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Blocking T cell co-stimulation in primary Sjögren's syndrome: rationale, clinical efficacy and modulation of peripheral and salivary gland biomarkers

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ABSTRACT

There is accumulating evidence that patients with primary Sjögren's syndrome (pSS) display aberrant CD4+ T cell responses, both in the peripheral compartment and in the inflamed salivary glands. CD4[+] T cell abnormalities are also critically associated with B cell hyper activation, one of the hallmarks of disease, which is linked with disease severity and evolution to lymphoma. T cell activation and the cross-talk between T and B cells are tightly regulated by the balance between co-stimulatory pathways, such as the interactions between CD80/CD86:CD28, CD40:CD40L and ICOS:ICOSL, and co-inhibitory signals, including the immunoregulatory CTLA-4 protein. Evidence from patients with pSS as well as data from animal models of the disease suggests that these pathways play a critical role in pSS pathogenesis and their targeting could be exploited for therapeutic purposes. In this review, we first summarise the evidence implicating aberrant T cell co-stimulation and co-inhibition in driving the disease before focusing on the results of recent randomised controlled trials (RCTs) with compounds able to block T cell co-stimulation and enhance T cell co-inhibition. Despite a clear biological effect on downstream B cell activation has been observed in patients treated with CTLA-4-Ig (abatacept) and with monoclonal antibodies targeting CD40 and ICOSL, the clinical efficacy of this approach has so far yielded mixed results; while the anti-CD40 monoclonal antibody iscalimab showed significant improvement in systemic disease activity compared to placebo, two large RCTs with abatacept and a phase IIa RCT with an anti-ICOSL monoclonal antibody (prezalumab) failed to reach their primary endpoints. Although the discrepancies between biological and clinical efficacy of targeting T cell co-stimulation on pSS remain unresolved, several factors including drug bioavailability and receptor occupancy, patient stratification based on T-cell related biomarkers and the choice of study outcome are likely to play an important role and form the basis for further work towards the quest for a disease-modifying biologic therapy in pSS.

Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disease which predominantly affects the exocrine glands and is characterised by lymphocytic infiltration of both the salivary and lacrimal glands, with progressive destruction and loss of function of the glands and the manifestation of sicca symptoms. However, systemic disease with involvement of other organs also occurs frequently (1). Although the exact mechanism leading to the glandular inflammation is not completely understood, both innate and adaptive immunity are involved in the disease pathogenesis, the former earlier on, possibly via activation of epithelial cells by viruses, whilst adaptive immunity plays a predominant role later, with the involvement of both T and B cells and the perpetuation of a chronic immune response (2). B cell hyperactivity is the cardinal feature of pSS, evidenced by raised immunoglobulins, circulating autoantibodies, alteration of B cell sub-populations, and increased risk of B-cell lymphoma (3,4). Enhanced B cell activation is thought to be related to the extra-glandular manifestations of the disease and is driven by T cells (5). T cells and B cells comprise the vast majority of the mononuclear cells infil-

trating the salivary and lacrimal glands (>90% of infiltrating mononuclear cells). In the early stages, the inflammatory infiltrates are dominated by CD4+ T helper cells, with B cell accumulation occurring at later stages (6). Different subsets of CD4+ T cells are involved: interferon-gamma (IFN-γ) producing Th1 cells and IL-17-producing Th17 cells infiltrate the glands at an early stage, contributing to the formation of segregated B and T cell aggregates (7, 8). In approximately 30–40% of cases, lymphocytic infiltrates are organised in structures similar to secondary lymphoid organs, defined ectopic lymphoid structures (ELS) (9, 10). These structures contain IL-21-producing T follicular helper (Tfh) cells, which support B cell activation and ectopic germinal centre (GC) formation, ultimately leading to local formation of memory B cells and plasma cells (9, 10).

