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Author manuscript

Clin Immunol. Author manuscript; available in PMC 2018 September 01.

Published in final edited form as:

Clin Immunol. 2017 September ; 182: 4–13. doi:10.1016/j.clim.2017.04.003.

Innate immunity in Sjögren's syndrome

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Abstract

Sjögren's syndrome (SS) is an autoimmune disease of exocrine tissue that primarily affects women. Although patients typically experience xerostomia and xerophthalmia, numerous systemic disease manifestations are seen. Innate immune hyperactivity is integral to many autoimmune diseases, including SS. Results from SS mouse models suggest that innate immune dysregulation drives disease and this is a seminal event in SS pathogenesis. Findings in SS patients corroborate those in mouse models, as innate immune cells and pathways are dysregulated both in exocrine tissue and in peripheral blood. We will review the role of the innate immune system in SS pathogenesis. We will discuss the etiology of SS with an emphasis on innate immune dysfunction. Moreover, we will review the innate cells that mediate inflammation in SS, the pathways implicated in disease, and the potential mechanisms governing their dysregulation. Finally, we will discuss emerging therapeutic approaches to target dysregulated innate immune signaling in SS.

Keywords

Sjögren's syndrome; Innate immunity; A253; submandibular gland; toll-like receptor

1. Introduction

Sjögren's syndrome (SS) is a debilitating autoimmune disease in which the immune system targets exocrine gland tissue. Like many other autoimmune diseases, SS displays a strong female predilection [1]. The disease occurs in two forms: primary (pSS) and secondary (sSS). Patients with pSS often experience loss of salivary and lacrimal flow, and exhibit serious systemic disease manifestations as well [2]. In sSS, patients have a diagnosis of SS as well as another autoimmune connective tissue disease, such as lupus erythematosus (SLE) or rheumatoid arthritis (RA) [1]. Most patients with SS experience salivary hypofunction, and this can lead to rampant dental decay and difficulty in chewing and swallowing [3]. Currently, there are no approved therapies that address disease etiology and patient management is palliative.

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Although the cause of SS remains poorly understood, it is characterized by exaggerated innate and adaptive immune responses. The adaptive immune system is comprised of B and T cells and has been studied extensively in SS [4, 5]. B and T cells are activated pathogenically, and abnormalities in both populations are seen in glandular tissue and systemically in SS mouse models and patients [6]. Specifically, alterations in normal CD4+ T helper subset ratios are observed [7, 8]. Activated T cells produce inflammatory cytokines that drive B cell differentiation and class switching. Numerous B cells abnormalities are found in SS patients. For example, SS patients have autoantibodies with diverse specificities and are at high risk of developing B cell lymphomas [4, 9–11]. Thus, the adaptive immune system is a key contributor to SS pathology.

The adaptive immune system is often activated by signals generated by the innate immune response. Innate immunity provides the "first line" of defense, and serves to recognize Pathogen-Associated Molecular Patterns (PAMPs). These microbial components are capable of eliciting rapid and robust inflammatory responses [12]. There are many different types of receptors that bind PAMPs, termed pattern recognition receptors (PRRs). Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are examples of PRRs that are activated by PAMPs [13]. Initially, it was thought that PRRs were important only in pathogen defense [14]; however, host-derived molecules can also activate innate immunity, and this is an established disease mechanism in autoimmunity [15–18]. While the innate immune system plays an important role in SS pathogenesis [19–22], the way in which it is activated is poorly understood. This review will provide an overview of the role of innate immunity in disease initiation and the specific cells and pathways that contribute to innate activation in SS. In addition, we will discuss current therapeutic approaches to mitigate activation of innate immunity.

2. Innate immune activation in salivary tissue precedes the adaptive in SS mouse models

Since markers of early SS disease in humans are not well defined, mouse models are instrumental in establishing early events that mediate exocrine gland dysfunction in SS [23]. Microarray studies performed on the C57BL/6.NOD-*Aec1Aec2* pSS mouse model prior to lymphocytic infiltration of the submandibular gland (SMG) and lacrimal tissue identified differential expression of numerous genes associated with innate immunity [24–26]. Studies in SMG tissue from these animals revealed upregulation of TLR3, and interferon (IFN)- and apoptosis-related genes [24, 25]. In addition, several chemokines were elevated in the lacrimal gland prior to the arrival of lymphocytes, including *Ccl8* and *Ccl9*. Increased expression of the B cell chemoattractant *Tnfsf13* (*APRIL*) was also noted [26].

Since genes associated with innate immunity are dysregulated prior to the arrival of lymphocytes, these data suggest an important role for the salivary and lacrimal gland epithelium in disease development. An elegant study in *IκB-ζ* deficient mice provides support for an initiating role of epithelium in SS, as epithelial cells in this model are crucial for the onset of inflammation in lacrimal tissue, and enhanced apoptosis in the epithelium

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precedes lymphocyte infiltration [27]. Thus, several lines of evidence suggest that pathology in the exocrine glands is initiated by innate immune dysfunction in SS.

