T helper subsets in Sjögren’s syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review

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Abstract

IgG4-related disease (IgG4-RD) is a systemic disease characterized by the elevation of serum IgG4 and infiltration of IgG4-positive plasma cells in multiple target organs, including the pancreas, kidney, biliary tract and salivary glands. In contrast, Mikulicz’s disease (MD) has been considered a subtype of Sjögren’s syndrome (SS) based on histopathological similarities. However, it is now recognized that MD is an IgG4-RD distinguishable from SS and called as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). Regarding immunological aspects, it is generally accepted that CD4+ T helper (Th) cells play a crucial role in the pathogenesis of SS. Since it is well known that IgG4 is induced by Th2 cytokines such as interleukin (IL)-4 and IL-13, IgG4-DS is speculated to be a unique inflammatory disorder characterized by Th2 immune reactions. However, the involvement of Th cells in the pathogenesis of IgG4-DS remains to be clarified. Exploring the role of Th cell subsets in IgG4-DS is a highly promising field of investigation. In this review, we focus on the selective localization and respective functions of Th cell subsets and discuss the differences between SS and IgG4-DS to clarify the pathogenic mechanisms of these diseases.

Keywords: T helper subset, IgG4-related disease, IgG4-related dacryoadenitis and sialoadenitis, Sjögren’s syndrome, Cytokine

Sjögren’s syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands with concomitant autoantibody production and destruction of the glandular tissue. Patients typically experience symptoms of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). Because of its characteristic lymphocytic infiltration and destruction of the salivary and lacrimal glands, SS is considered to be an ideal disease for studying patterns of cytokine production at the site of organ-specific autoimmune damage [1]. SS occurs alone as primary SS, or as secondary SS when underlying other connective tissue diseases [2]. Immunohistochemical studies demonstrated that the salivary glands are predominantly infiltrated by CD4+ T helper (Th) cells at an early stage of SS, and these cells are therefore thought to play a crucial role in the induction and/or maintenance of the disease [3]. In advanced stage, B cells predominate and these infiltration extends to occupy the acinar epithelium and further progress to hypergammaglobulinemia and B cell lymphoma [4]. Recent studies have suggested a central role of the epithelium in orchestrating the immune reaction by expressing HLA antigens, adhesion and costimulatory molecules, cytokines, and chemokines. Therefore, SS has been proposed as an etiological term “autoimmune epithelitis” [4–7], and it is of interest to examine the involvement of interaction between CD4+ Th cells and the epithelium in the initiation and progression of the disease process. Th cell populations comprise functionally distinct subsets characterized by specific patterns of cytokines and transcription factors. At least six Th subsets exist: Th0, Th1, Th2, Th17, regulatory T (Treg), and follicular helper T (Tfh) cells [8], which are suggested to be involved in the pathogenesis of SS [9–12].

On the other hand, Mikulicz’s disease (MD) has been considered to be a subtype of SS based on histopathological similarities between the two diseases [13]. However, MD has a number of differences compared with typical SS including: 1) difference of gender distribution (MD occurs in both men and women, while SS occurs mainly in women); 2) persistent enlargement of lacrimal and salivary glands; 3) normal or mild salivary secretion dysfunction; 4) good responsiveness to corticosteroid treatment; 5) hypergammaglobulinemia and low frequency of anti SS-A and SS-B antibodies by serological analyses; and 6) multiple GC formation in
glandular tissue (Table 1). Previously, we reported that SS was characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while MD showed non-periductal lymphocytic infiltration with hyperplastic GCs and mild destruction of the acini (Fig. 1) [14]. Fifteen of 66 patients with SS (23%) and 12 of 20 patients with MD (60%) showed ectopic GC formation in labial salivary glands (LSGs). Patients with MD showed a significantly higher frequency, higher number and larger size of GCs compared with SS patients [15]. In addition, Yamamoto et al. [16–18] reported that patients with MD had elevated levels of serum IgG4 and infiltrating IgG4-positive plasma cells in the gland tissues. Similar findings have been observed in autoimmune pancreatitis (AIP) [19], sclerosing cholangitis [20], tubulointerstitial nephritis [21], Ridel’s thyroiditis [22] and Küttner’s tumor [23]. These diseases are now referred to as IgG4-related disease (IgG4-RD) [24,25]. We recently described the concept of IgG4-RD and provided up-to-date information regarding this emerging disease entity [26]. Recent studies have referred to MD as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS) [15,27] (Table 2).

