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REVIEW ARTICLE

Unique Phenotypes and Functions of Follicular Helper T Cells and Regulatory T Cells in Sjögren's Syndrome

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Abstract: Sjögren's syndrome (SS) is a T cell-mediated autoimmune disease of the systemic exocrine glands, such as salivary and lacrimal glands. A variety of T-cell subpopulations maintain immune tolerance in the thymus and periphery through complex immune responses including cellular and humoral immunity. The T-cell subpopulations exhibiting abnormal or unique phenotypes and impaired functionality have been reported to play important roles in the cellular mechanisms of autoimmunity in SS patients and animal models of SS. In this review, we focused on follicular helper T cells related to antibody production and regulatory T cells to control immune tolerance in the pathogenesis of SS. The unique roles of these T-cell subpopulations in the process of the onset or development of SS have been demonstrated in this review of recent publications. The clinical application of these T-cell subpopulations will be helpful for the development of new techniques for diagnosis or treatment of SS in the future.

Keywords: Autoimmunity, follicular helper T cell, regulatory T cell, Sjögren's syndrome, phenotypes, exocrine.

1. INTRODUCTION

Sjögren's Syndrome (SS) is an autoimmune disease that targets exocrine glands, such as lacrimal and salivary glands. The primary clinical symptoms are sicca syndrome, including dry eyes and mouth [1, 2]. In addition, SS often accompanies other systemic autoimmune diseases, such as rheumatoid arthritis and lupus [3, 4]. Because SS has a complex pathogenesis, fundamental treatments for this disease have not yet been established [5].

SS is generally considered to be a T cell-mediated autoimmune disorder of the salivary and lacrimal glands. Several autoantigens in SS in humans and in mouse models of SS have been reported [6-9], and the relationship between autoreactive T cells and autoantigens in SS has been described. Moreover, anti-SSA and anti-SSB autoantibodies are widely known to be clinically useful autoantibodies in SS [10]. It appears that T- and B-cell responses during the onset or development of SS are differentially and intricately regulated by the interaction and communication of a variety of immune cells.

Follicular helper T (Tfh) cells are specialized providers of T cell help to B cells and are essential for Germinal Center (GC) formation, affinity maturation, and production of high-affinity antibodies [11-13]. Many studies have demonstrated that Tfh cells influence various immune responses, including autoimmunity and infection, in addition to other T-helper cell subsets [14, 15]. Tfh cells are increased in the peripheral blood and target organs in SS patients, together with enhanced memory B and GCB cells [16-19]. Therefore, Tfh cells have been highlighted for understanding the pathogenesis of SS.

Regulatory T (T_{reg}) cells are one of the central players in complex immune responses required to maintain immune homeostasis [20-22]. In particular, increasing evidence suggests that T_{reg} cells can suppress and control the autoimmune response to protect the body from autoimmune diseases in humans and in animal models [23-25]. It is also well known that T_{reg} cells play potent roles in the onset and development of SS [26, 27].

In this review focusing on the pathogenesis of SS, the significant involvement of Tfh and T_{reg} cells is highlighted to understand the precise molecular mechanisms of the pathogenesis of this disease.

2. ABNORMAL T-HELPER CELL SUBSETS IN SS

$CD4^+$ T helper cells play a crucial role in the pathogenesis of SS (Fig. **1**). A large population of $CD4^+$ T cells and small numbers of B cells, $CD8^+$ T cells, macrophages, and dendritic cells infiltrate the target organs, such as salivary and lacrimal glands, during the early stages of SS [28, 29]. With aging and disease progression, the infiltration of B cells or plasma cells increases in the autoimmune lesions. The

