T cell-Associated Cytokines in the Pathogenesis of Sjöögren’s Syndrome

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Abstract

Sjögren’s syndrome (SjS) is a systemic autoimmune disease that primarily targets salivary and lacrimal glands. SjS affects 2-4 million people in the US alone and greatly affects the life quality of the afflicted individuals. Autoreactive effector T cells are central executors and orchestrators in the pathogenic processes of SjS by mediating target organ inflammation and destruction and by facilitating B cell responses and autoantibody production. A variety of cytokines that are produced by effector T cells or capable of directly affecting effector T cells are elevated in the target organs and serum of SjS patients and mouse models of SjS, as discussed later in this review. Functional studies performed with mouse models of SjS that are deficient in specific cytokine genes demonstrate that IFN-γ, IL-4 and IL-17, signature cytokines for Th1, Th2 and Th17 effector cells, are all essential for the full development and onset of SjS by modulating the differentiation, expansion and function of self-reactive T and B cells and by directly affecting the homeostasis and biological activities of the target tissues [14-19]. In this review, we summarized the recent progress on the expression and functions of cytokines in the pathogenesis of SjS, with specific focus on those derived from T cells and/or directly affecting T cell responses.

Introduction

Sjögren’s syndrome (SjS) is a systemic autoimmune disease that primarily affects salivary and lacrimal glands. SjS affects 2-4 million people in the US alone, with 90% of the patients being women. SjS is characterized by progressive lymphocytic infiltration of salivary and lacrimal glands and generation of autoantibodies that include anti-SSA/Ro, SS-B/La and other exocrine gland-specific autoantibodies, which together lead to impaired secretory function [1-3]. The primary clinical symptoms are xerostomia (dry mouth) and keratoconjunctivitis (dry eyes), which are presented as difficulty swallowing, chewing or talking, sandy or burning sensations in the eyes, dry or burning feelings at lips, nose and throat, and as a result, a higher incidence of dental caries. In addition, patients often suffer from dryness of gastrointestinal tract, vagina, lung and skin, and from other extra-glandular symptoms such as chronic fatigue, fibromyalgia, muscle and joint pain, nephritis and peripheral neuropathy [1,3,4]. Finally, they have a much higher risk of developing B cell lymphoma than the general population and people with other autoimmune disorders [1,3,4]. SjS is usually chronic, progressive and at times debilitating, thereby greatly affecting the life quality of the patients. SjS can occur as primary SjS (pSjS) or secondary SjS, which is associated with other connective tissue diseases [2,5]. The current diagnosis of SjS is conducted according to the Revised European-American Criteria for the Classification of SjS [6], which entails histological analysis of a minor salivary gland biopsy for lymphocytic infiltration, presence of serum anti-SSA and/or SSB autoantibodies, presence of oral and ocular symptoms, as well as oral and ocular tests for saliva and tear production.

Although the etiology of SjS remains elusive, accumulating evidence indicates that both genetic factors and environmental triggers, such as viral infections, sex hormone changes and tissue injuries, contribute to the initiation of autoimmune process in SjS [3,7-10]. Both self-reactive T and B cells play crucial roles for the development and onset of SjS by driving exocrine gland inflammation and autoantibody production [3,5,11-13]. Cytokines are powerful orchestrators and effectors of the innate and adaptive immune responses. The differentiation of distinct effector T cells subsets, T helper (Th) 1, Th2, Th17 and Th follicular helper (TFH) cells, are instructed or influenced by various cytokines. Each effector T cell subset in turn produces a group of signature cytokines, which execute specialized effects on target tissues or pathogens and often simultaneously propel the further differentiation and expansion of the same effector subset. Many cytokines have been shown to be elevated in the target organs and serum of SjS patients and mouse models of SjS, as discussed later in this review. Functional studies performed with mouse models of SjS that are deficient in specific cytokine genes demonstrate that IFN-γ, IL-4 and IL-17, signature cytokines for Th1, Th2 and Th17 effector cells, are all essential for the full development and onset of SjS by modulating the differentiation, expansion and function of self-reactive T and B cells and by directly affecting the homeostasis and biological activities of the target tissues [14-19]. In this review, we summarized the recent progress on the expression and functions of cytokines in the pathogenesis of SjS, with specific focus on those cytokines that are either produced by effector T cells or directly affecting T cell responses.

