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Concise report

Absence of up-regulation for a proliferation-inducing ligand in Sjögren's sialadenitis lesions

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Abstract

Objective. To determine whether a proliferation-inducing ligand (APRIL) has a role in the survival of plasma cells infiltrating salivary glands from SS patients.

Methods. We performed immunological staining for APRIL in minor salivary glands from SS with a pair of antibodies specifically recognizing APRIL-producing cells and secreted APRIL.

Results. Despite high leucocyte infiltration, APRIL-producing cells, identified as neutrophils, were rare in SS salivary glands. Keratinocytes from the adjacent oral epithelium also produced APRIL, but we never detected significant levels of secreted APRIL in SS salivary glands. We obtained similar results with B-cell lymphomas associated with SS. In fact, there was no significant difference in APRIL production and the level of secreted APRIL in these pathological samples compared with normal corresponding tissues.

Conclusion. The combined observation that APRIL production is not up-regulated in lesions from SS patients, and that secreted APRIL is not retained in these lesions, indicates that plasma cells frequently present in SS lesions may not rely on APRIL for survival, as they do in other rheumatic diseases.

Key words: Sjögren's syndrome, Inflammation, TNF, A proliferation-inducing ligand, Plasma cells.

Introduction

A proliferation-inducing ligand (APRIL, TNFSF13) is one of the last members cloned from the TNF superfamily [1, 2]. APRIL modulates late steps of humoral immune responses by inducing immunoglobulin (Ig) switch [3–6], and transmitting a survival signal into plasmablast/plasma cells (PCs) [7–9]. Owing to this role, APRIL may be implicated in autoimmune diseases with a humoral component, such as the rheumatic diseases, RA, SLE and SS [10]. In terms of expression, APRIL is up-regulated in patients' sera suffering from RA, SLE and SS [11]. In these diseases, APRIL may have a local role for PC infiltrating lesions. Consistent with this, cells from the myeloid lineage, including dendritic cells [11], macrophages and neutrophils [12], produce APRIL in synovium from RA, confirming the APRIL up-regulation detected in the SFs of patients [11]. For SLE, one report indicated that PCs infiltrating kidneys from patients produce APRIL aberrantly [13]. These prompted the test of APRIL, and the closely related B-cell activation factor from the TNF family (BAFF, TNFSF13B) [14], antagonism in RA and SLE. Recent Phase Ib reports indicated a promising reduction in B cell numbers and Ig levels in both RA and SLE [15, 16]. APRIL expression in SS lesions has still not been studied. Here, we studied APRIL expression in SS minor salivary glands, and compared it with normal tissues.

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Materials and methods

Patients

Lip biopsies performed for routine diagnosis were obtained from 14 patients (11 women and 3 men) with a mean age of 53 years (range 25–82 years), fulfilling at least four of the six European Community Criteria defining SS.

These criteria include ocular symptoms, oral symptoms, evidence of KCS, focal lymphocytic sialadenitis of minor salivary glands, instrumental evidence of salivary gland involvement and presence of autoantibodies to Ro/SSA and/or to La/SSB [17]. Disease duration ranged from 0 to 4 years. Eight and four patients had circulating anti-SSA/SSB Igs and hyper IgG, respectively. At the time of the biopsy, patients had a focus score ranging from 2 to 6, and were not undergoing any immunomodulatory treatment. Four patients with a mean age of 60 years (range 47–69 years) affected by secondary SS subsequent to RA ($n = 4$) were also studied. Five patients with an occular adnexal marginal zone B-cell lymphomas associated with SS were also analysed. Normal salivary gland specimens were obtained from individuals undergoing surgery for benign lip lesions. All experiments were performed in agreement with local ethics committees and patients' informed consent. The study was approved by the Geneva Hospital Ethical Committee.