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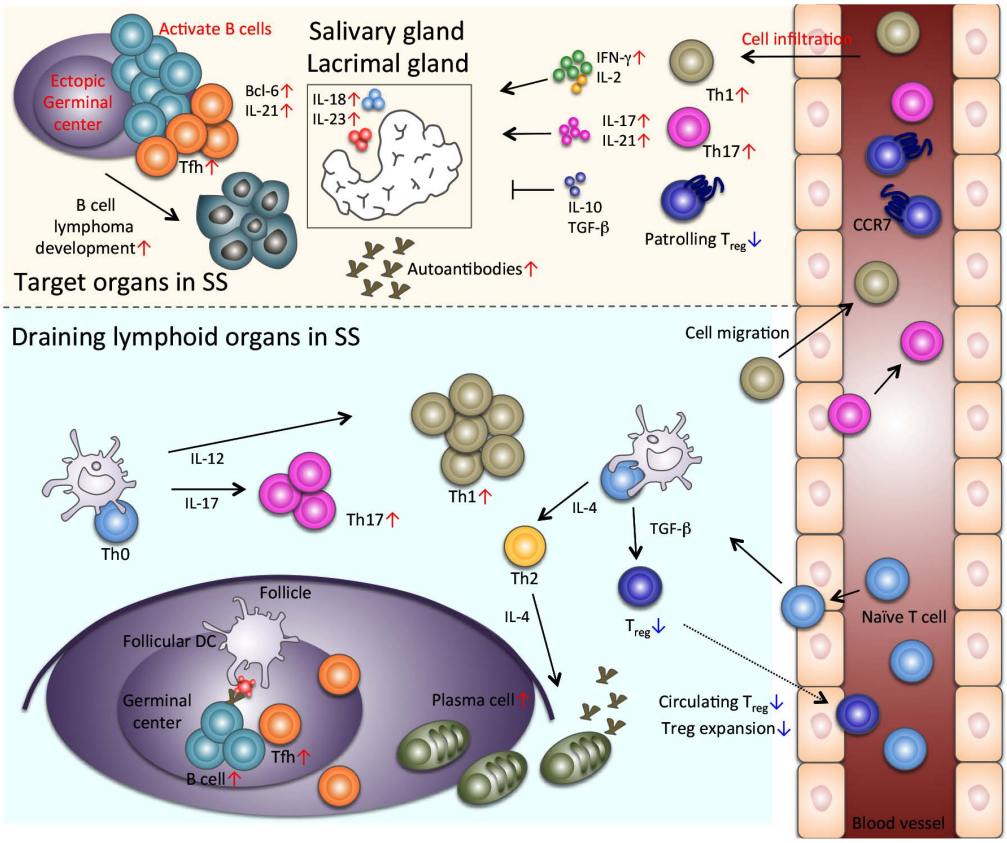

Fig. (1). Tfh cells and T_{reg} cells in SS. Tfh cells are found in ectopic GC of autoimmune lesions in SS. Increased infiltrates of Th1 and Th17 cells are detected in the target tissues of SS. Patrolling T_{reg} cells are significantly decreased in the target organs. In the draining lymphoid tissues of SS, Th1 and Th2 cells are increased. Expansion of T_{reg} cells is impaired, and circulating T_{reg} cells are reduced in SS.

majority of infiltrating $CD4^+$ T cells exhibits a memory and/or activated phenotype in the salivary glands of SS patients [30-32]. The pathogenesis of SS is mediated by the Th1 derived responses [33, 34]. Moreover, elevated numbers of IFN-γ positive $CD4^+$ T cells are detected in the salivary glands of SS patients, and intracellular cytokine analysis demonstrated the polarization of a Th1 phenotype [35]. Furthermore, interleukin (IL)-2 and IFN-γ are consistently detected in the target tissues form SS patients [36], whereas IL-4 and IL-5 are only detected in patients with high levels of B-cell accumulation in the salivary glands [37]. Several studies have evaluated cytokine profiles produced by a variety of cell types in SS patients. Among them, IL-10, IL-6, and Transforming Growth Factor beta (TGF-β) are also consistently detected in all patients, whereas IL-12 mRNA is only detected in some of the patients [38, 39]. Further, many reports demonstrated that the pathogenesis of SS in animal models is associated with Th1 cytokine-producing cells [38, 39].

Th17 cells are characterized by production of the proinflammatory cytokine, IL-17 and have been implicated in various immune responses, such as autoimmunity [40-44]. Th17 cells are observed in the autoimmune lesions of the salivary gland tissues of SS patients [27, 45]. In addition, IL-17 levels are significantly elevated in the sera of SS patients compared with that of the controls [46, 47]. Because Th17 cells also can produce IFN-γ, Th17 cells seem to play a critical role in the IFN-γ-mediated pathogenesis of SS. Furthermore, IL-18 and IL-23 produced by salivary epithelial cells also contribute to the pathogenesis of SS, activating or regulating Th17 cells [45].

