Sjögren’s syndrome (SjS)-like disease of mice: the importance of B lymphocytes and autoantibodies

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1. ABSTRACT

Sjögren’s syndrome (SjS) is a systemic autoimmune disease in which an immunological attack against the salivary and lacrimal glands results, respectively, in severe dry mouth and dry eye diseases. Although a CD4+ T lymphocyte population is an integral component in the pathogenesis of SjS, recent studies have focused on the importance the B lymphocyte plays in both the pre-clinical and clinical phases of the disease process. To understand the molecular and cellular mechanisms involved in SjS, numerous mouse models that mimic major clinical manifestations of the human disease have been developed. Studies have begun to define the genetics, the nature of the autoimmune response towards the salivary and lacrimal glands, as well as the possible mechanisms for effecting glandular dysfunction, thereby establishing insights to new intervention therapies. Not surprisingly, the B cell is taking center stage. Here, we present an in-depth discussion of how B cell populations may be involved in orchestrating or determining exocrine gland dysfunction.
B cells and autoantibodies in Sjögren’s syndrome

2. INTRODUCTION

2.2. SjS in humans

2.2.1. General features - what constitutes SjS?

SjS is a human disease characterized by exocrine gland dysfunction resulting from an autoimmune response (see (1-10)). Primary SjS is characterized generally by a chronic autoimmune attack against both the lacrimal and salivary glands, while secondary SjS is marked by a similar process in the presence of another autoimmune disease, most often rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or scleroderma (1, 11). In addition to the apparent primary sites of SjS, i.e., the lacrimal and salivary glands, other tissues that may become affected include the entire GI tract, skin, the lungs, the vasculature, kidneys, bladder and the vagina. Involvement of the musculature often leads to fibromyalgia-like symptoms and patients often complain of both chronic physical and mental fatigue. As with many autoimmune connective tissue diseases, there exists a sexual dimorphism in SjS with women affected 10- to 20-times more frequently than men, suggesting a role for sex hormones in disease susceptibility, possibly related to the relative balance between estrogen and androgen (12-16).

While keratoconjunctivitis sicca (dry eyes) and xerostomia (dry mouth) are assessed by specific tests for changes in exocrine gland flow rates and biochemical/enzymatic changes in protein composition and function, the recent European-American Consensus Group criteria for diagnosis of SjS strongly recommends detection of infiltrating lymphocytes within a minor salivary gland, determined by a histopathological analysis of a labial gland lip biopsy (17). Far less commonly performed is an analysis of a lacrimal gland biopsy (18). Nevertheless, based on studies in both humans and animal models, infiltrates appear generally as peri-ductal foci within the glandular architecture of the lacrimal and salivary glands consisting of CD4⁺ T cells, CD8⁺ T cells, B cells and macrophages. T cells are thought to exhibit a preferential (or restricted) antigen receptor repertoire, while the overall infiltrating cells express various cytokines (including IL-1β, IL-6, IL-10, TNF-alpha, and IFN-gamma) whose significance in the autoimmune pathology has yet to be determined (19-22). Serological evaluations have shown the frequent presence of rheumatoid factor, elevated immunoglobulin levels (hypergammaglobulinemia) and anti-nuclear autoantibodies, especially anti-SS-A/Ro and anti-SS-B/La antibodies. Additional autoantibodies react with numerous cellular components of the exocrine glands, probably representing spreading epitopes (23-33). Of late, intense interest has revolved around antibodies to the muscarinic acetylcholine type-3 receptor (M3R) (34-42).

2.1.2. SjS – is there a genetic predisposition?

SjS shows a weak tendency toward familial aggregation which, together with the presence of common autoantibodies in SjS patients, suggests that genetic factors are operative in disease susceptibility (43). While environmental triggers responsible for initiating SjS are unclear, intrinsic genes contributing to disease susceptibility are thought to be critical for development of autoimmunity. Although the best established hereditary markers in disease susceptibility are those encoded by genes of the major histocompatibility complex (MHC), previous associations of SjS with HLA-DR most likely reflect the presence of anti-nuclear SS-A/Ro and SS-B/La autoantibodies (44). Furthermore, studies from different ethnic groups have yielded inconsistent results, suggesting only weak MHC class II associations for SjS (45). More importantly, family studies suggest autosomal genes not linked to HLA and/or immunoglobulin genes are an important element of autoimmune exocrinopathy susceptibility (46), a conclusion consistent with studies in NOD mice, an animal model of SjS, where non-MHC genes clearly control susceptibility (47-49).

2.1.3. SjS – what are its consequences?

SjS is only one of many dry mouth/dry eye diseases, but at the same time considered one of the more severe forms of these conditions. Xerostomia and keratoconjunctivitis sicca result respectively from basic changes in the saliva and tear flow rates, the composition of saliva and tears, and/or combinations thereof. Underlying causes of xerostomia include the natural aging process, use of medications, asthma and mouth breathing, chemotherapy, radiation therapy, autoimmune attack against secretory tissues/glands of the mouth, thyroid dysfunction, kidney dialysis and/or stroke. Likewise, underlying causes of xerophthalmia include the natural aging process, physical injury, surgical procedures, meibomian gland dysfunction and/or autoimmune attack against one or more of the multiple secretory tissues/glands of the eye. Because saliva is a critical factor in oral health, patients with dry mouth can present with increased caries and tooth decay, increased oral microbial infections, halitosis, cracked lips and bleeding gums, taste disturbances, difficulty in eating and swallowing, and even difficulty in talking. In addition, patients complain of dysphagia, epigastric pain and dyspepsia due in part to decreased saliva, low levels of epidermal growth factor (EGF), as well as poor nutritional uptake. Adding insult to injury, between 4-10% of patients with SjS will develop non-Hodgkin’s malignant B cell lymphomas (50-53). While considerable emphasis is placed on manifestations resulting from xerostomia, the manifestations from xerophthalmia brought on by decreased tear fluid secretion in conjunction with an increase in tear fluid evaporation are just as debilitating. Complaints from patients with dry eyes include burning, grittiness, itching, fatigue, blurred vision and, surprisingly, watery eyes resulting from increased reflex tear secretions. Over time there is eye surface deterioration and ulceration, leading to small red-appearing eyes with crusts in the ciliae, debris in the tear film, meibomitis, mucus strands adhering to the corneal surfaces, reduced light reflectivity and irregular blinking. In general, these manifestations of dry mouth and dry eyes, especially in SjS, appear to correlate with a loss of exocrine cell mass, an onset of exocrine cell senescence or refractivity, and loss of neural regulation of ocular secretory function (18).

While diagnosis of SjS is based, in part, on subjective patient symptoms, a number of specific clinical tests are also critical. For xerostomia, these include: (a) dessicated buccal epithelium, (b) reduced production of

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either stimulated or unstimulated saliva flow, (c) reduced amylase activity, (d) reduced EGF levels, (e) detection of anti-nuclear autoantibodies (ANAs), especially anti-SS-A/Ro and anti-SS-B/La, and (f) presence of leukocyte foci within minor salivary gland biopsies. For keratoconjunctivitis sicca, these include: (a) break-up time test which measures the ability of pre-corneal tear films to maintain their integrity, (b) Schirmer-1 test which measures tear flow rates, (c) Rose-Bengal (or lissamine green) dye test which shows staining of desiccated epithelial cells lacking mucous protection, (d) lysozyme and lactoferrin enzyme activity measurements, and rarely (e) a lacrimal gland biopsy to determine the presence of leukocyte infiltrates. Despite this battery of tests, both the diagnosis and the underlying mechanisms of autoimmune exocrinopathy are difficult to assess due to the fact that SjS patients usually present when the autoimmune process is at or near its end-stage. Thus, at the present time, a direct cause of SjS remains elusive and correlative, and this limits development of pre-disease biomarkers, intervention therapies, as well as an ultimate prevention and cure for the illness. As a result, it has been important to turn to animal models of SjS, not only to define the pathophysiological processes, but also to identify appropriate biomarkers predictive of SjS.

2.2. Mouse models of SjS

2.2.1. SjS-like disease of mice – is it an antibody-mediated process?

Over the past two decades, a variety of mouse models exhibiting various aspects of SjS, whether spontaneous or experimentally induced, has been intensively investigated in an attempt to identify the nature of this autoimmune disease. Typically, these mouse models show lymphocyte infiltration of the exocrine glands, increased expression of pro-inflammatory cytokines, generation of unique autoantibodies (especially ANAs, anti-alpha-fodrin, and anti-muscarinic acetylcholine type-3 receptor (M3R) antibodies), and eventually decreased saliva flow rates. Strains that have been extensively studied include NZB/NZW F1-hybrids, MRL/lpr, NOD/LtJ and NFS/sldl. More recently, several new strains have been added, including the IQI/Jic mouse and C57BL/6.NOD-Aec1/Aec2 congenic line, as well as the Id3 gene knock-out (KO) mouse, the aromatase gene KO mouse, and the Baff transgenic mouse. A listing of these various models along with their general disease profiles is presented in Table 1. While each strain has been reported to resemble features of SjS in human patients, none recapitulates completely the pathological characteristics of the human disease.