Th1-Associated Cytokines

IFN-γ

IFN-γ, the hallmark cytokine of Th1 and cytotoxic CD8 T cells, plays a pivotal role in cellular immunity and host defense against intracellular pathogens and tumor [20]. IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by Th1 cell and CD8+ cytotoxic T cells (CTL) during antigen-specific adaptive immune response. Among its plethora of biological effects, IFN-γ can activate macrophages and NK cells, enhance MHC expression and antigen presentation, induce expression of many chemokines and adhesion molecules in both immune and non-immune cells, promote differentiation of Th1 cells and induce apoptosis of various tissue and cell types [21,22]. IFN-γ has been shown to play a pathogenic role in mouse models of type 1 diabetes [23,24], systemic lupus erythematosus (SLE) [25,26], rheumatoid arthritis (RA)

The salivary glands and saliva from patients with primary SjS exhibit elevated levels of IFN-γ compared to non-SjS subjects [29,30]. Patients with primary SjS demonstrate an increased Th1 response over Th2 response in the salivary glands, saliva and serum, compared to non-SjS sicca patients [31,32]. Moreover, the Th1 response shows positive association with lymphocytic infiltration of the salivary glands [33]. In non-obese diabetic (NOD) mice, a widely used model for type 1 diabetes and secondary SjS, IFN-γ-deficiency abolishes multiple pre-immunological abnormalities of the salivary glands, thus preventing the subsequent tissue-specific autoimmunity and clinical onset of SjS [15]. Mechanistically, IFN-γ has been reported to induce death and secretory dysfunction in salivary gland cells as well as enhance the abilities of epithelial cells and antigen presenting cells (APCs) in the salivary glands to present antigens and activate T cells [5,34,35]. Furthermore, our own study and another report have shown that IFN-γ induces expression of chemokines CXCL9 and -10, both of which are ligands for CXCR3, in a human salivary gland cell line and in primary salivary gland epithelial cells from SjS patients [36]. Consistent with a potential role of CXCR3 ligands in SjS pathogenesis, CXCL9, -10 and -11 are significantly increased in salivary gland lesions and tears of SjS patients [29,36,37]. Antagonizing CXCL10 activity in MRL/lpr mice, a model of secondary SjS, during the early stage of disease development significantly reduces mononuclear cell infiltration of salivary glands [38]. These findings suggest that induction of CXCR3 ligands is crucial mechanism by which IFN-γ promotes effector T cell recruitment to target tissues and initiates local autoimmune responses. Definitive in vivo studies, especially loss-of-function studies, are required to determine the functions of endogenous IFN-γ in the immunological phase of the SjS development and in the persistence of SjS after disease onset. Moreover, additional effects of this cytokine in SjS, such as those affecting macrophage and NK cell function and inducing additional chemokines, also await further investigation.

IL-12

IL-12, produced mainly by macrophages and dendritic cells (DCs), is a critical promoter of the differentiation of IFN-γ-producing T cells, both Th1 and effector CD8 T cells [39,40]. IL-12 potently induces and facilitates cellular immune responses by promoting proliferation, cytotoxic activity and IFN-γ production from effector T cells and NK cells [22,39,40]. IL-12 treatment decreases the frequency of regulatory T cells (Tregs) and downregulates Foxp3 levels in these cells [41]. Hence, IL-12 engages multiple mechanisms to promote immune activation. Averrantly enhanced IL-12 activity is implicated in the pathogenesis of many immune-mediated diseases, including psoriasis, Crohn’s disease, RA and type 1 diabetes [42-44].