3. TFH CELLS IN SS

Recently, Tfh cells have been identified as a $CD4^+$ T-cell subset capable of activating B cells in the lymphoid organs [11-14]. Tfh cells play a crucial role in the formation and maintenance of the GCs of secondary lymphoid organs and the regulation of B-cell differentiation of memory B cells and plasma cells [11-14]. Tfh cells may also contribute to B-cell activation, as a hallmark of SS is the frequent association of Tfh cells with autoantibody secretion (Fig. **1**). In addition, GC formation may be of critical importance for further clarification of the disease pathogenesis (Fig. **1**).

Tfh cells highly express the CXC Chemokine Receptor 5 (CXCR5), which is critical for homing and signaling [14, 48]. Moreover, the phenotype of Tfh cells includes the expression of the surface receptor inducible T cell co-stimulator and programmed cell death protein 1 (PD-1) as well as the nuclear transcriptional repressor B-cell lymphoma 6 (Bcl-6) [14, 49]. In addition, IL-21 is another key cytokine produced by Tfh cells [50]. IL-21 plays an important role in B-cell differentiation and antibody production, in combination with other cytokines [14].

T cell-mediated autoimmune diseases have been well studied and demonstrated using several SS animal models and patients [33, 51-53]. However, it is unclear whether the B cell-dependent mechanisms contribute to the onset of auto-

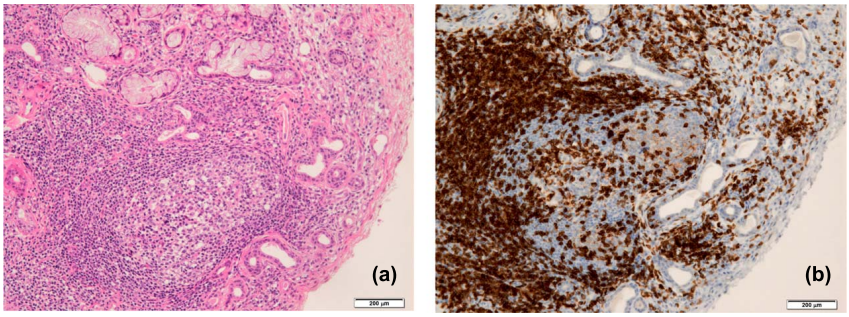

Fig. (2). Ectopic GC formation in the salivary gland tissue from SS patients. (**a**) Inflammatory lesions including CG in the lip biopsy tissue from a SS patient is shown by histological staining with hematoxylin and eosin. A lot of lymphocytes infiltrate extensively in the salivary gland tissue with destruction of acinar cells. (**b**) $CD3^+$ T cells in lip biopsy tissue from a SS patient are shown by immunohistochemistry. Scale bar: 200 μm.

immunity. Thus, there is little evidence for understanding the pathogenesis of autoimmune diseases *via* B cell-mediated mechanisms. However, autoantibodies from different autoimmune diseases are probably related to the severity or symptoms of the disease [54, 55]. In this context, Tfh cells play an important role in the B-cell autoimmune responses. The presence of peripheral Tfh cells is one of the biomarkers of autoimmune diseases, such as myasthenia gravis, autoimmune thyroiditis, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, type 1 diabetes, inflammatory bowel disease, and SS in both humans and animal models [17, 56-63].

The ectopic GC formation is observed in the salivary gland tissues of SS patients by histological analysis (Fig. **2a**). $CD3^+$ T cells including Tfh cells infiltrate within GC in addition to the accumulation out side GC in salivary gland tissue from SS patients (Fig. **2b**). Ectopic GC formation has been associated with development and outcome of B cell lymphoma [64-66]. In addition, a previous study demonstrated that a large number of Tfh cells were detected in the peripheral blood of SS patients at the time of disease onset, with aberrations of serum anti-Ro/SSA and anti-La/SSB levels. Moreover, $CD4^+CXCR5^+$Tfh cells are significantly elevated in the salivary gland tissues and peripheral blood of SS patients, together with aberrant B cells and plasma cells. This suggests that $CD4^+CXCR5^+$Tfh cells contribute to the pathogenesis of SS by promoting the maturation of B cells [61].