3. Genetic studies in pSS patients identify polymorphisms in innate immune genes

Although there is a need for continued genetic studies in SS patients, polymorphisms in genes associated with innate immunity are identified by several groups [28–32]. There are more than 15 robust susceptibility loci identified for SS and many of these are shared with SLE [33]. Similar to SLE, patients with SS display increased expression of type I and type II IFN-regulated genes in both salivary tissue and peripheral blood [19, 30, 34–38]. While the underlying causes of IFN activation remain poorly understood, it is possible that viruses and immune complexes could induce expression of these genes [39]. Alternatively, activating mutations within genes that regulate IFN pathways themselves may contribute to the IFN gene signature observed in SS patients. One of the most definitive genome-wide association studies in pSS patients published to date identified single nucleotide polymorphisms in many IFN-inducible genes that are implicated in innate immunity, specifically HLA alleles, *STAT4*, *IRF5*, *IL-12A,* and *TNIP1* [21]. However, the underlying genetic abnormalities that govern SS pathogenesis remain poorly understood and further work is needed to define the pathways and regulatory networks that contribute to disease.

It is important to point out that assessment of IFN gene expression and activity carries clinical significance. Studies classifying minor salivary gland tissue from pSS patients on the basis of high and low IFN activity revealed patients with high IFN activity displayed a higher prevalence of leukopenia and hyperglobulinemia. Moreover, these patients had higher anti-nuclear antibody (ANA) titers and anti-Ro (SSA) autoantibodies, and also showed higher focus scores in salivary tissue [37]. A corroborating study of monocytes from pSS patients demonstrated the presence of a type I IFN signature was associated with higher disease activity and elevated autoantibody levels [40]. Finally, expression of MxA protein in monocytes and whole blood samples was shown to be a reliable biomarker to stratify pSS patients on the basis of type I IFN activity [41]. Since approximately 60% of pSS patients exhibit a type I IFN signature and have elevated IFN activity [37, 40, 41], these data suggest that classifying patients on this basis is relevant not only for clinical trial recruitment but also for patient management decisions.

4. Evidence for activation of innate immunity by environmental stimuli

The innate immune system is activated by environmental insults and these likely play an important role in the initiation and progression of disease. Studies in SS patients suggest viral infection may contribute to disease initiation and chronicity, as cytomegalovirus (CMV), Epstein Barr virus (EBV), and hepatitis C virus (HCV) are implicated in SS pathogenesis [42–45]. Corroborating work shows that some SS patients display an IFN gene signature (*vide supra*), and IFNα is secreted by plasmacytoid dendritic cells (pDCs) in response to viral agonists in salivary tissue in SS [19, 39, 46, 47]. In addition, studies in mouse models provide evidence for a virally-mediated disease etiology, as mice infected with murine CMV (mCMV) or HCVδ virus develop a SS-like disease [48, 49]. Moreover,

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an elegant genetic study in mice shows salivary hypofunction is induced by type I IFN signaling [50]. Finally, administration of a synthetic analog of double-stranded RNA (dsRNA) (typically produced by viruses) exacerbates disease manifestations in SS-prone mice [51, 52]. While further studies are needed to establish the role of viral infection in SS conclusively, current evidence suggests that viruses mediate disease pathogenesis in at least a subset of patients.

5. Innate immune cells in SS disease

Many types of innate cells are implicated in SS. Inappropriate activation of innate immune pathways occurs within exocrine tissue as well as systemically [19, 27, 53]. DCs, macrophages, salivary epithelial gland cells (SGECs) and natural killer (NK) cells are among the best characterized innate cells in SS, and the role of these cells in disease is discussed below.

5.1. Dendritic Cells

DCs play an important role in SS, as they function as antigen presenting cells in ectopic germinal centers in salivary tissue [54]. Studies show DCs are decreased in peripheral blood and elevated in salivary tissue of pSS patients as compared to controls [19, 55, 56]. Evidence suggests that follicular DCs contribute to ectopic lymphoid neogenesis in salivary tissue in SS, as these cells promote expansion of T cells and are also associated with somatic hypermutation of B cells [57].

A specific DC subset, termed plasmacytoid DCs (pDCs), is associated with B cell infiltration in SS salivary tissue [58]. Plasmacytoid DCs are activated by TLRs and secrete numerous pro-inflammatory cytokines, including IFNα [59]. Mice injected systemically with an inflammatory stimulus exhibit salivary gland dysfunction that correlates with increased frequency of pDCs in the gland [60]. Importantly, a recent study suggests that these cells may also serve a tolerogenic role in SS, as pDCS and IFNα promote the expansion of regulatory T cells that have specificity for the SS autoantigen La [61]. Thus, further studies are needed to determine whether pDCs are primarily pathogenic or protective in disease.

5.2. Macrophages

Several studies suggest an important role for macrophages in salivary and lacrimal gland pathology. In the NOD model of SS, macrophages are recruited to salivary gland tissue prior to lymphocytes, suggesting that macrophage infiltration is an early disease event that promotes further immune cell chemotaxis [62]. Moreover, macrophages increase in salivary tissue with disease progression in SS patients [20]. Salivary gland macrophages have pathogenic potential in SS, as these cells express IL-18 and CXCL13, and patients with significant salivary macrophage infiltration tend to have enlarged salivary glands [63, 64]. In addition, many macrophage-associated genes are identified in severely inflamed salivary tissue from SS patients [65].

Of clinical importance, macrophages mediate dry eye in the *Aire* deficient mouse model for SS [66]. Notably, systemic macrophage depletion improves tear production in this model, demonstrating a definitive pathogenic role for these cells in disease [66]. Interestingly, IL-12

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expression by murine macrophages is enhanced by the SS autoantigen Ro52 [67]. This suggests a possible mechanism for inflammatory cytokine secretion by macrophages in SS, as Ro52 is elevated in peripheral blood from SS patients [68]. Thus, work in SS mouse models and pSS patients provides evidence that macrophages promote an inflammatory phenotype and also contribute to exocrine gland dysfunction in disease.

