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Early BAFF receptor blockade mitigates murine Sjögren's syndrome: Concomitant targeting of CXCL13 and the BAFF receptor prevents salivary hypofunction

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ABSTRACT

Sjögren's syndrome (SS) is a debilitating autoimmune disease. Patients with SS may develop xerostomia. This process is progressive, and there are no therapeutics that target disease etiology. We hypothesized BAFF receptor (BAFFR) blockade would mitigate SS disease development, and neutralization of CXCL13 and BAFF signaling would be more efficacious than BAFFR blockade alone. We treated NOD/ShiLtJ SS mice with soluble BAFF receptor (BAFFR-Fc) or anti-CXCL13/BAFFR-Fc in combination, prior to the development of clinical disease. Our results show treatment with BAFFR-Fc reduced peripheral B cell numbers and decreased sialadenitis. In addition, this treatment reduced total serum immunoglobulin as well as IgG and IgM specific anti-nuclear autoantibodies. NOD/ShiLtJ mice treated with BAFFR-Fc and anti-CXCL13 antibody were protected from salivary deficits. Results from this study suggest blockade of CXCL13 and BAFFR together may be an effective therapeutic strategy in preventing salivary hypofunction and reducing autoantibody titers and sialadenitis in patients with SS.

1. Introduction

Sjögren's syndrome (SS) is an autoimmune disease in which the immune system targets exocrine gland tissue [1]. Both the adaptive and innate immune systems are crucial to the progression of SS [2]. Inflammatory cells are observed in salivary and lacrimal tissue, and this lymphocytic infiltration may contribute to loss of glandular function [3]. B cell dysfunction is well documented in SS, both locally and systemically. SS is characterized by the presence of numerous autoantibodies, including those directed against Ro (SSA), La (SSB), nuclear autoantigens, and rheumatoid factor (RF) [4,5]. Since the etiology of SS is unknown, there are no therapeutics that target disease pathogenesis. Currently, treatment is palliative, and SS patients may experience significant morbidity related to xerostomia and xerophthalmia. These include loss of teeth due to dental caries, difficulty speaking and chewing, and deficits in vision. Thus, it is important to identify therapies that mitigate inflammation and loss of exocrine secretions in SS patients.

SS is characterized by lymphocytic infiltration of salivary tissue, termed focal lymphocytic sialadentitis (FLS) [3]. In SS, the percentage of the infiltrating salivary gland lymphocytes that are B cells increases with the degree of glandular inflammation [6]. B cells within salivary tissue likely contribute to SS pathogenesis, as they produce autoantibodies [7,8], and differences in immunoglobulin (Ig) repertoires are observed between salivary and peripheral blood B cells [9]. Moreover, memory B cells are increased in the salivary tissue of SS patients [10]. Systemic B cell abnormalities are also observed in SS. For example, there is a decrease in unswitched memory B cells, altered chemokine receptor expression, and evidence for dysregulated B cell development and selection [9,11–13].

Abbreviations: ANA, anti-nuclear autoantibodies; BAFF, B-cell activating factor of the tumor necrosis factor family; BAFFR, BAFF receptor; cLN, cervical lymph node; FLS, focal lymphocytic sialadenitis; M3R, muscarinic 3 acetylcholine receptor; RF, rheumatoid factor; SMG, submandibular gland; SS, Sjögren's syndrome; TACI, transmembrane activator and CAML interactor.

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B cells are regulated by complex cell–cell interactions and signals transduced by soluble mediators. B-cell activating factor of the TNF family (BAFF, also called BLyS, TALL-1, THANK, and zTNF4) is implicated in several autoimmune disorders, including SS [14]. BAFF is secreted mainly by macrophages, monocytes, and dendritic cells, and is also produced by non-myeloid cells such as salivary gland epithelial cells (SGECs) [15,16]. BAFF directs B cell maturation, development, and survival. BAFF also mediates Ig production and class switching [15]. BAFF is upregulated by interferon (IFN)-γ, interleukin (IL)-10 and CD40 ligand (CD40L) produced during inflammation and infection [17]. BAFF is the only cytokine known to activate the BAFF receptor (BAFFR), which is expressed by circulating B and T cells [18,19]. Studies in mice demonstrate a crucial role for BAFF in B cell survival. Accordingly, mice genetically deficient in *BAFF* or *BAFFR* show reduced peripheral B cell numbers [20,21]. Since BAFF plays a central role in maintenance of these B cells, dysregulation of this cytokine contributes to the persistence of autoreactive B cells [22]. It is important to note that *BAFF* transgenic mice develop SS- and lupus-like diseases. Moreover, patients with SS have elevated BAFF levels in salivary tissue, sera, and saliva [14,23–27]. Thus, BAFF is clearly important in SS pathogenesis in both murine models and SS patients.

The chemokine CXCL13 also plays an important role in B cell physiology and is increased in SS. CXCL13 is secreted by follicular stromal cells such as follicular dendritic cells and marginal reticular cells [28]. CXCL13 binds the G protein coupled receptor CXCR5 that is expressed predominantly by peripheral B cells and T follicular helper cells [29]. CXCL13 directs B cell chemotaxis, and is increased in both murine and human SS [30–36]. Of note, blockade of CXCL13 signaling results in a modest reduction in lymphocytic infiltration of salivary tissue in SS mice [30,37]. Thus, these data suggest CXCL13 may be integral to SS pathogenesis.