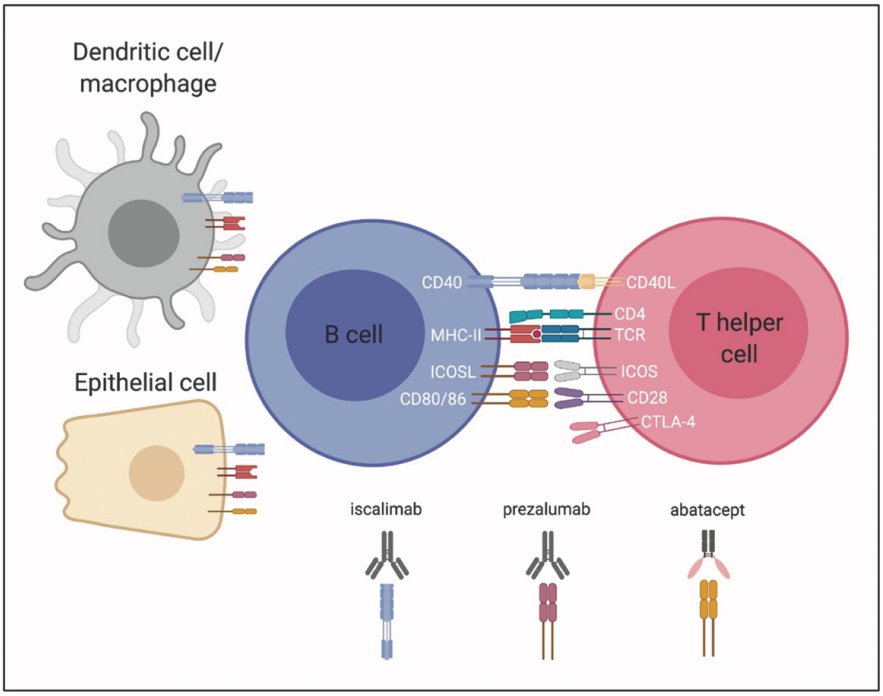

Fig. 1. Co-stimulatory pathways involved in the pathogenesis of primary Sjögren's syndrome. Co-stimulatory pathways that are involved in T-cell dependent B cell activation in pSS are illustrated. Other conventional (dendritic cell/macrophage) and unconventional (epithelial cell) antigen presenting cells that contribute to T cell activation in pSS are also depicted. So far, drugs that target CD40 (anti-CD40; iscalimab), ICOSL (anti-ICOSL; prezalumab), or CD80/CD86 (CTLA4-Ig; abatacept) have been clinically evaluated in patients with pSS.

Role of co-stimulatory and co-inhibitory molecules in pSS

The activation and proliferation of B and T cells is a crucial event in the pathogenesis of pSS, with three families of costimulatory molecules involved in the interaction between antigen presenting cells (APCs), including B cells, and T cells: i) immunoglobulin (Ig) super-family, mainly the B7 family including both activating signals, such as CD80/CD86 and inducible T cell co-stimulator ligand (ICOSL) on APCs binding CD28 and inducible T cell co-stimulator (ICOS) respectively on T cells, and inhibitory signals, such as CTLA-4, also interacting with CD80/CD86 (11); ii) Tumour necrosis factor (TNF) / TNF receptor (TNFR) family, such as CD40 on APCs binding CD40L on T cells, and iii) cell adhesion molecules, such as LFA-1, a β2 integrin on T cells, binding inter-cellular adhesion molecule-1 (ICAM-1) on endothelial cells and APCs mediating T cell migration to sites of inflammation (12, 13) (Fig. 1).

B7 family molecules

T helper (CD4+) cells recognise antigens presented by APCs via the interaction between the T cell receptor (TCR) and MHC class II molecules on APCs, but require a second signal, that is usually provided by CD28 interaction with CD80/CD86. In addition to conventional APCs, salivary gland (SG) epithelial cells in pSS may also function as APCs and induce the activation of immune cells via an aberrant expression of co-stimulatory molecules such as CD80/CD86 (14, 15).

Because the second signal is essential for T cell activation, the blockade of costimulatory molecules represents a valuable therapeutic option to control hyper activation of the immune system in pSS.

For example, anti-CD86 antibodies that block the interaction between CD28 and CD80/CD86 were shown to improve inflammation in lacrimal and SG of a pSS mouse model (NFS/sld mutant) (16). However, blocking CD80/86 also interferes with CTLA-4, a structural homologue of CD28, that antagonises CD28 and functions as a negative regulator of T cell activation (17,18). Additionally, CTLA-4 is abundantly expressed by regulatory T (Treg) cells, therefore strategies that block CD80/CD86 also lead to the impairment of Treg cells (19, 20). Accordingly, CTLA-4 blockade has been shown to exacerbate autoimmunity (21). Selective blockade of CD28 without affecting CTLA-4 is suggested as an effective strategy for modulating immune responses by preventing the maturation of pathogenic effectors while preserving the suppressive function of Tregs (22). In the context of pSS, adenoviral delivery of CTLA-4-Ig to submandibular glands to block CD80/CD86:CD28 interaction improved sialadenitis in a mouse model of pSS (23).