5.3. Salivary Gland Epithelial Cells

Saliva is produced within salivary acini, asymmetrical epithelial cells that are highly polarized. Cell polarity is controlled by cell-to-cell and the cell-to-extracellular matrix (ECM) interactions [69]. Decreased salivary flow is a hallmark of SS patients [2]. Studies in SS patients and mouse models suggest that this is due, at least in part, to loss of polarity in the salivary epithelium [70].

Tight junctions (TJ) mediate cell-to-cell interactions and dysregulation of salivary TJs is implicated in SS pathogenesis. TJ proteins establish a barrier between the apical and basolateral regions of the cell. In healthy individuals, TJs localize to the apical side of the epithelium. TJs are composed of complex branching networks of transmembrane proteins [71]. Claudins and occludins are TJ proteins that facilitate cell-to-cell interactions through their extracellular domains. TJs also require intracellular anchoring proteins such as ZO-1 [71]. In salivary tissue from SS patients, the apical localization of ZO-1 and occludin is maintained, but the expression of both is decreased. In addition, claudin proteins are redistributed to the basolateral surface, as evidence of dysregulated cell polarity [70]. Of note, the pro-inflammatory cytokines IFNγ and tumor necrosis factor α (TNFα) induce TJ disorganization in both human and rat acinar cells that is reminiscent of that seen in SS patients [72, 73]. Both IFNγ and TNFα may be secreted as part of the innate immune response [74]. These data suggest that the loss of TJ integrity in salivary epithelium in SS may be mediated by innate immune activation and this likely contributes to decreased saliva production in SS patients [72].

The histopathologic hallmark of SS is the presence of periductal lymphocytic infiltrates in the salivary gland [75, 76]. Since these cells are in close proximity, interactions between the epithelium and immune cells likely contribute to disease pathogenesis. In fact, both cultured SGECs and salivary gland tissue secrete numerous inflammatory mediators and express co-stimulatory markers that shape the immune response, including TLRs, MHC class I and II molecules, B-cell activating factor (BAFF), IL-1, IL-6, and IL-18, TNFα [47, 75, 77]. Moreover, epithelial cell apoptosis is essential for the development of inflammation in a SS mouse model [27]. Finally, data suggest that apoptosis and necrosis of SGECs leads to a vicious cycle of aberrant immune signaling that culminates in the recruitment, activation, and differentiation of B and T cells resulting in chronic salivary inflammation [75]. Therefore, SGEC function is compromised as a result of the inflammatory milieu in SS salivary tissue and salivary epithelium itself contributes directly to the innate immune hyperactivity observed in SS.

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5.4. Natural Killer cells

Historically, NK cells were recognized as key mediators of anti-viral and anti-tumor responses. NK cells express TLRs and communicate with DCs in shaping adaptive immunity [78, 79]. Emerging data show NK cells contribute to SS pathogenesis, although the precise role of these cells in disease remains unclear. Recent work using a mCMV model of SS found NK cells induced CD4+ T cell apoptosis in salivary tissue during chronic infection that was mediated by NK cell expression of TNF-related apoptosis inducing ligand (TRAIL) [80]. Subsequent experiments in *TRAIL* deficient mice revealed that persistent mCMV infection led to salivary gland inflammation reminiscent of SS disease. Moreover, *TRAIL*–/– animals showed reduced tear and salivary flow and diminished anti-Ro autoantibodies [80]. These data suggest that NK cells play a crucial role in mitigating SS development [80].

In contrast to findings in mice, NK cells correlate with salivary gland inflammation in pSS patients [31]. NK cells express NCR3/NKp30, an activating receptor that promotes type II IFN secretion and facilitates cross talk with DCs. Genetic polymorphisms in the promoter region of NCR3/NKp30 are associated with diminished transcription and protection from pSS [31]. Importantly, NKp30 expression and activity are increased in pSS patients as compared to controls. SGECs express NKp30 ligands, activation of which causes secretion of Th1 cytokines [31]. In contrast to findings in salivary tissue, peripheral blood NK cell numbers are diminished, and NK cell killing activity and activating receptor expression is significantly reduced in pSS patients as compared to healthy controls [81].

While the reasons for the disparate findings in mouse models and pSS patients are unclear, the initiation of SS is multifactorial [6, 82]. While viruses are thought to contribute to SS development, the disease etiology is complex (*vide supra*). It is possible that NK cells exacerbate disease in some individuals, but are protective when the disease is driven primarily by viral infection. Alternatively, NK cells may be protective in early disease and pathogenic once disease becomes advanced. Thus, further studies are needed to clarify the role of NK cells in disease initiation and progression.

6. Mechanisms of innate immune activation in SS

6.1. Toll-like receptors

TLRs are crucial for innate immune activation [14]. TLRs are upregulated in salivary tissue and peripheral blood from SS patients and mouse models [25, 77, 83–87]. Therefore, signals transduced by TLR ligation may represent a sustained event in SS pathogenesis. While several TLRs are dysregulated in SS, we will limit our discussion to those that are best characterized in this disease.

