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Original article

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

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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 subpopulations 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.

Introduction

Primary SS (p-SS) is a chronic autoimmune disease characterized by keratoconjunctivitis sicca and dryness. The pathogenesis of p-SS is considered to be multifactorial [1]. Focal lymphocytic gland infiltration, with up-regulation of Th1 and Th17 cytokine expression [2–6] as well as B-lymphocyte hyperactivity [6, 7] are hallmark characteristics of the disease. Salivary gland epithelial cells of patients with p-SS are also thought to play an important pathogenic role, as suggested by the increased epithelial expression of several inflammatory cytokines in the histopathological lesions (reviewed in [8]).

IL-34 is a recently described pro-inflammatory cytokine that specifically and independently, by colony stimulating factor 1 (CSF-1), binds to the CSF-1 receptor promoting the phosphorylation of extracellular signal-regulated kinases (ERK)1/2 [9, 10]. IL-34 was originally described as a cytokine that specifically increases the growth and differentiation of CD14^{+} monocytes in peripheral blood mononuclear cells (PBMCs) [9].

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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 $CD14^{high}CD16^{-}$ population and two minor $CD14^{bright}CD16^{+}$ and $CD14^{dim}CD16^{+}$ subpopulations [18]. These monocyte subpopulations are known to display distinct phenotypes, functions and behaviour in inflammatory human diseases. $CD14^{bright}CD16^{+}$ 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, $CD14^{dim}CD16^{+}$ monocytes represent a subset that patrols 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 naïve 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 $\geqslant$50 lymphocytes. The focus score was reported as the number of foci per 4 mm^2 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 (Hs00174097_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 $\Delta\Delta C_t$ method. Levels of the target transcript were normalized to a GAPDH endogenous control, constantly expressed in both groups (ΔC_t). For $\Delta\Delta C_t$ values, additional subtractions were performed between p-SS samples and n-SS ΔC_t 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

Table 1 Baseline characteristic of patients and controls

	p-SS ($n = 20$)	n-SS ($n = 10$)	P
Age (years), median (range)	55 (29–67)	58 (31–70)	0.65
Female sex, *n* (%)	18 (90)	10 (100)	0.70
Disease duration, months (range)	99 (12–240)	88 (18–300)	0.44
ANA, % of patients	80	–	–
Anti-Ro and/or anti-LA antibodies, % of patients	58	–	–
RF, % of patients	60	–	–
ESR mm/h, mean (S.D.)	32 (8)	15 (4)	<0.001
CRP, mg/l, mean (S.D.)	12 (3)	6 (2)	<0.05

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 10^3 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 nM HEPES and 100 U/ml penicillin/streptomycin (all four products are from Euroclone Ltd, UK). PBMCs were isolated from heparinized blood samples from all patients and controls by Ficoll-Hypaque (Sigma) density-gradient centrifugation. Cell viability (trypan blue dye exclusion) was always >95%. Tissue and peripheral blood-derived cells were stained with the following monoclonal antibodies: anti-human-CD14-PE, anti-human-CD16-FITC (both from BD Biosciences, San Jose, CA, USA) and anti-human cytokeratin (clone MNF116, Dako, Glostrup, Denmark). Isotype-matched irrelevant antibodies were used as a negative control. Total cells were incubated with mAbs for 30 min on ice and washed twice in PBS containing 0.1% (w/v) NaN_3. Flow cytometry analysis was performed using a FACSCalibur (BD Biosciences). At least 50 000 events, gated on the monocyte region, were acquired for each sample.

Cell cultures with recombinant IL-34

PBMCs isolated from heparinized blood samples of five p-SS patients and five n-SS by centrifugation over Ficoll-Hypaque density gradients were evaluated for CD14 and CD16 expression. Cell suspensions were also cultured in 24 flat-bottom plate (Becton Dickinson Labware, Franklin Lakes, NJ, USA) at a density of 1×10^6 cells in 1 ml of RPMI-1640 medium with 10% FCS, 2 mM L-glutamine, 20 nM 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% CO_2. 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