IgG4 molecules are symmetrical homobivalent antibodies that can exchange half-molecules (heavy and light chain) specific for two different antigens ("Fab-arm exchange"), which results in losing the ability to cross-link antigens and to form immune complexes [28]. In addition, IgG4 also can bind the Fc fragment of other IgG molecule, particularly other IgG4 molecules ("Fc–Fc interactions"). These IgG4 Fc–Fc interactions proceed to Fab-arm exchange reaction and may contribute to the anti-inflammatory activity, which includes a poor ability to induce complement and cell activation caused by low affinity for C1q (Fig. 2) [29]. Another characteristic is that IgG4 is a Th2-dependent immunoglobulin and has low affinity for its target antigen, interleukin (IL-)4 directs naive human B cell immunoglobulin isotype switching to IgG4 and IgE production [30]. We previously reported that peripheral CD4+ Th cells from patients with IgG4-DS revealed a deviation in the Th1/Th2 balance to Th2 and elevated expression of Th2-type cytokines [15,31,32]. Therefore, IgG4-DS is suggested to have a Th2-predominant phenotype. This review article will emphasize recent studies seeking to understand the role of Th cell subsets in primary SS and IgG4-DS.

1. Cytokine profiles of CD4+ Th cells

1.1. Th1/Th2 paradigm

Th1 cells support cell-mediated immunity and produce IL-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, which induce inflammatory responses responsible for killing intracellular parasites and perpetuating autoimmune responses. However, excessive inflammatory responses can lead to uncontrolled tissue damage. Th2 cells produce IL-4, IL-5, and IL-13, which provide help for humoral immunity and promote IgE secretion and eosinophilic responses. Th2 responses can counteract Th1-mediated micobical action. Thus, the Th1/Th2 balance plays an important role in immunoregulation. In contrast, Th0 cells are characterized by the production of both Th1 and Th2 cytokines and are considered precursors of Th1 and Th2 cells. Several studies have revealed that autoimmune diseases are caused by disruption of the Th1/Th2 balance [33,34]. The relationship of Th1/Th2 imbalance to the pathogenesis of SS has been widely investigated. Polarized Th1 responses were associated with the immunopathology of SS [9]. High numbers of IFN-γ-positive CD4+ T cells were detected in the salivary glands of SS patients and intracellular cytokine analysis demonstrated the polarization of Th cells to a Th1 phenotype [35]. Furthermore, we reported that IL-2 and IFN-γ were consistently detected in all SS patients, while IL-4 and IL-5 were only detected in patients with high levels of B cell accumulation in the salivary glands [10,36]. Recently, Theander et al. [37] reported that the detection of GC-like structures (B cell accumulation) in LSG biopsy specimens from primary SS patients could be used as a highly predictive and easy-to-obtain marker for B cell lymphoma development. Taken together, these studies suggest that Th1 cytokines are essential for the initiation and/or maintenance of SS, whereas Th2 cytokines may be involved in disease progression, especially local B cell activation. Our clinical data was demonstrated that Th1 and Th2 cytokine concentrations were significantly higher in saliva from SS patients than from controls, and the levels of Th2 cytokines were closely associated with increased lymphocytic accumulation in LSGs. Thus, the measurement of cytokines in saliva may be useful for diagnosis and to reveal disease status [12].

IgG4-DS patients frequently have a history of bronchial asthma and allergic rhinitis with severe eosinophilia and elevated serum IgE levels [38]. It is well known that allergic immune responses are induced by allergen-specific Th2 cytokines, such as IL-4 and IL-13, which promote the secretion of IgG4 and IgE by B cells [39]. Recent studies indicated that Th2 immune reactions contributed to IgG4-DS [15,32,40] and IgG4-related tubulointerstitial nephritis [31,41]. The expression profile of cytokines suggested that IgG4-DS was characterized by a deviation of the Th1/Th2 balance to a Th2 phenotype and elevated expression of Th2 cytokines. Contrary to our results, Ohta et al. [42] reported a strong predominance of Th1 and cytotoxic type 1 cells in the salivary glands from IgG4-DS patients. They concluded that disruption of the Th1/Th2 balance might be due to differences in the specimens examined or the severity of the disease.