6.1.1. Cell surface TLRs: TLR2 and TLR4—TLR2 and TLR4 mediate pathology in many different autoimmune diseases, although their role in SS is incompletely understood [17, 18, 88]. Emerging evidence suggests TLR2 and TLR4 signaling is dysregulated locally and systemically in SS. Peripheral blood mononuclear cells from SS patients stimulated with agonists for TLR2 or TLR4 (peptidoglycan (PGN) or lipopolysaccharide (LPS), respectively) secrete higher levels of the pro-inflammatory cytokine IL-17 as compared to

those from healthy controls [85]. Moreover, both TLR2 and TLR4 transcripts are elevated in salivary tissue derived from SS mouse models and SS patients as compared to controls [77, 86], and stimulation of SGECs with LPS or PGN causes upregulation of the co-stimulatory markers ICAM-1 and MHC-1 [77].

Although TLR2 and TLR4 are functional in SGECs, key features of salivary epithelium that may contribute to SS disease have yet to be examined, including activation of specific signaling pathways and inflammatory cytokine production. It is noteworthy to mention that studies using the human submandibular gland cell line, HSG, ascribe a functional role to TLR4 [83]. However, these cells were recently determined to be a HeLa cell contaminant [89].

Therefore, we performed studies to examine the effects of TLR4 ligation on salivary cells. We sought to determine whether TLR4 was expressed and functional in the human SMG cell line A253 [90]. We examined A253 cells for expression of TLR4 and associated signaling molecules in the presence and absence of LPS stimulation. We found each of these genes are expressed constitutively and transcript levels are unchanged following LPS treatment (Figure 1A). We then assessed expression of TLR4 in A253 cells using flow cytometry. We found TLR4 is expressed on A253 cells, and stimulation with LPS causes a modest increase in surface expression (Figure 1B). We then stimulated cells for 24 hours (h) and performed western blotting for the signaling intermediate MyD88. We found MyD88 was expressed in A253 cells and expression was not affected by LPS treatment (Figure 1C). Finally, we sought to determine whether LPS activated a known downstream target of TLR4, the NFκB signaling pathway. As expected, we observed phosphorylation of NF-κB p65 following incubation with LPS. Maximal p65 phosphorylation was observed 30 minutes post stimulation (Figure 1D). Taken together, these data show human SMG cells express TLR4 and are responsive to the classical TLR4 agonist, LPS.

To determine whether LPS induces inflammation in A253 cells, we cultured cells in the presence or absence of LPS for 24 h. We harvested the supernatant and performed multiplex arrays for inflammatory cytokines and chemokines implicated in SS. We found A253 cells secrete IL-6, IL-12, Monocyte Chemotactic Protein-1 (MCP-1), Regulated on Activation, Normal T Expressed and Secreted (RANTES), and Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) constitutively. Secretion of IL-6, IL-12, RANTES, and GM-CSF was further enhanced following stimulation with LPS ($p = 0.01$, 0.01, 0.005, and 0.0002, respectively) (Figure 2). Although MCP-1 was also increased following LPS stimulation, the difference was not significant ($p = 0.14$). Thus, treatment of human salivary cells with LPS induces release of several inflammatory mediators that are implicated in SS pathogenesis.

Therefore, studies by our group and others show activation of TLR2 and TLR4 induces many of the same pro-inflammatory cytokines and chemokines that are upregulated in SS patients [8, 91–94], and ligation these receptors likely contributes to the chronic inflammatory landscape that drives pathology in this debilitating disease. These findings are consistent with those in other autoimmune connective tissue diseases, as TLR2 and TLR4 agonism exacerbates disease in SLE, RA, and systemic sclerosis [95].

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6.1.2. Endosomal TLRs: TLR3, TLR7, and TLR9—Several studies in mice and humans demonstrate an important role for TLR3 in SS pathogenesis. TLR3 is expressed by salivary epithelium of SS mice and healthy controls and is activated by dsRNA viruses [51]. Administration of the TLR3 agonist polyinosinic:polycytidyic acid (poly(I:C)) to SS-prone mice causes loss of salivary flow and upregulation of numerous inflammatory cytokines and chemokines, including type I IFN. Moreover, sialadenitis is increased in SS-prone mice treated with poly(I:C) as compared to controls, supporting a viral etiology for SS [51, 52] (*vide supra*).

Studies in human cells corroborate those in mice. TLR3 is expressed and functional in primary culture human SGECS, as stimulation of cells with the TLR3 ligand poly(I:C) causes upregulation of ICAM-1, CD40 and MHC class I [77]. Further studies using human salivary epithelium support a role for TLR3 ligation in glandular apoptosis [84, 96]. Importantly, activation of TLR3 expressed by SGECs induces secretion of BAFF and promotes the synthesis of Ro52, an autoantigen included in the diagnostic criteria for SS [97–99]. Data from mouse models and SS patients show that both local and systemic BAFF expression is integral to SS pathogenesis. BAFF is crucial to maintain peripheral B cells and high BAFF levels allow enhanced survival of autoreactive B cells [100, 101]. The BAFF transgenic mouse model develops loss of salivary flow and salivary gland inflammation similar to SS patients [102]. Moreover, BAFF levels are elevated in pSS patients and are even higher than those observed in patients with SLE or RA [102]. Finally, evidence suggests that elevated BAFF levels contribute to loss of B cell tolerance and the development of B cell lymphomas in SS patients [103, 104].

The role of other endosomal TLRs in SS is less well understood. TLR7 and TLR9 are elevated in SS salivary tissue [87]. A recent study found that there are no differences in B cell expression of TLR7 and TLR9 between SS patients and healthy controls [105]. However, B cells from pSS patients secrete increased amounts of several pro-inflammatory cytokines when stimulated with agonists for TLR7 or TLR9, suggesting that TLR signaling in B cells may contribute to the chronic inflammatory landscape observed [106]. Interestingly, a recent study found that when pSS patients were segregated on the basis of type I IFN expression, pDCs and monocytes from the IFN-expressing subset expressed elevated levels of TLR7, whereas IFN negative patients expressed normal levels of TLR7 [107]. Thus, these studies suggest that endosomal TLRs contribute to SS and may be particularly important in IFN positive patients, although further work is required to understand the way in which TLR signaling pathways mediate pathology in SS.