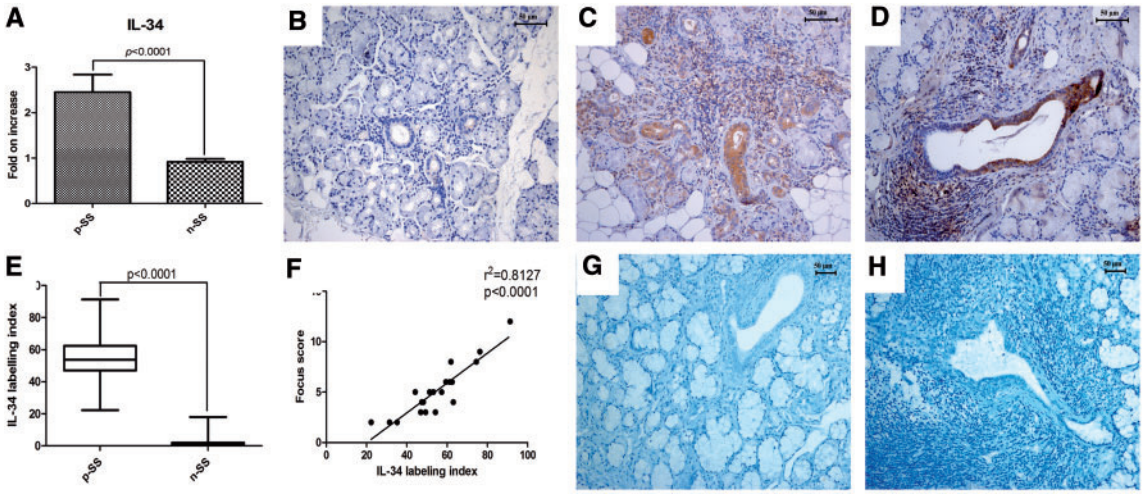
Analyses were performed using GraphPad Prism software (version 5.0, GraphPad Software, La Jolla, CA, USA). Multiple groups were analysed using one-way analysis of variance and pairwise comparisons using the Mann–Whitney *U* test, with Bonferroni's correction to adjust significance levels for multiple 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 cytoplasmic 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

FIG. 1 IL-34 is overexpressed in the salivary glands of patients with p-SS.



(**A**) Relative expression of IL-34 m-RNA in minor salivary glands of p-SS patients ($n = 20$), normalized for GAPDH and plotted as fold of increase over control tissues (n-SS) ($n = 20$). Results represent the mean ± S.E.M. (**B**–**E**) Representative immunostaining of salivary gland biopsies from patients and controls stained with IL-34 antibody in n-SS (**B**) and p-SS patients (**C** and **D**). Significant staining of IL-34 was observed only in p-SS patients (**C** and **D**) ($n = 20$) compared with n-SS ($n = 10$) (**B**). IL-34 expression was essentially observed in infiltrating mononuclear cells and ductal epithelial cells (**C** and **D**). (**E**) IL-34 labelling indexes of ductal epithelial cells in p-SS and n-SS. Data are shown as box plots. Each box represents the 25th–75th percentiles. Lines inside the boxes represent the median. Lines outside the boxes represent the 10th and 90th percentiles. (**F**) Correlation of IL-34 with focus scores. IL-34 expression in ductal epithelial cells by IHC was correlated with the focus score of minor salivary glands of p-SS. (**G** and **H**) Representative sections of n-SS and p-SS patients stained with control antibody.

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 ($r^2 = 0.8127$, $P < 0.0001$) (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 $CD14^{dim}CD16^{+}$, $CD14^{bright}CD16^{+}$ and $CD14^{+}CD16^{-}$ populations. Among salivary gland mononuclear cells from p-SS patients only, the $CD14^{bright}CD16^{+}$ 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).