Chemokines are important for leukocyte activation and chemotaxis. Interactions between chemokines and chemokine receptors promote the selective local infiltration of specific cells into inflamed areas. Furthermore, chemokines are intimately involved in maintenance of the Th1/Th2 balance and immune responses in cardiac allograft rejection [43], atopic keratoconjunctivitis [44], and cutaneous lupus erythematosus [45]. Chemokines also play a key role in lymphoid neogenesis in target organs [46]. Immunohistochemical staining in our studies indicated that Th2-type chemokines including macrophage-derived chemokine (MDC)/CCL22 and thymus and activation regulated chemokine (TARC)/CCL17, natural ligands for CCR4 on Th2 cells, were detectable in and around the ductal epithelial cells and GCs, while CCR4 was expressed on infiltrating lymphocytes in LSGs in both SS and IgG4-DS patients. Thus, interactions of CCR4 with MDC and TARC may play a critical role in the accumulation of Th2 cells and subsequently, the progression of SS and IgG4-DS [12,32]. In contrast, interferon gamma induced protein 10 (IP-10)/CXCL10, natural ligand for CXCR3 on Th1 cells, was detected in and around the ductal epithelial cells, while CXCR3 was only expressed on infiltrating lymphocytes in LSGs from SS patients [47].
The Th1/Th2 paradigm was recently expanded by the identification of Th17 cells, a subset of CD4⁺ T cells characterized by their ability to produce IL-17. Several studies have reported that IL-17 was detected in epithelial and infiltrating mononuclear cells in LSGs from patients with SS. In addition, Th17 cells are “tissue seeking” and intimately involved in the initiation of SS [48]. Youinou et al. [49] reported that Th17 cells orchestrate autoreactive GCs. However, our previous data in selectively extracted lesions from LSGs by laser capture microdissection showed that the expressions of Th17-related molecules in infiltrating lymphocytes outside ectopic GCs were higher than inside ectopic GCs [36]. Interestingly, a subset of Th17/Th1 cells identified in the gut of Crohn’s disease patients may co-express IFN-γ and IL-17 [50]. Both Th1 and Th17 cells were involved in the pathogenesis of SS [51], and the early induction of a CD4⁺ Th1/Th17 pathway caused the systemic release of IL-17 in mice [52]. Our previous data suggest that both Th1 and Th17 cells present around the ductal epithelial cells might be of critical importance in the initiation of SS. Furthermore, the destruction of epithelial by Th1 and Th17 cells are thought to play a key role in germinal center formation and IgG4 production in IgG4-DS. SS is characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while IgG4-DS shows non-periductal lymphocytic infiltration with hyperplastic GCs and mild destruction of the acini. Abbreviations: GC, germinal center.

### 1.2. Th17 cells

The Th1/Th2 paradigm was recently expanded by the identification of Th17 cells, a subset of CD4⁺ T cells characterized by their ability to produce IL-17. Several studies have reported that IL-17 was detected in epithelial and infiltrating mononuclear cells in LSGs from patients with SS. In addition, Th17 cells are “tissue seeking” and intimately involved in the initiation of SS [48]. Youinou et al. [49] reported that Th17 cells orchestrate autoreactive GCs. However, our previous data in selectively extracted lesions from LSGs by laser capture microdissection showed that the expressions of Th17-related molecules in infiltrating lymphocytes outside ectopic GCs were higher than inside ectopic GCs [36]. Interestingly, a subset of Th17/Th1 cells identified in the gut of Crohn’s disease patients may co-express IFN-γ and IL-17 [50]. Both Th1 and Th17 cells were involved in the pathogenesis of SS [51], and the early induction of a CD4⁺ Th1/Th17 pathway caused the systemic release of IL-17 in mice [52]. Our previous data suggest that both Th1 and Th17 cells present around the ductal epithelial cells might be of critical importance in the initiation of SS. Furthermore, the destruction of epithelial by Th1 and Th17 cells are thought to play a key role in germinal center formation and IgG4 production in IgG4-DS. SS is characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while IgG4-DS shows non-periductal lymphocytic infiltration with hyperplastic GCs and mild destruction of the acini. Abbreviations: GC, germinal center.

