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FcRL4$^+$ B-cells in salivary glands of primary Sjögren's syndrome patients

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ABSTRACT

Fc receptor-like protein 4 (FcRL4) is normally expressed on a small subset of mucosa-associated B-cells, as well as on mucosa-associated lymphoid tissue (MALT) lymphoma B-cells. Primary Sjögren's syndrome (pSS) patients have an increased risk of developing MALT lymphomas, preferentially in the parotid glands. For this reason we studied here by immunohistochemistry and mRNA analysis whether FcRL4 expressing B-cells are present in salivary gland tissue (labial and parotid) of pSS patients (n = 54) and non-pSS sicca patients (n = 16) and whether parotid gland MALT lymphomas in pSS patients (n = 49) also express this receptor. We also studied the effect of treatment (rituximab and abatacept) on the presence of FcRL4$^+$ B-cells, and whether numbers in labial gland biopsies at time of diagnosis of pSS can predict whether patients are at risk for MALT lymphoma development.

We demonstrate that FcRL4$^+$ cells are present in salivary gland tissue of pSS patients where they are closely associated with ductal epithelial cells forming lymphoepithelial lesions. The glandular FcRL4$^+$ cells are highly proliferative, genuine PAX5$^+$ B-cells. These FcRL4$^+$ B-cells are far more frequent in parotid gland than in labial gland tissue ($p = 0.003$). We further show that expression of FcRL4 is present in pSS-related parotid MALT lymphomas. The FcRL4 mRNA expression level in parotid MALT lymphoma is increased compared to parotid gland tissue of pSS patients without lymphoma ($p = 0.017$). However, numbers of FcRL4$^+$ B-cells in labial gland biopsies taken at the time of pSS diagnosis, are not predictive for later development of MALT lymphoma. Reduction of parotid gland FcRL4$^+$ B-cells by rituximab, but not abatacept is accompanied by restoration of the glandular epithelium, illustrating the crosstalk between these B-cells with the ductal cells.

In conclusion, intraepithelial FcRL4$^+$ B-cells are present in the salivary glands of pSS patients. These cells are likely involved in the epithelial changes seen in pSS. Their enrichment in parotid glands may explain why MALT lymphomas in pSS patients preferentially develop at this specific location.

1. Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disease affecting exocrine glands leading to dryness of mouth and eyes [1]. Histologically, salivary glands of pSS patients harbor lymphocytic infiltrates that are arranged around striated ducts. The interaction of lymphocytes and the ductal epithelial cells is further emphasized by the formation of lymphoepithelial lesions (LELs), formerly called epimyoepithelial islands. These LELs are composed of proliferative metaplastic epithelial cells and intraepithelial lymphocytes. Interestingly, LELs are more pronounced in parotid glands than in labial glands [2–4].

A hallmark of pSS pathogenesis is hyperactivity of B-cells, which is amongst others reflected by the elevated risk of non-Hodgkin lymphoma development. This serious complication occurs in 5–10% of pSS patients [5,6]. These lymphomas are almost exclusively B-cell lymphomas, mostly of the mucosa-associated lymphoid tissue (MALT) type (>60%) and preferentially develop in the parotid gland [6–8]. It is not clear why MALT lymphomas arise predominantly at this location, since all minor and major salivary glands are affected in pSS.

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Parotid MALT lymphomas often contain distinct LELs that are infiltrated and surrounded by neoplastic B-cells with a centrocyte-like or monocytoid appearance, sometimes admixed with neoplastic plasma cells [9,10]. Falini et al. demonstrated that exclusively marginal zone lymphomas, including major salivary gland MALT lymphomas and a subset of DLBCL (diffuse large B-cell lymphoma) express the inhibitory Fc receptor-like protein 4 (FcRL4/IRTA1/CD307d) [11]. DLBCL can develop, occasionally as transformation from MALT lymphoma in pSS patients as well [9,12]. In healthy individuals, FcRL4 is expressed on a small subset of highly proliferative memory B-cells in mucosal tissues (tonsils, Peyer's patches), where they are concentrated near and within the epithelial surfaces. FcRL4$^+$ B-cells are rarely found in blood, spleen and peripheral lymph nodes [13,14]. This association with the epithelium at mucosal sites is in concordance with the notion that FcRL4 is a receptor for IgA and strongly argues that FcRL4$^+$ B-cells exert an important role in mucosal immune responses [15]. In response to chronic antigen stimulation FcRL4 dampens activation by the B-cell receptor (BCR) and enhances innate Toll-like receptor (TLR) activation [15,16]. Upon differentiation from FcRL4$^+$ B-cells towards plasma cells, FcRL4 expression is lost.

An increased number of FcRL4$^+$ B-cells is found in reactive lymphadenitis caused amongst others by toxoplasmosis, HIV and mononucleosis [13,14]. In the autoimmune disease rheumatoid arthritis, FcRL4$^+$ B-cells are found in synovial fluid and synovial tissue, where they are located beneath the synovial lining and around blood vessels [17].

In this study we assessed the presence and localization of FcRL4$^+$ B-cells in salivary gland tissue of pSS and control patients as well as in pSS-associated MALT lymphomas.

