Regulatory immune cells: a review of the novel paradigm of primary Sjogren's syndrome

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ABSTRACT

Primary Sjögren's Syndrome (pSS) is an autoimmune disease that mostly affects women. Patients with pSS experience dry mouth and eyes in addition to signs of systemic disease. pSS was considered a Th1 autoimmune disease for many years. However, in various studies, it has been shown that dysregulation of regulatory cells play critical role in the pathogenesis of the disease. This review focuses on studies supporting this view and answers questions about the role of regulatory cells in the pathogenesis of pSS.

Keywords: Arthritis, rheumatoid, regulatory T-cells, regulatory B-cells, primary Sjogren's syndrome, follicular regulatory T-cells

INTRODUCTION

Primary Sjögren's syndrome (pSS) is an exocrinopathy that is associated with lymphocyte infiltration in exocrine glands which results in progressive inflammation and tissue destruction. The glands generally affected are the lacrimal glands and salivary glands. However, lung, heart, kidney , nervous system, and lymphatic systems may be affected as part of extra-glandular pattern (1). Environmental factors, especially viruses, activate glandular vascular endothelial cells, the glandular epithelial cells and dendritic cells (DCs) in the stromal area. Along with the activation of DCs, type I and type II IFN pathways are activated and further IL-12 production results in IFN-gamma production by NK and TH1 cells (2). Vascular endothelial cells also secrete nitric oxide (NO) which decrease the secretion of exocrine glands (3). As a result of decrease in secretions, clinical ocular symptoms (keratoconjunctivitis sicca) and oral symptoms (dry mouth) are seen in pSS. There is no etiology directed treatment of pSS for today. Symptomatic therapies such as glucocorticoids, cyclophosphamide, conventional disease-modifying antirheumatic drugs (DMARDs), rituximab make up the backbone of therapy (4).

Disease mechanism through IFN-gamma pathway is also supported by genetic, epigenetic and immunological alterations. Interaction of IFN-gamma pathway with both innate and acquired immunity takes shape via the B lymphocytes. In addition to B lymphocyte custody, T cells also contribute to immunopathogenesis of pSS. Recent studies have shown that regulatory cells have critical functions in the evolution of pSS pathology as effector immune cells (2,3-5). In this review we summarized the functions and proofs of regulatory cells and in pSS.

REGULATORY B CELLS

B cells take part a major place in the pSS pathogenesis by production of autoantibodies, cytokines which cause apoptosis in epithelial cells in exocrine glands (3). Regulatory B (Breg) cells are immunosuppressive cells that promote immunological tolerance. Breg cells suppress immunopathology by inhibiting the expansion of helper T cells, and another proinflammatory lymphocytes through the production of interleukin-10 (IL-10), IL-35 and transforming growth factor β (TGF-β) (3,6). The suppression mechanisms of Breg cells are shown in **Figure 1**. Interleukin 10 (IL-10) production classically defines a Breg, but the stability and/or plasticity of this population is not yet well understood by studies. It is known that more studies are needed to understand Breg plasticity (7).
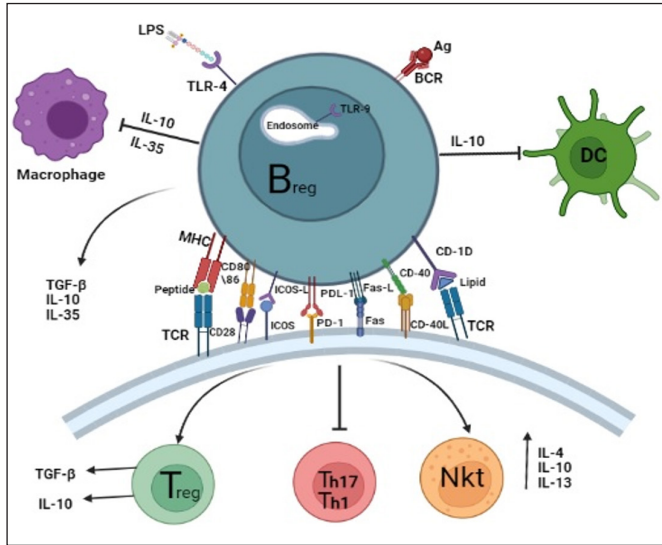
Figure 1. Breg cells suppression mechanisms

Breg cells form 0.2 to 0.6% of B cells and this ratio reveals that its regulatory properties are quite strong. The phenotype of Bregs is CD19+CD24hiCD38hi. Three different types of Bregs identified in pSS. IL-10-producing Bregs which suppress Th17 and Tfh cells augmenting the autoimmune inflammation are found to be decreased in pSS (2). GrB-producing Bregs that cause apoptosis in target cell via perforin, are increased in pSS. IL-35 producing Bregs act as IL-10 producing Bregs that both suppress Th1 and Th17 and also induce Tregs. It is not clear whether IL-35 producing Bregs increased or decreased in pSS.