Immunohistochemistry

Immunohistochemistry (IHC) analyses were performed on formalin-fixed paraffin-embedded tissues. Tissue reactivity for Stalk-1 and Aprily-8 antibodies was performed with an antigen-retrieval step consisting of a microwave treatment in 0.01 M citrate buffer of pH 6, as previously described [18]. The anti-CD138 [gamma immunoglobulin 1 (IgG1), clone MI15] and anti-elastase (IgG1, clone NP57) were from Dako-Cytomation (Glostrup, Denmark). Peroxydase-conjugated secondary reagents have all been described previously [18]. For multi-colour IF, phycoerythrin-conjugated anti-mouse IgG1 goat serum (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) and alexa 488-conjugated anti-rabbit Ig goat serum (Molecular Probes, Leiden, The Netherlands) were used. 4′,6′-diamidino-2-phenylindole staining was included in the merged images. Images were visualized under light or fluorescent microscopy with Axiophot 1 (Carl Zeiss AG, Berlin, Germany), captured with an axiocam (Carl Zeiss AG) colour Charge-Coupled device camera and treated on a Pentium III computer with axioVision software (Carl Zeiss AG). Stalk-1-stained cells and CD138$^+$ PCs were numerated as previously described [12] for a 4 mm^2 section. Statistical analyses were performed with a Mann–Whitney test. Quantification of the Stalk-1 signal in epithelium was performed as previously described in tonsils [9].

Results

APRIL expression in minor salivary glands from patients with SS

We first assessed, by IHC, the presence of APRIL-producing cells and secreted APRIL in salivary glands from patients with SS. Cells producing APRIL, detected by the Stalk-1 antibody, were rare, despite high infiltration of leucocytes (Fig. 1A), and there was no staining with Aprily-8, the antibody detecting secreted APRIL. Few of the cells producing APRIL in the parenchyma lesion had a segmented nucleus, and we identified them as elastase-expressing neutrophils in two-colour IF staining (Fig. 1B). There was no CD68$^+$ macrophage that produced APRIL in these SS lesions. Keratinocytes from the adjacent oral epithelium were the main producers of APRIL (Fig. 1C). APRIL-producing cells were located in the upper layers of this epithelium, similar to the epithelium from tonsil [9]. Epithelial cells secreted all the APRIL they produced, since they were not stained with Aprily-8, consistent with the efficient APRIL secretion observed in previously studied tissues, including tonsil, small intestine and diverse tumour lesions [19]. The interstitial tissue around salivary glands did not retain APRIL secreted by the epithelium. The latter observation is consistent with the known absence of communication between these two tissues. We previously reported APRIL up-regulation in some B-cell lymphomas, both at the level of APRIL production and accumulation of secreted APRIL on tumour cells [18, 20, 21]. Hence, we also studied B-cell lymphomas that can be associated with SS [22]. We observed a similar paucity of APRIL-producing cells and absence of secreted APRIL in these tumour cases (data not shown). Taken together, these show that APRIL expression is weak in autoimmune and tumoural SS.

APRIL expression in minor salivary glands from patients with SS harbouring PC accumulation

As discussed above, the primary role of APRIL is to sustain PC survival. PCs frequently accumulate in minor salivary glands from SS patients [23], and ectopic germinal centres (GCs) have also been described in some SS lesions [24]. Hence, APRIL might be more predominant in these cases. We studied six SS cases with accumulating PCs (496 ± 319, ranging from 209 to 926/4 mm^2), and one of these cases even showed an ectopic GC. In such a case, PCs localized outside the GC in the interstitial space among salivary glands (Fig. 2). At that site, we detected neither APRIL-producing cells nor secreted APRIL. This shows that PCs accumulate in SS salivary glands in areas devoid of detectable APRIL.

