients with p-SS.

Relative expression of IL-23 (A) and IL-17 (B) m-RNA in minor salivary glands of p-SS patients, plotted as the fold increase over control tissues (n-SS). Results represent the mean ± S.E.M. In p-SS patients, IL-34 expression was significantly correlated with the levels of both IL-23p19 (C) and IL-17 (D).

References

15 Manoussakis MN, Boui S, Korkolopoulou P et al. Rates of infiltration by macrophages and dendritic cells and expression of interleukin-18 and interleukin-12 in the chronic inflammatory lesions of Sjögren’s syndrome: correlation...
with certain features of immune hyperactivity and factors associated with high risk of lymphoma development. 


20 Cros J, Cagnard N, Woillard K et al. Human CD14(dim) monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. Immunity 2010;33:375–86.