IL-21 is a key regulator of B-cell activation and is primarily secreted by Tfh cells. Previous reports have indicated that the number of Tfh cells is significantly increased in the peripheral blood and that the expression of the IL-21/IL-21 receptor is elevated in the salivary glands of SS patients [17, 67]. Other studies have also suggested that IL-21 plays a pathogenic role in the onset or development of other autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis [68-70]. On the other hand, salivary gland epithelial cells are capable of promoting Tfh-cell differentiation and IL-21 secretion through the production of IL-6 and inducible T cell co-stimulator ligand expression [71]. Increased serum IL-21 levels in SS patients are associated with systemic disease activity [72]. Furthermore, *IL-21* and *IL-21 receptor* gene polymorphisms are associated with an increased susceptibility to several autoimmune diseases [73-76].

Bcl-6 expression in T cells has been reported to be essential for the formation of Tfh and GC B cells [14, 49]. Recent studies have described the mRNA expression levels of *Bcl-6* to be significantly higher in ectopic GC of the salivary gland tissues from SS patients [77]. In addition to CXCR5, CD84 and PD-1 expression were also detected on infiltrating lymphocytes in the salivary gland tissues of SS patients [77].

4. T_{reg} CELLS IN SS

T_{reg} cells are a unique subset of T cells that play an important role in the maintenance of immune tolerance [78, 79]. The expression of the transcription factor forkhead box p3 (Foxp3) is the genetic hallmark of T_{reg} cells [80, 81]. Moreover, naturally occuring T_{reg} (nT_{reg}) cells arise as a discrete and largely stable lineage in the thymus [21, 82]. The nT_{reg} subset exhibits a T-cell Receptor (TCR) repertoire that is distinct from those of $Foxp3^-$conventional T cells and induced T_{reg} (iT_{reg}) cells [83]. In contrast to nT_{reg} cells, iT_{reg} cells can be formed from naïve $CD4^+$ T cells in the presence of TGF-β and IL-2 outside the thymus [79, 84]. Studies using animal models have demonstrated that the adoptive transfer of iT_{reg} cells generated from naïve T cells can prevent the onset of autoimmune diseases [85-87]. Thus, the number and function of T_{reg} cells, including nT_{reg} and iT_{reg} cells, are maintained in our body to prevent and control the breakdown of immunological tolerance (Fig. **1**).

A simple animal model of Inflammatory Bowel Disease (IBD) has been well characterized by the adoptive transfer of $CD25^-$ naïve T cells into lymphopenic mice, such as recombination-activating $gene^{-/-}$, severe combined immunodeficiency, or irradiated mice [88, 89]. Considerable evidence suggests that an altered balance between T_{reg} cells and T effector cells in the intestinal microenvironment contributes to the onset or development of IBD [90, 91]. In addition, several studies have shown that T_{reg} cells are also present within non-lymphoid sites in the periphery, including autoimmune lesions, infectious sites, and tumor tissues [92-94]. Depletion of T_{reg} cells from normal mice by injecting anti-CD25 antibodies can induce the spontaneous development of various autoimmune lesions [95-97]. Tissue-resident T_{reg} cells in non-lymphoid sites other than peripherally circulating T_{reg} cells also contribute to the maintenance of local immune tolerance.

The numbers of peripheral T_{reg} cells in SS patients is significantly reduced compared with healthy controls [98]. In

contrast, it was reported that $CD4^+CD25^{high}$ T_{reg} cells are not impaired in primary SS patients based on experiments involving an *in vitro* suppression assay [99]. In addition, the frequency of $Foxp3^+$ T_{reg} cells in the salivary gland tissues from SS patients correlates with the inflammation grade and certain risk factors for the development of lymphoma [26].

We previously established a murine model for SS in NFS/*sld* mutant mice that were thymectomized 3 days after birth [6, 100]. Neonatal thymectomy in certain strains of mice results in the spontaneous development of inflammatory lesions resembling human autoimmune diseases in the thyroid gland, ovaries, kidneys, testes, and stomach [101-106]. The ratio of T_{reg} cells to effector T cells in the SS model was significantly lower than that of control mice [107]. In addition, the *in vitro* induction of iT_{reg} cells by TGF-β using naïve T cells from the SS mouse model was severely impaired [107]. Moreover, T_{reg} cells derived from mice with SS exhibited an IFN-γ-producing Th1-like phenotype [107]. An *in vivo* transfer of T_{reg} cells from the SS model was not sufficient to provide protection from the onset of autoimmune lesions in the murine model of SS [107]. These findings suggest that the abnormal expansion, differentiation, and inflammatory cytokines producing by T_{reg} cells contribute to the pathogenesis of SS (Fig. **1**).