One of the more interesting and well-studied models of SjS is the NOD mouse which closely mimics the human disease (see Table 2). A major strength of this model has been the ability to study a large number of congenic partner gene KO strains, e.g., NOD-scid, NOD.Ifn-gamma-, NOD.IIL2-, NOD.IL4-, NOD.IIl10-, NOD.Igma-, and NOD.Stat6-, permitting investigations into the role of individual genes in the development and onset of SjS-like disease in this model (54-57). Taken together, these studies suggested that the disease could be divided into three distinct consecutive phases (57-60), as presented in Figure 1. In phase 1 (initiation of glandular pathology), a number of aberrant genetic, physiological and biochemical activities associated with retarded salivary gland organogenesis and increased acinar cell apoptosis occur sequentially prior to and independent of detectable autoimmunity (60). In phase 2 (onset of autoimmunity believed to result from the acinar cell apoptosis), leukocytes expressing pro-inflammatory cytokines infiltrate the exocrine glands, establishing lymphocytic foci, first of T cell clusters followed by recruitment of B lymphocytes (61, 62). In phase 3 (onset of clinical disease), loss of salivary and lacrimal gland secretory functions occurs, most likely as the result of IgG subclass autoantibodies reactive with the M3Rs (36, 54, 63, 64). While the onset of SjS-like disease in NOD mice is independent of the appearance of ANAs in the sera, Scofield et al. (65) have recently shown that immunization of BALB/c mice with a peptide derived from the sequence of Ro antigen induces a SjS-like disease, pointing to the importance of individual antibodies in the onset of exocrine gland dysfunction. Interestingly, the recently derived IQI/Jic mouse line appears to mimic the disease profile of the NOD mouse (66).

A critical observation derived from studies with NOD mice is the important role of B lymphocytes in the development and onset of disease. First, NOD.Igmu-/- (mu gene knockout) mice lacking mature B cells fail to develop glandular dysfunction despite exhibiting peripheral T cell activation and considerable T cell infiltration of the salivary and lacrimal glands (64). Second, NOD.IL4-/- mice lacking the ability to produce the cytokine IL-4 also fail to develop glandular dysfunction despite exhibiting both T and B cell activation in the periphery, T and B cell infiltration of the salivary and lacrimal glands, and the production of ANAs (56). Third, NOD.IL4-/- (Il4 gene knockout) mice fail to produce IgG1 antibodies reactive with the muscarinic acetylcholine receptors, a deficiency that appears to be circumvented following an adoptive transfer of T cells isolated from NOD.Igmu-/- mice or injections of recombinant IL-4 cytokine protein (56). Considering these observations, it is not surprising that passive transfer of serum IgG from human SjS patients or from diseased NOD mice, but not healthy human subjects or pre-diseased animals, into NOD.Igmu-/- mice results in a temporary loss of saliva secretion (64). Furthermore, serum IgG fractions from human SjS patients, but not healthy human subjects, can competitively inhibit the binding of the muscarinic receptor agonist, [3H]-quinuclidinyl benzilate, to salivary gland membranes (64).

The disease profile observed in NOD.IL4-/- mice raises several questions pertinent to the role of the B lymphocyte in development and onset of SjS-like disease. First, do B lymphocytes act as antigen-presenting cells (APCs) for initiation of the SjS-like autoimmune response, as has been proposed for initiation of type 1 diabetes in NOD mice? Second, do the B lymphocyte populations in NOD mice respond abnormally to IL-4, resulting in circumvention of normal homeostatic mechanisms that would prevent over-proliferation, survival and escape from
### Table 1. Mouse strains used in the study of Sjögren’s Syndrome

<table>
<thead>
<tr>
<th>Mouse strains</th>
<th>Characteristics</th>
<th>Disease Manifestations / Phenotypes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZB/W ([NZB x NZW]F1]</td>
<td>Naturally occurring mouse model by crossing NZB and NZW</td>
<td>Lacternal gland involvement that shows greater percentage of B cells compared with MRL/pr mouse</td>
<td>207</td>
</tr>
<tr>
<td>MRL/pr</td>
<td>Mutation in /pr that encodes Fas protein</td>
<td>Diffuse lymphocytic infiltration of glands, presence of autoantibodries (against ssDNA, RNP, IgG), but no loss of secretion or destruction of anti-M3R antibodies</td>
<td>77</td>
</tr>
<tr>
<td>NOD</td>
<td>Spontaneous insulitis &amp; diabetes</td>
<td>SjS-like disease phenotype with loss of secretion, anti-M3R antibodies, &amp; focal lymphocytic infiltration</td>
<td>208, 209</td>
</tr>
<tr>
<td>NOD/1.1J</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• NOD-congenics</td>
<td>NOD with H-2d from C57BL/10</td>
<td>SjS-like disease phenotype without diabetes</td>
<td>47, 209</td>
</tr>
<tr>
<td>• NOD.B10-H2b</td>
<td>NOD with H-2b from C3H.Q</td>
<td>Severity of saladenitis greater than that of NOD</td>
<td>210, 211</td>
</tr>
<tr>
<td>• NOD.P</td>
<td>NOD with H-2f from C3H.NB</td>
<td>Severity of saladenitis lesser than that of NOD</td>
<td>210, 211</td>
</tr>
<tr>
<td>• NOD-scid</td>
<td>Homozygous mutation in scid locus (no functional lymphocytes)</td>
<td>No disease phenotype, but abnormal organogenesis</td>
<td>57, 212</td>
</tr>
<tr>
<td>• NOD.Igmu−</td>
<td>No functional B-lymphocytes</td>
<td>SjS-like disease phenotype, but with normal salivary flow</td>
<td>64</td>
</tr>
<tr>
<td>• NOD.IL4</td>
<td>No IL-4 cytokine production</td>
<td>SjS-like disease phenotype, but with normal salivary flow</td>
<td>54, 56</td>
</tr>
<tr>
<td>• NOD.IFN-gamma−</td>
<td>No IFN-gamma production</td>
<td>Lack of a SjS-like disease phenotype, normal organogenesis and normal salivary &amp; lacrimal gland function</td>
<td>55</td>
</tr>
<tr>
<td>• NOD.IFN-gammaR−</td>
<td>No IFN-γ receptor</td>
<td>Lack of a SjS-like disease phenotype, normal organogenesis and normal salivary &amp; lacrimal gland function</td>
<td>55</td>
</tr>
<tr>
<td>• NOD.scid.IFN-gamma−</td>
<td>No functional lymphocytes or IFN-gamma</td>
<td>Lack of a SjS-like disease phenotype, normal organogenesis and normal salivary &amp; lacrimal gland function</td>
<td>55</td>
</tr>
<tr>
<td>C57BL/6.NOD-Aec1:Aec2</td>
<td>C57BL/6 carrying Aec1 (Id3) genetic region on Chr 3 and Aec2 (Idd5) genetic region on Chr 1</td>
<td>SjS-like disease phenotype in a C57BL/6 genetic background</td>
<td>48, 49</td>
</tr>
<tr>
<td>IQU/jc</td>
<td>Inbred strain originating from ICR with SjS-like disease in the absence of diabetes</td>
<td>Anti-kallikrein-1 and -13 antibodies</td>
<td>65, 161</td>
</tr>
<tr>
<td>NFA/sld</td>
<td>Autosomal recessive gene with sublingual gland differentiation arrest</td>
<td>Anti-alpha fodrin antibodies</td>
<td>213</td>
</tr>
<tr>
<td>Id3 gene KO</td>
<td>No Id3 (basic helix-loop-helix transcription factor) production, a dominant negative inhibitor of gene expression</td>
<td>Impaired TCR-mediated T cell selection, loss of secretion and presence of Anti-Ro and anti-La antibodies</td>
<td>174</td>
</tr>
<tr>
<td>Aromatase gene KO</td>
<td>Estrogen deficiency due to absence of enzyme catalyzing the conversion of testosterone to estradiol</td>
<td>B cell hyperplasia in the BM and spleen, and anti-alpha-fodrin antibodies</td>
<td>214</td>
</tr>
<tr>
<td>Alysophosphafia (aly/aly)</td>
<td>Homogamous mutation in aly (alysophosphafia) gene</td>
<td>Conserved CDR3 in TCR of infiltrating T cells in the lacrimal glands, salivary glands and kidneys</td>
<td>215</td>
</tr>
<tr>
<td>GVHD (graft-vs-host disease)</td>
<td>GVHD induced by the injection of DBA/2 spleen cells into non-irradiated (C57BL/6 × DBA/2) F1 mice</td>
<td>Lymphocytic infiltrations with a majority of T cells</td>
<td>216</td>
</tr>
<tr>
<td>BAFF Transgenic</td>
<td>Over-expression of the B-cell survival factor, BAFF (BLYS)</td>
<td>Lymphocytic infiltrations with a majority of B cells, leading to loss of secretion by 15-17 months of age</td>
<td>67</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Immunized with short peptides of 60 kDa Ro antigen</td>
<td>Lymphocyte infiltration of salivary gland, production of anti-Ro antibodies, and loss of secretion</td>
<td>65</td>
</tr>
</tbody>
</table>

### Table 2. Comparison of general symptoms of Sjögren's syndrome patients and NOD mice

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sjögren’s Syndrome</th>
<th>NOD mice 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal sialadenitis</td>
<td>(Yes) 5</td>
<td>Yes</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased tear &amp; saliva flow rates</td>
<td>Yes</td>
<td>Variable 5</td>
</tr>
<tr>
<td>Altered proteins in tears &amp; saliva</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pro-inflammatory cytokine production</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Anti-Ro/SS-A, Anti-La/SS-B</td>
<td>Yes</td>
<td>Probably not 6</td>
</tr>
<tr>
<td>• Anti-DNA (ANAs)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>• Anti-beta2GPI</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>• Anti-beta-adrenergic receptor</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>• Anti-type-3 muscarinic ACh receptor</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Keratoconjunctivitis sicca</td>
<td>Yes</td>
<td>(?)</td>
</tr>
<tr>
<td>Ocular epithelium desiccation (Rose-Bengal Dye)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Break-up time testing</td>
<td>Decreased 7</td>
<td>(?)</td>
</tr>
<tr>
<td>Lysosome &amp; Lactoferrin activity</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Stomatitis sicca</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Buccal epithelium desiccation</td>
<td>Yes</td>
<td>(?)</td>
</tr>
<tr>
<td>Serine protease activity against PSP</td>
<td>(?)</td>
<td>Yes</td>
</tr>
<tr>
<td>Amylase &amp; EGF activity</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
</tbody>
</table>