APRIL expression in normal salivary glands

We next studied healthy salivary glands. Notably, the expression of APRIL was very similar, at the level of the production and concentration of secreted APRIL. Cells from the upper layers of the oral epithelium also produced APRIL (Fig. 3A, upper panel), to a level comparable with SS lesions as assessed by a quantitative analysis of the Stalk-1 staining (Fig. 3A, bottom panel). Interstitial APRIL-producing neutrophils were barely detectable, and secreted APRIL was again virtually absent from this tissue (data not shown). Quantitative comparative study between pathological (autoimmune and tumoural) and healthy tissues indicated that the number of APRIL-producing cells was only slightly increased in 5/14 autoimmune SS lesions, but the entire SS cohort did not show any statistically significant up-regulation compared with healthy salivary glands (Fig. 3B). The values obtained here for APRIL-producing cells infiltrating SS lesions were

Fig. 1 Oral epithelium produces APRIL, but no retention of secreted APRIL in salivary glands from SS lesions. (**A**) Serial sections of salivary glands from SS patients were immunostained with control Ig (cIg), Stalk-1 and Aprily-8 (Ap-8). Pictures are representative of 14 primary SS lesions. (**B**) High magnification (×63) of a Stalk-1-stained cell in the parenchyma of a salivary gland from SS (left panel). APRIL-producing cells (Stalk-1 staining, green) express elastase (red staining) (right panel). Single and merged pictures from two-colour IF are representative of four SS lesions. (**C**) Serial sections of the adjacent oral epithelium from SS lesions were immunostained as in (**A)**. Pictures are representatives of five biopsies from SS lesions with epithelium presence.

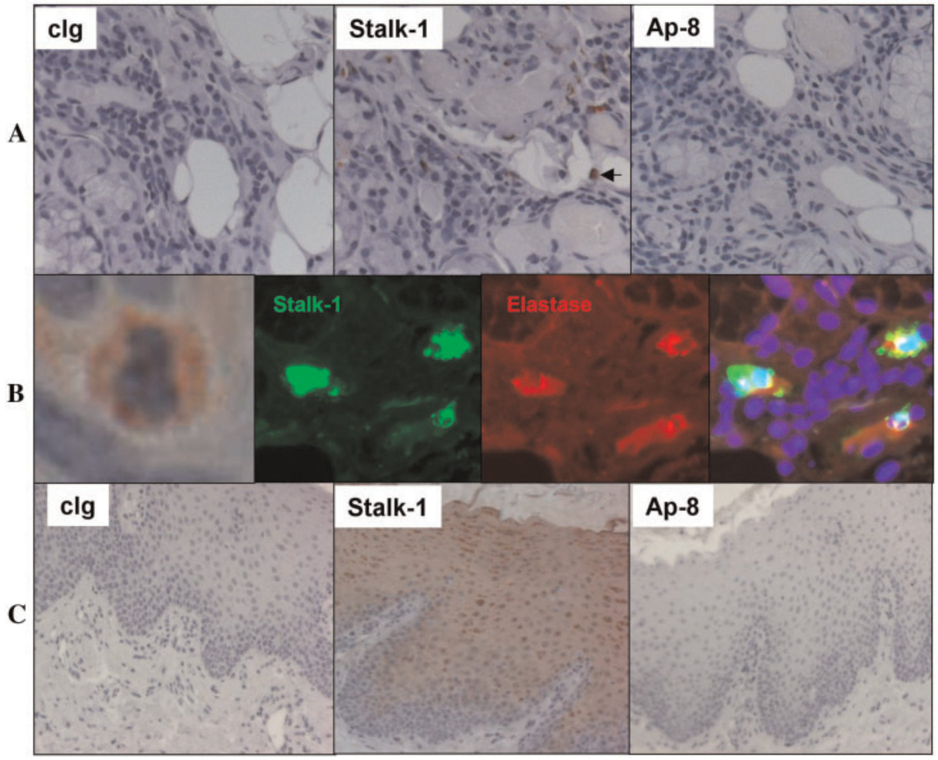
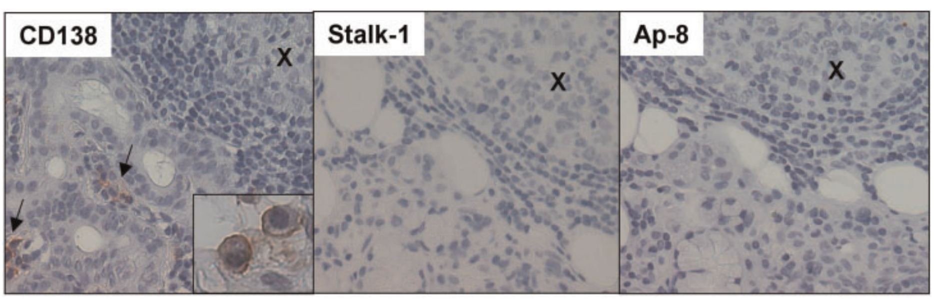