C-C-chemokine receptor 7 (CCR7)-deficient mice have been well characterized as one of the models of SS [108]. Autoimmune lesions are observed in the lacrimal and salivary glands in $CCR7^{-/-}$ mice, similar to that observed in SS [108]. The enhanced immune response observed in $CCR7^{-/-}$ mice is caused by the defective lymph node positioning of T_{reg} cells and consequent suppressor function impairment of the T_{reg} cells [109]. In addition, our study demonstrated that CCR7 particularly controls the patrolling functions of Treg cells by regulating their migration into target organs [93]. Furthermore, we found that the migratory function of *CCR*KO T_{reg} cells was impaired in response to sphingosine 1-phosphate (S1P), suggesting that CCR7 participates in the molecular mechanism underlying the migratory function of peripheral T_{reg} cells through S1P and one of its receptors, $S1P_1$ [110].

The impaired migratory response of $CCR7^{-/-}$ T_{reg} cells in response to S1P occurs because of a defective association between $S1P_1$ and a G-coupled protein [110]. In addition, TCR- and $S1P_1$-mediated Ras-related C3 botulinum toxin substrate 1, extracellular signal-related kinase, and c-Jun phosphorylation required for activator protein 1 (AP-1) transcriptional activity were significantly impaired in $CCR7^{-/-}$ T_{reg} cells [110]. We also detected an abnormal nuclear localization of Foxp3 following the abrogation of c-Jun and Foxp3 interaction in the nucleus of *CCR7*KO T_{reg} cells [110]. These results indicate that CCR7 controls the migratory function of T_{reg} cells through $S1P_1$-mediated AP-1 signaling. This pathway is regulated through the interaction of CCR7 with Foxp3 in the nucleus, thereby protecting the body from autoimmunity. Moreover, the histopathological findings of the salivary gland tissues from SS patients revealed that the number of $CCR7^+Foxp3^+$ patrolling T_{reg} cells in the healthy control samples was significantly increased compared with SS patients [93]. This finding indicates that $CCR7^+$ T_{reg} cells patrol within the target organs of the salivary and lacrimal glands to protect against autoimmune lesions.

Recently, it has been reported that an IL-17-producing $CD161^+CD25^-CD4^+$ T-cell subpopulation as effector cells and a $CD161^+CD25^+CD4^+$ T-cell subpopulation as regulatory cells in peripheral blood mononuclear cells (PBMCs) from SS patients are related to the clinical severity of the pathogenesis of SS [111]. Compared with healthy controls, a significant increase in the number of $CD161^+CD25^+CD4^+$ T cells was also observed in PBMCs from SS patients [111]. In addition, the function of this unique regulatory cell population in SS patients is more impaired than that of $CD161^-CD25^+CD4^+$ T_{reg} cells [111].

5. RELATIONSHIP BETWEEN TFH AND T_{reg} CELLS

Although IL-2 inhibits the differentiation and development of Tfh cells, the differentiation, maintenance, and function of T_{reg} cells are promoted by IL-2 signaling [112, 113]. However, CXCR5-expressing T follicular regulatory cells control Tfh and GC B cell responses [114-116]. In addition, the depletion of Treg cells impairs the differentiation of influenza virus-specific Tfh cells [117]. T_{reg} cells may promote antigen-specific GC responses by controlling excessive IL-2 signaling, which inhibits the differentiation of Tfh cells. However, direct evidence of the relationship between Tfh and T_{reg} cells remains to be defined. In particular, it is unclear whether the direct or indirect interaction between Tfh and T_{reg} cells influences the onset or development of autoimmune diseases, such as SS.

CONCLUSION

SS is caused by multiple factors via the complex interaction between the immune system and the target organs. Although the precise mechanisms of the onset or development of SS remain unclear, the phenotypes or functions of Tfh and T_{reg} cells in human SS patients and animal models of SS are abnormal or unique (Fig. **1**). As these cells contribute to the regulation of SS pathogenesis, the clinical defection or manipulation of their cells would be expected to aid in the development of new diagnosis techniques or treatment strategies for SS.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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