6.1.3. Activation of TLRs in SS—Although TLRs are upregulated in salivary tissue from SS patients and mouse models [25, 77, 83–87], it is not clear whether TLR activation occurs as a result of endogenous or exogenous triggers, and whether these are produced within exocrine tissue or are expressed systemically. Because high rates of chronic bacterial infection are not reported for the adult SS population, it is unlikely that the ligands that activate TLRs in SS are primarily pathogen-derived. Thus, it is probable that alternative mechanisms drive TLR activation in SS, such as sterile inflammation mediated by host-derived ligands.

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These endogenous ligands, termed damage or danger-associated molecular patterns (DAMPs), include heat shock and ECM proteins [108]. Inflammation caused by DAMPs is considered to be "sterile," because it arises as a result of tissue damage and is independent of microbial infection [108, 109]. Molecules that serve as DAMPs are normally sequestered from the immune system. However, when tissue integrity is compromised they are released in soluble form, thereby allowing for TLR engagement. Numerous studies show activation of TLRs by DAMPs has pathologic consequence in autoimmunity [95]. Specifically, the ECM molecules biglycan and tenascin-c are implicated in SLE and RA pathogenesis, respectively [16, 18]. While DAMP-mediated inflammation is not well studied in SS, it is likely an important driver of inflammation [110]. The ECM molecules laminin, fibronectin, and heparin sulfate are dysregulated in SS salivary tissue, which may result in heightened TLR signaling [25, 111, 112]. Thus DAMP-induced inflammation may contribute to innate immune activation that is characteristic of SS [110].

6.2. Inflammasome-mediated inflammation

The inflammasome is a key component of the innate immune system. Inflammasomes are activated by microbial components, toxins, and mediators that are released following cellular damage [113]. Inflammasome assembly is dependent on NLR family members [114]. There are several types of inflammasomes that are named according to the specific NLR protein utilized, such NLRP1, NLRP3, and AIM2 [115]. Upon ligation, inflammasomes activate caspase proteins, ultimately culminating in the secretion of IL-1 and IL-18 [115]. Of significance to SS pathogenesis, the NLRP3 inflammasome is activated by concomitant stimulation of TLRs and purinergic $P2X_4$/$P2X_7$ receptors [15]. Inflammasome-related genes are elevated in human SS salivary tissue, including $P2X_7$, NLRP3 and caspase-1, and this expression correlates with focal lymphocytic sialadenitis and the presence of anti-Ro autoantibodies [116]. Corroborative studies in SS mouse models show capase-1 is elevated and active in SS salivary tissue in early disease [25, 117]. Both IL-1 and IL-18 are upregulated in murine and human SS, indicating possible inflammasome hyperactivity in disease [25, 63, 116, 118–121]. Finally, studies in *$P2X_7$ receptor* (*$P2X_7R$*) deficient mice show that these animals are protected from salivary inflammation, and local delivery of a $P2X_7$R agonist to *$P2X_7R$* sufficient mice induces salivary inflammation [122]. Taken together, these studies suggest that inflammasome activation in salivary tissue may be a key event in SS pathogenesis.

6.3. Noncoding RNAs

Emerging evidence supports a role for noncoding RNAs in SS, as both short and long noncoding RNAs (lncRNAs) are potent regulators of innate immunity [123, 124]. LncRNAs function at every level of gene regulation [125, 126], and are implicated in many different pathoses ranging from autoimmunity to cancer [127, 128]. LncRNAs regulate many aspects of innate immunity including cytokine and chemokine expression, apoptosis, and antigen presentation [129]. While the role of lncRNAs in SS is largely speculative at present, a recent study profiled these RNAs in the salivary tissue of pSS patients and healthy controls. This study identified more than 1000 lncRNAs that were differentially expressed in pSS salivary tissue. Of note, expression of specific lncRNAs showed strong correlations with SS disease manifestations including the presence of rheumatoid factor (RF), anti-La (SSB)

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autoantibodies, immunoglobulin (Ig) A, and IgM titers [130], suggesting a pathogenic role for certain lncRNAs in disease.

MicroRNAs (miRNAs) are short non-coding RNAs that play important regulatory roles in post-transcriptional gene expression. They act by inhibiting gene expression or by promoting gene degradation [131]. It is estimated that miRNAs regulate between 30 and 90% of genes in the genome, as a single miRNA may regulate hundreds of genes [132, 133]. Aberrant miRNA expression is implicated in numerous autoimmune diseases including SS [132–136].

A study using minor salivary gland tissue from pSS patients identified miRNAs that are strongly associated with sialadenitis [134]. Significantly, this study revealed 9 miRNAs that are differentially expressed in pSS patients with reduced salivary production [134]. More recent work shows several miRNAs are increased in minor salivary gland tissue, SGECs, monocytes and peripheral blood mononuclear cells from SS patients [137–142]. Importantly, several studies suggest miRNAs contribute to innate immune dysregulation in SS, as numerous miRNAs are elevated in monocytes derived from SS patients as compare to controls and miRNA-target pathway predictions suggest these may suppress TGFβ signaling [142]. Moreover, the miRNA MiR-146a shows increased expression in both glandular tissue and peripheral blood mononuclear cells of SS patients, and overexpression of MiR-146a in the human monocyte cell line THP-1 diminishes pro-inflammatory cytokine secretion, suggesting a possible regulatory role for this miRNA in SS pathogenesis [137]. Thus, while studies point to a functional role for noncoding RNAs in disease pathogenesis, further work is needed to determine the means by which these regulate the innate immune response in SS.

