T cell-dependent B cell hyperactivity in primary Sjögren's syndrome

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IS THE T FOLLICULAR REGULATORY / T FOLLICULAR HELPER CELL RATIO IN BLOOD A BIOMARKER FOR ECTOPIC LYMPHOID STRUCTURE FORMATION IN SJÖGREN'S SYNDROME?

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Comment on 'Blood T Follicular Regulatory Cells / T Follicular Helper Cells ratio Marks Ectopic Lymphoid Structure Formation and PD-1+ ICOS+ T Follicular Helper Cells Indicate Disease Activity in Primary Sjögren's Syndrome' by Fonseca et al. (2018)

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We read with great interest the article by Fonseca et al [1], that was published in a recent issue of Arthritis & Rheumatology. The authors elegantly showed that T follicular regulatory (Tfr) cells were enriched in blood as well as in matched minor salivary gland (MSG) biopsies from patients with primary Sjögren's syndrome (pSS). They also showed that the Tfr/Tfh ratio in blood was increased in pSS compared to non-SS sicca patients. Interestingly, this Tfr/Tfh ratio in blood correlated with ectopic lymphoid structure formation in MSG tissue. To our opinion the authors did, however, not show a direct correlation between aberrant Tfr/Tfh ratios and ectopic lymphoid structure formation among pSS patients. In essence their study showed that in pSS patients the Tfr/Tfh ratio in blood was correlated with numbers of infiltrating lymphocytes, as assessed by flow cytometric analysis of MSG cell suspensions. Additionally, the authors showed that the Tfr/Tfh ratio in blood was increased in patients with focal sialoadenitis (FSA) (defined in their study as a focus score ≥1), compared to patients without FSA. Of note, this comparison was made irrespective of a diagnosis of pSS, which implicated that the majority of patients without FSA were non-SS sicca patients.

We assessed the number of circulating Tfr cells and Tfh cells in a larger inception cohort of 98 sicca patients clinically suspected of pSS. MSG biopsies of all patients were assessed in detail by histopathological analysis. Forty-four patients were classified as pSS (43 females, mean age 53, mean ESSDAI 7), and 54 patients as non-SS sicca patients (46 females, mean age 48). Of the 44 pSS patients, 80% were naive for treatment with corticosteroids or disease-modifying anti-rheumatic drugs. Consistent with the findings by Fonseca et al [1], frequencies of Tfr cells and the Tfr/Tfh ratio in blood were significantly increased in pSS compared to non-SS sicca patients (Figure 1A). In contrast to what has been suggested by Fonseca et al [1], we could not demonstrate in this larger inception cohort that pSS patients with a focus score ≥1 in MSG tissue had a higher Tfr/Tfh ratio in blood than pSS patients with a focus score <1. (Figure 1A). Moreover, neither focus score nor area of the $CD45^+$ infiltrate was correlated with the blood Tfr/Tfh ratio (Figure 1B). The Tfr/Tfh ratio was also not associated with ultrasonographic score of the major salivary glands (sUS) (Spearman's ρ=-0.04, P=0.831), while sUS was significantly associated with focus scores in both labial and parotid gland biopsies [2]. Thus, although our data also show that pSS patients have higher Tfr/Tfh ratios in blood, we found no association between this ratio in blood and glandular inflammation.

Besides increased levels of Tfr cells and the Tfr/Tfh ratio in blood, we also observed a significant increase in the frequency of activated ($PD\text{-}1^+ICOS^+$) Tfh cells in pSS compared to non-SS sicca patients (Figure 1C), while Fonseca et al. only observed a tendency towards higher frequencies of activated Tfh cells. Nonetheless, similar to the observations of Fonseca et al [1], we found that frequencies of activated Tfh cells in blood were associated with ESSDAI scores in pSS patients (Figure 1D). In addition, we observed that frequencies of activated Tfh cells correlated with Clinical ESSDAI