4 Discussed in text, 5 Biopsies of lacrimal glands not often performed, 6 Male and female mice differ, depending on severity of disease in glands, males have more severe, lacrimal gland disease, females more severe salivary gland disease, 7 Possibly detected as part of ANAs, but very rare in NOD background mice
negative selective pressures? Third, is there an autoimmune B cell population present in NOD mice that is activated by IL-4 to produce a specific subclass of IgG antibody that can inhibit normal acinar cell function? Whereas sorting out the possibility that the B-cell population can act as APCs might be difficult because the autoimmunity is generally characterized as a type II hypersensitivity (or antibody-mediated) response, recent studies indicate that IL-4 may be important in both survival of M3R (auto)-reactive B cells and in their IgG isotype switching to promote production of IgG1 anti-M3R autoantibody (56). This latter concept is supported by observations from studies using BAFF-transgenic mice in which there is an over-production of BAFF. These BAFF-transgenic mice exhibit enhanced B cell proliferation and survival, as well as altered differentiation patterns, and develop an autoimmune condition resembling SLE (67). Interestingly, by 4 months of age, these mice develop a secondary pathology reminiscent of SjS characterized by severe sialadenitis, decreased saliva production, and destruction of the submandibulary glands. Infiltrates within the salivary glands of BAFF-transgenic mice appear to be a marginal zone (MZ) B cell population, a population increased in the spleen of BAFF-transgenic mice and thought to participate in the maintenance of germinal centers in the target tissue and subsequent antibody production in SjS (68).

A role for aberrant lymphocyte survival is also supported by studies using the MRL/lpr mouse model (69). MRL/lpr mice carry a mutation in the Fas gene that impairs the Fas/FasL apoptotic pathway. This defect leads to diminished apoptosis, resulting in an abnormal accumulation of lymphocytes, including B cells, and possibly other cells (70). While the MRL/lpr mouse has been mostly used for the study of SLE, it manifests an autoantibody pattern which partially overlaps with that seen in SjS, including anti-dsDNA, anti-ssDNA, ANA and rheumatoid factor (71). In addition, nearly 30% of mice develop anti-52 KDa SS-A/Ro antibodies, 6% develop anti-60 KDa SS-A/Ro antibodies, and 6% develop anti-SS-B/La antibodies (72). MRL/lpr mice also develop sialadenitis of the submandibular, parotid and lingual glands and dacryoadenitis of the lacrimal glands (73). The infiltrates are generally comprised of CD4+ T cells, with lesser numbers of CD8+ T cells and B cells. In addition, there are scattered foci of macrophages and dendritic cells (74). However, the glandular infiltrates appear diffuse and not as the compact, tightly packed foci found in NOD mice. Although lymphocyte infiltrations may cause destruction of exocrine gland tissues, perhaps by iNOS / nitric oxide (NO) (75), tumor necrosis factor (TNF)-alpha and/or various cytokines (76), anti-M3R antibodies have not been observed in MRL/lpr mice, and salivary secretion is normal (77). To put these observations in perspective, it is important to note that, in the SjS-like disease process of NOD mice, a high rate of acinar cell apoptosis occurs in the submandibular glands around 2 months of age, or approximately 2-4 weeks prior to onset of detectable leukocytic infiltration of the salivary glands, and this process is associated with an up-regulation of Fas/Fas-ligand expression by acinar cells (60).

2.2.2. Abnormal exocrine gland development – is it a basis for SjS-like autoimmunity?

In studies of neonatal NOD mice, Cha et al. (60) identified a distinct retardation of acinar cell proliferation
and maturation in the salivary glands up to 3 days post-partum as compared to mice not predisposed to development of SjS-like disease. Concomitant with this delayed organogenesis was an over-expression of interferon (IFN)-gamma in the affected glands. As one function of this cytokine is to retard replication of viruses, the effect can also slow down cellular growth. With this in mind, NOD. Ifn-gamma^-/- and NOD. Ifn-gammaR^-/- (Ifn-gamma and Ifn-gammaR knockout) mice were examined and found to have normal acinar cell proliferation and maturation, as well as normal development of their salivary glands (55). Most impressively, both NOD. Ifn-gamma^-/- and NOD. Ifn-gammaR^-/- mice failed to develop any aspect of SjS-like disease of the salivary glands, including acinar cell apoptosis around 8-10 weeks of age and subsequent leukocyte infiltration of the salivary glands normally observed 10 weeks and beyond. The concept that the delay in acinar cell maturation may prevent expression of cellular antigens at the critical time of self-tolerance resulting from clonal deletion remains an intriguing issue for further study. Thus, it is interesting that the NFS/sld mouse with an autosomal genetic defect that arrests sublingual gland maturation exhibits several characteristics of SjS-like disease, especially the aberrant proteolysis of alpha-fodrin and the production of anti-alpha-fodrin autoantibodies (29).

2.2.3. SjS-like disease – is there genetic susceptibility?

There is little doubt that genetic manipulations of a variety of mouse can either result in the appearance of various disease traits mimicking SjS, delay development of, or prevent the onset of pre-clinical and clinical disease. This is obvious in such mouse lines as MRL/lpr, NFS/sld, the Buff-KI transgenic, the Id3-KO transgenic, and Aromatase-KO transgenic. A remaining question, however, is whether we can identify those regions of the mouse genome that predispose mice to develop SjS-like disease. NOD mice provide an excellent opportunity to investigate this issue because a large collection of congenic mice are available defining the diabetes susceptibility (Idd) loci that predispose this strain to autoimmune type 1 diabetes (T1D). Unlike the genetic predisposition for T1D in both humans and NOD mice which is dependent on specific genes mapping to the major histocompatibility complex (MHC), the genetic predisposition for SjS-like disease in NOD mice appears independent of, or only weakly dependent on, MHC-associated genes. The first indication involved the studies of the congenic strain, NOD.B10-H2^d, in which the NOD MHC I-A^d Idd1 T1D susceptibility locus is replaced by MHC I-A^b (47). These mice, while failing to exhibit insulitis and development of diabetes, continue to show a complete SjS-like syndrome including salivary and lacrimal gland dysfunction. Thus, NOD.B10-H2^d mice were advanced as the first naturally-occurring model for primary SjS (47).

Replacing other diabetes susceptibility loci in the NOD mouse (e.g., Idd10, Idd9, Idd13, and so forth), while lowering the incidence of insulitis and diabetes, proved to have little effect on its SjS-like disease. However, when both the Idd3 and Idd5 loci were replaced with the corresponding genetic intervals derived from C57BL/6 mice, the severity of the biological markers of epithelial cell pathology was reduced and the loss of secretory function reversed (48, 49). In a reverse approach, introducing both the Idd3 and Idd5 genetic regions derived from NOD mice into the SjS non-susceptible C57BL/6 mouse resulted in the appearance of SjS-like disease, confirming the contributions of these two genetic loci to development and onset of disease (48, 49). In follow-up studies, we have now narrowed the genetic region of Idd3 to a centromeric segment approximately 20 cM in size (unpublished data). Furthermore, we have found that the pre-clinical, non-immune aspects of the disease (e.g., increased expression of caspase, matrix metalloproteinase and PSP proteolytic enzymes, increased apoptosis, as well as altered protein secretions) are associated with the Idd5 locus of chromosome 1, while the immunological aspects of the disease (e.g., appearance of activated T and B lymphocytes, increased expression of pro-inflammatory cytokines, appearance of autoantibodies, and loss of secretory function) are associated with Idd3 of chromosome 3. However, recapitulation of the full disease profile requires genes within both these genetic loci. This SjS-susceptible C57BL/6 congenic mouse, currently carrying our designation C57BL/6.NOD-Acr1Aec2 (where Acr1 corresponds to Idd3 and Aec2 corresponds to Idd5), offers many genetic advantages over the NOD mouse model while maintaining the NOD SjS-like disease profile in the absence of susceptibility for diabetes.

3. ROLE OF B LYMPHOCYTES IN SJS-LIKE DISEASE OF MICE

3.1. Development of germinal center (GC)-like lymphocytic foci in the exocrine glands

3.1.1. Lymphocytic foci – are they exocrine gland germinal centers?

Considerable attention has been focused on attempts to define the organization of the immune cell infiltrates, referred to as lymphocytic foci (or lymphoepithelial sialadenitis lesions), which appear in salivary and lacrimal glands with the onset of disease. We and others have reported that these lymphocyte infiltrations within the submandibular and lacrimal glands of NOD mice are composed predominantly of CD4+ T cells with lesser numbers of CD8+ T cells and B cells (61). Subsequently, we have found that these observations appear to be misleading due to the procedures used to isolate the infiltrating cells from the exocrine tissues for flow cytometric analyses. We now believe that vigorous digestion and subsequent purification steps may account for the loss of many fragile cells, thereby leading to possible erroneous conclusions. Recently, we have revisited this question using immunofluorescent staining of paraffin-embedded salivary and lacrimal tissues that not only maintain the native structure of the glands, but retain the cellular organizations. Results strongly indicate that lymphocytic foci are dynamic entities whose cellular compositions and organizations change dramatically as they mature. Thus, to state that the major cell type is a CD4+ T cell grossly over-simplifies the real situation.

