Abatacept treatment of patients with primary Sjögren's syndrome results in a decrease of germinal centres in salivary gland tissue

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Received on May 26, 2016; accepted in revised form on September 2, 2016.

Key words: Sjögren's syndrome, abatacept, germinal centre, ESSDAI, parotid gland

Funding: this study was financially supported by Bristol Myers Squibb, Rueil Malmaison, France, who also supplied study medication.
There was no involvement of this funding source in study design, data collection, analysis and interpretation and writing of this report.

Competing interests: H. Bootsma is the recipient of an unrestricted grant and also receives consultancy fees from BMS; F.G.M. Kroese has received consultancy fees from BMS; the other co-authors have declared no competing interests.

ABSTRACT

Objective. *The aim of this study was to assess the histopathological changes in parotid gland tissue of primary Sjögren's syndrome (pSS) patients treated with abatacept.*

Methods. *In all 15 pSS patients included in the open-label Active Sjögren Abatacept Pilot (ASAP, 8 abatacept infusions) study parotid gland biopsies were taken before treatment and at 24 weeks of follow up. Biopsies were analysed for pSS-related histopathological features and placed in context of clinical responsiveness as assessed with EULAR Sjögren's syndrome disease activity index (ESSDAI).*

Results. *Abatacept treatment resulted in a decrease of germinal centres (GCs)/mm² (p=0.173). Number of GCs/mm² at baseline was associated with response in the glandular domain of ESSDAI (Spearman ϱ=0.644, p=0.009). Abatacept treatment did not reduce focus score, lymphoepithelial lesions, area of lymphocytic infiltrate, amount of CD21⁺ networks of follicular dendritic cells, and numbers of CD3⁺ T-cells or CD20⁺ B-cells. Number of IgM plasma cells/mm² increased (p=0.041), while numbers of IgA and IgG plasma cells/mm² were unaffected during abatacept treatment.*

Conclusion. *Abatacept affects formation of GCs of pSS patients in parotid glands, which is dependent on co-stimulation of activated follicular-helper-T-cells. Herewith, local formation of (autoreactive) memory B-cells is inhibited. Presence of GCs at baseline predicts responsiveness to abatacept in the ESSDAI glandular domain.*

Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disease characterised by chronic inflammation of exocrine glands, histomorphologically seen as periductal infiltrates predominantly consisting of T- and B-cells. Lymphocytic infiltration of the epithelium of striated ducts leads to formation of lymphoepithelial lesions (LELs) which are more pronounced in parotid than in labial glands (1). Besides periductal infiltrates, there is a plasmacytosis with an increased number of IgG expressing plasma cells (2). A subset of pSS patients develop germinal centres (GCs) in ectopic lymphoid infiltrates of the glands. Presence of GCs is associated with more active disease and is considered to be a predictor for malignant lymphoma development (3-5).

There are no approved therapeutic interventions for pSS yet, but promising results with biological disease modifying anti-rheumatic drugs, *e.g.* abatacept, are reported. Abatacept was shown to be effective and safe in open-label studies in pSS (6, 7). This fully human biological binds to CD80/CD86 on antigen presenting cells and hereby blocks the CD28-mediated co-stimulation of CD4⁺ T-cells (8). In pSS patients, systemic disease activity is assessed with the EULAR Sjögren's Syndrome Disease Activity (ESSDAI) score. Abatacept treatment resulted in a decrease of ESSDAI with most prominent beneficial clinical effects in the glandular, articular, constitutional and biological domains. Saliva production remained stable during treatment (6).

It is not clear yet what the effect of abatacept treatment is on the inflammatory process in salivary glands, in the context of responsiveness to treatment. Therefore, the aim of this study was to assess the histopathological changes in parotid gland tissue of early and active pSS patients treated with abatacept. This evaluation was performed in a standardised fashion that was shown before to identify biomarkers useful for personalised medicine (9).

Materials and methods

Patients

In all pSS patients (for characteristics see supplementary Table I) included in the open-label Active Sjögren Abatacept Pilot (ASAP) study (n=15) (6), a parotid gland biopsy was taken within 12 months before and 24 weeks after the initiation of abatacept treatment. Patients received abatacept infusions (≈10 mg/kg) on days 1, 15, 29 and every 4 weeks thereafter (Bristol Myers Squibb, France). ESSDAI scores were used for rating disease activity (10). Patients gave their consent and ethics approval was obtained from the Institutional Review Board of the University Medical Center Groningen (METc 2009.371).