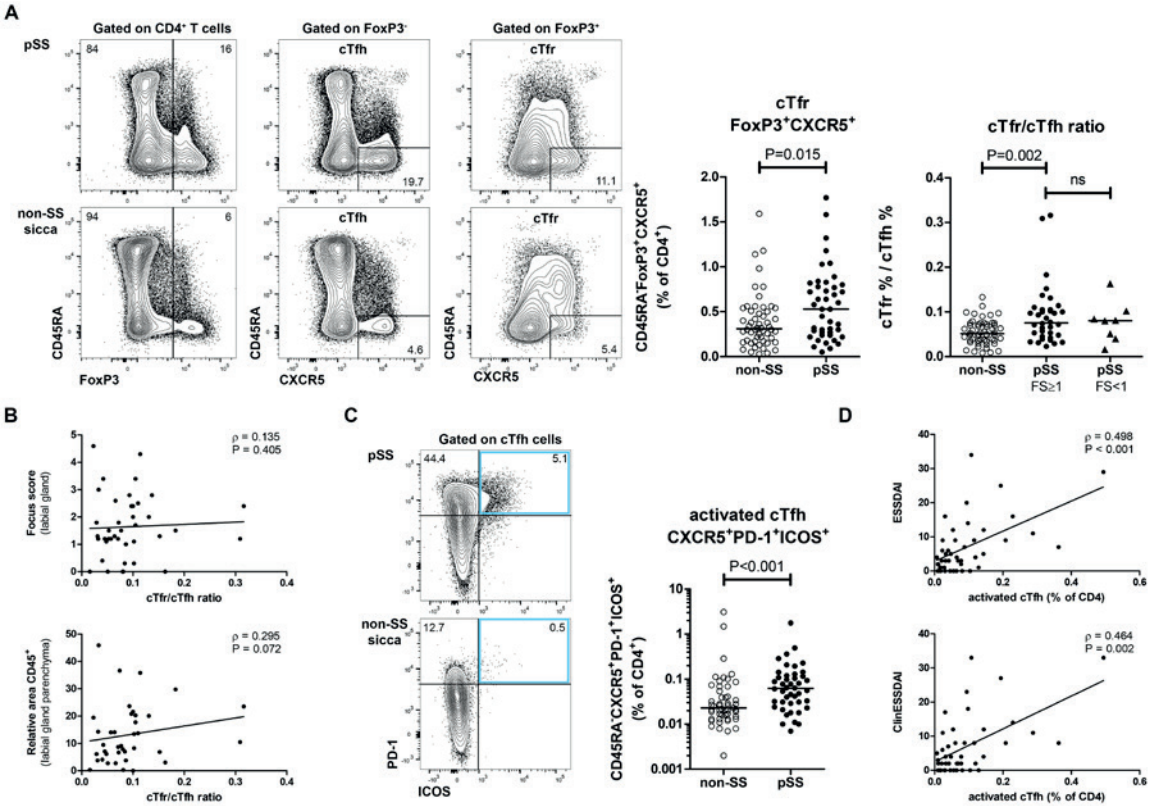

FIGURE 1. A, Gating strategy for circulating T follicular helper (cTfh) cells and T follicular regulatory (cTfr) cells, frequencies of cTfr cells, and the cTfr/cTfh ratio in 54 non-SS sicca patients, 36 pSS patients with FS≥1 and 8 pSS patients with FS<1. **B**, Correlations between the cTfr/cTfh ratio, FS in labial gland sections (n=40), and the relative area of parenchyma positively stained for CD45 in labial gland sections (n=38), in pSS patients. **C**, Gating strategy for activated Tfh cells, and frequencies of this cell subset in blood from 54 non-SS sicca and 44 pSS patients. Light blue box shows gate for activated cTfh cells. **D**, Correlations between activated Tfh cell frequencies, ESSDAI scores, and ClinESSDAI scores in blood from 44 pSS patients. Horizontal lines indicate the median. Circles indicate individual subjects. FS = focus score (number of foci per 4 mm^2 parenchyma); ρ = Spearman's Rho correlation coefficient; ESSDAI = EULAR Sjögren's syndrome disease activity index.

(ESSDAI without the biological domain [3]) scores (Figure 1C), indicating that the correlation is not only based on activity in the biological domain (e.g., hypergammaglobulinemia). Support for an association between activated Tfh cells and disease activity also comes from our previous study, in which circulating Tfh cells in pSS patients were studied before and after treatment with abatacept [4]. In that study we observed a significant decrease in activated Tfh cells in blood during treatment. Furthermore, the reduction of ICOS expression by the remaining Tfh cells correlated significantly with the decrease in ESSDAI scores [4].

In conclusion, the data presented by Fonseca et al. provides evidence that Tfr and Tfh cells are important players in pSS pathogenesis [1]. Likely, these cells are involved in B cell hyperactivation that characterizes this disease, but levels of these cells in blood may not necessarily reflect the presence of ectopic lymphoid tissue in the salivary glands. Importantly, all available data do indicate that Tfh cells contribute significantly to systemic disease activity in pSS, and emphasize that these cells are an important target for treatment.

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