As presented in Figure 2, immunofluorescent staining of paraffin-embedded sections of glandular tissues freshly explanted from NOD mice of different ages depicts
Figure 2. Immunofluorescent staining of paraffin-embedded sections of glandular tissues freshly explanted from female NOD/LtJ mice at 8 weeks (A), 16 weeks (B), and 24 weeks (C) showing the changing CD3+ T cells (green) and B220+ B cells (red) profiles within the lymphoepithelial sialadenitis lesions. Panel A magnification = 400X; Panel B magnification = 200X; and Panel C magnification = 100X.

a rapidly changing cellular composition of the lymphocytic foci. During the early stages of lymphocyte infiltration into the salivary glands, small, densely-packed foci appear that are mostly composed of CD3+ T cells virtually free of detectable B cells. As the foci mature, an increasing number of B220+ B cells are detected which histologically appear to surround T cell cores. Over time, the number of T cells decrease relative to the B cells and become more dispersed throughout the B cell areas. However, the lifespan of B lymphocytes within the lymphocytic foci appears short-lived as salivary glands from mice >40 weeks of age show loci once again dominated by T cells (unpublished observation).

An important observation from studies using NOD mice, first reported by van Blokland and colleagues (74), is that dendritic cells (DCs) and possibly macrophages appear to be the first cells to migrate into the glands, resulting in the activation of epithelial cells to express adhesion molecules and produce chemokines that attract T cells, and subsequently additional macrophages and B cells. Most BM8+ macrophages are found to occupy areas around the periphery of the foci and T-cell regions (74). We have recently shown by immunohistochemistry that F4/80 macrophages are also scattered throughout the foci (unpublished data). However, careful examination shows that these well-defined lymphoepithelial sialadenitis-like foci do not appear to mimic ectopic germinal centers. It is still uncertain if these ectopic lymphocytic foci have any biological significance in terms of the clinical manifestation of SjS, but their absence generally results in loss of progression of disease into the clinical phase. It has been reported that the lymphocytic infiltrates are made up of clonally expanded T cells with the TCR repertoire of Vbeta8.1,2, Vbeta6, and Vbeta4 (78). However, it is also unknown if localized or systemic activation results in effector molecules for the clinical development of SjS. Our findings have clearly demonstrated that the infiltrates are mostly B220+ B cells (75%) and a smaller percentage of CD3+ T cells when the disease appears to be most progressive; however, to fully understand the nature of the organization of these lymphocytic foci, additional work is needed to clarify what subpopulations of B220+ B cells and T cells are present in the infiltrates.

3.2. Characteristics of B cells and B cell development in SjS

The life-history of B lymphocytes is now a well-studied and well-documented area on which many reviews have been written (79-82). While it is recognized that T cells play an important role in regulating B cell activation, maturation and subclass antibody production, considerable attention has recently been focused on the role of B lymphocytes in the development and onset of SjS in humans and SjS-like disease in mice. This is due, in part, to reports documenting a number of apparent developmental abnormalities in B cells for both patients and mouse models. However, the stage(s) at which this abnormal B cell behavior is induced and/or how it is maintained remain unknown but probably represent multi-factorial processes, since multiple environmental factors dictate the fate and behavior of B cells, as will be detailed in this section.

3.2.1. Marginal zone (MZ) and B-1 B cells – are they important in SjS?

As previously discussed, mice over-expressing BAFF develop SLE-like disease with significant increases
B cells and autoantibodies in Sjögren’s syndrome

in the number of MZ B cells (67). Interestingly, when these mice are age 16-18 months, they exhibit a SjS-like disease with a subset of B cells in the salivary glands that resemble MZ B cells (68). Similar to Graves disease in which MZ cells infiltrate the thyroid gland (83), NOD mice also develop infiltrations in their thyroid glands (84). While it is not known if these MZ B cells play any role in the pathogenesis of autoimmunity, they may provide interesting insights into how chronic stimulation of auto-reactive B lymphocytes within a tissue eventually induces transformation to lymphomas, as hyperproliferation of MZ B cells is hypothesized to be the cause and source of transformed B cells that often arise in SjS patients and are capable of developing into non-Hodgkin B cell lymphomas (85). Dependent on location, MZ B cells can be induced to transform into splenic MZ lymphomas, nodal MZ lymphomas, and/or extra-nodal MALT lymphomas (85). Therefore, this particular subset of B cells appears to be an important connection between autoimmunity and tumorigenesis in SjS patients.

For B-1 cells, a vast majority are found in the peritoneal and pleural cavities of the mouse, but only 5% of this B cell population is present in the spleen and virtually none are present in the peripheral lymph nodes (86). B-1 cells can be further separated into B-1a cells that express membrane CD5 molecules or B-1b cells that are CD5 negative. The development and survival of B-1 cells are critically dependent on the strength of B cell receptor signaling. Numerous studies in mice involving the genetic disruption (either by deletion or over-expression) of molecules that contribute to the signal transduction pathways of the BCR greatly effects the development of B-1 cell populations. For example, genetic alterations that enhance BCR signaling, e.g., deletion of CD22 or CD72, and over-expression of CD19, lead to significant increases in the number of B-1 cells. In contrast, genetic manipulations that lower BCR signaling, e.g., deletion of CD19, CD21/35 or Vav-1, reduce the number of B-1 cells (87). Therefore, these observations indicate the importance of maintaining homeostasis of BCR signaling to ensure appropriate development, survival or even expansion of this B cell population.

B-1 cells manifest many unique characteristics important to proper B cell function, including longevity with high potential for self-renewal, refractoriness to activation by ligand binding to BCRs, lack of somatic mutation, and restriction of N insertions that result in limited immunoglobulin gene repertoires (88). Since B-1 cells arise from fetal precursors, they express limited Ig gene repertoires, mostly restricted to “natural” IgM, where the term “natural” indicates the production of IgM subclass of antibody in the absence of stimulation by exogenous antigens (89). Therefore, B-1 cells produce antibodies that are predominantly autoreactive, but highly polyreactive with numerous naturally or evolutionarily conserved pathogen-associated carbohydrate antigens, such as phosphorycholine (PC) (90), phosphatidyl choline (PC) (91) and lipopolysaccharide (LPS) (92), indicating B-1 cells are critical for protection against environmental antigens (93,96).

A correlation between the functions of B-1 cells and systemic rheumatic autoimmune diseases has drawn considerable attention. Several reports have demonstrated that in both humans and mice there is a significant correlation between the frequency of B-1 cells and the eventual development of autoimmune diseases (97, 98). Interestingly, dramatic increases in the number of B-1 cells are found in human patients with SjS (99) and RA (100). While B-1 cells are present in minor labial glands of SjS patients (101), it is not certain whether B-1 cells can initiate autoimmunity or provide protection against it. In the latter case, it is important to note the similarity between B-1 cells and anergic B cells, including failure of BCR-antigen stimulation to induce Ca ++ influx (102), expression of low levels of CD5 (103), and expression of high levels of the transcription factor NFATc (104, 105). Therefore, it has been speculated that B-1 cells may induce tolerance of potentially autoreactive B cells rendering them anergic or nonpathogenic. On the other hand, B-1 cells are often autoreactive, producing autoantibodies with low affinity compared to conventional B cells, thereby possibly being involved in initiating autoimmunity (88). A smaller number of this B-1 cell population, however, can migrate to and reside in the germinal centers where they will receive signal from helper T cells, the consequence of which is an induction of class switching and somatic hypermutation (106). This newly emerged set of B-1 cells can then give rise to high affinity autoantibodies that are potentially pathogenic.

### 3.2.2. The B cell receptor – is there a restricted expression in SjS-like disease?

A hallmark feature of B cells in SjS, is the relatively restricted B cell receptor (BCR) repertoires found on exocrine gland-infiltrating B cells, as well as the MALT-associated B cell lymphomas that develop in a subset of SjS patients. In a recent study by Kaschner et al. (107) involving three patients, a significant over-representation of specific V Light-chains were observed. Four V Lambda genes (2A2, 2B2, 2C and 7A) represented 56% of all functional V LAMBdAs. In the productive V Kappa repertoires, three genes (L12, 012/02, and B2) represented 43% of all V Lambda Kappas. Interestingly, V Kappa A27, a gene frequently found on autoantibodies, rheumatoid factor and lymphomas in SjS patients, was identified at an increased frequency of 29% in the parotid gland compared to only 8% in the peripheral blood. In addition, significant enrichment of V Kappa A19 and V LAMBdA2E, specifically the clonal expansion of V Kappa A27- J kappa 5 and V Kappa A19-J kappa 2 has also been reported in the parotid glands of SjS patients (108). Since B cells take advantage of receptor editing to escape apoptosis and prevent recognition of self-antigen, and because there is a marked decrease in receptor editing observed in primary SjS patients, this restricted BCR V-region usage may be a result of a defect in receptor editing. However, also influencing this restricted V-region usage may be the reported depletion of memory B cells from the peripheral blood with a concomitant elevated level of antigen-activated B cells in the parotid glands (107), an observation implying a defect in selection. This peripheral memory B cell population, characterized as CD19+/CD27+, was found to have a mutational frequency of 8.6% in SjS patients in the
immunoglobulin VH transcripts compared to 4.3% in normal healthy controls, as well as an elevated mutational frequency in Cmu transcripts for multiple Ig heavy chain isotypes (109). Not addressed by these data is whether the restricted use of BCR repertoires is due to general innate selection mechanisms for B cell survival or an antigen-driven expansion and survival of a restricted receptor-bearing B cell population.