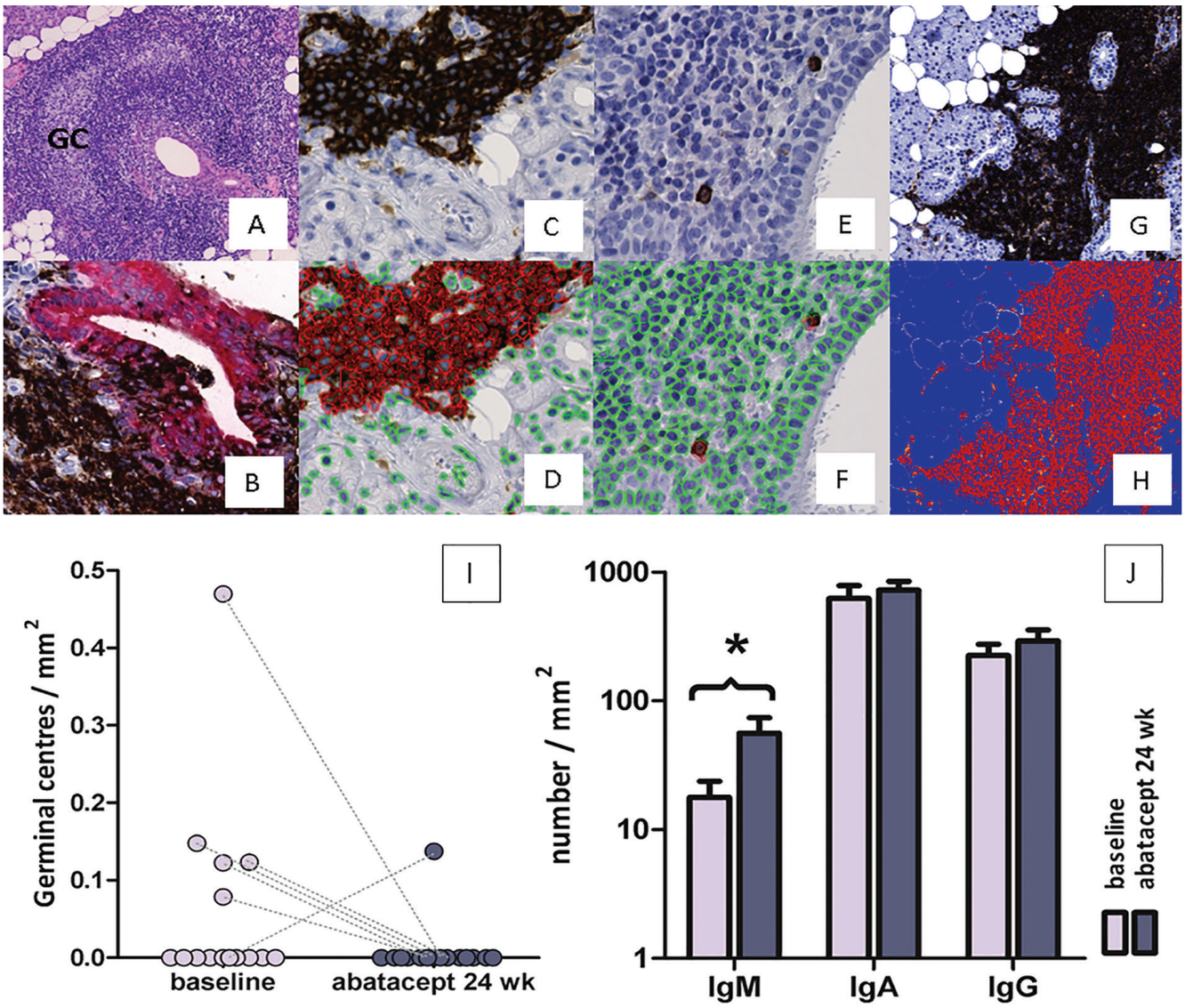

Fig. 1. (Immuno-)histological analysis of parotid gland tissue of pSS patients treated with abatacept.
Biopsy from a parotid gland showing **A**) germinal centre (HE) **B**) LEL (CD20 in brown and CK 8/18 in red) **C**) CD20$^+$ B-cells **D**) analysis of sample shown in C with HistoQuest **E**) IgM$^+$ plasma cells **F**) analysis of sample shown in E with HistoQuest **G**) CD45$^+$ lymphocytes **H**) measurement of relative area of CD45$^+$ lymphocytic infiltrate with Aperio ImageScope of sample shown in G. **I**) Number of GCs in parotid gland tissue decline after treatment with abatacept. **J**) Plasma cells in parotid gland tissue before and after treatment of abatacept. IgM plasma cells/mm^2 increase after abatacept treatment (*Wilcoxon Signed Rank, p=0.041). Values presented in mean and SD.