Fig. 2 Absence of APRIL expression in SS lesions infiltrated by PCs. Serial sections of an SS lesion with GC formation and PC generation were immunostained with an anti-CD138, Stalk-1 and Ap-8. GC light zone (×). Representative clusters of $CD138^+$ cells (↓). Insert represents a ×100 magnification of a $CD138^+$ cell.

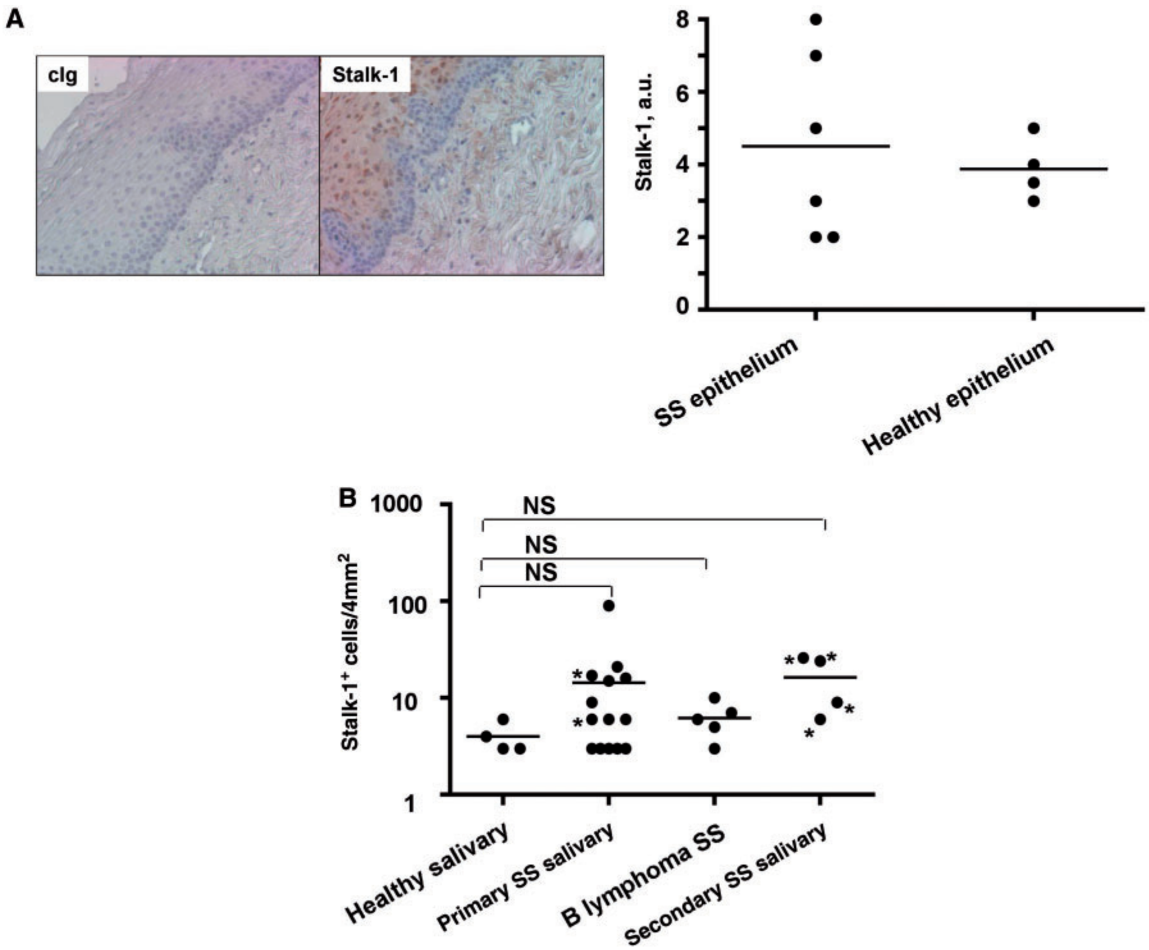


~10-fold less than the one we previously observed in RA synovium [12]. To further compare these two rheumatic diseases, we also studied salivary glands from secondary SS subsequent to RA. These lesions were as low as primary SS lesions for APRIL-producing cells, and were also devoid of secreted APRIL (data not shown). Taken together, this demonstrates the absence of APRIL up-regulation in salivary glands from SS despite high infiltration.

Discussion

The present study demonstrates that APRIL production in SS lesions was low. We observed this with

Fig. 3 Quantification of APRIL production in pathological and healthy salivary glands. (**A**) Healthy salivary glands were immunostained with cIg and Stalk-1. The adjacent epithelium is shown (upper panel). Intensity of the Stalk-1 staining in the indicated epithelium was quantified and reported in arbitrary units (a.u.) (lower panel). (**B**) The number of Stalk-1-stained cells is shown for healthy salivary glands ($n = 4$), primary SS salivary glands ($n = 14$), B-cell lymphoma associated with SS ($n = 5$) and secondary SS salivary glands following RA ($n = 4$). SS lesions infiltrated by PCs (*). NS: not significant.



primary SS, secondary SS subsequent to RA and B-cell lymphoma associated with SS. This is in marked contrast with the high APRIL production that can be observed in RA and B-cell lymphoma lesions. Furthermore, pathological salivary glands did not retain secreted APRIL, as opposed to RA. In fact, levels