### 1.3. Regulatory T cells

Treg cells, identified by the expression of Foxp3, are essential for the maintenance of immunological self-tolerance and immune homeostasis to prevent the development of various inflammatory diseases. It achieves this either by direct contact with effector immune cells and/or by secreting anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β. Treg cells exert their effects through the modulation of both T and B cell responses. Two subsets of Treg cells, CD4⁺ CD25⁺ Foxp3⁺ Treg cells [53] and IL-10—producing Tr1 cells [54] are crucial for regulating effector T cell functions. CD4⁺ CD25⁺ Foxp3⁺ Treg cells can prevent
Mice with defects in Treg cell generation often develop T cell-mediated systemic autoimmune responses that affect multiple organs. Kolkowski et al. [56] demonstrated that salivary glands in SS constitutively expressed IL-10 and TGF-β. Other studies reported a significant reduction of Tregs in LSGs and peripheral blood from SS patients that might be involved in the pathogenesis of salivary gland destruction [57,58]. In contrast, Gottenberg et al. [59] reported increased Treg cell numbers in the peripheral blood of SS patients. Therefore, it is unclear whether Tregs are involved in the pathogenesis of SS. According to recent data, Foxp3+ T-regulatory cell frequency in the salivary glands of SS patients correlates with inflammation grade and certain risk factors for lymphoma development [60]. While in early and moderate infiltrations a compensatory control of Tregs in response to Th17 expansion seems to occur, in advanced SS lesions Tregs may fail to control the immune mediated tissue injury [7,61]. Increased levels of Treg cells in salivary glands from SS patients might suggest negative feedback is more active than in healthy subjects. Therefore, Treg cells might be not involved in the initiation of disease.

Zen et al. [62] reported that significant numbers of CD4+ CD25+ Foxp3+ Tregs infiltrated the affected tissues in cases of autoimmune pancreato-cholangitis (AIPC), which is one of IgG4-RD. Furthermore, another study demonstrated that IL-21 increased the number of GCs formed in serum and high levels of IL-21 receptor were present on the surface of most B cells [67]. We previously observed that Tfh-related molecules, CXCR5 and B-cell lymphoma 6 protein (Bcl-6), were highly expressed on infiltrating lymphocytes in SS lesions from both SS and IgG4-DS patients [15,36]. These results provide strong support for Tfh cells in the progression of disease as a lymphoproliferative disorder, particularly in the growth and activation of ectopic GC formation (Fig. 3).

IL-21 was mainly produced by Th2 and Th17 cells in addition to Tfh cells [68,69]. Interestingly, high IL-21 expression was only detected outside ectopic GCs in patients with IgG4-DS in our immunohistological analyses. The expression patterns of Th2-related molecules (IL-4, CCR4 and c-Maf) in LSGs were similar to that of IL-21 in patients with IgG4-DS. In contrast, Th17-related molecules were rarely expressed in patients with IgG4-DS. Furthermore, IL-21 positively correlated with the number of GCs formed in LSGs from patients with IgG4-DS [15]. Taken together, these findings suggest that excessive IL-21 production by Th2 cells in salivary glands from IgG4-DS patients might induce Bcl-6 expression in B cells resulting in multiple GC formation. Furthermore, IL-21 directly inhibited IL-4-induced IgE production [70], and IgG4 class switching was induced by co-stimulation with IL-4 and IL-21 in humans and mice [71]. In addition, IL-21 induced IL-10 production by mitogen-stimulated peripheral blood mononuclear cells in humans [72]. Therefore, we speculate that IL-21 correlates with IL-4 and IL-10 for IgG4 class switching. In the current study, we found that IL-21 positively correlated with the IgG4/IgG ratio in immunohistochemically positive cells

2. Role of IL-21 in SS and IgG4-DS

2.1. Follicular helper T cells

Tfh cells were recently identified as a unique Th phenotype, expressing high levels of CXCR5, a chemokine receptor [64]. Several studies reported that Tfh cells control the functional activity of effector Th cells and promote ectopic GC formation by IL-21, which contributed to impaired B cell differentiation [65,66]. Once GCs are formed, Th cells are required for their maintenance and the regulation of B cell differentiation into plasma cells and memory B cells. Several studies in SS patients demonstrated that IL-21 was increased in serum and high levels of IL-21 receptor were present on the surface of most B cells [67]. Furthermore, IL-4 and IL-21 receptors knockout mice have greatly reduced IgG responses, indicating that IL-21 co-operates with IL-4 to regulate humoral immune responses [68]. We previously observed that Tfh-related molecules, CXCR5 and B-cell lymphoma 6 protein (Bcl-6), were highly expressed on infiltrating lymphocytes in ectopic GCs of LSG lesions from both SS and IgG4-DS patients [15,36]. These results provide strong support for Tfh cells in the progression of disease as a lymphoproliferative disorder, particularly in the growth and activation of ectopic GC formation (Fig. 3).