7. Therapeutic Approaches Targeting Innate Immunity May Be Efficacious

Many therapeutics that reduce activation of the innate immune response are currently in clinical trials for the treatment of RA, SLE, and inflammatory bowel disease (IBD) [95, 108, 143–145]. A summary of emerging innate immune drug targets, effects and study phase is provided in Table 1 [95, 144, 146–158]. Given the centrality of innate-driven inflammation in SS, inhibition of receptors and pathways that mediate this dysregulation may constitute a successful strategy to reduce local and systemic pathology in this disease.

Inhibition of TLR-mediated activation holds therapeutic promise, as drugs that target TLR2 and TLR4 directly are in clinical trials for several autoimmune diseases [95]. In addition, blockade of TLR signaling intermediates show promise for the treatment of RA and IBD [108]. These drugs may also be efficacious in the treatment of SS, as these receptors and pathways are implicated in both local and systemic disease ([77, 85] and Figure 2). Therefore, targeting of TLRs may prevent progressive systemic inflammation and preserve glandular function.

Blockade of cytokines generated by innate immune interactions may also mitigate SS disease manifestations. While pSS patients commonly experience salivary and lacrimal dysfunction, fatigue is also a common disease manifestation [159]. Importantly, a recent randomized clinical trial examined the effect of IL-1 inhibition on fatigue in 26 patients with pSS. While this study did not achieve its primary endpoint, more patients that received IL-1

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blockade saw a 50% reduction in fatigue as compared to those that received the placebo [156], suggesting inhibition of IL-1 may be beneficial in SS. Moreover, studies are ongoing to evaluate the efficacy of IL-6 and BAFF inhibition in pSS patients [152]. Given the importance of these cytokines in B cell maturation and survival [100, 101, 160], inhibition of such may diminish pathogenic autoantibody production and may even reduce malignant transformation of autoreactive B cells.

While systemic administration of drugs that inhibit innate immune activation may be necessary for some patients with SS, local delivery of therapeutics directly to the salivary tissue may be beneficial for others. Systemic drug administration will likely reduce inflammation, but will also attenuate the host response to pathogens. Therefore, local delivery of drugs may be advantageous, as the salivary tissue could be targeted without widespread immunosuppression. Accordingly, salivary duct cannulation is an efficacious approach in animal models and further studies are needed to determine whether this approach is feasible for SS patients [161, 162]. Thus, therapeutics that target local or systemic innate immune hyperactivity will likely result in improved patient management and amelioration of SS disease.

8. Conclusion

In summary, the innate immune response is crucial for SS pathogenesis, as it is implicated in disease initiation, chronicity, and likely contributes to the development of B cell lymphomas. Numerous cell types in peripheral blood as well as in exocrine tissue participate in the hyperactive innate immune response in SS. Emerging data suggest that noncoding RNAs also mediate innate immune dysfunction in the context of SS. Therefore, therapeutics that target innate immune inflammation will likely prove an effective therapeutic strategy for patients afflicted with this debilitating disease.

Acknowledgments

Funding for this study was provided by the University at Buffalo School of Dental Medicine Start-up Funds (JMK).

Abbreviations

ANA	Anti-nuclear antibodies
BAFF	B-cell activating factor
CMV	Cytomegalovirus
DAMP	Damage or danger-associated molecular pattern
DC	Dendritic cell
EBV	Epstein Barr virus
ECM	Extracellular matrix
HCV	Hepatitis C virus

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GM-CSF	Granulocyte macrophage colony-stimulating factor
IBD	Inflammatory bowel disease
IFN	Interferon
LncRNA	Long noncoding RNAs
LPS	Lipopolysaccharide
mCMV	Murine cytomegalovirus
MCP-1	Monocyte chemotactic protein-1
MiRNA	Micro RNA
NK	Natural killer
NLR	Nucleotide-binding oligomerization domain (NOD)-like receptor
PAMP	Pathogen-associated molecular pattern
pDC	plasmacytoid dendritic cell
PGN	Peptidoglycan
Poly(I:C)	Polyinosinic:polycytidyic acid
PRR	Pattern recognition receptor
pSS	primary Sjögren's syndrome
RA	Rheumatoid arthritis
RANTES	Regulated on activation, normal T expressed and secreted
SGEC	Salivary gland epithelial cell
SLE	Systemic lupus erythematosus
SMG	Submandibular gland
SS	Sjögren's syndrome
sSS	Secondary Sjögren's syndrome
TJ	Tight junction
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis inducing ligand