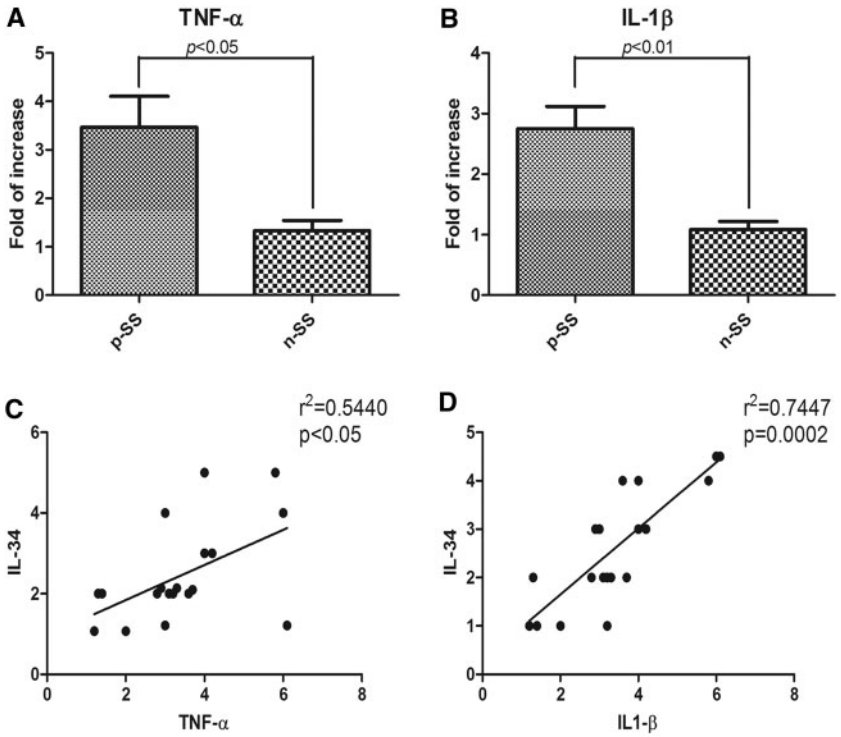
The number of $CD14^{bright}CD16^{+}$ monocytes in the inflamed salivary glands appeared to be directly correlated with the expression of IL-34, suggesting a role of IL-34 in the expansion of this monocyte population ($r^2 = 0.9420$, $P = 0.006$) (data not shown).

Differently from salivary glands, among PBMCs only the $CD14^{dim}CD16^{+}$ 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 $CD14^{bright}CD16^{+}$ 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 $CD14^{bright}CD16^{+}$ 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

FIG. 2 TNF-α and IL-1β expression in the salivary glands of patients with p-SS.



Relative expression of TNF-α (**A**) and IL-1β (**B**) m-RNA in minor salivary glands of p-SS patients ($n = 20$) normalized for GAPDH and plotted as fold of increase over control tissues (n-SS) ($n = 20$). Results represent the mean ± S.E.M. In p-SS patients, IL-34 expression was significantly correlated with the levels of TNF-α (**C**) and IL-1β (**D**).

classic monocytes $CD14^+CD16^-$, $CD14^{bright}CD16^+$ and $CD14^{dim}CD16^+$. 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 $CD14^{dim}CD16^+$ cells (Fig. 4A–F) and expansion of $CD14^{bright}CD16^+$ 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 $CD14^{bright}CD16^+$ is correlated with IL-23 expression

$CD14^{bright}CD16^+$ 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).