Like CD28, ICOS provides a positive T-cell activation signal and is specifically involved in the activation of T-cell dependent B-cell activation (11). Recently, ICOS and its ligand, ICOSL, together with CD28 signalling were identified as the most up-regulated pathways in pSS SG, in particular in the presence of ectopic GC (24). The development of functional GCs is an antigen-driven process relying on cognate interactions between Tfh cells and B cells, critically dependent on interactions between ICOS- ICOSL, CD40-CD40L, and the release of IL-21 (25). ICOS signalling activated through ICOSL ligation, aber-

rantly expressed on pSS SG epithelial cells (26), along with IL-6, support naive CD4+ T cell differentiation into Tfh cells *in vitro* (25). These findings imply a contributing role of SG epithelial cells to the observed expansion of Tfh cells in pSS peripheral blood and SG (27–30), with increased IL-21 production in both compartments sustaining B cell hyper-activation. A non-depleting, blocking anti-ICOS monoclonal antibody down-modulated IL-21, TNF-α, IL-6 and IL-8 in pSS SG organ culture experiments, supporting the critical role of ICOS-ICOSL pathway in the activation of T-cell related pro-inflammatory pathways (24).

TNF/TNF receptor family molecules

The CD40-CD40L costimulatory pathway is also critically involved in the generation of humoral immune responses to T-dependent antigens (31, 32). Its role has been widely investigated in systemic rheumatic diseases [reviewed in (33)] with pathogenic autoantibodies, including pSS (34, 35). CD40 is constitutively expressed on APCs but also on other non-immune cells such as SG ductal epithelial cells and endothelial cells that likewise show aberrant expression of other co-stimulatory molecules as already discussed for CD80/CD86, and therefore may function as APCs (36-38). Triggering of SG epithelial cells through CD40 ligation can upregulate adhesion molecules such as ICAM1 (38). CD40L upregulation by infiltrating SG T cells (38), or soluble CD40L (39), might sustain T cell activation in pSS through CD40-CD40L costimulatory pathway in secondary lymphoid tissue and SG tissue. Notably, blockade of CD40-CD40L pathway with anti-CD40L inhibited sialadenitis and reduced the numbers of infiltrating lymphocytes in the non-obese diabetic (NOD) mouse model of pSS (40, 41).

Given the multifaceted involvement of co-stimulatory and co-inhibitory pathways in autoimmune diseases in general and specifically in pSS, it is not surprising that these pathways provide several potential therapeutic targets. Below we describe current clinical trials which target some of these molecules.

Clinical efficacy, peripheral and salivary gland biomarkers modulation of abatacept in patients with pSS

Abatacept (CTLA-4-Ig) is a fully human co-stimulation modulator that selectively targets the CD80/CD86:CD28 co-stimulatory pathway (Fig. 1). Three small open-label studies suggested clinical efficacy of abatacept in pSS (42-44). Two studies assessed systemic disease activity, using the ESSDAI, and showed significant improvement (43, 44). Salivary flow rates were assessed in all three studies, showing either improvement or stabilisation (42-44). In the ASAP-II trial, multiple patient-reported outcomes were assessed and fatigue and health-related quality of life improved by abatacept (43).

Based on the promising results from open-label studies, two large RCTs with abatacept followed. In both studies the primary endpoint was a significant difference in ESSDAI score between abatacept and placebo arms. Disappointingly, both studies did not meet their primary endpoint, although significant biological effects (see below) and subtle changes in patient-reported outcomes, including sexual function, were observed (45, 46). Large response rates (50–60%) for the primary outcome were observed in the placebo-treated groups, suggesting that the ESSDAI is influenced by non-specific treatment effects. Because of acknowledged deficiencies in ESSDAI as a primary study endpoint (45, 47, 48) a composite endpoint is currently being developed for pSS within the NECESSITY consortium. Preliminary analyses show that abatacept is superior to placebo based on a composite endpoint (CRESS: composite of relevant endpoints for SS) that includes ESSDAI, ESSPRI, serology, and functional tests of the SGs and eyes (49). Ongoing validation of the composite endpoint, assessment of histologic outcomes and identification of responders to abatacept should provide further insights into the clinical effectiveness of this drug for treating (subgroups of) pSS patients.