3.2.3. The B cell receptor/co-receptor system – does it play a role in development of SJS?

Activation of BCR-associated signaling pathways is critical to the generation of humoral immunity as these signaling pathways determine B cell proliferation, differentiation, selection, survival and eventually function. B cell function, however, is also regulated by B cell surface co-receptor molecules, which include CD19, CD21, CD22 and complement component C3d. These co-receptors modulate the intensity, quality, and duration of the BCR signal transduction pathways. The BCR complex is made up of membrane-bound immunoglobulin (Ig) associated with CD79a and CD79b, two heterodimers of Igα and Igβ. Following interaction of the BCR with a ligand, protein tyrosine kinases (PTKs), including Syk and Btk of the Tec family and Lyn, Fyn, Btk, and Lck of the Src family, are activated. In addition, the BCR is fine-tuned through a crosstalk between PTKs, protein tyrosine phosphatases and adapter proteins such as B cell linker (BLNK) and BAM32 (B lymphocyte adapter molecule of 32 kDa) (see references (110-112) for a comprehensive discussion of this signal transduction pathway). At the same time, the co-receptor molecules modify the intrinsic intracellular signal transduction threshold by adjusting the strength of the signals needed to initiate BCR-mediated activation. CD19, CD21 and CD22, are functionally linked with Lyn, Vav, and SHP1 in a common signal transduction pathway that is initiated by BCR binding to its ligand.

The ligand for CD19 remains undefined; however, CD19 can form a non-covalent quaternary complex on the surface that includes CD21, CD81 and CD225. CD81 is a member of tetra-spans family that is involved in regulation of cell growth, mobility and signaling. CD81 is physically associated with CD225, whose precise function is unknown but thought to be involved in anti-proliferative activity regulated by interferon and B cell growth (111). CD21 is the receptor for C3d, a cleavage product of C3 that forms covalent bonds with foreign antigen and immune complexes. It is also a receptor for Epstein Bar Virus (EBV), long considered to be involved in SJS. The ability of C3d-antigen complexes to cross-link the CD19/CD21/BCR complex provides a possible link between innate and adaptive immunity (113), functioning as a positive regulator of B cell activation because signals generated by the co-ligation of CD19/CD21 and BCR are additive. Co-ligation of BCR and CD19/CD21 by a C3d-antigen complex lowers the threshold for B cell activation, thus lowering the amount of antigen required to activate B cells (114). Currently, there are at least two models for the CD19/CD21 complex in its function as a regulator of transmembrane signals. The “co-stimulatory” model postulates that transmembrane signaling results from cross-linking between the BCR and CD19/CD21 by the simultaneous binding of C3d-antigen complexes. The “response regulator” model postulates that the BCR independently transmits the transmembrane signal, whereas CD19 merely regulates intrinsic levels of Lyn and Vav phosphorylation and activation. In this model, an association between CD21 and C3d-antigen complexes promotes the intrinsic functions of CD19 (111).

In opposition to CD19/21, CD22 plays an important inhibitory role in B cell activation. The cytoplasmic domain of CD22 contains six tyrosines located in the three Immunoreceptor Tyrosine-based Inactivation Motifs (ITIMs) and two ITAMs, suggesting the potential for both negative and positive signaling. Phosphorylated CD22 recruits the phosphotyrosine phosphatases, SHP1 and SHIP, to limit BCR signaling (115); thus, CD19/CD21 and CD22 are reciprocally regulated by each other. SHP-1 and SHIP, when bound to CD22, dephosphorylates CD19 and BCRs thereby down-regulating CD19 and BCR function. In addition, SHIP can convert PIP3 to PI3,4-P2 which down-regulates the PI3K-dependent pathway (116).

3.2.4. The B cell receptor/co-receptor system – is it altered in SjS-like disease-susceptible NOD mice?

In light of the above discussion, a major question is whether B lymphocytes and pathogenic autoantibodies play a major role in SJS (and other diseases like SLE) due in part to a breakdown in B lymphocyte self-tolerance (112, 117). One mechanism that controls survival, activation and proliferation of B cells is through cross-linking of B cell receptors and their co-receptors, especially CD19 and CD21. Cross-linking of BCRs and co-receptors that results in the hyperproliferation of B cells involves C3d (118). To investigate this issue in greater detail, we have turned to the NOD.B10-H2b mouse, our model of primary SJS exhibiting many of the immunological manifestations observed in SJS patient (47). We have shown that NOD.B10-H2b mice exhibit reduced apoptosis, hyper-proliferation and over-activation of B lymphocytes starting around 10 weeks of age. The result is the production of both organ-specific and organ-nonspecific autoantibodies.

In initial studies, we reasoned that inactivation of complement component C3 might prevent this over-reactivity of B cells and reduce the severity of the SJS-like disease in NOD.B10-H2b mice by preventing production of C3d and reducing the cross-linking of BCRs and their CD19/CD21 co-receptors. Treatment of NOD.B10-H2b mice with cobra venom factor (CVF), known to deplete C3 from circulation, while not preventing the aberrant physiological activities of pre-clinical disease (e.g., activation of unique serine protease), reduced the severity of lymphocyte infiltration into the salivary glands, the production of autoantibodies, and the degree of salivary gland dysfunction (119). Interestingly, this reduction in clinical disease severity correlated with significant reduction in the co-expression of CD19/CD20 and CD21 on the B cell subpopulations. No major changes were noted in the CD22 expression levels of CD19 B cell subpopulations (119).
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Although the results of this study revealed a direct correlation between C3-depletion, loss of CD19<sup>hi</sup>/CD21<sup>hi</sup> B cell subpopulations and reduced autoimmunity in CVF-treated NOD.B10-H<sup>2</sup> mice pointing to the possible importance of C3d cross-linking between the BCR and its co-receptors, other explanations are also possible. First, it may not be possible to rule out any involvement of the membrane-attack complex formation since NOD mice are C5-deficient (120). Second, the importance of C3 in both innate and adaptive inflammatory responses cannot be underestimated. C3a is a critical mediator of inflammatory responses, especially in recruiting monocytes/macrophages to the site of cell injury. Furthermore, C3 products can bind to or form complexes with antigens to facilitate inflammatory and immunological responses, in part through binding to specific complement receptors, such as CR1/CR2 present on follicular dendritic cells (FDCs). Such localization of antigen on FDCs in secondary lymphoid tissues promotes germinal center formation, B cell retention, survival and activation within germinal centers, as well as subsequent antibody formation (121-123). Thus, reduced ectopic germinal center-like formation in the salivary glands of CVF-treated animals could be due to reduced levels of functional C3 affecting FDC secretion of chemokines that would normally recruit B lymphocytes to the germinal centers.

The role of complement in either development or severity of SjS has not been easily defined, resulting in conflicting reports appearing over the past two decades. Molina et al. (124) reported that complement levels in SjS patients with neutrophil inflammatory vascular disease were decreased, while Thomsen et al. (125) reported increased levels in primary SjS patients. Both of these reports, however, differed from the earlier study by Fischbach et al. (126) indicating that SjS patients exhibited normal levels of complement. Our current concept of why C3 might be important in SjS focuses primarily on the role that C3d plays in cross-linking BCRs with the co-receptors CD19/CD21 molecules or CD21/CD35 through CR1/CR2. As stated above, C3d fragments, when bound to antigen, provide powerful secondary signals within B lymphocytes by co-ligating the CD19/CD21 complex with BCR, thereby greatly lowering the signaling threshold of B cells. This, in turn, enhances the BCR-associated signal transduction pathways, effectively enhancing the response of B cells to antigen (123). In support of this concept, NOD.B10-H<sup>2</sup> mice that naturally produce B cells with high CD21/CD35 expression levels lose this subpopulation of B cells following treatment with CVF to deplete functional C3 levels. Whether loss of this population is through apoptosis during B cell development or a specific down-regulation of these C3d receptors remains to be determined.

3.3 Co-stimulatory molecules regulating B cell activities

3.3.1 Differentially expressed genes in exocrine glands – do they depict impending disease?

Taking advantage of the C57BL/6.NOD-Aec1Aec2 mouse model, we have compared the gene expression profiles of submandibular glands derived from 8 and 12 week old mice with those derived from 8 week old C57BL/6 mice using oligonucleotide microarrays, thereby focusing on genes differentially expressed in the pre- or early-disease stages. Of interest has been the identification of altered gene expressions occurring between 8 and 12 weeks of age. One temporal inverse relationship discovered by our microarray involves trafl3 versus trafl6 gene expression. At 8 weeks, trafl6 gene expression is up-regulated, but down-regulated at 12 weeks, whereas trafl3 gene expression is down-regulated at 8 weeks and up-regulated at 12 weeks. In a recent study, Häcker et al. (127) provided evidence that these two TRAF molecules are involved in controlling diverse signaling pathways. Specifically, TRAF3 is essential for the induction of type 1 IFNs and the cytokine IL-10, most likely through the recruitment of TBK-1/NAK. Interestingly, our microarray data indicate that both IL10ra and IL10rb genes are up-regulated at 12 weeks of age (unpublished data). In addition, TRAF3 appears to be an important downstream transduction signal for BAFF-BAFFR (128), APRIL-BCMA (129), CD70-CD27 (130), CD40L-CD40 (128) and LIGHT-HVEM (131), five systems thought to play a role in the pathogenesis of SjS. Since early signs of an overt autoimmune attack against the submandibular glands of C57BL/6.NOD-Aec1Aec2 mice can be observed by 12 weeks of age, including the appearance of leukocytes, it is important to note that three genes of the TNF superfamily, 