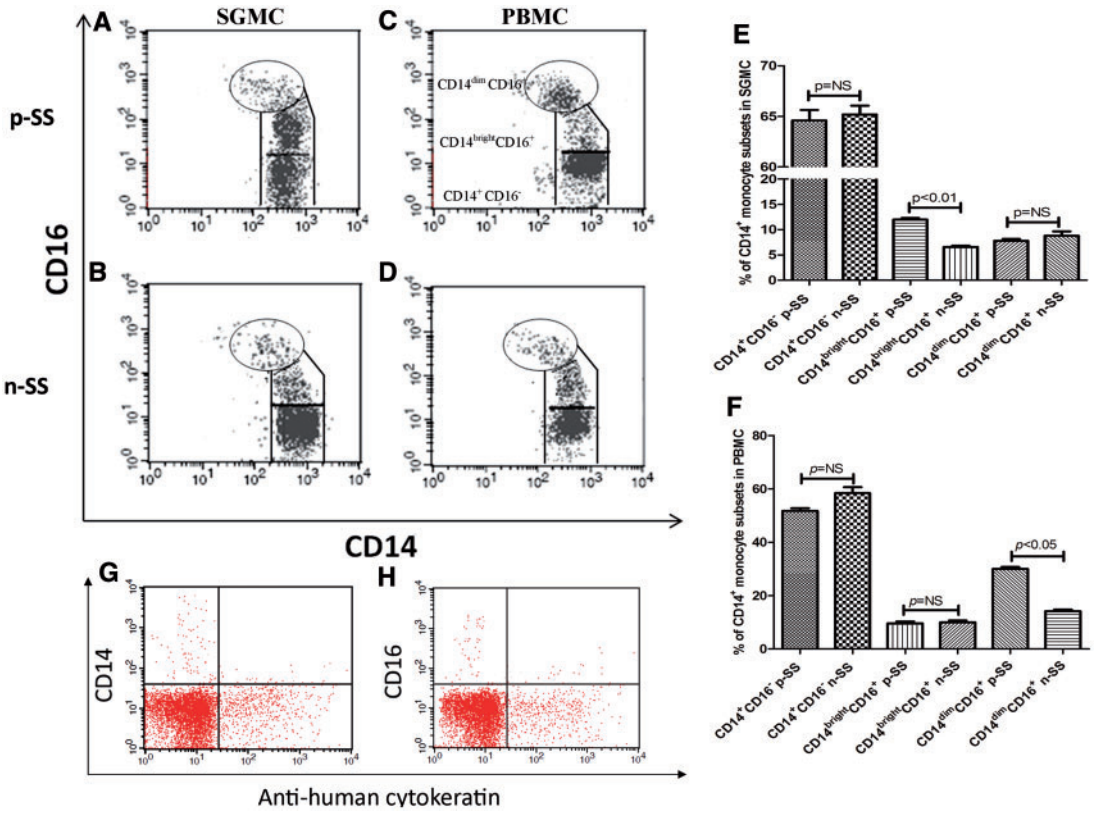
Discussion

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 $CD14^{bright}CD16^+$

FIG. 3 Monocyte subset percentages in peripheral blood and salivary glands of patients with p-SS and controls (n-SS).



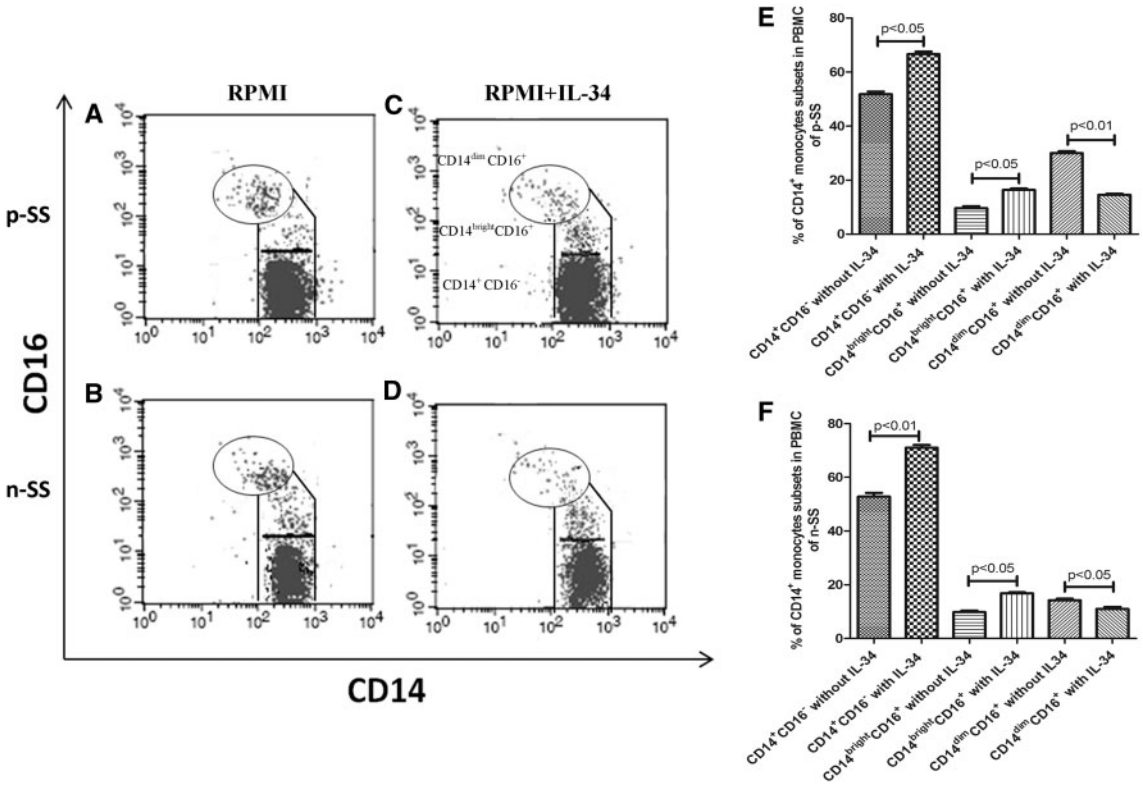
Freshly isolated peripheral blood (PBMC) and salivary gland (SGMC) mononuclear cells from 10 patients with p-SS and 10 n-SS controls were stained with anti-human CD14 and anti-human CD16 monoclonal antibodies and analysed by flow cytometry. Representative dot-plot analysis of SGMCs from patients (**A**) and controls (**B**). Representative dot-plot analysis of PBMC from patients (**C**) and controls (**D**). Percentages of the different monocyte subsets obtained from the salivary glands (**E**) and peripheral blood (**F**) of p-SS patients and controls. (**G** and **H**) Representative dot-plot analysis of SGMCs from patients stained for CD14/CD16 and pan-cytokeratin (as a marker of epithelial cells). The expression of CD14 (**G**) and CD16 (**H**) was never observed among epithelial cells.