Various studies have shown that regulatory B cells are effective in the pathogenesis of pSS and in disease control. In one study, the percentage of regulatory B cell subsets showed significant differences in pSS patients with active and inactive disease. CD19+CD24hiCD38hiIL-10+ cells were found to be strikingly higher in the whole pSS group crosschecked to healthy controls which suggests that they are effective in disease regulation and pathogenesis (8). In another study 21 pSS patients with low disease activity and 21 healthy controls were compared and IL-10 producing regulatory B cell frequency is not decreased and these cells are functional (9). There is opposite findings that do not support this view, for example Lin et al. (10) showed that CD24hiCD38hi Breg cells from pSS patients could not effectively suppress follicular helper T cell differentiation due to reduced IL-10 production, arguing that Bregs are dysfunctional. The reason for the ineffectiveness of the regulatory properties was affirmed that the selected patients were inactive patients and used various immunosuppressive drugs. Overall, it can be resulted in that the levels of regulatory B cell phenotypes studied are different in patients with clinically inactive pSS and active pSS. All these results suggest that Breg regulatory functions are important in pSS progress.

Currently approved treatments of pSS is mainly symptomatic treatment and the treatment of organ involvement are antirheumatic drugs or targeted therapies adapted from other rheumatologic diseases. Earlier studies on pSS focusing on targeting the B cells have been reported to fail in efficacy but recent phase IIb study of anti-B cell-activating factor (BAFF) receptor antibody ianalumab showed positive findings in subjects with moderate to severe pSS (11). BAFF has an inhibitor role on Bregs (12). BAFF inhibition inversely has potential to influence the effector B cells negatively, and Bregs cell function positively, thus, while autoantibodies secreted by effector B cells would decrease, immunsupression by Bregs will increase which results in a double hit to autoimmune pSS.

REGULATORY T CELLS

Regulatory T cells (Tregs), were originally identified by the high surface expression of the alpha chain of the IL-2 receptor (IL-2Rα, CD25) and contain forkhead box protein P3 (FoxP3) transcriptional factor as a marker. The phenotype of Tregs is CD4+CD25hiFOXP3+. Their ability to inhibit autoimmunity originates from suppressive activity against autoreactive lymphocytes through cell-to-cell contact or the soluble mediators including IL-10 and transforming growth factor β (TGF-β) (13). Most of the peripheral Treg cells originate from the thymus and these cells are called "thymus-derived Treg (tTreg)" cells (14-17). In addition, Treg cells can differentiate from naive CD4+ T cells in the periphery, and these Treg cells are called "peripherally derived Treg (pTreg)" cells. Also, it can differentiate after stimulation in the presence of TGF-β and IL-2 in the periphery in vitro and these cells are called "iTreg cells" (18,19). FoxP3 expression plays an important role in Treg plasticity. it is also known that intracellular metabolites and metabolic pathways regulate the expression of Foxp3 and the functional plasticity of Treg cells. (20-22).

The immunopathogenesis of pSS has not yet been fully clarified. There are many studies on the role of Tregs in the pathogenesis of pSS. In one study of pSS patients, Tregs were investigated by immunohistochemical staining (IHC) from lip salivary gland and by flow cytometric assay from peripheric blood. Also mRNA expression of Foxp3 is analysed by real-time polymerase chain reaction (rt-PCR) in salivary gland tissue. At result Tregs relative expresions in peripheric blood and Foxp3 genetic expression in salivary gland tissues of patients with pSS were significantly decreased when compared with healthy controls (23). Other studies also confirmed these findings (24,25). Also, there are contrasting studies which showed that

circulating Tregs were increased (26,27) or remained similar in pSS when compared to healthy controls (8,28,29). Alunna et al. (30), found higher relative expression of CD4+CD25lowGITR+ cells in milder Sjögren's syndrome patients than in healthy controls. Clonal expansion of this subset of Treg cells attenuated the activity of effector T cells that results in milder disease. Christodoulou et al. (28) reported also Treg frequency in salivary gland tissue positively correlated with disease inflammation grade. In another study, CD161+CD25+CD4+ Treg subpopulation, which have regulatory properties with IL-17 production was increased when compared with healthy controls. Furthermore, the function of this regulatory subset in SS patients is related to the clinical severity of the pathogenesis of pSS (31).

Studies have shown that Treg cells help in the regulation of pathogenesis in pSS patients. It is thought that the presence of these cells may help the development of new diagnostic techniques or treatment methods in pSS patients.