of APRIL expression in SS lesions were very similar to corresponding healthy tissues. The absence of APRIL up-regulation observed here is consistent with the predominant presence of lymphoid cells in SS infiltrates [25], over myeloid cells, the latter constituting the most important source of APRIL in tissues [19]. Our present results imply that the elevated level of APRIL reported in patients' sera is unlikely to originate from lesions, but rather from other sites of APRIL production. Since precursor and mature granulocytes are both producing APRIL in the bone marrow and blood, respectively [12, 18], our results indicate that an increased myelopoiesis, already reported for other rheumatic diseases such as SLE [26], may explain up-regulation of circulating APRIL in SS patients. Taken together, the present report indicates that PCs present in SS may not be dependent on APRIL for survival in lesions. To our knowledge, this is the first *in situ* demonstration of PCs residing in a tissue devoid of APRIL. Such an observation warrants further investigations with the study of more SS lesions with ectopic GCs, as well as SS lesions developing at other mucosa-associated lymphoid tissue (MALT) sites, due to the APRIL key role for PCs in healthy MALTs [9]. It should also be noted that our present report does not reccomend not using APRIL targeting reagents for the treatment of SS; rather it indicates that these approaches will primarily affect bone marrow-homed PCs secreting autoantibodies systemically.

Rheumatology key messages

- Salivary glands from SS do not harbour APRIL up-regulation.
- Plasma cells infiltrating salivary glands from SS do not rely on APRIL for survival.
- APRIL antagonism in SS may still be valuable by targeting lymphoid organ-resident plasma cells.

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