IL-12 levels are elevated in the target organs of patients with SjS, with macrophages and DCs being the main cellular sources [45,46]. A marked increase in IL-12 mRNA and protein levels is detected at the early stage of the disease development in mouse models of SjS compared to control mice [47,48]. Moreover, thyroid gland-specific IL-12-transgenic mice with SJL genetic background exhibit SjS-like salivary gland inflammation, anti-SSB/La autoantibody production and salivary gland secretory dysfunction [49]. However, loss-of-function studies in mouse disease models are still needed to define the functional importance of endogenously produced IL-12 in the pathogenesis of SjS.

IL-18

IL-18, a member of IL-1 family of pro-inflammatory cytokines, mediates a variety of inflammatory responses in the context of inflammation, infections and autoimmunity through affecting both immune and non-immune cells and both innate and adaptive immune systems [50-52]. One major effect of IL-18 on adaptive immunity is to enhance IL-12-induced Th1 responses [51,53]. IL-18 facilitates the development of autoimmune and inflammatory diseases, including pulmonary inflammatory disease, RA, type 1 diabetes and IBD [52-55]. IL-18 is detected in the serum, salivary glands and saliva of SjS patients and its levels correlate with the severity of the disease among the patients [56-58]. Moreover, expression of IL-18 by salivary gland-infiltrating macrophages and DCs shows positive correlation with degree of leukocyte infiltration and lymphoma development in patients with primary SjS [45]. Similarly to IL-12, the in vivo pathogenic role of IL-18 in SjS still awaits future determination.

TNF-α

Tumor necrosis factor-alpha (TNF-α) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reactions such as fever, cell death, sepsis, cachexia and inflammation. It is produced by many cell types including Th1 cells and CD8 effector cells and implicated in the pathogenesis of various autoimmune and rheumatic diseases. SjS patients have higher salivary TNF-α level than those from non-SjS patients [32]. TNF-α enhances the surface expression of Ro (SS-A) and La (SS-B) on human keratinocytes, two important autoantigens involved in SjS and SLE [59]. Moreover, in vitro studies showed that TNF-α, alone or cooperating with IFN-γ, induces apoptosis and secretory dysfunction of salivary gland cells [34,60,61]. However, despite these lines of evidence suggesting a role of TNF-α in SjS pathogenesis, the in vivo functional studies are still lacking. Furthermore, a randomized, double-blind and placebo-controlled clinical trial of infliximab, a neutralizing monoclonal antibody against TNF-α that showed promising therapeutic effects on a number of autoimmune or inflammatory disorders including SLE, did not demonstrate any efficacy in treating SjS [62]. Therefore, TNF-α may not be an indispensable pathogenic factor for SjS or it may play a more complicated role than simply promoting the disease.

Th2-Associated Cytokines

IL-4

IL-4, originally characterized as an anti-inflammatory cytokine, is the hallmark cytokine of Th2 immune response. IL-4 amplifies Th2 differentiation, mediates asthma and allergy and provides critical stimulating signals for the growth and function of activated B cells [63,64]. Meanwhile, IL-4 inhibits the activation of Th1 response and cellular immunity, which largely accounts for its anti-inflammatory function [65,66]. Provision of exogenous IL-4 protects against development of type 1 diabetes and RA [67,68]. However, IL-4 is required for the development of systemic autoimmune diseases SLE, in that it promotes target organ inflammation and pathologies and facilitates production of IgG1 and IgE immunoglobulins [25,69].