(Immuno-)histological staining and evaluation of parotid gland biopsies

(Immuno-)histological processing and staining was performed as previously described (see also Fig. 1) (9). Primary antibodies are listed in supplementary Table II. Staining was performed on serial sections, except for CD21. For practical reasons, staining for CD21 was performed on sections from the same paraffin blocks, but sectioned in a separate session. Haematoxylin and eosin (HE) stained sections were used to assess FS and number of GCs/mm^2. Dual staining with CD20 and CK8/18 was used to determine number of LELs/mm^2. Severity of LELs was scored as previously described (9). Evaluation was performed independently and blinded (B.V., E.H.) resolving discrepancies by consensus. Relative area of CD45$^+$ lymphocytic infiltrate and CD21$^+$ follicular dendritic cell (FDC) networks was measured using ImageScope v. 12.0 (Aperio Technologies, USA). Numbers of CD20, CD3, IgA, IgG, IgM positive cells were assessed using HistoQuest v. 3.5.3.0171 (Tissuegnostics, Austria) (9). The parenchyma of the whole biopsy was examined, excluding intraparenchymal connective,- and fat tissue.

Statistical analysis

Wilcoxon Signed Rank test was used to test differences between groups. Spearman correlation coefficient was calculated for correlations (IBM SPSS Statistics v. 22).

Results

Focus score, relative area of CD45$^+$ lymphocytic infiltrate, number of CD20$^+$ B-cells/mm^2 and CD3$^+$ T-cells/mm^2, CD21$^+$ FDC networks and total

Table I. Histopathologic results of parotid gland tissue evaluation in pSS patients (n=15) treated with abatacept.

Variable	Baseline	Abatacept wk 24	*p*-value (Wilcoxon Signed Rank)
Histopathological pSS parameters			
Area of parotid parenchyma	5.3 (4.3-8.1)	6.5 (4.9-8.9)	0.820
Focus score (number of foci of ≥50 lymphocytes/4 mm^2)	3.1 (1.5-5.0)	3.2 (0.9-4.1)	0.173
Germinal centres (n./mm^2)	0.06 (0.13)*	0.009 (0.04)*	0.173
Lymphoepithelial lesions (n./mm^2)	0.24 (0-0.56)	0.27 (0-0.61)	0.583
Area of CD21$^+$ FDC networks	0.063 (0.006-0.539)	0.030 (0.004-0.244)	0.334
Area of CD45$^+$ lymphocytic infiltrate	9.0 (5.8-38.0)	15.3 (10.4-33.1)	0.649
B-cells (n./mm^2)	1187 (687-3128)	752 (268-3677)	0.394
T-cells (n./mm^2)	1109 (892-2510)	1119 (425-2971)	0.427
Total plasma cells (n./mm^2)	1271 (566-1939)	2161 (1177-2887)	0.125

Values are presented as median (IQR) unless otherwise specified.*Average (SD). *p*-value <0.05 was considered statistically significant.

plasma cell population, were all unaffected by abatacept (Fig. 1, Table I). Also structure and regeneration of the ductal epithelium, as reflected by number and severity of LELs, was not improved (supplementary Fig. 1). In contrast to these findings, number of GCs/mm^2 was reduced by abatacept treatment. At baseline, GCs were present in parotid gland biopsies of five patients and GCs were absent in all these patients after treatment (Fig. Ii). In one patient GCs were absent at baseline, but detected after abatacept treatment. This patient was the only patient in whom ESSDAI had increased after treatment and thus can be considered as non-responder. In all other patients ESSDAI had decreased upon treatment and in these patients the decline in GCs/mm^2 was statistically significant (p=0.043). The ESSDAI glandular domain was higher in patients with GC activity than patients without GC activity at baseline (1.2 *vs*. 0.6). The number of GCs/mm^2 at baseline was associated with improvement in the ESSDAI glandular domain (Spearman ϱ=0.644, p=0.009), but not with other ESSDAI domains. Numbers of IgA and IgG plasma cells/mm^2 remained stable, while numbers of IgM plasma cells/mm^2 increased (p=0.041, Fig. Ij). However, relative proportion of IgM plasma cells remained still low after treatment (4.8%).