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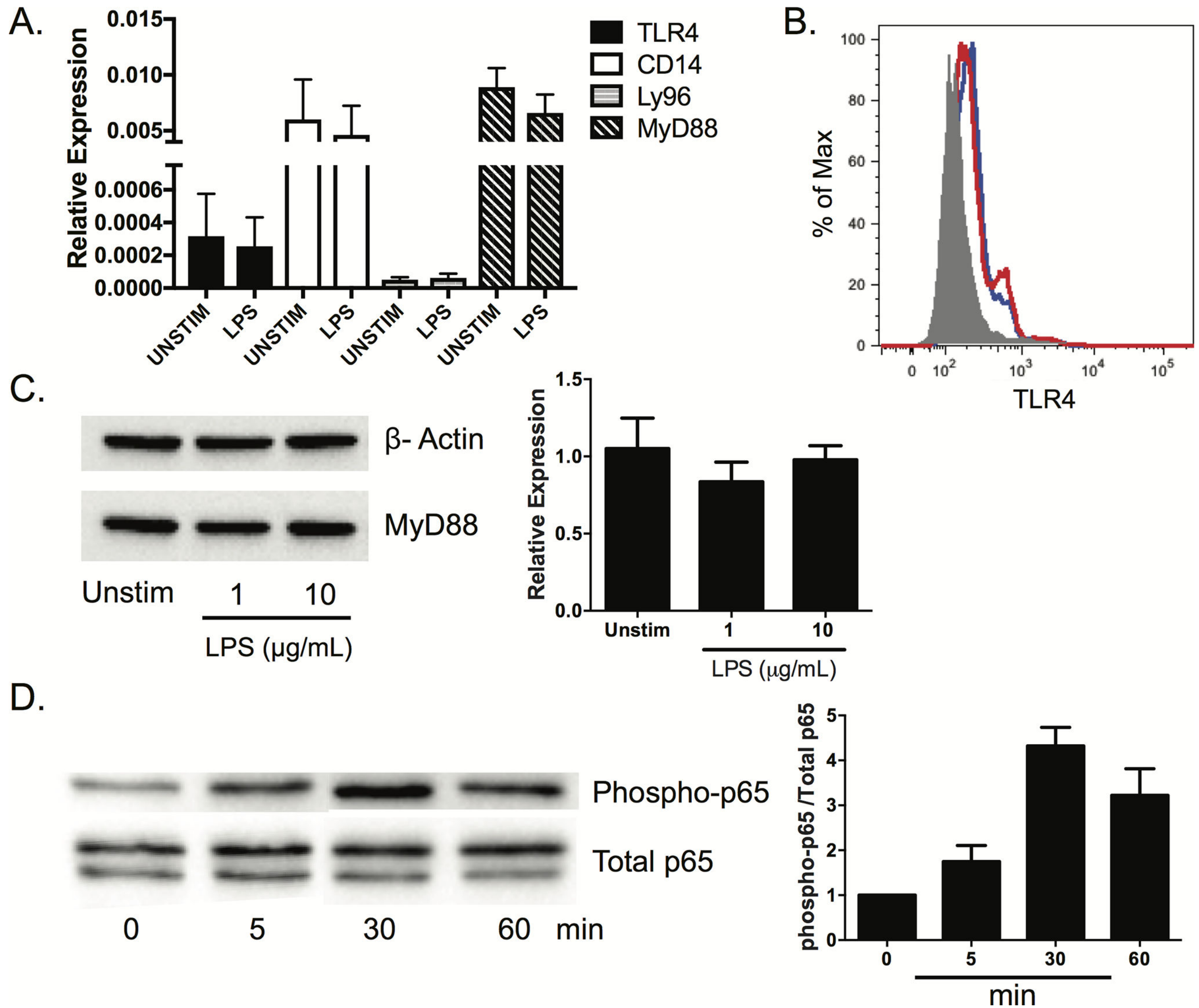

Figure 1. LPS activates TLR4 signaling in A253 cells

A253 cells were purchased from American Type Culture Collection. Cells were cultured in McCoy's 5A (Modified) Media containing 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in 6 well plates. (A) A253 cells (3.5 × 10^5) were harvested, and relative TLR4, Ly96, CD14, and MyD88 transcript levels were quantified by real time PCR. Each sample was analyzed in duplicate and normalized expression relative to GAPDH is shown. (B) A253 cells were harvested and cultured in the presence or absence of LPS (10 μg/mL) derived from *Salmonella typhimurium* for 24 h. Tissue was fluorescently stained with isotype control (grey shading) or TLR4 antibody. The red line represents unstimulated cells and the blue line indicates LPS treated cells. (C) A253 cells (3.0 × 10^5) were incubated in the presence or absence LPS (10 μg/mL) for 24 h. Cells were lysed and western blots performed for MyD88. All samples were normalized to GAPDH. (D) A253 cells (3.0 × 10^5) were stimulated with LPS (10 μg/mL) for the indicated times. Phosphorylated p65 was normalized to total p65 levels. Results of at least three independent experiments are shown (min = minutes).