IL-4 has been detected in the salivary glands of a portion of SjS patients, especially those with higher degree of B cell accumulation in the target organs [33,70]. Deficiency of IL-4 or STAT6 gene in NOD and NOD.B10-H2b mice abolishes the production of IgG1-type anti-M3R antibody, the crucial autoantibody causing the secretory dysfunction and xerostomia [14,17,48]. Moreover, whereas the sera from NOD. B10-H2b mice can cause salivary secretory dysfunction upon transfer to C57BL/6 mice, those from STAT6-deficient NOD.B10-H2b mice fail
CD3+CD4-CD8- T cells are also expanded in the peripheral blood salivary glands of SjS patients [84,86,88]. In addition, IL-17-producing and their receptors within lymphocytic infiltrates and ductal areas in gland and serum of patient with primary SjS [84-87]. Moreover, demonstrated in the corresponding mouse models [39,78-83]. RA, Crohn’s disease and psoriasis, and an essential role of IL-23-IL-17 autoimmune or inflammatory diseases, including multiple sclerosis, increase in IL-17 and IL-23 levels was reported in patients of various inflammation, once attributed mainly to Th1 cells [39,78-81]. An the fundamental mechanisms causing autoimmunity and chronic expansion of Th17 cells [39,79,81]. A rapidly growing knowledge in recent years about the pro-inflammatory functions of IL-17 and IL-23-Th17 pathway has greatly advanced our understanding about the fundamental mechanisms causing autoimmune and chronic inflammation, once attributed mainly to Th1 cells [39,78,81]. An increase in IL-17 and IL-23 levels was reported in patients of various autoimmune or inflammatory diseases, including multiple sclerosis, RA, Crohn’s disease and psoriasis, and an essential role of IL-23-IL-17 pathway in the development and onset of these diseases has been demonstrated in the corresponding mouse models [39,78-83].

Elevated IL-17 and IL-23 levels are reported in salivary gland and serum of patient with primary SjS [84-87]. Moreover, immunohistochemistry analysis has shown IL-17 and IL-23 proteins and their receptors within lymphocytic infiltrates and ductal areas in salivary glands of SjS patients [84,86,88]. In addition, IL-17-producing-CD3-CD4-CD8- T cells are also expanded in the peripheral blood and salivary gland infiltrates in patients with SjS [89]. Inhibition of IL-17 activity by adenovirus-mediated expression of a soluble IL-17R:Fc fusion protein impedes the development of SjS-like disease in C57BL/6:NOD-ld-Aec1Aec2 mice, a model of primary SjS, as indicated by diminished tissue-inflammation and autoantibody production and improved secretory function [19]. Conversely, adenovirus-mediated overexpression of IL-17 in non-SjS-prone C57BL/6 mice induces the development of SjS-like disease based on multiple disease parameters [18], including tissue-inflammation and antinuclear autoantibody production. Interestingly, overexpression of IL-17 results in the activation of B cell antibody production, the underlying mechanisms for which requires further investigation. Collectively, these findings define a crucial pathogenic function of IL-17 in SjS and identify it as a potential therapeutic target for this disease.

**IL-13**

IL-13 is originally characterized as a Th2 cytokine as it can be produced by these effector T cells and has overlapping effects with IL-4 on macrophages, B cells and inflammation [71-73]. IL-13 enhances the activities of many cell types, such as B cells, fibroblasts and mast cells, and as a result plays pivotal roles in allergic asthma, anti-helminth immunity and tissue fibrosis [71]. IL-13 levels are increased in patients with rheumatic disease RA and SLE and correlated with disease severity [74,75]. IL-13 mRNA has been detected in the exocrine glands of SjS patients [33,76]. A recent report demonstrates the presence of IL-13-producing T cells in salivary gland draining lymph nodes in Id3-deficient mice, a mouse model of primary SjS, but not in wild-type control mice [77]. Id3-deficient mice demonstrate an increase in mast cells in the salivary glands, which may contribute to the salivary gland dysfunction. Blockade of IL-13 activity in Id3-KO mice with a neutralizing antibody improves salivary gland secretory function, which is associated with a reduction of mast cells in the target organs [77]. This study has presented important evidence that IL-13 facilitates the development of secretory dysfunction, possibly by affecting mast cells.