Despite disappointing data from the larger randomised trials, abatacept treatment affects multiple peripheral biomarkers associated with disease activity in pSS. Serum IgG, free light chains (FLC), and RF levels decreased significantly after 24 and 48 weeks of treatment. The largest adjusted difference was seen for RF (43, 45, 46, 50). RFs consist mainly of RF-IgM, which are short-lived antibodies. Although IgM antibodies are often produced in a T cell-independent manner, survival of RF+ B cells and RF production are amplified by costimulatory signals from T cells (51, 52). Furthermore, precipitating RFs linked to immune complex formation have undergone somatic hypermutation (53, 54), which is considered to be a T cell-dependent process and presumably occurs during recall responses. These results indicate that T cell co-stimulation blockade with abatacept reduces T cell-dependent B cell hyperactivity and formation of RF-secreting B cells in particular. We speculate that the formation of pathogenic RFs by somatic hypermutation is also inhibited.

In addition to total IgG, FLC and RF, other relevant peripheral biomarkers affected by abatacept are circulating T follicular helper (cTfh) cells, peripherally-induced T regulatory (pTreg) cells and serum CXCL13 levels (46, 55). CXCL13 is a lymphoid chemokine critically involved in ectopic germinal centre formation in pSS SGs (10). CXCL13 is under normal circumstances involved in lymphocyte homing and positioning within follicles and is produced by follicular DCs, stromal cells, and GC-Tfh cells (56). In non-lymphoid tissues, T peripheral helper (Tph) cells are also a potential source of CXCL13 (57). In addition to a reduction in CXCL13, numbers and frequencies of cTfh cells were significantly lower and ICOS expression on these cells was diminished after 24 weeks of abatacept treatment (55). The decrease in ICOS expression on cTfh cells correlated with lowering of ESSDAI scores over time. Together with cTfh cells, pTreg cells were also reduced by abatacept, while Tph cells were not (55). Whether the decrease in pTreg cells implicates lower immune suppressive capacity is not known.

In line with results from the ASAP-II study, cross-sectional studies showed

that frequencies of activated cTfh cells correlate positively with ESSDAI scores (27, 58). Serum CXCL13 also correlate with ESSDAI scores, and with lymphoma development in pSS (59). Furthermore, serum CXCL13 closely correlates with immunopathological lesions in pSS SG including the inflammatory focus score and the presence of ectopic lymphoid structures (59, 60).

While multiple peripheral biomarkers are affected by abatacept, histopathologic effects seem rather limited. Adler and colleagues found a decrease in lymphocytic foci in labial gland tissue after 24 weeks of abatacept treatment, but this was not confirmed in parotid gland tissue by a post-hoc analysis of the ASAP-II trial (61). Despite a lack of change in the degree of infiltration in parotid glands, the number of germinal centres was reduced by abatacept (61). Glandular expression of ICOS was also significantly decreased, while IL-21 expression remained unchanged (55). A possible explanation for the modest effect of abatacept on glandular T cells is that effector Th cells, including Tfh cells and other IL-21-producing cells, in the inflamed glandular tissue are not fully dependent on CD28-mediated co-stimulation for their maintenance and proliferation. However, new formation of effector T cells from naïve precursors might be inhibited and this could be beneficial in particular at early stages of the disease. Ongoing histologic and transcriptomic evaluation of SG tissue collected from two recent RCTs will hopefully provide conclusive evidence on the glandular effects of abatacept in pSS.

Blockade of CD40/CD40L and ICOS/ICOSL co-stimulation: clinical efficacy and modulation of peripheral and salivary glands biomarkers

As described earlier in this review, CD40/CD40L (CD154) and ICOS/ICOSL interactions play a critical role in physiological T-cell dependent B-cell activation, immunoglobulin class-switching as well regulation of germinal centre responses in secondary lymphoid organs (62, 63). Both pathways are also upregulated in the peripheral and SG compartment of pSS patients likely contributing to pSS pathogenesis (24, 64-66). Two recent phase II randomised double blinded clinical trials blocking CD40/CD40L (NCT02291029) and ICOS/ICOSL (NCT02334306) costimulatory pathways have been completed in pSS patients reporting mixed results in terms of clinical response and amelioration of laboratory parameters of pSS immune cell dysregulation.