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Expression Change</th>
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<tbody>
<tr>
<td>tnfrsf19</td>
<td>up-regulated</td>
</tr>
<tr>
<td>tnfrsf13</td>
<td>BAFF receptor</td>
</tr>
<tr>
<td>tnfsf14</td>
<td>LIGHT</td>
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</tbody>
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were up-regulated in the submandibular glands at 12 weeks as compared to 8 weeks of age, suggesting active signaling processes occurring within infiltrating immune cells, the salivary gland epithelial and/or neural cells per se. BAFF, as discussed below, is known to regulate lymphocyte survival and activation (132). BAFF binds to three receptors, BAFF receptor (BAFF-R), transmembrane activator and cytophilin ligand interactor (TACI), as well as B cell maturation antigen (BCMA) known to be closely associated with the development of germinal centers in the salivary glands of SjS patients (133, 134). An up-regulation of Nik, IKK and NF-κB down-stream of TACI and BCMA or Ppard, Cnnt1, Poll and Dyrk1 associated with proliferation may account for the increased B cell maturation and prolonged survival observed in both SjS patients and C57BL/6.NOD-Aec1Aec2 mice. Interestingly, a recent microarray analysis of minor salivary gland tissue derived from human SjS patients also found up-regulated BCMA (Tnfrsf17) expression (135) consistent with our data from the mouse model. Similarly, LIGHT, which binds to the TR2 receptor of CD68-positive macrophages, induces the phosphorylation of IkB and nuclear translocation of NF-κB (136, 137). One potential consequence of LIGHT signaling, as observed in rheumatoid arthritis (137), might be the up-regulation of matrix metalloprotease (MMP)-9 expression, and MMP9 has been shown to be actively induced in the submandibular glands of NOD mice during the disease state (138, 139). Lastly, an up-regulation of tnfsf19 (Taj/TROY) may indicate an involvement of neural tissue, and studies suggest that the parasympathetic neural system along with the muscarinic acetylcholine type-3 receptor are probable targets of the autoimmune process (63).

3.3.2 The CD70 – CD27 system

The CD70 / CD27 co-stimulatory system appears to play an important role in the activation of both T and B
lymphocytes, especially at the level of T cell priming during contact with DCs as well as subsequent T cell proliferation and differentiation during T-T and T-B cell interactions. During initiation of an immune response, CD70 expression on DCs is induced following ligand binding of their Toll-like receptors (TLRs) and/or CD40 membrane-bound molecules. Interaction of these CD70 molecules with CD27 receptors on T lymphocytes, together with either mitogen or TNF-α stimulation, induces rapid expression of CD70 on T cells, in particular CD4+ T cells, which enhances the CD70 – CD27 interactions between CD4+ and CD8+ T cells. In addition, these activated T cells, in part via their CD27 molecules, can interact with B cell populations whose expression of CD70, similar to that of DCs, is up-regulated by ligand binding to their TLRs and/or CD40 molecules in conjunction with BCR antigen-recognition. As B lymphocytes become activated, they up-regulate expression of CD27, primarily in the centroblast stage as shown for mice. Studies using CD27-deficient mice suggest that this up-regulation of CD27 is important for formation of germinal centers and IgG synthesis. Interestingly, the abnormal B cell stimulation observed in SLE has been reported to be dependent, in part, on an over-expression of T cell-associated CD70 that synergizes with an elevated synthesis of IL-10 to induce hyper-production of IgG. Since expression of both the CD70 – CD27 system and the cytokine IL-10 are up-regulated in our mouse models, it seems likely that a similar mechanism may underly the hypergammaglobulinemia observed in SjS.

### 3.3.3. The BAFF - APRIL system

In recent years, considerable attention has focused on two members of the TNF supergene family, BAFF (aka, BLys, TALL-1, THANK and zTNF4 and APRIL (aka, TALL-2, TRDL-1 and TNFSF13a), and their receptors, BAFF-R (BAFF receptor), BCMA (B cell maturation antigen), and TACI (transmembrane activator and CAML interactor) (141, 142). Both BAFF and APRIL exist as homotrimers and can be found either as membrane bound proteins or as soluble factors following cleavage by proteases, in particular furin family (143). BAFF is expressed by monocytes, macrophages, neutrophils, follicular dendritic cells (FDCs), DCs, activated T cells, and myeloma B cells, whereas APRIL is expressed by macrophages, DCs, activated T cells, and B cell lymphoma (144). Expression of both molecules is up-regulated by various factors, including type I and II IFN, CD40L, LPS and peptidoglycan, especially in DCs and macrophages (145). Both IFN-gamma and IL-10 can stimulate release of BAFF from DCs and macrophages in vitro (146), while IL-4 acts as a negative regulator to down-regulate BAFF expression (147).

While BAFF is thought to enhance survival and proliferation of peripheral B cells in a way that is critical for their maturation process, APRIL is thought to mediate B cell proliferation, in part, due to induction of anti-apoptotic proteins. However, adding to the complexity, both BAFF and APRIL have alternate splice isoforms, delta-BAFF and TWEAK, respectively, that mediate distinct biological functions (148, 149). Transgenic mice expressing 8-BAFF exhibit reduced numbers of B cells with normal T cell-dependent antibody responses and serum immunoglobulin levels, in contrast to transgenic mice over-expressing BAFF. In addition, delta-BAFF tends to form heteromultimers with BAFF resulting in inhibition of Ig secretion. Like delta-BAFF, TWEAK can bind to its isoform partner, APRIL, to create a membrane-bound fusion protein called TWE-PRIL protein consisting of an intracellular, transmembrane and stalk region from TWEAK and the receptor-binding domain from APRIL. The resulting fusion protein, therefore, recognizes the same receptors as APRIL and BAFF, respectively, to inhibit their actions of BAFF and APRIL (TWE-PRIL) participate in regulating B cell proliferation, survival and maturation, if not differentiation, especially of marginal zone B cells (151), resulting in the up-regulation of transcription factor Pax5 activity and the B cell co-receptors CD19, CD21/35 and CD23 (152-154).

On the surface, it is clear that a correlation exists between over-expression of BAFF / APRIL and B cell hyperproliferation in SLE and SjS. Elevated production of BAFF in transgenic mice has been shown to result in many features of SLE, including increased levels of serum immunoglobulin, high titers of anti-DNA antibodies and rheumatoid factors, as well as the presence of circulating immune complexes (67). These mice also exhibit B cell hyperplasia, supporting the role of BAFF in B cell survival and proliferation (155). Interestingly, as these mice age, they develop a SjS-like disease characterized by a severe sialadenitis with MZ B cells, loss of salivary gland tissue and a decrease in stimulated saliva production. However, anti-Ro/SS-A and anti-La/SS-B were not detected (68), differing from reports showing a correlation between the level of BAFF and titers of anti-Ro/SS-A, anti-La/SS-B in sera of SJS patients(134).

### 3.4. Autoantibodies

#### 3.4.1. Auto-antibodies – biomarkers or effectors of SjS-like disease?

In recent years, the nature of various autoantigens detected in connective tissue disorders has been identified using an array of molecular approaches. Many autoantigens have proven to be intracellular enzymes and regulatory factors required for cellular function, e.g., gene replication, transcription, RNA processing and protein synthesis (156). Interestingly, these molecules have little in common in terms of structure, sub-cellular localization or biological function. More importantly, few data are available indicating that antibodies directed against these molecules have any direct effect in eliciting a pathological consequence, for example, loss of fluid secretion by exocrine glands. Nevertheless, antibodies targeting nuclear proteins (ANAs) in SjS, especially the ribonuclear proteins Ro/SS-A and La/SS-B (157), have long been used as a diagnostic marker of disease in both humans and animal models. Different immuno-fluorescence patterns of ANAs have, over time, led to the identification of other nuclear antigens targeted by autoantibodies, such as Sm, dsDNA, the nuclear mitotic apparatus (NuMA), proteasomes, mitotic chromosomal autoantigens (MCAs), and poly-(ADP-ribose) polymerase (32).
Autoantibodies detected in SjS patients and various animal models are not limited to targeting nuclear proteins. One well-studied autoantigen is the intracellular cytoskeletal protein alpha-fodrin, targeted by proteolytic enzymes in the salivary glands of both SjS patients and NFS/sld mice (158). In addition, as NFS/sld mice age, they develop autoantibodies against ssDNA, IgG1 and IgG2a subclass immunoglobulins (RF) and type II collagen (159). Other examples of intracellular autoantigens are tissue kallikrein-1 (Kik-1) and kallikrein-13 (Kik-13), detected in the sera of IQI/Jic mice affected with SjS (~12 wks of age). However, only Kik-13 was shown to induce a proliferative response by splenic T cells (160). IQI/Jic is an inbred strain that originated, like the NOD strain, from the ICR mouse colony in Japan(161) and exhibits spontaneous SjS-like disease that mimics that of NOD mice (66). The fact that Kik-13 is highly expressed in the salivary gland ductal cells may explain target tissue specificity of SjS as it is consistent with periductal infiltration of immune cells. Furthermore, since only reduced forms of Kik-13 are recognized by sera of IQI/Jic mice, one might conclude that cryptic epitopes are involved, not unlike the case proposed for the potential development of autoantibody responses to PSP in NOD mice (162). Lastly, both SjS patients and NOD mice form autoantibodies reactive with islet cell autoantigen-69 (ICA-69) expressed in pancreatic islets, the brain and both salivary and lacrimal glands (163). Disruption of the ICA69 locus in the NOD mouse prevents lacrimal gland disease and greatly reduces salivary gland, suggesting that immunoreactivity against ICA-69 might play a role in disease progression (163).