**Th17-Associated Cytokines**

**IL-17A**

IL-17, a potent pro-inflammatory cytokine that has been in the center of investigation in the field of autoimmunity and inflammation, is the hallmark cytokine produced by Th17 cells. It plays important roles in host defense against bacterial and fungal infections and has been shown to be the predominant pathogenic player in numerous autoimmune and inflammatory diseases [78-80]. IL-23, a member of IL-12 family of cytokines that comprises the p40 subunit of IL-12, has been shown to be a crucial cytokine for induction, stabilization and expansion of Th17 cells [39,79,81]. A rapidly growing knowledge in recent years about the pro-inflammatory functions of IL-17 and IL-23-Th17 pathway has greatly advanced our understanding about the fundamental mechanisms causing autoimmune and chronic inflammation, once attributed mainly to Th1 cells [39,78,81]. An increase in IL-17 and IL-23 levels was reported in patients of various autoimmune or inflammatory diseases, including multiple sclerosis, RA, Crohn’s disease and psoriasis, and an essential role of IL-23-IL-17 pathway in the development and onset of these diseases has been demonstrated in the corresponding mouse models [39,78-83].

**IL-21**

IL-21 is a T cell-derived, pleiotropic cytokine belonging to the common cytokine receptor γ chain (γc)-dependent cytokine family [94,95]. IL-21 is produced by TFH, Th17 and NKT cells, with its receptor expressed on T cells and various types of immune cells and non-hematopoietic cells [94-96]. IL-21 can directly promote plasma cell differentiation and enhance germinal center B cell response, potentiate Th17 cell differentiation and enhance effector and memory CD8 T cell survival and function [79,81,96,97]. Rapidly growing evidence in recent years has identified IL-21 as a crucial new player in the pathogenesis of a number of autoimmune diseases, including IBD, SLE, type 1 diabetes and psoriasis [98,99]. IL-21 also has anti-inflammatory and immune-suppressive effect by promoting the generation of IL-10-producing type 1 regulatory T (Tr1) cells [100,101].

Patients with SjS have significantly elevated serum IL-21 levels, which are positively correlated with levels of IgG1. Immunohistochemistry analyses show that lymphocytic foci in the salivary glands from SjS patients express high levels of IL-21 compare to the controls [102]. Moreover, IL-21-producing T cells are detected in salivary glands in SjS patients [103]. A recent report shows that local suppression of IL-21 in submandibular glands, achieved by lentivirus mediated expression of IL-21 shRNA, reduces target organs inflammation and improves salivary gland secretory function and thus impedes the development of SjS in NOD mice [104]. The effect of IL-21 is associated with an impaired TFH response, which might result in subsequent diminished B cell antibody production. Thus, IL-21 is a newly characterized pathogenic player in SjS with direct impact on both B and T cells.

**IL-10**

IL-10, initially classified as a Th2 cytokine, is a potent anti-inflammatory cytokine that suppresses both innate and adaptive...
immune response [105,106]. IL-10 inhibits the maturation, function and cytokine production of antigen presenting cells and also can directly suppress T cell differentiation into pro-inflammatory Th1 and Th17 subsets [106-108]. Consequently, IL-10 prevents the development of IBD, restrains the development of RA and experimental autoimmune encephalomyelitis, and limits tissue damage in response to certain microbial infections and chemical stimulants [107,109,110]. IL-10 can be produced by macrophages, Th1 cells, Tregs and various effector T cell subsets [105,106]. IL-10 has certain stimulatory effects on B cells, promoting their survival and proliferation and enhancing plasma cell generation, antibody production and isotype switching [107,111,112].

IL-10 levels are elevated in the saliva of SJ/S patients and correlated with severity of xerophthalmia and xerostomia [113]. Increased serum IL-10 levels are reported in patients with primary SJ/S, which are positively associated with the levels of IgG1 and immune cell-infiltration [114]. These lines of evidence suggest that IL-10, despite of its well-documented immune-suppressive effects, may play a pathogenic role in SJ/S. Indeed, transgenic expression of IL-10 in salivary and lacrimal glands leads to SJ/S-like lymphocytic infiltration and enhanced apoptosis of glandular cells, which are underpinned by the effect of IL-10 to upregulate Fas ligand expression in CD4 T cells [115]. IL-10-deficiency in NOD mice markedly reduces both insulitis and sialadenitis [116]. The pro-inflammatory properties of IL-10 in SJ/S may be attributed to additional mechanisms, such as upregulation of ICAM-1 expression in target tissues and acquisition of pro-inflammatory function in the presence of excess IFN-α [117,118]. These mechanisms have been demonstrated in the cases of type 1 diabetes and SLE, in which IL-10 exerts pathogenic effects, likely in a fashion that depends on the stage of the disease and the cell types that produce IL-10 [117,118]. Future investigations are warranted to thoroughly delineate the complex function of IL-10 in the pathogenesis of SJ/S.