Since BAFF and CXCL13 both direct B cell function, it is not surprising that these cytokines act synergistically to regulate B cell activity. Studies in humans show BAFF increases the chemotactic response of B cells to CXCL13, and this effect is more pronounced in memory B cells than naïve. Importantly, blockade of BAFFR abrogates this migration [38]. To determine whether BAFFR neutralization alone or in combination with CXCL13 blockade mitigates SS disease development, we inhibited CXCL13 and BAFFR signaling in the NOD/ShiLtJ (NOD) model of SS. Animals were treated prior to disease development continuously until the time that they would normally develop disease. We found that salivary gland inflammation, total serum antibody and ANA specific IgG and IgM autoantibody titers were decreased in animals given BAFFR alone. Animals that received concomitant CXCL13 and BAFFR blockade also exhibited reduced salivary gland inflammation, total serum antibody and ANA specific IgG autoantibody titers. In addition, these animals also had diminished IgM titers and did not lose salivary flow. Results from this study suggest that neutralization of CXCL13 and BAFFR mediated signaling may be an effective therapeutic strategy in SS.

2. Materials and methods

2.1. Mice

Female NOD/ShiLtJ (NOD) mice (age 3 weeks) were purchased from Jackson Labs. All animals were cared for and handled in accordance with NIH and IACUC guidelines.

2.2. Serum collection

For murine studies, sera were harvested immediately following euthanasia. Blood was collected by retro-orbital eye bleed or cardiac puncture following euthanasia in accordance with IACUC protocols.

2.3. Assessment of saliva production

Pilocarpine HCl (0.3 mg/100 μL) was injected intraperitoneally (Sigma-Aldrich), and saliva was collected for 10 min. Saliva was immediately placed on ice, centrifuged briefly, and quantified using a pipette. Saliva was stored at −80 °C until use.

2.4. CXCL13 and BAFFR neutralization

2.4.1. Reagents

Anti-CXCL13 antibody (MAb 5378) and soluble BAFFR-Fc were generously provided by Vaccinex. IgG2a isotype control and anti-CXCL13 antibodies were generated and validated as previously described [30]. To make the soluble BAFFR-Fc reagent, the murine BAFFR gene was obtained from Open Biosystems (accession #BC104127, clone ID: 40044559). PCR primers were designed to amplify the region corresponding to amino acid residues 10–71. The resultant PCR product was cloned into an expression vector encoding a signal peptide, and was placed in-frame with a 3′ amino acid linker sequence followed by the mouse IgG2a Fc domain (hinge-CH2-CH3). CHO cells were transfected with this construct using polyethylenimine max transfection reagent (Polysciences, Inc.), and the culture supernatant harvested. BAFFR-Fc was purified by affinity chromatography using POROS MabCapture protein A resin (Life Technologies). The molecular weight of BAFFR-Fc is approximately 32 kDa, and the theoretical isoelectric point is 5.76. The protein was eluted with 0.1 M glycine (pH 2.7). We performed buffer-exchange on the BAFFR-Fc protein into 20 mM Tris (pH 8.0) using PD-10 desalting columns (GE Healthcare Life Sciences). The protein was then loaded onto POROS HQ anion exchange resin (Life Technologies) and eluted fractions with successive 95 mM, 140 mM, 165 mM, and 1 M NaCl step gradients. The 140 mM elution fraction was used for further processing. BAFFR-Fc was concentrated using Amicon Ultra-15 30 K filters (Millipore) and buffer-exchanged into PBS (pH 7.2) using PD-10 desalting columns. The protein was passed through a Mustang E filter (Pall Corporation) to remove endotoxin. Finally, the product was sterile-filtered through a 0.2 μm syringe filter (Pall Corporation). A PyroGene Recombinant Factor C endotoxin assay (Lonza) was performed to verify endotoxin removal. Size exclusion chromatography was carried out using a TSKgel G3000SWXL column (TOSOH Bioscience) to confirm removal of aggregates and contaminants as additional quality control steps.

2.4.2. Validation of BAFFR-Fc

2.4.2.1. BAFF ELISAs. Microtiter plates were coated with murine BAFF (0.5 μg/mL, R&D systems). Serial dilutions of commercially available recombinant murine BAFFR-Fc (R&D systems) or BAFFR-Fc (Vaccinex) were performed. Biotinylated goat anti-mouse BAFFR (100 ng/ml, R&D systems) was added, followed by incubation with Streptavidin-HRP (10 μg/mL, Jackson ImmunoResearch). ELISAs were developed with TMB substrate and read at 450/570 nm.

2.4.2.2. Proliferation assays. Splenocytes were isolated from 8 to 10 week old female C57BL/6 mice and cultured in 96-well microtiter plates (1×10^5 cells per well) in RPMI1640 with 10% FBS. The cells were treated with recombinant murine BAFF (0.01 μg/mL, R&D systems) and goat anti-mouse IgM (10 μg/ml Jackson ImmunoResearch) in the presence or absence of commercially available recombinant murine BAFFR-Fc (10 μg/ml, R&D systems) or BAFFR-Fc (10 μg/ml, Vaccinex) for 4 days. Cells were incubated with alamar blue at 37 °C for 4 h, and fluorescence read at 530/590 nm.

2.4.3. Administration protocol

NOD females were injected with 100 μg each of isotype control IgG2a antibody, BAFFR-Fc, and anti-CXCL13 antibody/BAFFR-Fc in combination. Animals were injected three times weekly for 12 weeks beginning at 4 weeks of age. Antibody was administered by intraperitoneal injection. All animals were euthanized at 16 weeks of age. Sera and saliva were collected as described above. Spleen, cervical lymph nodes (cLNs), and salivary tissue were harvested.