Discussion

We assessed histopathological changes in parotid gland tissue in relation to abatacept treatment in early and active pSS patients. These changes were evaluated in a standardised fashion, that was previously shown to be able to identify biomarkers that predict responsiveness to rituximab treatment (9). Importantly, we observed that GCs disappeared completely in parotid gland tissue of patients with GCs at baseline (33%). Furthermore, number of IgM producing plasma cells increased, while other histopathological parameters measured did not change upon abatacept treatment. These observations are in line with our previous findings that during abatacept therapy secretion of stimulated whole saliva did not deteriorate (6). Also Adler *et al*. (7) observed that foci/mm^2, CD3$^+$ T-cells, CD20$^+$ B-cells and total number of plasma cells in labial (rather than parotid) glands were unaffected by abatacept. However, in their study, two major elements of pSS histopathology, namely GCs and LELs, were not analysed. We demonstrated for the first time that abatacept abrogated ectopic, histomorphologically-defined, GCs in a human autoimmune disease. Other studies had shown in a murine model for rheumatoid arthritis that the proportion of flow-cytometry-defined (GL7$^+$Fas$^+$) GC B-cells was reduced by abatacept treatment in lymph nodes draining affected joints (11). Abatacept did not decrease the amount of CD21$^+$ FDC networks (supplementary Fig. 2). Although these networks are a prerequisite for GC formation in the foci, presence of these networks does not imply per se that GCs are also present (12). Since abatacept does not affect the FDC networks, this observations suggests that absence of GCs cannot be attributed to a disorganised microenvironment. For formation and perpetuation of GCs, GC B-cells require co-stimulatory signals from T-follicular-helper-cells (T_{fh}-cells) for their development and maintenance. T_{fh}-cells are also involved in pSS pathogenesis (13). T_{fh}-cells are elevated in blood from pSS patients and are also present in glandular tissue (14). In pSS patients T_{fh}-cells appear to be in a hyperactivated state as reflected by elevated ICOS-levels on circulating T_{fh}-cells [Verstappen, unpublished observations]. We have shown that abatacept selectively reduces the (elevated) proportion and number of circulating T_{fh}-cells in the peripheral blood of pSS patients to levels of healthy controls, and also normalises the ICOS-levels [Verstappen, unpublished obervations]. A reduction in T_{fh}-cell activity after abatacept treatment might lead to decreased GC activity in inflamed salivary glands. Presence of GCs in labial glands is associated with higher FS and percentages of patients positive for autoantibodies (RF, anti-SSA/SSB) (3, 4). Whether patients with GCs also exhibit higher systemic disease as reflected by higher ESSDAI remains to be explored. In our (small) group of pSS patients there was no difference in total ESSDAI scores between patients with and without GCs in their biopsy, but there was a difference detectable considering the ESSDAI glandular domain.

The characteristic epitheliotropic autoimmune inflammation in pSS is histologically seen as LELs of striated ducts. LELs are composed of proliferative metaplastic epithelial cells in association with intra-epithelial lymphocytes and are more pronounced in parotid than in labial glands (1), making analysis of these structures more easy and reliable in parotid tissue. We have shown here that numbers and severity of LELs are not influenced by abatacept. This finding indicates that the cross-talk between epithelial cells and intra-epithelial lymphocytes, thought to be responsible for formation and maintenance of LELs

(9), is independent of CD28-mediated co-stimulation.

Our data showed that numbers of IgG plasma cells/mm^2 remained stable in parotid gland tissue after abatacept treatment. Part of these IgG plasma cells comprise autoantibody producing cells (15). These findings support the notion that (autoreactive) plasma cells located at inflamed sites are long-lived cells that are not replaced by newly generated plasma cells in a T($_{fh}$)-cell dependent fashion. Indeed our previous B-cell depletion studies showed that in parotid glands IgG-producing B-cell clones can persist for at least one year (16). A small proportion of long-lived plasma cells may express CD28 which is needed for their maintenance and survival (17). CD28 expressing plasma cells have, however, not yet been detected in parotid glands of pSS patients (unpublished data). Despite the fact that numbers of IgG (and IgA) producing plasma cells remained constant in parotid glands, serum IgG levels and autoantibody (anti-SSA/SSB) titers decreased during treatment (6). We suggest that this decrease is due to loss of (autoreactive) IgG producing plasma cells located elsewhere in the body. These cells are either CD28-expressing plasma cells that require CD28 stimulation for their survival or require T-cell dependent and CD28-mediated co-stimulation for their generation. In contrast to isotype switched plasma cells, there was an increase in numbers of IgM plasma cells/mm^2 in parotid tissue. IgM plasma cells are generally considered to be short-lived and their formation can partially occur in a T-cell independent fashion. The increase in IgM plasma cell numbers during abatacept treatment is in line with this T-cell independency. Why their numbers increase is unclear. We speculate that blocking T-cell help, drives the B-cells towards differentiation of unswitched IgM plasma cells, at the expense of IgG or IgA plasma cells.

To conclude, abatacept treatment results in a reduction of GCs in parotid gland tissue of pSS patients, probably due to inhibition of local T-cell dependent B-cell activation. Likely, the selective decrease in (activated) T_{fh}-cells plays an important role in reduction of GCs.

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