Model for the Role of Cytokine Network in SJ/S Pathogenesis

As described in this review, findings from human SJ/S patients and various in vitro and in vivo studies using mouse SJ/S models have provided evidence implicating the functions of a number of T cell-derived or T cell-affecting cytokines in the development and onset of this disease. Among them, IL-4, IL-13, IFN-γ, IL-17, IL-21 and IL-10 have been convincingly shown to play distinct but essential roles in the pathogenic processes of this disease through in vivo loss-of-function studies using mouse SJ/S models. The roles of many other cytokines, including IL-12, IL-23, IL-18, TNF-α and IL-22, are strongly suggested but remain to be determined by in vivo loss-of-function studies. We propose a model delineating the specific functions and contributions of some of these cytokines in various pathogenic events leading to the onset of SJ/S (illustrated in Figure 1). Th1 cytokine IFN-γ and TNF-α are crucial for the initial induction of salivary gland tissue apoptosis, auto-antigen release and activation of APCs and exocrine gland epithelial cells. Moreover, IFN-γ and TNF-α induce production of various chemokines, such as macrophage chemotactic protein, CXCR3 ligands and CCL20 [119] from exocrine gland cells and APCs, which initiate and reinforce the recruitment of macrophages, Th1, Th17 and other T cell subsets to the exocrine glands. IL-17 produced by Th17 cells in the exocrine glands induces or potentiates production of inflammation cytokines, such as IL-1 and TNF-α, and chemokines, such as MIP3-α, from exocrine gland cells. It also induces production of matrix metalloproteinases from tissue cells to enhance tissue damage. Moreover, IL-17 may also directly affect B cell responses. At a later phase of SJ/S development, IL-4 produced by Th2 cells provides crucial help to B cells in their activation, proliferation and isotype switch to produce IgG1 type anti-M3R antibodies that contribute to secretory dysfunction of salivary gland cells. Th2-derived IL-13 facilitates the secretory dysfunction, possibly by enhancing the proliferation and activation of mast cells and histamine release from these cells. However, how mast cells promote secretory dysfunction requires further characterization. Also around this phase or at an even later stage, IL-21 produced by Th17 cells or other effector T cell subsets enhances the germinal center (GC) B cell responses to worsen the secretory dysfunction, and further positively reinforce the TFH response. IL-21 may also have additional effects on T cell responses. In such a fashion, the coordinated effects of multiple T cell cytokines mediate distinct pathogenic events, propelling the development of various tissue pathologies and leading to the eventual onset of SJ/S (Figure 1).

Many aspects proposed in this model need to be tested in future by in vivo functional studies in mouse models and by in vitro functional studies using T cells from SJ/S patients. Furthermore, the precise effects and the stage-dependent functions of many cytokines, the underlying mechanisms for the effects of these cytokines and the interactions, cross-regulation and redundancy among cytokines and Th subsets in the development and persistence of SJ/S are either completely uncharacterized or only partially delineated, and therefore await in-depth investigations in future.

Conclusion

Cytokines are crucial effectors and modulators of the autoimmune responses that lead to the onset of SJ/S. They are also promising targets for treatment and prevention of this disease. The recent advancement in our understanding about the functions of SJ/S-associated cytokines, achieved by utilizing both human samples and mouse models, will guide future studies that elucidate the regulation of cytokine expression and interaction between different cytokines in the context of SJ/S, and fully determine the precise pathological effects of these cytokines in the development, progression and systemic manifestation of SJ/S. These accomplishments will provide crucial insights and tools for the development of better diagnostic criteria and more effective and specific therapeutic strategies to combat this prevalent, high-impact autoimmune disease.
References


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