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Fig. 2. Unique structure of IgG4 antibody. A, IgG4 antibody consists of two heavy chains and two light chains. B, Fc fragment of IgG4 can interact with the Fc fragment of another IgG4 molecule. C, Exchange of half-molecules (Fab-arm exchange) results in IgG4 combining two different specificities in a single molecule (bispecific antibody).
suggesting that IL-21 might also be involved in the class switching of IgG4 in IgG4-DS [73].

2.2. Innate immunity in IgG4-DS

Macrophages act as cells in the immune response to foreign invaders of the body, by presenting pathogenic antigens to antigen-specific Th cells. Historically, they have been classified into two distinct macrophage phenotypes, "classically activated" pro-inflammatory (M1) and "alternatively activated" anti-inflammatory (M2) macrophages [74]. M2 macrophages are activated by IL-4, produce high levels of IL-10 and are important for debris scavenging, wound healing and fibrosis. These polarized macrophage populations can also contribute to systemic diseases [75]. Watanabe et al. [76] demonstrated that abnormal innate immune responses induced via Toll-like receptor signaling in macrophages might enhance Th2 immune responses and the immunopathogenesis of IgG4-RD. Our current studies observed that IgG4-DS patients showed predominant infiltration by M2 macrophages that secreted IL-10 and IL-13 in salivary glands.

Dendritic cells (DCs) are professional antigen presenting cells that bridge innate and adaptive immunity. Expression of

Fig. 3. Schematic model of Th cell network in SS. Th1 and Th17 cells are involved in early stages of disease, while Th2 and Tfh cells are associated with GC formation in the late stage. Abbreviations: Th, T helper; Tfh, follicular helper T.

Fig. 4. Schematic model of Th cell and innate immune network in IgG4-DS. Th2, Treg, and Tfh cells play key roles in GC formation and IgG4 production. Dendritic cells and macrophages promote Th2 immune reaction by IL-33 as well as BAFF and APRIL. Abbreviations: Treg, regulatory T; BAFF, B cell activating factor belonging to the tumor necrosis factor family; APRIL, a proliferation-inducing ligand.
DC-derived TNF-family ligands such as a proliferation-inducing ligand (APRIL) and B cell activating factor belonging to the tumor necrosis factor family (BAFF) is induced by innate immune signals to promote the differentiation and activation of plasma cells [77]. In IgG4-RD patients, BAFF and APRIL levels were significantly higher than in healthy individuals [78]. BAFF and APRIL may contribute to progressive plasma cell infiltration and ectopic GC formation in the target organs of patients with IgG4-RD. In addition, BAFF and APRIL enhance IgG4 and IgE class switching in the presence of IL-4 [79]. Th2 cytokine production was increased in the tissues of patients with autoimmune pancreatitis [80]. Therefore, BAFF and APRIL may contribute to the pathogenesis of IgG4-RD in concert with Th2 cells. Although IgG4-RD was considered to be a Th2-dependent disease [40, 41, 81], the mechanism of Th2 polarization has yet to be elucidated. IL-33 is a recently identified cytokine that directly stimulates ST2, IL-33 receptor, expressed by Th2 cells to produce IL-4, IL-5, and IL-13 [82]. Moreover, the genetic polymorphism of IL-33 in humans is associated with allergic diseases [83]. Our current studies suggest that IL-33 production by DCs and M2 macrophages might play a key role in Th2 cytokine production and the pathogenesis of IgG4-D (Fig. 4).

3. Conclusions

Research accumulated in recent years makes it increasingly clear that the immunological backgrounds are entirely different between SS and IgG4-RD. However, additional research is required to elucidate further the pathogenesis of IgG4-D, especially the development of a mouse model of IgG4-D. Although Glucocorticoids are the standard treatment for IgG4-RD, Yamamoto et al. [84] reported that the relapse rate of IgG4-D during steroid therapy is 26.8%. A more thorough understanding of the complex mechanisms of IgG4-D, especially the role of Th subset-related cytokines, could lead to the development of novel pharmacological strategies aimed at disrupting the cytokine network and inhibiting the initiation and/or progression of IgG4-D. Finally, it should be noted that while this thesis focuses primarily on T cells, that there have recently been other extensive reviews and hypotheses published on Sjögren’s syndrome, reflecting its increased interest not only to basic immunologists, but also to rheumatologists [4, 85–116].

Competing interests

The authors declare no competing interests.

Author contributions

All authors provided substantial contributions to discussions of content, and to reviewing and editing the manuscript before submission. M Moriyama researched the data and wrote the article.

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