FOLLICULAR REGULATORY T CELLS (TFR)

Lately defined follicular regulatory T (Tfr) cells, a subset of Treg cells, are known to control the function of T follicular cells and Germinal Center (GC) reactions by regulating T follicular helper (Tfh) cell mediated B cell responses after antigenic exposure. Tfr cells inhibit B cells via CTLA4, an inflammatory cytokine producion via Tfh cells (32). Tfr cells have the properties of Treg cells, regulating Germinal Center responses and antibody production. The regulating Germinal Center responses and antibody production shown schematically in **Figure 2**. They are identified as FoxP3+ CD4+ T cells that express chemokine receptor CXCR5 (33,34). Tfr cells have lower expression of CXCR5 when compared to the Tfh cells (32).
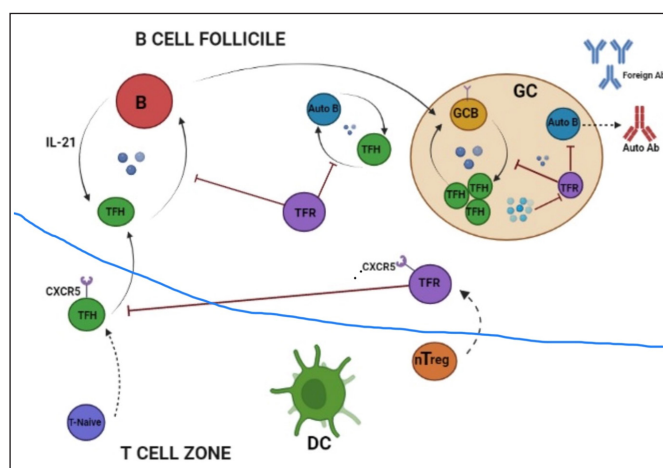

Figure 2. Schematic view of regulating Germinal Center responses and antibody production.

CXCR5 expression on Tregs is dependent on Bcl-6. These CXCR5(+) Bcl-6(+) T(reg) cells are absent in the thymus, but CXCR5(-) Foxp3(+) can be regenerated from natural T(reg) precursors. (33-35) Upregulation of CXCR5 results in TFH cells and mature TFR allows migration to germinal centers. Also, Tfr cells can migrate to GC independently of CXCR5. Tfr cells that migrate to the germinal center become equipped to regulate larger germinal center reactions, including affinity maturation of antibodies and differentiation of plasma cells (36,37). It is reported that humoral immunesuppression capacity of Tfr was inferior compared to conventional Treg cells (38).

Fonseca et al. (39) reported increased levels of Tfr cells and Tfr/Tfh ratio in peripheral blood of pSS subjects. In another study Fonseca et al. also reported strong correlation between circulating Tfr cells and lymphocytic infiltration in minor salivary glands of pSS subjects. They stated that the ratio of cTfr/cTfh as a marker for the diagnosis of pSS could be a biomarker for the diagnosis of pSS (40). Contrary to Fonseca's study Verstappen et al. (41) found circulating Tfr/Tfh ratio was increased in pSS patients but this increase was not associated with gland inflammation. Kim et al. (42) investigated subsets of Tfh and Tfr cells in the blood and relation of these subsets with disease activity, glandular inflammation, and autoantibody responses in 18 pSS patients compared to HCs. They found that blood Tfr and Tfh cell ratios were increased in pSS patients compared to HCs. These data prove the presence of Tfhr cells with regulatory functions in the peripheral blood and salivary gland tissues of pSS subjects. Nevertheless, the role of Tfr cells in the pathogenesis of pSS is still controversial. However, the functional capabilities of Tfr cells in SG tissue and peripheral blood requires more research.

CONCLUSION

In the pathogenesis of pSS, damage to salivary and lacrimal gland epithelium results in pro-inflammatory cytokine secretion, organised B and T cell infiltration. Autoreactivity exocrine gland antigens drives more T cell activation and many inflammatory cytokines which trigger more T cells and B cells. Increased B cell activation and autoantibody production causes progressive tissue damage. This cycle of activation and reaction that repeats itself over and over again. Regulatory cells are critical brakes for this endless activation mechanisms. Tregs, Bregs and fTr cells control the overactivated immune response. Dysregulation or deficiency in Treg cells expansion and differentiation contribute Th1-like or Th17 inflammatory phenotype. It has been shown that the density of Treg cells in the blood and exocrine glands of pSS patients depends on the disease activity,

and the disease activity increases in cases where the density is low or Treg cells are dysfunctional. The Tfr cells as a subset of Tregs, extend the suppression effect of Tregs into GC. They are antagonistic to Tfh cells that additionally inhibit autoantibody production, somatic hypermutation, and class switch recombination of B cells. Bregs attenuate cytokine production from monocytes and T cells. They inhibit Th1, Th17 and CD8+T cell responses, transfom naive CD4+ cells into Tregs and suppress inflammation by soluble mediators IL-10, TGF-β and IL-35.

It is important to systematically evaluate the correlation among the count of circulating Treg, Breg and Tfr cells and markers of the diagnosis, severity of disease, treatment efficacy, and prognosis of pSS. Also, the correlation among circulating - tissue regulatory cells should be clarified in pSS. Phenotypically and functional distinct regulatory cells cooperate in pSS in order to limit inflammation and overbuild immune homeostasis.

ETHICAL DECLARATIONS

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