An intriguing question in autoimmune diseases like SjS pertains to how intracellular components, i.e., self-proteins, become recognized and presented as dominant neo-antigens by immune cells. The recent reviews and journal articles by Rosen et al. (164-166) advance cellular apoptosis as an initial event. In this work, Rosen and colleagues describe how molecules within the subcellular compartment are redistributed in apoptotic cells. Small membrane blebs could be shown to contain 52-kDa Ro and other molecules, such as calreticulin, normally present within the ER lumen. Nuclear antigens also exhibit redistribution during apoptosis, showing an increase in localization of 60-kDa Ro/SS-A, La/SS-B, the snRNPs, Ku and PARP as a rim around the condensing chromatin (166, 167). Such clustering of potential autoantigens occurs during apoptosis, but not during necrosis (168, 169). Although the degree of acinar cell apoptosis in the salivary and lacrimal glands is not well-established or universally accepted, proteolysis, phosphorylation, glutathiolation, transglutamination, citrullination, and/or formation of novel protein-protein or protein-nucleic acid complexes probably play a role in the alteration of molecular structures permitting exposure of neo- or cryptic epitopes to the immune system (168, 170, 171).

The importance of T lymphocytes in the activation, proliferation and differentiation of antigen-reactive B cells in autoimmune-prone mice is nicely demonstrated in Id3-gene KO mice (172-174). The Id proteins bind basic helix-loop-helix transcription factors and function as dominant negative inhibitors of gene expression. Id3 is an immediate early-response gene regulating growth and is involved directly in TCR-mediated T cell selection during T cell development in the thymus. In the absence of T cells, these Id3-deficient mice failed to exhibit development of a SjS-like disease. One fascinating observation in this mouse model is the appearance of secretory dysfunction as early as 6 weeks of age, a time point prior to other visible disease symptoms. Since Id3 is known to be a Smad4-dependent TGF-beta responsive gene whose pathway is important for salivary gland development (173), the possibility exists that organogenesis of the salivary glands may be impaired in this Id3-gene KO mouse resulting in aberrant antigen presentation and activation of autoreactive lymphocytes. We have speculated that delayed organogenesis of the salivary glands in NOD mice is critical for subsequent development of SjS-like disease in the NOD mouse (55, 60). Since Id3-deficient mice with various genetic backgrounds develop a SjS-like disease characterized by the synthesis of anti-Ro/SS-A and anti-La/SS-B antibodies at approximately one year of age (174), whereas NOD mice are not known to make anti-Ro/SS-A and anti-La/SS-B antibodies, multiple mechanisms must be imprinting the antibody responses in these different mouse models.

3.4.2. Anti-muscarinic acetylcholine type-3 receptor autoantibodies – are they effectors of glandular dysfuntion?

Although the loss of saliva and tear flow in SjS was initially believed to be a consequence of acinar cell apoptosis elicited by cytotoxic T lymphocytes, an interesting paradigm shift has occurred based on studies showing the requirement for B lymphocytes and immunoglobulin. First, B cell deficient NOD.Igmu-/- mice fail to develop secretory dysfunction, and second, IgG from SjS patients can induce a reversible stimulation or inhibition of salivary function when infused into NOD-scid mice (64). Accumulating evidence suggests that disturbances in lymphocyte homeostasis, including ectopic germinal center formation in the target tissue and/or aberrations of cellular signaling regulated by B cell activating factor (BAFF), are present in SjS (6, 68, 109, 133, 134, 175-177). As shown in both transgenic mice over-expressing BAFF and patients with SjS, B cell hyperactivity may lead to excessive immunoglobulin production and prolonged B cell expansion that eventually lead to the monoclonal expansion of B cells and transformation to B-cell lymphomas in a subset of patients (51, 52). However, intrinsic defects in the B cell compartment associated with SjS may play a role in the generation of SjS autoantibodies with diversified prevalence and specificity, as evidenced by the wide array of autoantigens targeted. Thus, identification of which autoantibodies that directly cause dryness in SjS patients is essential for understanding the pathogenesis and onset of clinical disease.

Of the many autoantibodies identified in SjS patients to date, the association between anti-M3R autoantibodies and secretory dysfunction seems most intuitive since the M3R is the major receptor mediating secretion in the salivary and lacrimal glands in response to
parasympathetic stimuli. Studies strongly indicate that serum or purified IgG from SjS patients can down-regulate carbachol-evoked bladder muscle contraction by 50%, while anti-idiotypic antibodies can neutralize this inhibition of cholinergic neurotransmission (35, 38, 178). Furthermore, anti-idiotypic antibodies were able to neutralize autoantibodies that inhibit cholinergic neurotransmission (178). Similarly, studies using the human salivary gland ductal cells, HSG, showed that pretreatment of the cells with SjS IgG for 12 or 24 hours reduced the magnitude of subsequent carbachol-induced intracellular calcium release (37).

In a recent study, Cha et al. (36) reported that M3R desensitization occurs in mice with anti-M3R autoantibodies, as revealed in a comparison of carbachol-evoked responses in NOD mice >20 weeks of age versus either age-matched C57BL/6 or antibody-negative 8-10 week old NOD mice. These observations, therefore, would be consistent with the hypothesis that chronic stimulation by anti-M3R antibody induces an inhibitory effect on M3Rs. Importantly, NOD mice with overt SjS-like disease initially responded well to pilocarpine-induced stimulation; however, this stimulation was down-regulated following chronic injections. We currently interpret these results as an augmented desensitization of M3Rs in the presence of anti-M3R autoantibodies. Extrapolating these data to a clinical setting, chronic intake of pilocarpine might enhance saliva secretion initially, yet may induce eventually a more rapid desensitization in human patients positive for anti-M3R autoantibodies.

Another interesting observation is the mixed response profiles obtained when bladder strips isolated from normal, healthy C57BL/6 mice were incubated with sera from a number of different SjS patients. While a few sera enhanced smooth muscle contraction in comparison with either normal sera or Krebs physiological buffer, other sera inhibited the contractions. This could represent effects of different antibody titers, length of incubation time, and/or different titers of a pathogenic subset of anti-M3R autoantibodies. In light of recent studies (discussed below), SjS-like disease may be dependent on anti-M3R autoantibodies of a specific isotype (54). If this is true, then it will be necessary to determine if its inhibitory activity on acinar cell secretion is dependent on the constant region of an immunoglobulin subclass or relies on a variable region whose specificity is shaped by the constant region.

3.5. Role of IL-4 in onset of SjS-like disease of NOD mice

3.5.1. Onset of SjS-like disease in NOD mice – is it dependent on IL-4?

In recent studies, we investigated the role of the cytokine IL-4 in the development of SjS-like disease of NOD mice using two IL4-gene KO mouse lines, NOD.IL4−/− and NOD.B10-H2b.IL4−/−. Two critical characteristics of SjS-like disease were found to be absent in the IL4-gene KO mice. First, there was no temporal loss of pilocarpine/isoproterenol-stimulated saliva flow rates. As a consequence, the protein concentrations of the saliva remained within normal ranges, as did the measured amylase activities. Second, there was no temporal increase in the number of IgM- and IgG1-positive B lymphocytes at the age when the autoimmune response would be developing rapidly (unpublished data). Taken together, these phenotypic characteristics suggest that NOD.IL4−/− and NOD.B10-H2b.IL4−/− mice express a similar disease phenotype, solely dependent on the role of IL-4 in development of the SjS-like autoimmune exocrinopathy. As a cytokine directly involved in B lymphocyte growth, proliferation, survival and differentiation, as well as regulation of IgG isotype switching (179-185), IL-4 plays many important roles in immune responses, several of which are clearly critical to the pathogenesis of SjS-like disease of the NOD mouse.

One interesting point in these studies was the fact that a temporal increase in the relative number of B lymphocytes in the spleens of disease-prone NOD.B10.H2b mice (from 44.3% to 51.5%) could be detected due primarily to an increase in IgM- and IgG1-positive B cells. Further, this increase in B cells correlated directly with the activation of the autoimmune response and onset of salivary gland dysfunction. A similar increase in the splenic B cell population did not occur in NOD.B10-H2b.IL4−/− mice (remaining constant at 43.2% versus 41.3%, respectively). Thus, the absence of a functional IL4 gene appeared to reverse the process of B cell proliferation and survival, correlating with retention of normal secretory function.

A second interesting point was the possible time-dependent expression of IL-4 for development and onset of the SjS-like disease in these NOD mice. First, detection of IL-4 mRNA expression in parental NOD.B10-H2b mice is restricted to a time around 12 weeks of age. Second, onset of secretory dysfunction in NOD.B10-H2b.IL4−/− mice subsequent to adoptive transfer of T cells from NOD.B10-H2b.Gfp mice occurred if the adoptive transfers were carried out prior to 12 weeks of age and/or disease activation, but not if carried out at 16 weeks of age after disease activation. Consistent with these observations, gene expression profiles for the IL-4-associated STAT6 and IRS-PI3K signal transduction pathways in the B cell population of NOD.B10-H2b.IL4−/− mice at 12 weeks of age were reduced compared to those in B cells of disease-prone NOD.B10-H2b mice. These two pathways are important in cell proliferation and survival, as well as isotype switching from IgM to IgG1 (186, 187).

A third point of interest regarding the role of immunoglobulins in SjS-like disease was the general observation that the degree of leukocytic infiltration within the submandibular and lacrimal glands of NOD.B10-H2b mice occurs at an earlier age and becomes more intense, as measured by the number of foci and the area of infiltration, than that observed in NOD.B10-H2b mice. Despite the fact that the submandibular glands can be nearly overrun by infiltrating lymphocytes in NOD.B10-H2b.IL4−/− mice, the capacity to secrete normal levels of saliva persists, opposite of what is observed in parental NOD/Lt or NOD.B10-H2b mice, where onset of glandular dysfunction occurs concomitantly with the lymphocyte infiltration. These differences strongly suggest a major role for a systemic soluble factor, like IgG, in the onset of xerostomia rather than a lymphocyte-acinar cell contact. Although IL-4...
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Figure 3. Proposed cellular inter-relationships between the development of autoimmunity and subsequent transformation of reactive B cells to extra-nodal MALT-like B lymphomas and high grade B lymphomas.

may merely control IgG isotype switching, it is also possible that it permits survival and persistence of a unique autoimmune B cell subpopulation expressing self-reactive BCRs.  