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 ($CD14^{bright}CD16^+$ and $CD14^{dim}CD16^+$) according to the level of CD14 expression. These cells exhibited distinct phenotype and function [18]. $CD14^{dim}CD16^+$ monocytes appear to be essentially involved in the patrolling of blood vessels and are characterized by high migratory but limited phagocytic potential [20]. Conversely, $CD14^{bright}CD16^+$ 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 $CD14^{dim}CD16^+$ 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 $CD14^{bright}CD16^+$ population. In contrast to salivary glands, in the peripheral blood of p-SS patients $CD14^{dim}CD16^+$ 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 $CD14^{bright}CD16^+$ subset under the stimulation of local specific immunological triggers. In our study, increased IL-34 expression was correlated with the expansion of $CD14^{bright}CD16^+$ 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 $CD14^{bright}CD16^+$ monocytes and the local expression of IL-34. In this regard, in addition to the

FIG. 4 Monocyte subset percentages after *in vitro* incubation of PBMCs of patients with p-SS and controls (n-SS) with IL-34.



Freshly isolated PBMCs from five patients with p-SS and five n-SS controls were incubated with IL-34 and then stained with anti-human CD14 and anti-human CD16 monoclonal antibodies and analysed by flow cytometry. Representative dot-plot analysis of PBMCs obtained from p-SS patients after stimulation with medium (**A**) or IL-34 (**B**). Representative dot-plot analysis of PBMCs from n-SS control subjects after stimulation with medium (**C**) or IL-34 (**D**). Mean percentages of different monocyte subsets derived from peripheral blood of p-SS patients (**E**) and n-SS controls (**F**) after incubation with rIL-34 or with medium only; results represent the mean ± S.E.M.

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 $CD14^{bright}CD16^+$ monocyte subset. In RA peripheral blood, $CD14^{bright}CD16^+$ 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 $CD14^{bright}CD16^+$ 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 $CD14^{bright}CD16^+$ 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.

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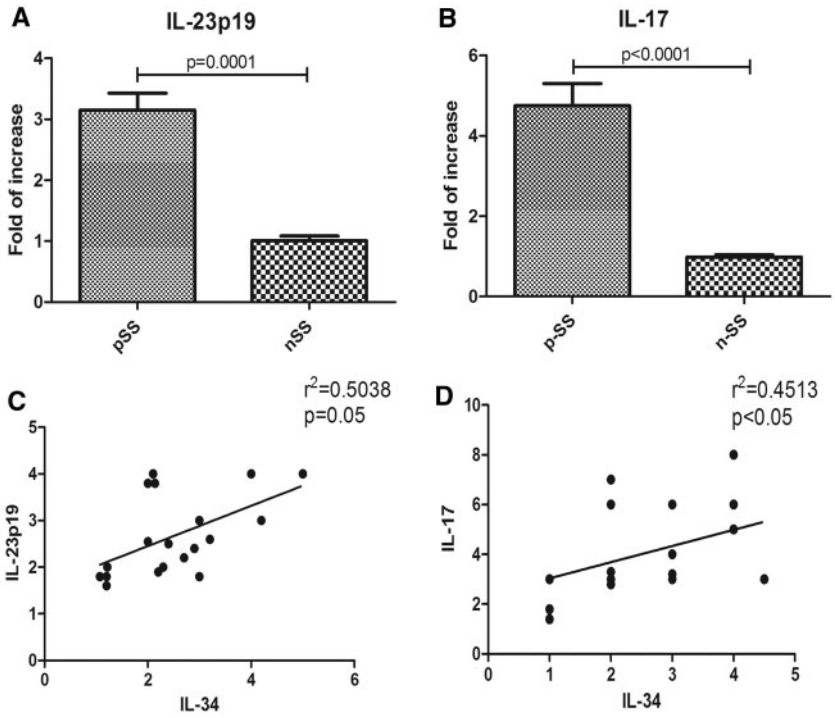
Disclosure statement: The authors have declared no conflicts of interest.

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**).

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