In the first trial, a phase II, 2:1 randomised double blinded clinical trial, which has been recently reported in full (67), iscalimab (CFZ533), a novel antagonistic and non-depleting anti-CD40 monoclonal antibody (Fig. 1) was tested in pSS patient with a baseline ESSDAI ≥6 either as multiple subcutaneous 3 mg/kg injections (cohort 1; n=12) or 10 mg/kg intravenous doses (cohort 2; n=32). While no improvement in ESSDAI was observed in cohort 1, a significant reduction in disease activity compared with placebo was reported in cohort 2, with a baseline-adjusted difference of 5.2 ESSDAI points at week 12, which is above the defined 3 points for minimal clinically important improvement (MCII) in ESSDAI (68). Of interest, even a greater difference (6.1 points) was observed in clinical ESSDAI (*i.e.* ESSDAI without the biological domain) at week 12 compared with placebo, suggesting that ESSDAI improvement with iscalimab was not primarily driven by a strong effect on biological parameters. Accordingly, a sub-analysis of individual ESSDAI domains revealed a significant reduction in some domains with the highest observed difference in the articular domain. There was, however, a marked imbalance in articular domain involvement between the groups at baseline. The lack of efficacy in the 3 mg/kg subcutaneous dosing regimen was dependent on substantially lower than expected drug plasma concentrations compared to pharmacokinetic data in healthy volunteers, suggesting significantly higher CD40 expression in pSS resulting in low target occupancy and below what would be required for efficient suppression of T-cell-dependent GC responses. Additional improvement in secondary endpoints was also observed including ESSPRI (but below the defined 1 point MCII), short-form (36) healthy survey, multidimensional fatigue inventory, patient and investigator assessments using VAS as well as some measurement of salivary and ocular exocrine function. In terms of biomarkers of B cell activation, RF significantly decreased in the iscalimab group whilst anti-SSA/Ro and anti-SSB/La IgG levels were only modestly affected. Although no assessment of SG immunopathology was reported, circulating CXCL13 was significantly and rapidly decreased after 10 mg/kg intravenous iscalimab treatment compared with placebo. This is of particular interest as serum CXCL13 closely correlates with immunopathologic features of pSS, as previously discussed (60). Intriguingly, while biological effects of abatacept and iscalimab show strong similarities, the clinical efficacy of abatacept seems inferior. Whether this is due to the difference in drug target or differences between study populations (*e.g.* proportion of patients with articular involvement), remains to be investigated.

In the second trial, a phase IIa, 1:1 randomised, placebo-controlled study, which has only been reported in abstract form (69), prezalumab (AMG557/MEDI5872), a blocking non-depleting anti-ICOSL monoclonal antibody (Figure 1), was administered at 210mg to 32 pSS patients (original recruitment target n=42) once weekly for 3 weeks, then every 2 weeks for 9 weeks. ESSDAI ≥6 and serologic evidence of B cell hyperactivity (defined as IgG≥16 g/L or RF positivity) were the main inclusion criteria for this study. The mean ESSDAI score at day 99 decreased from 11.8 to 8.0 in the prezalumab group, thus on average above the 3 points MCII for ESSDAI, and by 2.3 points in the placebo group but this difference was not statistically significant, with the trial failing its primary endpoint. In terms of peripheral biomarkers of B cell activation, IgM-, IgG- and IgA-RF levels all decreased significantly with MEDI5872 but not with placebo suggesting that the dose used in this study achieved biological efficacy. This was confirmed in repeated SG biopsies before and after treatment whereby a significant differ-

ence in the number of infiltrating $CD4^+$/$ICOS^+$ Tfh-like cells was observed at day 99 in the prezalumab compared to the placebo group, an effect due to both reduction in the treatment group and increase in the placebo group of this cell subset. Conversely, no difference in the $PD1^+$/$ICOS^+$ Tfh-like cell subsets or in plasma cells was detectable in SG tissue after treatment. While blocking ICOSL has yielded disappointing results, it might be possible that direct targeting of ICOS together with additional co-stimulatory molecules on T cells or in combination with direct B cell targeting (70), may provide superior clinical and biological efficacy. In this regard, promising early data in pSS patients, albeit limited to *in vitro* experiments on peripheral blood mononuclear cells, indicated a strong biological response with down-modulation of key inflammatory patients upon incubation with ALPN-101, a dual CD28/ICOS antagonist (71).

Conclusions

Recent improvements in our understanding of the function of several T cell co-stimulatory and co-inhibitory molecules within the normal immune response but also in the pathogenesis of pSS provided new therapeutic opportunities for a disease that still lacks a definitive immunomodulatory treatment. Clinical trials with drugs targeting different co-stimulatory molecules have provided unique opportunities to further elucidate the pathogenic mechanisms of pSS. However, the discrepancy observed in several RCTs between evident biological effects of blocking T cell co-stimulation and the mixed and often disappointing clinical efficacy, advocated further efforts in defining better clinical outcomes, understanding the heterogeneity of the clinical phenotypes, identifying biomarkers of disease response and resistance and devising strategies for careful patient selection and stratification to maximise potential benefits.

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