3.5.2. SjS-like disease in NOD mice – is it IgG subclass-specific?  
Despite the multi-faceted activities of IL-4, there is no indication to date that IL-4 plays any other role in the loss of secretory function of NOD mice than enhancing synthesis of an IgG1 isotypic anti-M3R autoantibody. The concept that the effector molecule of clinical disease may be restricted to IgG1 isotypic anti-M3R autoantibodies is supported by the observation that sera of both NOD.B10-H2b and NOD.B10-H2b.IL4-/-/ mice contain IgG2a/c, IgG2b and IgG3 anti-mM3R autoantibodies (detected on transfected CHO cells expressing the mouse M3R), but only the sera of NOD.B10-H2b mice contain anti-M3R autoantibodies of IgG1 isotype. In addition, adoptive transfer into NOD.B10-H2b.IL4-/-/ mice of T cells capable of IL-4 synthesis at either 4 or 10 weeks of age, but not at 16 weeks of age, resulted in production of IgG1 isotypic anti-M3R autoantibodies and subsequent decline in salivary secretory activity. Whether the other isotypic anti-M3R autoantibodies, especially those of the IgG3 isotypic, contribute to disease still needs to be determined. Furthermore, why the NOD mouse develops a humoral response against the exocrine gland acinar cells and autoantibodies reactive with the M3R remains unknown; however, we have speculated that delayed organogenesis of the submandibular glands in mice of the NOD genetic background may permit the retention of anti-self B lymphocytes reactive with membrane-associated proteins, like M3R, due to delayed expression (60). This would result in circumvention of clonal deletion and/or self-tolerance (188, 189). Alternatively, activation of the autoimmune response may occur through delayed expression of antigens such as endogenous neo-antigens or viral proteins (190, 191).

3.6. SjS as a cross-over disease  
SjS is considered a lymphoproliferative disorder in which a B lymphocyte infiltrate of the salivary and lacrimal glands, while initiating as a polyclonal response, selectively expands into monoclonal B cell populations that in about 5-10% of patients transform to mucosa-associated lymphoid tissue (MALT)-associated B cell lymphomas (192). In some patients, there is a gradual progression from low grade MALT lymphomas to high grade lymphomas (53). The polyclonal and monoclonal proliferations of autoreactive B lymphocytes in SjS lead to the production of organ-specific and organ-nonspecific autoantibodies that correspond to the progression of the disease development (157). Transformation of B cells is thought to be the consequence of constant antigenic stimulation of B cells, possibly in conjunction with the inactivation of molecular systems, like p53, and concomitant activation of bcl2 (193). This proposed process is pictured in Figure 3.

Histologically, the salivary gland infiltrates appear as lymphoepithelial sialadenitis lesions in SjS patients as well as patients having gastric MALT lesions associated with Helicobacter pylori (194). Because many salivary gland-associated MALT lymphomas tend to express similar immunoglobulin heavy and light chain variable genes, a feature not observed as readily in other tissue-associated MALT lymphomas, it is assumed that the B lymphocyte populations in SjS that eventually undergo transformation recognize a restricted number of similar epitopes while establishing GC-like structures with expanded marginal zones. As stated earlier, MALT B cell lymphomas are believed to arise from the MZ B cell populations expressing CD19, CD20 and CD22, but usually not CD5 and CD10. Since MALT lymphomas, in general, remain fairly localized, they may be dependent on continuous antigen stimulation of the BCR complex. Interestingly, correlations between the formation of lymphoepithelial sialadenitis and infection with certain viruses, e.g., retroviruses and hepatitis-C virus, have been
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advanced (195, 196). Recent data have suggested a potentially important role of BAFF/APRIL signaling in the transformation of B lymphocytes. BAFF may be produced by malignant B cells as an autocrine factor that is essential for survival (197, 198). On binding to cellular receptors, BAFF/APRIL can activate NF-kB, phosphatidylinositol-3 (PI-3) kinase (AKT), and mitogen-activated protein kinase (MAPK) pathways that enhance cell proliferation and survival. In addition, BAFF/APRIL up-regulates anti-apoptotic molecules (specifically Mcl-1 and Bcl-2) to evade programmed cell death (199-201). Under these conditions, and in conjunction with a constant antigenic stimulation, there is an increased likelihood of B cell transformation. In support of this concept, serum levels of BAFF is elevated in patients with systemic autoimmune diseases and non-Hodgkin B cell lymphomas. In addition, the expression levels of BAFF receptors (i.e., BCMA, TACI, and BAFF-R) are highly correlated with the transformation stage of B cells and severity of disease (197). Unfortunately, this issue has not been studied in any detail in mouse models of SjS-like disease, although Batten et al. (202) have shown that BAFF transgenic mice lacking TNF-α exhibited a 35% greater frequency of developing B cell lymphoma.

4. PERSPECTIVES AND COMMENTS

4.1. Role of B lymphocytes in SjS

In summary, SjS is considered a lymphoproliferative disorder that progresses through various stages of disease, possibly best defined using mouse models like the NOD. Although the infiltrates observed in the salivary and lacrimal glands begin as collections of CD3 T lymphocytes, they quickly involve an increasing number of B lymphocytes thought to initiate as a polyclonal lymphocyte infiltration that over time selectively expand into ever increasingly monoclonal B cell populations. Within a few patients, these chronically stimulated B lymphocytes can transform to low grade MALT lymphomas (192) and even progress into high grade lymphomas (53). The polyclonal and monoclonal proliferations of autoreactive B lymphocytes lead to a state of hypergammaglobulinemia characterized by the production of organ-specific and organ-nonspecific autoantibodies corresponding, for the most part, to the progression of disease development (157). The presence of autoantibodies against intracellular ribonuclear proteins, e.g., anti-Ro/SS-A and anti-La/SS-B, has major implications for diagnostic biomarkers and patient classification (17), while the precise role in the pathogenesis of others, such as anti-alpha-fodrin (29), anti-carbonic anhydrase (31), anti-nuclear specificities (203), and especially anti-M3R (204, 205), is still unknown. The possibility that anti-muscarinic receptor autoantibodies may be effectors of SjS is supported by the studies of Robinson et al. (46) wherein IgG fractions of sera obtained from SjS patients or NOD mice with overt disease suppressed stimulated salivary flow rates when infused into normal healthy mice, and Brayer et al. (36) wherein the inability to produce anti-M3R antibodies of the IgG1 subclass correlated with the absence of clinical disease.

In general, whether SjS is a T cell or B cell disease is a matter of perspective. All autoimmune diseases appear to require activation of T cells, whether directly by self antigens or indirectly by environmental antigens that mimic self-antigens. However, the clinical phases of many autoimmune diseases rely on the production of autoantibodies by B cells, perhaps best depicted by Graves disease. Based on the studies emerging from NOD mice and other mouse models of SjS, the onset of clinical SjS-like disease appears to be both a time-dependent and an IgG-subclass dependent event, supporting the importance of both T and B cells. A serious question raised by studies in mouse models still requiring investigations in both mice and humans is whether T and B cells effect clinical disease at different stages, as T cells tend to dominate the glandular lesions early and late while B cells tend to dominate at an intermediate phase. Thus, like the T cell, the B cell cannot be regarded merely as a by-stander in SjS. As such, B cell development is stringently regulated by several mechanisms, including receptor editing, apoptosis and anergy, populations of autoreactive B lymphocytes can escape tolerance-inducing mechanisms. These autoreactive B cells can become hyper-proliferative, capable of evading apoptosis, sensitive to activation and eventually mature to produce autoantibodies (112). In addition, hyperproliferation of B lymphocytes contributes to an approximately 4-17 fold increase in the production of gammaglobulins compared with normal individuals (206). Clearly, then, future therapies for SjS could target multiple aspects of B cell activity.

4.2. Importance of mouse models in the study of B lymphocytes in SjS

It is important to remember that, from a genetics perspective, any one inbred mouse model represents, at best, a single individual with a disease process dictated by a specific genetic pre-disposition and an immunological system shaped by years of antigenic influences. Considering the complexity of a disease like SjS, it is unlikely that the NOD mouse, or any of the other mouse strains used as models of SjS, will present a complete picture of the various underlying mechanisms of the disease and the possible genetic and environmental modulators that result in the wide variations in patients presenting with SjS. Nevertheless, a compilation of the various factors involved in the development and onset of disease should begin to unravel the nuances of the biological processes that regulate this particular autoimmune response. Already, multiple correlations between SjS in humans and SjS-like disease in various mouse models have been identified that suggest some common bases. These include genetics as well as pathophysiological and immunological aspects. Further studies using the mouse models currently available and under construction should increase this knowledge base, which in the end may lead to a surprisingly simple intervention therapy, despite our current concept of the complexity of SjS.

5. ACKNOWLEDGMENTS

We wish to thank Dr. Jue-hua Gao, Dr. Smruti Kiledar and Ms. Janet Cornelius for their contributions to many of the studies reported herein. This work was supported in part by PHS grants DE55304, DE013769,
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DE014344, DE015152 and AI47483 from the National Institutes of Health.

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Key Words: Autoantibodies, Autoimmune exocrinopathy, B lymphocytes, Mouse models, NOD mice, Sjögren’s syndrome, Review

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