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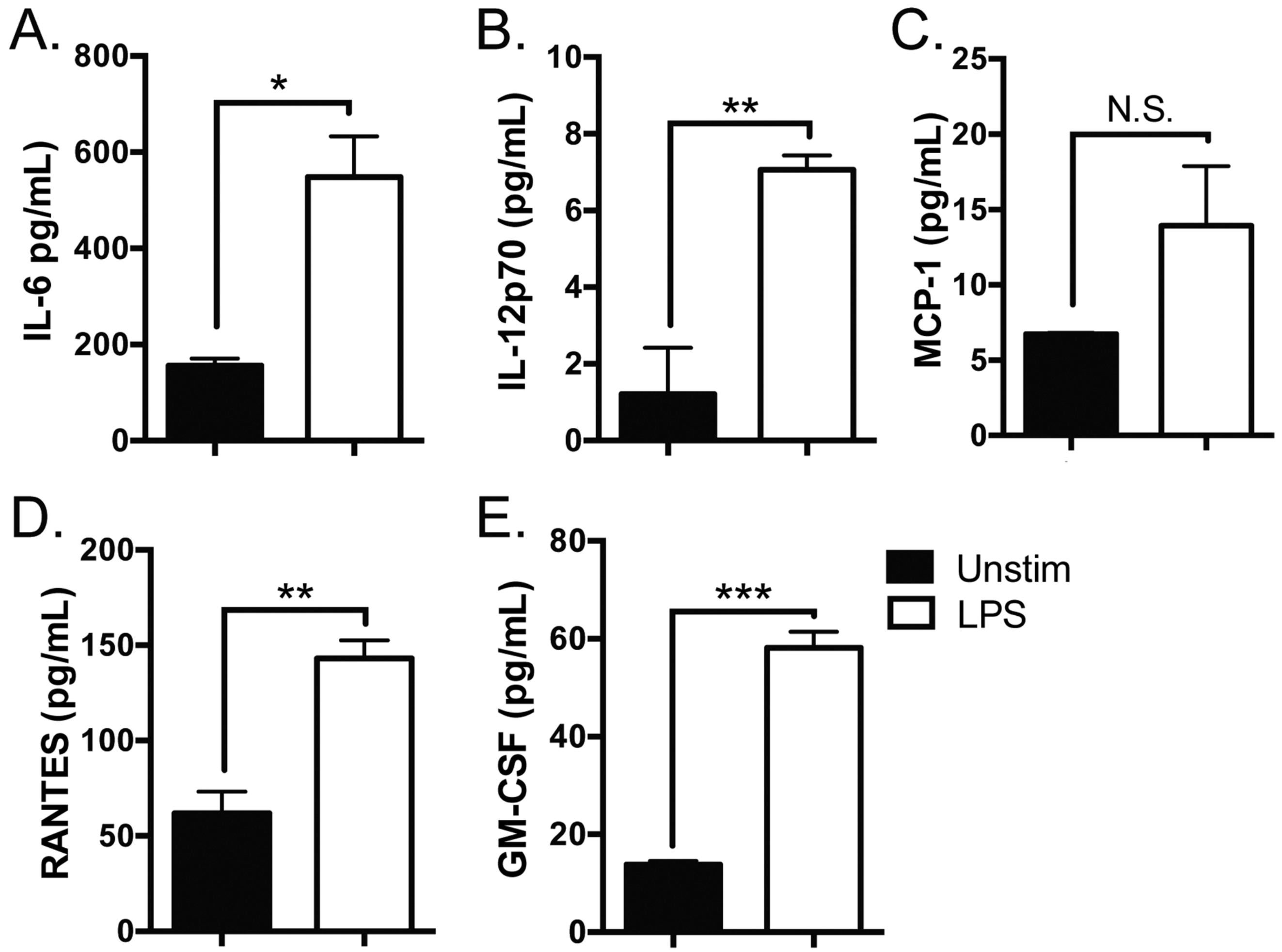

Figure 2. LPS induces inflammatory cytokine secretion in A253 cells

A253 cells (3.0×10^5) were cultured in the presence or absence of LPS (10 μg/mL) for 24 h, and the supernatant harvested. (A) IL-6, (B) IL-12, (C) MCP-1, (D) RANTES, and (E) GM-CSF were assessed by multiplex array. All samples were analyzed in triplicate and results of three independent experiments are shown (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, N.S. = non significant).

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Table 1

Emerging innate immune therapeutic targets for SS and other autoimmune diseases

Drug	Target	Disease	Effect	Study/Trial Type	Refs
TLR pathway inhibitors					
DV1079 (IRS 954)	TLR7/TLR9	SLE	Reduced autoantibody levels, proteinuria and kidney damage in a murine model	Pre-clinical	[95]
ML 120B	IKK-2	Murine arthritis	Reduced inflammation	Pre-clinical	[147]
PHA-408	IKK-2	Human synovial fibroblasts and rat arthritis models	Reduced inflammation	Pre-clinical	[148]
ST2825	MyD88	SLE	Blockade of antibody production *in vitro*	Pre-clinical	[149]
VGX-1027	p38 MAPK/NF-κB	RA and SLE	Improved clinical and histopathological disease signs in a murine model of lupus	Phase I	[146, 157,158]
Cpn10 (heat-shock protein 10)	TLR4	SLE and MS	Decrease in inflammatory cytokines	Phase II	[95, 146, 150]
RDP58	MyD88/IRAK4/TRAF6	Ulcerative colitis	Improvement in histology scores	Phase II	[95, 144]
IMO-8400	TLR7/TLR8/TLR9	Plaque Psoriasis	Decrease in psoriasis area and severity index	Phase II	[146]
MIS416	TLR9/NOD2	MS	Study results unavailable	Phase I & II	[146, 151]
Cytokine Blockade					
Infliximab	TNFα	SS	Improvement in visual analog score, fatigue, and dryness	Phase II	[152]
Etanercept	p75 TNFR	SS	Subjective or objective improvement in 2 out of 3: dry eyes, dry mouth, and IgG levels	Phase II	[146, 152]
Belimumab	BAFF	SS	Improvement in EULAR SS Disease Activity Index and EULAR SS Patient Reported Index scores	Phase II	[146, 152, 153]
VAY736	BAFF-R	SS	Study results unavailable	Phase II	[146, 155]
Anakinra	IL-1R	SS	Reduced fatigue	Phase II	[146, 156]