2. Material and methods

2.1. Patients

Salivary gland biopsies were collected from 54 patients diagnosed with pSS. All patients fulfilled the American-European Consensus Group (AECG) criteria for pSS and were selected because they had not developed a malignant lymphoma during follow up (median 9.2 year, IQR 4.4–12.6 year). Thirty biopsies were taken from the parotid gland and 24 from the minor salivary (labial) glands. As controls served parotid gland biopsies (n = 8) and labial gland biopsies (n = 8) obtained from non-pSS sicca patients (complaints of dryness, not fulfilling the AECG criteria). Clinical data of the pSS patients and sicca patients are presented in Table 1. Furthermore, 5 parotid glands from individuals with no complaints of dryness were included in this study. Of these five patients, who had undergone surgery for pleomorph adenoma (n = 3) or warthin tumor (n = 2) the resection margins of the parotid gland were used. To explore whether FcRL4$^+$ cells are affected by rituximab or abatacept, parotid gland biopsies from pSS patients enrolled in the placebo-controlled rituximab trial (18 rituximab, 9 placebo) [18,19] and open label abatacept study (n = 15) [20,21] were also analyzed.

In addition, tissue samples from the parotid gland of 49 pSS patients diagnosed with parotid MALT lymphomas were obtained. These pSS patients were either diagnosed or referred for parotid MALT lymphoma at the University Medical Center Groningen (Supplementary Fig. 1). Biopsy material stored at other hospitals was requested for re-evaluation. Clinical data at time of diagnosis of MALT lymphoma are presented in Table 1. From 10 parotid MALT lymphoma patients, a diagnostic labial salivary gland biopsy (for the diagnosis of pSS) prior to lymphoma development was available for analysis as well.

2.2. Histological evaluation of salivary gland biopsies

Hematoxylin and eosin (HE) stained sections were used to assess focus score (FS) and presence of LELs. FS was based on the number of periductal infiltrates of ≥50 lymphocytes (foci)/4 mm^2 parenchyma. In case of multiple large confluent foci an arbitrary maximal FS of 12 was used (1 pSS patient) [22]. LELs were defined as a cross section of a striated duct with infiltration of lymphocytes

Table 1
Characteristics of pSS patients diagnosed with parotid MALT lymphoma, pSS patients and non-pSS sicca patients.

Variable	pSS parotid (n = 30)	Sicca parotid (n = 8)	pSS labial (n = 24)	Sicca labial (n = 8)	pSS parotid MALT lymphoma (n = 49)	pSS pre-lymphoma labial (n = 10)
Patient characteristics						
Age year diagnose, median (IQR)	47 (33–62)[b]	56 (46–64)[b]	56 (38–68)[b]	44 (40–62)[b]	53 (43–64)[c]	46 (32–61)[b]
Female, n(%)	30 (100%)	7 (88%)	22 (92%)	7 (88%)	45 (92%)	10 (100%)
ΔpSS-MALT lymphoma year, mean ± SD	–	–	–	–	2.6 ± 5.13	5.3 ± 1.5
Ann Arbor/Musshoff stage[a]						
Localized disease, n(%)	–	–	–	–	29 (59%)	–
Locally disseminated, n(%)	–	–	–	–	19 (39%)	–
Disseminated disease, n(%)	–	–	–	–	1 (2%)	–
Laboratory assessments						
SSA positive, n(%)	26 (87%)	0 (0%)[d]	20 (83%)	1 (13%)	43 (92%)[g]	10 (100%)
SSB positive, n(%)	17 (57%)	0 (0%)[d]	9 (38%)	0 (0%)	32 (68%)[g]	6 (60%)
RF elevated, n(%)	25(83%)	0 (0%)[e]	14 (61%)[f]	0 (0%)	44 (94%)[g]	10 (100%)
ANA positive, n(%)	26 (87%)	3 (43%)[d]	22 (96%)[f]	3 (38%)	46 (98%)[g]	10 (100%)
Histopathological parameters						
Focus score, median (IQR)	2.4 (1.8–3.7)	0 (0–0.7)	2.9 (1.6–4.2)	0 (0–0.5)	–	1.8 (0.6–3.0)
Presence LELs, n(%)	28 (93%)	0 (0%)	8 (33%)	0 (0%)	48 (98%)	4 (40%)

Between the pSS patients with a labial gland biopsy and pSS patients with a parotid gland biopsy there was no significant differences in age at time of pSS diagnosis.

[a] Staging of parotid MALT lymphoma based on the Ann Arbor classification with modification by Musshoff [35,36]. Localized disease: lymphoma located in one or more salivary glands. Locally disseminated: lymphoma localized in one or more salivary glands with one or more enlarged regional lymph nodes (>1 cm). Disseminated disease: localization of lymphoma in one or more salivary glands, with one or more enlarged regional lymph nodes (>1 cm) and/or bone marrow, spleen, liver or other extra nodal site than the salivary gland, or localization of lymphoma in multiple extra nodal sites. LELs: lymphoepithelial lesions, IQR: Interquartile range.

[b] Age at diagnosis of pSS or non-pSS sicca.

[c] Age at diagnosis of MALT lymphoma.

[d] n = 7.

[e] n = 6.

[f] n = 23.

[g] n = 47.